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Editors

Clinical Cardiogenetics

Second Edition

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Preface

Dear colleague,

Five years have passed since the release of the first edition of Clinical Cardiogenetics. In the highly dynamic field of cardiogenetics, revolutionary changes occur almost on a day-to-day basis. Therefore, a second edition has been long overdue. With contributions from worldwide leading experts in their fields, we feel this textbook will provide an up-to-date and useful reference for those interested in the field of cardiogenetics. Since the first edition, the field has changed dramatically by advances in technological possibilities such as the wide availability of next-generation sequencing allowing for sensitive and rapid analysis of a large number of disease-associated genes simultaneously. In addition, powerful new tools, such as the CRISPR-Cas9 system allowing rapid gene editing, have led to a revolution in understanding the molecular basis of genetic disorders. Increasingly, the outcome of genetic testing influences management and follow-up of patients with hereditary disorders, for example, in screening for intracranial aneurysms in a patient with an *ACTA2* or *TGFBR1* mutation. Another example of the advances in the field is provided by the exciting study by Green et al. who show that inhibition of sarcomere contractile function by a small molecule in transgenic mice supressed the development of ventricular hypertrophy, cardiomyocyte disarray and myocardial fibrosis and attenuated hypertrophic and profibrotic gene expression.[1] Their study indicates that, in the near future, alteration of the progression of HCM, a disease estimated to affect 1 in 500 people worldwide, may be feasible, whereas contemporary pharmacological treatment only provides symptom relief. The emergence of these novel techniques also poses questions in the fields of medical ethics, community genetics, and challenges in the interpretation of the ever increasing amount of variants of unknown clinical significance found by genetic screening in index patients and their relatives. We expect the second edition will provide the reader the means to cope with these challenges in their day-to-day work.

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[1] E.M. Green et al. Science 351(6273):617–21, 2016.

Contents

Part I

Genetics

Introduction to Molecular Genetics

1

Jan D.H. Jongbloed, Ronald H. Lekanne Deprez, and Matteo Vatta

Abstract

In the last decades, molecular genetics has been rapidly integrated into the diagnostics of cardiovascular diseases. At first to solve well-defined familial cases, but with the recent developments of Next Generation Sequencing (NGS) also to identify genetic components involved in complex genetic cardiac diseases and to implement personalized genomics into routine patient care. In this introductory chapter several aspects of molecular genetics will be described and discussed. Firstly, the molecular basics of DNA, RNA and proteins and the different types of genetic mutations and their effects at the level of these different molecules will be addressed in the sections "DNA, RNA and proteins" and "Genetic Mutations". As the mode of inheritance of mutations as well as the specific outcomes in mutation carriers may differ, several aspects related to this is being discussed in the "Genes in families and populations" section. Although NGS is becoming the most widely used technique to identify mutations, still several other techniques are being applied and in the "Molecular Genetic Techniques" section an overview of all currently used methods is provided. With the use of the aforementioned techniques often large amounts of data are being produced and careful analysis and interpretation of these data to dissect 'noise' from truly relevant information is of utmost importance. The section "Analysis and Interpretation" will focus on this. The use of molecular genetics already led to the identification of significant numbers of genes underlying cardiovascular diseases, however still more are to be discovered and approaches to do this are being described in the "Finding New disease genes" section. Finally, in the section "Clinical Genetic Diagnostics" the integration of molecular genetics in daily clinical genetic patient care is being addressed.

Introduction

In recent years, comprehensive developments in genetics vastly improved our knowledge of inherited human diseases, including genetic cardiac disorders. While previously only one to several candidate genes could be studied to search for putative disease-causing mutations, currently whole exome sequencing (WES) and whole genome sequencing (WGS) enable the analysis of all variants within a personal genome, including those predisposing to disease. Important in this respect is the fact that the sequencing of a full genome can now be performed in reasonable time and at a relatively low cost, as the $1000 genome is nowadays within reach. Moreover, this and other genotyping techniques provide the

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possibility to identify variants, modifiers, which have an effect on disease development by either leading to more severe symptoms or protecting carriers of pathogenic mutations from getting seriously ill. Interestingly, these developments also confronted the clinical genetics community with the challenges of handling "big data" and building bioinformatical tools and methods to extract the truly relevant ones from the wealth of variants identified using these techniques. The identification of disease-associated genes in unsolved families no longer requires linkage (-like) techniques. Moreover, with the rapidly growing numbers of reported disease-associated genes and gene variants, we are challenged to distinguish the definitely disease causing ones from (relatively) innocent variants. The latter demands intelligent genomic *in silico* solutions, as well as "old-fashioned" wet, but preferably high-throughput laboratory experimental setups to functionally study the effect of identified variants. Gene and variant curation remains one of the important goals for the near future. Logically, in the field of inherited cardiac disorders, to perform this properly, close collaboration between cardiologists (and other clinical specialists involved), clinical geneticists/genetic counselors, and laboratory specialists is essential. In the following paragraphs, the above-mentioned topics and challenges will be addressed.

DNA, RNA and Proteins

All information necessary to "build" a human is secured within genomic and mitochondrial DNA (deoxyribonucleic acid) and the variation on that theme therefore too. The simple concept, however, of parts of the DNA (the genes) being transcribed into RNA molecules that provide the prescriptions to produce proteins that then form the building blocks of this complex organism is no longer valid. In reality, the situation is much more complex. Both other genetic factors, like regulatory RNAs (ribonucleic acids) and imprinting processes, as well as nongenetic factors like metabolites and other environmental influences have important effects on the final outcome of the information captured in genes. In this paragraph, the basic principles of the molecular factors involved will be described and their interplay discussed.

DNA molecules are composed of four nucleotides, which all consist of a deoxyribose and a phosphate group, together forming the sugar-phosphate backbone of the molecule, and vary in the base side chains adenine (A), guanine (G), cytosine (C), or thymine (T) (see Fig. 1.1). These nucleotides are connected via phosphodiester linkages and the resulting nucleic acid strands entwine each other in an antiparallel fashion, leading to the very stable double-helix structure

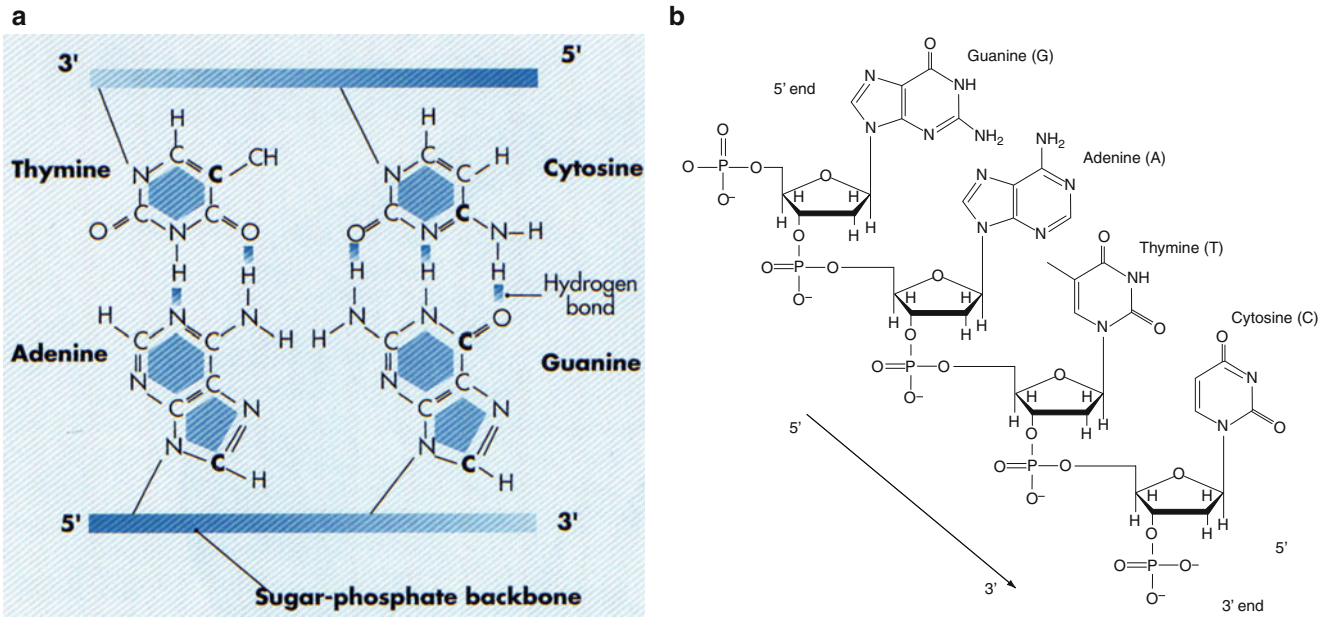

Fig. 1.1 Structure of DNA. A base (C, T, A, or G) combined with a deoxyribose and a phosphate group is called a nucleotide. These nucleotides are polymerized through phosphodiester linkage. DNA is read from the 5′ to the 3′ end. (**a**) The four bases that make-up the actual DNA code. Alanine always pairs with thymine with two hydrogen bonds and cytosine always pairs with guanine using three hydrogen bonds. (**b**) Chemical structure of DNA, showing the sugar backbone and the polymerization through phosphodiester linkage, as DNA is read in 5′–3′ direction, the code of this stretch of DNA would read GATC. (**c**) DNA double-helix structure, the base pairs in the middle are aligned around the helical axis. The major and minor grooves are the result of imperfect winding of the helix. Adapted from Clinical Cardiogenetics edition 2011

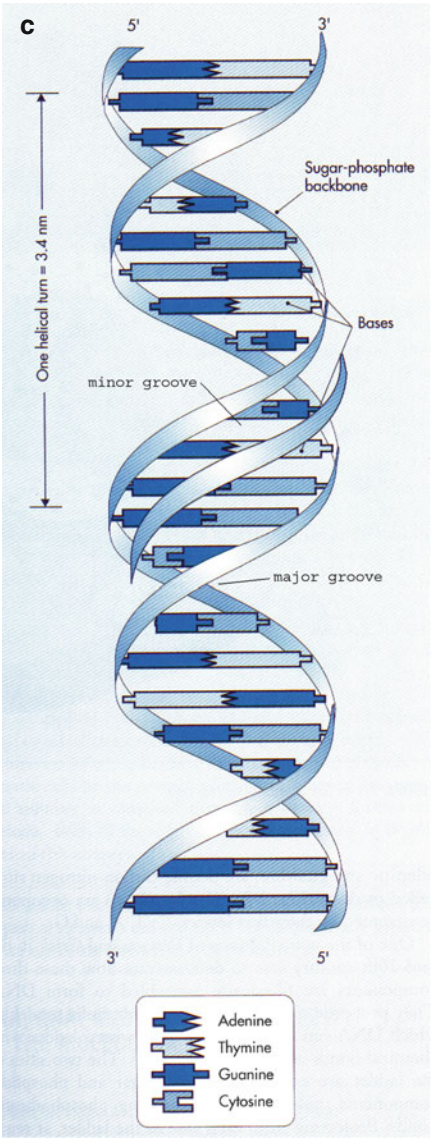

Fig. 1.1 (continued)

first described by Watson and Crick in 1953. The antiparallel pairing, meaning that these strands run in opposite directions (5′–3′ vs. 3′–5′), of the nucleotide strands is mediated via hydrogen bonds between adenine and thymine or guanine and cytosine bases. As AT pairs are formed by two hydrogen bonds and CG pairs by three, the latter bonds are stronger. When the total DNA of a human cell would be placed in a row, it would form a thin stretch that measures between 2 and 3 m. This long DNA fiber has to fit in the nucleus of the cell, which in humans has an average diameter of 6 µm and therefore has to be very condensed. This condensation is realized within chromosomes in which the antiparallel DNA molecules are tightly packed with the help of histone molecules. Each human cell contains two pairs (diploid) of 23 different chromosomes (homologues) that vary in size, chromosome 1 being the largest and chromosomes 21 and 22 being the smallest. Of these, 22 chromosome pairs, the autosomes, are present in both male and female cells, while the sex chromosomal (the 23rd chromosome) content differs: male cells carry one X and one Y chromosome (XY), while female cells contain an X chromosome pair (XX). In general, the full genetic content is maintained in every cell, and to accomplish this, the DNA has to be duplicated before every cell division. A complex of proteins is involved in unpacking chromosomes, unwinding the helical DNA structure to make the antiparallel strands accessible for this process and subsequent replication of the DNA molecules, resulting in two copies of the original strand. During this replication process, mistakes occur, and in spite of the presence of DNA repair mechanisms correcting such mistakes, on average 1 in 10^9 mismatches remain, potentially resulting in mutations. During cell division within the human organism, these mutations (somatic mutations) generally only affect the specific tissue in which they arise; however, sometimes causing serious nonhereditary diseases, for example, cancer. When occurring during sexual reproduction, such mutation (germline mutations) may be transmitted to the next generation and form the origin of a hereditary disease in the offspring. Moreover, while chromosomes stay diploid (two copies per cell) during somatic cell division, they become haploid (one copy per cell) during gametogenesis (the production of sperm or egg cells) and in the course of that process, recombination occurs. This is the exchange of genetic material between homologous chromosomes. This process is an important driving force for evolutionary diversity, however, may also result in mutations at the chromosomal level. For more information on the variety of mutations underlying human diseases: see section “Genetic Mutations.”

It is important to note that only ~2 % of the human genome encodes proteins and this part of the genetic information is contained within the coding exons of genes. The genome consists of up to 30,000 different genes. These genes generally consist of “upstream” 5′ promoter sequences, regulatory and/or stabilizing 5′ UTR (untranslated region) containing exons, alternating coding exons and noncoding introns, regulatory/stabilizing 3′UTR exons and “downstream” 3′ sequences (Fig. 1.2). Notably, the 5′UTR and 3′UTR sequences lie within the first and last exon or exons, respectively, and also exons containing only UTR sequences without protein-encoding parts can be present. The promoter region contains sequences that are recognized by the transcription machinery and transcription-modulating factors that, in collaboration with RNA polymerase, are responsible for the transcription of genes. During transcription, which starts at the transcription start site lying within the first nucleotides of the 5′ UTR, the full sequence between the transcription start site and the polyadenylation signal at the end of the 3′ UTR is being transcribed into pre-

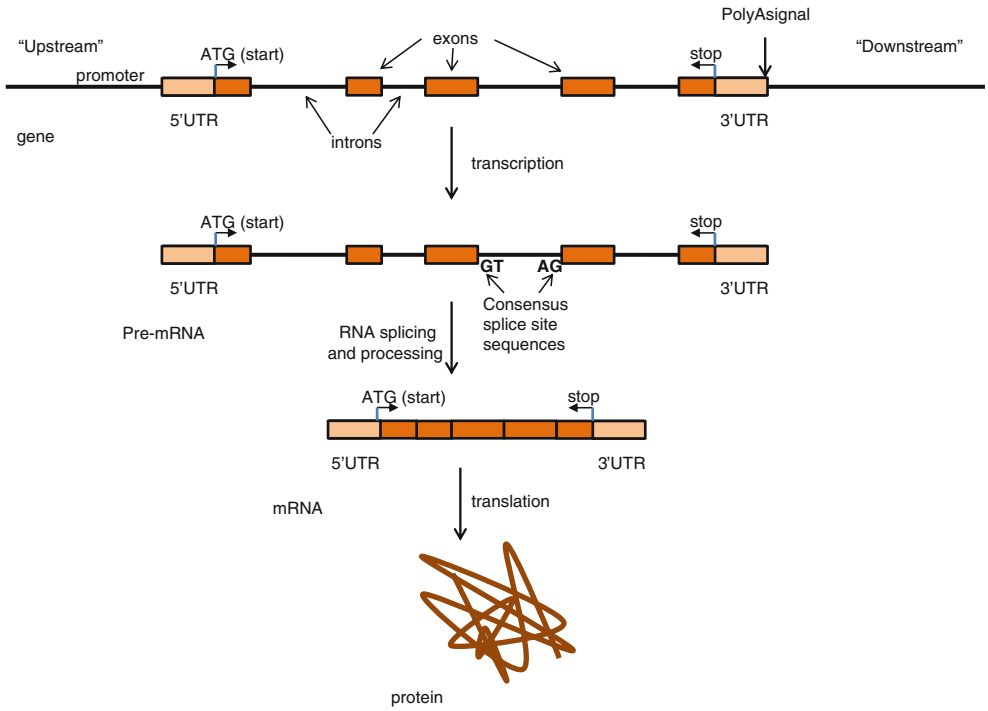

Fig. 1.2 Organization of a human gene and its processing via pre-mRNA and mRNA into protein. For explanation of the different features and aspects shown, see the main text

Table 1.1 The genetic code

	T		C		A		G	
T	TTT	Phe (F)	CTT	Leu (L)	ATT	Ile (I)	GTT	Val (V)
	TTC	Leu (L)	CTC	Leu (L)	ATC	Ile (I)	GTC	Val (V)
	TTA	Ser (S)	CTA	Leu (L)	ATA	Ile (I)	GTA	Val (V)
	TTG	Leu (L)	CTG	Leu (L)	ATG	Met (M)	GTG	Val (V)
C	TCT	Ser (S)	CCT	Phe (F)	ACT	Thr (T)	GCT	Ala (A)
	TCC	Ser (S)	CCC	Phe (F)	ATT	Thr (T)	GCC	Ala (A)
	TCA	Ser (S)	CCA	Phe (F)	ATA	Thr (T)	GCA	Ala (A)
	TCG	Ser (S)	CCG	Phe (F)	ACG	Thr (T)	GCG	Ala (A)
A	TAT	Tyr (Y)	CAT	His (H)	AAT	Asn (N)	GAT	Asp (D)
	TAC	Tyr (Y)	CAC	His (H)	AAC	Asn (N)	GAC	Asp (D)
	TAA	Stop (X)	CAA	Gln (Q)	AAA	Lys (K)	GAA	Glu (E)
	TAG	Stop (X)	CAG	Gln (Q)	AAG	Lys (K)	GAG	Glu (E)
G	TGT	Cys (C)	CGT	Arg (R)	AGT	Ser (S)	GGT	Gly (G)
	TGC	Cys (C)	CGC	Arg (R)	AGC	Ser (S)	GGC	Gly (G)
	TGA	Stop (X)	CGA	Arg (R)	AGA	Arg (R)	GGA	Gly (G)
	TGG	Trp (W)	CGG	Arg (R)	AGG	Arg (R)	GGG	Gly (G)

The 64 genetic triplets and the respective amino acids. In the upper row, the first nucleotide of each codon is depicted (at the DNA level; in RNA, the thymine (T) would be a uracil (U)). In the most left column, the second nucleotide of each codon is depicted. Amino acids are indicated with both their three letter and single letter codes: *Ala* alanine, *Arg* arginine, *Asn* asparagine, *Asp* aspartic acid, *Cys* cysteine, *Gln* glutamine, *Glu* glutamic acid, *Gly* glycine, *His* histidine, *Ile* isoleucine, *Leu* leucine, *Lys* lysine, *Met* methionine, *Phe* phenylalanine, *Ser* serine, *Stop* codon, *Thr* threonine, *Trp* tryptophan, *Tyr* tyrosine, *Val* Valine

mRNA (see also Fig. 1.2). For this purpose, the part of the DNA molecule on which a particular gene is located is unwinded, making it accessible for the previously mentioned transcription machinery. The resulting pre-mRNA molecule is the antiparallel copy of a gene (so the complementary sequence), a situation comparable to DNA being duplicated during replication. Therefore, pre-mRNA molecules are composed comparable to DNA molecules, except for the base thymine being replaced for uracil and ribose being used for the sugar backbone instead of deoxyribose. The pre-mRNA molecule still contains intronic sequences and these are spliced out and the mRNA is further processed by another complex machinery, the mRNA-processing complex, within the nucleus (see also Fig. 1.2). The resulting mature mRNA, now only

consisting of regulatory 5′and 3′ UTR sequences and exonic sequences encoding the amino acid sequence of the respective protein, is then transported to the cytosol. In the cytosol, ribosomes, complexes of proteins and ribosomal RNAs (rRNAs), are responsible for translating the amino acid encoding part of the mRNA molecule into protein. Proteins contain 20 different amino acids and each of these is encoded by nucleotide triplets (codons). In total, 64 different codons encode these 20 amino acids, meaning that most amino acids are encoded by more than one triplet (see Table 1.1). The exceptions are phenylalanine (F), tryptophan (W), and methionine (M), which are encoded by one triplet only. Importantly, the codon of the latter is also recognized as start codon at which translation of the protein is initiated. Moreover, three codons encode so-called stop codons indicating the end of an amino acid sequence. During protein translation, tRNA molecules operate as amino acid carriers and recognize the respective triplets encoding these amino acids. By binding to specific codons on the mRNA within a ribosome, the respective amino acid can be linked to the previous amino acid of a growing peptide. By repeating this and thus linking subsequent amino acids to each other, a complete protein is being produced. During or after completion of this process, the protein will fold into its final conformation, unless it first has to be transported to other cellular organelles, like the endoplasmic reticulum (ER), mitochondria, etc. Notably, often also post-translational modifications of proteins occur, like glycosylation of proteins in the ER and the Golgi complex.

As mentioned earlier, only a small part (2 %) of DNA is actually encoding proteins. In contrast, about 25 % of the DNA codes for introns, the sequences separating subsequent exon sequences, which generally are longer than exons. The remainder of the genome may have different functions: sequences can have regulatory roles (i.e., promoter and enhancer sequences), can be involved in stabilizing and/or maintaining the DNA (e.g., binding modules for histones) or play a role in replication and recombination processes. Part of the DNA may also simply be the remains of ancient evolutionary events and have no function anymore. For example, a considerable number of so-called pseudogenes are known that resemble parts of genes or sometimes full genes that are, however, not being processed into functional proteins, even though these pseudogenes sometimes only differ from the functional gene for only a limited number of nucleotides. It is also increasingly recognized that the genome encodes a wide range of noncoding RNAs that, however, do play functional roles. Among these are tRNAs and rRNAs, but also many more recently identified catalytic and regulatory RNAs, like small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), microRNAs (miRNAs), transcription initiation RNAs (tiRNAs), splicing RNAs (spliRNAs), and long noncoding RNAs (lncRNAs) [36]. miRNAs were shown to recognize specific nucleotide sequences within 3′ UTRs and by doing so modulating protein synthesis, while lncRNAs are believed to be involved in the regulation of differentiation and development. For many of these functional RNAs, their precise roles still have to be elucidated. Moreover, yet unknown new functions of DNA sequences may still be revealed in the near future. Finally, it is very likely that also variations in the different noncoding, but functional sequences do contribute to the spectrum of disease-causing mutations.

Genetic Mutations

As mentioned previously, during DNA replication and recombination processes, errors occur and, in spite of the existence of repair mechanisms, these errors can form the basis of somatic or germline mutations. Moreover, mutations can also occur as the result of chemical modification due to the effects of sunlight (UV), smoking, air pollution, radioactivity, and/or simply the chemical instability of DNA. While somatic mutations may lead to disease in individuals (like the development of cancer), germline mutations (those that did arise during gametogenesis) may be the basis of inherited disease, like those in cardiogenetics. Mutations differ in size, varying from only one nucleotide being changed up to the deletion or duplication of a complete chromosome (like that of chromosome 21 leading to the well-known Down syndrome, including cardiac abnormalities). Different types of mutations are described in the following sections. In section "Molecular Genetic Techniques," techniques used to identify these mutations will be addressed.

Mutations affecting only one nucleotide can have different outcomes. This of course depends on the nucleotide change itself, but also whether the affected base is located within regulatory, coding, or intronic sequences. When considering mutations in coding sequences, these can sort different effects at the amino acid level. The substitution of one nucleotide can result in an amino acid change, a missense mutation that impairs the function of the protein either because the activity of the protein is affected, but with the production of an intact molecule (gain or loss of function mutations), or because it leads to misfolding or unfolding and thus instability of the protein. In the latter case, this most likely leads to an approximately 50 % reduction in the production of that protein. Notably, the other half of produced protein is the result of the transcription of the intact ("wild type") gene copy on the other chromosome and subsequent translation of its mRNA product. However, when this amount is insufficient for proper functioning of the protein, this can have immediate or long-term deleterious effects. This phenomenon is known as haploinsufficiency. The same basically accounts to single-nucleotide substitutions leading to an amino acid-encoding triplet being changed into a stop codon-encoding triplet, which at the protein level result in a nonsense mutation (see below for other examples leading to haploinsufficiency). This introduction of a premature stop codon (also called truncating mutation) was for years by definition considered pathogenic. However, the large-scale sequencing efforts of the last decennium have shown that every human genome actually contains several dozens of truncating mutations without being disease causing. And in relation to that, it has been shown that

such mutations exert no or only mild effects in a significant number of disease genes, while, in contrast, missense mutations in the same gene may have significant effects. When truncating mutations are tolerated, this is most likely related to the availability of an intact gene copy on the other chromosome still resulting in the production of sufficient protein. Of course in cases where both gene copies are mutated, the situation is different and this is almost always deleterious. Although most nucleotide substitutions in intronic or regulatory sequences are most likely benign, it is rather difficult to predict the pathogenic nature of such mutations, except for those close to the exon–intron boundaries within consensus sequences that are being recognized by the RNA-splicing machinery. The latter mutations are frequently deleterious as these often lead to the skipping of exons, either resulting in the deletion of a significant part of the protein or a reading frame shift often leading to haploinsufficiency. Finally, with respect to the previously mentioned alterations, some intronic/regulatory mutations or (silent or missense) coding mutations that seem nondeleterious at the protein level may introduce an alternative (cryptic) splice site also resulting in aberrant RNA splicing.

In addition to single-nucleotide substitutions, the deletion or insertion (indels) of one or more nucleotides occurs also quite regularly. When this affects nucleotide triplets within reading frames, it will delete or add the encoding amino acids. This may have effects similar to that of missense mutations, leading to stable but dysfunctional proteins or instable and therefore degraded proteins. However, when the number of deleted/inserted nucleotides does not equal three or a multitude of that it will lead to a disruption of the normal reading frame and thus a frame shift mutation. At the protein level, this will result in a completely different protein sequence (and structure, in the unlikely case that this would lead to a stable product) from that position or more frequently to the loss of protein. The latter because the frame shift frequently leads to the introduction of a premature termination codon and, thus, resulting in haploinsufficiency. The presence of a premature stop codon (as a result of a frame shift or nonsense mutations) is often already recognized during the process of (pre-) mRNA processing and the respective RNA molecule is degraded. This process is known as "nonsense-mediated decay" (NMD) and a large complex of proteins is involved. Important to note is that premature stop codons that are positioned at the 3′ end of coding sequences do escape this NMD pathway, leading to the production of a truncated protein (however with a limited number of amino acids being truncated; this applies to both nonsense and frame shift mutations) or an extended protein of which the C-terminal amino acid sequence is divergent from the normal sequence, starting from the position of the respective mutation (in this case only applying to frame shift mutations). As a general "rule of thumb," this applies to premature stop codon introducing mutations in the last coding exon and in the 3′ 50–55 nucleotides of the second to last exon. Important to realize is that with both Sanger sequencing and nowadays routinely used next-generation sequencing (NGS) applications, the possibility to accurately identify larger indels (>10–15 nucleotides, depending on the technique used) is limited. Standardized methods to identify deletion or insertions that are larger than 10–15 nucleotides and those that encompass one to several exons, as described below, are currently limited.

In addition to single-nucleotide mutations and small indels, larger insertions, duplications, or deletions may cause disease. First of all and already mentioned earlier, this may concern the deletion or duplication of one to several exons within a gene. Generally, this will also result in haploinsufficieny, as the affected gene will not be (properly) expressed. For example, the deletion of only exon 3 of the *RYR2* gene was shown to cause cardiomyopathy, dilated (DCM), or left ventricular noncompaction (LVNC) and catecholaminergic polymorphic ventricular tachycardia (CPVT) [4]. Moreover, the deletion of several coding exons of the *PKP2* gene were shown to be involved in the development of arrhythmogenic right ventricular cardiomyopathy (ARVC) [48]. These types of mutations are often detected by using the multiple ligation probe amplification (MLPA) technique ("Molecular Genetic Techniques"). Deletions or duplications, however, often include one or more genes, so-called microdeletions or duplications. The pathogenicity of microdeletions is well established (e.g., the deletion of the region including the *TAB2* gene leading to dominantly inherited congenital heart defects: [75]), whereas that of duplications is more debated. As long as the insertion of DNA sequences that are duplicated do not affect the functionality of the gene duplicated or that of a gene or region in which it is inserted, duplications can be harmless. Identifying such mutations is generally done by array-comparative genomic hybridization (CGH), single-nucleotide polymorphism (SNP)-array technologies and/or by performing copy number variation (CNV) analysis of NGS-derived data ("Molecular Genetic Techniques"). Finally, deletions/duplications of a large part of a chromosome or full chromosomes can also underlie cardiac diseases, however, often as part of a broad spectrum including other clinical features. The already mentioned Down syndrome (trisomy of chromosome 21) is a well-known example. Moreover, translocation of parts of chromosomes during gametogenesis result in the deletions and concomitant duplications of parts of chromosomes that may lead to an imbalanced situation in the resulting oocytes or spermatozoids and as a result of that a child with a severe clinical phenotype, including cardiac abnormalities (often congenital heart defects (CHDs)). These aberrations can be identified using "old-fashioned" karyotyping and fluorescence *in situ* hybridization (FISH) methods, QF-PCR (quantitative fluorescent polymerase chain reaction), or with CNV analyses of NGS-derived data, including analysis of sequences that were affected by the translocation event.

Genes in Families and Populations

In the previous paragraph, different kinds of mutations, from those only affecting one specific nucleotide up to full chromosome duplications or deletions, have been described. When such mutations arise during sexual reproduction, these

can be transmitted to following generations and, thus, form the basis of inherited diseases. In general, this does not apply to very large deletions/duplications, as these often have rather drastic effects, often already resulting in a spontaneous abortion. In the following sections, different aspects related to the inheritance of mutations (or variations; in particular when concerning multifactorial diseases) are described.

Modes of Inheritance

Depending on which chromosome or gene is affected by mutations and how, different ways of inheritance are recognized. Most well-established genetic diseases are Mendelian-inherited diseases; those caused by mutations on autosomal or sex chromosomal genes. These mutations can be inherited in a dominant or recessive manner. Important in this respect is that the terms dominant and recessive describe the inheritance of genetic disease and not the genes or mutations themselves. When dominantly inherited, carrying the mutation on one allele (one chromosome) is sufficient to cause disease, and as a result, children of mutation carriers have a 50 % chance of inheriting the same mutation. When such mutation causes disease mainly after the reproductive age, it can be transmitted through pedigrees for numerous generations. In contrast, recessively inherited mutations result in disease development only when both alleles are affected. In general, this implicates that patients having both alleles mutated, inherited a mutation from both parents (each carrying one copy of the mutated allele) without being affected. Such mutations are often also transmitted through numerous generations and through populations, and only causing disease in the rare situation where two mutations come together in one individual. This is seen relatively frequently in situations where consanguinity is involved: the parents of an affected child being closely or more distantly (sometimes some generations ago) related. In this case, it most likely concerns homozygous mutations: the same mutation on both alleles. In more rare situations, compound heterozygous mutations may be encountered: different, deleterious mutations on the two alleles (i.e. two chromosomes) but in the same gene. In addition to the situation of parents not being affected in case of recessively inherited diseases, this also applies to cases were mutations are actually "*de novo*". This means that these did arise early in the development of the embryo, or in the reproductive cells of the father or the mother and were transmitted to the affected child. Of course, when the disease course is not too severe, this can be the start of a dominantly inherited disease in following generations.

In the situations described above, mutations are generally located on the autosomes and not on the sex chromosomes (X or Y). Mutations can, however, also be present on the X or Y chromosomes. Y-chromosome inheritance is quite uncommon and has until now never been described for heart-related diseases. X-linked cardiac diseases are often recognized by the fact that female carriers do not exhibit disease, or only mildly, while their sons are severely affected. Logically, this mode of inheritance is often recognized in pedigrees because of affected male family members being connected via mildly or unaffected females. If consanguinity is present in these families, a homozygous, X-linked mutation may cause severe disease in females too. Notably, the exception to this is the situation in which male carriership is actually lethal, while female carriers of one X-linked mutation are being affected (comparable to the above-described dominant inheritance on autosomes). As mitochondria also contain a genome that is duplicated independently from the nuclear genome and transmitted to new mitochondria, mutations in mitochondrial DNA can also be transmitted to next generations and mitochondrial inheritance is being recognized too. Important to note is that mitochondrial diseases also show a maternal segregation pattern (like X-linked diseases) and recognizing this inheritance patterns is being complicated by the fact that mitochondria contain multiple genome copies that are not necessarily all mutated. Actually, mitochondrial-inherited diseases are non-Mendelian diseases, as are multifactorial (polygenic) diseases. The latter will be discussed in more detail in section "Multifactorial Inheritance".

Penetrance and Disease Expressivity

It is important to realize that carrying a mutation does not necessarily mean that disease will develop. In particular in dominantly inherited diseases, the phenomenon of reduced penetrance is often present. When the respective disease is 90 % penetrant, this means that 90 % of carriers develop disease. Often penetrance is age dependent, that is, symptoms of disease develop during the course of life. Moreover, disease expressivity can differ considerably. For example, the founder mutation c.40_42delAGA; p.(Arg14del) in the *PLN* gene predisposes to ACM and/or DCM [68, 69]. In a cohort of over 400 people in the Netherlands carrying this deletion, the phenotype ranges from severe outcomes as sudden cardiac death (SCD) or heart transplantation in young adults to fully healthy elderly individuals in their 70s even though performing quite extensive sport activities. Moreover, the same mutation can have different outcomes. For example, families have been described in which a mutation in a cardiomyopathy gene resulted in dilated, hypertrophic, or noncompaction cardiomyopathy in different family members. Both reduced penetrance and differences in disease expressivity may be explained by either nongenetic, environmental factors, and genetic factors, that is, secondary mutations and/or modifiers. Although the effect-size of such genetic factors is still difficult to establish or predict, current genome wide association studies (GWAS), and in particular, NGS approaches now enable the search for these genetic variations.

Genetic Heterogeneity

With the increasing possibilities to detect underlying genetic mutations in inherited diseases, we have seen an exponential

growth in the number of identified disease genes, also within the field of cardiogenetics. As a result, it has become evident that a disease or disease type is rarely explained by a single underlying gene. Often several genes can be involved. For example, currently, more than 50 genes have been identified that are associated with dilated cardiomyopathy (DCM). In combination with the fact that mutations in the yet known genes only explain half of the patients suffering from the inherited form of this disease, this underscores that many DCM genes are still to be uncovered. Moreover, it is also increasingly recognized that mutations in the same gene can result in different phenotypes (which is different from the same mutation being differently expressed, as discussed earlier). Again within the cardiomyopathies, significant "genetic overlap" is observed, as mutations in one gene can underlie different cardiomyopathy subtypes, some even being associated with every subtype known. Furthermore, genes can even be involved in several cardiac diseases. For example, mutations in the *SCN5A* gene are known to cause cardiomyopathy, but also channelopathies like Brugada syndrome or long QT syndrome or conduction disease. Likewise, in addition to leading to cardiomyopathies, mutations in the *LMNA* or *DES* genes can also underlie generalized muscular diseases (i.e., limb girdle, desminopathy), or, in the case of *LMNA*, noncardiac disorders like lipodystrophy (caused by specific heterozygous mutations) or Hutchinson–Gilford progeria syndrome (in which a specific heterozygous *LMNA* mutation is the cause). Finally, several syndromes, like Noonan or Danon syndrome, may include cardiac manifestations, sometimes dependent on the underlying gene defect and/or mutation.

Multifactorial Inheritance

So far we only described monogenetic diseases. However, in a significant number of cases, the disease is not purely monogenic and considering multigenic or multifactorial inheritance is more appropriate. This can vary from digenic inheritance in which two genes together underlie disease up to polygenic diseases in which many variants, individually giving rise to very small effect sizes, together increase the chances of developing disease or multifactorial disease where a combination of genetic variants and environmental factors underlie disease expressivity. Of course it is rather difficult, if at all possible, to identify all components of such multigenic diseases, even with all the novel genetic technologies and concomitant bioinformatical tools that have recently become available. Moreover, even when all could be identified, estimating both the individual and the total effect is almost impossible. In addition, variations that actually have protective effects (and quantifying those) and that thus modify the negative effects of the putatively disease-associated ones exist. Likewise, it is also increasingly recognized that monogenetic diseases are actually not really monogenetic and that several other genetic factors may modify disease (both negatively and positively), although with the major disease gene mutation being the one asserting the strongest effect.

Despite the difficulties described earlier with interpreting these results, numerous studies to identify causal variants involved in polygenic diseases, as well as modifying factors in more Mendelian-inherited disease have been performed in the last decades. In this respect, also within the field of cardiogenetics, many GWAS have been performed to find SNPs that are associated with cardiological traits. For this purpose, large groups of individuals suffering from the same disease, at least several hundreds, have to be analyzed using arrays containing a large amount of SNPs in order to identify variants that show a statistically relevant association with the respective trait. In addition, when detected, such association is often only valued after being confirmed in an independent large cohort (preferably from another ethnic origin). As discussed earlier, the relevance of such findings for the individual patient is debatable; however, the results may help in understanding and further elucidating underlying molecular pathways and mechanisms and the identified genes linked with these variants could serve as potential drug targets. As these type of analyses are limited to the SNPs captured on these arrays, this approach may only identify variants in the direct genetic neighborhood of the affected gene that is linked to the phenotype studied, but not the actual variant. Therefore, with all the new NGS-based technologies available, the sequencing of thousands of exomes and genomes now enables the identification of many more of such variants. However, the wealth of data that have already become available indicates that every individual is carrying many private variants (even truncating mutations that are not always clinically relevant) and distinguishing the irrelevant ones from the relevant ones in polygenic disease will be a tremendous challenge. On the other hand, all ongoing whole exome and genome-sequencing activities will help in separating relevant from irrelevant genetic information in the future.

Molecular Genetic Techniques

From Past to Future

The last 20 years have seen unprecedented advances in technology and the development of innovative techniques in molecular genetics for the discovery of genes related to human diseases as well as the application of such knowledge to molecular genetic diagnostics as a powerful tool in patient care. Year 2016 marks the 20th anniversary from the publication of the first comprehensive genetic map of the human genome based on 5264 microsatellites [8]. Although a "Report of the DNA committee and catalogues of cloned and mapped genes, markers formatted for PCR and DNA polymorphisms" was previously published in 1991 (Williamson et al. [76]), the 1996 map represented the

most powerful tool in the hand to human geneticists for employment of PCR-based polymorphic dinucleotide and tetranucleotide microsatellite markers for pursuing gene mapping via linkage analysis. In addition, it provided the possibility to study structural chromosomal alterations such as loss of heterozygosity (LOH) as commonly occurs in cancer, in which one copy of a tumor suppressor gene is deleted due to various rearrangements [12]. Furthermore, these highly polymorphic markers provided the possibility to detect copy-neutral LOH, uniparental disomy (UPD) [26] as well as microsatellite instability (MSI) resulting from abnormal DNA mismatch repair (MMR) [71]. Molecular genetics and cytogenetics have since witnessed an exciting 20 years of technical and technological progress, which will be discussed in the following paragraphs.

Cytogenetics

Cytogenetics is the branch of genetics that studies the number, structure and function of the chromosomes, which contains the highly organized and packed nuclear DNA of the cell. The employment of the karyotyping technique allows researchers to define the structure of the normal complete chromosome set and identify alterations linked to biological abnormalities. In particular, the application to human genetics and clinical practice led to the discovery of defects in chromosome number (aneuploidy) in a cell leading to human diseases, such as trisomy 21 (Down syndrome), trisomy 13 (Patau syndrome), and trisomy 18 (Edwards syndrome). Furthermore, karyotype analysis by Mary F. Lyon of somatic cells led to uncovering the process of X-chromosome inactivation (Xi) in female mouse [30] and in 1962 in female human subjects [31]. This pivotal observation paved the way for future investigations in the field of epigenetic regulation. In addition to aneuploidy, the use of karyotype analysis using the G-banding technique permitted the identification of even more subtle aberrations, such as the constriction at the subtelomeric position of chromosome X forming the basis for the discovery of the cytogenetic mechanism of the Fragile X syndrome, the most common inherited cause of intellectual disability in males [29]. The investigation of the subtle changes in banding pattern was instrumental in determining not only the loss of the genetic material, but also its exchange between chromosomes. The study of the karyotypes from patients with various diseases, allowed the identification of syndromes caused by the transfer of a piece of a chromosome detached from its original location and translocating to another chromosome resulting in the exchange of genetic material. In some cases, depending on the genes involved, this mechanism can lead to the constitutively activation of fusion (chimeric) gene product leading to novel aberrant function. Many forms of leukemia may stem from this very mechanism.

The examples mentioned above underscore the profound impact in clinical diagnosis and management derived from the application of the karyotype analysis, also defined as traditional cytogenetics. However, despite much advances in the DNA molecule stretching and in the banding technique, karyotyping suffers from a lack of resolution, preventing the detection of submicroscopic chromosomal aberrations, in particular deletions and duplications of genetic material. Although normally utilized by cytogenetic laboratories, the answer to the need in increasing the resolution came from molecular genetic approaches, such as fluorescent in situ hybridization (FISH), comparative genomic hybridization (CGH) and SNP arrays. In a significant number of cases, the use of CGH or SNP array technologies allowed the identification of submicroscopic genomic rearrangements leading to genomic architecture aberrations associated with the occurrence of genetic diseases. Such genomic architecture aberrations could be the result of mispairing of highly homologous repetitive sequences called low-copy repeats (LCR), which are segmental duplications of DNA blocks ranging from 1 to 400 kb, leading to recurrent chromosomal rearrangement [58]. The plasticity of the human genome does not only depend on the LCRs, but other mechanism can lead to interstitial or subtelomeric deletion/duplication causing CNV, which represents a much greater source of structural diversity of the human genome than previously expected [14, 22, 42]. However, in most cases, when a clear clinical diagnosis is established and a specific defect, such as a microscopic deletion or duplication is a known mechanism of the disease, a more targeted approach such as FISH, which does not include a genomewide analysis such as conventional karyotyping, CGH or SNP array, can be employed. The development of fluorescent molecular probes pairing to a specific genomic region, exploiting the high complementarity to a given DNA sequence, is the basis of FISH and allows the detection or lack thereof of a specific signal in an interphase or metaphase chromosome preparation. Probably, the first (in 1993) and best known cardiovascular disease in which FISH was used and is still currently widely employed in clinical practice is the 22q11 deletion syndrome, previously known as DiGeorge syndrome and velocardiofacial syndrome, among others [9]. Currently, the most commonly used genomewide cytogenetic approaches are array-based, and due to the higher resolution, these techniques are capable of detecting rather small genomic aberrations. These techniques are mostly used to identify mutations associated with CHDs or syndromes involving cardiovascular abnormalities.

Molecular DNA Techniques

Among the technical advances in molecular biology, which have been widely utilized in genetic research and in clinical molecular diagnostics, two represent absolute milestones: Sanger sequencing, developed in 1977 by Frederick Sanger [53], and the PCR developed in 1983 by Kary Mullis [37]. PCR is a process used to exponentially amplify a specific

DNA sequence, through sequential repetitive cycles, at several orders of magnitude starting even from a single DNA molecule and leading to millions of copies of the desired DNA target. PCR found immediate applications in biological research and molecular diagnostics including DNA cloning for sequencing, forensic genetic fingerprinting, and diagnosis in infectious diseases, evolutionary biology and quantitative gene expression analysis [20, 21, 50]. A natural application of PCR was the ability to provide the rapid availability of sufficient DNA target for sequencing, compared to previous laborious DNA isolation techniques. In addition, the exploitation of the exponential phase of the PCR along with the employment of fluorescently labeled primers, probes or dyes, led to the development of a real-time quantitative PCR (qPCR) method for the accurate and sensitive detection and quantification of nucleic acids [15]. Among the various applications in clinical practice, an important role was played by qPCR in the detection of infective agents load in endomyocardial specimen from patients with myocarditis [5] or in post-heart and lung transplant subjects at risk of rejection [10]. The latest development of such technology led to digital PCR (dPCR), which can be used to provide the direct absolute quantification of the initial nucleic acids sample amount by partitioning the specimen into a large number of separated microwells or emulsion reactions, allowing for a large data points collection and more sensitive measurement of the target nucleic acid amount [62] including small deletions and duplications, as well as CNV in a manner independent on the number of amplification cycles [39].

Another frequently used molecular technique is multiplex ligation-dependent probe amplification (MLPA). MPLA is extensively employed in clinical diagnostics for the detection of CNV and exon-based small and large deletion/duplication analysis [57]. MLPA represents a derivative of multiplex PCR that allows multiple targets to be amplified simultaneously using various pairs of forward and reverse oligonucleotide primer probes recognizing adjacent target sites on the DNA. Only when both probes perfectly anneal to their respective targets, they can be ligated into a complete probe and amplified into a fluorescently labeled PCR product of unique length that can be separated and identified by capillary electrophoresis [57]. The comparison of the detected fluorescent peak patterns between the test sample and a reference sample can provide a quantitative ratio of each amplicon stemming from the relative amount of the target sequence along with the relative quantity of each product obtained [57].

In addition to the numerous applications of PCR and all its derivatives, PCR has become the perfect partner of the Sanger technique, which is the chain-termination method for sequencing DNA molecules, Sanger sequencing exploits the inability of dideoxy nucleotides to allow another DNA base to be added to the newly synthesized DNA strand. This enables the rapid and accurate determination of the sequence composition of long stretches of DNA such as the 16,569 base pairs of the human mitochondrial genomic DNA [1], the 48,502 base pairs of the bacteriophage λ (Sanger F et al. [54]), and the complete and accurate sequence of the 3 billion DNA base pairs that compose the human genome [17, 18].

Next-Generation Sequencing

For several decades, Sanger sequencing has been the gold standard for the determination of variants in nucleic acids in both biological research and clinical molecular diagnostics. However, the pace of gene discoveries along with unveiling the increasing genetic heterogeneity in most diseases, and especially in primary cardiomyopathies and arrhythmia syndromes overcame the ability to target all most relevant genes to increase the detection rate and improve molecular diagnosis. Since the announcement of the completion of the first draft of the Human Genome Project in 2003, it became apparent that the throughput employed until then was insufficient to address the emerging challenges in genetics and genomic research and clinical practice. In particular, the high demand of cost-efficient methods for large-scale sequencing projects pushed toward the development of high-throughput sequencing or next-generation sequencing (NGS) technologies that provide massive parallel sequencing, producing millions of sequences simultaneously. The technique was initially applied to determine gene expression levels by counting the number of individual mRNA molecules produced by each gene. The NGS technology evolved between the end of the 1990s and the beginning of the twenty-first century with the advent of the 454 pyrosequencing machine for DNA sequencing, which drastically reduced the cost of sequencing compared to Sanger produced 20 million bases (20 Mbp) worth of data [49]. In the following years, the throughput increased almost exponentially. Almost simultaneously, another platform, the Genome Analyzer (GA) entered the market based on the sequencing by synthesis (SBS) technology using reversible dye-terminators chemistry generating up to 50 billion bases (50 Bbp) of usable data per run [32]. Another platform based on the SBS technology is the single-molecule real-time (SMRT) sequencing in which the DNA is synthesized in zero-mode wave-guides (ZMWs) a nanophotonic confinement structure consisting of a hole in an aluminum shell film deposited on a clear silica substrate in which a single DNA polymerase enzyme is attached to the bottom of a ZMW with a single molecule of DNA as a template where fluorescently labeled nucleotides are added to the growing DNA molecule allowing reads of up to 60,000 nucleotides or more, with average read lengths of 5000 bases [25]. In addition to the aforementioned technologies, various companies developed a vast number of machines able to yield up to 600 Gbp bases of data with increasing read length and coverage depth with the capacity to sequence a human genome in about 24 h, 20 exomes in a day, or 30 transcriptomes (RNA sequencing) samples in as little as five hours. Other technologies include

sequencing by oligo ligation detection, employed by the SOLiD platform, and the semiconductor technology used by the Ion Torrent sequencer, which is based on the detection of hydrogen ions that are released during the polymerization of DNA. Of great interest, methods are recently commercialized, such as nanopore sequencing, which relies on the retrieval of electrical signals from nucleotides passing through nanopores changing conformation of the pore and the ion flow going through it according to the shape, size, and length of the DNA sequence. The major limitation for large-scale application is that the nanopore sequencing cannot achieve single-nucleotide resolution yet, thus preventing its use in clinical diagnostics [6, 60]. Depending on the desired application, NGS can be applied to target a panel of candidate genes, known to be associated with a specific disease or spectrum of disorders, or can be used to capture the entire 35 Mbp of the coding sequence collection, whole exome sequence (WES) or even the 3 Gbp of the WGS. Several parameters have to be considered in designing NGS experiments, such as the read modality, which means obtaining a sequence read from one end of a DNA fragment (single-end read) or from both (pair-end read). The single-end read usually is faster, cheaper and usually enough for applications such as RNAseq (gene expression profile) or ChIPseq (chromatin immunoprecipitation paired with massively parallel DNA sequencing to identify the binding sites of DNA-associated proteins). Pair-end read sequencing, however, provides more accurate mapping of the DNA fragment to the reference genome, making it an excellent approach for clinical diagnostics and allowing the resolution of structural rearrangements such as deletions, insertions, and inversions. The application of NGS in either research or clinical diagnostics does offer the flexibility to determine the extent of nucleic acid sequence to cover (panels, WES, WGS, RNAseq, ChiPSeq, methylome, etc.). However, NGS has technical limitations, which are intrinsic to the modality utilized for the test. In all applications, but WGS, after fragmenting the nucleic acid molecules, specific probes to target or capture the desired sequences have to be employed. There are mainly two capture modalities currently used in NGS: amplicon based, which use oligonucleotide probes as PCR primers for amplicons, while the hybridization-based approach allows the nucleic acid fragments to be used for further enrichment and clonal amplification [52]. Each capturing modality provides various levels of sequence complexity and uniformity, along with differences in depth of coverage. Constitutional genetic disorders, such as cardiovascular diseases, can be effectively detected using a minimum of 100X average coverage (approximately 100 reads from both directions), being derived mostly from constitutional variants. However, for somatic cancer genetics applications, much higher depth of coverage is usually required due to the genetic heterogeneity in tumor samples along with normal tissue. Furthermore, the choice of the sequencing platform also has an important impact on the ability to detect different types of variants and each platform comes with unique advantages as well as technical limitations in variant detection, which can be partially resolved by the use of appropriate software for alignment and mutation calling that normalizes for these errors [52]. Recently, investigators have compared amplicon based with hybridization capture-based methods, and apparently, hybridization capture-based methods resulted in better sequencing complexity and uniformity, lower false-positive and false-negative rates for single-nucleotide variants (SNVs), although the latter issue can be corrected by modifying the parameters, such as minimum variant frequency or minimum read coverage necessary for a base call [52]. In the case of clinical molecular diagnostic resequencing, the raw data obtained from the various sequencing machines need to be processed bioinformatically and assembled into contigs reconstructing the entire DNA fragment clonally amplified during the sequencing process. In addition, once the sequence fragments have been aligned in contigs, they also have to be mapped against a reference genome sequence, called assembly. An important issue in bioinformatics is the filtering of repetitive sequence, segmental duplications and pseudogenes that can be spread all over the genome. In many cases, if a sequence cannot be uniquely assigned to a specific genomic location, it is filtered out loosing part of the information. Following this, the next steps in the procedure involves the detection of each variant compared to the reference sequence, and its placement with respect to the coding, splicing, or intronic sequence, a process called variant annotation. It is only after all variants annotations have been completed that the laborious and complex process of variant interpretation begins (see section "Analysis and Interpretation").

For many decades, primary cardiac diseases, such as cardiomyopathies or channelopathies, have been regarded as purely monogenic Mendelian disorders characterized by high locus and allelic heterogeneity, incomplete penetrance, and clinical variability. However, recent findings challenged this view and a significant fraction of cardiovascular patients with suspected genetic origin analyzed for large gene panels, WES or WGS presented with the detection of multiple pathogenic variants suggesting the occurrence of a more complex genetic interaction, leading to a challenging interpretation of the clinical relevant role of each identified variant [70]. Recently, the American College of Medical Genetics and Genomics (ACMG) has published the standard guidelines for the interpretation of sequence variants [45] weighing strong genetic data, such as linkage analysis and cosegregation of the variants in large pedigrees, or solid and comprehensive functional characterization of the variants along with the frequency in large controls databases, amino acid conservation analysis, *in silico* damage prediction, association studies data, and other "indirect" suggestive parameters [45]. More details on this are being provided in section "Analysis and Interpretation".

Despite all the existing limitations and technical challenges, the increasing utilization of massive parallel sequencing

technologies will continue to allow the unveiling of an increasing level of complexity in genetic causes of human diseases along with an impressive amount of data, which will permit, in a nondistant future the swift interpretation of genetic variants.

Analysis and Interpretation

Variant Classification

In the past few years, sequencing technology has changed rapidly with the development of high-throughput sequencing methods, which are collectively referred to as NGS. In the past, Sanger sequencing has been the gold standard in molecular diagnostics. Because this test is relatively labor intensive and rather expensive, it was not suitable for diseases that show extensive genetic heterogeneity. It was therefore either applied to diseases in which mutations in a small number of genes could explain the phenotype or in cases of genetic heterogeneous diseases to analyze only the most prevalent disease genes. The recent technological developments in high-throughput sequencing and computing have resulted in accurate sequencing at much lower costs, which enabled testing for targeted gene panels of over 100 disease genes, as well as exomes and even genomes ("Next-Generation Sequencing"). The limiting factor in deciding on the content of the test is no longer the size of the gene or its relative contribution but more on its relevance for the disease. Inaccurate variant–disease associations represent a challenge for clinical variant interpretation and because the amount of information about genes and mutations is growing daily, there is a continuous need for the reassessment of previously classified genetic variants. In daily practice, however, this is virtually impossible. Instead, it is recommended to reassess the variant if it is identified in another index patient or when the patient or family in which the mutation was originally found revisits the cardiogenetic clinic, or as a result of discussion on classification of a variant between different laboratories where the variant was found. When this reassessment results in another classification that affects medical care, all previously identified gene carriers should be informed about this.

Molecular genetic testing is highly relevant. Finding a pathogenic mutation may be of help to identify family members at risk, or those that do not need cardiological surveillance any more. In addition, knowing the underlying gene mutation may guide clinical treatment in some cases. Therefore, accurate variant assessment is very important but not easily accomplished as relevant information is not always available or accessible at the time of interpretation. Moreover, no standard, comprehensive, and efficient variant classification method is available that is approved and shared by the community. However, variant interpretation should be as uniform as possible, and therefore, medical genetic laboratories have developed guidelines for interpretation, including those from American (ACMG) Dutch (VKGL), and British (ACGS) Clinical Molecular Genetics Societies [45, 73, 74]. Important to note is that this classification is only relevant for the interpretation of variants that cause a (suspected) inherited Mendelian disease and not intended to be used for the interpretation of pharmacogenomics, somatic or multigenic non-Mendelian diseases. In addition, care must be taken in interpreting variants in candidate disease genes (GUS: genes of uncertain significance) which will be detected when exomes and genomes are studied.

Classification of variants. It is of great importance that diagnostic laboratories use a consistent method to report variants. Therefore, the diagnostic genetic community has decided to use the guidelines available from the Human Genome Variation Society (HGVS: http://varnomen.hgvs.org). Clinical reports should include sequence references(s) (or genome build when genomic coordinates are used) to ensure unambiguous naming of the variant at the DNA and protein level ("c." for coding DNA sequence, "p." for coding protein and "g." for genomic sequence). There is a general agreement on using a classification system based on five variant classes. These are; class 1: certainly not pathogenic or benign ("common" polymorphisms and therefore not reported), class 2: unlikely pathogenic mutations or likely benign (diagnosis not confirmed molecularly and often not reported), class 3: unknown or uncertain pathogenicity or significance (does not confirm or exclude diagnosis), class 4: likely pathogenic (consistent with the diagnosis); class 5: (certainly) pathogenic (result confirms the diagnosis). Some laboratories subdivide class 3 variants, also known as VUS, even further into VUS-favor benign, VUS-unknown and VUS-favor pathogenic. Often this is done for internal use and not reported to the referring physician. The term "likely" is used when the professional believes that the variant is about 90 % benign (class 2) or pathogenic (class 4). To obtain an appropriate classification, the following major aspects are taken into account: frequency and number of alleles in patient and control populations, degree of segregation with disease, functional evidence, predicted protein effect and comparison with the established spectrum of pathogenic variation in a gene.

Basically, variant interpretation consists of two parts. In the first part, mutation-specific features are calculated and scored based on *in silico* (computational) predictive programs, many of them being available on the internet (see [45, 73]) and frequency data from "control" databases, for example, from the Exome Aggregation Consortium (ExAC). The second part is using already available information from the literature or online databases or can be obtained by performing additional studies of a particular variant. Examples of this are as follows: how often has this variant been found before related to this disease, does the variant cosegregate with the

disease, has it functionally been analyzed and did the variant occur *de novo* (not present in both biological parents). Many genome diagnostic labs use Alamut (http://www.interactive-biosoftware.com/alamut.html), a commercially licensed software package that allows a user-friendly environment for variant review, visualization and interpretation combining several protein effect prediction programs and "control" and disease databases. In addition, it can be used as a database for the analyzed samples in the laboratory. Many other commercial applications for the annotation and data interpretation are available as well (see Oliver GR et al. [38]).

In Silico Predictive Programs In general, the algorithms can be divided into tools that predict whether a missense change could result in a change or loss of protein function (e.g., PolyPhen-2, SIFT, MutationTaster, Grantham score) or those that predict whether there is an effect on splicing (e.g., Human Splice Finder, NetGene 2). In general, most programs for missense variant prediction are about 70 % accurate when examining known disease variants [46], [65]. Although many prediction tools are based on different algorithms, they have similarities in their underlying methodology. All tools depend on criteria such as biochemical consequences of the amino acid substitution and evolutionary conservation. The predicted classification must not be considered definitive but should be considered as one aspect of a more extensive investigation (i.e., moderate evidence). Splice prediction tools in general have a greater sensitivity (about 90 %; fraction or percentage of splice site mutations successfully identified) than specificity (about 70 %; fraction or percentage of neutral mutations successfully identified) and should only be used as a first indication ([16], [72]). If possible, RNA studies should be performed to prove that the variant indeed interferes with splicing. This should be performed in an appropriate and validated tissue or cell type. Only nucleotide changes that disrupt the (essential) dinucleotide consensus splice sites (located in the intron at the intron/exon boundary, that is, positions +/−1 and +/−2) can be considered to disrupt the splicing without functional RNA analysis when most splice prediction tools predict the loss of splicing. However, whether this variant will be considered (truly) pathogenic depends on aspects like the role of the gene in disease and whether these type of mutations have been reported before as pathogenic in this specific disease.

Frequency data from "control" databases. Determining the frequency of a variant in the general (or control) population is useful in judging its potential pathogenicity, preferably by using ethnically-matched controls. For this, several publically available population databases as well as other resources (in house, publications) are being used. The already mentioned ExAC database (http://exac.broadinstitute.org) is at present the largest with frequency data from over 60,000 exomes. An allele frequency higher than expected for the disease is in general considered as strong support for a benign interpretation. Several exceptions to this rule are, however, known in the literature like the common South Asian c.3628-41_3628-17del mutation in *MYBPC3* that is associated with cardiomyopathies and occurs with a frequency of 3.1 % in South Asian (ExAC) and even up to 8 % in certain Indian populations [7]. The absence of a variant in a large general control population indicates only a moderate piece of evidence for pathogenicity of a variant, because also many benign ones are "private" (i.e., unique to an individual or family).

Occurrence of the variant in disease databases. Data sharing of variants has shown to be of great value for classifying VUSs. For this reason, diagnostic labs make use of mutation/disease databases, like HGMD: Human Gene Mutation Database (http://www.hgmd.org), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar), and Locus-Specific Databases (LOVD; http://www.lovd.nl) [11, 23, 59] and published literature. However, these data should be used with caution. When using these databases, it is important to know the quality of the data (e.g., Sanger validated vs. low-quality next-generation sequencing), how often the database is updated and source reliability and independence of the data. Notably, dated literature may contain older nomenclature and classification. Many previously published disease-associated variants turned out to be present in population-based exome data and made the pathogenicity of these variants dubious [2, 47]. A variant is statistically more likely pathogenic if it occurs in affected individuals more frequently than expected by chance. For this, the likelihood of random occurrence (e.g., through case–control studies using Fisher's exact or chi-square test) or odds ratios (ORs, http://www.hutchon.net/confidor.htm, http://www.easycalculation.com/statistics/odds-ratio) are being calculated. In general, variants with a modest Mendelian effect size will have an OR of 3 or greater, while highly penetrant variants will have very high ORs (up to 13). When interpreting ORs, it is important to take the confidence interval (CI) around the OR into account. For instance, if the CI includes 1.0 (e.g., OR = 2.5, CI = 0.9–7.4), there is little confidence in the assertion of association [45].

Because NGS results in exponential growth of the number of variants, data sharing has become even more important. Therefore, clinical laboratories should submit variants to existing databases or should create their own sharing databases. These databases should offer the possibility to record each unique detection of the same variant to keep track of the prevalence of the disease in the general population. At present, sharing of data is unfortunately still in its infancy due to issues like insufficiently automated submissions, curation of data, patient privacy issues and the traceability of the origin of submission. For organizational reasons, as well as to record population and/or geographic-specific information, Weiss et al. [74] recommended that diagnostic labs should start with national data

sharing initiatives that could merge into an international database. Not only sharing of the mutation is important but also sharing of accurate and detailed phenotypic data. This is far from optimal at present and requires good collaboration between clinic and laboratory- and software-supported approaches.

Cosegregation with the disease. Significant cosegregation of a variant with the disease in families is a strong indication for pathogenicity. However, it should be realized that the presence of partial (age-related) penetrance, phenocopies (affected family members with the disease due to a nongenetic or different genetic cause) and nonpaternity can result in erroneous interpretation. Accurate clinical evaluation and communication of this to the laboratory is of crucial importance for a reliable interpretation. In addition, the segregation of a particular variant with a phenotype in a family is evidence for linkage of the locus to the disorder but not necessarily evidence of pathogenicity of the variant itself, that is, any variant in linkage disequilibrium with the causative variant will segregate with the disease. Cosegregation in multiple distantly related family members, multiple families from diverse ethnic background and sequencing of the complete gene provide stronger proof that another variant is not involved. Linkage analysis methods have been published [3, 64] and recently a more **si**mplified method for **s**egregation **a**nalysis (SISA, [35]). As a rule of thumb, 10 informative segregations can provide a significant LOD score (>3.0), providing significant evidence for linkage between a genetic locus and the disease. In general, extended families with cardiogenetic diseases are rare, and therefore, significant LOD scores difficult to obtain, although LOD scores of multiple independent families carrying the same mutation, but not genealogically linked, can be added together. Notably, fewer informative segregation may be sufficient in combination with other supporting data. In addition, lack of segregation of a variant with a phenotype provides strong evidence against pathogenicity.

Functional studies. A reliable functional test is regarded as an important piece of evidence to confirm pathogenicity. However, such assays are rarely available as part of routine diagnostic services. In addition, it is important to assess the validity of a functional test with respect to how closely it reflects the biological situation. Other factors to consider are, for example, validation (robustness, reproducibility) of the assay and specimen integrity including storage and transport. In general, direct analysis on patient material provides the strongest functional evidence. *In vitro* studies can be useful (e.g., patch clamp studies for ion-channels) but may not represent the biological situation completely and therefore does not always provide direct proof for causality but is only moderate evidence.

De novo variants. A *de novo* observation in an exome or genome as such is no longer considered unequivocal evidence for pathogenicity because all individuals are expected to have approximately one *de novo* variant in their exome or 100 in their genome. A *de novo* variant (not present in both biological parents) is considered strong support for pathogenicity when the phenotype of the patient is in line with the gene's disease association and the family history of disease is consistent with *de novo* inheritance. This means unaffected parents for a dominant disorder but more than one sibling can be affected if one of the parents is a germline mosaic.

Other aspects to consider. Genes may have multiple transcripts, some of which can be tissue specific and associated with different phenotypes. When multiple mutations are identified in a single gene, the phase (i.e., in *cis* on the same chromosome or in trans on homologous chromosomes) of the variant is of importance, in particular for recessive disorders. Variant spectrum (i.e., which type of mutation gives rise to which disease) is important to take into account as well. For some genes, truncating variants (e.g., resulting in loss of function) are the primary type of pathogenic variants, as is the case for *MYBPC3* mutations in HCM, whereas in other genes missense (e.g., resulting in a dominant-negative effect) mutations are the primary type of pathogenic variants, as is the case for *MYH7* mutations in HCM. In addition, index patients could have multiple variants that can contribute to more severe disease [24]. These aspects need to be considered and further complicate variant interpretation.

Data Quality Issues and NGS

NGS methods are being used by genome diagnostics laboratories worldwide because of their fast, efficient and relatively cheap analysis of diseases that show genetic heterogeneity (gene panels, exomes, in the future genomes). Many NGS platforms are available and are being adjusted and improved constantly presenting their users with new challenges, both at the technical, data management, interpretation (see section "Variant Classification") and counseling level. With the handling of NGS data, a skilled and dedicated bioinformatician as part of the analysis team in any diagnostic laboratory is mandatory. Before NGS can be used in diagnostics all methods and equipment should be validated. Previously discussed guidelines (e.g., [33, 74]) are being used to allow more standardization and agreement about quality issues and number of genes that needs to be analyzed for a particular disease. Analyzing more genes for a disease does not necessarily mean a higher yield but can instead result in more uncertainty (e.g., identification of more VUSs or obtaining unsolicited findings) for the patient [41]. A condense summary of these guidelines will be given below. Before doing that we will discuss the major components of a NGS analytical pipeline [38]. It all starts by deciding what should be analyzed. One can choose for a targeted panel, which consists of the DNA from only the proven candidate genes for a disease and is obtained by an amplicon-based strategy (PCR-based enrichment of target region) or sequence capture (hybridization based) [34, 51]. WES is also an option. For this, DNA from all coding exons using a sequence capture are isolated. Finally, the whole genome can be sequenced (WGS, not yet in diagnostics). For this, no DNA enrichment is involved.

WES and WGS can be analyzed in different ways using different analysis models, for example, trio analysis for detecting *de novo* mutations or specific approaches for autosomal dominant or recessive inheritance. In addition, filtering of a specific gene panel (virtual analysis) can be used. Each approach has its own quality issues that should be considered, and none of these pretend to give a "complete" analysis, although in the mind of patients who have their "genome" analyzed, this may be suggested. It is the task of the referring clinician to clearly explain the shortcomings of the test to the patient (as a part of the pretest counseling). The conditions for including a gene into a panel or what type of analysis is preferred for a certain disease should ideally be dealt with at the community level in a multidisciplinary way and is not common practice yet. To guarantee uniform and transparent molecular testing between clinical genetic laboratories, it is recommended that a "core disease gene" list and/or "diagnostic routing" for genetic diseases will be defined and maintained [33, 74]. Mutation analysis in these "core disease genes" should warrant a sequencing quality that matches current practice (i.e., high sensitivity and specificity). Sometimes this could mean that gaps should be sequenced with another sequencing method (e.g., Sanger sequencing).

The (enriched) DNA is subsequently used for NGS. Three parts can be distinguished in the process from DNA sequencing to interpretation of NGS data. The first part is the sequencing on the sequencing instrument (e.g., MiSeq, Ion Torrent). Most instruments are also able to convert the raw signals generated by the sequencing instrument into nucleotide bases with associated quality scores in a so-called FASTQ file. The second part involves several methods that operate together to detect genomic aberrations from quality-scored sequence data and consists of several stages from which alignment of reads to the human reference genome, flagging or filtering duplicate reads (probably PCR artifacts) and variant calling are the most important. Alignment problems due to repetitive genomic regions and pseudogenes can result in the loss of sequence data as a result of relatively short read lengths generated by most NGS technologies [67]. The final output is often a VCF (Variant Call Format) file. This file is used in the final stage, which consists of result interpretation by annotating (classifying), the variants to determine their biological significance by trained clinical laboratory geneticists using homemade pipelines or commercial programs like Cartagenia and AlaMut (see also section "Variant Classification").

In order to deliver high-quality diagnostics, it is important to realize the limitations of the different NGS platforms and enrichment methods (if any) used to isolate the DNA to be sequenced. Not only for the laboratory but also for the referring clinician, it is important to know which quality issues need to be considered. In general, the sensitivity of NGS depends largely on the horizontal and vertical coverage of the genomic regions of interest (Table 1.2). In this respect, one should distinguish between raw coverage and informative coverage (Table 1.2): the first is the actual number of times a certain position, that is, nucleotide, has been observed (including low-quality calls), whereas the latter reflects the true informative value of all positions within a gene target (only high-quality calls are included). This informative coverage can be calculated on the basis of a predefined set of filtering criteria: uniqueness of mapping, mapping quality of the read, position of the base within the read and the number of individual start sites represented by the reads (independent samplings from the pool). The informative coverage is per definition at best equal to, but generally lower than, the raw coverage. By only using these informative reads, variant calling efficiency and accuracy have enhanced tremendously, thereby reducing the minimal coverage needed per nucleotide. The minimal vertical informative coverage is dependent on the platform and the strategy used. In general, the Dutch laboratories recommend at least 20–30× coverage; read depths that range from 15 to 20 are left to the expert interpretation of the laboratory specialist. In addition, the laboratory must be able to guarantee that reported variants are associated with the analyzed patient by using a SNP control test or confirmation on a second (independent) DNA sample to rule out sample swaps. Other aspects that influence data quality are the way in which target enrichment is performed. Amplicon-based strategies can result in losing important sequence information because of allelic dropout (due to rare polymorphisms), whereas an important drawback of hybridization-based enrichment strategies is reduced coverage in GC-rich regions [34, 51]. To make this issue more transparent [33], proposed a rating system (A, B, or C) for NGS diagnostics. With a type-A test reserved for genes or gene panels analyzed with the highest possible quality, that is, no sequence gaps in the target region are allowed. In a type-B test, the lab describes exactly which regions are sequenced with the highest possible quality and which not (only for some regions sequence gaps are filled with another sequencing method). The type-C test solely

Table 1.2 Definitions and used terminology (see also [74])

Target	Selected template region that needs to be investigated
Raw coverage	Percentage of reads that map to reference genome
Horizontal coverage	Percentage of target mapped reads that are on or near the target
Vertical coverage	Read depth, uniquely mapped reads at a specific locus
Informative coverage	Uniquely mapped (high quality) reads, excluding duplicate reads
Core disease gene list	Disease genes that are considered essential to establish a molecular diagnosis (i.e., genes containing proven pathogenic mutations that explains the disease)
Diagnostic routing	Flowchart, indicating the routing of genetic tests within the laboratory for a specific disease, can be a combination of different techniques

relies on the quality of NGS sequencing (no additional Sanger (or other) sequencing is offered) as is the case for exomes. If a test is based on exome of genome sequencing, then it should be realized that with the current possibilities quality issues related to insufficient sequence depth of many regions should be accepted. Rehm [43] stated this in the following way: "although exome and genome sequencing are often referred to as 'whole' exome or genome sequencing, these services might better be called 'hole' exome and genome sequencing". Nevertheless in cases of extreme locus heterogeneity (e.g., in pediatric cardiac diseases), WES can be the best choice because the reduced sensitivity per gene compared with conventional sequencing may be compensated by the large number of genes that can be included in this test, resulting in a higher diagnostic yield (i.e., the number of patients that receive a molecular confirmation of a given clinical diagnosis).

Reporting of Results

The writing of diagnostic reports is challenging, as they should be as concise as possible but contain the essential information in understandable wording and according to international diagnostic standards ISO15189, ACMG [27, 28, 44, 56], CMGS [66], or RCPA [61]. In this section, the most important issues will be discussed. Further details about this subject can be found in the diagnostic standards referred above and other guidelines [45, 73, 74]. In summary, a report should contain all of the essential elements of the test performed, an interpretation, relevant references, methodology, proposed follow-up tests if appropriate and disclaimers. The mutations should be described using HGVS nomenclature (see also section "Classification of Variants"). It is important that test characteristics (e.g., minimal vertical coverage, the average vertical coverage per gene, the complete gene list and analyzed gene parts, the data analysis pipeline and version and diagnostic routing) are included or provided in an alternative manner. In Fig. 1.3, an example letter from a diagnostic laboratory is shown including all relevant test characteristics in the appendix. It is strongly recommended to use the 5 class system for reporting variants (see section "Classification of Variants"). Class 1 variants are generally not reported as this could lead to misinterpretation outside of the laboratory. Local policy will determine whether class 2 variants are reported. In general, class 4 and 5 variants are always reported. Reporting of class 3 variants depends on the DNA test performed. Class 3 variants are reported when targeted panels containing proven candidate genes are analyzed but often not reported when a class 3 variant is detected in a gene of uncertain significance (GUS) [74]. Class 3 variants have the potential to cause confusion and should therefore be communicated to adequately trained clinicians. Most of the time this would be a clinical geneticist or genetic counselor. As indicated above, not all variants will be reported to the referring clinician, but all variants are recorded within the laboratory. In addition, it is not essential to document all lines of evidence obtained in a report because this can be confusing to the clinician, but complete records must be stored in the laboratory.

Finding New Disease Genes

As exemplified in the coming chapters, many genes associated with various cardiovascular diseases have been identified in the past decennia. Nevertheless, all these genes still do not explain all known genetic cardiac disorders and more genes still have to be uncovered. Up to the current NGS era, in general new genes were discovered by studying large families in which many affected family members were available to be genetically analyzed. In order to identify the most likely disease-associated region within a family polymorphic markers, often dinucleotide repeats (and in some cases repeats of three or more nucleotides) that differ in length between individuals, covering the full genome were being used. By mapping these markers in all affected family members and comparing with nonaffected family members, so-called linkage analysis (or also known as haplotype-sharing analysis), it was possible to identify a set of linked markers shared by all affected, and most likely harboring the causal mutation in this family. Often this region still contained many possible candidate genes in which each had to be analyzed by Sanger sequencing to get to the true disease gene. Of course, genes known to be related to the heart or for which expression in cardiac tissue was shown were the ones prioritized for this. In case of suspected recessive-inherited disease, mostly because of known consanguinity in the respective family, polymorphic markers were also mapped but limited to the affected child (and often also the parents). In such analysis, the underlying genetic cause was supposed to be identical on both alleles (homozygous), and thus, (a) region(s) with a large number of subsequent identical markers were analyzed for mutations. This so-called homozygosity mapping has, however, been successful in only a limited part of all inherited cardiac disorders. When high-density SNP arrays became available, linkage-like analyses and homozygosity mapping adapted to using such arrays as these had higher resolution, thereby in most cases reducing the size of the linked/homozygous region, and limiting the amount of candidate genes to be Sanger sequenced. Another way to identify new disease genes was by comparing the deleted (or occasionally duplicated) regions on the same chromosomal region in a number of patients with the same phenotype and deducing the minimal deleted region shared. By including as many of such patients as possible, the regions could be considerably minimized to one containing only several genes. By subsequently sequencing these putative candidate genes in another group of patients with the same or a very comparable phenotype, but in which no deletion of the respective region could be found, the identification of causal point mutations in one of these genes would pinpoint to the actual disease gene in the deleted region of the initial patient cohort.

a

To: Consultant Clinical Geneticist

Date:
Letter ID:
Our reference: FFxxxxx,

Dear colleague,

Enclosed the results of the DNA-analysis you requested:

Name:	A. Patient
Date of birth:	……
Gender:	……
Date application:	……
Indication(s):	Confirmation of clinical diagnosis of Dilated Cardiomyopathy
Performed diagnostics:	Next-generation sequence analysis of the cardiomyopathy panel (50 genes)
Diagnosed materials:	16Dxxxx (DNA from EDTA blood)

Result

Heterozygous mutation c.40_42del was found in the PLN gene (reference sequence NM_002667.3). No other (pathogenic) mutations detected in the analyzed genes by Next Generation Sequencing (see appendix for method and quality).

Conclusion

Patient is heterozygous for the mutation c.40_42del; p.(Arg14del) in the PLN gene. This result confirms the clinical diagnosis of dilated cardiomyopathy in this patient. Based on the findings indicated in the remarks section the mutation is classified as a class 5 mutation.

Classification system:

Class 1: (certainly not pathogenic), class 2: (unlikely pathogenic) mutations, class 3: unknown pathogenicity, class 4: likely pathogenic; class 5: (certainly) pathogenic.

Remarks

The nucleotide change c.40_42del results in a deletion of three nucleotides predicting a deletion of amino acid Aginineat position 14 (p.(Arg14del) in the PLN protein. The deleted amino acid (Arg14) is strongly conserved. In literature this mutation has been described before in DCM families (de Witt MM et al., J Am Coll Cardiol 2006;48:1396-1398; Haghighi K et al., Proc Natl Acad Sci 2006;103:1388-93). Introduction of this mutated gene in transgenic mice results in a similar phenotype and co-transfection in HEK-293 cells shows strong inhibition of the sarcoplasmic reticulum Ca2+-ATPase (de Witt MM et al., J Am Coll Cardiol 2006;48:1396-1398). This mutation is a well-known founder mutation in The Netherlands (van der Zwaag PA et al., EurJ Heart Fail. 2012;14(11):1199-207).

Genetic testing of at risk family members is possible.

All coding exons of the 50 genes (45 "core genes" for cardiomyopathy plus ALPK3, FHL2, HCN4, PRDM16 enTTN), including the 20 flanking intron nucleotides have been analyzed. The presence of larger deletions or insertions, or mutations located outside the analysed fragments can not be excluded. The "Dutch core disease genes" in a diagnostic test represent the genes which are considered as essential for establishing a reliable and accurate molecular diagnosis (see Weiss MM et al, Human Mutat 2013; 34:1313-1321). In the Netherlands all diagnostic laboratories agreed to analyze at least these 45 core genes in cardiomyopathy index patients requested for genetic testing.

Yours sincerely,

clinical molecular geneticist clinical molecular geneticist

The Genome Diagnostics laboratory … is EN-ISO15189:2012 accredited (…. Accreditation Council, M174); Nomenclature according to http://www.hgvs.org/mutnomen/ . Our conclusions are based on the assumption that the tubes were correctly labelled and that the information provided in the pedigree is correct. We accept no responsibility for errors involving incorrect interpretation and/or translation of our letters.

Fig. 1.3 Example letter (*A*) including quality issues in the appendix (*B*) sent to a referring clinical geneticist that request targeted NGS analysis for dilated cardiomyopathy

b **Appendix**

Method and quality

Machine: MiSeq
MiSeq experiment nr: ….
Chemistry: MiSeq Reagent Kit v2, 2 x 150 bp
Analysis programs: BWA-MEM(0.7.5), GenomeAnalysisTK-2.8-1-g932cd3a, Cartagenia v4.2.2 (r13768).
Enrichment of the target regions: Nimblegen SeqCapeasy choice (OID…..+ OID….) version CMv12.
Minimal coverage (minimal MAPQ20 and BaseQ20): 30 reads.
Average vertical coverage (minimal MAPQ20 and BaseQ20): 787 (±372)
Sanger sequencing for regions with a coverage < 30 reads.
Sample swaps ruled out with a SNP control test.
We do not report variants occurring in > 0,3% of control alleles (NHLBI Exome Sequencing Project), silent mutations without a clear effect on splicing (as predicted by the programs in AlaMut), class 1 (certainly not pathogenic) and class 2 (unlikely pathogenic) mutations.
Based on our validation experiments we determined that the sensitivity of our combined test (NextGenand Sanger sequencing) for nucleotide substitutions and deletions, insertions and duplications up to 68 nucleotides is >99%.

Genes with reference sequences:
1. ACTC1 (NM_005159.4), 2. ACTN2 (NM_001103.2), 3. ALPK3 (NM_020778.4), 4. ANKRD1 (NM_014391.2), 5. BAG3 (NM_004281.3), 6. CALR3 (NM_145046.3), 7. CAV3 (NM_033337.3), 8. CRYAB (NM_001885.1), 9. CSRP3 (NM_003476.3), 10. CTNNA3 (NM_013266.2), 11. DES (NM_001927.3), 12. DSC2 (NM_024422.3, NM_004949.3), 13. DSG2 (NM_001943.3), 14. DSP (NM_004415.2), 15. EMD (STA) (NM_000117.2), 16. FHL1 (NM_001159702.2, NM_001159701.1, NM_001159699.1), 17. FHL2 (NM_201555.1), 18. GLA (NM_000169.2), 19. HCN4 (NM_005477.2), 20. JPH2 (NM_020433.4, NM_175913.3), 21. JUP (NM_021991.2), 22. LAMA4 (NM_002290.4, NM_001105206.2, NM_001105208.2), 23. LAMP2 (NM_002294.2, NM_013995.2, NM_001122606.1), 24. LDB3 (NM_007078.2, NM_001080116.1), 25. LMNA (NM_170707.3, NM_001257374.1, NM_005572.3), 26. MIB1 (NM_020774.2), 27. MYBPC3 (NM_000256.3), 28. MYH6 (NM_002471.3), 29. MYH7 (NM_000257.2), 30. MYL2 (NM_000432.3), 31. MYL3 (NM_000258.2), 32. MYOZ2 (NM_016599.3), 33. MYPN (NM_032578.2), 34. NEXN (NM_144573.3), 35. PKP2 (NM_004572.3), 36. PLN (NM_002667.3), 37. PRDM16 (NM_022114.3), 38. PRKAG2 (NM_016203.3), 39. RBM20 (NM_001134363.1), 40. SCN5A (NM_198056.2, NM_001160160.1), 41. TAZ (NM_000116.3), 42. TCAP (NM_003673.3), 43. TMEM43 (NM_024334.2), 44. TNNC1 (NM_003280.2), 45. TNNI3 (NM_000363.4), 46. TNNT2 (NM_000364.2, NM_001001430.1), 47. TPM1 (NM_000366.5, NM_001018005.1, NM_001018020.1), 48. TTN (N2-B (NM_003319.4; all coding exons), N2A (NM_133378.4; all coding exons), Novex-3 (NM_133379.3; all coding exons), Novex-1 (NM_133432.3; all coding exons), Novex-2 (NM_133437.3; all coding exons), transcript variant IC (NM_001267550.1; 335 of the 362 all coding exons were analyzed)), 49. TTR (NM_000371.3), 50. VCL (NM_014000.2).

Fig. 1.3 (continued)

Nowadays, NGS is generally being employed to find new disease genes. Comparably to the situation in previous described linkage/haplotype sharing analyses, now several affected family members are being analyzed by WES of GWS after which the identified exome or genome wide variation is being compared. In order to perform a proper comparison, first all variants that are rather frequent in the general population (>1 %) are removed from the analysis. Only then the list of remaining variants will be short enough to enable the detection of the shared, potentially pathogenic variant. However, this is still not an easy task and predictions on pathogenicity of the different shared candidate variants are required to identify the truly pathogenic variant. Combining this with an additional linkage-like approach will facilitate the search for the causal mutation, as the variation in the shared region or regions identified by the linkage method can then be prioritized. The same of course accounts to homozygosity mapping in combination with NGS and subsequent hunting for deleterious mutations in the homozygous region of patients supposedly affected with a recessive inherited disease. Moreover, performing NGS in a cohort of patients with the same phenotype, but no deletion/duplication detected via array-based approaches, and then zooming in on regions that have been shown to be deleted in a comparable patient cohort could also result in identifying nucleotide mutations in such patients. Of course, the above-described methods can also be exploited to identify causal genes in patients that show the same phenotype and come from the same geographic region, but are not known to be related, with the underlying idea that they might share a founder mutation (and thus in the end are related). This could be done by using NGS data only, but will probably be more successful in combination with a haplotype-sharing approach. Finally, in case of very rare diseases, the comparison of NGS data of several, certainly not related patients (often even originating from different ethnic backgrounds) is being used to find new disease genes, however in that case with the assumption that these patients do not share the same mutation, but different drastic mutations, in the

same gene. It is important to realize with respect to this type of analyses that not all patients included in such efforts necessarily share only one disease gene, and extensive computational work is needed to get to the once that do.

Clinical Genetic Diagnostics

In the last decades, clinical genetic testing has become increasingly incorporated in the clinical care for patients and their families with inherited cardiac diseases, and genetic analyses often considerably contributing to the diagnostic work-up for these patients. With the recent developments in WGS possibilities, clinical genetic diagnostics may even develop toward "genotype first" approaches, meaning that patients suspected of having a genetic cardiac disease will first undergo genetic screening, preferably by performing WGS, to identify putative pathogenic mutations in cardiac-related genes, before other diagnostic testing will be performed. It can be expected that WGS will be performed rapidly enough in the near future that waiting for the result to guide further diagnostics, and prevent unnecessary examinations, treatments, and interventions, may become general practice. In this respect, rapid WGS was already used to reach genetic diagnoses within several days, including that of Long QT syndrome ([55], Priest et al. [40]). Moreover, with the continuing aggregation of large amounts of exome- and genome-sequencing data and concomitant developments in interpreting the data, personalized genetic medicine will become a reality soon as well. However, these methods are not yet within reach for every individual and will thus only be applied in particular cases, and more targeted approaches are currently daily practice and will therefore be discussed in more detail below.

In the past, when only individual genes were screened sequentially genetic screening was mostly terminated as soon as a likely pathogenic or pathogenic mutation was identified, precluding the identification of a second mutation of which it is known that these may explain the clinical phenotype in ~5–10 % of cases. The early application of targeted NGS approaches within the field of cardiogenetics relates to the fact that many candidate genes were already known to be involved in different cardiac diseases but was certainly also endorsed because of the extensive genetic heterogeneity within as well as the genetic overlap between these disorders. Although the latter would support the screening of all known cardiac-related genes within one experiment in every cardiac patient, most laboratories started with developing gene-panel approaches targeting genes involved in a specific disease or disease type, and which gene-panel used was guided by the clinical diagnosis/suspicion. This had to do with the labs being unfamiliar with both the technique and the putative outcome of genes irregularly screened. And in addition with the costs involved in using larger panels, as these would increase because of more materials and equipment being used, but also due to more (bioinformatical) processing time required. Currently, genome diagnostic labs do offer targeted NGS analyses for most diseases, among which cardiac diseases. The number of genes targeted may differ between one gene (e.g., the screening of largest known human gene *TTN*, in which truncating mutations explain between 15 % and 25 % of inherited DCM cases is being offered as a stand-alone NGS test in a number of laboratories) up to several hundreds. Unfortunately, the exact content of these gene panels differs between labs. It is therefore of major importance that specialists worldwide continuously interact and reach a consensus on which genes would be mandatory to be included in disease specific panels. Of note, the exact content may of course be influenced by regional differences. In this respect, all Dutch genome diagnostic laboratories proposed that labs should agree on and use a list of "core disease genes" for every genetic disease that should be included in a diagnostic test to establish a reliable and molecular diagnosis [74]. Therefore, the five labs in the Netherlands that provide cardiogenetic NGS tests decided on a core list of 45 cardiomyopathy genes that are included in their gene panels, despite differences in the remainder of the content of their gene panels. It is important to realize, however, that some labs may offer NGS applications targeting only a relatively small subset of genes associated with a specific disease type, while these may be included in larger panels in other genetic testing facilities. On the other hand, often also the possibility is offered to sequence a larger gene panel, but subsequently filter bioinformatically for a subpanel that is related to the specific disorder. The advantage of this approach is that this provides the possibility to again analyze the data using other or no filtering when the disease-specific filter failed to identify causal mutations. Importantly, in addition to proposing the use of core disease gene lists, the Dutch genome diagnostic labs in the same manuscript published general best practice guidelines for the use of NGS applications in clinical settings to ensure high-quality diagnostics. Likewise, this was for example done by the ACMG [44] and EuroGentest and the European Society of Human Genetics [33].

The reason to initially offer NGS application in which disease-associated genes were specifically enriched, and subsequently, sequenced was due to insufficient sequencing quality and coverage in WES data. However, due to significant improvements in both vertical and horizontal coverage of, in particular, disease-associated genes in recently introduced WES applications, a substantial number of clinical genetic testing laboratories are now moving toward diagnostic WES with subsequent *in silico* filtering for disease-specific genes. Comparable to the possibility mentioned

above to analyze subpanels within enriched larger gene panels and subsequently apply other filtering or "open" the gene panel, the same can be done with WES data, without being limited to the enriched genes. Moreover, this also enables "opening the exome," including the sequences of genes not (yet) related to the disease and by allowing the hunt for new disease genes. In spite of the already mentioned improvements in WES, WGS would still be the most preferred method, as this will provide more evenly distributed coverage and shorter sequencing time. Since this approach is becoming cheaper, WGS will most likely be the most preferred option for the near future and several studies have proven its added value in clinical practice [13, 40, 55].

One of the advantages of the more evenly distributed sequencing data coming from WGS is that it is well suitable for quantitative analyses, in order to identify deletions and duplications [13, 77], as the normalization of data to enable the identification of aberrations in coverage indicating such deletions/duplications is easier than with WES or disease panel-specific enrichment approaches. Nevertheless, computational methods to perform this in the latter NGS data have been developed and have found their way into clinical diagnostics recently as well [19, 63]. Although these methods do not yet assure detection of all deletions/duplications, it may provide more information than previously used tests like MLPA and Q-PCR or array-based approaches, as they either focus on a limited set of genes only, or have limitations in detecting smaller deletions, respectively. Nevertheless, currently, these techniques are often still used in addition to NGS applications, certainly when these type of mutations in specific genes can be expected, for example using the respective MLPA when deletions in the *LMNA* gene are to be suspected in patients with cardiomyopathy and conduction disease. In addition to the use of these methods in parallel with NGS, other "old-fashioned" techniques are still being used in clinical genetic diagnostics. Sanger sequencing is still the preferred method to perform predictive testing in family members at risk after NGS has identified the disease-causing mutation in the respective index patient. Moreover, in numerous cases, it is still more cost-effective to first perform Sanger sequencing of the most prevalent gene, or sometimes the only gene yet known to be involved in that disease before using a gene panel, WES or WGS. However, as the logistics of near future genome diagnostic labs will almost fully be focused on NGS applications, one may anticipate that even those rare cases will all be screened by NGS first followed be targeting the respective gene(s) *in silico*. As long as WGS is not routinely introduced, in addition to applying the current NGS methodologies, several cytogenetic techniques, like karyotyping, FISH analyses, and array-CGH, are still widely used within the field of CHDs, syndromal cardiac diseases, or multiple congenital anomalies/mental retardation (MCA/MR) syndromes including cardiac abnormalities. Finally, the introduction of other techniques or approaches, like the TLA technology to detect disease-causing mutations outside coding of near-coding sequences or using RNA sequencing instead of DNA sequencing, will be implemented in diagnostics in the near future as well.

References

1. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG. Sequence and organization of the human mitochondrial genome. Nature. 1981;290:457–65.
2. Andreasen C, Nielsen JB, Refsgaard L, Holst AG, Christensen AH, Andreasen L, Sajadieh A, Haunsø S, Svendsen JH, Olesen MS. New population-based exome data are questioning the pathogenicity of previously cardiomyopathy-associated genetic variants. Eur J Hum Genet. 2013;21:918–28.
3. Bayrak-Toydemir P, McDonald J, Mao R, Phansalkar A, Gedge F, Robles J, Goldgar D, Lyon E. Likelihood ratios to assess genetic evidence for clinical significance of uncertain variants: hereditary hemorrhagic telangiectasia as a model. Exp Mol Pathol. 2008;85:45–9.
4. Bhuiyan ZA, van den Berg MP, van Tintelen JP, Bink-Boelkens MT, Wiesfeld AC, Alders M, Postma AV, van Langen I, Mannens MM, Wilde AA. Expanding spectrum of human *RYR2*-related disease: new electrocardiographic, structural, and genetic features. Circulation. 2007;116:1569–76.
5. Calabrese F, Thiene G. Myocarditis and inflammatory cardiomyopathy: microbiological and molecular biological aspects. Cardiovasc Res. 2003;60:11–25.
6. dela Torre R, Larkin J, Singer A, Meller A. Fabrication and characterization of solid-state nanopore arrays for high-throughput DNA sequencing. Nanotechnology. 2012;23:385–08.
7. Dhandapany PS, Sadayappan S, Xue Y, Powell GT, Rani DS, Nallari P, Rai TS, Khullar M, Soares P, Bahl A, Tharkan JM, Vaideeswar P, Rathinavel A, Narasimhan C, Ayapati DR, Ayub Q, Mehdi SQ, Oppenheimer S, Richards MB, Price AL, Patterson N, Reich D, Singh L, Tyler-Smith C, Thangaraj K. A common *MYBPC3* (cardiac myosin binding protein C) variant associated with cardiomyopathies in South Asia. Nat Genet. 2009;41:187–91.
8. Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J. A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature. 1996;380:152–4.
9. Driscoll DA, Salvin J, Sellinger B, Budarf ML, McDonald-McGinn DM, Zackai EH, Emanuel BS. Prevalence of 22q11 microdeletions in DiGeorge and velocardiofacial syndromes: implications for genetic counselling and prenatal diagnosis. J Med Genet. 1993;30:813–7.
10. Guiver M, Fox AJ, Mutton K, Mogulkoc N, Egan J. Evaluation of CMV viral load using TaqMan CMV quantitative PCR and comparison with CMV antigenemia in heart and lung transplant recipients. Transplantation. 2001;71:1609–15.
11. Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. Hum Mutat. 2011;32:557–63.
12. Futreal PA, Söderkvist P, Marks JR, Iglehart JD, Cochran C, Barrett JC, Wiseman RW. Detection of frequent allelic loss on proximal chromosome 17q in sporadic breast carcinoma using microsatellite length polymorphisms. Cancer Res. 1992;52:2624–7.
13. Gilissen C, Hehir-Kwa JY, Thung DT, van de Vorst M, van Bon BW, Willemsen MH, Kwint M, Janssen IM, Hoischen A, Schenck A, Leach R, Klein R, Tearle R, Bo T, Pfundt R, Yntema HG, de Vries BB, Kleefstra T, Brunner HG, Vissers LE, Veltman JA. Genome sequencing identifies major causes of severe intellectual disability. Nature. 2014;511:344–7.

14. Goidts V, Cooper DN, Armengol L, Schempp W, Conroy J, Estivill X, Nowak N, Hameister H, Kehrer-Sawatzki H. Complex patterns of copy number variation at sites of segmental duplications: an important category of structural variation in the human genome. Hum Genet. 2006;120:270–84.
15. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res. 1996;6:986–94.
16. Houdayer C, Caux-Montcoutier V, Krieger S, Barrois M, Bonnet F, Bourdon V, Bronner M, Buisson M, Coulet F, Gaildrat P, Lefol C, Léone M, Mazoyer S, Muller D, Remenieras A, Révillion F, Rouleau E, Sokolowska J, JP V, Lidereau R, Soubrier F, Sobol H, Sevenet N, Bressac-de Paillerets B, Hardouin A, Tosi M, OM S, Stoppa-Lyonnet D. Guidelines for splicing analysis in molecular diagnosis derived from a set of 327 combined in silico/in vitro studies on *BRCA1* and *BRCA2* variants. Hum Mutat. 2012;33:1228–38.
17. International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. Nature. 2004; 431:931–45.
18. Istrail S, Sutton GG, Florea L, Halpern AL, Mobarry CM, Lippert R, Walenz B, Shatkay H, Dew I, Miller JR, Flanigan MJ, Edwards NJ, Bolanos R, Fasulo D, Halldorsson BV, Hannenhalli S, Turner R, Yooseph S, Lu F, Nusskern DR, Shue BC, Zheng XH, Zhong F, Delcher AL, Huson DH, Kravitz SA, Mouchard L, Reinert K, Remington KA, Clark AG, Waterman MS, Eichler EE, Adams MD, Hunkapiller MW, Myers EW, Venter JC. Whole-genome shotgun assembly and comparison of human genome assemblies. Proc Natl Acad Sci U S A. 2004;101:1916–21.
19. Johansson LF, van Dijk F, de Boer EN, van Dijk-Bos KK, Jongbloed JD, van der Hout AH, Westers H, Sinke RJ, Swertz MA, Sijmons RH, Sikkema-Raddatz B. CoNVaDING: Single Exon Variation Detection in Targeted NGS Data. Hum Mutat. 2016;37:457–64.
20. Jovanovich S, Bogdan G, Belcinski R, Buscaino J, Burgi D, Butts EL, Chear K, Ciopyk B, Eberhart D, El-Sissi O, Franklin H, Gangano S, Gass J, Harris D, Hennessy L, Kindwall A, King D, Klevenberg J, Li Y, Mehendale N, McIntosh R, Nielsen B, Park C, Pearson F, Schueren R, Stainton N, Troup C, PM V, Vangbo M, Woudenberg T, Wyrick D, Williams S. Developmental validation of a fully integrated sample-to-profile rapid human identification system for processing single-source reference buccal samples. Forensic Sci Int Genet. 2015;16:181–94.
21. Klein D. Quantification using real-time PCR technology: applications and limitations. Trends Mol Med. 2002;8:257–60.
22. Komura D, Shen F, Ishikawa S, Fitch KR, Chen W, Zhang J, Liu G, Ihara S, Nakamura H, Hurles ME, Lee C, Scherer SW, Jones KW, Shapero MH, Huang J, Aburatani H. Genome-wide detection of human copy number variations using high-density DNA oligonucleotide arrays. Genome Res. 2006;16:1575–84.
23. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, Gu B, Hart J, Hoffman D, Hoover J, Jang W, Katz K, Ovetsky M, Riley G, Sethi A, Tully R, Villamarin-Salomon R, Rubinstein W, Maglott DR. ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res. 2016;44:D862–8.
24. Lekanne Deprez RH, Muurling-Vlietman JJ, Hruda J, Baars MJ, Wijnaendts LC, Stolte-Dijkstra I, Alders M, van Hagen JM. Two cases of severe neonatal hypertrophic cardiomyopathy caused by compound heterozygous mutations in the *MYBPC3* gene. J Med Genet. 2006;43:829–32.
25. Levene MJ, Korlach J, Turner SW, Foquet M, Craighead HG, Webb WW. Zero-mode waveguides for single-molecule analysis at high concentrations. Science. 2003;299:682–6.
26. Lindor NM, Karnes PS, Michels VV, Dewald GW, Goerss J, Jalal S, Jenkins RB, Vockley G, Thibodeau SN. Uniparental disomy in congenital disorders: a prospective study. Am J Med Genet. 1995;58:143–6.
27. Lubin IM, Caggana M, Constantin C, Gross SJ, Lyon E, Pagon RA, Trotter TL, Wilson JA, McGovern MM. Ordering molecular genetic tests and reporting results: practices in laboratory and clinical settings. J Mol Diagn. 2008;10:459–68.
28. Lubin IM, McGovern MM, Gibson Z, Gross SJ, Lyon E, Pagon RA, Pratt VM, Rashid J, Shaw C, Stoddard L, Trotter TL, Williams MS, Amos Wilson J, Pass K. Clinician perspectives about molecular genetic testing for heritable conditions and development of a clinician-friendly laboratory report. J Mol Diagn. 2009;11:162–71.
29. Lubs HA. A marker X chromosome. Am J Hum Genet. 1969;21: 231–44.
30. Lyon MF. Gene action in the X-chromosome of the mouse (Mus musculus L.). Nature. 1961;190:372–3.
31. Lyon MF. Sex chromatin and gene action in the mammalian X-chromosome. Am J Hum Genet. 1962;14:135–48.
32. Mardis ER. Next-generation sequencing platforms. Annu Rev Anal Chem. 2013;6:287–303.
33. Matthijs G, Souche E, Alders M, Corveleyn A, Eck S, Feenstra I, Race V, Sistermans E, Sturm M, Weiss M, Yntema H, Bakker B, Scheffer H, Bauer P. Guidelines for diagnostic next-generation sequencing. Eur J Hum Genet. 2016;24:2–5.
34. Mamanova L, Coffey AJ, Scott CE, Kozarewa I, Turner EH, Kumar A, Howard E, Shendure J, Turner DJ. Target-enrichment strategies for next-generation sequencing. Nat Methods. 2010;7:111–8.
35. Møller P, Clark N, Mæhle L. A simplified method for Segregation Analysis (SISA) to determine penetrance and expression of a genetic variant in a family. Hum Mutat. 2011;32:568–71.
36. Morris KV, Mattick JS. The rise of regulatory RNA. Nat Rev Genet. 2014;15:423–37.
37. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol. 1986;51(Pt 1): 263–73.
38. Oliver GR, Hart SN, Klee EW. Bioinformatics for Clinical Next Generation Sequencing. Clin Chem. 2015;61:124–35.
39. Pinheiro LB, Coleman VA, Hindson CM, Herrmann J, Hindson BJ, Bhat S, Emslie KR. Evaluation of a droplet digital polymerase chain reaction format for DNA copy number quantification. Anal Chem. 2012;84:1003–11.
40. Priest JR, Ceresnak SR, Dewey FE, Malloy-Walton LE, Dunn K, Grove ME, Perez MV, Maeda K, Dubin AM, Ashley EA. Molecular diagnosis of long QT syndrome at 10 days of life by rapid whole genome sequencing. Heart Rhythm. 2014;11:1707–13.
41. Pugh TJ, Kelly MA, Gowrisankar S, Hynes E, Seidman MA, Baxter SM, Bowser M, Harrison B, Aaron D, Mahanta LM, Lakdawala NK, McDermott G, White ET, Rehm HL, Lebo M, Funke BH. The landscape of genetic variation in dilated cardiomyopathy as surveyed by clinical DNA sequencing. Gene Med. 2014;16:601–8.
42. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, González JR, Gratacòs M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. Global variation in copy number in the human genome. Nature. 2006;444:444–54.
43. Rehm HL. Disease-targeted sequencing: a cornerstone in the clinic. Nat Rev Genet. 2013;14:295–300.
44. Rehm HL, Bale SJ, Bayrak-Toydemir P, Berg JS, Brown KK, Deignan JL, Friez MJ, Funke BH, Hegde MR, Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Commitee. ACMG clinical laboratory standards for next-generation sequencing. Gene Med. 2013;15:733–47.
45. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Gene Med. 2015;17:405–24.
46. Riera C, Lois S, Dom C, Fernandez-Cadenas I, Montaner J, Rodrıguez-Sureda V, de la Cruz X. Molecular damage in Fabry

disease: Characterization and prediction of alpha-galactosidase A pathological mutations. Proteins. 2015;83:91–104.
47. Risgaard B, Jabbari R, Refsgaard L, Holst AG, Haunsø S, Sadjadieh A, Winkel BG, Olesen MS, Tfelt-Hansen J. High prevalence of genetic variants previously associated with Brugada syndrome in new exome data. Clin Genet. 2013;84:489–95.
48. Roberts JD, Herkert JC, Rutberg J, Nikkel SM, Wiesfeld AC, Dooijes D, Gow RM, van Tintelen JP, Gollob MH. Detection of genomic deletions of *PKP2* in arrhythmogenic right ventricular cardiomyopathy. Clin Genet. 2013;83:452–6.
49. Ronaghi M, Karamohamed S, Pettersson B, Uhlén M, Nyrén P. Real-time DNA sequencing using detection of pyrophosphate release. Anal Biochem. 1996;242:84–9.
50. Ross RS, Chien KR. The polymerase chain reaction (PCR) and cardiovascular diagnosis. Trends Cardiovasc Med. 1991;1:1–5.
51. Rossetti S, Hopp K, Sikkink RA, Sundsbak JL, Lee YK, Kubly V, Eckloff BW, Ward CJ, Winearls CG, Torres VE, Harris PC. Identification of gene mutations in autosomal dominant polycystic kidney disease through targeted resequencing. J Am Soc Nephrol. 2012;23:915–33.
52. Samorodnitsky E, Jewell BM, Hagopian R, Miya J, Wing MR, Lyon E, Damodaran S, Bhatt D, Reeser JW, Datta J, Roychowdhury S. Evaluation of Hybridization Capture Versus Amplicon-Based Methods for Whole-Exome Sequencing. Hum Mutat. 2015;36:903–14.
53. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A. 1977;74:5463–7.
54. Sanger F, Coulson AR, Hong GF, Hill DF, Petersen GB. Nucleotide sequence of bacteriophage lambda DNA. J Mol Biol. 1982;162:729–73.
55. Saunders CJ, Miller NA, Soden SE, Dinwiddie DL, Noll A, Alnadi NA, Andraws N, Patterson ML, Krivohlavek LA, Fellis J, Humphray S, Saffrey P, Kingsbury Z, Weir JC, Betley J, Grocock RJ, Margulies EH, Farrow EG, Artman M, Safina NP, Petrikin JE, Hall KP, Kingsmore SF. Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units. Sci Transl Med. 2012;4:154ra135.
56. Scheuner MT, Hilborne L, Brown J, Lubin IM. A report template for molecular genetic tests designed to improve communication between the clinician and laboratory. Genet Test Mol Biomarkers. 2012;16:761–9.
57. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res. 2002;30:e57.
58. Sharp AJ, Locke DP, McGrath SD, Cheng Z, Bailey JA, Vallente RU, Pertz LM, Clark RA, Schwartz S, Segraves R, Oseroff VV, Albertson DG, Pinkel D, Eichler EE. Segmental duplications and copy-number variation in the human genome. Am J Hum Genet. 2005;77:78–88.
59. Stenson PD, Mort M, Ball EV, Shaw K, Phillips A, Cooper DN. The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. Hum Genet. 2014;133:1–9.
60. Stoddart D, Heron AJ, Mikhailova E, Maglia G, Bayley H. Single-nucleotide discrimination in immobilized DNA oligonucleotides with a biological nanopore. Proc Natl Acad Sci U S A. 2009;106:7702–7.
61. Suthers G. Guidelines for reporting molecular genetic tests to medical practitioners. 2009. http://www.rcpa.edu.au//Home.htm
62. Sykes PJ, Neoh SH, Brisco MJ, Hughes E, Condon J, Morley AA. Quantitation of targets for PCR by use of limiting dilution. Biotechniques. 1992;13:444–9.
63. Tan R, Wang Y, Kleinstein SE, Liu Y, Zhu X, Guo H, Jiang Q, Allen AS, Zhu M. An evaluation of copy number variation detection tools from whole exome sequencing data. Hum Mutat. 2013;35:899–907.
64. Thompson D, Easton DF, Goldgar DE. A full-likelihood method for the evaluation of causality of sequence variants from family data. Am J Hum Genet. 2003;73:652–5.
65. Thusberg J, Olatubosun A, Vihinen M. Performance of mutation pathogenicity prediction methods on missense variants. Hum Mutat. 2011;32:358–68.
66. Treacy RJL and Robinson DO. Draft Best Practice Guidelines for Reporting Molecular Genetics results. 2013 http://www.cmgs.org/BPGs/Best_Practice_Guidelines.htm.
67. Treangen TJ, Salzberg SL. Repetitive DNA and next generation sequencing: computational challenges and solutions. Nat Rev Genet. 2012;13:36–46.
68. van der Zwaag PA, van Rijsingen IA, Asimaki A, Jongbloed JD, van Veldhuisen DJ, Wiesfeld AC, Cox MG, van Lochem LT, de Boer RA, Hofstra RM, Christiaans I, van Spaendonck-Zwarts KY, Lekanne dit Deprez RH, Judge DP, Calkins H, Suurmeijer AJ, Hauer RN, Saffitz JE, Wilde AA, van den Berg MP, van Tintelen JP. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. Eur J Heart Fail. 2012;14:1199–207.
69. van Rijsingen IA, van der Zwaag PA, Groeneweg JA, Nannenberg EA, Jongbloed JD, Zwinderman AH, Pinto YM, Dit Deprez RH, Post JG, Tan HL, de Boer RA, Hauer RN, Christiaans I, van den Berg MP, van Tintelen JP, Wilde AA. Outcome in phospholamban R14del carriers: results of a large multicentre cohort study. Circ Cardiovasc Genet. 2014;7:455–65.
70. Vatta M, Spoonamore KG. Use of genetic testing to identify sudden cardiac death syndromes. Trends Cardiovasc Med. 2015;25:738–48.
71. Vilar E, Mork ME, Cuddy A, Borras E, Bannon SA, Taggart MW, Ying J, Broaddus RR, Luthra R, Rodriguez-Bigas MA, Lynch PM, You YQ. Role of microsatellite instability-low as a diagnostic biomarker of Lynch syndrome in colorectal cancer. Cancer Gene Ther. 2014;207:495–502.
72. Vreeswijk MP, Kraan JN, van der Klift HM, Vink GR, Cornelisse CJ, Wijnen JT, Bakker E, van Asperen CJ, Devilee P. Intronic variants in *BRCA1* and *BRCA2* that affect RNA splicing can be reliably selected by splice-site prediction programs. Hum Mutat. 2009;30:107–14.
73. Wallis Y, Payne S, McAnulty C, Bodmer D, Sistermans E, Robertson K , Moore D, Abbs S, Deans Z and Devereau A. 2013. Practice guidelines for the evaluation of pathogenicity and the reporting of sequence variants in Clinical Molecular Genetics. http://www.acgs.uk.com/media/774853/evaluation_and_reporting_of_sequence_variants_bpgs_june_2013_-_finalpdf.pdf
74. Weiss MM, Van der Zwaag B, Jongbloed JD, Vogel MJ, Brüggenwirth HT, Lekanne Deprez RH, Mook O, Ruivenkamp CA, van Slegtenhorst MA, van den Wijngaard A, Waisfisz Q, Nelen MR, van der Stoep N. Best practice guidelines for the use of next-generation sequencing applications in genome diagnostics: a national collaborative study of Dutch genome diagnostic laboratories. Hum Mutat. 2013;34:1313–21.
75. Weiss K, Applegate C, Wang T, Batista DA. Familial *TAB2* microdeletion and congenital heart defects including unusual valve dysplasia and tetralogy of fallot. Am J Med Genet A. 2015;167A:2702–6.
76. Williamson R, Bowcock A, Kidd K, Pearson P, Schmidtke J, Ceverha P, Chipperfield M, Cooper D.N., Coutelle C, Hewitt J, Klinger K, Langley K, Beckmann J, Tolley M, Maidak B, Hewett D, Linch C, Maslen G. Report of the DNA committee and catalogues of cloned and mapped genes, markers formatted for PCR and DNA polymorphisms. Cytogenet Cell Genet 1990;55:457-778.
77. Zhao M, Wang QQ, Jia P, Zhao Z. Computational tools for copy number variation (CNV) detection using next-generation sequencing data: features and perspectives. BMC Biochem. 2013;14(Suppl 11):S1.

Clinical Genetics

2

J.J. van der Smagt and Jodie Ingles

Abstract

Clinical geneticists are medical doctors that combine general knowledge of medicine with specific expertise in genetics, genetic diagnosis, and genetic aspects of disease. Genetic counselors are masters-level university-trained health professionals who deal in the psychosocial and genetic aspects of familial diseases. Both are specifically trained in communicating the implications of genetic information and genetic disease to patients and their families. Clinical geneticists and genetic counsellors often work together with cardiologists to ensure a high standard of care for families with cardiogenetic diseases.

Introduction

With the rapid advances in genetic knowledge, and more specifically genetic knowledge of human disease, many physicians will not find the time to keep up with the pace. Even though new generations of doctors are much better trained, both in genetics and in quickly acquiring adequate information from (Internet) databases, cooperation between "organ specialists" and "genetic specialists" seems to be the best option for the near future. Importantly, many genetic diseases are relatively rare, so that individual specialists will only encounter patients with a specific genetic disease on an occasional basis. This makes it difficult for them to obtain sufficient experience in providing adequate genetic information, in addressing the genetic questions of both patients and their families, and eventually in interpreting the often complex genetic test results.

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Genetics has become increasingly important to the field of cardiology [1, 2]. There is increasing awareness that some cardiac disorders occur in families and that important genetic factors play a role in disease causation. This holds true not only for monogenic disorders such as hypertrophic cardiomyopathy, congenital long QT syndromes, and catecholaminergic polymorphic ventricular tachycardia, but also for more common complex disorders such as coronary artery disease, hypertension, and diabetes. In the latter group of disorders, many different additive genetic and environmental contributions, each of relatively small effect size, are hypothesized to be disease causing. Important progress has been made in understanding the molecular background predisposing to different types of cardiac disease.

Meanwhile, in clinical genetic practice, focus has shifted from primarily reproductive issues (parents wanting to know the risk of recurrence after the birth of a child with a mental handicap or serious congenital abnormality, for example, a congenital heart defect) to include the assessment of risk of genetic disease, occurring later in life, in individuals with a positive family history. This started in neurology with individuals at risk of mostly untreatable neurodegenerative disease, like Huntington's chorea, wanting to know their genetic status in order to make future plans. Subsequently, genetic diagnosis entered the field of oncology, where it has become an increasingly important tool in identifying individuals at high risk of getting cancer. Of course, in the field of oncology, genetic testing has important medical implications, as

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individuals at risk may opt for increased cancer surveillance and preventive treatment strategies may be devised, based on genetic information.

Cardiology is another discipline of medicine where large-scale so-called presymptomatic testing of healthy at risk individuals has become available for some of the primary electrical heart diseases and cardiomyopathies. In particular, following the sudden cardiac death of a young person, post-mortem genetic testing (i.e., extraction of DNA from post-mortem frozen blood or tissue sections for genetic analysis) can play an important role in clarifying the cause of death and risk to family members. Although for most disease entities, family studies have not yet actually been proven to be beneficial, identifying those individuals at risk seems a logical first step in the development of preventive strategies. However, genetics of cardiac disease is complicated, for example, by vast genetic heterogeneity (many different genetic causes result in clinically identical disease) and the fact that test results may be difficult to interpret. Cooperation between cardiologists and clinical geneticists is, therefore, of great importance.

In this chapter, basic concepts in genetics and important issues that have to be considered in case of genetic testing are discussed.

The Clinical Genetic Intake

For those cardiologists involved in caring for families with genetic cardiac disorders, it is important to gain some experience in constructing pedigrees and recording family histories.

Family History

History taking will be more time-consuming than usual as, besides the regular cardiac anamnesis, detailed information on several family members has to be obtained [3]. Usually, information on three (sometimes four) generations is considered sufficient. Whenever possible, information should be collected on first-degree relatives (parents, siblings, and children), second-degree relatives (grandparents, uncles/aunts, and nephews/nieces), and third-degree relatives (first cousins). On average, they share 50 %, 25 %, and 12.5 % of their DNA with the index patient. Information on past generations may be sparse or even misleading as many conditions could not be correctly diagnosed in the past, whereas in contrast, younger generations will be less informative as they may not have lived long enough yet for disease symptoms to become manifest. Therefore, information on more distant relatives, like first cousins, from the same generation as the index patient may prove essential.

The reliability of the information obtained through family history taking will vary from case to case. In general, accuracy decreases with the decreasing degree of relationship. As a general rule, it is wise to confirm important information by checking medical records, whenever possible. If this involves family members, their written consent to retrieve these records will be required.

While taking a family history, it is important to be as specific as possible. People may leave out vital information when they do not think that it is important. Possible cardiac events should be specifically asked for, and approximate ages at which they occurred should be recorded. Of course, also the circumstances in which the event took place have to be noted. Depending on the nature of the condition under investigation, it may be necessary to ask for specific events, like diving or swimming accidents in case of suspected long QT syndrome type 1. It is useful to keep in mind that syncope resulting from arrhythmias may in the past have been diagnosed as seizures or epilepsy and that sudden death of an infant could have been documented as a sudden infant death syndrome (SIDS) [4, 5]. General questions about the entire family can be asked to elicit any additional information, such as "are there any other family members who have the same heart condition as yourself?" and "are there any individuals who have died suddenly or at a young age?"

If family members are under cardiac surveillance elsewhere, it is prudent to record this, and if individuals are deceased as a result of a possible cardiac event always inquire whether autopsy has been performed. Information on consanguinity is often not readily volunteered and should be specifically asked for. Depending on the nature of the disorder under investigation, it may also be important to inquire about medical conditions not specifically involving the heart. For example, when investigating a family with possible autosomal dominant dilated cardiomyopathy, it would be prudent to also ask for signs of skeletal muscle disease in family members.

Pedigree Construction

Drawing a pedigree is a helpful tool in assessing any familial disorder. Presenting family history information in a pedigree allows to quickly visualize family structure and asses the possible inheritance patterns [6, 7]. In addition, a drawn pedigree will make it more easy to see which, and how many, family members are at risk for cardiac disease, and who should be contacted. The symbols commonly used for pedigree construction are represented in Fig. 2.1.

Nowadays, different software packages exist for pedigree construction. These packages have the advantage that it is easier to update pedigrees and that pedigrees can be more easily added to other digital medical files. Frequently, the software also offers options that are valuable for genetic research.

However, the great advantage of pen and paper is that the pedigree can be constructed while taking the family history, thus ensuring that no important family members are overlooked.

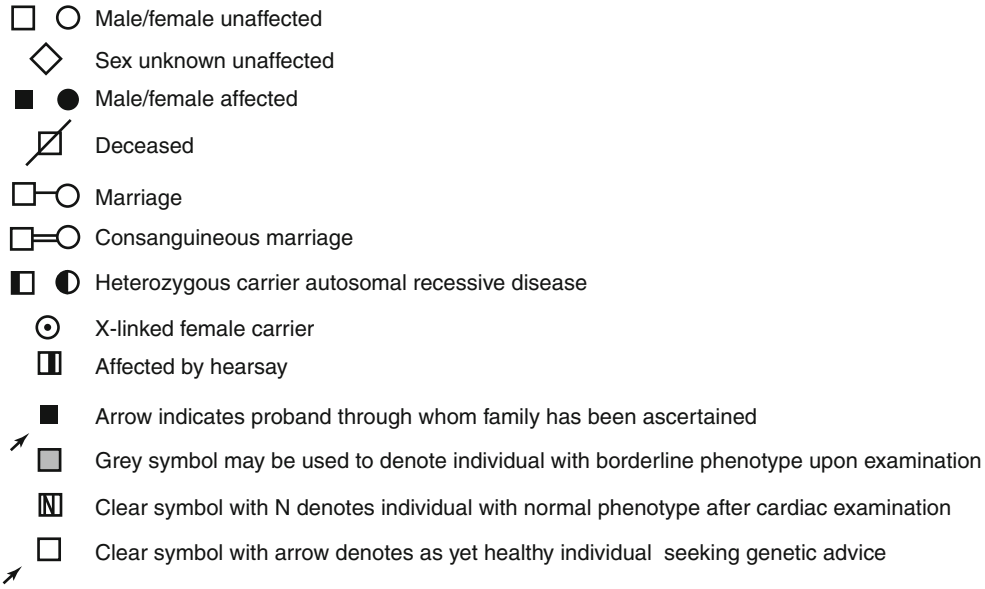

Fig. 2.1 Symbols used to denote individuals in a pedigree

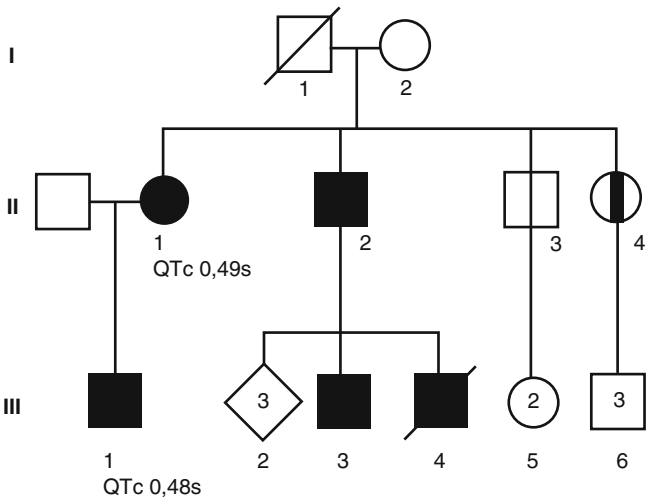

Fig. 2.2 Example of a small pedigree like one could draw up, while taking a family history, during consultation of a family suspected of long QT syndrome type 1. Footnotes with this pedigree could be: III-1 index patient (27 October 2000), syncope while playing soccer, spontaneous recovery, QTc 0.48 s, repolarization pattern compatible with LQT type I. I-1: no medical information, died in unilateral car accident at age 32. II-1: (02 March 1975), no symptoms, QTc 0,49 sec. II-2 (10 May 1977) known with seizures as a child. II-4: (13 June 1979) said to have fainted during exercise more than one occasion. III-4 sudden death, while swimming at age 12 years. No other persons known with seizures, syncope or sudden death known in the family

A few tips and tricks (see Fig. 2.2):

- Start drawing your pedigree on a separate sheet of paper. Start with your index patient in the middle of the paper and go from there.
- Add a date to your pedigree.
- Numbering: By convention, generations are denoted by a Roman numeral, whereas individuals within a generation are identified by an Arabic numeral. In this way, each individual can be identified unambiguously by combining the two numbers (II-3, III-1, etc.). Additional information on a specific individual can be added in a footnote referring to this identification number.
- The most important clinical information can be directly added to the pedigree (see Fig. 2.2).
- Record approximate dates (e.g., birth year or 5-year interval), not ages. Add age at time of death.
- Especially in case of a suspected autosomal recessive disorder, names and places of birth of all grandparents of the index patient should be recorded (usually in a footnote). Consanguinity is unlikely when paternal and maternal grandparents come from very different areas and may be more common in certain ethnic groups. If birthdates are also available this could facilitate genealogical studies in search of consanguinity.
- Levels of evidence: for individuals that are probably affected based on heteroanamnestic information, but whose medical records have not yet been checked, the symbol "affected by hearsay" (Fig. 2.1) can be used.
- For counseling reasons, add information on both sides of the family. Unexpected additional pathology may be of importance to your patient and his or her offspring.

Basic Concepts in Inherited Disease

A single copy of the human genome contains over three billion base pairs and is estimated to contain 20,000–25,000 protein coding genes [6]. Genes are transcribed into messenger RNA in the nucleus. Subsequently, the noncoding parts of genes (introns) are spliced out to form the mature messenger RNA, which is in turn translated into protein in the

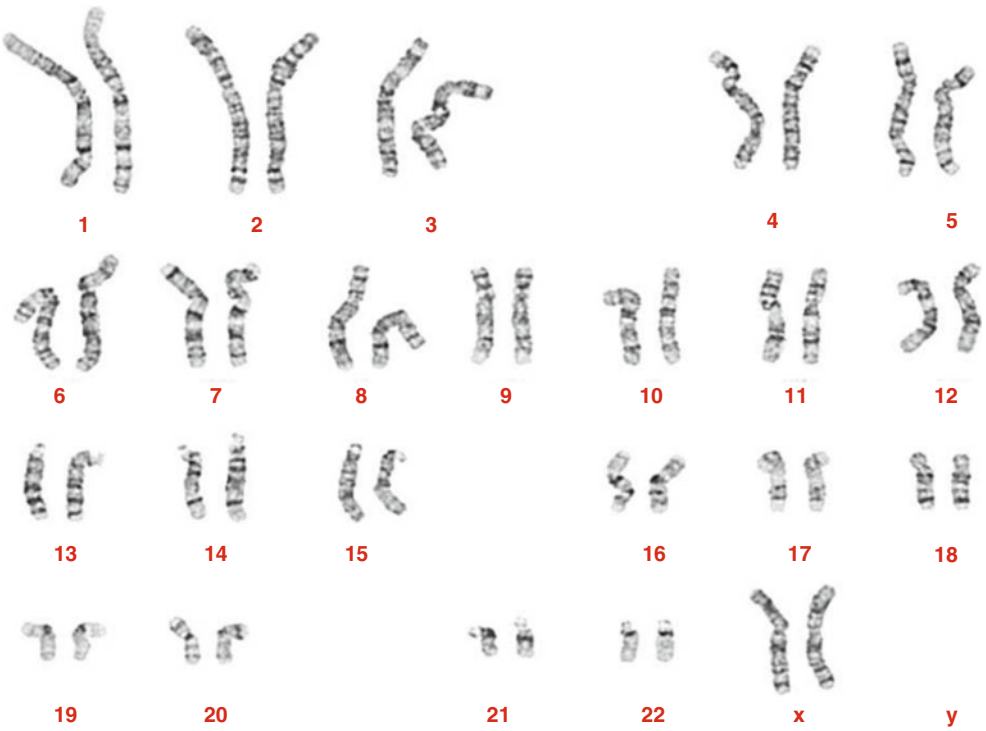

Fig. 2.3 Normal female karyogram (46, XX): the way the chromosomes are shown, when DNA is visualized through a light microscope

cytosol. Proteins consist of chains of amino acids. Each amino acid is coded by one or more combinations of three nucleotides in the DNA.

Less than 3 % of DNA is protein coding. The remainder codes for RNA genes, contains regulatory sequences, or consists of DNA of undetermined function, sometimes misleadingly referred to as "junk DNA."

DNA is stored on 23 chromosome pairs (Fig. 2.3), present in the nucleus of each cell; 22 pairs of autosomes and one pair of sex chromosomes. During gametogenesis (the production of oocytes and sperm cells), meiosis takes place ensuring that only one copy of each pair is transmitted to the offspring. Since chromosomes are present in pairs, humans are diploid organisms. They have two complete copies of DNA, one copy contributed by the father and one by the mother. Therefore, each gene at each locus is present in two copies. These are usually referred to as the two alleles of that specific gene.

The exceptions to this rule are the sex chromosomes, as males have only one X-chromosome and one Y-chromosome, the first being inherited from the mother, the latter from the father. Thus, males have only a single copy of most X-linked genes. In addition to the nuclear DNA, small circular DNA molecules are present in the mitochondria in the cytoplasm. Many copies of this mtDNA will be present per cell. The mtDNA is exclusively inherited from the mother. Oocytes may contain up to 100,000 copies of mtDNA. MtDNA only codes for 37 genes, all involved in mitochondrial function.

Mitosis and Meiosis

Two types of cell divisions exist: mitosis and meiosis. Mitosis ensures the equal distribution of the 46 chromosomes over both daughter cells. In order to accomplish this, first, the DNA on each chromosome has to be replicated. At cell division, each chromosome consists of two identical DNA chromatids (sister chromatids), held together at a single spot: the centromere. To ensure orderly division, the DNA in the chromosome has to be neatly packaged (a process called condensation). This is when chromosomes actually become visible through a microscope. Prior to cell division, a bipolar mitotic spindle develops, the completely condensed chromosomes move to the equator of the cell, the nuclear membrane dissolves and microtubular structures develop reaching from both poles of the spindle to the centromere of each chromosome. Subsequently, the centromeres divide and the sister chromatids are pulled to opposite poles of the dividing cell. Cell division results in two daughter cells, each with 46 unreplicated chromosomes and exactly the same nuclear genetic information as the original cell (Fig. 2.4)

Meiosis is a specialized cell division that is necessary to finish the process of gametogenesis. The goal is to produce gametes that contain only 23 unreplicated chromosomes. The vital steps of meiosis are outlined in Fig. 2.5. One of the hallmarks of meiosis is that both replicated chromosomes of each pair come in close apposition to each other and actually exchange genetic material before meiosis takes place. This

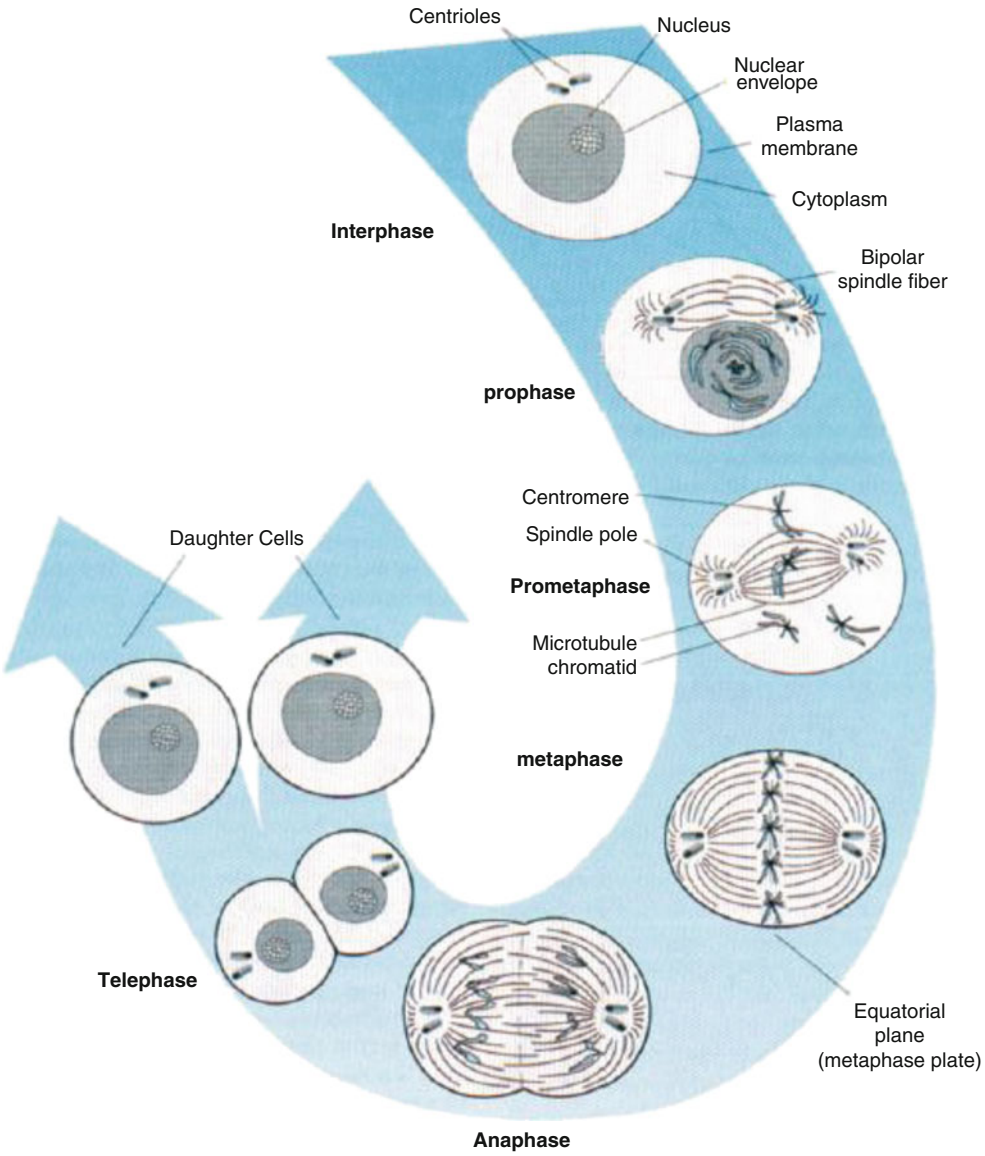

Fig. 2.4 Different stages of mitosis, leading to two daughter cells with exactly the same nuclear DNA content. (Reprinted with permission Jorde, Carey, Bamshad, White, Medical Genetics third edition, Mosby Elsevier 2006)

more or less random process is called homologous recombination. Recombination ensures that each individual is able to produce an infinite number of genetically different offspring. Apart from ensuring genetic diversity, recombination is also necessary for proper segregation of the homologous chromosomes during meiosis I. During male meiosis, the X and Y are able to function as a chromosome pair, thus ensuring proper segregation of sex chromosomes. They can recombine at the tip of their short arms.

Chromosomal Abnormalities

Mutations may affect single genes, but also the genomic architecture at a larger scale can be affected. Such aberrations, when visible through a microscope, are called chromosomal abnormalities. Humans have 22 pairs of autosomes and one pair of sex chromosomes. Abnormalities can be divided into numerical (any deviation from 46 chromosomes) and structural defects (abnormal chromosomes). A whole set of 23 extra chromosomes is called triploidy. It results from fertilization or meiotic error. Children with triploidy die before or immediately after birth. A single extra chromosome is called a trisomy. They most often result from meiotic error. Only three autosomal trisomies are potentially viable: trisomy 21 (Down syndrome), trisomy 18, and trisomy 13. All three have a high chance of being associated with congenital heart defects.

In structural chromosome abnormalities, a distinction is made between balanced and unbalanced defects. In balanced defects, chromosome parts are displaced, but there is no visible extra or missing chromosome material. Balanced rearrangements are most often not associated with an abnormal phenotype, but they may predispose to unbalanced offspring. Unbalanced chromosome abnormalities have a very high risk of being associated with mental retardation and birth

defects. As heart development is a very complex process, probably involving hundreds of genes, chances are that this process will be disturbed in one way or another in case of a visible chromosomal abnormality. Indeed, heart defects are very frequent in children with structural chromosomal abnormalities.

Smaller abnormalities are not readily visible through the microscope and will be missed unless specific techniques are applied to detect them. Still, so-called microdeletions may contain a large number of genes and are often associated with heart defects. Examples of microdeletion syndromes associated with heart defects are the 22q11.2 (velo-cardio-facial/DiGeorge) deletion syndrome, Williams–Beuren syndrome, 1p36 deletion syndrome and Wolf–Hirschhorn syndrome.

As a general rule, regardless whether a visible chromosome abnormality or a microdeletion is involved, the resulting heart defect will usually not occur as an isolated feature. Often associated birth defects, developmental delay and/or abnormal growth will be present. Therefore, it is in this category of heart defect patients with additional anomalies that a chromosome abnormality has to be considered. Nowadays, classical karyotyping (looking at chromosomes through a microscope) is most often replaced by SNP arrays (with a much higher resolution) that are able to detect copy number of hundred thousands of single SNPs across the genome. Therefore, microdeletions and microduplications will be easily detected, without ordering any specific test to detect them.

In contrast to mutation analysis, chromosome analysis by classical karyotyping requires dividing cells for the chromosomes to become visible through a microscope. Usually, white blood cells or cultured fibroblasts are used for chromosome analysis.

Inheritance Patterns

A genetic component plays a role in many diseases. Usually, the genetic contribution to disease is appreciated when either

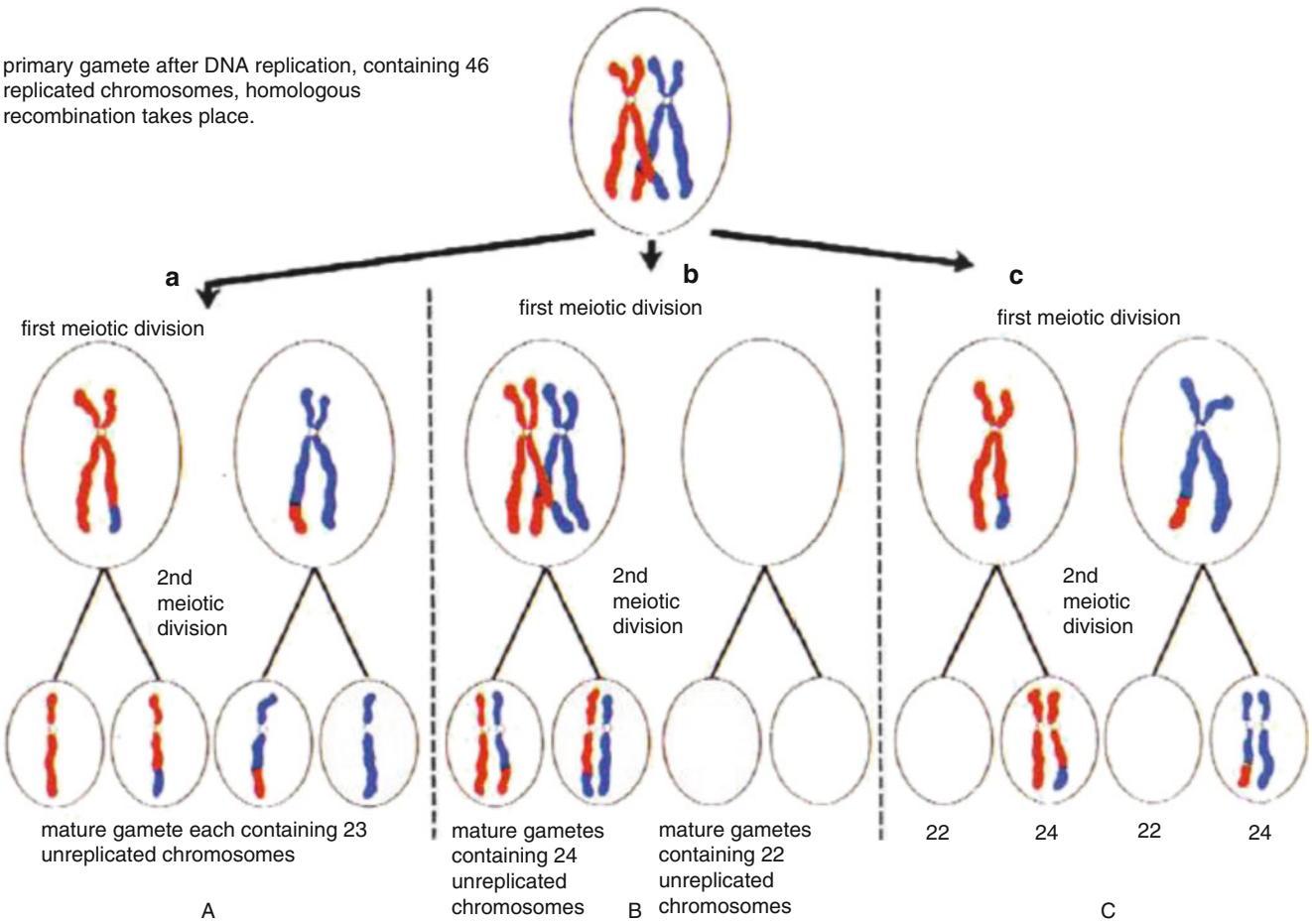

Fig. 2.5 Meiosis: (**a**) demonstrates the normal stages of meiosis (after division one cell contains 23 replicated chromosomes – 22 autosomes and 1 sex chromosome). (**b**) Demonstrates nondisjunction in meiosis 1 (the most frequent cause of for instance Down syndrome). (**c**) Demonstrates nondisjunction in meiosis 2. Appreciate the effect of recombination in the mature gamete, in this way each grandparent contributes to both copies of each autosome of his/her grandchild (**b**). (Adapted from Langman Inleiding tot de embryologie Bohn Scheltema & Holkema 9e herziene druk 1982)

a clear pattern of inheritance or significant familial clustering of a disease is noted [7].

Classical genetic disease follows a recognizable Mendelian inheritance pattern. These disorders are called monogenic disorders as a mutation at a single locus conveys a very strong risk of getting the disease. Sometimes, indeed, everybody with a specific mutation develops the disease (this is called complete penetrance). In that case, the influence of environmental factors or contributions at other genetic loci seems negligible. In practice, however, most monogenic diseases display considerable variation in disease manifestation, severity, and age at onset (clinical variability), even within a single family (where every affected person has the same mutation). Especially in autosomal dominant disease, the chance of developing clinical manifestations of disease when a specific pathogenic mutation is present is often far less than 100 % (incomplete or reduced penetrance). However, such clinically unaffected mutation carriers may foster severely affected children when they transmit the mutation to their offspring. So, even in so-called monogenic diseases many other genetic and nongenetic factors can usually modify clinical outcome.

Whereas monogenic diseases are often relatively rare, there is a clear genetic contribution to many common disorders such as coronary artery disease, hypertension, and hypercholesterolemia. In the vast majority of patients, these diseases are explained by the combined additive effect of unfavorable genetic variants at multiple different loci and environmental factors (anything nongenetic), eventually causing disease. Polygenic disease, multifactorial disease, and complex genetic disease are all terms used to denote this category of diseases. When looking at pedigrees apparently, nonrandom clustering within the family can often be noted, however, without a clear Mendelian inheritance pattern. Very common complex disorders may mimic autosomal dominant disease, whereas in less common disorders like, for example, congenital heart defects, a genetic contribution is very likely although the majority of cases will present as sporadic cases without a positive family history. Importantly, frequent complex genetic diseases may have less common monogenic subtypes like for example FH (familial hypercholesterolemia) as a result of mutations in the LDL receptor or MODY (maturity onset diabetes in the young), which are examples of monogenic subtypes of diseases that most often have a complex etiology.

Single Gene Disorders: Mendelian Inheritance

In single-gene disorders, inheritance patterns can be explained in terms of Mendelian inheritance. Of importance in the first place is whether the causative gene is on one of the autosomes or on one of the sex chromosomes, more specifically on the X-chromosome (the Y-chromosome contains very few disease-related genes and will not be discussed further).

The second distinction to be made is whether mutations in the gene follow a dominant or recessive mode of inheritance.

Autosomal Dominant Inheritance

Autosomal dominant disease is caused by dominant mutations on one of the autosomes. Dominant mutations already cause disease when only one of both alleles is mutated. Most individuals with dominant disease are heterozygous for the mutation (they have one mutated and one normal allele). Heterozygous carriers of such a mutation have a high risk of clinically expressing disease symptoms. It is the most common form of inheritance in monogenic cardiac disease. It is characterized by (see Fig. 2.6):

- Equal chance of males and females being affected.
- Individuals in more than one generation are usually affected (unless a new mutation has occurred).
- Father-to-son transmission can occur.
- On average, 50 % of offspring will be affected (assuming complete penetrance).

Although this inheritance pattern is rather straightforward, in practice precise predictions are often complicated by issues of penetrance and variable expression (see paragraph on penetrance and variable expressivity).

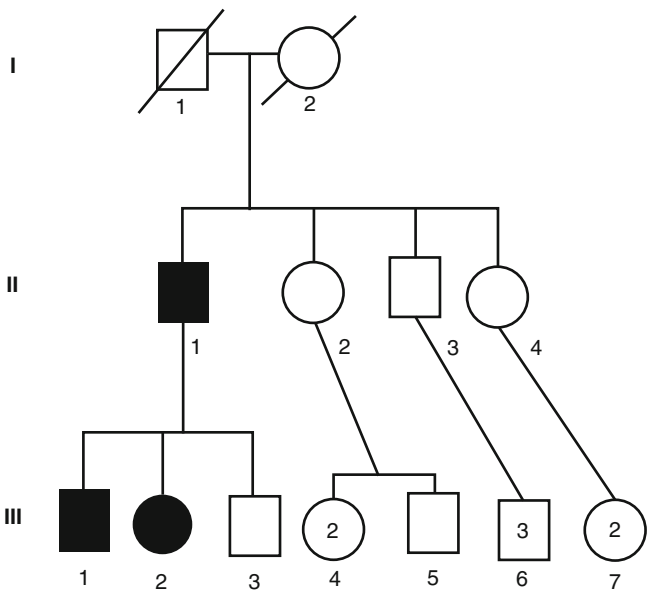

Fig. 2.6 Example of a small autosomal dominant pedigree, the observed male to male transmission (II-1 > III-1) excludes X-linked dominant inheritance. If we assume this disorder has full penetrance, the novo mutation must have occurred in II-1

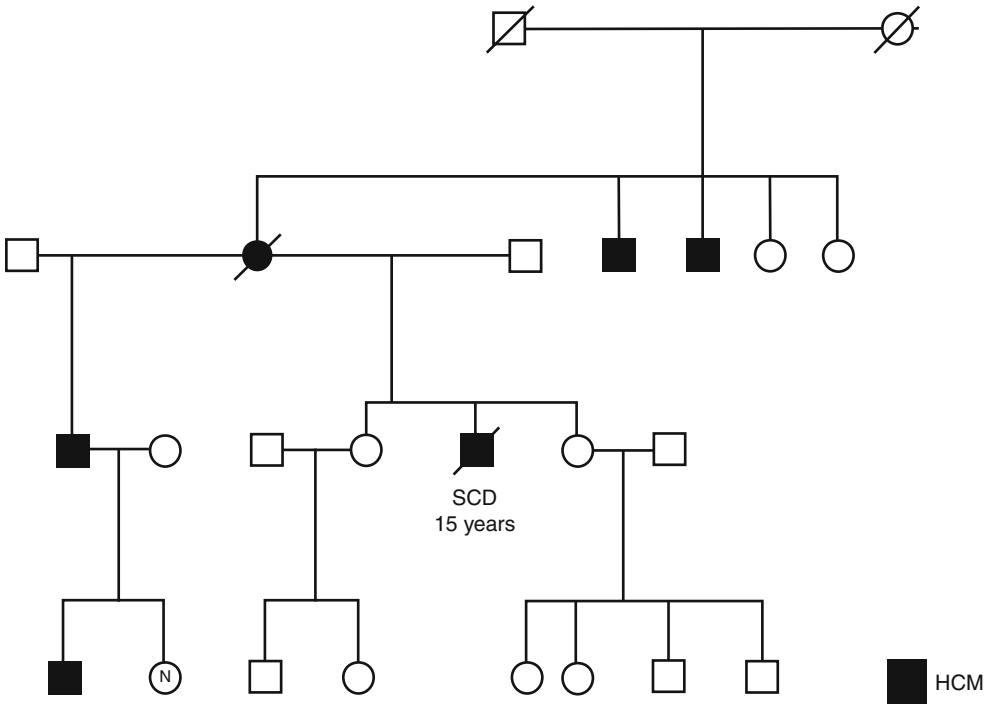

Fig. 2.7 Example of a hypertrophic cardiomyopathy (HCM) pedigree showing autosomal dominant inheritance. The proband was a 15-year-old boy who suffered a sudden cardiac death (SCD), as a result other family members were identified on family screening

An example in cardiogenetics is hypertrophic cardiomyopathy, and a typical pedigree demonstrating autosomal dominant inheritance is shown below. The vast majority of monogenic cardiogenetic diseases are inherited in an autosomal dominant fashion, like most cardiomyopathies and the most common forms of congenital long QT syndromes and CPVT (Fig. 2.7).

Autosomal Recessive Inheritance

In recessive inheritance, disease occurs only when both alleles of the same gene are mutated.

Affected patients carry mutations on both the paternal and maternal allele of a disease gene. New mutations are rarely encountered. Therefore, it is reasonable to assume that both healthy parents are heterozygous carriers of one mutation. These healthy individuals are often called "carriers." It is reasonable to assume that each person is carrier of one or more disease-associated autosomal recessive mutations.

Affected patients can be homozygous (the same mutation on both alleles of the gene) or compound heterozygous (different mutations on the two alleles of the gene) for the mutation. If consanguinity is involved, a single mutation that was present in the common ancestor is transmitted to the patient by both parents, leading to the homozygous state.

In most cases, but not always autosomal recessive conditions are limited to a single sibship (see Fig. 2.8). If vertical transmission of an autosomal recessive disease occurs, this is called "pseudodominance." Pseudodominance can be encountered in case of consanguinity in multiple generations or in case of a very high population frequency of healthy heterozygous carriers. Autosomal recessive inheritance is characterized by the following:

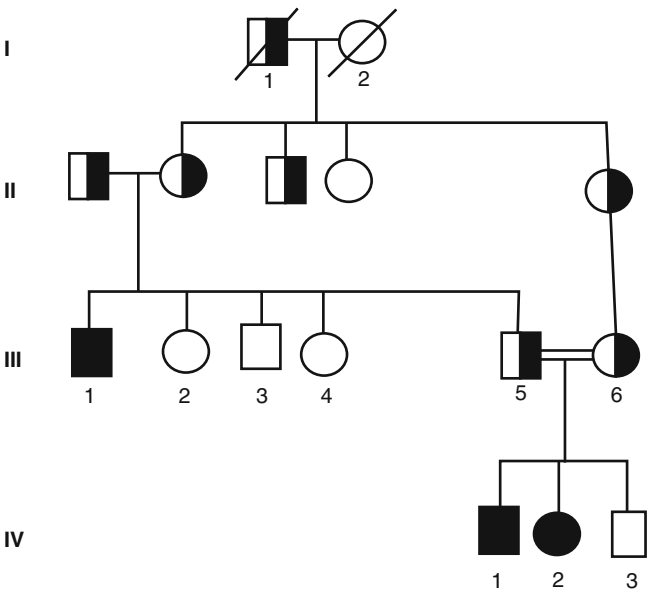

Fig. 2.8 Example of an autosomal recessive pedigree, illustrating the role of consanguinity in AR disease. III-5 and III-6 are first cousins. IV-1 and IV-2 inherited both their mutated alleles from a single heterozygous grandparent (in this case I-1). In this pedigree, heterozygous carriers are indicated, usually heterozygous carriers for AR disorders can only be unambiguously identified by DNA analysis

- Equal chance of males and females to be affected.
- Parents of patients are usually healthy carriers.

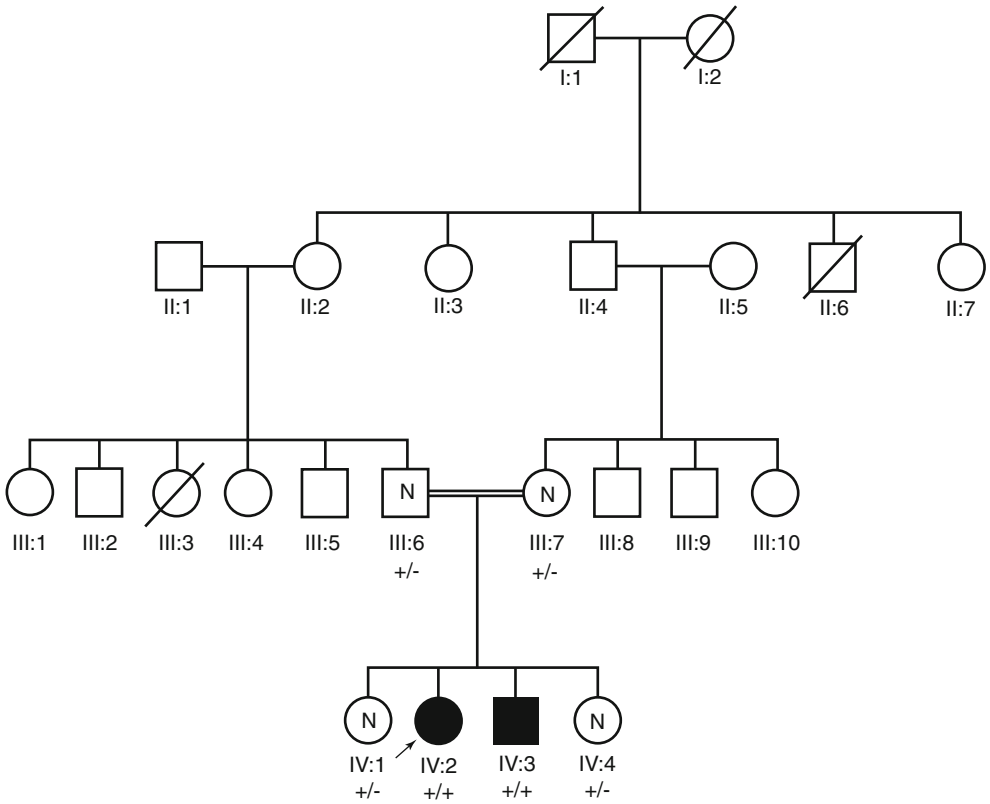

Fig. 2.9 Example of a recessively inherited form of hypertrophic cardiomyopathy (TNNI3 Arg162Trp). This family presented after the symptomatic presentation of IV:2 who subsequently required cardiac transplantation due to a severe restrictive phenotype. Her brother (IV:3) suffered a resuscitated cardiac arrest while awaiting cardiac investigation. Both parents and siblings were heterozygous carriers and showed no clinical evidence of disease (REF: Gray et al. [28])

- The chance that the next child (a sib) will be affected is 25 %.
- Affected individuals are usually limited to a single sibship.
- The presence of consanguinity in the parents favors, but does not prove, the autosomal recessive inheritance mode.

While autosomal recessive inheritance is rare among cardiogenetic diseases, there are reports including this family shown below (Fig. 2.9).

X-Linked Recessive Inheritance

X-linked disorders are caused by mutations on the X-chromosome. The X-chromosome does not contain "female-specific" genes. As females have two X-chromosomes and males only one, in X-linked disorders, usually, a difference in disease expression will be noted between males and females.

In X-linked recessive inheritance (see Fig. 2.10),

- No male-to-male transmission occurs.
- Heterozygous females are usually healthy.
- All daughters of affected males will be usually healthy carriers.
- Sons of carrier women have a 50 % chance of being affected.
- Daughters of carrier women have a 50 % chance of being a healthy carrier.

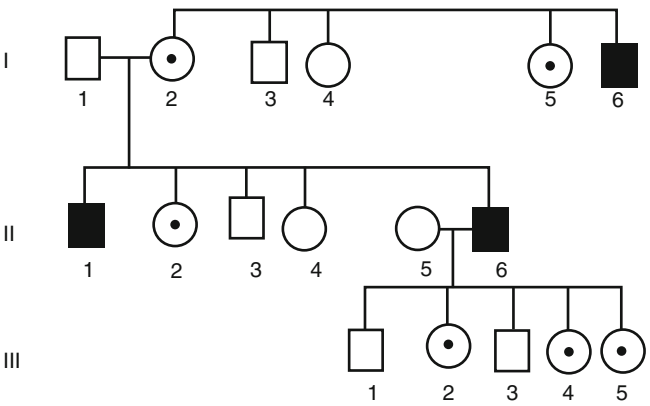

Fig. 2.10 Example of an X-linked recessive disorder, the disease is transmitted via apparently healthy heterozygous females, only hemizygous males manifest the disease. All daughters of an affected male will be carriers. Carrier females are indicated with dots within circles, usually DNA analysis will be required to unambiguosly identify carrier females

It should be noted that in female somatic cells only one X-chromosome is active. The other X-chromosome is inactivated. This process of *X-inactivation* (called lyonization) is random, occurs early in embryogenesis and remains fixed, so that daughter cells will have the same X-chromosome inactivated as the cell they were derived from. Usually, in a given female tissue, approximately half of the cells will express the paternal X-chromosome and the other half of the cells, the

maternal X-chromosome. However, for a variety of reasons, significant deviations of this equal distribution of active X-chromosomes may occur (called *skewing of X-inactivation*). Naturally, this may influence disease expression in case of X-linked disease. For example, if the X-chromosome containing an X-linked recessive mutation is expressed in over 90 % of cells in a given tissue, disease may develop in a female like it does in males.

X-Linked Dominant Inheritance

In X-linked dominant disorders, heterozygous females are most likely to be affected. However, on average, these heterozygous females are often less severely affected than hemizygous males. Exceptions, however, do exist.

Some X-linked dominant disorders may be lethal in hemizygous males like, for instance, the Oculo-Facio-Cardio-Dental syndrome that is associated with congenital heart defects. Hemizygous males will miscarry, leading to a reduced chance of male offspring in affected females.

The characteristics of X-linked dominant inheritance are (see Fig. 2.11):

- No male-to-male transmission occurs.
- Heterozygous females are affected.
- All daughters of an affected male will also be affected.
- Affected females have a 50 % chance of having affected children.

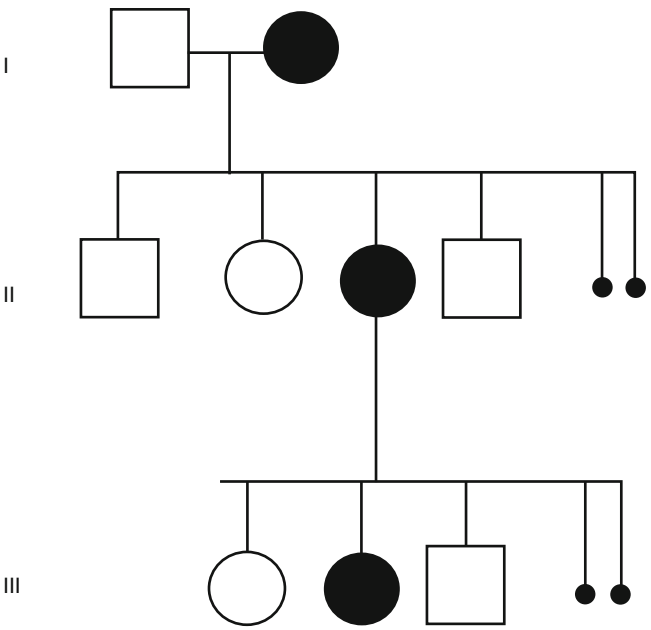

Fig. 2.11 Example pedigree of an X-linked dominant disorder with early lethality in males. Affected males that are conceived will miscarry (leading to skewed sex ratios in offspring). The black dots in the pedigree represent miscarriages. Based on such a limited pedigree, definite distinction from autosomal dominant inheritance would be impossible

Especially in X-linked disorders, the distinction between dominant and recessive disease may be blurred, with some heterozygous females being not affected at all, while others are affected to the same degree as hemizygous men. In cardiogenetics, several X-linked disorders are known where heterozygous females may be asymptomatic, but also run a high risk of significant disease, like for instance in Fabry disease. In Duchenne muscular dystrophy, considered to be an X-linked recessive disorder, females only very rarely develop severe skeletal muscle weakness, but they are at considerable risk for dilation of the left ventricle and should be monitored by a cardiologist (Fig. 2.12).

Non-Mendelian Inheritance

In fact, any deviation from the classical rules of Mendel could be categorized under the heading of non-Mendelian inheritance. Such deviations can, for example, result from genome disorders (*de novo* deletions or duplications of larger stretches of DNA or even whole chromosomes), epigenetic

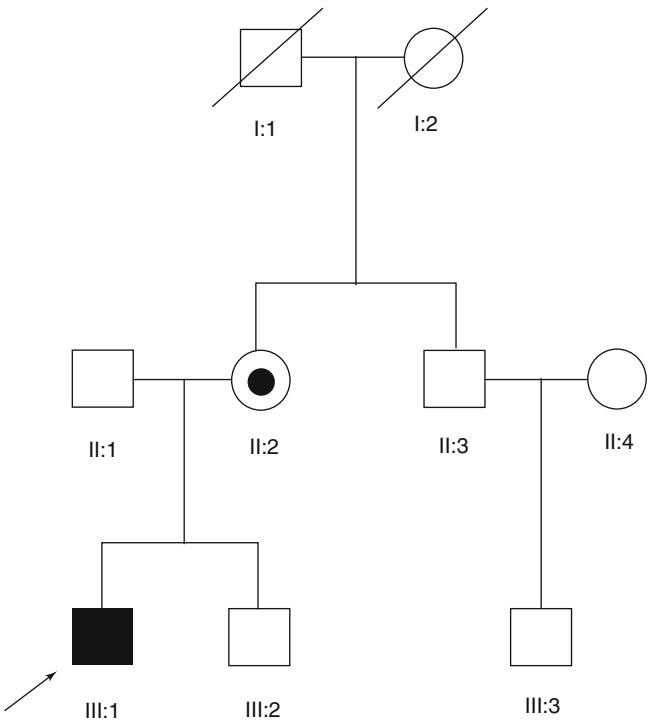

Fig. 2.12 Example of an X-linked dominantly inherited condition, Danon disease. This is a rare disease caused by mutations in the *LAMP2* gene and is a known HCM phenocopy (i.e., causing clinical characteristics similar to HCM). In this family, the proband (III:1) presented with severe concentric left ventricular hypertrophy (LVH) aged 12 years. His mother (II:2) was found to be a carrier of the causative loss of function variant in *LAMP2* though had no clinical evidence of disease herself. Other family members have not been assessed. Female carriers of *LAMP2* mutations can be affected, though often less severely than males.

factors (these are factors not changing the actual DNA code, but change the way in which specific genes are expressed) and unstable mutations (trinucleotide repeat mutations such as in myotonic dystrophy that may expand over generations and lead to a more severe phenotype in subsequent generations). However, for sake of brevity only multifactorial inheritance and maternal (mitochondrial) inheritance will be briefly discussed.

Multifactorial Inheritance

Although genetic factors very often contribute to disease, most of the time this will not be in a monogenic fashion. The majority of disorders are caused by a complex interplay of multiple unfavorable genetic variants at different loci in combination with environmental (nongenetic factors). The genetic variants involved may each by themselves have a limited effect. It is the additive effect of multiple factors that eventually will lead to disease, hence the name multifactorial inheritance. In this paragraph, no distinction is made between multifactorial inheritance and polygenic inheritance (no important environmental contribution). In general practice, such a distinction is most often of no importance unless specific environmental factors can be identified that can be influenced. Hereditability is a measure used to indicate the contribution of inherited factors to a multifactorial phenotype. In animal studies, heritability can be calculated, as both environment and genetic composition of the animals can be controlled. In man, hereditability can only be estimated indirectly.

In multifactorial inheritance, sometimes clustering of a condition within a family may be observed that cannot be easily explained by chance. Especially in common diseases like diabetes or hypertension (when the underlying genetic variants occur at high frequency in the population), this clustering may mimic Mendelian inheritance. However, in more rare disorders like, for example, in congenital heart disease, an identified patient may well be the only affected one in the family. Still, family members may be at increased risk of a congenital heart defect.

Many continuous traits like, for example, blood pressure, can be explained in terms of the additive effect of multiple deleterious or protective genetic and environmental factors. In case of hypertension, the sum of all these factors would be defined as disease liability, which is distributed as a Gaussian curve in the population. At the right side of the curve (highest liability), those with hypertension are found. Their close relatives who will share many of the predisposing genetic (and possibly also environmental factors) with the hypertensive patient, will usually have a higher than average disease liability; however, they may not meet with the clinical criteria for hypertension. For discontinuous traits like, for example, congenital heart defects, a threshold model has been proposed (Fig. 2.13). If disease susceptibility exceeds the threshold level, disease will arise. Again, the liability of close relatives of a patient with a heart defect will be, on average, closer to the disease threshold than that of unrelated individuals, but most of them will not exceed the threshold and therefore will have anatomically normal hearts.

It is important to realize that some disorders are more multifactorial than others. Sometimes mutations at a single locus will not be sufficient to cause disease, but have a strong effect. If a mutation in such a major gene is present, little else has to go wrong for disease to occur. Therefore, strict separation between Mendelian and multifactorial disease is artificial. Indeed, genes that are involved in rare monogenetic variants of a disease may also play a role in the more common multifactorial forms of the disease.

The following characteristics can be applied to multifactorial inheritance:

- Familial clustering may occur, but usually no Mendelian inheritance pattern can be identified.
- Recurrence risks for family members are in general lower than in monogenic disease.
- Risk of disease rapidly falls with decreasing degree of relationship to the index patient.
- Risk may be higher for relatives of severely affected patients.

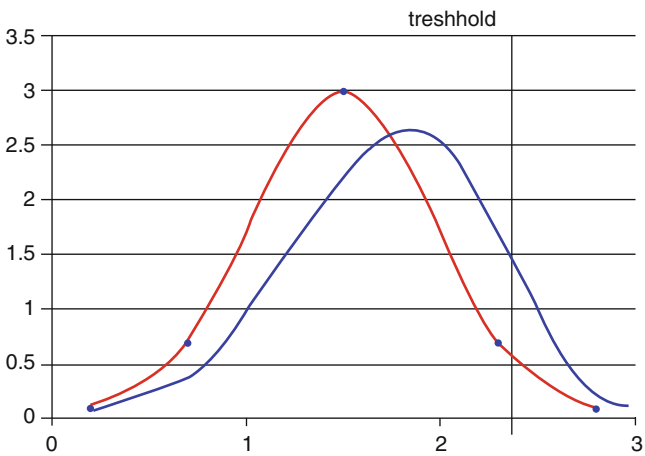

Fig. 2.13 Example of a liability distribution of a discontinuous multifactorial trait (a congenital heart defect) in a given population. The red curve is for the general population. The area under the red curve to the right of the threshold represents the proportion of individuals with CHD in the general population. The blue curve is for first degree relatives of a patient with CHD. Since CHD is a discontinuous trait (it is either present or absent) a threshold is introduced. Everybody with a liability exceeding the threshold will have CHD. Liability for CHD will be determined by the additive effect of unfavorable genetic and environmental factors. As a result of shared unfavorable factors, the liability curve for first-degree relatives has shifted to the right explaining the fact that a larger proportion of first-degree relatives will be affected with CHD in comparison to the general population, whereas the majority of relatives has no CHD, as their liability does not exceed the threshold

- Risk estimations are usually based on empirical (observational) data.
- These risks are not fixed risks, like in Mendelian disease. New disease cases in a family may indicate a higher genetic load, and therefore a higher risk for relatives.

At this point in time, the use of predictive genetic testing in multifactorial disease is limited, as usually only a small part of morbidity can be explained by the genetics variants that have thus far been identified for these disorders. As these variants have by themselves only a small effect, the odds of getting the disease, once an unfavorable variant has been identified, are small. Still, commercial genetic tests, supplying risk profiles for many common conditions, based on genetic profiles, are readily available via the internet. Such risk predictions may be imprecise and differ substantially between different test providers.

Although exceptions may exist, predictive genetic testing in multifactorial disease is not likely to play a role of importance in genetic counseling in the near future. In contrast, genetic tests for common disorders may play a role in clinical practice in the near future, for example, in risk stratification, and in identifying groups that are eligible for specific treatments.

Maternal (Mitochondrial) Inheritance

Mitochondria are present in most cells in different numbers and are the principle providers of energy by means of the respiratory chain. Mitochondria contain small circular DNA molecules of their own (*mtDNA*). These molecules are only 16,569 base pairs in length and code for only 37 genes. Thirteen polypeptides of the respiratory chain are encoded by the mitochondrial DNA, whereas the remainder (the majority) are encoded by the nuclear DNA. The rest of the mitochondrial genes play a role in mitochondrial translation (transfer RNAs and ribosomal RNAs).

Somatic cells typically contain 1000–10,000 mtDNA molecules (two to ten molecules per mitochondrion). Mitochondrial DNA replication is under nuclear control and suited to meet with the energy requirement of the cell. It is not associated with cell division like the nuclear DNA. When a cell divides, mitochondria randomly segregate to daughter cells within the cytoplasm. Oocytes may contain up to 100,000 copies of mtDNA, whereas sperm cells usually contain only a few hundreds. Moreover, these paternal copies do not enter the oocyte at fertilization. Therefore, the paternal contribution to the mtDNA is negligible, and mtDNA is inherited exclusively via the mother, hence the concept of maternal inheritance.

Whereas nuclear genes are present in two copies per cell, mitochondrial genes are present in thousands of copies. In maternally inherited disease, in a specific tissue, a significant part of the mtDNA copies may carry a similar mtDNA mutation, whereas the remainder of the copies is normal (wild type). This phenomenon is called heteroplasmy. Here, again, a threshold is important, that is determined by the specific energy requirement of the tissue. If the percentage of mutated mtDNA becomes so high that the energy requirement cannot be fulfilled, this may result in mitochondrial disease. If a mutation is present in all mtDNA molecules in a specific tissue, this is called homoplasmy. The mechanism that leads to homoplasmy of certain mtDNA mutations is not yet fully understood.

Mitochondrial DNA differs in many aspects from nuclear DNA. In contrast to nuclear DNA, most of the mtDNA codes for genes. Therefore, any random mutation in the mtDNA is much more likely to disrupt an actual gene than is the case in the nuclear genome. DNA repair mechanisms to repair acquired DNA damage, as are present in the nucleus, are lacking, leading to accumulation of mtDNA mutations, for example, in aging. On the other hand, since mtDNA genes are present in hundreds to thousands of copies per cell, acquired mutations rarely lead to recognizable mitochondrial disease. Only a minute fraction of mtDNA mutations will become "fixed" and will subsequently be transmitted to offspring

It is important to realize that maternal inheritance is not equivalent to mitochondrial disease. As most of the proteins active in the mitochondrion are encoded by nuclear genes, mitochondrial diseases may be inherited in other fashions, most often in an autosomal recessive manner.

Mitochondrial disease affects many tissues, although tissues with the highest energy requirements (muscle, brain) are most often involved. Cardiac muscle may be involved in different mitochondrial conditions. Sometimes a cardiomyopathy may be the first or most prominent manifestation of a mutation in the mtDNA.

The following characteristics apply to maternal inheritance:

- Men and women are affected with similar frequencies; however, only females transmit the disease to offspring.
- Phenotypes may be extremely variable (and unpredictable) as a result of different levels of heteroplasmy in different tissues.
- The percentage of mutated mtDNA in one specific tissue may not accurately predict the level of heteroplasmy in other tissues. This is a major problem for example in prenatal diagnosis.
- Affected females are likely to transmit mutated mtDNA to all of their offspring, but nonpenetrance will result if the threshold for disease expression is not reached.

New (*De Novo*) Mutations

Mutations can occur at any time both during gametogenesis or regular cell division. If a detected mutation is present in neither

of the parents (i.e., if it is absent in the blood of both parents), the mutation is called "*de novo.*" *De novo* mutations may have arisen in the sperm or egg cell or may even have occurred after conception. Mutation rates in genes (the number of mutations per gene per generation) are on average very low, in the order of 10^{-5} to 10^{-7}. Therefore, if in an isolated patient, a *de novo* mutation in a candidate gene for the disorder is being found, it is usually regarded as a pathogenic mutation.

It should be realized that most new mutations will go unnoticed. When they are situated in noncoding DNA or in recessive genes, they will have no immediate effect, whereas new mutations in important dominant genes may be lethal and may therefore not be ascertained.

Mosaicism

When mutations (or chromosomal abnormalities) arise shortly after conception, mosaicism may result. Mosaicism is defined as the presence of genetically different cell populations (usually an abnormal and a normal cell line) within a single individual. The importance of mosaicism in relation to cardiac disease is that (at least in theory) mutations that are not detected in the blood of the affected individual may be present in the heart. Preliminary observations suggest that this may be important in some types of congenital heart disease.

Germline mosaicism is a special type of mosaicism, where a population of precursor spermatocytes or oocytes carries a specific mutation that is not detected in other tissues. As a result of germline mosaicism, a healthy (apparently noncarrier) parent may unexpectedly transmit the same disease mutation to several offspring. The classic observation of germline mosaicism is in Duchenne muscular dystrophy, where apparently noncarrier females may give birth to more than one affected son with exactly the same dystrophin mutation. However, germline mosaicism may occur in any disorder including cardiac disorders and, therefore, it should be considered a possibility in any apparently de novo mutational event.

On Penetrance and Variable Expressivity

The penetrance of a specific mutation refers to its ability to cause a disease phenotype. In monogenic disease mutations may show 100 % penetrance. For instance, most pathogenic dystrophin mutations will cause Duchenne muscular dystrophy in all hemizygous males. However, especially in autosomal dominant disease, penetrance is often reduced; not everybody with the mutation actually becomes ill. Whether or not disease symptoms develop may be dependent on a constellation of other genetic (genetic background) or environmental factors, such as lifestyle. Disease penetrance is not necessarily identical to having actual clinical complaints. Especially in cardiogenetics, many clinically asymptomatic individuals with, for example, a hypertrophic cardiomyopathy or long QT syndrome may have easily noticeable abnormalities on ECG or echocardiography. Such individuals may not realize their genetic status, but they cannot be regarded as true nonpenetrants. Usually, they should be under cardiac surveillance and often preventive treatment will be indicated. Penetrance, in this way, is to some extent dependent on how well individuals have been examined for disease symptoms. If true nonpenetrance occurs, genetic diagnosis may be the only means to identify individuals that may transmit the disease to their offspring. For decisions with respect to patient care, it is more useful to look at penetrance of specific phenotypic traits, for example, the chance of a ventricular arrhythmia in case of a *KCNQ1* mutation in long QT syndrome type 1.

In congenital heart disease, penetrance is fixed, as the disease is either present or not. In diseases that manifest themselves later in life this is not true. For instance, in an autosomal dominant inherited cardiomyopathy penetrance at the age of 10 may be low, whereas at the age of 60 most individuals with the genetic defect will have developed disease manifestations. In this case, there is age-dependent penetrance. Of course, this will influence risk estimations based on clinical observation. At the age of 10, a child of cardiomyopathy patient from this family may still have an almost 50 % chance of having inherited the familial mutation despite a normal cardiac evaluation, while at the age of 60, a normal cardiac evaluation severely reduces the chance of the mutation being present. If sound scientific data are available on penetrance, these can be used in genetic counseling and decision making. However, unfortunately, this is often not the case.

Variable expression is used to indicate the presence of variation in disease symptoms and severity in individuals with a similar mutation. For example, in desmin myopathy, some individuals may mainly suffer from skeletal myopathy, whereas in others from the same family cardiac manifestations may be the principle determinant of the disease.

Genotype–Phenotype Correlations

This term refers to the extent to which it is possible to predict a phenotype (i.e., clinical disease manifestation) given a specific genotype and vice versa. In an era where presymptomatic genetic testing becomes more and more customary, this is an issue of great importance. If it were possible to predict phenotype based on genotype with great accuracy, this would lend additional legitimacy to genetic testing, especially if early intervention would change disease course. Indeed,

there have been claims that, for example, hypertrophic cardiomyopathy caused by mutations in the gene-encoding cardiac troponin T (*TNNT2*) has a higher potential for malignant arrhythmias than mutations in some other genes [8, 9]. Also within a given gene, some mutations may have a stronger pathogenic effect than others.

Without doubt significant genotype–phenotype correlations do exist, but it is prudent to regard such claims with caution, as some of them may also be the result of ascertainment and publication bias. From a clinical point of view, it is obvious that if intrafamilial (where every affected individual has the same mutation) variation in disease severity and penetrance is considerable, little can be expected of phenotype predictions based on the presence of this family-specific mutation alone. As a result of the difficulty in establishing straightforward genotype–phenotype correlations, the role of genetic information in cardiac risk stratification protocols has been limited thus far. Nowadays, there are a few exceptions in genetic cases of DCM, where specific classes of mutations in LMNA and PLN seem to be associated with adverse outcome. In these selected cases, precise genetic diagnosis may influence clinical decision making for example with regard to ICD therapy [10, 11].

The reverse situation needs also to be considered. Clinical history and clinical data such as, for example, T-wave morphology in patients suspected of having a form of long QT syndrome are very helpful in selecting the genes that should be analyzed first [12]. In the long QT syndromes genotype–phenotype correlations can be used in practice: clinical parameters suggest a specific genotype and subsequently, genotype-specific therapy can be instituted. Accurate clinical information may improve the yield of genetic testing and may decrease costs and time needed for these analyses.

Basic Concepts in Population Genetics

Population genetics studies genetic variation and genetic disease in the context of populations. Here, a population is defined as the group of individuals that are likely to get offspring together, and the genetic diversity that is contained within this group. Populations are not only delimited by geographical boundaries such as borders, rivers, mountains, islands, but also by religious, ethnic, and cultural differences.

Some insights from population genetics are important to the field of clinical genetics and necessary for understanding genetic phenomena that are relevant to clinical practice like, for instance, founder effects. Two important population genetic "laws" predict the distribution of neutral genetic variation (i.e., the Hardy–Weinberg equilibrium) and the frequency of disease mutations (mutation-selection equilibrium), respectively.

Hardy–Weinberg Equilibrium

The Hardy–Weinberg equilibrium predicts that the relative frequency of different genotypes at a locus within a population remains the same over generations. For an autosomal gene G with two alleles A and a with an allele frequency of p and q, respectively, the possible genotypes AA, Aa, and aa will occur with a frequency of p^2, 2pq, and q^2. As there are only two alleles for G, $p + q = 1$.

However, for the Hardy–Weinberg equilibrium to be true, many assumptions have to be made. The population has to be infinitely large, there has to be random mating with respect to G, there has to be no selection against any of the G genotypes, no new mutations occur in G, and there is no migration introducing G alleles into, or removing G alleles from the population. Clearly no situation in real life will ever satisfy all these criteria.

The Hardy–Weinberg equilibrium is a neutral equilibrium. Small deviations from the expected genotype frequencies occur by chance (genetic drift) and over multiple generations a significant difference in genotype frequency (when compared to the original equilibrium) may become apparent. There is no driving force correcting such chance deviations. As a matter of fact a new Hardy–Weinberg equilibrium is established with each generation.

In real life, new mutations do occur and often selection does exist against disease-associated alleles, causing them to disappear from the gene pool. However, mutation rates for recessive disorders are extremely small and selection pressure is low, as selection works only against the homozygous affected. Therefore, in autosomal recessive disorders, the Hardy–Weinberg equilibrium can be used to calculate carrier frequencies for recessive disorders if the frequency of the disorder in the population (q^2) is known. Because of the limitations mentioned above, such calculations have to be regarded as estimates and interpreted with caution.

Mutation–Selection Equilibrium

To understand the dynamics of disease causing (not neutral) alleles, another equilibrium is of importance: the mutation-selection equilibrium. New disease alleles will arise with a given frequency as a result of new mutations, but when diseased individuals are less likely to reproduce, they also disappear again from the gene pool. Therefore, the equilibrium that predicts the frequency of disease alleles is a function of the mutation rate, the reproductive fitness, and the mode of inheritance of the disease.

The easiest example is a severe congenital heart defect as a result of a new autosomal dominant mutation. If this heart defect is lethal, reproductive fitness is nil, and the population frequency of the mutated autosomal dominant gene would be identical to the mutation frequency. In, for example, long QT

syndrome type 1, most mutation carriers, however, will reproduce, but reproductive fitness is somewhat reduced as a result of some affected individuals dying from arrhythmias at a young age [13]. Here, the actual frequency of the disease allele is much larger than the mutation frequency, as most disease alleles will be inherited. Still, if no new mutations would occur, the disease would eventually die out as a result of reduced fitness.

Mutation-selection equilibrium is more stable than the Hardy–Weinberg equilibrium. If for some reason more new mutations arise than expected, selective pressure increases as well since there are more affected individuals to target, moving the equilibrium again in the direction of the original state. However, if reproductive fitness increases significantly as a result of improved therapies, eventually a new equilibrium with a higher population frequency of the mutated allele will be established.

Founder Mutations

If a population descends from a relatively limited number of ancestors, the genetic variation is largely dependent on the variation that was present in this small group of ancestors. If by chance a rare disease allele was present in one of these "founders," this disease allele may achieve an unusual high frequency in this founder population, which is not found in other populations. This is especially true if selection against the mutation is small, so that the mutation is not easily eliminated from the gene pool.

For example, in the Netherlands, over 20 % of hypertrophic cardiomyopathy is caused by a single c.2373_2374insG mutation in the MYBPC3 gene [14]. In order to prove that this is indeed a founder mutation and not a mutation that has occurred *de novo* more than once, it was established that the mutation in each patient lies on an identical genetic marker background (haplotype), which must have been present in the founder. If the mutation had occurred many times de novo it would have been expected to be associated with different haplotypes.

Founder effects, like the one described above, can help explain why certain diseases are more frequent in some populations than others. Moreover, it is important to be aware of these mechanisms as they can aid in devising efficient strategies for molecular diagnosis in specific populations.

Genetic Isolates

Genetic isolates are small, closed communities within a larger population where people tend to marry among each other. Consanguinity is more likely and even if this is not the case, genetic variation within an isolate is much more limited, because of the absence of new genes contributing to the gene pool. As a result, some genetic diseases may have a much higher frequency within an isolate than in the population as whole, while in contrast other genetic diseases may be virtually absent. Therefore, it may be important to realize whether or not a specific patient comes from a genetic isolate.

Consanguinity

Consanguineous marriages are very common in some cultures and unusual in others [15]. Marriages between first cousins are most frequent. They share 12.5 % of their DNA, derived from their common ancestor. In some cultures, uncles are allowed to marry their nieces. Such second-degree relatives share 25 % of their DNA. This situation, from a genetic point of view, is no different from double first cousins that have all four grandparents in common and, therefore, also share 25 % of their DNA.

Consanguinity may have significant social and economical advantages, especially in low-income societies. However, the genetic risks cannot be ignored, but they are highly dependent on the degree of relationship. The problem with consanguinity arises from the reduction to homozygosity in offspring of consanguineous parents. If both parents carry the same recessive mutation in their shared DNA, there is a 25 % risk of the mutation being homozygous in each child. Therefore, consanguinity mainly increases the likelihood of autosomal recessive disease. The chance that a recessive disorder is caused by consanguinity increases with decreasing frequency of the disorder. In other words, the relative risk increase as a result of consanguinity is highest for the rarest recessive disorders. For example, thus far a rare form of catecholaminergic polymorphic ventricular tachycardia (CPVT) as a result of an autosomal recessive mutation in the CASQ2 gene has only been found in consanguineous families [16]. In addition, one also has to be aware of the fact that autosomal dominantly inherited disease may also run in consanguineous families. If offspring has inherited both affected alleles, the clinical picture is often severe and lethal at an early age. Examples have been found long in QT syndrome and hypertrophic cardiomyopathy [17, 18].

If consanguinity occurs frequently within a population, the population becomes inbred. In such a population, for any genetic locus, the frequency of heterozygotes will be lower than expected under Hardy–Weinberg equilibrium (because of reduced random mating). This will lead to overestimation of carrier frequencies.

In multifactorial disease, consanguinity may play a role as well, although less conspicuous than in autosomal recessive disease. Shared predisposing genetic variants, present in heterozygous form in the parents, have a 25 % chance of being present in homozygous form in the offspring, thus increasing the likelihood for multifactorial disorders.

Information on consanguinity is not always volunteered and should be specifically asked for. Sometimes consanguinity is present, but not known to the family. Most individuals have little information on relatives dating further than three generations back. If ancestors from both parents are from the same small isolated community, consanguinity may still be suspected. When of importance, genealogical studies may be used to substantiate this.

Genetic Testing

Any test to identify a genetic disease can be considered a genetic test. Genetic testing using DNA analysis is available for an increasing number of cardiac diseases and conditions that are associated with cardiovascular disease in a wider context. Two important differences between genetic DNA tests, when compared to other diagnostic tests, need mentioning. First, DNA tests usually have health implications that last a life time, while the genetic defect in itself is not amenable to treatment. Second, the implications of genetic test results often are not limited to the patient in front of you, but also are of concern to family members including future offspring. The family and not the individual patient could be regarded as the "diagnostic unit" in genetic disease. As a result of these notions, DNA testing is and should only be offered as part of a genetic counseling procedure in order to assure that patients fully understand the scope of the tests that are being performed. This is especially true for monogenic disease and tests for very high risk genes.

Genetic Counseling

Genetic counseling is a two-way communication process aiming at helping patients with genetic disease or at (perceived) increased risk of genetic disease, and their relatives, to understand the genetic risk and decide on a suitable course of action [19]. Genetic counseling is offered by trained medical or paramedical professionals. Its goals are as follows:

- To help patients and their family members comprehend medical facts (diagnosis, symptoms, complications, course, variation, and management)
- To help patients and their family members understand the basic facts of the genetic contribution to their disorder, where this is relevant for communicating risks to specific family members and recurrence risks in (future) children
- To make them understand the options available to deal with risks and recurrence risks (preventive treatments, lifestyle adjustments, reproductive options, prenatal diagnosis)
- To help individuals choose a suitable course of action in view of their individual risk of disease, goals, personal and cultural values, and religious beliefs, and to facilitate this course of action
- Support individuals and their families in making the best possible adjustment to their disease condition or to their increased risk of genetic disease

Most common counseling situations for cardiac disorders can be grouped into one of the three categories mentioned below. All three have their own dynamics and major issues:

- Parents who have a child with a congenital heart defect, a syndrome that has important cardiovascular implications, or other cardiac disease. They want to be informed about prognosis, recurrence risk to other children, and the possibility of prenatal diagnosis.
- Patients that have a cardiac defect or cardiac disease themselves and have questions about genetic aspects and prognosis. They may also be concerned about risk to family members, most often (future) children and/or sibs.
- Those who have been referred because of a positive family history for cardiac disease or suspicion thereof, or have a family history for sudden cardiac death at a young age. They come for information on their personal risk, questions about the usefulness of presymptomatic cardiac evaluation and, if possible, they may opt for presymptomatic genetic testing.

Some basic principles of genetic counseling:

- Nondirectiveness. Historically, nondirectiveness is an important hallmark of genetic counseling. The counselor provides adequate information and support. The counselee decides. This notion stems from time that genetic counseling was mainly concerned with reproductive issues. Naturally, counselors should have no say in the reproductive decisions made by their clients. Also, in presymptomatic testing of late onset neurodegenerative disease, where medical interventions to change disease course are virtually absent, maximum nondirectiveness should be applied in counseling.
- However, with a changing focus in medical genetics to disorders that are, at least to some extent, amenable to early intervention or preventive treatment, the applicability of nondirective genetic counseling becomes less obvious. For example, in long QT syndrome type 1, where β-blocker therapy has been proven to be effective in symptomatic patients, nondirective counseling seems less indicated [20]. In practice, in cardiogenetics, a balance that respects both patient autonomy, and assures that the appropriate medical decisions are made, should be sought for.
- Informed consent. Informed consent is not unique to clinical genetics or genetic counseling. However, some institutions will require written informed consent prior to

DNA testing, especially if presymptomatic testing of apparently healthy individuals is concerned. This is no rule of thumb and may vary based on individual insights and local differences in the medico-legal situation.

- Privacy issues. These are also not unique to genetic medicine, but may be more urgent in this discipline. Genetic information may have a huge impact on insurability and career options. The extent to which this is true is largely dependent on legislation dealing with genetic discrimination, which varies between countries. However, a danger of discrimination on genetic grounds always exists. Therefore, maximum confidentiality of genetic information should be assured. Providing genetic information to third parties, without written permission from the individual involved, would be defendable only in case of a medical emergency. In contrast, genetic information is much harder to keep confidential because DNA is shared by relatives that are likely to benefit significantly from this information. When appropriate, permission to use genetic information for the benefit of relatives should be actively acquired by the genetic counselor. Especially in families that communicate insufficiently, clinical geneticists may encounter problems with confidentiality and find themselves confronted with conflicting duties.

Cardiac Genetic Counseling

The most notable feature of cardiogenetic diseases is the risk of sudden cardiac death. Every aspect of this devastating event defines the unique role of cardiac genetic counselors [21]. These include involvement in prevention strategies, such as helping patients to make lifestyle modifications and coordinating clinical screening of at-risk relatives. Furthermore, the cardiac genetic counselor may be involved in key treatment strategies such as implantable cardioverter defibrillator therapy, thorough investigation of the family history, and in many cases dealing with a grieving family who have unexpectedly lost a loved one. Given the importance of clinical screening of first-degree relatives as a primary prevention strategy, cardiac genetic counselors frequently provide assistance in coordinating clinical surveillance of at-risk family members. Being the first point of call for many families, it is also important for cardiac genetic counselors to educate patients about how frequently clinical screening should occur, and often to dispel misinformation regarding family members who have been told in the past that they never need to return for clinical assessment. A diagnosis of many of these diseases will also impose exercise restrictions, particularly relating to competitive sports, and this can be devastating to many patients making adjustment to their disease much more difficult. This is particularly relevant in the younger population where disqualification from sports participation can often lead to major psychosocial consequences. Similarly, patients deemed at increased risk of SCD who are advised to undergo implantation of an ICD experience a range of emotions, and the role of the cardiac genetic counselor to provide information and emotional support can be significant. Many of the families seen in the specialized cardiac genetic clinics will have direct experience with SCD, often having lost a close relative. In such cases, the grief of the family will be in the forefront and make the provision of information and adjustment to new diagnoses far more difficult.

Cardiac Genetic Testing

Next-generation sequencing technologies have paved the way for testing of a vast number of genes, with a typical cardiac gene panel now comprising 50–200 genes. Many of the genes included in these panels have only minimal evidence of disease association or causation (i.e., accounting for less than 5 % of disease). Use of such panels has significantly increased the yield of genetic diagnosis in cardiomyopathy families. However, as could be expected, it has also resulted in an enormous expansion of the detection of VUSs (VUS=variant of unknown significance) (ref. Pugh et al. Genetics in Medicine 2014). Despite databases filled with sequence data of over 60,000 controls from different populations (ExAC.broadintitute.org) and the availability of different *in silico* prediction algoritms, these VUSs at this moment often cannot yet be satisfactorily resolved. Whole exome (WES; sequencing of the entire coding region of the genome) and whole genome sequencing (WGS; sequencing of the entire genome) are powerful tools for research and gene discovery, and becoming increasingly common in the commercial setting. Coupled with rapidly decreasing costs, and wider access and uptake, the complexity of the results generated when a cardiogenetic gene test is now ordered goes beyond the basic expertise and scope of current practices.

Genetic testing is an important component of cardiogenetic disease management. Commercial genetic tests are available for most and increasing uptake among patients has contributed to a vastly improved knowledge of the genetic basis of these diseases. The incredible advances in genetic technologies have translated to more rapid, comprehensive and inexpensive genetic tests, completely changing the landscape of genetic testing in recent years. While there are enormous challenges, mostly relating to interpretation of variants, the value of a genetic diagnosis should not be underestimated. In almost all cases, the single greatest utility is for the predictive genetic testing of family members.

With increasing number of uncertain variants identified after cardiac genetic testing, determining methods to ensure the highest yield of causative variants is important. One key consideration is defining the clinical phenotype both in the individual patient, and the family. A complete cardiogenetic

Table 2.1 Outcomes of cardiac genetic testing (Table modified from Ingles et al. Heart Rhythm. 2014 PMID: 24632221)

Possible outcome	Consequences for the proband	Consequences for the family
No variants of potential clinical importance identified	An indeterminate gene result does not exclude a cardiac genetic disease, but reassessment of the phenotype should be considered	Predictive genetic testing cannot be offered to the family. At-risk relatives are advised to be clinically assessed according to current guidelines
Variant of uncertain significance identified (VUS)	Efforts to delineate pathogenicity of the variant are required, including cosegregation studies involving phenotyped family members	While pathogenicity of a variant is under question, it cannot be used to inform clinical management of family members. Predictive genetic testing cannot be offered. At-risk relatives are advised to be clinically assessed according to guidelines
Pathogenic mutation identified (pathogenic or likely pathogenic)	Confirm clinical diagnosis, limited therapeutic and prognostic application except in familial long QT syndrome	Predictive genetic testing of asymptomatic family members is available following genetic counseling
Multiple pathogenic mutations identified	Confirm clinical diagnosis and potentially explain a more severe clinical phenotype	Complex inheritance risk to first-degree relatives must be discussed. Predictive genetic testing of asymptomatic family members is available following genetic counseling
Incidental or secondary pathogenic mutation identified	Action regarding incidental or secondary findings must be discussed with the proband pretest	Genetic counseling to determine clinical and genetic impact to family members is available

evaluation is required, which includes being certain of the clinical diagnosis in the proband. The highest yields from genetic testing are often based on patient cohorts with confirmed disease. A word of caution with respect to pathogenicity seems justified: a variant is not causative because it has been published as such. Many publications do provide neither adequate information on segregation (does the variant cosegregate with disease in the family?), nor functional data supporting pathogenicity. The fact that a variant has been reported in a series of 50 cardiomyopathy patients and was absent in 400 ethnically matched controls cannot now be regarded as sufficient evidence for pathogenicity. Indeed, large-scale population sequencing efforts demonstrate that some mutations believed to be causal occur at a far too high population frequency to be actual monogenic causal pathogenic mutations (ref: C.Andreasen et al. Eur J. Human Genet 2013PMID:23299917).

The outcomes of cardiac genetic testing are summarized in Table 2.1.

Pre and Posttest Genetic Counseling

Cardiac genetic counseling is a key component of the multidisciplinary approach to care for families with cardiogenetic diseases, and this is not more evident than in the setting of cardiac genetic testing. With increasing complexity of cardiac genetic results, ensuring individuals are well informed prior to testing is a challenging but critical task.

Genetic test results should not be considered a binary (yes/no) outcome, but rather a carefully considered result along a continuum from benign, to VUS, likely pathogenic, and pathogenic. The genetic test result is therefore a probabilistic one, in which the weight of evidence for pathogenicity determines the probability of the specific variant being disease-causing. Conveying this result to the family can be a challenge, but the basic principles of pretest counseling essentially remain unchanged with the ultimate goal of ensuring a full understanding of the process and consequences of genetic testing. There is often inherent uncertainty of the gene result and therefore discussion with a health professional who can effectively explain what this means to the family is preferable [22]. There should be an understanding that the identification of a VUS may require initiation of further family investigation to clarify pathogenicity, and indeed, a detailed family history at this point will give information about whether this is even possible. Furthermore, it should be highlighted clearly that there is a small chance new information will become available in the future that may change lead to reclassification of the result. Trained genetic counselors are skilled in delivering complex information in a sensitive manner and should play a key role in the testing process.

Interpreting Genetic Test Results

Although nowadays most genetic tests are based on direct mutation testing, interpretation of the results is not always straightforward. Without going into great depths on this subject, it may be appropriate to spend a few lines on this subject. Mutations can basically have effects in three different

ways. They can cause loss of normal protein function. This is called haploinsufficiency. They can cause gain or change of normal protein function, or they can make the protein become toxic, if normal metabolism is disturbed. For example, loss of function mutations in the SCN5a gene causes Brugada syndrome and progressive conduction disease, whereas gain of function mutations in the same gene underlies long QT syndrome type 3.

If nonsense mutations (leading to a stop codon) or frameshift mutations (leading to disruption of the reading frame, which usually causes a premature stop) occur, one can be confident that this will lead to haploinsufficiency, unless the truncation is very close to the C-terminus of the gene. As a result of a process called nonsense-mediated messenger RNA decay, only very little truncated protein will be produced. Most splice mutations (especially those disturbing the reading frame) and larger rearrangements of genes will also lead to haploinsufficiency. In certain genes where haploinsufficiency is known to be a mechanism of disease, these changes will almost certainly be considered pathogenic. In cardiogenetic diseases, this can include MYBPC3 mutations in HCM- and TTN-truncating mutations in familial DCM. Truncating mutations in MYH7 are an example of changes not expected to impact the protein, since loss of function is not thought to be the underlying mechanism of disease for this gene.

Missense mutations (mutations changing only one amino acid in the protein) may lead to both loss of protein function or gain/change of function. Especially in case of structural proteins, where different protein molecules act together to form a structure, missense mutations may be more deleterious than truncating mutations, as the mutated proteins are incorporated into the structure and disrupt it. This is called a dominant negative effect.

However, many of the missense mutations detected may actually be rare variants without significant effect on protein function. Therefore, if a new missense mutation in a candidate gene for a specific disorder is identified it will often be difficult to predict whether or not it is the actual causative mutation, that is, VUS (Table 2.1). Unfortunately, in clinical practice, VUSs occur rather frequently and cannot always be satisfactorily resolved. Some factors for consideration when classifying pathogenicity of a variant are shown below in Table 2.2. Assumptions made regarding the pathogenic potential of missense mutations are therefore often provisional. It is important to realize this when using genetic information in clinical practice. Overinterpreting missense variants for pathogenic mutations is harmful in several ways, as on the one hand some individuals without a genetic predisposition to the disease will be stigmatized and unnecessarily kept under surveillance, while on the other hand, the actual causative mutation will go unnoticed and individuals may be released from surveillance, based on incorrect genetic information. The fact that a specific missense mutation has been published as a pathogenic mutation in the literature cannot always be regarded as sufficient evidence (one has to go back to the original publications and weigh the evidence).

Predictive Testing and the Dynamics of Family Studies

Predictive, presymptomatic, or cascade genetic testing is performed on family members with no apparent evidence of clinical disease to find out whether or not they have inherited the gene variant. Often predictive testing takes place in the context of family studies. In family studies, specific individuals are targeted for evaluation based on a positive family history for genetic disease. Both predictive testing and family studies are unique features of clinical genetics practice.

Predictive DNA Testing

Predictive DNA testing is usually performed for monogenic disorders with important health risks. Demonstrating that an individual has not inherited the family-specific mutation usually reduces risks to population level and also risks for offspring will be normalized. However, if the mutation is indeed identified, this does not automatically mean that the individual will get the disease. In many cardiac disorders, penetrance is significantly reduced. In general, the presence of the familial mutation will not allow for predictions on severity of the disease or age of onset. Most genetic cardiac disorders show significant locus heterogeneity, that is, many different genes are associated with a similar phenotype. Besides, molecular heterogeneity (the number of different mutations in a gene) is immense. Therefore, as a paradigm, predictive genetic testing in a family is only possible if a causative family-specific mutation has been identified in the index patient.

It is important to emphasize that predictive testing does not necessarily involve DNA testing. A cardiologist performing echocardiography in a symptom free sib of a hypertrophic cardiomyopathy patient is involved in both a family study and predictive testing. The detection of, even a very mild, hypertrophy of the interventricular septum, that as yet does not need treatment, will have serious consequences for this person. The adverse consequences (see next paragraph) of predictive testing based on concealed cardiac symptoms are no different from those associated with predictive DNA testing. Therefore, in a case like this, the same standards of genetic counseling should be applied prior to echocardiography.

In families where DNA studies have been unsuccessful, family studies will have to rely solely on phenotype and therefore on cardiac evaluation. An important difference between family studies based on phenotype and those based

Table 2.2 Key criteria used in determining pathogenicity of variants

Key criteria	Description	Tools/approach
The absence of rarity in general population	The variant has not been reported in general population databases. In 2016, the Exome Aggregation Consortium (reports >60,000 exomes giving allele frequencies by ethnicity. The absence of the variant in a large number of healthy controls is compatible with pathogenicity, but merely confirms that the variant is indeed rare	ExAC database *ExAC.broadinstitute.org*
Variant previously reported in disease population	Many classification criteria will require the variant to have been previously reported in >3 unrelated probands with the same phenotype. Public access databases such a NCBI ClinVar encourage laboratories and research groups to upload their details and experience of certain variants. Other disease specific databases exist	ClinVar website *clinvar.com* ARVD/C genetic variant database *arvcdatabase.info*
De novo event	If the variant has arisen spontaneously at conception, (i.e., *de novo*) this can be regarded as very strong evidence in favor of pathogenicity. As mutation frequencies are exceedingly low, the chance that a new mutation would occur in the studied candidate gene just by coincidence is negligible	Genetic and clinical testing of both parents is necessary. Where there is question over paternity, this should be confirmed by haplotype analysis following discussion with the family. In recent times, due to occurrence of egg donor *in vitro* fertilization options, it may also be necessary to confirm maternity with a family. Paternity/maternity issues are sensitive discussion topics and should be carefully approached
Segregation with multiple affected family members	Proving that a variant cosegregates with the disease phenotype in a family can provide definite evidence for pathogenicity, but only if the family is large enough (about ten informative meiosis would be required). This is often not the case. In families where even two or more affected relatives can be shown to carry the variant, this will provide lower level supportive evidence for pathogenicity and should be pursued where possible Absence of the variant in an affected family member is, of course, strong evidence against pathogenicity	Clinical and genetic testing of relatives is needed to gather segregation data. In general, we collect DNA samples from affected relatives only since unaffected relatives are not informative (i.e., we know there is incomplete penetrance among cardiogenetic diseases). When segregating an uncertain variant in a family, the individual should understand the importance of the variant is unknown. Often the approach is to request the DNA as a research sample, where there is no expectation to get a result. If pathogenicity can be adequately confirmed then the family members will be invited to the clinical service to undergo predictive genetic testing
Variant causes loss of function of a gene known to result in a phenotype by this mechanism	As described above, loss of function in a handful of genes can be very strong evidence of pathogenicity	This rule does not apply to all genes
In silico tools and conservation scores supportive of a deleterious role	There are a multitude of *in silico* prediction software and conservation scores, no weight should be placed on a single score and in general whether a number of these are in support of a deleterious impact can be used as low level evidence to support pathogenicity	Polyphen2 *genetics.bwh.harvard.edu/pph2* Polyphen HCM *genetics.bwh.harvard.edu/hcm* SIFT (Sorting Intolerant from Tolerant) *sift.jcvi.org* CADD Score (Combined Annotation Dependent Depletion) *cadd.gs.washington.edu*

on genotype arises when nonpenetrance or age-dependent penetrance occurs. In that case, of course, a genetic test will be more sensitive to demonstrate the predisposition especially in young individuals. In conditions with age-dependent penetrance, it may be prudent to re-evaluate individuals with a 50 % prior chance of having inherited the genetic defect after a couple of years.

Adverse Consequences of Predictive Testing

Predictive testing may offer important medical and psychosocial benefits to the individuals tested. However, it should be realized that, in contrast to this, predictive testing can also have negative psychological and socioeconomic repercussions [23]. Individuals may perceive themselves as less

healthy, even when no disease symptoms can yet be demonstrated. Coming to terms with knowledge about one's own genetic predisposition, feelings of guilt toward children that are now also at increased risk, forced lifestyle changes and difficulty with choices regarding, for example, reproductive issues may cause a lot of distress and anxiety. Importantly, knowing that one has the predisposition for a serious late onset disorder is likely to complicate qualifying for, for example, life or health insurance or might interfere with career options. Last but not least, predictive testing can complicate family relationships, especially if some family members want to be tested while others decline testing. Test results of one person may also yield risk information with regard to other family members that may not want to know this. Therefore, predictive testing should not be embarked on without giving these issues serious thought. Opting for predictive testing should be a well-considered and autonomous decision of the individual involved. Pressure on individuals to undergo testing, for instance, by insurance companies or employers would be absolutely unethical.

Predictive Testing in Minors

Minors cannot make their own well-informed decisions with regard to predictive testing. It is a paradigm in clinical genetics not to perform predictive genetic testing in minors if there is no direct and important medical benefit [24]. Late-onset disorders, or disorders that are not amenable to preventive treatment, are not to be tested in healthy children [24]. In some countries, predictive genetic testing in minors is subject to specific restrictive legislation.

However, in many cardiac disorders like, for example, long QT syndromes, preventive therapy should be instituted at an early age. In such cases, postponing testing until children can make their own autonomous decisions is often not a realistic option. Thus, predictive genetic testing of minors can certainly be indicated. In the Netherlands, for example, predictive genetic testing of minors for cardiac disorders is performed in centers for cardiogenetics, according to a protocol that also involves participation of a psychologist or specialized social worker. It should be noted that parents who have their children tested for heritable arrhythmia syndromes are likely to experience major distress and anxiety [25]. This may influence the handling of their children and moreover parental anxiety is likely to lead to anxiety in children.

Although, as a rule of thumb, predictive testing in children is only performed if treatment or surveillance is possible and necessary, there may be exceptions to this rule that have to judged on a case-by-case basis. The bottom line is that testing has to be in the interest of the child. For example, should a child from a hypertrophic cardiomyopathy family be talented enough to seriously pursue a professional career in sports, it would be unfair to postpone testing, thereby depriving the child from the possibility to choose another career at an earlier stage.

Conducting Family Studies

The way individuals are selected for evaluation in a family study primarily depends on the mode of inheritance of the disease. Cardiogenetic family studies most often involve autosomal dominant conditions, in which affected individuals are likely to occur in several generations and both males and females may be affected. Family studies are conducted using the "cascade method." As soon as a new disease carrier has been identified, his or her first-degree relatives become the next targets for study. When parents of a disease carrier are deceased, it will often be difficult to determine whether the condition has been inherited from the mother or from the father. The possibility also remains that the disease predisposition was inherited from neither parent, but resulted from a *de novo* mutational event. A decision will have to be made whether to stop here or to pursue the family study further to aunts, uncles, and often first cousins at both sides. This decision depends in part on the medical information available on the parents and more distant relatives. Moreover, the magnitude of the risk for severe events that is associated with the familial disease, knowledge on the frequency of the familial character of the disease, and the availability of therapies that influence this risk are important issues when deciding how far family studies should be pursued.

The major justification for family studies is to unambiguously identify those individuals that run an increased risk of disease, in order to institute preventive therapies or closely monitor these individuals and enroll them in risk stratification protocols.

However, sometimes the targeted family members themselves may not be at high risk for serious disease anymore. Contacting them may still be justified if there is a considerable chance that the predisposition to a treatable disease has been transmitted to their children. This may, for example, be the case in elderly individuals from long QT syndrome families that never experienced arrhythmias themselves. Demonstrating the predisposition in them will not necessarily lead to treatment, but exclusion of the predisposition will render further testing unnecessary for all of his or her children. The medical benefits for elderly tested individuals may be limited, but also the socioeconomic dangers of predictive testing may be less urgent in older individuals, as they will usually already have insurance and careers.

In case of a disorder that is not amenable to treatment, only reproductive counseling can be offered to family members that turn out to have the genetic predisposition. For personal reasons family members may opt for predictive testing. Uncertainty regarding genetic status may by itself be a major cause of distress and anxiety. However, if no clear medical benefits are to be expected, family studies should only be initiated on specific demand of the relatives themselves.

Prenatal Diagnosis

Prenatal diagnosis can be requested for a number of different reasons. Termination of pregnancy may be the ultimate consequence once it has been established that the fetus has a very serious debilitating genetic disorder. However, the goal of prenatal diagnosis may also be to aid in planning peripartum medical interventions, or help parents to emotionally prepare for the birth of a child with a birth defect. Parents with a previous child with a congenital heart defect will qualify for specialized ultrasound in subsequent pregnancies. Depending on the severity and type of heart defect that is detected at ultrasound, parents may decide to terminate the pregnancy or to deliver in a center where appropriate neonatal intensive care is available. On rare occasions, even fetal therapy can be applied; for instance, some fetal tachyarrhythmias can be treated by putting the mother on medication.

Prenatal diagnosis can be divided in invasive diagnosis and noninvasive imaging studies, mainly prenatal ultrasound. Invasive prenatal diagnosis involves obtaining chorionic villi (placental cells), amniocytes (fetal cells present in amniotic fluid), or rarely cord blood, for genetic and, sometimes, protein or metabolite studies. The invasive procedures are associated with a small albeit significant risk of pregnancy loss. Therefore, these should be undertaken only if the prenatal diagnosis will have medical consequences. Like in predictive genetic testing, prenatal DNA diagnosis for cardiac disorders will only be possible if the family-specific mutation has been identified beforehand.

Except for ultrasound diagnosis in pregnancies of couples to whom an earlier child with a congenital heart defect has been born, requests for prenatal diagnosis are infrequent in cardiogenetic practice. However, requests for prenatal diagnosis should always be taken seriously and the reasons should be explored. Frequently, other issues like feelings of guilt, fear of disapproval from friends or relatives, uncertainty about postnatal follow-up, and so on may be found to underlie such requests.

Preimplantation genetic diagnosis (PGD) is a technique in which *in vitro* fertilization (IVF) is combined with genetic diagnosis prior to implantation of the embryo into the womb. As genetic diagnosis has to be performed on one or two embryonal cells instead of millions of white blood cells, PGD is technically much more demanding. PGD may be an alternative to couples that are opposed to pregnancy termination, but would not be able to reproduce knowing that their child is at high risk of serious genetic disease. Success rates of PGD are limited by the limitations of the IVF procedure and the fact that after genetic testing fewer viable embryos may be left for implantation. PGD has been performed for a limited number of disorders that may have major cardiac consequences like, for instance, Marfan syndrome or myotonic dystrophy [26, 27].

Besides prenatal diagnosis, which is performed in selected cases because of increased risk of genetic disease, also prenatal screening programs exists. In principle all pregnant women are eligible for prenatal screening programs that may be differently set-up in different countries. In most western countries nowadays prenatal ultrasound screening is offered to pregnant women at around 20 weeks of gestation. As congenital heart defects occur at high frequency in the general population, many more heart defects will be found by chance during ultrasound screening than by using other methods of prenatal diagnosis, even if the sensitivity of ultrasound screening may be relatively poor.

The Cardiogenetics Out-patient clinic

Since the care for individuals with genetic cardiac disease and their relatives requires both cardiologic and genetic expertise, in the multidisciplinary outpatient clinics for cardiogenetics are advocated as the ideal model of care. In these outpatient clinics that are operating within the university hospitals, cardiologists, pediatric cardiologists, clinical geneticists, molecular geneticists, genetic nurses, psychologists, and/or social workers cooperate to provide integrated health care for this specific patient group. This is of benefit to patients because the number of hospital visits can usually be reduced and also to health care providers because of easier communication. Besides, from a data collection and research point of view, centralization of patients with inherited cardiac disease also has obvious advantages. It will be immediately clear that most of the regular care for this patient group will have to remain with cardiologists working in regional or local hospitals. With an estimated prevalence of 1 in 500 for hypertrophic cardiomyopathy, it would not only be unnecessary to follow all these patients in out-patient clinic for cardiogenetics, but it would also be impossible. This implicates that a general awareness of the genetic aspects of cardiac disease among cardiologists is needed.

References

1. Hofman N, van Langen I, Wilde AM. Genetic testing in cardiovascular diseases. Curr Opin Cardiol. 2010;25 .e-published
2. Cowan J, Morales A, Dagua J, Hershberger RE. Genetic testing and henetic counseling in cardiovascular genetic medicine: overview and preliminary recommendations. Congest Heart Failure. 2008;14:97–105.
3. Morales A, Cowan J, Dagua J, Hershberger RE. Family history: an essential tool for cardiovascular genetic medicine. Congest Heart Failure. 2008;14:37–45.
4. Arnestad M, Crotti L, Rognum TO, et al. Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. Circulation. 2007;115:361–7.

5. Berul CI, Perry JC. Contribution of long-QT syndrome genes to sudden infant death syndrome: is it time to consider newborn electrocardiographic screening? Circulation. 2007;115:294–6.
6. Kingston HM. Genetic assessment and pedigree analysis. In: Rimoin DL, Connor JM, Pyeritz RE, Korf BR, editors. *Emery and Rimoin's principles and practice of medical genetics*. 4th ed. London: Churchill Livingstone; 2002.
7. Turnpenny P, Ellard S. *Emery's elements of medical genetics*. 13th ed. London: Churchill Livingstone; 2008.
8. McKenna WJ, Thierfelder L, Suk HJ, Anan R, O'Donoghue A, et al. Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy. N Engl J Med. 1995;332:1058–64.
9. Gimeno JR, Monserrat L, Pérez-Sánchez I, et al. Hypertrophic cardiomyopathy. A study of the troponin-T gene in 127 spanish families. *Rev Esp Cardiol*. 2009;62:1473–7.
10. IAW van Rijsingen et al. J. Am. Coll Cardiol. 2012.
11. IAW van Rijsingen et al. Circulation Cardiovascular genetics 2014.
12. Van Langen IM, Birnie E, Alders M, Jongbloed RJ, Le Marec H, Wilde AA. The use of genotype-phenotype correlations in mutation analysis for the long QT syndrome. J Med Genet. 2003;40:141–5.
13. Schwartz PJ, Priori SG, Spazzolini C, et al. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. Circulation. 2001;103:89–95.
14. Alders M, Jongbloed R, Deelen W, et al. The 2373insG mutation in the MYBPC3 gene is a founder mutation, which accounts for nearly one-fourth of the HCM cases in the Netherlands. Eur Heart J. 2003;24:1848–53.
15. Othman H, Saadat M. Prevalence of consanguineous marriages in Syria. J Biosoc Sci. 2009;41:685–92.
16. Lahat H, Pras E, Olender T, et al. A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. Am J Hum Genet. 2001;69:1378–84.
17. Hoorntje T, Alders M, van Tintelen P, et al. Homozygous premature truncation of the HERG protein: the human HERG knockout. Circulation. 1999;100:1264–7.
18. Zahka K, Kalidas K, Simpson MA, et al. Homozygous mutation of MYBPC3 associated with severe infantile hypertrophic cardiomyopathy at high frequency among the Amish. Heart. 2008;94:1326–30.
19. Resta R, Biesecker BB, Bennett RL, et al. A new definition of Genetic Counseling: National Society of Genetic Counselors' Task Force report. J Genet Couns. 2006;15:77–83.
20. Vincent GM, Schwartz PJ, Denjoy I, et al. High efficacy of beta-blockers in long-QT syndrome type 1: contribution of noncompliance and QT-prolonging drugs to the occurrence of beta-blocker treatment "failures". Circulation. 2009;119:215–21.
21. Ingles et al. Heart Rhythm 2011 PMID: 21767518.
22. Ingles et al. Heart Rhythm 2014. PMID: 24632221.
23. Hendriks KS, Hendriks MM, Birnie E, et al. Familial disease with a risk of sudden death: a longitudinal study of the psychological consequences of predictive testing for long QT syndrome. Heart Rhythm. 2008;5:719–24.
24. European Society of Human Genetics. Genetic testing in asymptomatic minors: recommendations of the European Society of Human Genetics. Eur J Hum Genet. 2009;17:720–1.
25. Hendriks KS, Grosfeld FJ, Wilde AA, et al. High distress in parents whose children undergo predictive testing for long QT syndrome. Comput Geneal. 2005;8:103–13.
26. Piyamongkol W, Harper JC, Sherlock JK, et al. A successful strategy for preimplantation genetic diagnosis of myotonic dystrophy using multiplex fluorescent PCR. Prenat Diagn. 2001;21:223–32.
27. Spits C, De Rycke M, Verpoest W, et al. Preimplantation genetic diagnosis for Marfan syndrome. Fertil Steril. 2006;86:310–20.
28. Gray B, Yeates B, Medi C, Ingles J, Semsarian C. Homozygous mutation in the cardiac troponin I gene: clinical heterogeneity in hypertrophic cardiomyopathy. Int J Cardiol. 2013;168(2):1530–1.

Part II

Cardiomyopathies

Introduction Hereditary Cardiomyopathies

3

Paul A. van der Zwaag and Maarten P. van den Berg

Abstract

Cardiomyopathies are myocardial disorders in which the heart muscle is structurally and functionally abnormal in the absence of coronary artery disease, hypertension, valvular disease, and congenital heart disease sufficient to explain the observed myocardial abnormality. We describe five main cardiomyopathy subtypes as outlined by the European Society of Cardiology: hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic cardiomyopathy, restrictive cardiomyopathy and non-compaction cardiomyopathy. All show substantial overlap, both clinically and genetically. For each cardiomyopathy subtype, multiple disease genes have been identified and mutations in several genes can cause different subtypes. In addition to hereditary cardiomyopathies, several non-genetic causes can result in cardiomyopathy, which can be very difficult to distinguish clinically.

All hereditary cardiomyopathies are characterized by incomplete penetrance, meaning that some mutation carriers will remain unaffected during their entire life, and variable expression, i.e. the type and severity of the disease can vary widely, even within families. The availability of diagnostic and management guidelines for different cardiomyopathies should improve the outcome of these patients and aid the clinician to identify the many rare presentations of cardiomyopathies, that all can be part of a wider spectrum of multi-organ, systemic or syndromic diseases.

Cardiomyopathies are defined by the European Society of Cardiology (ESC) Working Group on Myocardial and Pericardial Diseases as myocardial disorders in which the heart muscle is structurally and functionally abnormal in the absence of coronary artery disease, hypertension, valvular disease, and congenital heart disease sufficient to explain the observed myocardial abnormality [1]. In other words, a cardiomyopathy is disease of the heart muscle "itself," but the requirement "sufficient" is crucial since minor structural disease is acceptable. For instance, the finding of mild concomitant coronary artery disease does not preclude the diagnosis of DCM, and by the same token mild hypertension does not necessarily preclude the diagnosis of HCM. Conversely, patients with a genetic predisposition to a cardiomyopathy can more easily display myocardial disease caused by environmental factors such as hypertension, medication or intoxication, excessive exertion, etc., illustrating that a mutation is in essence merely a risk factor, but by itself often insufficient to cause the disease. Cardiomyopathy can be part of a systemic disease (e.g., amyloidosis), syndrome (e.g., Noonan syndrome), muscular disease (e.g., limb girdle muscular dystrophy), or an isolated cardiac disorder.

Like the definition of cardiomyopathy, the classification of subtypes of cardiomyopathy has always been a matter of hot debates, reflecting the complexity of the topic and the plethora of clinical pictures and entities as well the ever-

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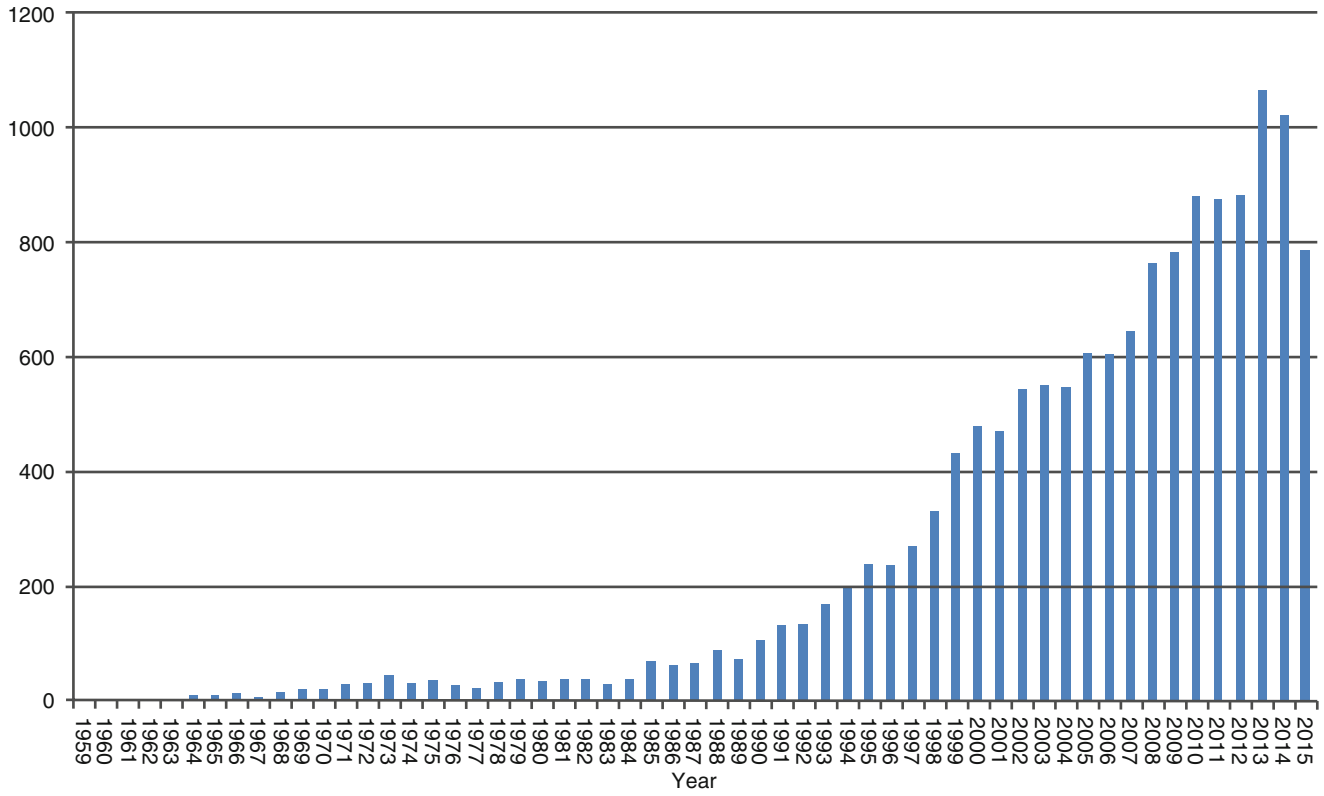

Fig. 3.1 The number of PubMed indexed papers on "Cardiomyopathy" and "Genetics," on the *y*-axis, is shown per year, on the *x*-axis. The drop in 2015 could be explained by electronic publications that have not been indexed yet

increasing knowledge on the underlying mechanisms of disease, including new insights acquired by molecular genetics. Indeed, the number of PubMed indexed papers on "Cardiomyopathy" and "Genetics" has greatly increased over the years (Fig. 3.1). Of note, the classification of cardiomyopathies by ESC differs substantially from the American Heart Association (AHA) classification. The AHA focuses more on the underlying mechanism and first distinguishes "Genetic" and "Acquired" forms of cardiomyopathies, and by the same token the Americans, for instance, also consider ion channel disorders to be forms of (genetic) cardiomyopathy [2]. In contrast, the vantage point of ESC Working Group on Myocardial and Pericardial Diseases is the clinical phenotype as presented to the attending physician, in particular the findings as obtained with echocardiography. Putting it simply: is there a phenotype of left ventricular hypertrophy versus dilatation and systolic dysfunction versus restriction versus arrhythmias and right ventricular involvement? In this chapter, we largely adhere to the European classification and we thus discuss five different subtypes of cardiomyopathy: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic cardiomyopathy (ACM), restrictive cardiomyopathy (RCM), and noncompaction cardiomyopathy (NCCM). Over the years however, we have learned that there is substantial overlap between these cardiomyopathies, both clinically and genetically. For example, severe HCM can progress to systolic dysfunction with dilatation of the left ventricle [3]. If not diagnosed before the dilatation started, this could be therefore misdiagnosed as DCM and not as HCM. Likewise, DCM is often accompanied by a certain measure of hypertrophy, and in particular, in the setting of limited dilatation of the left ventricle and/or limited systolic dysfunction, the distinction with HCM can be arbitrary. Also, the term "ACM" was coined to account for the fact arrhythmogenic right ventricular cardiomyopathy is often accompanied by some degree of left ventricular dilatation and/or dysfunction [4]. In fact, left dominant forms have been reported with features otherwise typical for arrhythmogenic right ventricular cardiomyopathy, including fibro-fatty replacement [5]. The next step in the European classification is the distinction between hereditary and nonhereditary forms of cardiomyopathy. For instance, cardiomyopathy with phenotype of hypertrophy ("HCM") can also be due to obesity or AL amyloidosis. Likewise, cardiomyopathy with phenotype of dilatation and systolic dysfunction ("DCM") can also be due to myocarditis, anthracycline cardiotoxicity, etc. However, for the purpose of this chapter, these forms of nonhereditary cardiomyopathy will not be discussed further. All hereditary cardiomyopathies are characterized by incomplete and age-related penetrance. The proportion of individu-

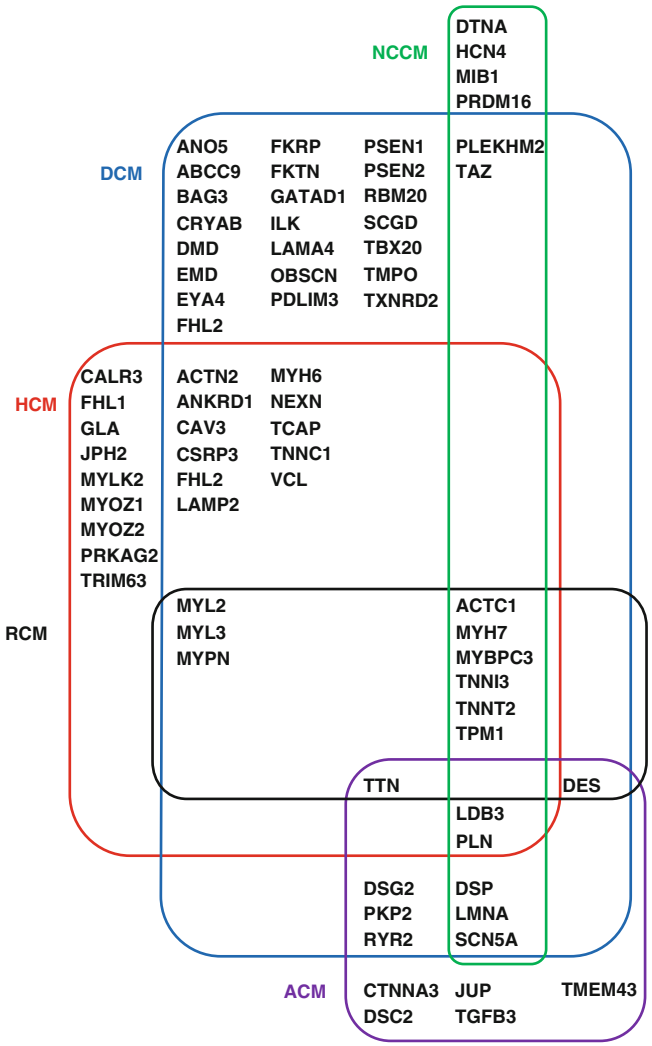

Fig. 3.2 Genetic heterogeneity and overlap in genes causing cardiomyopathies. This figure shows the genes underlying hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic cardiomyopathy (ACM), restrictive cardiomyopathy (RCM), and noncompaction cardiomyopathy (NCCM) (Modified from Van der Zwaag PA. Genetic and clinical characterisation of arrhythmogenic cardiomyopathy. Doctoral Thesis, 2012 [http://irs.ub.rug.nl/ppn/352159146])

als carrying a pathogenic mutation with associated clinical symptoms increases with age, but virtually never reaches 100 %. This incomplete penetrance implies that some mutation carriers will remain unaffected during their entire life. The onset of symptoms is usually after adolescence or in early adulthood, but children with severe forms of cardiomyopathy have been described. Some of these cases have been associated with multiple mutations [6, 7]. The type and severity of the disease and sometimes even cardiomyopathy subtype can vary widely, even within families, a concept that is called variable expression.

Hereditary cardiomyopathies are not only clinically variable, but the genetic background is also heterogeneous. For each cardiomyopathy, multiple disease genes have been identified, and mutations in several genes can cause different cardiomyopathy subtypes (Fig. 3.2) However, despite this large overlap and numerous important exceptions, some broad distinctions can be made. HCM is usually caused by mutations in genes encoding constituents of the sarcomere and the same holds true for NCCM, whereas ACM is usually due to mutations in genes encoding constituents of the desmosome. In contrast, DCM is indeed genetically highly heterogeneous (Fig. 3.3) [8]. The number of genes associated with cardiomyopathies continues to increase and will continue to do so, given the many genetically unsolved cases, including familial ones (Fig. 3.4).

Hypertrophic Cardiomyopathy

HCM is the most prevalent cardiomyopathy, affecting an estimated 1 in 500 individuals worldwide. Thereby it does not fulfill the criterion for a rare disease, which is defined as prevalence below 1:2000. HCM is the most common cause of sudden cardiac death (SCD) below the age of 35. Familial HCM has long been recognized, mostly with an autosomal dominant pattern of inheritance, and the first mutation was identified in 1990 by the group of Seidman et al. in the gene encoding the sarcomeric protein β-myosin heavy chain (*MYH7*) [9, 10]. Soon afterward, mutations in several other sarcomere genes were identified in HCM families, resulting in the concept of genetic HCM as a disease of the sarcomere.

The ESC HCM guideline provides clear tools for the diagnostic work-up, treatment, and risk stratification of patients with HCM. In adults, HCM is defined by a wall thickness ≥15 mm in one or more LV myocardial segments – as measured by any imaging technique [echocardiography, cardiac magnetic resonance imaging (CMR) or computed tomography (CT)] – that is not explained solely by loading conditions. Both genetic and nongenetic disorders can present with lesser degrees of wall thickening (13–14 mm), justifying further investigations and in assumed genetic cases also familial screening [11]. In sarcomeric HCM, the LV hypertrophy is usually asymmetric and most prominent at the interventricular septum. Concentric hypertrophy is more frequent in metabolic disorders and also suggestive of hypertension. In its workup, several causes should be excluded before considering HCM to be sarcomeric. Pathology studies show that typical myocardial disarray distinguishes sarcomeric HCM from secondary LV hypertrophy or, for example, Fabry's disease, where myocardial cells are expanded with vacuolar spaces. The myocardial fibrosis can be appreciated on CMR studies, showing late gadolinium enhancement and these abnormalities form a substrate for arrhythmias.

Several red flags should raise awareness that the observed ventricular hypertrophy in a patient may not be a sarcomeric

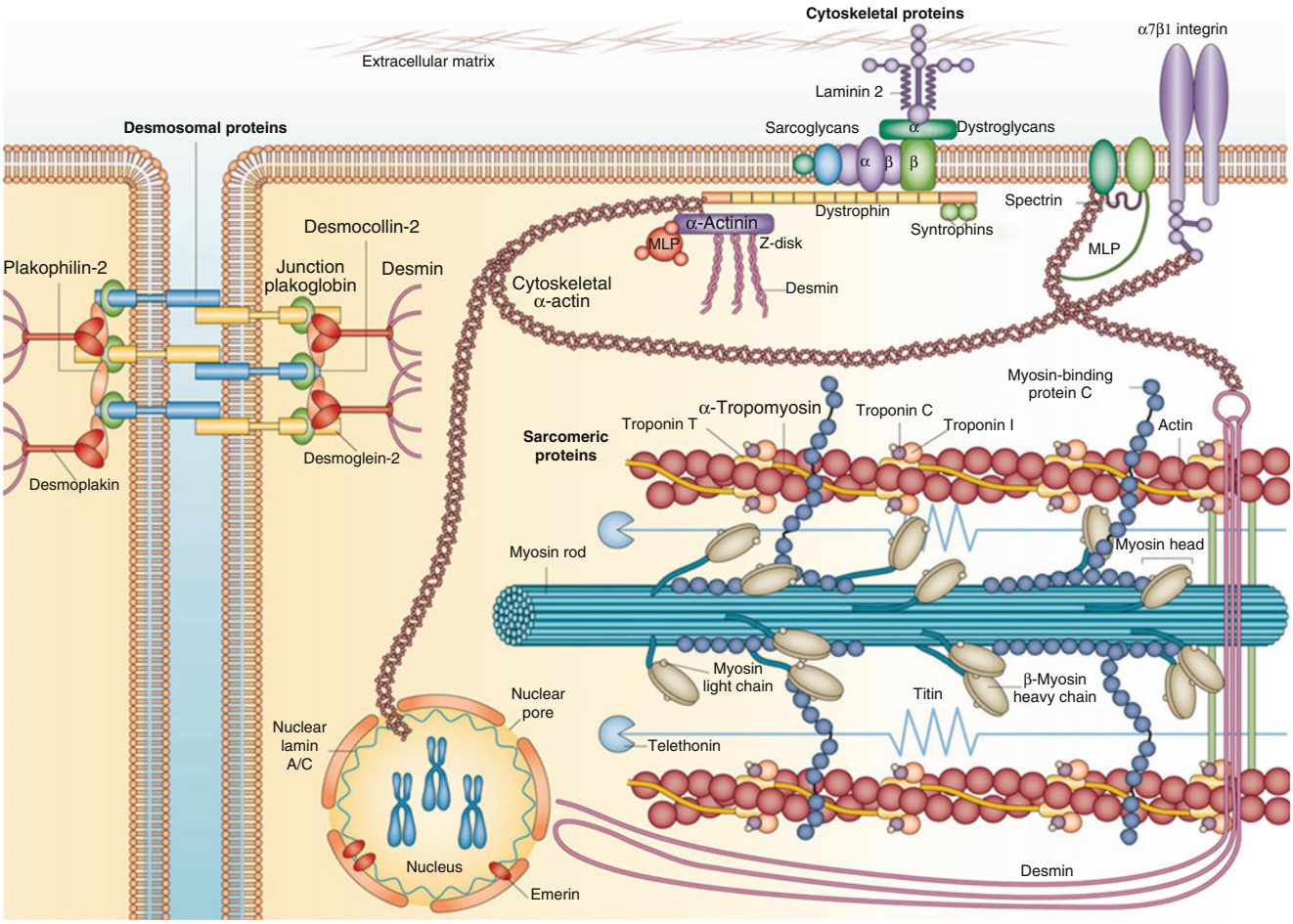

Fig. 3.3 The main proteins involved in cardiomyopathies. Indicated are the sarcomeric proteins, which form the contractile apparatus; the desmosomal proteins, which form the desmosome and connect one cell to another; the cytoskeletal proteins, which connect the extracellular matrix to the cells, and connect various transmembrane proteins to the sarcomere and the nucleus; and the nuclear envelope proteins. Mutations in the encoding genes lead to aberrant function of the respective proteins and to one of the cardiomyopathy subtypes. The sarcoplasmic reticulum, where phospholamban controls SERCA2a, a Ca^{2+} pump, is not shown in this figure [8]. [Obtained with permission from SpringerNature]

disorder and/or limited to the heart [12]. Childhood onset, involvement of other organs, a family history suggestive of an X-linked or autosomal recessive inheritance pattern, and concentric hypertrophy all warrant further investigation into metabolic, syndromic, and systemic diseases. In addition, several electrocardiographic abnormalities may suggest specific diagnoses other than sarcomeric HCM, for instance, a short PR-interval, AV-block, low voltages or very high voltages and extreme left ventricular hypertrophy (LVH). Metabolic causes include Pompe's disease, Danon disease, and Fabry's disease [13]. Noonan syndrome and LEOPARD syndrome are both RASopathies that can display HCM. RASopathies are a group of syndromes caused by mutations in genes that encode components or regulators of the Ras/mitogen-activated protein kinase (MAPK) pathway [14]. A systemic condition that can mimic sarcomeric HCM is cardiac amyloidosis, which by itself can be inherited as well due to mutations in the transthyretin (*TTR*) gene [15].

The ESC HCM guidelines recommend that patients undergo a standardized clinical evaluation to estimate the 5-year risk of SCD using the HCM Risk-SCD model. This model takes into account several variables, such as age, echocardiographic and Holter abnormalities and family history, to determine risk categories for recommending ICD implantation. This online calculator can be found at http://doc2do.com/hcm/webHCM.html

Of all cardiomyopathies, the yield of genetic testing is the highest in HCM. In a recent study by the Mayo Clinic, multivariate analysis identified a number of positive predictors for a positive genetic test in a cohort of >1000 unrelated HCM patients, tested for nine genes. Illustrative of the role of environmental factors is the fact that the presence of hypertension was identified as a negative predictor. A history of hypertension lowered the chance of a positive genetic test from 14 % without any positive or negative predictors to 6 %. The overall genetic yield in this cohort was 34 % [16].

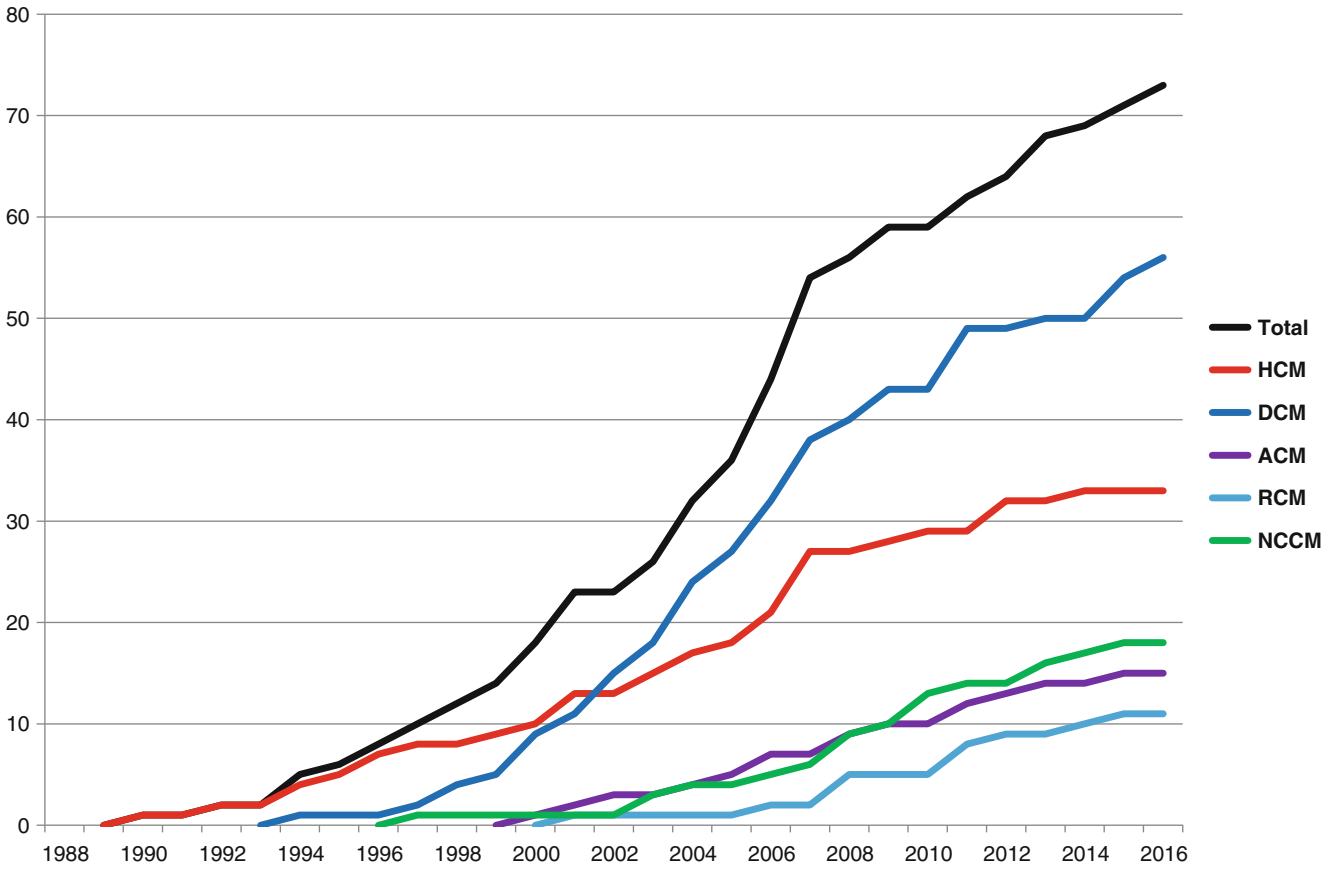

Fig. 3.4 Total number of genes associated with one or more cardiomyopathy subtype reported since 1990. The total number of associated cardiomyopathy genes is shown by the black line. Per cardiomyopathy subtype, the novel-associated genes are listed since the first publication in 1990 of a mutation in the *MYH7* gene in HCM by Geisterfer-Lowrance et al. [10]. Since many genes have been associated with multiple cardiomyopathy subtypes, the line of the total number of associated genes is not the sum of all lines of the cardiomyopathy subtypes

Dilated Cardiomyopathy

The criteria provided by Mestroni et al. are often used for the diagnosis of DCM in cardiogenetics [17]. DCM is thus characterized by systolic LV dysfunction (ejection fraction <45 % or fractional shortening <25 %) in combination with LV dilatation (LV end-diastolic volume/dimension >117 % (2SD + 5 %) of the predicted value corrected for age and BSA). As alluded to earlier, in case a patient presents with DCM, underlying (nongenetic) diseases should be excluded first. By and large, in about 50 % of cases such underlying disease/etiology can be identified [18]. In the remaining 50 % of cases ("idiopathic" DCM), about one third is likely attributable to a genetic defect, that is, a mutation in one of the genes implicated in DCM. DCM is genetically the most heterogeneous of all cardiomyopathies; at least 50 different genes have been implicated in DCM. The first were the same sarcomeric genes as identified in HCM, and it was shown that DCM-causing mutations had different molecular properties when compared to mutations that cause HCM, for example, calcium handling. The yield per gene has been low until the identification of titin (*TTN*) mutations as most prevalent cause of DCM in 2012 [19]. Titin was previously implicated in DCM, but as the largest gene, consisting of 363 exons, it was for a long time technically challenging and too costly to screen the gene for mutations. Like in the setting of HCM, there are red flags that may suggest a specific genetic defect [12]. DCM with AV-block is thus suggestive of a mutation in *LMNA* (the gene encoding lamin A/C, the major constituents of nuclear lamina) or in *DES* (the gene encoding the intermediate filament desmin) [20, 21], whereas DCM with extremely low voltage is a hallmark of the Dutch *PLN* p.Arg14del founder mutation [22]. In addition, the clinician should be aware of and look for extracardiac features like learning difficulties and deafness, which may point to mitochondrial disease or muscle weakness which is among others suggestive of a mutation in *LMNA* or *DES*.

As a general rule, therapeutic management of patients with DCM should be performed according to the ESC Guidelines for heart failure, including device therapy (CRT, ICD) [23]. However, in selected cases, knowledge of the underlying genetic defect should be considered in terms of

therapeutic choices, in particular timing of ICD implantation. Whereas ICD implantation should generally be considered in cases of left ventricular ejection fraction <35 %, patients with DCM due to a *LMNA*-mutation have a high risk for malignant arrhythmias even in the setting of relatively preserved left ventricular ejection fraction and they should receive an ICD when ejection fraction drops below 45 % when an additional risk factor is present, that is, nonmissense mutation, male sex, and the presence of nonsustained VTs [24]. The same holds true for patients with ACM due to the Dutch *PLN* p.Arg14del founder mutation: a left ventricular ejection fraction <45 % portends a poor prognosis regarding malignant arrhythmias and cardiac arrest/sudden death and these patients should also be considered for ICD implantation [25]. On the other hand, the available data suggest that DCM due to *TTN*-mutations may behave more benign, (Jansweijer et al. accepted) in terms of both malignant arrhythmias and progression to advanced heart failure. These examples show that genetics may have a considerable impact on clinical management in individual patients with DCM, in terms of not only establishing a specific diagnosis but also regarding therapeutic choices.

Arrhythmogenic Cardiomyopathy

ACM was first described in 1982 as "right ventricular dysplasia" by Marcus et al. [26]. These patients were characterized by life-threatening ventricular arrhythmias, originating from the right ventricle (RV). The cause of the disease was believed to be a developmental defect of the myocardial tissue of the RV. However, the finding that the RV myocardium is subject to cell death and is subsequently replaced by fibrous and fatty tissue, interfering with electrical conduction of the heart and results in arrhythmias, later led to the concept of arrhythmogenic right ventricular cardiomyopathy (ARVC). In addition to this classic form of ARVC, biventricular and left-dominant forms have been recognized, and therefore nowadays, the term "ACM" is increasingly used [27, 28]. The estimated prevalence in the general population ranges from 1 in 1000 to 1 in 5000, and men are more frequently affected than women [29].

The current diagnostic criteria were published in 2010 as the modification of the original task force criteria in 1994, comprising six different categories, including structural and histological findings, depolarization and repolarization abnormalities, arrhythmias, and family history [30, 31]. ACM is considered a disease of the cardiac desmosome (Fig. 3.3) following the identification of mutations in two genes encoding desmosomal proteins, plakoglobin (*JUP*) and desmoplakin (*DSP*), in syndromic forms of ACM called Naxos disease and Carvajal disease, respectively [32, 33]. Plakophilin 2 (PKP2) is the most prevalent mutated gene in ACM. Mutations in several nondesmosomal genes, however, have also been identified, including PLN and TMEM43 [22, 34]. ACM is a nice example of the added value of genetics in terms of providing insight into the pathophysiology.

In 2015, an international task force published a consensus statement on the treatment and management of ACM. These recommendations deal with electrophysiological studies, lifestyle changes such as restriction from competitive sports, pharmacotherapy, catheter ablation, and ICD implantation. These recommendations are useful for all health care professionals dealing with ACM, especially those who are not very familiar with this rare disease [35].

Restrictive Cardiomyopathy

RCM is characterized by impaired filling of the ventricles in the presence of normal wall thickness and normal systolic function. RCM can be part of systemic, inflammatory or storage diseases, but it also occurs in isolated form and is often associated with sarcomeric mutations (Fig. 3.2) [36]. RCM is the least common cardiomyopathy subtype and can be observed in a variety of cardiac or multiorgan diseases such as Löffler's endocarditis, amyloidosis, sarcoidosis, hemochromatosis, and Fabry's disease [37]. Furthermore, RCM is found in patients who have undergone radiotherapy, for instance, for Hodgkin's disease [38].

As for other sarcomeric cardiomyopathies, different cardiomyopathy subtypes have been observed within families, for RCM mostly in combination with HCM [39]. There is no consensus regarding diagnostic criteria for RCM, but in a case of heart failure with abnormal diastolic function but preserved contractility, and no signs of other cardiomyopathy subtypes such as dilatation or hypertrophy, the diagnosis should be considered. Of the cardiomyopathies, RCM has the worst prognosis, especially in children, as a cardiac transplant is often necessitated within only a few years after diagnosis.

The relatively common scenario of a severely affected child with RCM of apparent healthy parents is usually attributable to the fact that the sarcomeric mutation occurred *de novo*. Mutations in the gene encoding the sarcomeric contractile protein troponin I (*TNNI3)* appear to be the most frequent [40, 41]. As in DCM, the presence of a conduction defect, especially AV-block, in patients with RCM could unmask a *DES* mutation [42].

Non-compaction cardiomyopathy

NCCM, also referred to as left ventricular noncompaction (LVNC), is an enigmatic disorder. Although it is a rare form of cardiomyopathy, it is relatively often diagnosed as a

chance finding, in particular during echocardiography, performed for another reason. The wall of the left ventricle is thus found be "noncompacted," that is, in addition to the normal compacted wall (outer layer) there is an inner layer of meshy myocardium with trabeculations and recesses. According to the Jenni criteria, the ratio of the thickness of the noncompacted layer and the compacted layer should exceed two (end-systolic) at parasternal short-axis view on echocardiography for NCCM to be diagnosed [43]. However, this echocardiographic definition is highly arbitrary since there is no gold standard for NCCM, which is also exemplified by the fact that other criteria are used when CMR is performed. In terms of the etiology, there also is much controversy. NCCM can be part of a syndrome (e.g., Barth syndrome), a congenital heart defect (e.g., Epstein's anomaly), or it can present as an isolated hereditary cardiomyopathy. But even in the latter case, the picture is not always clear and there is often overlap with DCM and HCM. Moreover, in families with, for instance, HCM, some individuals may present with NCCM. This close association is supported by the fact the most commonly implicated genes in HCM and NCCM are the same, that is, *MYH7* and *MYBPC3* [44]. Another important observation is that individual patients may progress from one form of cardiomyopathy to another, in particular from NCCM to DCM. NCCM is thus a striking example of the clinical and genetic heterogeneity of cardiomyopathies and taking all this into consideration some investigators even question whether NCCM should be considered a separate entity. However, for the clinician taking care of the patients with alleged NCCM some points should be kept in mind. Due to the recesses, there is a chance for thrombus formation and embolic events and anticoagulation therapy should be considered, in particular in case of concomitant left ventricular dysfunction. In addition, some patients with NCCM may develop heart failure and there is also a propensity for malignant arrhythmias, and as a general rule, the guidelines for heart failure (including device therapy) should be applied. As such, NCCM is not always a benign chance finding and it may have serious consequences for the patient, and it is reason for family screening.

Given the complexity of cardiomyopathies in terms of the phenotype, extracardiac (organ) involvement, genetics, and etiology, a group of experts have recently proposed a new classification, the MOGE(S) classification [45]. They identified five attributes:

M. The "Morphofunctional" notation provides a descriptive diagnosis of the phenotype: for instance, DCM (M_D), HCM (M_H), etc.
O. "Organ involvement" is notated as heart only (O_H) or involvement of other organs for instance, kidney (O_K), skeletal muscle (O_M), etc.
G. "Genetics" provides information on the mode of inheritance, for instance, autosomal dominant (G_{AD}), X-linked (G_{XL}), etc.
E. "Etiology" adds to the description of the underlying cause, including the gene/mutation, for instance, in case of HCM ($E_{G,MYH7}$) or DCM due to myocarditis secondary to Epstein–Barr virus infection ($E_{V,EBV}$)
S. "Heart failure Stage" pertains to AHA/ACC stage of heart failure (A to D) and the NHYA functional class (I to IV), for instance, $S_{A\text{-}I}$

In the article, the authors provide numerous examples of how this classification works. Just to name one: in a male with Fabry's disease with a phenotype of "HCM," with involvement of kidney, due to a mutation in GLA, the gene encoding galactosidase-α, the MOGE(S) annotation would be: $M_H\ O_k\ G_{X\text{-}L}\ E_{G\text{-}GLA\ +}$ For further details, the reader is referred to this very interesting paper. The practical implications of the MOGES-classification have already been shown in several subsequent papers, including a study by Hazebroek et al. [46]. These authors have applied the MOGE(S) classification in a group of patients with DCM and using the classification they convincingly showed the importance of gene–environment interaction in terms of clinical outcome. These are big steps forward in unraveling the many intricacies of cardiomyopathies, but much work is still to be done.

Take Home Message

- The five main cardiomyopathy subtypes, HCM, DCM, ACM, RCM, and NCCM, show substantial overlap, both clinically and genetically. For each cardiomyopathy subtype, multiple disease genes have been identified and mutations in several genes can cause different subtypes.
- All hereditary cardiomyopathies are characterized by incomplete penetrance, meaning that some mutation carriers will remain unaffected during their entire life, and variable expression, that is, the type and severity of the disease can vary widely, even within families. The availability of diagnostic and management guidelines for different cardiomyopathies should improve the outcome of these patients and aid the clinician to identify the many rare presentations of cardiomyopathies, that all can be part of a wider spectrum of multiorgan, systemic or syndromic diseases.

References

1. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2008;29:270–6.
2. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure

and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation. 2006;113:1807–16.
3. ten Cate FJ, Roelandt J. Progression to left ventricular dilatation in patients with hypertrophic obstructive cardiomyopathy. Am Heart J. 1979;97:762–5.
4. Sen-Chowdhry S, Morgan RD, Chambers JC, McKenna WJ. Arrhythmogenic cardiomyopathy: etiology, diagnosis, and treatment. Annu Rev Med. 2010;61:233–53.
5. Sen-Chowdhry S, Syrris P, Prasad SK, Hughes SE, Merrifield R, Ward D, et al. Left-dominant arrhythmogenic cardiomyopathy an underrecognized clinical entity. J Am Coll Cardiol. 2008;52:2175–87.
6. Jeschke B, Uhl K, Weist B, Schroder D, Meitinger T, Dohlemann C, et al. A high risk phenotype of hypertrophic cardiomyopathy associated with a compound genotype of two mutated beta-myosin heavy chain genes. Hum Genet. 1998;102:299–304.
7. Lekanne Deprez RH, Muurling-Vlietman JJ, Hruda J, Baars MJ, Wijnaendts LC, Stolte-Dijkstra I, et al. Two cases of severe neonatal hypertrophic cardiomyopathy caused by compound heterozygous mutations in the MYBPC3 gene. J Med Genet. 2006;43:829–32.
8. Wilde AA, Behr ER. Genetic testing for inherited cardiac disease. Nat Rev Cardiol. 2013;10:571–83.
9. Jarcho JA, McKenna W, Pare JA, Solomon SD, Holcombe RF, Dickie S, et al. Mapping a gene for familial hypertrophic cardiomyopathy to chromosome 14q1. N Engl J Med. 1989;321:1372–8.
10. Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE, et al. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. Cell. 1990;62:999–1006.
11. Authors/Task Force members, Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, et al. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: the Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). Eur Heart J 2014;35:2733-2779.
12. Rapezzi C, Arbustini E, Caforio AL, Charron P, Gimeno-Blanes J, Helio T, et al. Diagnostic work-up in cardiomyopathies: bridging the gap between clinical phenotypes and final diagnosis. A position statement from the ESC Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2013;34:1448–58.
13. Wicks EC, Elliott PM. Genetics and metabolic cardiomyopathies. Herz. 2012;37:598–610.
14. Gelb BD, Roberts AE, Tartaglia M. Cardiomyopathies in Noonan syndrome and the other RASopathies. Prog Pediatr Cardiol. 2015;39:13–9.
15. Ruberg FL, Berk JL. Transthyretin (TTR) cardiac amyloidosis. Circulation. 2012;126:1286–300.
16. Bos JM, Will ML, Gersh BJ, Kruisselbrink TM, Ommen SR, Ackerman MJ. Characterization of a phenotype-based genetic test prediction score for unrelated patients with hypertrophic cardiomyopathy. Mayo Clin Proc. 2014;89:727–37.
17. Mestroni L, Rocco C, Gregori D, Sinagra G, Di Lenarda A, Miocic S, et al. Familial dilated cardiomyopathy: evidence for genetic and phenotypic heterogeneity. Heart Muscle Disease Study Group. J Am Coll Cardiol. 1999;34:181–90.
18. Felker GM, Thompson RE, Hare JM, Hruban RH, Clemetson DE, Howard DL, et al. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. N Engl J Med. 2000;342:1077–84.
19. Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, Christodoulou D, et al. Truncations of titin causing dilated cardiomyopathy. N Engl J Med. 2012;366:619–28.
20. Arbustini E, Pilotto A, Repetto A, Grasso M, Negri A, Diegoli M, et al. Autosomal dominant dilated cardiomyopathy with atrioventricular block: a lamin A/C defect-related disease. J Am Coll Cardiol. 2002;39:981–90.
21. Taylor MR, Slavov D, Ku L, Di Lenarda A, Sinagra G, Carniel E, et al. Prevalence of desmin mutations in dilated cardiomyopathy. Circulation. 2007;115:1244–51.
22. Van der Zwaag PA, van Rijsingen IA, Asimaki A, Jongbloed JD, van Veldhuisen DJ, Wiesfeld AC, et al. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. Eur J Heart Fail. 2012;14:1199–207.
23. McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Bohm M, Dickstein K, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur Heart J. 2012;33:1787–847.
24. van Rijsingen IA, Arbustini E, Elliott PM, Mogensen J, Hermans-van Ast JF, van der Kooi AJ, et al. Risk factors for malignant ventricular arrhythmias in lamin a/c mutation carriers a European cohort study. J Am Coll Cardiol. 2012;59:493–500.
25. van Rijsingen IA, van der Zwaag PA, Groeneweg JA, Nannenberg EA, Jongbloed JD, Zwinderman AH, et al. Outcome in phospholamban R14del carriers: results of a large multicentre cohort study. Circ Cardiovasc Genet. 2014;7:455–65.
26. Marcus FI, Fontaine GH, Guiraudon G, Frank R, Laurenceau JL, Malergue C, et al. Right ventricular dysplasia: a report of 24 adult cases. Circulation. 1982;65:384–98.
27. Corrado D, Basso C, Thiene G, McKenna WJ, Davies MJ, Fontaliran F, et al. Spectrum of clinicopathologic manifestations of arrhythmogenic right ventricular cardiomyopathy/dysplasia: a multicenter study. J Am Coll Cardiol. 1997;30:1512–20.
28. Sen-Chowdhry S, Syrris P, Ward D, Asimaki A, Sevdalis E, McKenna WJ. Clinical and genetic characterization of families with arrhythmogenic right ventricular dysplasia/cardiomyopathy provides novel insights into patterns of disease expression. Circulation. 2007;115:1710–20.
29. Basso C, Corrado D, Marcus FI, Nava A, Thiene G. Arrhythmogenic right ventricular cardiomyopathy. Lancet. 2009;373:1289–300.
30. McKenna WJ, Thiene G, Nava A, Fontaliran F, Blomstrom-Lundqvist C, Fontaine G, et al. Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Task Force of the Working Group Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology. Br Heart J. 1994;71:215–8.
31. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke DA, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the Task Force Criteria. Eur Heart J. 2010;31:806–14.
32. McKoy G, Protonotarios N, Crosby A, Tsatsopoulou A, Anastasakis A, Coonar A, et al. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). Lancet. 2000;355:2119–24.
33. Norgett EE, Hatsell SJ, Carvajal-Huerta L, Cabezas JC, Common J, Purkis PE, et al. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. Hum Mol Genet. 2000;9:2761–6.
34. Merner ND, Hodgkinson KA, Haywood AF, Connors S, French VM, Drenckhahn JD, et al. Arrhythmogenic right ventricular cardiomyopathy type 5 is a fully penetrant, lethal arrhythmic disorder caused by a missense mutation in the TMEM43 gene. Am J Hum Genet. 2008;82:809–21.
35. Corrado D, Wichter T, Link MS, Hauer R, Marchlinski F, Anastasakis A, et al. Treatment of arrhythmogenic right ventricular cardiomyopathy/dysplasia: an international task force consensus statement. Eur Heart J. 2015;36:3227–37.

36. Mogensen J, Arbustini E. Restrictive cardiomyopathy. Curr Opin Cardiol. 2009;24:214–20.
37. Kushwaha SS, Fallon JT, Fuster V. Restrictive cardiomyopathy. N Engl J Med. 1997;336:267–76.
38. Adams MJ, Lipsitz SR, Colan SD, Tarbell NJ, Treves ST, Diller L, et al. Cardiovascular status in long-term survivors of Hodgkin's disease treated with chest radiotherapy. J Clin Oncol. 2004;22:3139–48.
39. Mogensen J, Kubo T, Duque M, Uribe W, Shaw A, Murphy R, et al. Idiopathic restrictive cardiomyopathy is part of the clinical expression of cardiac troponin I mutations. J Clin Invest. 2003;111:209–16.
40. Kaski JP, Syrris P, Burch M, Tome-Esteban MT, Fenton M, Christiansen M, et al. Idiopathic restrictive cardiomyopathy in children is caused by mutations in cardiac sarcomere protein genes. Heart. 2008;94:1478–84.
41. Mogensen J, Hey T, Lambrecht S. A systematic review of phenotypic features associated with cardiac troponin I mutations in hereditary cardiomyopathies. Can J Cardiol. 2015;31:1377–85.
42. Arbustini E, Pasotti M, Pilotto A, Pellegrini C, Grasso M, Previtali S, et al. Desmin accumulation restrictive cardiomyopathy and atrioventricular block associated with desmin gene defects. Eur J Heart Fail. 2006;8:477–83.
43. Jenni R, Oechslin E, Schneider J, Attenhofer Jost C, Kaufmann PA. Echocardiographic and pathoanatomical characteristics of isolated left ventricular non-compaction: a step towards classification as a distinct cardiomyopathy. Heart. 2001;86:666–71.
44. Hoedemaekers YM, Caliskan K, Michels M, Frohn-Mulder I, van der Smagt JJ, Phefferkorn JE, et al. The importance of genetic counseling, DNA diagnostics, and cardiologic family screening in left ventricular noncompaction cardiomyopathy. Circ Cardiovasc Genet. 2010;3:232–9.
45. Arbustini E, Narula N, Dec GW, Reddy KS, Greenberg B, Kushwaha S, et al. The MOGE(S) classification for a phenotype-genotype nomenclature of cardiomyopathy: endorsed by the World Heart Federation. J Am Coll Cardiol. 2013;62:2046–72.
46. Hazebroek MR, Moors S, Dennert R, van den Wijngaard A, Krapels I, Hoos M, et al. Prognostic relevance of gene-environment interactions in patients with dilated cardiomyopathy: applying the MOGE(S) classification. J Am Coll Cardiol. 2015;66:1313–23.

Hypertrophic Cardiomyopathy

4

Imke Christiaans and Perry M. Elliott

Abstract

Hypertrophic cardiomyopathy (HCM) is the most common monogenetic heart disease affecting over 1 in 500 people worldwide. The hallmark of the disease is left ventricular hypertrophy (LVH) in the absence of abnormal loading conditions that may cause hypertrophy. The disease can present at any age and is highly variable in clinical expression. Patients can remain asymptomatic throughout their life, but HCM is also associated with premature mortality from heart failure, stroke, and sudden cardiac death (SCD). Therapy is mainly directed toward relief of symptoms caused by heart failure and left ventricular outflow tract obstruction. Clinical risk stratification can identify patients at high risk for SCD and is used to select patients for preventative therapy with implantable cardioverter defibrillators.

Because of the hereditary nature of the disease, first degree relatives are advised to undergo periodic cardiac evaluation for the presence of LVH. In about half of all patients, a disease causing mutation can be detected in one of the genes encoding for sarcomeric proteins. Detection of a disease causing mutation allows predictive genetic testing in relatives, and facilitates identification of relatives at risk of developing HCM and associated disease-related complications. Although there is no evidence of a clear benefit of early pharmacological treatment in asymptomatic relatives carrying a mutation, they can still benefit from primary prevention strategies.

Introduction

Hypertrophic cardiomyopathy (HCM) has been described in medical literature for several centuries. The resurgence of anatomy in the Renaissance allowed further study of disease and dissections of victims of sudden death revealed bulky hearts [1]. Nowadays, HCM is still a major cause of sudden cardiac death (SCD) in the young, and the most common monogenetic heart disease affecting at least one in 500 persons worldwide [2–10]. This chapter discusses the epidemiology, diagnosis, pathophysiology, and therapy of HCM and reviews topics specific to cardiogenetic diseases. It is intended to be of help for all involved in the care for patients and families with HCM.

Diagnosis

The clinical diagnosis of HCM rests on the detection of a hypertrophied left ventricle. Hypertrophy is defined as a left ventricular wall thickness ≥15 mm in adults and >2 SD than the predicted mean (z-score >2) in children measured using any imaging technique and in the absence of cardiac or systemic diseases characterized by increased ventricular afterload such as aortic valve stenosis and systemic arterial hypertension [11–16]. Clinical diagnostic criteria in relatives of patients with unequivocal disease are less stringent; the

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Table 4.1 Nonsarcomeric causes of HCM

Categories	Examples
Metabolic disorders	Anderson-Fabry disease, Danon disease, PRKAG2 gene mutation-related HCM, Pompe disease, carnitine disorders
Mitochondrial disorders	MELAS, MERFF
Neuromuscular diseases	Friedreich's ataxia, FHL1 gene mutation-related HCM, DES gene mutation-related HCM
Malformation syndromes	Noonan syndrome, LEOPARD syndrome, Costello syndrome, CFC syndrome
Infiltrative diseases	Amyloidosis
Endocrine diseases	Pheochromocytoma, acromegaly, maternal diabetes
Other	Chronic use of certain drugs (anabolic steroids, tacrolimus, hydroxychloroquine)

diagnosis can be made if left ventricle wall thickness is ≥13 mm [16, 17]. Minor abnormalities including diastolic dysfunction, mitral leaflet elongation, myocardial clefts, and incomplete systolic anterior motion (SAM) of the mitral valve can also be seen as an early or mild expression of the disease. The diagnosis of HCM can be difficult when there is coexisting pathology such as hypertension, or in the elderly and obese, and in elite athletes.

Left ventricular hypertrophy (LVH) can be caused by many genetic and nongenetic disorders (Table 4.1). Relatively common metabolic diseases include Anderson-Fabry disease, which is X-linked [18] and mitochondrial disorders [19]. Although neuromuscular disorders are more often associated with dilated or restrictive cardiomyopathy, LVH can be a part of the disease spectrum in a minority of diseases [20]. LVH is also common in syndromic disorders with multiple congenital abnormalities including Noonan and LEOPARD syndrome [21]. Amyloidosis – both genetic and nongenetic forms – can cause thickening of the left ventricle in older patients with or without noncardiac symptoms [22, 23].

Disease Presentation: Histopathology, the Cardiac Sarcomere, Pathophysiology, and Symptoms

Histopathology

The British pathologist Robert Donald Teare made the first modern pathological description of HCM in 1958 [24]. Although bulky hearts and hypertrophy in sudden death victims had been described centuries earlier [1] and subaortic stenosis (a former name for HCM) was reported in the nineteenth century, [25, 26] Teare was the first to describe the asymmetrical pattern of LVH and the characteristic disordered arrangement of muscle fibers at microscopic examination, now known as myocardial disarray (Fig. 4.1) [24]. This cellular disarray is also associated with disordered arrangement of the myofilaments within cells. Several studies have shown that myocardial disarray is not necessarily confined to the thickened parts of the left ventricle [27]. Other prominent histopathological features include myocardial fibrosis and abnormalities in the intramyocardial small vessels in which vessels show thickening of the media and a decrease in luminal size [28].

The Cardiac Sarcomere and Pathophysiology

The sarcomere is the fundamental contractile unit of the myocardium. It is composed of the thick (myosin) and thin (actin) myofilaments and proteins involved in the cytoarchitecture of the sarcomere located at the Z-disc and M-band (Fig. 4.2). Contraction is initiated by electrical depolarization of the cardiomyocyte which, through the opening of voltage gated calcium channels and the sarcolemmal ryanodine receptor, causes a rise in cytosolic calcium. Calcium binds to troponin C, inducing an allosteric conformational change in troponin I and T that is transmitted to tropomyosin, causing a transition from a "blocked" to a "closed" state, which then exposes the myosin binding sites of actin and allow cross-bridges to occur ("open" state). The myosin heavy chain head then interacts with the exposed actin binding sites, which triggers the release of ADP and inorganic phosphate from its nucleotide binding pocket. This occurs simultaneously with the power stroke, resulting in force development and shortening of the sarcomere. Following this, ATP binds to the nucleotide binding pocket of the myosin heavy chain head, which detaches from actin and myosin then hydrolyzes ATP into ADP and inorganic phosphate again, re-starting the cycle [29]. Calcium is not only a key element regulating both cardiac contraction and relaxation, but it also plays an important role in the early pathogenesis of HCM. It is hypothesized that many sarcomeric protein gene mutations cause calcium to accumulate within the sarcomere which disrupts normal contraction and relaxation of the sarcomere. This leads to a reduction of calcium re-uptake and eventually to reduced stores in the sarcoplasmic reticulum and increased calcium sensitivity. Myocytes in mice with HCM also display an inefficient use of ATP. This in combination with the increased calcium sensitivity triggers a remodeling process resulting in hypertrophy of cardiomyocytes. The increased mass of cardiomyocytes and inefficient use of ATP lead to an increased energy demand. When this energy demand cannot be met, ischemia can result in premature myocyte death and replacement fibrosis [29, 30].

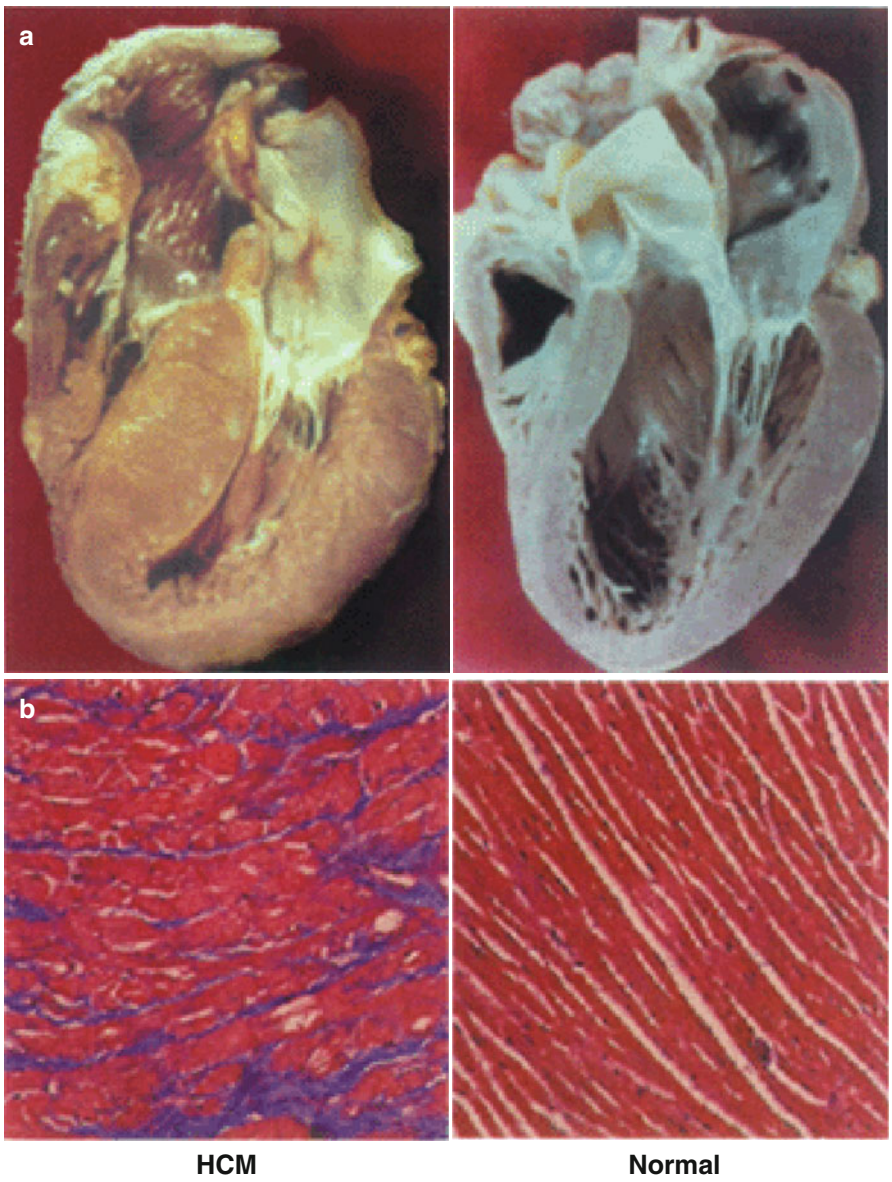

Fig. 4.1 (**a**) Normal heart and heart with hypertrophic cardiomyopathy. (**b**) Heart muscle on microscopy with normal fiber pattern and myocardial disarray in a heart with hypertrophic cardiomyopathy (Figure derived from: Hypertrophic cardiomyopathy: From gene defect to clinical disease. Chung, MW, Tsoutsman, T, Semsarian, C. Cell Research (2003) 13, 9–20)

LVH can appear at virtually any age, but studies suggest that most patients develop hypertrophy in adolescence or by the third decade of life [31–34]. Extreme hypertrophy (arbitrarily defined as a maximum left ventricular wall thickness of ≥30 mm) is uncommon in older patients. This might be explained by a higher rate of premature cardiac death in younger patients with severe LVH, meaning that patients with extreme LVH are underrepresented in the subgroup of older patients (the so-called healthy survivors phenomenon). Alternatively, progressive wall thinning may occur in older patients, 5–10 % of whom progress to a so-called end-stage which is characterized by systolic dysfunction, dilation of the left ventricle, and wall thinning [32, 35]. Patients with this end-stage hypokinetic evolution are younger at first evaluation, and more often have a family history of HCM or SCD than patients who do not develop these features [36].

The most common location for LVH is the anterior part of the interventricular septum, giving rise to an asymmetrical pattern of hypertrophy. This is typically associated with reverse curvature of the interventricular septum with a crescent-shaped LV cavity. In some older patients, a more localized form associated with a sigmoidal septal shape can be observed, which is less frequently caused by mutations in cardiac sarcomere protein genes [37]. Other patterns of LVH, for example, concentric and apical hypertrophy are less common (Fig. 4.3).

Symptoms

The clinical course of HCM is highly variable, but most people with HCM are asymptomatic and probably have a normal

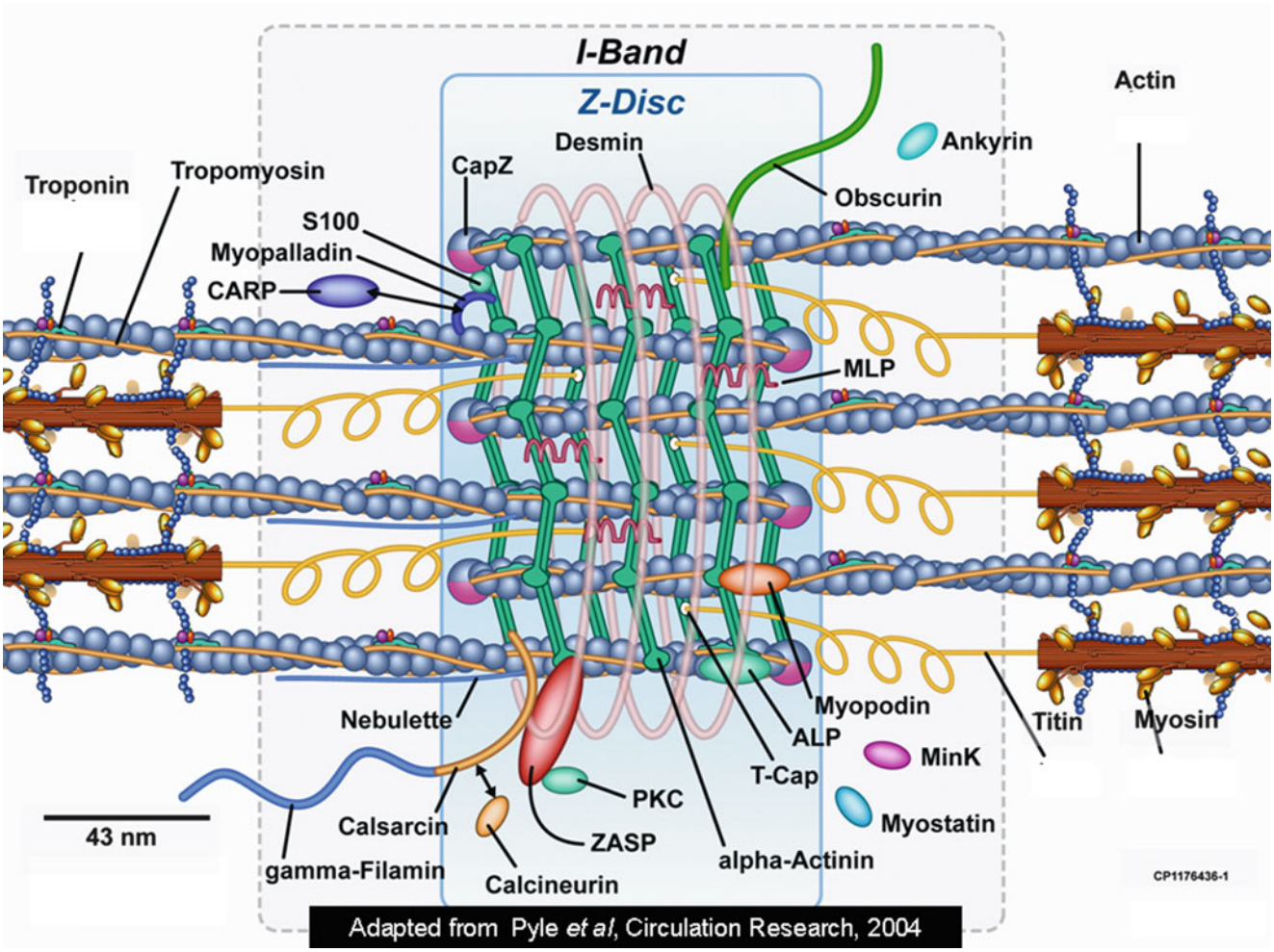

Fig. 4.2 Main sarcomeric proteins (Figure derived from: Familial hypertrophic cardiomyopathy: Basic concepts and future molecular diagnostics. Rodrigues JE, McCudden CR, Willis MS. Clin Biochem 2009; 42: 755–765)

lifespan. In many individuals, disease is detected incidentally because of a heart murmur, an abnormal ECG or during screening of a family in which a relative has HCM. Other patients present with symptoms including fatigue, dyspnea, chest pain, palpitations, and syncope. Rarely, SCD and embolic stroke are the first presenting symptoms. In infants, tachypnea, poor feeding, sweating, and failure to thrive can be presenting symptoms.

Exertional dyspnea is primarily caused by diastolic dysfunction or dynamic left ventricular outflow tract obstruction [38, 39]. Symptoms may become more disabling in the presence of atrial arrhythmia which occurs in more than 20 % of patients and is associated with advanced age, heart failure symptoms and an increased left atrial size [40]. Patients with atrial fibrillation have an increased risk of heart failure-related death and stroke [40–43].

Dynamic left ventricular outflow tract obstruction (LVOTO) – defined as a peak pressure gradient of 30 mmHg or more identified by Doppler echocardiography – is present in approximately 20–30 % of patients with HCM under resting conditions. It may also be provoked by physical maneuvers that reduce left ventricular filling (standing from a squatting position or Valsalva maneuver) or increase myocardial contractility. The cause of LVOTO is SAM of the mitral valve leaflets (Fig. 4.4) [37, 41–44]. The presence of LVOTO is a strong predictor of disease progression and death due to heart failure and stroke [42].

Clinical Management

Management in patients with HCM is directed toward control of symptoms and prevention of disease-related complications (see section, "Treatment of Symptoms and Prevention of Complications"), risk stratification for SCD (see section, "Risk Stratification for SCD") and the screening of relatives (see section, "Screening of Relatives: Genetic Counseling and Testing").

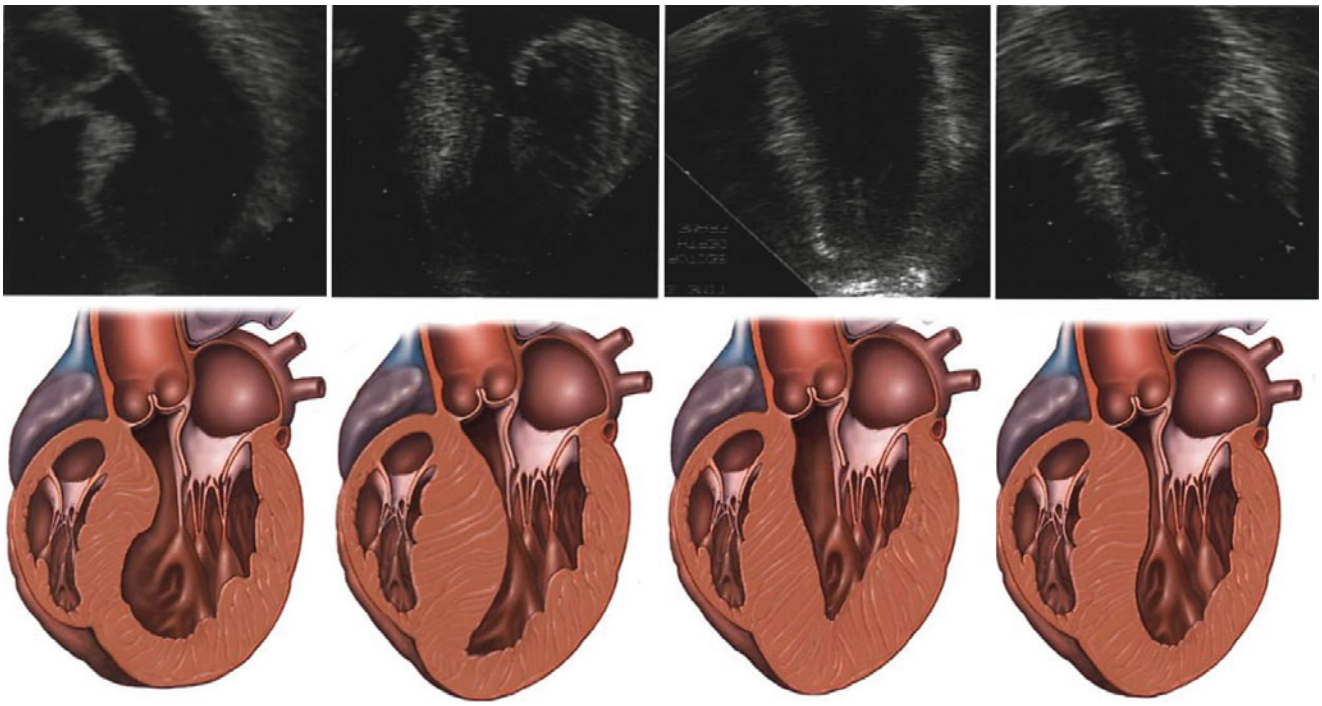

Fig. 4.3 Hypertrophic cardiomyopathy septal morphological subtypes based on standard echocardiography long-axis views taken at end diastole (*top*). From *left* to *right*, sigmoid, reverse curve, apical, and neutral subtypes (Figure adapted from: Echocardiography-guided genetic testing in hypertrophic cardiomyopathy: septal morphological features predict the presence of myofilament mutations. J. Binder, S.R. Ommen, B.J. Gersh, S.L. Van Driest, A.J. Tajik, R.A. Nishimura and M.J. Ackerman, *Mayo Clin. Proc.* **81** (2006), 459–467)

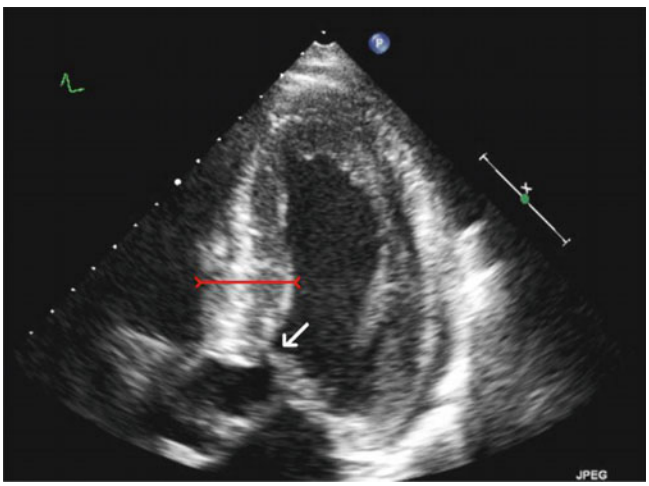

Fig. 4.4 Apical four chamber echocardiography in a HCM patient. *Red* arrow indicates the hypertrophic interventricular septum (>25 mm) and the *white* arrow indicates systolic anterior motion of a mitral valve leaflet in the *left* ventricular outflow tract

Treatment of Symptoms and Prevention of Complications

Symptoms of dyspnea, angina, syncope, and fatigue are appraised and treated appropriately [16, 38]. However, prophylactic pharmacological treatment in asymptomatic patients has not been proved to be effective in preventing progression of the disease. Negative inotropic agents like beta-blockers and calcium antagonists can relieve symptoms in patients with and without left ventricular outflow tract obstruction. Beta-blockers decrease the heart rate thereby prolonging diastole and increasing passive ventricular filling. They also decrease left ventricular contractility and myocardial oxygen demand ameliorating microvascular angina. Verapamil also has favorable effects on symptoms by improving ventricular relaxation and filling. In patients with symptoms caused by LVOTO, disopyramide, a negative inotropic and type I-A antiarrhythmic drug can be used to decrease systolic anterior movement of the mitral valve and mitral regurgitation. In end-stage HCM, conventional anti-heart failure pharmacotherapy (ACE inhibitors, angiotensin-II receptor blockers, diuretics, digitalis, beta-blockers or spironolactone) can be used to alleviate symptoms from systolic failure [16, 38].

There are several invasive options for patients with symptomatic LVOTO refractory to pharmacological treatment. In experienced centers, ventricular septal myectomy in which a trough of muscle is removed from the interventricular septum via an aortic incision has low operative mortality and provides long lasting improvement in symptoms [45, 46]. An alternative for some patients with symptomatic LVOTO is percutaneous alcohol septal ablation in which

alcohol is selectively injected in the septal perforator branch of the left anterior descending coronary artery [47]. Both procedures can be effective but the reduction in outflow gradient and symptom relief is, on average, better following surgery [46, 48].

Indirect evidence suggests an association between exercise and SCD. Intense physical activity (e.g., sprinting) or systematic isometric exercise (e.g., heavy lifting) should be discouraged, and all patients should be advised to avoid intense competitive sports and professional athletic careers [16].

Risk Stratification for SCD

In the past, HCM was considered a highly symptomatic disease with a poor long-term prognosis [32, 49–54]. Data supporting this view, however, came from highly selected patient populations of severely affected patients treated in tertiary referral centers [55, 56]. Recent reports with less referral bias indicate overall annual mortality rates of 1–2 % (SCD and heart failure-related death). Annual mortality from SCD alone is 0.4–1 % [57–63].

Although the overall risk of SCD for HCM patients is small in absolute terms, a small subset of patients is at much higher risk of SCD. Identification of these individuals offers the prospect of primary prophylaxis with implantable cardioverter defibrillators (ICD) to prevent sudden death. Patients surviving ventricular fibrillation or symptomatic sustained ventricular tachycardia are at high risk of a subsequent arrhythmic event and should receive an ICD if their anticipated life expectancy is >1 year. For patients without such a history, guidelines recommend the systematic evaluation of a number of clinical risk factors that can be used to estimate the risk of potentially fatal ventricular arrhythmia. In the ESC guidelines on HCM, it is advised to estimate a 5-year risk of SCD using a specifically designed risk model (HCM Risk-SCD) (www.escardio.org/guidelines-surveys/esc-guidelines/Pages/hypertrophic-cardiomyopathy.aspx), which is based on a set of prognostic clinical variables (Fig. 4.5) [16, 64]. These clinical variables are derived from clinical and family history, echocardiography (or cardiac MRI), 48-h ambulatory ECG, and an exercise test. An ICD is not recommended if the estimated 5-year risk of SCD is <4 % and other clinical features potentially associated with a risk of SCD are absent. The HCM Risk-SCD model has not been evaluated in HCM patients <16 years, in mutation carriers without cardiac hypertrophy, in professional athletes, in patients following myectomy or alcohol septal ablation, or in patients with cardiac hypertrophy caused by rare phenocopies such as Anderson-Fabry disease.

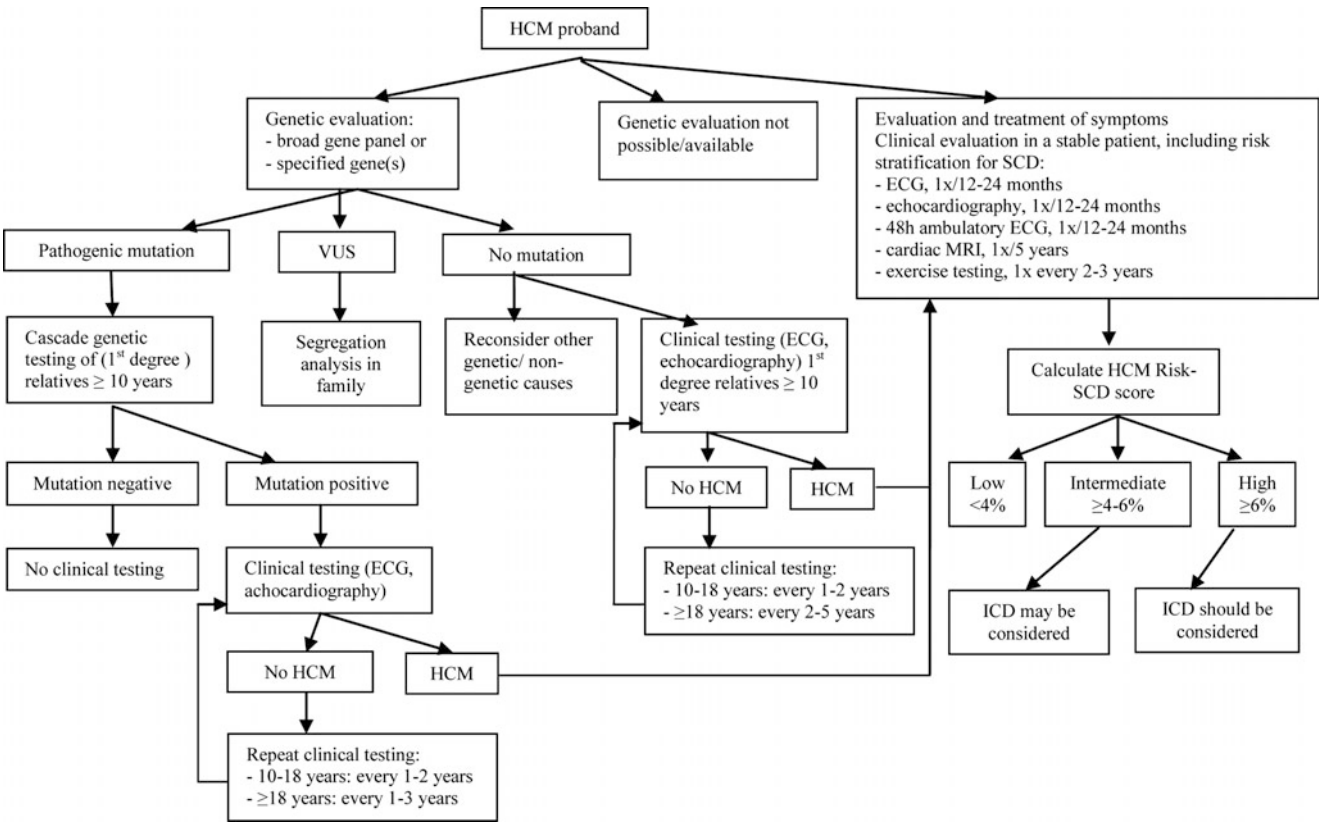

Fig. 4.5 Flowchart for clinical and genetic evaluation of HCM patients and their relatives

Risk stratification in children with HCM is even more challenging, but major risk factors for SCD include severe LVH, unexplained syncope, nonsustained ventricular tachycardia, and a family history of sudden death. Cardiac hypertrophy in children is considered severe if ≥30 mm or a *Z*-score ≥6. In the presence of two or more of these risk factors, an ICD should be considered. If only one risk factor is present, an individual approach with consideration of the risks and benefits of ICD implantation for the child and family should be followed [16].

In mutation carriers without LVH, the risk of SCD is probably very low and clinical evaluation consists of ECG and echocardiography once every 2–5 years in adults and once every 1–2 years in children between 10 and 18 years [16, 65]. If HCM becomes manifest during follow-up, clinical assessments in mutation carriers should also include an assessment of the risk of SCD according to the HCM Risk-SCD model.

Genetics of HCM and Genetic Testing

HCM is inherited as an autosomal dominant trait. In more than half of the HCM patients, the disease causing mutation can be identified currently [66–74]. Mutations can be located in many genes, but are most often found in the genes encoding sarcomeric proteins (Table 4.2, Fig. 4.2). Sarcomeric genes can be divided into genes encoding for myofilament proteins [14, 15, 67, 68, 71–73, 75–78] and genes encoding for Z-disc proteins [79–84].

Most patients with HCM are heterozygous for a sarcomeric protein gene mutation, but in 3–5 % of cases, patients carry two mutations in the same gene (different alleles – compound heterozygous or very rarely homozygous) or in different genes (digenic). This is generally associated with a more severe phenotype with younger age of onset (often <10 years) and more adverse events suggesting a gene-dosage effect [68, 72, 74, 85–89]. The two most frequently mutated genes are *MYBPC3* and *MYH7,* encoding the sarcomeric proteins cardiac myosin-binding protein C and beta myosin heavy chain, respectively (Table 4.2). Both proteins are major components of the sarcomeric thick filament. In contrast to *MYH7* and most of the other genes associated with HCM, 70 % of *MYBPC3* mutations are nonsense or frameshift and should result in truncated proteins [90, 91] suggesting haploinsufficiency. In the other genes missense mutations are most frequent, which create a mutant protein that interferes with normal function (dominant negative effect).

Since the discovery of the first genes for HCM, many papers on genotype–phenotype correlations have been written. At first specific mutations, mainly in the *MYH7* gene, were described that were associated with a "malignant" phenotype (decreased survival) [92–94]. So-called benign mutations were reported in families with normal longevity, as well [92, 93, 95–100]. These suggested "malignant" and "benign" mutations have been contradicted in many subsequent studies, but there are still only limited data on genotype–phenotype relations [73, 92, 96, 98, 101–103]. Moreover, genetic studies have revealed that not all mutation carriers are clinically affected, using standard echocardiography. This suggests the existence of other genetic or epigenetic influences on gene expression. Associations have been found with polymorphisms in genes for the angiotensin II type 1 and type 2 receptors and in the promoter region of the calmodulin III gene [104–106].

Nonsarcomeric genes have been associated with specific phenotypes which include besides HCM almost always a distinct noncardiac syndromic phenotype, like the *PTPN11* gene in Noonan syndrome and the *LAMP2* gene in Danon disease. However, mutations in the *GLA* gene, associated with Anderson-Fabry disease, can give rise to HCM without systemic symptoms of Anderson-Fabry disease, especially in women [107]. *PRKAG2* is another nonsarcomeric gene which can present with an exclusively cardiac phenotype that includes pre-excitation and conduction disease (Table 4.1) [108].

De novo mutations and germline mosaicism occur very rarely in HCM [109–113]. In certain countries/populations, founder mutations have been identified, in which haplotype analysis suggests a common ancestor. These founder mutations often comprise a large part (10–25 %) of the detected mutations in these countries. Founder mutations have been found in the Netherlands [114, 115], South Africa [116], Finland [117], Italy [67], Japan [31], India [118], and in the Amish population of the USA [119].

As in other genetic diseases, identified mutations in HCM patients can be pathogenic (disease causing), silent polymorphisms, or unclassified variants of which the pathogenic effect is still unclear, also called variants of unknown significance (VUS). According to guidelines of the Association of Clinical Genetic Science (http://www.acgs.uk.com/media/774853/evaluation_and_reporting_of_sequence_variants_bpgs_june_2013_-_finalpdf.pdf), mutations can be classified into five subtypes: class 1 clearly not pathogenic, class 2 unlikely to be pathogenic, class 3 unknown significance, class 4 likely to be pathogenic, and class 5 clearly pathogenic. Because pathogenicity is not completely clear for class 3 and 4 mutations, the laboratory should state in their report that follow-up studies, including segregation analysis in the family, are needed to clarify the significance of a variant. Class 3 and 4 mutations should not be used for genetic cascade screening in healthy relatives [65, 74, 120]. Nonsense or frameshift mutations are most often pathogenic because they are predicted to result in a C-terminal truncated protein, which is likely to be non-

Table 4.2 Genes associated with HCM and their detection rate [67, 68, 71–74, 79–84]

Gene	Name	Detection rate
Sarcomeric		
Myofilament		
MYBPC3	Myosin-binding protein C	13–32 %
MYH7	Beta myosin heavy chain	4–25 %
TNNT2	Troponin T2	0.5–7 %
TNNI3	Cardiac troponin I	<5 %
MYL2	Myosin light chain 2	<5 %
MYL3	Myosin light chain 3	<1 %
TPM1	Alpha tropomyosin	<1 %
ACTC	Alpha actin	<1 %
TNNC1	Troponin C	<1 %
Z-disc		
ACTN2	Alpha-2 actinin	4–5 %
CSRP3	Cysteine- and glycine-rich protein 3	
LBD3 (or ZASP)	Lim domain-binding 3	
TCAP	Titin-cap (Telethonin)	
VCL	Vinculin	
TTN	Titin	
MYOZ2	Myozenin 2	<1 %
Nonsarcomeric[a]		**Phenotype**
PRKAG2	AMP-activated protein kinase gamma 2	LVH/pre-excitation (Wolf-Parkinson-White syndrome)/ conduction disturbances
LAMP2	Lysosome-associated membrane protein 2	Danon disease
GLA	Alpha galactosidase	Anderson-Fabry disease
PTPN11	Protein-tyrosine phosphatase nonreceptor-type 11	Noonan, Leopard, CFC syndrome
KRAS2	Kirsten rat sarcoma viral oncogen homolog	Noonan, Leopard, CFC syndrome
SOS1	Son of sevenless homolog 1	Noonan syndrome
BRAF1	V-RAF murine sarcoma viral oncogen homolog B1	CFC syndrome
MAP2K1	Mitogen-activated protein kinase kinase 1	CFC syndrome
MAP2K2	Mitogen-activated protein kinase kinase 2	CFC syndrome
HRAS	Harvey rat sarcoma viral oncogene homolog	Costello syndrome
GAA	Glucosidase alpha acid	Pompe disease
GDE	Glycogen debrancher enzyme	Glycogen storage disorder III
FXN	Frataxin	Friedreich's ataxia
TTR	Transthyretin	Amyloidosis I
Mitochondrial DNA		LVH "plus"

[a]Because of specific phenotype, mutation detection rate is not provided

functional. Moreover, due to the presence of two quality control mechanisms, the nonsense-mediated mRNA decay degrading nonsense (truncated) mRNAs [121] and the ubiquitin-proteasome degrading aberrant proteins, [122] truncating mutations most often do not result in the formation of protein at all and therefore lead to haploinsufficiency of the protein encoded by the mutated allele in the cells. Missense mutations create a mutant protein that either interferes with normal function (dominant negative effect) or assumes a new function. Sometimes it remains unclear if a missense mutation results in a protein with no or abnormal function.

The amino acid substitution in missense mutations can give some indication of pathogenicity. Missense mutations at codons conserved between species and isoforms are more likely to be pathogenic than mutations at poorly conserved regions. Different *in silico* methods have been developed to assess not only conservation, but also changes in protein structure, chemical and biophysical characteristics and interactions. Ideally, these *in silico* predictive tools should be validated by comparison to a gold standard. Gold standards can be functional assays, frequently found mutations (e.g., founder mutations) or segregation with the clinical phenotype. Each of these potential standards, however, has their strengths and weaknesses

[120, 123, 124]. In addition, unclassified variants and even polymorphisms in HCM-associated genes and other genes (e.g., in the renin-angiotensin-aldosterone system [125, 126]) may have phenotype modifying effects. In most countries functional analysis of uncertain variants and modifiers in HCM patients is currently performed in research setting only and not used in clinical decision making [120].

The main reason for genetic testing in HCM is to enable genetic cascade testing in relatives. This is a more cost-efficient way of detecting relatives at risk of HCM compared to clinical testing [127, 128]. An incomplete understanding of genotype–phenotype relationships means that for most mutations, the genetic test result does not influence management. Exceptions may include double mutations that tend to have a more severe phenotype and nonsarcomeric diseases such as Anderson-Fabry disease for which there are specific therapies.

Genetic testing in HCM has shifted from testing of a few genes in order of mutation frequency towards the use of large panels of cardiomyopathy associated genes, including those associated with common nonsarcomeric conditions. Testing large gene panels is a form of next generation sequencing and has a higher yield of pathogenic mutations and a short turn-around time. The major disadvantage is that the chance of finding a VUS is greater [129]. The yield of VUS (class 3 mutations) is around 20 % for gene panels testing between 40 and 50 genes, and increases to around 30 % if likely pathogenic mutations (class 4 mutations) are also considered to be VUS [74]. In patients with signs of specific nonsarcomeric genetic causes of HCM genetic testing can be more targeted. With new developments in diagnostic DNA testing and also increasing knowledge on tolerated DNA variants, we constantly have to re-evaluate which test is best suited for which patient.

Screening of Relatives: Genetic Counseling and Testing

Consensus documents and guidelines on HCM encourage screening of relatives as identification of a disease causing mutation in a HCM patient (the proband) enables the screening of relatives by means of predictive DNA testing [16, 38, 65]. DNA testing for HCM, and especially predictive DNA testing in relatives, is becoming more common in most developed countries, but is still uncommon in non-Western countries because health insurance does not cover the costs of DNA testing, and/or because genetic counseling is unavailable. DNA testing is therefore sometimes only available in a research setting. Instead, most of these countries use clinical modalities such as echocardiography and ECG to screen relatives on the presence of disease (Fig. 4.5).

It is advised that, before genetic testing, pre-test genetic counseling is performed by a professional trained for this specific task working in a multidisciplinary team. This holds for relatives as well as for probands. Healthy children are advised to undergo clinical or predictive genetic testing starting from age ten, unless young children have been affected in their family or a nonsarcomeric genetic cause has been identified in their family [16, 65].

In a family where a definite pathogenic mutation (class 5 mutation) is detected the most cost-efficient way to identify relatives at risk for HCM is by genetic cascade testing. First degree relatives are often informed by the proband, sometimes using a family letter composed by the physician, about the possibility of predictive genetic testing. They can make an appointment for counseling where the pros and cons of genetic testing are discussed [130, 131]. Relatives without the familial HCM mutation can be discharged from cardiac follow-up. Relatives who carry the familial HCM mutation are, according to international guidelines, advised to undergo regular cardiac evaluation to evaluate the presence of LVH (once every 1–3 years by ECG and echocardiography in adults and once every year in children) and/or the risk for SCD [16, 38, 65]. During follow-up, LVH (manifest disease) can present at any age. The presence of risk factors for SCD in mutation carriers is associated with an increased risk of SCD if HCM is manifest (i.e., if LVH is present). In mutation carrying relatives (still) without manifest HCM, risk assessment for SCD is no longer advised, because the risk of SCD is very small [16, 132].

If no mutation can be detected in a proband with HCM or DNA-diagnostics is not possible, first degree relatives of HCM patients (and of SCD victims in the family) still have a risk to develop the disease and so are also advised to undergo regular cardiac evaluations (an ECG and echocardiography once every 1–2 years between 10 and 18 years and once every 2–5 years >18 years) assessing the presence of hypertrophy or ECG abnormalities directing toward HCM [16, 38, 65].

If a variant of unknown significance (class 3 or 4 mutation) is detected in a proband, one can try to perform segregation analysis in the family. This can be done by genetic testing of all affected relatives, but often there are not enough available affected relatives to allow a mutation to be reclassified as clearly pathogenic. Testing nonaffected relatives is of less value because of the age-dependent and incomplete disease penetrance of HCM. Another option is to offer relatives a combination of cardiac evaluation and genetic testing. However, it is still unclear if this results in more variants being reclassified while relatives can experience negative psychological effects if they find out to be a carrier of the variant even when they do not have a phenotype. If segregation is not possible or does not result in a reclassification of the DNA variant, first degree relatives are advised to undergo regular cardiac evaluations just as in families where no mutation has been detected [16].

Take Home Messages

- HCM is the most common monogenetic heart disease and the most important cause of sudden death at young age.
- The hallmark of HCM is unexplained LVH.
- Clinical risk markers can identify patients at high risk for SCD and who might benefit from treatment with an implantable defibrillator.
- In 50–60 % of patients, a causal gene mutation can be identified allowing predictive genetic testing of relatives, thereby providing a better way to differentiate being relatives at risk for HCM and associated SCD and relatives not at risk.

References

1. Coats CJ, Hollman A. Hypertrophic cardiomyopathy: lessons from history. Heart. 2008;94(10):1258–63.
2. Basso C, Calabrese F, Corrado D, Thiene G. Postmortem diagnosis in sudden cardiac death victims: macroscopic, microscopic and molecular findings. Cardiovasc Res. 2001;50(2):290–300.
3. Drory Y, Turetz Y, Hiss Y, Lev B, Fisman EZ, Pines A, et al. Sudden unexpected death in persons less than 40 years of age. Am J Cardiol. 1991;68(13):1388–92.
4. Maron BJ. Sudden death in young athletes. N Engl J Med. 2003;349(11):1064–75.
5. Hada Y, Sakamoto T, Amano K, Yamaguchi T, Takenaka K, Takahashi H, et al. Prevalence of hypertrophic cardiomyopathy in a population of adult Japanese workers as detected by echocardiographic screening. Am J Cardiol. 1987;59(1):183–4.
6. Maron BJ, Peterson EE, Maron MS, Peterson JE. Prevalence of hypertrophic cardiomyopathy in an outpatient population referred for echocardiographic study. Am J Cardiol. 1994;73(8):577–80.
7. Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE. Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA study. Coronary artery risk development in (young) adults. Circulation. 1995;92(4):785–9.
8. Morita H, Larson MG, Barr SC, Vasan RS, O'Donnell CJ, Hirschhorn JN, et al. Single-gene mutations and increased left ventricular wall thickness in the community: the Framingham Heart Study. Circulation. 2006;113(23):2697–705.
9. Zou Y, Song L, Wang Z, Ma A, Liu T, Gu H, et al. Prevalence of idiopathic hypertrophic cardiomyopathy in China: a population-based echocardiographic analysis of 8080 adults. Am J Med. 2004;116(1):14–8.
10. Semsarian C, Ingles J, Maron MS, Maron BJ. New perspectives on the prevalence of hypertrophic cardiomyopathy. J Am Coll Cardiol. 2015;65(12):1249–54.
11. Klues HG, Schiffers A, Maron BJ. Phenotypic spectrum and patterns of left ventricular hypertrophy in hypertrophic cardiomyopathy: morphologic observations and significance as assessed by two-dimensional echocardiography in 600 patients. J Am Coll Cardiol. 1995;26(7):1699–708.
12. Maron BJ, Spirito P, Wesley Y, Arce J. Development and progression of left ventricular hypertrophy in children with hypertrophic cardiomyopathy. N Engl J Med. 1986;315(10):610–4.
13. Wigle ED, Sasson Z, Henderson MA, Ruddy TD, Fulop J, Rakowski H, et al. Hypertrophic cardiomyopathy. The importance of the site and the extent of hypertrophy. A review. Prog Cardiovasc Dis. 1985;28(1):1–83.
14. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, et al. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology working group on myocardial and pericardial diseases. Eur Heart J. 2008;29(2):270–6.
15. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary working groups; and Council on Epidemiology and Prevention. Circulation. 2006;113(14):1807–16.
16. Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, et al. 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy: the task force for the diagnosis and management of hypertrophic cardiomyopathy of the European Society of Cardiology (ESC). Eur Heart J. 2014;35(39):2733–79.
17. McKenna WJ, Spirito P, Desnos M, Dubourg O, Komajda M. Experience from clinical genetics in hypertrophic cardiomyopathy: proposal for new diagnostic criteria in adult members of affected families. Heart. 1997;77(2):130–2.
18. Elliott P, Baker R, Pasquale F, Quarta G, Ebrahim H, Mehta AB, et al. Prevalence of Anderson-Fabry disease in patients with hypertrophic cardiomyopathy: the European Anderson-Fabry disease survey. Heart. 2011;97(23):1957–60.
19. Limongelli G, Masarone D, D'Alessandro R, Elliott PM. Mitochondrial diseases and the heart: an overview of molecular basis, diagnosis, treatment and clinical course. Future Cardiol. 2012;8(1):71–88.
20. Limongelli G, D'Alessandro R, Maddaloni V, Rea A, Sarkozy A, McKenna WJ. Skeletal muscle involvement in cardiomyopathies. J Cardiovasc Med (Hagerstown). 2013;14(12):837–61.
21. Wilkinson JD, Lowe AM, Salbert BA, Sleeper LA, Colan SD, Cox GF, et al. Outcomes in children with Noonan syndrome and hypertrophic cardiomyopathy: a study from the Pediatric Cardiomyopathy Registry. Am Heart J. 2012;164(3):442–8.
22. Quarta CC, Kruger JL, Falk RH. Cardiac amyloidosis. Circulation. 2012;126(12):e178–82.
23. Ruberg FL, Berk JL. Transthyretin (TTR) cardiac amyloidosis. Circulation. 2012;126(10):1286–300.
24. Teare D. Asymmetrical hypertrophy of the heart in young adults. Br Heart J. 1958;20(1):1–8.
25. Chevers N. Observations on the diseases of the orifice and valves of the aorta. Guys Hosp Rep. 1842;7:387–442.
26. Vulpian A. Contribution à l'étude des rétrécissements de l'orifice ventriculo-aortique. Arch Physiol. 1868;3:456–7.
27. Maron BJ, Wolfson JK, Roberts WC. Relation between extent of cardiac muscle cell disorganization and left ventricular wall thickness in hypertrophic cardiomyopathy. Am J Cardiol. 1992;70(7):785–90.
28. Maron BJ, Wolfson JK, Epstein SE, Roberts WC. Intramural ("small vessel") coronary artery disease in hypertrophic cardiomyopathy. J Am Coll Cardiol. 1986;8(3):545–57.
29. Ahmad F, Seidman JG, Seidman CE. The genetic basis for cardiac remodeling. Annu Rev Genomics Hum Genet. 2005;6:185–216.
30. Tsoutsman T, Lam L, Semsarian C. Genes, calcium and modifying factors in hypertrophic cardiomyopathy. Clin Exp Pharmacol Physiol. 2006;33(1–2):139–45.

31. Kubo T, Kitaoka H, Okawa M, Matsumura Y, Hitomi N, Yamasaki N, et al. Lifelong left ventricular remodeling of hypertrophic cardiomyopathy caused by a founder frameshift deletion mutation in the cardiac Myosin-binding protein C gene among Japanese. J Am Coll Cardiol. 2005;46(9):1737–43.
32. Maron BJ. Hypertrophic cardiomyopathy: a systematic review. JAMA. 2002;287(10):1308–20.
33. Elliott PM, Gimeno Blanes JR, Mahon NG, Poloniecki JD, McKenna WJ. Relation between severity of left-ventricular hypertrophy and prognosis in patients with hypertrophic cardiomyopathy. Lancet. 2001;357(9254):420–4.
34. Maron BJ, Piccininno M, Casey SA, Bernabo P, Spirito P. Relation of extreme left ventricular hypertrophy to age in hypertrophic cardiomyopathy. Am J Cardiol. 2003;91(5):626–8.
35. Maron BJ, Casey SA, Hurrell DG, Aeppli DM. Relation of left ventricular thickness to age and gender in hypertrophic cardiomyopathy. Am J Cardiol. 2003;91(10):1195–8.
36. Biagini E, Coccolo F, Ferlito M, Perugini E, Rocchi G, Bacchi-Reggiani L, et al. Dilated-hypokinetic evolution of hypertrophic cardiomyopathy: prevalence, incidence, risk factors, and prognostic implications in pediatric and adult patients. J Am Coll Cardiol. 2005;46(8):1543–50.
37. Binder J, Ommen SR, Gersh BJ, Van Driest SL, Tajik AJ, Nishimura RA, et al. Echocardiography-guided genetic testing in hypertrophic cardiomyopathy: septal morphological features predict the presence of myofilament mutations. Mayo Clin Proc. 2006;81(4):459–67.
38. Maron BJ, McKenna WJ, Danielson GK, Kappenberger LJ, Kuhn HJ, Seidman CE, et al. American College of Cardiology/European Society of Cardiology clinical expert consensus document on hypertrophic cardiomyopathy. A report of the American College of Cardiology Foundation task force on clinical expert consensus documents and the European Society of Cardiology Committee for practice guidelines. J Am Coll Cardiol. 2003;42(9):1687–713.
39. Sanderson JE, Gibson DG, Brown DJ, Goodwin JF. Left ventricular filling in hypertrophic cardiomyopathy. An angiographic study. Br Heart J. 1977;39(6):661–70.
40. Olivotto I, Cecchi F, Casey SA, Dolara A, Traverse JH, Maron BJ. Impact of atrial fibrillation on the clinical course of hypertrophic cardiomyopathy. Circulation. 2001;104(21):2517–24.
41. Autore C, Bernabo P, Barilla CS, Bruzzi P, Spirito P. The prognostic importance of left ventricular outflow obstruction in hypertrophic cardiomyopathy varies in relation to the severity of symptoms. J Am Coll Cardiol. 2005;45(7):1076–80.
42. Maron MS, Olivotto I, Betocchi S, Casey SA, Lesser JR, Losi MA, et al. Effect of left ventricular outflow tract obstruction on clinical outcome in hypertrophic cardiomyopathy. N Engl J Med. 2003;348(4):295–303.
43. Olivotto I, Maron BJ, Montereggi A, Mazzuoli F, Dolara A, Cecchi F. Prognostic value of systemic blood pressure response during exercise in a community-based patient population with hypertrophic cardiomyopathy. J Am Coll Cardiol. 1999;33(7):2044–51.
44. Elliott PM, Gimeno JR, Tome MT, Shah J, Ward D, Thaman R, et al. Left ventricular outflow tract obstruction and sudden death risk in patients with hypertrophic cardiomyopathy. Eur Heart J. 2006;27(16):1933–41.
45. Morrow AG, Reitz BA, Epstein SE, Henry WL, Conkle DM, Itscoitz SB, et al. Operative treatment in hypertrophic subaortic stenosis. Techniques, and the results of pre and postoperative assessments in 83 patients. Circulation. 1975;52(1):88–102.
46. Brown ML, Schaff HV. Surgical management of obstructive hypertrophic cardiomyopathy: the gold standard. Expert Rev Cardiovasc Ther. 2008;6(5):715–22.
47. Faber L, Meissner A, Ziemssen P, Seggewiss H. Percutaneous transluminal septal myocardial ablation for hypertrophic obstructive cardiomyopathy: long term follow up of the first series of 25 patients. Heart. 2000;83(3):326–31.
48. Sorajja P, Valeti U, Nishimura RA, Ommen SR, Rihal CS, Gersh BJ, et al. Outcome of alcohol septal ablation for obstructive hypertrophic cardiomyopathy. Circulation. 2008;118(2):131–9.
49. Elliott PM, Poloniecki J, Dickie S, Sharma S, Monserrat L, Varnava A, et al. Sudden death in hypertrophic cardiomyopathy: identification of high risk patients. J Am Coll Cardiol. 2000;36(7):2212–8.
50. Maki S, Ikeda H, Muro A, Yoshida N, Shibata A, Koga Y, et al. Predictors of sudden cardiac death in hypertrophic cardiomyopathy. Am J Cardiol. 1998;82(6):774–8.
51. McKenna W, Deanfield J, Faruqui A, England D, Oakley C, Goodwin J. Prognosis in hypertrophic cardiomyopathy: role of age and clinical, electrocardiographic and hemodynamic features. Am J Cardiol. 1981;47(3):532–8.
52. Priori SG, Aliot E, Blomstrom-Lundqvist C, Bossaert L, Breithardt G, Brugada P, et al. Task force on sudden cardiac death of the European Society of Cardiology. Eur Heart J. 2001;22(16):1374–450.
53. Swan DA, Bell B, Oakley CM, Goodwin J. Analysis of symptomatic course and prognosis and treatment of hypertrophic obstructive cardiomyopathy. Br Heart J. 1971;33(5):671–85.
54. Koga Y, Itaya K, Toshima H. Prognosis in hypertrophic cardiomyopathy. Am Heart J. 1984;108(2):351–9.
55. Maron BJ, Spirito P. Impact of patient selection biases on the perception of hypertrophic cardiomyopathy and its natural history. Am J Cardiol. 1993;72(12):970–2.
56. Elliott PM, Gimeno JR, Thaman R, Shah J, Ward D, Dickie S, et al. Historical trends in reported survival rates in patients with hypertrophic cardiomyopathy. Heart. 2006;92(6):785–91.
57. Cecchi F, Olivotto I, Montereggi A, Squillatini G, Dolara A, Maron BJ. Prognostic value of non-sustained ventricular tachycardia and the potential role of amiodarone treatment in hypertrophic cardiomyopathy: assessment in an unselected non-referral based patient population. Heart. 1998;79(4):331–6.
58. Adabag AS, Casey SA, Kuskowski MA, Zenovich AG, Maron BJ. Spectrum and prognostic significance of arrhythmias on ambulatory Holter electrocardiogram in hypertrophic cardiomyopathy. J Am Coll Cardiol. 2005;45(5):697–704.
59. Maron BJ, Casey SA, Poliac LC, Gohman TE, Almquist AK, Aeppli DM. Clinical course of hypertrophic cardiomyopathy in a regional United States cohort. JAMA. 1999;281(7):650–5.
60. Kofflard MJ, Waldstein DJ, Vos J, Ten Cate FJ. Prognosis in hypertrophic cardiomyopathy observed in a large clinic population. Am J Cardiol. 1993;72(12):939–43.
61. Cannan CR, Reeder GS, Bailey KR, Melton LJI, Gersh BJ. Natural history of hypertrophic cardiomyopathy. A population-based study, 1976 through 1990. Circulation. 1995;92(9):2488–95.
62. Kyriakidis M, Triposkiadis F, Anastasakis A, Theopistou A, Tocta R, Barbetseas J, et al. Hypertrophic cardiomyopathy in Greece: clinical course and outcome. Chest. 1998;114(4):1091–6.
63. Maron BJ, Olivotto I, Spirito P, Casey SA, Bellone P, Gohman TE, et al. Epidemiology of hypertrophic cardiomyopathy-related death: revisited in a large non-referral-based patient population. Circulation. 2000;102(8):858–64.
64. O'Mahony C, Jichi F, Pavlou M, Monserrat L, Anastasakis A, Rapezzi C, et al. A novel clinical risk prediction model for sudden cardiac death in hypertrophic cardiomyopathy (HCM risk-SCD). Eur Heart J. 2014;35(30):2010–20.

65. Charron P, Arad M, Arbustini E, Basso C, Bilinska Z, Elliott P, et al. Genetic counselling and testing in cardiomyopathies: a position statement of the European Society of Cardiology working group on myocardial and pericardial diseases. Eur Heart J. 2010;31(22):2715–26.
66. Ackerman MJ, VanDriest SL, Ommen SR, Will ML, Nishimura RA, Tajik AJ, et al. Prevalence and age-dependence of malignant mutations in the beta-myosin heavy chain and troponin T genes in hypertrophic cardiomyopathy: a comprehensive outpatient perspective. J Am Coll Cardiol. 2002;39(12):2042–8.
67. Girolami F, Olivotto I, Passerini I, Zachara E, Nistri S, Re F, et al. A molecular screening strategy based on beta-myosin heavy chain, cardiac myosin binding protein C and troponin T genes in Italian patients with hypertrophic cardiomyopathy. J Cardiovasc Med (Hagerstown). 2006;7(8):601–7.
68. Ingles J, Doolan A, Chiu C, Seidman J, Seidman C, Semsarian C. Compound and double mutations in patients with hypertrophic cardiomyopathy: implications for genetic testing and counselling. J Med Genet. 2005;42(10):e59.
69. Van Driest SL, Vasile VC, Ommen SR, Will ML, Tajik AJ, Gersh BJ, et al. Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. J Am Coll Cardiol. 2004;44(9):1903–10.
70. Van Driest SL, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ. Yield of genetic testing in hypertrophic cardiomyopathy. Mayo Clin Proc. 2005;80(6):739–44.
71. Erdmann J, Daehmlow S, Wischke S, Senyuva M, Werner U, Raible J, et al. Mutation spectrum in a large cohort of unrelated consecutive patients with hypertrophic cardiomyopathy. Clin Genet. 2003;64(4):339–49.
72. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, et al. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. Circulation. 2003;107(17):2227–32.
73. Van Driest SL, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ. Sarcomeric genotyping in hypertrophic cardiomyopathy. Mayo Clin Proc. 2005;80(4):463–9.
74. Alfares AA, Kelly MA, McDermott G, Funke BH, Lebo MS, Baxter SB, et al. Results of clinical genetic testing of 2,912 probands with hypertrophic cardiomyopathy: expanded panels offer limited additional sensitivity. Genet Med: Off J Am Coll Med Genet. 2015;17(11):880–8.
75. Bonne G, Carrier L, Bercovici J, Cruaud C, Richard P, Hainque B, et al. Cardiac myosin binding protein-C gene splice acceptor site mutation is associated with familial hypertrophic cardiomyopathy. Nat Genet. 1995;11(4):438–40.
76. Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE, et al. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. Cell. 1990;62(5):999–1006.
77. Watkins H, Conner D, Thierfelder L, Jarcho JA, MacRae C, McKenna WJ, et al. Mutations in the cardiac myosin binding protein-C gene on chromosome 11 cause familial hypertrophic cardiomyopathy. Nat Genet. 1995;11(4):434–7.
78. Watkins H, McKenna WJ, Thierfelder L, Suk HJ, Anan R, O'Donoghue A, et al. Mutations in the genes for cardiac Troponin T and {alpha}-Tropomyosin in hypertrophic cardiomyopathy. N Engl J Med. 1995;332(16):1058–65.
79. Posch MG, Thiemann L, Tomasov P, Veselka J, Cardim N, Garcia-Castro M, et al. Sequence analysis of myozenin 2 in 438 European patients with familial hypertrophic cardiomyopathy. Med Sci Monit. 2008;14(7):CR372–CR4.
80. Osio A, Tan L, Chen SN, Lombardi R, Nagueh SF, Shete S, et al. Myozenin 2 is a novel gene for human hypertrophic cardiomyopathy. Circ Res. 2007;100(6):766–8.
81. Vasile VC, Will ML, Ommen SR, Edwards WD, Olson TM, Ackerman MJ. Identification of a metavinculin missense mutation, R975W, associated with both hypertrophic and dilated cardiomyopathy. Mol Genet Metab. 2006;87(2):169–74.
82. Theis JL, Bos JM, Bartleson VB, Will ML, Binder J, Vatta M, et al. Echocardiographic-determined septal morphology in Z-disc hypertrophic cardiomyopathy. Biochem Biophys Res Commun. 2006;351(4):896–902.
83. Bos JM, Poley RN, Ny M, Tester DJ, Xu X, Vatta M, et al. Genotype-phenotype relationships involving hypertrophic cardiomyopathy-associated mutations in titin, muscle LIM protein, and telethonin. Mol Genet Metab. 2006;88(1):78–85.
84. Hayashi T, Arimura T, Itoh-Satoh M, Ueda K, Hohda S, Inagaki N, et al. Tcap gene mutations in hypertrophic cardiomyopathy and dilated cardiomyopathy. J Am Coll Cardiol. 2004;44(11):2192–201.
85. Ho CY, Lever HM, DeSanctis R, Farver CF, Seidman JG, Seidman CE. Homozygous mutation in cardiac troponin T: implications for hypertrophic cardiomyopathy. Circulation. 2000;102(16):1950–5.
86. Lekanne Deprez RH, Muurling-Vlietman JJ, Hruda J, Baars MJ, Wijnaendts LC, Stolte-Dijkstra I, et al. Two cases of severe neonatal hypertrophic cardiomyopathy caused by compound heterozygous mutations in the MYBPC3 gene. J Med Genet. 2006;43(10):829–32.
87. Richard P, Isnard R, Carrier L, Dubourg O, Donatien Y, Mathieu B, et al. Double heterozygosity for mutations in the beta-myosin heavy chain and in the cardiac myosin binding protein C genes in a family with hypertrophic cardiomyopathy. J Med Genet. 1999;36(7):542–5.
88. Richard P, Charron P, Leclercq C, Ledeuil C, Carrier L, Dubourg O, et al. Homozygotes for a R869G mutation in the beta -myosin heavy chain gene have a severe form of familial hypertrophic cardiomyopathy. J Mol Cell Cardiol. 2000;32(8):1575–83.
89. Girolami F, Ho CY, Semsarian C, Baldi M, Will ML, Baldini K, et al. Clinical features and outcome of hypertrophic cardiomyopathy associated with triple sarcomere protein gene mutations. J Am Coll Cardiol. 2010;55(14):1444–53.
90. Carrier L, Bonne G, Bahrend E, Yu B, Richard P, Niel F, et al. Organization and sequence of human cardiac myosin binding protein C gene (MYBPC3) and identification of mutations predicted to produce truncated proteins in familial hypertrophic cardiomyopathy. Circ Res. 1997;80(3):427–34.
91. Richard P, Villard E, Charron P, Isnard R. The Genetic Bases of Cardiomyopathies. J Am Coll Cardiol. 2006;48(9, Supplement 1):A79–89.
92. Marian AJ, Mares Jr A, Kelly DP, Yu QT, Abchee AB, Hill R, et al. Sudden cardiac death in hypertrophic cardiomyopathy. Variability in phenotypic expression of beta-myosin heavy chain mutations. Eur Heart J. 1995;16(3):368–76.
93. Anan R, Greve G, Thierfelder L, Watkins H, McKenna WJ, Solomon S, et al. Prognostic implications of novel beta cardiac myosin heavy chain gene mutations that cause familial hypertrophic cardiomyopathy. J Clin Invest. 1994;93(1):280–5.
94. Hwang TH, Lee WH, Kimura A, Satoh M, Nakamura T, Kim MK, et al. Early expression of a malignant phenotype of familial hypertrophic cardiomyopathy associated with a Gly716Arg myosin heavy chain mutation in a Korean family. Am J Cardiol. 1998;82(12):1509–13.
95. Marian AJ, Roberts R. Molecular genetic basis of hypertrophic cardiomyopathy: genetic markers for sudden cardiac death. J Cardiovasc Electrophysiol. 1998;9(1):88–99.
96. Watkins H, Rosenzweig A, Hwang DS, Levi T, McKenna W, Seidman CE, et al. Characteristics and prognostic implications of myosin missense mutations in familial hypertrophic cardiomyopathy. N Engl J Med. 1992;326(17):1108–14.

97. Roberts R, Sigwart U. New concepts in hypertrophic cardiomyopathies, part II. Circulation. 2001;104(18):2249–52.
98. Fananapazir L, Epstein ND. Genotype-phenotype correlations in hypertrophic cardiomyopathy. Insights provided by comparisons of kindreds with distinct and identical beta-myosin heavy chain gene mutations. Circulation. 1994;89(1):22–32.
99. Consevage MW, Salada GC, Baylen BG, Ladda RL, Rogan PK. A new missense mutation, Arg719Gln, in the beta-cardiac heavy chain myosin gene of patients with familial hypertrophic cardiomyopathy. Hum Mol Genet. 1994;3(6):1025–6.
100. Coviello DA, Maron BJ, Spirito P, Watkins H, Vosberg HP, Thierfelder L, et al. Clinical features of hypertrophic cardiomyopathy caused by mutation of a "hot spot" in the alpha-tropomyosin gene. J Am Coll Cardiol. 1997;29(3):635–40.
101. Havndrup O, Bundgaard H, Andersen PS, Larsen LA, Vuust J, Kjeldsen K, et al. The Val606Met mutation in the cardiac beta-myosin heavy chain gene in patients with familial hypertrophic cardiomyopathy is associated with a high risk of sudden death at young age. Am J Cardiol. 2001;87(11):1315–7.
102. Epstein ND, Cohn GM, Cyran F, Fananapazir L. Differences in clinical expression of hypertrophic cardiomyopathy associated with two distinct mutations in the beta-myosin heavy chain gene. A 908Leu----Val mutation and a 403Arg----Gln mutation. Circulation. 1992;86(2):345–52.
103. Van Driest SL, Ackerman MJ, Ommen SR, Shakur R, Will ML, Nishimura RA, et al. Prevalence and severity of "benign" mutations in the beta-myosin heavy chain, cardiac troponin T, and alpha-tropomyosin genes in hypertrophic cardiomyopathy. Circulation. 2002;106(24):3085–90.
104. Deinum J, van Gool JM, Kofflard MJ, Ten Cate FJ, Danser AH. Angiotensin II type 2 receptors and cardiac hypertrophy in women with hypertrophic cardiomyopathy. Hypertension. 2001;38(6):1278–81.
105. Osterop AP, Kofflard MJ, Sandkuijl LA, Ten Cate FJ, Krams R, Schalekamp MA, et al. AT1 receptor A/C1166 polymorphism contributes to cardiac hypertrophy in subjects with hypertrophic cardiomyopathy. Hypertension. 1998;32(5):825–30.
106. Friedrich FW, Bausero P, Sun Y, Treszl A, Kramer E, Juhr D, et al. A new polymorphism in human calmodulin III gene promoter is a potential modifier gene for familial hypertrophic cardiomyopathy. Eur Heart J. 2009;30(13):1648–55.
107. Monserrat L, Gimeno-Blanes JR, Marin F, Hermida-Prieto M, Garcia-Honrubia A, Perez I, et al. Prevalence of Fabry disease in a cohort of 508 unrelated patients with hypertrophic cardiomyopathy. J Am Coll Cardiol. 50(25):2399–403.
108. Gollob MH, Green MS, Tang AS, Roberts R. PRKAG2 cardiac syndrome: familial ventricular preexcitation, conduction system disease, and cardiac hypertrophy. Curr Opin Cardiol. 2002;17(3):229–34.
109. Rai TS, Ahmad S, Bahl A, Ahuja M, Ahluwalia TS, Singh B, et al. Genotype phenotype correlations of cardiac beta-myosin heavy chain mutations in Indian patients with hypertrophic and dilated cardiomyopathy. Mol Cell Biochem. 2009;321(1–2):189–96.
110. Forissier JF, Richard P, Briault S, Ledeuil C, Dubourg O, Charbonnier B, et al. First description of germline mosaicism in familial hypertrophic cardiomyopathy. J Med Genet. 2000;37(2):132–4.
111. Watkins H, Thierfelder L, Hwang DS, McKenna W, Seidman JG, Seidman CE. Sporadic hypertrophic cardiomyopathy due to de novo myosin mutations. J Clin Invest. 1992;90(5):1666–71.
112. Watkins H, Anan R, Coviello DA, Spirito P, Seidman JG, Seidman CE. A de novo mutation in alpha-tropomyosin that causes hypertrophic cardiomyopathy. Circulation. 1995;91(9):2302–5.
113. Cuda G, Perrotti N, Perticone F, Mattioli PL. A previously undescribed de novo insertion-deletion mutation in the beta myosin heavy chain gene in a kindred with familial hypertrophic cardiomyopathy. Heart. 1996;76(5):451–2.
114. Alders M, Jongbloed R, Deelen W, van den Wijngaard A, Doevendans P, Ten Cate F, et al. The 2373insG mutation in the MYBPC3 gene is a founder mutation, which accounts for nearly one-fourth of the HCM cases in the Netherlands. Eur Heart J. 2003;24(20):1848–53.
115. Michels M, Solima OII, Kofflard MJ, Hoedemaekers YM, Dooijes D, Majoor-Krakauer D, et al. Diastolic abnormalities as the first feature of hypertrophic cardiomyopathy in Dutch myosin-binding protein C founder mutations. J Am Coll Cardiol Img. 2009;2:58–64.
116. Moolman-Smook JC, De Lange WJ, Bruwer EC, Brink PA, Corfield VA. The origins of hypertrophic cardiomyopathy-causing mutations in two South African subpopulations: a unique profile of both independent and founder events. Am J Hum Genet. 1999;65(5):1308–20.
117. Jaaskelainen P, Miettinen R, Karkkainen P, Toivonen L, Laakso M, Kuusisto J. Genetics of hypertrophic cardiomyopathy in eastern Finland: few founder mutations with benign or intermediary phenotypes. Ann Med. 2004;36(1):23–32.
118. Dhandapany PS, Sadayappan S, Xue Y, Powell GT, Rani DS, Nallari P, et al. A common MYBPC3 (cardiac myosin binding protein C) variant associated with cardiomyopathies in South Asia. Nat Genet. 2009;41(2):187–91.
119. Zahka K, Kalidas K, Simpson MA, Cross H, Keller BB, Galambos C, et al. Homozygous mutation of MYBPC3 associated with severe infantile hypertrophic cardiomyopathy at high frequency among the Amish. Heart. 2008;94(10):1326–30.
120. Mogensen J, van Tintelen JP, Fokstuen S, Elliott P, van Langen IM, Meder B, et al. The current role of next-generation DNA sequencing in routine care of patients with hereditary cardiovascular conditions: a viewpoint paper of the European Society of Cardiology working group on myocardial and pericardial diseases and members of the European Society of Human Genetics. Eur Heart J. 2015;36(22):1367–70.
121. Garneau NL, Wilusz J, Wilusz CJ. The highways and byways of mRNA decay. Nat Rev Mol Cell Biol. 2007;8(2):113–26.
122. Mearini G, Schlossarek S, Willis MS, Carrier L. The ubiquitin-proteasome system in cardiac dysfunction. Biochim Biophys Acta. 2008;1782(12):749–63.
123. Chan PA, Duraisamy S, Miller PJ, Newell JA, McBride C, Bond JP, et al. Interpreting missense variants: comparing computational methods in human disease genes CDKN2A, MLH1, MSH2, MECP2, and tyrosinase (TYR). Human mutation. 2007;28(7):683–93.
124. Goldgar DE, Easton DF, Byrnes GB, Spurdle AB, Iversen ES, Greenblatt MS. Genetic evidence and integration of various data sources for classifying uncertain variants into a single model. Hum Mutat. 2008;29(11):1265–72.
125. Ortlepp JR, Vosberg HP, Reith S, Ohme F, Mahon NG, Schroder D, et al. Genetic polymorphisms in the renin-angiotensin-aldosterone system associated with expression of left ventricular hypertrophy in hypertrophic cardiomyopathy: a study of five polymorphic genes in a family with a disease causing mutation in the myosin binding protein C gene. Heart. 2002;87(3):270–5.
126. Perkins MJ, Van Driest SL, Ellsworth EG, Will ML, Gersh BJ, Ommen SR, et al. Gene-specific modifying effects of pro-LVH polymorphisms involving the renin-angiotensin-aldosterone system among 389 unrelated patients with hypertrophic cardiomyopathy. Eur Heart J. 2005;26(22):2457–62.
127. Ingles J, McGaughran J, Scuffham PA, Atherton J, Semsarian C. A cost-effectiveness model of genetic testing for the evaluation of

families with hypertrophic cardiomyopathy. Heart. 2012;98(8): 625–30.
128. Wordsworth S, Leal J, Blair E, Legood R, Thomson K, Seller A, et al. DNA testing for hypertrophic cardiomyopathy: a cost-effectiveness model. Eur Heart J. 2010;31(8):926–35.
129. Lubitz SA, Ellinor PT. Next-generation sequencing for the diagnosis of cardiac arrhythmia syndromes. Heart Rhythm. 2015;12(5): 1062–70.
130. Christiaans I, Birnie E, Bonsel GJ, Wilde AAM, van Langen IM. Uptake of genetic counselling and predictive DNA testing in hypertrophic cardiomyopathy. Eur J Hum Genet. 2008;16(10):1201–7.
131. van der Roest WP, Pennings JM, Bakker M, van den Berg MP, van Tintelen JP. Family letters are an effective way to inform relatives about inherited cardiac disease. Am J Med Genet A. 2009; 149a(3):357–63.
132. Christiaans I, Birnie E, Bonsel GJ, Mannens MM, Michels M, Majoor-Krakauer D, et al. Manifest disease, risk factors for sudden cardiac death, and cardiac events in a large nationwide cohort of predictively tested hypertrophic cardiomyopathy mutation carriers: determining the best cardiological screening strategy. Eur Heart J. 2011;32(9):1161–70.

Dilated Cardiomyopathy

5

J.A. Jansweijer, R. Hershberger, and K.Y. Van Spaendonck

Abstract

Dilated cardiomyopathy (DCM) is characterized by impaired left ventricular (LV) systolic function and left ventricular dilation. Remarkable progress has been made in understanding the genetic basis of idiopathic dilated cardiomyopathy (iDCM). Rare variants in >30 genes, some also involved in other cardiomyopathies, muscular dystrophy, or syndromic disease, perturb a diverse set of important myocardial proteins to produce a final DCM phenotype. Recommendations on genetic screening and cardiac screening are provided in this chapter.

Introduction

Dilated cardiomyopathy (DCM) is characterized by impaired left ventricular (LV) systolic function and left ventricular dilation. Of many clinically detectable causes, reviewed below, DCM used here refers to cardiomyopathy of unknown cause, where all usual clinically detectable causes have been excluded. DCM can be asymptomatic, but may present with heart failure, arrhythmia, embolus from mural thrombus, or sudden cardiac death. DCM, even when treated, has significant mortality. DCM is the most prevalent indication for heart transplantation.

The first classification of the cardiomyopathies was made in 1972 [1] and was reiterated by the World Health Organization (WHO) in 1980 [2], defining DCM as myocardial disease of unknown origin with impaired systolic function and dilation of the left ventricle. The American Heart Association (AHA) followed the updated 1995 WHO definition [3] when it defined DCM as a disease of the myocardium characterized by ventricular chamber enlargement and systolic dysfunction with normal LV wall thickness [4]. The European Society of Cardiology (ESC) narrowed the definition to a myocardial disorder with left ventricular dilatation and left ventricular systolic dysfunction in the absence of abnormal loading conditions, such as hypertension or valve disease, or coronary artery disease sufficient to cause global systolic impairment [5].

A revised definition of DCM is proposed in a recent position statement of the ESC, in which DCM is seen as a clinical spectrum with left ventricular dilatation, impaired left ventricular function, conduction disease, and arrhythmias. In this proposal, a new category of hereditary nondilated cardiomyopathy (HNDC) is introduced as part of this spectrum, defined as left ventricular of biventricular global systolic dysfunction without dilatation not explained by abnormal loading conditions or coronary artery disease [6]. Preclinical phases of DCM are recognized in this proposal, including isolated ventricular dilation, which has been observed as an early sign of DCM in relatives [7].

The prevalence of DCM is unknown. One early population-based study conducted from 1975 to 1984 yielded an age- and sex-adjusted incidence of 6 per 100,000 person-years and a prevalence of 1 in 2700 [8]. This number is undoubtedly a significant underestimate. A recent review used several approaches to conclude that DCM may be more than tenfold as prevalent, that is 1 in 250 [9].

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DCM can be classified into idiopathic DCM (iDCM), DCM secondary to other causes, and syndromic DCM, a disorder with both cardiac and extra-cardiac features. Familial (fDCM) is defined when two or more closely related family members have DCM with all usual clinically detectable causes excluded [9, 10]. The European guidelines have also included unexplained sudden death before the age of 35 in a first-degree relative of a DCM patient as criteria to establish fDCM [10]. Nongenetic DCM can be secondary to a number of causes, including ischemic, structural, endocrine, and environmental causes. Ischemic cause is excluded from idiopathic DCM definitions. Coronary artery disease should be excluded when assessing a DCM patient, especially in males over 40 years and females over 45 years, and even at younger ages in the presence of significant coronary risk factors that include a strong family history of premature myocardial infarction, hypercholesterolemia, or cigarette smoking.

DCM can also occur as neuromuscular or syndromic disease with extracardiac features, including metabolic, mitochondrial, and chromosomal disorders. This category is also genetic but differs from genetic DCM restricted to cardiac features with different genetic causes, disease courses, and epidemiology. Childhood-onset DCM has a more diverse etiology than adult-onset DCM and more frequently falls into the latter category.

In this chapter, the clinical aspects of DCM will first be covered. The focus will then shift to the clinical and molecular features of genetic DCM and family screening.

Clinical Presentation

Many DCM patients present from the fourth decade onwards [11], with presenting manifestations including heart failure (80–85 %), arrhythmia (15 %), and thromboembolism (1–2 %). DCM may also be diagnosed postmortem in patients who died suddenly. Childhood-onset DCM also exists but is less common and has a diverse etiology and broader differential diagnosis.

Before modern-era treatment, reported mortality of DCM was 66 % in two years [12]. There is no recent study that systematically assessed DCM mortality, but as heart failure mortality was comparable to DCM mortality before modern-era treatment, it seems general heart failure data can be used. These data show that over time, significant progress has been made, but that 5-year mortality is still as high as 50 % [13–16].

Etiology of DCM

DCM has a broad and mixed etiology with both genetic factors and nongenetic factors involved, an overview is given in Table 5.1. When no clinically detectable cause for disease can be identified, DCM is called idiopathic (iDCM). From this category, genetic cause can be most commonly identified, and family screening to detect familial DCM (fDCM) has been recommended.

Genetic DCM has an autosomal dominant inheritance pattern in most cases, typically with age-dependent penetrance and variable expression. Rare variants have been implicated in more than 50 genes. Despite the number of associated genes [17], and although recent advances in techniques have made it possible to test an increasing number of genes in patients in a diagnostic setting, a likely pathogenic genetic defect is identified in only around 40 % of cases [18–20]. Besides fDCM, apparently sporadic iDCM can also have a genetic cause [21–23], although whether this group has largely a genetic basis has not yet been resolved, and neither have its potential genetic mechanisms [9]. Part of this group may actually represent fDCM, underdiagnosed because of (age related) low penetrance, the availability of only small families or incomplete testing of relatives. The presence of more than one rare variant that may be relevant for disease in up to one-third of patients from one DCM cohort suggests that oligogenic mechanisms may be at play [24].

DCM as part of a disorder with both cardiac and extracardiac features can be due to a neuromuscular disorder, inborn metabolism disorder, and malformation disorder. This category of DCM is more common in childhood-onset DCM. The age of onset and prognosis [25] differ between various forms of childhood-onset DCM, as do the potentially involved genes and inheritance patterns. Table 5.1 includes some of the most relevant childhood-onset forms of DCM and the gene in which mutations cause disease. The origin of disease in this category of DCM remained elusive in two-thirds of these cases [25]. New techniques, like exome sequencing, are likely to have lowered this number, although there are no recent studies on childhood DCM that tested this assumption. In the rest of the chapter, the focus will be on adult-onset DCM.

Peripartum cardiomyopathy (PPCM) is defined as DCM without detectable clinical cause presenting during pregnancy or in the months following delivery [26]. Considerable debate remains regarding etiology, but a subset of PPCM has shown to have a genetic cause [27–29]. Still, a comprehensive understanding of the etiology of all PPCM patients remains uncertain.

Myocarditis is an inflammatory disease of the myocardium, of infectious or autoimmune origin but in many cases of unknown origin. It mostly resolves spontaneously, but in some cases progresses to DCM. It has been postulated that myocarditis triggers disease onset in genetic DCM, analogous to certain cardiotoxic chemotherapeutic agents that cause DCM [30, 31].

Table 5.1 Classification of DCM

Main classification	Subclassification/etiology with some examples
Nongenetic DCM	Ischemic Coronary artery disease (with or without infarction) Structural heart disease Valvular, congenital, pressure or volume overload Toxins Chemotherapy (anthracyclines, alkylating agents, trastuzumab), alcohol Infectious Viral (e.g., HIV), other infectious etiology (e.g., Lyme) Autoimmune SLE, noninfectious myocarditis Infiltrative Amyloidosis, sarcoidosis Endocrine Diabetes mellitus, hypo- and hyperthyroidism, Cushing/Addison disease Metabolic Thiamine or carnitine deficiency, hypocalcaemia, hypophosphataemia Other Tachycardia, Kawasaki
Genetic DCM	Familial DCM, (apparently sporadic) idiopathic DCM, PPCM
DCM as part of a disorder with noncardiac features	Neuromuscular Duchenne/Becker (*DMD*) Myotonic dystrophy (DMPK) Limb-girdle muscular dystrophy (*LMNA, SGCD, SGCB*) Laing myopathy (*MYH7*) Myofibrillar myopathy (*DES*) Syndromic/metabolic Carnitine deficiency (*SLC22A5*) Glycosylation disorders (*DOLK* or *PGM1*) Alstrom syndrome (*ALMS1*) Barth syndrome (*TAZ*) Mitochondrial MIDD (maternally inherited diabetes and deafness) Kearns–Sayre syndrome Chromosomal disorders 1p36deletion syndrome

DCM divided into three categories with subdivisions of each category and some examples of DCM causes

Clinical Diagnosis

The path from clinical symptoms of DCM as set out in the previous paragraph to diagnosis has been clearly described by the ESC Working Group on Myocardial and Pericardial diseases [32]. In every step in the diagnostic process, information can be gathered that steers diagnosis toward one or more of the many different causes of DCM. Clinical characteristics that point to specific hereditary forms of DCM are covered in paragraph 6.

The starting point after clinical presentation is a medical history and physical examination. The personal and medical history should include known medical causes of DCM, as well as assess environmental risk factors such as exposure to cancer chemotherapeutic agents, alcohol, or other drug abuse. The patient's family history should be recorded, focusing on family members with a history of cardiomyopathy, heart failure, sudden cardiac death, cardiac transplantation, pacemaker or defibrillator implantation, stroke at early age and skeletal muscle disease. If familial disease is present, the family history can help to determine the inheritance pattern of DCM and may identify features specific to the cause of DCM in the particular family. Physical examination of the patient should be directed not only toward cardiac symptoms, but also extra-cardiac symptoms, most importantly skeletal muscle weakness. In children, physical examination should also be pointed to dysmorphic or syndromic features typical of childhood-onset DCM. Because family history is known to be insensitive to detect familial DCM, it is recommended that first-degree family members undergo clinical screening to detect evidence of asymptomatic DCM, which is covered below (see paragraph 7).

Obtaining an electrocardiogram (ECG) is indicated in all cardiovascular evaluations, as a standard ECG may contain features pointing to structural or electrical abnormalities. Certain ECG findings are more common in some genetic types of DCM, like atrioventricular conduction disease in *LMNA* [33] or *DES* [34]-induced DCM.

Routine laboratory testing should be performed to assess disease severity and to identify other causes or exacerbating factors of left ventricular dysfunction (LVD) and arrhythmias. Specifically, signs of ischemic disease or myocarditis [35] may be present in the form of elevated cardiac enzymes (creatine kinase (CK), creatine kinase myocardial band (CK-MB), troponin), endocrine dysfunction can be identified (high or low thyroid hormone levels), and signs of infectious disease or metabolic dysfunction and nutritional deficiencies can be found. To exclude ischemic disease, a coronary angiogram (CAG) is needed.

Another essential step in assessment is cardiac imaging to fully characterize the degree of left ventricular dilatation and left ventricular systolic dysfunction. Echocardiography is the most widely available imaging modality, which can be easily used to assess the cardiac function and dimensions. Furthermore, echocardiography can implicate causes of left ventricular dysfunction, like valvular dysfunction, regional wall motion abnormalities suggestive of ischemic disease, myocarditis or Tako Tsubo cardiomyopathy, or a thick septum suggestive of infiltrative diseases like amyloidosis and sarcoidosis. Other findings, such as left ventricular noncompaction, augment the differential diagnosis.

Most echocardiographic authorities have considered the lower limits of normal of the left ventricular ejection fraction (LVEF) to be 50 %, from early M-mode echocardiographic studies [36] to more recent US consensus documents [37]. More recent larger population-based studies suggest that the lower limits of two standard deviations for adults is an LVEF of 52 % [38, 39]. The ESC, however, has defined DCM as a LVEF less than 45 %, although with modern imaging precision this definition may be too stringent, as individuals with LVEF between 45 % and 50 % are abnormal. The assessment of LV size has been more challenging, with initial efforts devised using M-mode (one dimensional) echocardiography for infants, children, and adults based on an equation derived from 93 younger and 136 older subjects [40]. This was updated with improved standards from a much larger cohort of 1099 adults without known cardiovascular disease, using a height- and gender-based approach [41]. With proliferation of two-dimensional echocardiography (2D echo), M-mode-derived 2D-guided measurements have been advocated. The recent US/European consensus statements use gender-based approaches and BSA to estimate LV dimensions [38].

Cardiac magnetic resonance (CMR) imaging can assess cardiac function and morphology with greater accuracy than echocardiography. CMR can also be used for tissue characterization, which is helpful in the diagnosis of both ischemic cardiac disease (late gadolinium enhancement) and myocarditis (late gadolinium enhancement, T1- and T2-weighted images) [42, 43], amyloidosis and sarcoidosis, and may be used to detect fibrofatty replacement commonly present in arrhythmogenic right ventricular cardiomyopathy (ARVC) that may present with left ventricular involvement.

Especially if the family history is suggestive of hereditary disease, diagnostic procedures should be supplemented with genetic testing. This will be covered in paragraphs 5 and 6. The presence of DCM in relatives from families with fDCM is assessed according to less stringent criteria than in the index patient. These criteria will be covered in paragraph 7.

Clinical Therapy

Clinical management of DCM is pointed toward reducing symptoms and mortality and should be in accordance with ESC and ACCF/AHA guidelines [44, 45]. In short, therapy comprises ACE-inhibitors or angiotensin receptor blockers, and beta-blockers to prevent or treat heart failure and reduce morbidity and mortality in all patients, aldosterone receptor antagonists in patients with NYHA class II–IV HF and a LVEF of 35 % or less to reduce morbidity and mortality, and diuretics to alleviate symptoms. In certain patients, further medical therapy may be considered. Any causes of DCM, like ischemic disease and abnormal loading conditions, including hypertension and valvular disease, should be treated. Patients on maximal medical therapy may be considered for device therapy, either for the reduction of symptoms (cardiac resynchronization therapy) or primary prevention of sudden cardiac death (SCD) to reduce mortality.

Depending on the cause of hereditary disease, one may deviate from guideline therapy. For instance, mutations in certain genes give rise to more arrhythmias than in other genes. Especially in *LMNA* mutations, the presence of various risk factors can guide early defibrillator device therapy [46].

Molecular Diagnostics

In fDCM, genetic testing can be performed to search for a genetic cause. In apparently sporadic iDCM cases, testing can also be considered as these patients have been shown to carry likely pathogenic variants as well [21, 22]. For the past two decades, genetic testing of a DCM proband was performed gene per gene, starting with the most prevalently affected genes and steered by clues in the clinical presentation pointing to a possibly affected gene. Sanger sequencing was used to identify most variant types, for certain variant types like partial exon deletions multiplex ligation-dependent probe amplification (MLPA) was used. In recent years, next-generation sequencing (NGS) has become widely available at acceptable and still declining cost, which makes the testing of gene panels, whole-exome sequencing (WES) and even whole-genome sequencing (WGS) feasible. Considering that the amount of genes in which mutations can cause DCM exceeds 50, application of NGS can considerably speed up

genetic testing in DCM patients. Furthermore, the most common genetic cause of DCM [20, 21, 24] is truncating mutations in *TTN*, a gene so large that NGS is necessary to make clinical testing possible. NGS gene panel results are available fast, although testing still takes longer than testing gene per gene by Sanger sequencing. Targeted NGS can substitute for Sanger sequencing and also seems sensitive enough to replace MLPA [47, 48]. In rare cases, in which red flags point toward a certain genetic subtype like for instance *LMNA*-caused DCM, testing a particular gene by Sanger sequencing can lead to even faster diagnosis. If signs of neuromuscular disease are present, testing of particular genes with neuromuscular involvement increases the diagnostic yield of genetic testing [22]. As the etiology of childhood-onset DCM more often includes syndromic/metabolic and neuromuscular disease, with other genes involved than tested in adult-onset DCM gene panels, the molecular diagnostic approach in these patients should be broader than in adult-onset DCM. Genetic testing in childhood-onset DCM may also include whole-exome/whole-genome sequencing (WES/WGS) in the patient and his or her parents to identify a causative mutation. Although literature on genetic testing in childhood-onset DCM is sparse, this method gives information on more genes than those in gene panels and enables the discovery of *de novo* mutations, novel disease genes [49] and may lead to the identification of an unexpected underlying disorder.

A concern of NGS gene panels (and even more of whole-exome/whole-genome sequencing) is that as more genes are tested, the percentage of identified variants of unknown significance (VUS) increases up to even 60 % when 46 genes are tested [50]. Interpretation of the genetic data is a challenge, especially since most pathogenic variants are unique to a patient or his or her family, the exception being for a few founder mutations. Ideally, pathogenicity of a variant is demonstrated by co-segregation of disease and variant. Alternatively, functional analysis from in vitro data on the variant or affected gene can give information on how likely it is for the variant to cause disease. Finally, various software tools can be used to classify variants by assessing the effect of amino acid changes, splicing effects, and evolutionary variation. These software-based approaches should be interpreted with care, however, since their calls are merely predictions, and for diagnostic purposes should only be used in concordance with other data.

Large reference databases, for instance the ExAC browser [51], have recently emerged and are highly functional, but large-scale sharing of identified variants in clearly defined DCM cohorts is lacking. This is especially the case for information on cosegregation of the variant. Also standardized and complete phenotype information is only scarcely available. The classification of variants has not been systematically applied between commercial testing laboratories or academic research groups. Further, variant classifications can also change over time, as more information on a particular variant is gathered, so to be of value, variant databases need to be updated regularly. Ideally, this requires continuous curation and worldwide collaboration. The US-based Clinical Genetics Resource (ClinGen) with its ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/) proposes to collect and collate such data and has recently started this effort [52].

Molecular Genetics

More than 50 genes have been associated with DCM, although not all have been robustly shown to cause DCM [17]. The most common genes with mutations in DCM are *TTN* and *LMNA*, but numbers vary per geographic region for example due to the prevalence of founder mutations [20–22, 24]. Most involved genes code for sarcomeric, Z-disk or cytoskeleton proteins, but in contrast to HCM and ARVC, genes encoding proteins of diverse cardiomyocyte structures, functions, and pathways can be affected (Table 5.2 and Table 5.3 and Fig. 5.1). The pathophysiology through which the various mutations and affected genes lead to the final DCM phenotype varies and is not known for all genes. Clinical overlap syndromes at times occur in patients who have signs of DCM and another cardiomyopathy. This is understandable from a genetic point of view, as many DCM genes have been associated with one or more other cardiomyopathies (e.g., *MYH7* in DCM and LVNC [53, 54], *DSP* and *PLN* in DCM and ARVC [55–57]). (See also the respective chapters on these cardiomyopathies).

Familial DCM often has an autosomal dominant pattern of inheritance, but recessive [58], X-linked [59], and mitochondrial [60] inheritance patterns have also been reported. Missense mutations are most common, although in-frame insertions and deletions, and frameshift, splice-site, and nonsense mutations also occur.

Disease penetrance of familial mutations is often incomplete, and in most cases, age-related, meaning that the probability that mutation carriers will develop the DCM phenotype increases with age. Even within one family, the phenotype in mutation carriers who do develop disease varies both in severity and in clinical characteristics. For instance, one relative may develop structural cardiomyopathy while the other only develops conduction disease or arrhythmia. The cause for incomplete disease penetrance and varying disease expression is uncertain. It is hypothesized that a multiple hit model, in which one or more common or rare variants and/or environmental factors modify expression of the familial mutation may explain this phenotypic variation [24]. Such a model implies a threshold for disease, requiring one or more factors to reach the threshold and

Table 5.2 Genes considered relevant or likely relevant in adult onset iDCM

Gene	Protein	Specific features	Inheritance
Sarcomere			
ACTC1 [67, 68]	Actin alpha 1	LVNC	AD
ACTN2 [69–72]	Alpha actinin 2	Heterogeneous phenotype, including LVNC, ventricular fibrillation, and sudden cardiac death (SCD)	AD
MYH7 [53, 54, 73, 74]	Myosin heavy chain beta	Early onset, left ventricular noncompaction (LVNC)	AD
MYPN [75, 76]	Myopalladin	Left ventricular hypertrophy (LVH)	AD
TNNC1 [77]	Slow troponin C	Not known	Unknown
TNNT2 [53, 77–80]	Troponin T	Possibly LVNC and LVH	AD
TPM1 [81, 82]	Tropomyosin 1	LVNC	AD
TTN [19, 21, 61, 62, 83]	Titin	Not known	AD
Cytoskeleton			
DES [34, 84–87]	Desmin	Myopathy or muscular weakness, conduction disease and arrhythmias, SCD	Unknown
DMD [59, 88–90]	Dystrophin	Myopathy	XL
LDB3 [91–94]	LIM domain-binding 3	LVNC	AD
Nuclear envelope			
LMNA [46, 64, 95]	Lamin A/C	Conduction disease, arrhythmias, skeletal muscle weakness, SCD	AD
Ion channel			
SCN5A [96–101]	Sodium channel	Conduction disease, arrhythmias	AD
Mitochondrial			
TAZ [102, 103]	Tafazzin	A predominantly proximal skeletal myopathy, growth retardation, LVH, LNVC, ventricular arrhythmias	XL
Spliceosome			
RBM20 [65, 66]	RNA Binding Motif Protein 20	Arrhythmias, SCD	AD
Sarcoplasmatic reticulum			
PLN [104, 105]	Phospholamban	Early onset and mortality, arrhythmias, ECG microvoltages	AD
Desmosomal			
DSP [55, 56, 106, 107]	Desmoplakin	Palmoplantar keratoderma, woolly hair, tooth agenesis, ARVC	AD/AR
Other			
BAG3 [108, 109]	BCL2-associated anthanogene 3	Not known	AD
EYA4 [110]	Eyes absent 4	Sensorineural hearing loss	AD
PSEN1 [111]	Presenilin 1	Alzheimer's disease	AD

cause disease. Environmental factors include general DCM risk factors, sex, blood pressure, activity level, drug and other toxicity exposure, and perhaps other specific physiologic or endocrine factors such as pregnancy or the postpartum state.

TTN

Truncations in the gene encoding for the sarcomeric protein titin, which is the largest protein in humans, seem to be the most common cause of fDCM, although it is not certain all truncating variants affecting *TTN* cause DCM. *TTN* mutations were first implicated in DCM in 2002 [61, 62], and the advent of NGS made testing for mutations possible on large scale in the past years. The first study to report on the prevalence of truncating *TTN* variants reported these variants in 25 % of fDCM patients [21]. Complicating matters, this study also reported truncating variants in *TTN* in healthy controls. As there are many isoforms of *TTN*, of which two are cardiac specific, not all truncating variants are disease causing. Filtering for variants that affect the cardiac isoforms still yields a prevalence of up to 22 % [19, 20] and dramatically decreases the number of identified variants in controls [19, 63]. Whether all truncating *TTN* variants are pathogenic in cardiac-specific isoforms has not yet been established. The role of missense variants in *TTN*, which average at 23 per individual, in any disease has as of yet not been evaluated systematically.

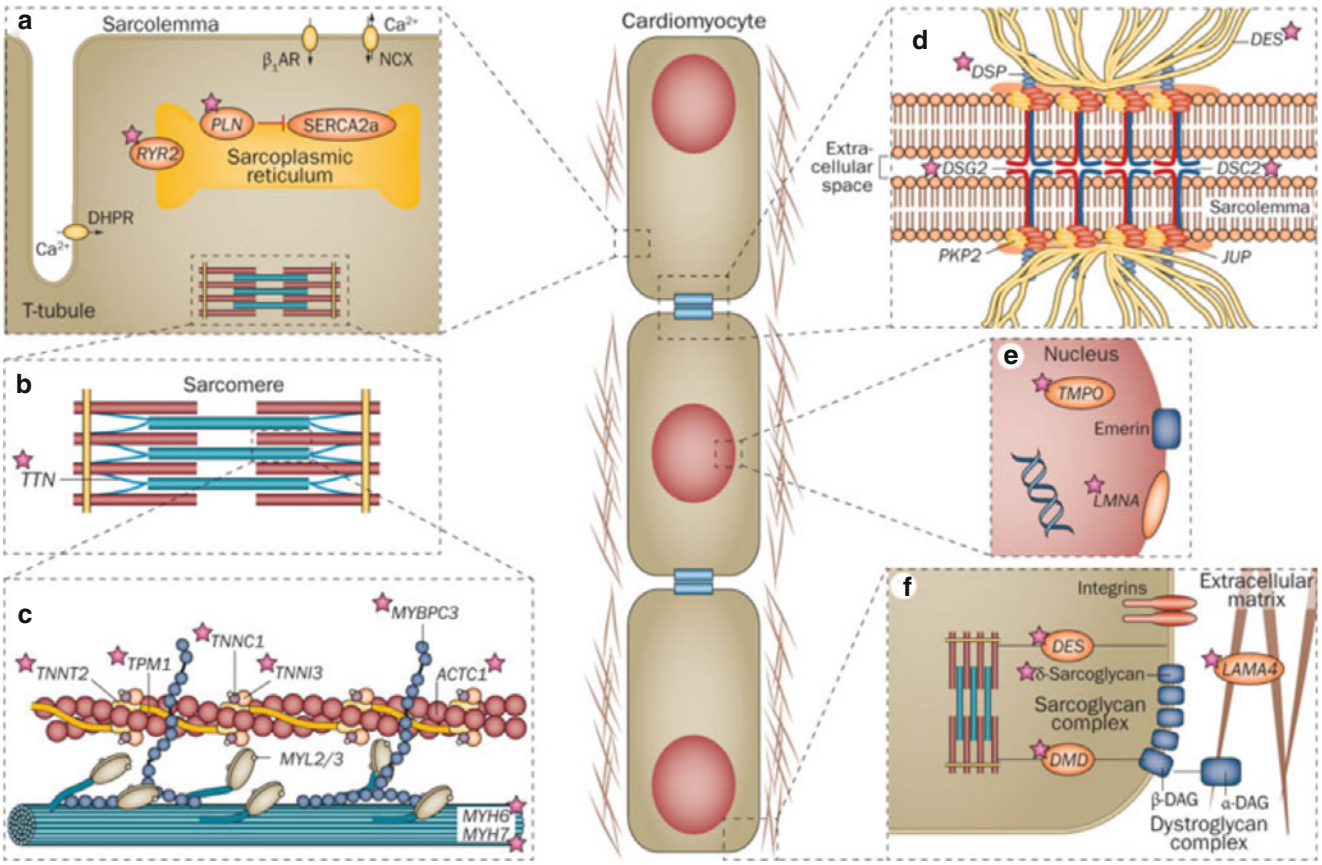

Fig. 5.1 Genes relevant to genetic DCM encode a diverse set of proteins. Some of the encoded proteins are illustrated in their cellular and molecular context, and those in which a DCM-associated mutation has been identified in the coding sequence are indicated by a red star. **A**. The cardiomyocyte membrane (sarcolemma), transverse (T)-tubule, and sarcoplasmic reticulum. Mutations in the genes that encode the ryanodine receptor and phospholamban (*RYR2, PLN*), which are important proteins in the regulation of intracellular calcium, are known to cause DCM. *B,C*. The sarcomere is the force-generating structure in cardiomyocytes, and is comprised of several proteins including cardiac actin (*ACTC1*), myosin- binding protein C (*MYBPC3*), myosin heavy chains (*MYH6, MYH7*), myosin light chains (*MYL2, MYL3*), tropomyosin (*TPM1*), cardiac troponin C, troponin I, and troponin T (*TNNC1, TNNI3*, and *TNNT2*) and titin (*TTN*). **D.** Desmosomal junctions, which assist in force transmission during muscle contraction. Variants in desmosomal proteins are associated with DCM, including desmin (DES), desmocollin-2 (DSC2), desmoglein-2 (DSG2) and desmoplakin (DSP). **E.** The cardiomyocyte nucleus. Mutations in *LMNA*, which encodes the lamin A and C filaments of the protein structure associated with the inner nuclear membrane, and *TMPO*, are associated with DCM. F. Cardiomyocyte membrane and extracellular matrix proteins. Mutations in *LAMA4*, an extracellular-matrix protein, have been found in patients with DCM. The proteins encoded by *DES* and *DMD* are connected to the sarcomere and inner nuclear membrane by cytoskeletal filament protein ACTG1. Abbreviations: β1AR, β1-adrenergic receptor; DAG, dystrophin-associated glycoprotein; DCM, dilated cardiomyopathy; DHPR, dihydropyridine receptor (voltage-gated L-type calcium channel); NCX, sodium/calcium exchanger; SERCA2a, sarcoplasmic/endoplasmic reticulum Ca2+ ATPase 2a. (*Adapted from Hershberger 2013 with permission*)

LMNA

Variants in *LMNA*, coding for a nuclear envelope protein, are known to cause a severe form of DCM that may be characterized more by electrical than structural abnormalities. *LMNA* variants were first shown to cause DCM in families in 1999 [64], a study in which the association with conduction disease and arrhythmias was already suggested. Long-term follow-up showed disease penetrance in individuals with a *LMNA* mutation is high and that these mutations are associated with malignant disease with high rates of heart failure and malignant arrhythmias [33]. Because of this, ICD implementation may be considered even if usual guideline criteria (e.g., specifying a left ventricular ejection fraction of less than 35%) have not been met [44, 45] and guided more by the presence of the risk factors. These risk factors are nonsustained ventricular tachycardia, ejection fraction of less than 45 % at first clinical contact, male sex, and nonmissense mutations [46].

Other Genes

As depicted in Tables 5.2 and 5.3, some genes are associated with certain clinical characteristics. The presence of these may guide the approach to genetic testing, although this is of decreased relevance since the advent of NGS as discussed in paragraph 5. In particular, some genes give rise to arrhythmias or conduction disease more often than others, with malignant

Table 5.3 Genes of uncertain relevance in adult onset iDCM

Gene	Protein	Specific features	Inheritance
Sarcomere			
TNNI3 [112]	Troponin I	Not known	AD
NEXN [113]	Nexilin	Not known	Unknown
Cytoskeleton			
VCL [114]	Metavinculin	Not known	Unknown
FKTN [115]	Fukutin	Early onset, muscle weakness	AR
Nuclear envelope			
LAMA4 [116]	Laminin alpha 4	Not known	AD
TMPO [117, 118]	Thymopoietin	Not known	Unknown
Desmosomal			
DSG2 [119]	Desmoglein-2	Not known	Unknown
Other			
GATAD1 [120]	GATA zinc finger domain-containing protein 1	Not known	AR
PSEN2 [111]	Presenilin 2	Less complete penetrance and milder disease than *PSEN1*. Alzheimer's disease	AD

phenotypes not only in patients with a mutation in *LMNA* as discussed earlier, but also in patients with a mutation in *RBM20* [65, 66].

Family Screening

Familial DCM and Affected Relatives

As set out in paragraph 3, taking a detailed family history of at least three generations is essential in patients with idiopathic DCM to detect evidence of familial disease. Furthermore, first-degree relatives should undergo clinical cardiac screening including a cardiac imaging study to assess ventricular size and function, as is further set out in the next section. Familial DCM is diagnosed when (1) two or more individuals in a family have DCM, or (2) if unexplained sudden death is documented before the age of 35 in a first-degree relative of a DCM patient [10].

The presence of DCM in relatives in a family with fDCM has been defined more liberally than in probands in the European literature. The usual DCM criteria of LVEF <45 % and LVEDD >117 % of the predicted value [40] are regarded major criteria, and several minor criteria are used including arrhythmias, conduction disease, sudden death and less stringent LVEF and LVEDD criteria. A relative is said to be affected in the presence of both major criteria, in the presence of left ventricular dilation >117 % and one minor criterion, or in the presence of three minor criteria. If a relative has another clear cause for DCM features, he or she is not considered as affected in the context of familial DCM [10]. In the US literature, the definition of DCM in family members has been the same as that for probands (LVEF <50 % with left ventricular enlargement) [121].

Considering the varying phenotypic expression of known pathogenic variants, and considerable age-dependent disease penetrance, even these criteria may be too strict and variant classification in fDCM families and cardiac evaluation of patients may profit from less stringent criteria. For this reason, in the proposed 2016 definition of DCM and HNDC [6], both major and minor criteria are defined less stringently. However, no large, prospectively collected, family-based DCM studies, accompanied by comprehensive genetic screening, have validated these clinical criteria. Thus, thoughtful, thorough and rigorous assembly of individual phenotype, pedigree and genetic information by informed clinicians continues to be the mainstay of detection, assessment and care for patients with possible genetic DCM.

Screening of Relatives

Presymptomatic evaluation for DCM allows for early diagnosis [122, 123] and intervention, which may decrease morbidity and mortality. Cardiac evaluation should be performed in first-degree relatives of all DCM probands. Even if the family history is negative, fDCM is regularly found after cardiac screening in first-degree relatives. Evaluation should at least include medical history, physical examination, ECG, and echocardiogram. Generally, in families with adult-onset fDCM, cardiac evaluation should be started in first-degree relatives at around 10–12 years of age and repeated every few years [124, 125]. The findings at evaluation in an individual and disease characteristics specific to a family may influence the age at which evaluation should start and how frequent evaluations should take place in individual cases. The steps described in the text are also set out in a flow chart (Fig. 5.2).

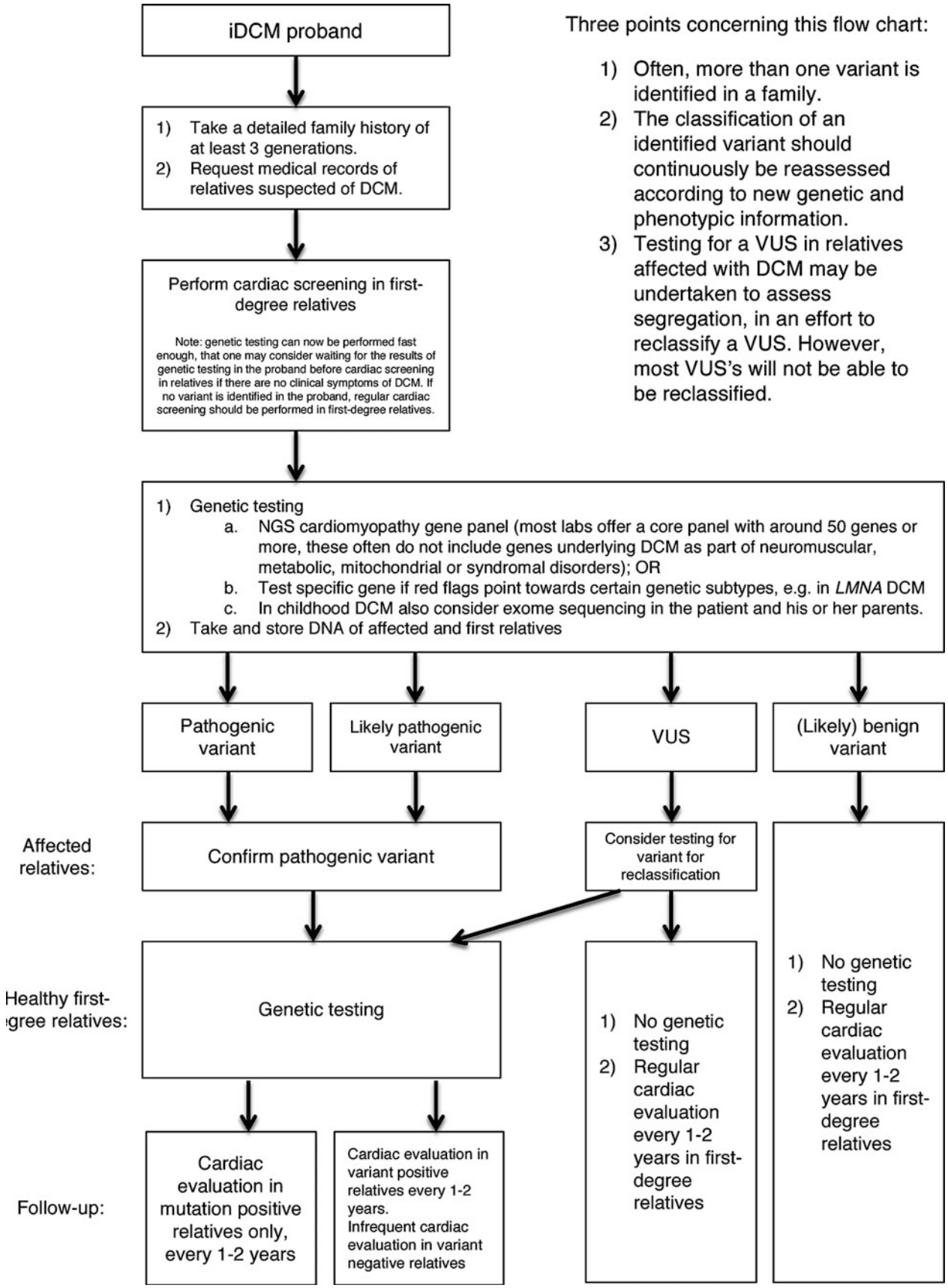

Fig. 5.2 Flow chart family screening steps/decisions

Genetic testing in the proband will in most cases be performed by NGS so many of the possibly involved genes can be tested. Often, laboratories will offer a cardiomyopathy gene panel of several dozen or more genes. When testing large numbers of genes, it is common that multiple rare variants in different DCM genes are identified. Variants may be categorized as pathogenic (also herein called a mutation), likely pathogenic, a variant of unknown significance (VUS), likely benign or benign.

If a pathogenic or likely pathogenic variant is identified in the proband, testing affected relatives to show that the variant segregates in all those with DCM validates the premise that the identified variant indeed is the disease-causing allele. Subsequently, healthy relatives can be tested for the mutation to include or exclude them for routine cardiac evaluation. Mutation positive relatives should be offered cardiac evaluation every 1–2 years. If the variant is still classified as only likely pathogenic, not only regular cardiac evaluation should be offered to variant positive relatives, but also low frequent cardiac evaluation to variant-negative relatives in case the identified variant is not the disease causing variant.

If a VUS is identified in the proband, testing affected relatives may be performed to test for cosegregation of the variant and DCM, although most families are too small to be informative enough to reclassify a VUS. If a VUS segregates with DCM, and the variant is reclassified as (likely) pathogenic, further genetic testing and clinical follow-up should be performed as described earlier. If a VUS remains classified as such, only regular cardiac evaluation and not genetic testing should be offered to first-degree relatives.

If a likely benign or benign variant is identified, no further genetic testing is indicated and regular cardiac evaluation can be restricted to first-degree relatives. The identification of a benign or likely benign variant does not exclude the possibility of a genetic cause, as in familial disease a mutation is identified in only 40 % of cases.

At times relatives meeting a formal iDCM diagnostic standard will not carry the familial mutation. In a series of 19 *LMNA* pedigrees, six were shown to have one or more family members who were negative for the familial *LMNA* variant, termed incomplete segregation pedigrees [95]. In two of the pedigrees, the *LMNA* variant-negative members have been shown to harbor pathogenic variants in other genes [126, 127]. Such findings as these raise the question of how frequently variants from more than one gene are relevant in DCM. Individuals who do not carry a familial variant but exhibit the family DCM phenotype have traditionally been termed phenocopies, for example, they show a resembling phenotype that has been considered due to another nongenetic cause. However, an alternative explanation is an oligogenic model, where more than one rare variant may be at play in an individual or a pedigree. This illustrates how important it is to test all affected relatives in a family and to perform cardiac evaluation in carriers, but also in noncarriers if a variant is not certainly pathogenic as described earlier and in the flow chart (Fig. 5.2).

It is essential that genetic counseling accompanies genetic testing. Further, since genetic testing and family evaluations touch ethical, medical, and psychosocial issues dealt with in clinical practice by most clinicians, it is recommended that genetic evaluations be performed by specialized cardiologists, clinical geneticists, and/or genetic counselors working in close collaboration. Patients and families should be informed that although genetic testing can identify relatives at risk, thereby enabling early diagnosis and treatment, in the majority of cases a causative mutation will not be found, and a VUS, uninformative for the family, may be identified. This latter situation where no actionable genetic information can be derived from testing is the case in more than half of the families with fDCM. For these families, testing cannot alleviate the uncertainty regarding genetic risk. Counseling should also cover that at times this uncertainty, perhaps exacerbated by continued clinical screening in asymptomatic individuals, may cause discomfort and undue concern. Also if a causative mutation is found, the relatives who test positive for the mutation but are yet unaffected may have undue anxiety about the uncertainty of their prognosis and the timing of possible disease onset, as penetrance may be incomplete so they will not develop disease, or if disease onset may be delayed for decades. This uncertainty may also influence individuals' eligibility for jobs, mortgages and life insurance, and have an impact on important life decisions like whether to have children and what profession to choose.

Summary

Take home messages

- DCM is a disease with complex genetic and environmental etiology and high mortality.
- Genetic causes for DCM are common. A family history should be taken in all idiopathic DCM probands and their first-degree relatives should be offered cardiac screening.
- If a (likely) pathogenic variant is identified in a DCM proband, affected relatives and subsequently also healthy first-degree relatives should be offered testing for that variant.
- The advent of next-generation sequencing allows for more comprehensive testing of DCM probands at the cost of more variants of unknown significance, increasing the need for careful variant interpretation.

References

1. Goodwin JF, Oakley CM. The cardiomyopathies. Br Heart J. 1972;34:545–52.
2. Report of the WHO/ISFC task force on the definition and classification of cardiomyopathies. Br Heart J. 1980;44:672–3.
3. Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, O'Connell J, Olsen E, Thiene G, Goodwin J, Gyarfas I, Martin I,

Nordet P. Report of the 1995 World Health Organization/ International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. Circulation. 1996;93:841–2.
4. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, Moss AJ, Seidman CE, Young JB. Contemporary definitions and classification of the cardiomyopathies: An American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functio. Circulation. 2006;113:1807–16.
5. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, Dubourg O, Kuhl U, Maisch B, McKenna WJ, Monserrat L, Pankuweit S, Rapezzi C, Seferovic P, Tavazzi L, Keren A. Classification of the cardiomyopathies: A position statement from the european society of cardiology working group on myocardial and pericardial diseases. Eur Heart J. 2008;29:270–6.
6. Pinto YM, Elliott PM, Arbustini E, Adler Y, Anastasakis A, Böhm M, Duboc D, Gimeno J, de Groote P, Imazio M, Heymans S, Klingel K, Komajda M, Limongelli G, Linhart A, Mogensen J, Moon J, Pieper PG, Seferovic PM, Schueler S, Zamorano JL, Caforio ALP, Charron P. Proposal for a revised definition of dilated cardiomyopathy, hypokinetic non-dilated cardiomyopathy, and its implications for clinical practice: a position statement of the ESC working group on myocardial and pericardial diseases. Eur Heart J. 2016;ehv727.
7. Michels VV, Moll PP, Miller FA, Jamil Tajik A, Chu JS, Driscoll DJ, Burnett JC, Rodeheffer RJ, Chesebro JH, Tazelaar HD. The frequency of familial dilated cardiomyopathy in a series of patients with idiopathic dilated cardiomyopathy. N Engl J Med. 1992;326:77–82.
8. Codd MB, Sugrue DD, Gersh BJ, Melton LJ. Epidemiology of idiopathic dilated and hypertrophic cardiomyopathy. A population-based study in Olmsted County, Minnesota, 1975–1984. Circulation. 1989;80:564–72.
9. Hershberger RE, Hedges DJ, Morales A. Dilated cardiomyopathy: the complexity of a diverse genetic architecture. Nat Rev Cardiol. 2013;10:531–47.
10. Mestroni L, Maisch B. Guidelines for the study of familial dilated cardiomyopathies. Eur Heart J. 1999;20:93–102.
11. Elliott P, Charron P, Blanes JRG, Tavazzi L, Tendera M, Konté M, Laroche C, Maggioni AP. European cardiomyopathy pilot registry: EURObservational research programme of the European society of cardiology. Eur Heart J. 2015;37:164–73.
12. Fuster V, Gersh BJ, Giuliani ER, Tajik AJ, Brandenburg RO, Frye RL. The natural history of idiopathic dilated cardiomyopathy. Am J Cardiol. 1981;47:525–31.
13. Levy D, Kenchaiah S, Glarson M, Benjamin EJ, Kupka MJ, Ho KKL, Murabito JM, Vasan RS. Long-term trends in the incidence of and survival with heart failure. N Engl J Med. 2002;347:1397–402.
14. Roger VL, Weston SA, Redfield MM, Hellermann-Homan JP, Killian J, Yawn BP, Jacobsen SJ. Trends in heart failure incidence and survival in a community-based population. JAMA. 2004; 292:344–50.
15. Jhund PS, MacIntyre K, Simpson CR, Lewsey JD, Stewart S, Redpath A, Chalmers JWT, Capewell S, McMurray JJV. Long-term trends in first hospitalization for heart failure and subsequent survival between 1986 and 2003. A population study of 5.1 million people. Circulation. 2009;119:515–23.
16. Stewart S, Ekman I, Ekman T, Oden A, Rosengren A. Population impact of heart failure and the most common forms of cancer: A study of 1 162 309 hospital cases in Sweden (1988 to 2004). Circ Cardiovasc Qual Outcomes. 2010;3:573–80.
17. Posafalvi A, Herkert JC, Sinke RJ, van den Berg MP, Mogensen J, Jongbloed JDH, van Tintelen JP. Clinical utility gene card for: dilated cardiomyopathy (CMD). Eur J Hum Genet. 2013;21:1–5.
18. Lakdawala NK, Funke BH, Baxter S, Cirino AL, Roberts AE, Judge DP, Johnson N, Mendelsohn NJ, Morel C, Care M, Chung WK, Jones C, Psychogios A, Duffy E, Rehm HL, White E, Seidman JG, Seidman CE, Ho CY. Genetic testing for dilated cardiomyopathy in clinical practice. J Card Fail. 2012;18:296–303.
19. Roberts AM, Ware JS, Herman DS, Schafer S, Baksi J, Bick AG, Buchan RJ, Walsh R, John S, Wilkinson S, Mazzarotto F, Felkin LE, Gong S, MacArthur JA, Cunningham F, Flannick J, Gabriel SB, Altshuler DM, Macdonald PS, Heinig M, Keogh AM, Hayward CS, Banner NR, Pennell DJ, O'Regan DP, San TR, de Marvao A, Dawes TJ, Gulati A, Birks EJ, Yacoub MH, Radke M, Gotthardt M, Wilson JG, O'Donnell CJ, Prasad SK, Barton PJ, Fatkin D, Hubner N, Seidman JG, Seidman CE, Cook SA. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. Sci Transl Med. 2015;7:270ra6.
20. Akinrinade O, Ollila L, Vattulainen S, Tallila J, Gentile M, Salmenperä P, Koillinen H, Kaartinen M, Nieminen MS, Myllykangas S, Alastalo T-PT-P, Koskenvuo JW, Heliö T. Genetics and genotype-phenotype correlations in Finnish patients with dilated cardiomyopathy. Eur Heart J. 2015;36:2327–37.
21. Herman DS, Lam L, Taylor MRGG, Wang L, Teekakirikul P, Christodoulou D, Conner L, DePalma SR, McDonough B, Sparks E, Teodorescu DL, Cirino AL, Banner NR, Pennell DJ, Graw S, Merlo M, Di Lenarda A, Sinagra G, Bos JM, Ackerman MJ, Mitchell RN, Murry CE, Lakdawala NK, Ho CY, Barton PJRR, Cook SA, Mestroni L, Seidman JGG, Seidman CE. Truncations of titin causing dilated cardiomyopathy. N Engl J Med. 2012;366:619–28.
22. Van Spaendonck-Zwarts KY, van Rijsingen IAW, van den Berg MP, Lekanne Deprez RH, Post JG, van Mil AM, Asselbergs FW, Christiaans I, van Langen IM, Wilde AAM, de Boer RA, Jongbloed JDH, Pinto YM, van Tintelen JP. Genetic analysis in 418 index patients with idiopathic dilated cardiomyopathy: overview of 10 years' experience. Eur J Heart Fail. 2013;15:628–36.
23. Hershberger RE, Norton N, Morales A, Li D, Siegfried JD, Gonzalez-Quintana J. Coding sequence rare variants identified in MYBPC3, MYH6, TPM1, TNNC1, and TNNI3 from 312 patients with familial or idiopathic dilated cardiomyopathy. Circ Cardiovasc Genet. 2010;3:155–61.
24. Haas J, Frese KS, Peil B, Kloos W, Keller A, Nietsch R, Feng Z, Müller S, Kayvanpour E, Vogel B, Sedaghat-Hamedani F, Lim W-K, Zhao X, Fradkin D, Köhler D, Fischer S, Franke J, Marquart S, Barb I, Li DT, Amr A, Ehlermann P, Mereles D, Weis T, Hassel S, Kremer A, King V, Wirsz E, Isnard R, Komajda M, Serio A, Grasso M, Syrris P, Wicks E, Plagnol V, Lopes L, Gadgaard T, Eiskjær H, Jørgensen M, Garcia-Giustiniani D, Ortiz-Genga M, Crespo-Leiro MG, Deprez RHLD, Christiaans I, van Rijsingen IA, Wilde AA, Waldenstrom A, Bolognesi M, Bellazzi R, Mörner S, Bermejo JL, Monserrat L, Villard E, Mogensen J, Pinto YM, Charron P, Elliott P, Arbustini E, Katus HA, Meder B. Atlas of the clinical genetics of human dilated cardiomyopathy. Eur Heart J. 2014. Eur Heart J. 2015 May 7;36(18):1123–35a.
25. Towbin JA, Lowe AM, Colan SD, Sleeper LA, Orav EJ, Clunie S, Messere J, Cox GF, Lurie PR, Hsu D, Canter C, Wilkinson JD, Lipshultz SE. Incidence, causes, and outcomes of dilated cardiomyopathy in children. JAMA. 2006;296:1867–76.
26. Sliwa K, Hilfiker-Kleiner D, Petrie MC, Mebazaa A, Pieske B, Buchmann E, Regitz-Zagrosek V, Schaufelberger M, Tavazzi L, Van Veldhuisen DJ, Watkins H, Shah AJ, Seferovic PM, Elkayam U, Pankuweit S, Papp Z, Mouquet F, McMurray JJV. Current state of knowledge on aetiology, diagnosis, management, and therapy of peripartum cardiomyopathy: a position statement from the Heart Failure Association of the European Society of Cardiology Working Group on peripartum cardiomyopathy. Eur J Heart Fail. 2010;12:767–78.
27. Van Spaendonck-Zwarts KY, Van Tintelen JP, Van Veldhuisen DJ, Van Der Werf R, Jongbloed JDH, Paulus WJ, Dooijes D, Van Den Berg MP. Peripartum cardiomyopathy as a part of familial dilated cardiomyopathy. Circulation. 2010;121:2169–75.
28. Van Spaendonck-Zwarts KY, Posafalvi A, van den Berg MP, Hilfiker-Kleiner D, Bollen IAE, Sliwa K, Alders M, Almomani R, van Langen IM, van der Meer P, Sinke RJ, van der Velden J, Van

Veldhuisen DJ, van Tintelen JP, Jongbloed JDH. Titin gene mutations are common in families with both peripartum cardiomyopathy and dilated cardiomyopathy. Eur Heart J. 2014;35:2165–73.
29. Ware JS, Li J, Mazaika E, Yasso CM, DeSouza T, Cappola TP, Tsai EJ, Hilfiker-Kleiner D, Kamiya CA, Mazzarotto F, Cook SA, Halder I, Prasad SK, Pisarcik J, Hanley-Yanez K, Alharethi R, Damp J, Hsich E, Elkayam U, Sheppard R, Kealey A, Alexis J, Ramani G, Safirstein J, Boehmer J, Pauly DF, Wittstein IS, Thohan V, Zucker MJ, Liu P, Gorcsan J, McNamara DM, Seidman CE, Seidman JG, Arany Z. Shared genetic predisposition in peripartum and dilated cardiomyopathies. N Engl J Med. 2016;374:233–41.
30. Van Den Berg MP, Van Spaendonck-Zwarts KY, Van Veldhuisen DJ, Gietema JA, Postma A, Van Tintelen JP. Familial dilated cardiomyopathy: another risk factor for anthracycline-induced cardiotoxicity? Eur J Heart Fail. 2010;12:1297–9.
31. Wasielewski M, van Spaendonck-Zwarts KY, Westerink N-DL, Jongbloed JDH, Postma A, Gietema JA, van Tintelen JP, van den Berg MP. Potential genetic predisposition for anthracycline-associated cardiomyopathy in families with dilated cardiomyopathy. Open Hear. 2014;1:e000116.
32. Rapezzi C, Arbustini E, Caforio ALP, Charron P, Gimeno-Blanes J, Heliö T, Linhart A, Mogensen J, Pinto Y, Ristic A, Seggewiss H, Sinagra G, Tavazzi L, Elliott PM. Diagnostic work-up in cardiomyopathies: bridging the gap between clinical phenotypes and final diagnosis. A position statement from the ESC Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2013;34:1448–58.
33. Pasotti M, Klersy C, Pilotto A, Marziliano N, Rapezzi C, Serio A, Mannarino S, Gambarin F, Favalli V, Grasso M, Agozzino M, Campana C, Gavazzi A, Febo O, Marini M, Landolina M, Mortara A, Piccolo G, Viganò M, Tavazzi L, Arbustini E. Long-term outcome and risk stratification in dilated cardiolaminopathies. J Am Coll Cardiol. 2008;52:1250–60.
34. Van Spaendonck-Zwarts KY, Van Hessem L, Jongbloed JDH, De Walle HEK, Capetanaki Y, Van der Kooi AJ, Van Langen IM, Van den Berg MP, Van Tintelen JP. Desmin-related myopathy. Clin Genet. 2011;80:354–66.
35. Lauer B, Niederau C, Kühl U, Schannwell M, Pauschinger M, Strauer BE, Schultheiss HP. Cardiac troponin T in patients with clinically suspected myocarditis. J Am Coll Cardiol. 1997;30:1354–9.
36. Pfisterer ME, Battler A, Zaret BL. Range of normal values for left and right ventricular ejection fraction at rest and during exercise assessed by radionuclide angiocardiography. Eur Heart J. 1985;6:647–55.
37. Radford MJ. ACC/AHA key data elements and definitions for measuring the clinical management and outcomes of patients with chronic heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Data Standards. Circulation. 2005;112:1888–916.
38. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt JU. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American society of echocardiography and the European association of cardiovascular imaging. Eur Heart J Cardiovasc Imaging. 2015;16:233–71.
39. Poppe KK, Doughty RN, Gardin JM, Hobbs FDR, McMurray JJV, Nagueh SF, Senior R, Thomas L, Whalley GA, Aune E, Brown A, Badano LP, Cameron V, Chadha DS, Chahal N, Chien KL, Daimon M, Dalen H, Detrano R, Akif Duzenli M, Ezekowitz JA, De Simone G, Di Pasquale P, Fukuda S, Gill PS, Grossman E, Hobbs FDR, Kim HK, Kuznetsova T, Leung NKW, Linhart A, McDonagh TA, McGrady M, McMurray JJV, Mill JG, Mogelvang R, Muiesan ML, Ng ACT, Ojji D, Otterstad JE, Petrovic DJ, Poppe KK, Prendergast B, Rietzschel E, Schirmer H, Schvartzman P, Senior R, Simova I, Sliwa K, Stewart S, Squire IB, Takeuchi M, Thomas L, Whalley GA, Altman DG, Perera R, Poppe KK, Triggs CM, Au Yeung H, Beans Picón GA, Poppe KK, Whalley GA, Anderson T, Dyck J, Ezekowitz JA, Chirinos JA, De Buyzere ML, Gillebert TC, Rietzschel E, Segers P, Van Daele CM, Doughty RN, Poppe KK, Walsh HA, Whalley GA, Izzo R, De Luca N, Trimarco B, De Simone G, Chadha DS, Goel K, Misra A, Chen PC, Chien KL, Lin HJ, Su TC, Detrano R, Cameron V, Richards AM, Troughton R, Mogelvang R, Skov Jensen J, Di Pasquale P, Paterna S, Akif Duzenli M, Hobbs FDR, Davies MK, Davis RC, et al. Ethnic-specific normative reference values for echocardiographic la and LV size, LV mass, and systolic function: The EchoNoRMAL study. JACC Cardiovasc Imaging. 2015;8:656–65.
40. Henry WL, Gardin JM, Ware JH. Echocardiographic measurements in normal subjects from infancy to old age. Circulation. 1980;62:1054–61.
41. Vasan RS, Larson MG, Levy D, Evans JC, Benjamin EJ. Distribution and categorization of echocardiographic measurements in relation to reference limits : the Framingham Heart Study: formulation of a height- and sex-specific classification and its prospective validation. Circulation. 1997;96:1863–73.
42. Friedrich MG, Sechtem U, Schulz-Menger J, Holmvang G, Alakija P, Cooper LT, White JA, Abdel-Aty H, Gutberlet M, Prasad S, Aletras A, Laissy JP, Paterson I, Filipchuk NG, Kumar A, Pauschinger M, Liu P. Cardiovascular magnetic resonance in myocarditis: a JACC white paper. J Am Coll Cardiol. 2009; 53:1475–87.
43. Ferreira VM, Piechnik SK, Dall'Armellina E, Karamitsos TD, Francis JM, Ntusi N, Holloway C, Choudhury RP, Kardos A, Robson MD, Friedrich MG, Neubauer S. T(1) mapping for the diagnosis of acute myocarditis using CMR: comparison to T2-weighted and late gadolinium enhanced imaging. JACC Cardiovasc Imaging. 2013;6:1048–58.
44. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJV, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WHW, Tsai EJ, Wilkoff BL. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2013;62:e147–239.
45. McMurray JJV, Adamopoulos S, Anker SD, Auricchio A, Böhm M, Dickstein K, Falk V, Filippatos G, Fonseca C, Gomez-Sanchez MA, Jaarsma T, Køber L, Lip GYH, Maggioni A Pietro, Parkhomenko A, Pieske BM, Popescu BA, Rønnevik PK, Rutten FH, Schwitter J, Seferovic P, Stepinska J, Trindade PT, Voors AA, Zannad F, Zeiher A. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart. Eur Heart J. 2012;33:1787–847.
46. van Rijsingen IAW, Arbustini E, Elliott PM, Mogensen J, Hermans-van Ast JF, van der Kooi AJ, van Tintelen JP, van den Berg MP, Pilotto A, Pasotti M, Jenkins S, Rowland C, Aslam U, Wilde AAM, Perrot A, Pankuweit S, Zwinderman AH, Charron P, Pinto YM. Risk factors for malignant ventricular arrhythmias in lamin a/c mutation carriers a European cohort study. J Am Coll Cardiol. 2012;59:493–500.
47. Wei X, Dai Y, Yu P, Qu N, Lan Z, Hong X, Sun Y, Yang G, Xie S, Shi Q, Zhou H, Zhu Q, Chu Y, Yao F, Wang JJ, He J, Yang YY, Liang Y, Yang YY, Qi M, Yang L, Wang W, Wu H, Duan J, Shen C, Wang JJ, Cui L, Yi X. Targeted next-generation sequencing as a comprehensive test for patients with and female carriers of DMD/BMD: a multi-population diagnostic study. Eur J Hum Genet. 2014;22:110–8.

48. Ceyhan-Birsoy O, Pugh TJ, Bowser MJ, Hynes E, Frisella AL, Mahanta LM, Lebo MS, Amr SS, Funke BH. Next generation sequencing-based copy number analysis reveals low prevalence of deletions and duplications in 46 genes associated with genetic cardiomyopathies. Mol Genet Genomic Med. 2016;4:143–51.
49. Almomani R, Verhagen JMA, Herkert JC, Brosens E, van Spaendonck-Zwarts KY, Asimaki A, van der Zwaag PA, Frohn-Mulder IME, Bertoli-Avella AM, Boven LG, van Slegtenhorst MA, van der Smagt JJ, van IJcken WFJ, Timmer B, van Stuijvenberg M, Verdijk RM, Saffitz JE, du Plessis FA, Michels M, Hofstra RMW, Sinke RJ, van Tintelen JP, Wessels MW, Jongbloed JDH, van de Laar IMBH. Biallelic truncating mutations in ALPK3 cause severe pediatric cardiomyopathy. J Am Coll Cardiol. 2016;67:515–25.
50. Pugh TJ, Kelly MA, Gowrisankar S, Hynes E, Seidman MA, Baxter SM, Bowser M, Harrison B, Aaron D, Mahanta LM, Lakdawala NK, McDermott G, White ET, Rehm HL, Lebo M, Funke BH. The landscape of genetic variation in dilated cardiomyopathy as surveyed by clinical DNA sequencing. Genet Med. 2014;16:601–8.
51. Exome Aggregation Consortium (ExAC) [Internet]. Available from: http://exac.broadinstitute.org
52. Rehm HL, Berg JS, Brooks LD, Bustamante CD, Evans JP, Landrum MJ, Ledbetter DH, Maglott DR, Martin CL, Nussbaum RL, Plon SE, Ramos EM, Sherry ST, Watson MS. ClinGen – the Clinical Genome Resource. N Engl J Med. 2015;372:2235–42.
53. Kamisago M, Sharma SD, DePalma SR, Solomon S, Sharma P, McDonough B, Smoot L, Mullen MP, Woolf PK, Wigle ED, Seidman JG, Seidman CE. Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. N Engl J Med. 2000;343:1688–96.
54. Klaassen S, Probst S, Oechslin E, Gerull B, Krings G, Schuler P, Greutmann M, Hürlimann D, Yegitbasi M, Pons L, Gramlich M, Drenckhahn JD, Heuser A, Berger F, Jenni R, Thierfelder L. Mutations in sarcomere protein genes in left ventricular noncompaction. Circulation. 2008;117:2893–901.
55. Rampazzo A, Nava A, Malacrida S, Beffagna G, Bauce B, Rossi V, Zimbello R, Simionati B, Basso C, Thiene G, Towbin JA, Danieli GA. Mutation in human desmoplakin domain binding to plakoglobin causes a dominant form of arrhythmogenic right ventricular cardiomyopathy. Am J Hum Genet. 2002;71:1200–6.
56. Yang Z, Bowles NE, Scherer SE, Taylor MD, Kearney DL, Ge S, Nadvoretskiy VV, DeFreitas G, Carabello B, Brandon LI, Godsel LM, Green KJ, Saffitz JE, Li H, Danieli GA, Calkins H, Marcus F, Towbin JA. Desmosomal dysfunction due to mutations in desmoplakin causes arrhythmogenic right ventricular dysplasia/cardiomyopathy. Circ Res. 2006;99:646–55.
57. Van Rijsingen IAW, Van Der Zwaag PA, Groeneweg JA, Nannenberg EA, Jongbloed JDH, Zwinderman AH, Pinto YM, Lekanne Dit Deprez RH, Post JG, Tan HL, De Boer RA, Hauer RNW, Christiaans I, Van Den Berg MP, Van Tintelen JP, Wilde AAM. Outcome in phospholamban R14del carriers results of a large multicentre cohort study. Circ Cardiovasc Genet. 2014;7:455–65.
58. Murphy RT, Mogensen J, Shaw A, Kubo T, Hughes S, McKenna WJ. Novel mutation in cardiac troponin I in recessive idiopathic dilated cardiomyopathy. Lancet. 2004;363:371–2.
59. Towbin JA, Hejtmancik JF, Brink P, Gelb B, Zhu XM, Chamberlain JS, McCabe ER, Swift M. X-linked dilated cardiomyopathy. Molecular genetic evidence of linkage to the Duchenne muscular dystrophy (dystrophin) gene at the Xp21 locus. Circulation. 1993;87:1854–65.
60. Zaragoza MV, Brandon MC, Diegoli M, Arbustini E, Wallace DC. Mitochondrial cardiomyopathies: how to identify candidate pathogenic mutations by mitochondrial DNA sequencing, MITOMASTER and phylogeny. Eur J Hum Genet. 2011;19:200–7.
61. Gerull B, Gramlich M, Atherton J, McNabb M, Trombitás K, Sasse-Klaassen S, Seidman JG, Seidman C, Granzier H, Labeit S, Frenneaux M, Thierfelder L. Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. Nat Genet. 2002;30:201–4.
62. Itoh-Satoh M, Hayashi T, Nishi H, Koga Y, Arimura T, Koyanagi T, Takahashi M, Hohda S, Ueda K, Nouchi T, Hiroe M, Marumo F, Imaizumi T, Yasunami M, Kimura A. Titin mutations as the molecular basis for dilated cardiomyopathy. Biochem Biophys Res Commun. 2002;291:385–93.
63. Akinrinade O, Koskenvuo JW, Alastalo T-P. Prevalence of titin truncating variants in general population. PLoS One. 2015; 10:e0145284.
64. Fatkin D, MacRae C, Sasaki T. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. N Engl J Med. 1999;341:1715–24.
65. Brauch KKM, Karst MML, Herron KJ, de Andrade M, Pellikka PA, Rodeheffer RJ, Michels VV, Olson TM. Mutations in ribonucleic acid binding protein gene cause familial dilated cardiomyopathy. J Am Coll Cardiol. 2009;54:930–41.
66. Li D, Morales A, Gonzalez-Quintana J, Norton N, Siegfried JD, Hofmeyer M, Hershberger RE. Identification of novel mutations in RBM20 in patients with dilated cardiomyopathy. Clin Transl Sci. 2010;3:90–7.
67. Olson TM, Michels VV, Thibodeau SN, Tai YS, Keating MT. Actin mutations in dilated cardiomyopathy, a heritable form of heart failure. Science. 1998;280:750–2.
68. Monserrat L, Hermida-Prieto M, Fernandez X, Rodríguez I, Dumont C, Cazón L, Cuesta MG, Gonzalez-Juanatey C, Peteiro J, Álvarez N, Penas-Lado M, Castro-Beiras A. Mutation in the alpha-cardiac actin gene associated with apical hypertrophic cardiomyopathy, left ventricular non-compaction, and septal defects. Eur Heart J. 2007;28:1953–61.
69. Mohapatra B, Jimenez S, Lin JH, Bowles KR, Coveler KJ, Marx JG, Chrisco MA, Murphy RT, Lurie PR, Schwartz RJ, Elliott PM, Vatta M, McKenna W, Towbin JA, Bowles NE. Mutations in the muscle LIM protein and alpha-actinin-2 genes in dilated cardiomyopathy and endocardial fibroelastosis. Mol Genet Metab. 2003;80:207–15.
70. Chiu C, Bagnall RD, Ingles J, Yeates L, Kennerson M, Donald JA, Jormakka M, Lind JM, Semsarian C. Mutations in alpha-actinin-2 cause hypertrophic cardiomyopathy. A genome-wide analysis. J Am Coll Cardiol. 2010;55:1127–35.
71. Bagnall RD, Molloy LK, Kalman JM, Semsarian C. Exome sequencing identifies a mutation in the ACTN2 gene in a family with idiopathic ventricular fibrillation, left ventricular noncompaction, and sudden death. BMC Med Genet. 2014;15:99.
72. Girolami F, Iascone M, Tomberli B, Bardi S, Benelli M, Marseglia G, Pescucci C, Pezzoli L, Sana ME, Basso C, Marziliano N, Merlini PA, Fornaro A, Cecchi F, Torricelli F, Olivotto I. Novel alpha-actinin 2 variant associated with familial hypertrophic cardiomyopathy and juvenile atrial arrhythmias: a massively parallel sequencing study. Circ Cardiovasc Genet. 2014;7:741–50.
73. Postma AV, Van Engelen K, Van De Meerakker J, Rahman T, Probst S, Baars MJH, Bauer U, Pickardt T, Sperling SR, Berger F, Moorman AFM, Mulder BJM, Thierfelder L, Keavney B, Goodship J, Klaassen S. Mutations in the sarcomere gene MYH7 in Ebstein anomaly. Circ Cardiovasc Genet. 2011;4:43–50.
74. Villard E, Duboscq-Bidot L, Charron P, Benaiche A, Conraads V, Sylvius N, Komajda M. Mutation screening in dilated cardiomyopathy: prominent role of the beta myosin heavy chain gene. Eur Heart J. 2005;26:794–803.
75. Duboscq-Bidot L, Xu P, Charron P, Neyroud N, Dilanian G, Millaire A, Bors V, Komajda M, Villard E. Mutations in the

Z-band protein myopalladin gene and idiopathic dilated cardiomyopathy. Cardiovasc Res. 2008;77:118–25.
76. Purevjav E, Arimura T, Augustin S, Huby AC, Takagi K, Nunoda S, Kearney DL, Taylor MD, Terasaki F, Bos JM, Ommen SR, Shibata H, Takahashi M, Itoh-satoh M, Mckenna WJ, Murphy RT, Labeit S, Yamanaka Y, Machida N, Park JE, Alexander PMA, Weintraub RG, Kitaura Y, Ackerman MJ, Kimura A, Towbin JA. Molecular basis for clinical heterogeneity in inherited cardiomyopathies due to myopalladin mutations. Hum Mol Genet. 2012;21:2039–53.
77. Mogensen J, Murphy RT, Shaw T, Bahl A, Redwood C, Watkins H, Burke M, Elliott PM, McKenna WJ. Severe disease expression of cardiac troponin C and T mutations in patients with idiopathic dilated cardiomyopathy. J Am Coll Cardiol. 2004;44:2033–40.
78. Menon SC, Michels VV, Pellikka PA, Ballew JD, Karst ML, Herron KJ, Nelson SM, Rodeheffer RJ, Olson TM. Cardiac troponin T mutation in familial cardiomyopathy with variable remodeling and restrictive physiology. Clin Genet. 2008;74:445–54.
79. Luedde M, Ehlermann P, Weichenhan D, Will R, Zeller R, Rupp S, Müller A, Steen H, Ivandic BT, Ulmer HE, Kern M, Katus HA, Frey N. Severe familial left ventricular non-compaction cardiomyopathy due to a novel troponin T (TNNT2) mutation. Cardiovasc Res. 2010;86:452–60.
80. Møller DV, Andersen PS, Hedley P, Ersbøll MK, Bundgaard H, Moolman-Smook J, Christiansen M, Køber L. The role of sarcomere gene mutations in patients with idiopathic dilated cardiomyopathy. Eur J Hum Genet. 2009;17:1241–9.
81. Olson TM, Kishimoto NY, Whitby FG, Michels VV. Mutations that alter the surface charge of alpha-tropomyosin are associated with dilated cardiomyopathy. J Mol Cell Cardiol. 2001;33:723–32.
82. Probst S, Oechslin E, Schuler P, Greutmann M, Boyé P, Knirsch W, Berger F, Thierfelder L, Jenni R, Klaassen S. Sarcomere gene mutations in isolated left ventricular noncompaction cardiomyopathy do not predict clinical phenotype. Circ Cardiovasc Genet. 2011;4:367–74.
83. Hinson JT, Chopra A, Nafissi N, Polacheck WJ, Benson CC, Swist S, Gorham J, Yang L, Schafer S, Sheng CC, Haghighi A, Homsy J, Hubner N, Church G, Cook SA, Linke WA, Chen CS, Seidman JG, Seidman CE. Titin mutations in iPS cells define sarcomere insufficiency as a cause of dilated cardiomyopathy. Science. 2015;349:982–6.
84. Li D, Tapscoft T, Gonzalez O, Burch PE, Quiñones MA, Zoghbi WA, Hill R, Bachinski LL, Mann DL, Roberts R. Desmin mutation responsible for idiopathic dilated cardiomyopathy. Circulation. 1999;100:461–4.
85. Otten E, Asimaki A, Maass A, Van Langen IM, Van Der Wal A, De Jonge N, Van Den Berg MP, Saffitz JE, Wilde AAM, Jongbloed JDH, Van Tintelen JP. Desmin mutations as a cause of right ventricular heart failure affect the intercalated disks. Heart Rhythm. 2010;7:1058–64.
86. Brodehl A, Dieding M, Klauke B, Dec E, Madaan S, Huang T, Gargus J, Fatima A, Šaric T, Cakar H, Walhorn V, Tönsing K, Skrzipczyk T, Cebulla R, Gerdes D, Schulz U, Gummert J, Svendsen JH, Olesen MS, Anselmetti D, Christensen AH, Kimonis V, Milting H. The novel desmin mutant p.A120D impairs filament formation, prevents intercalated disk localization, and causes sudden cardiac death. Circ Cardiovasc Genet. 2013;6:615–23.
87. Tesson F, Sylvius N, Pilotto A, Dubosq-Bidot L, Peuchmaurd M, Bouchier C, Benaiche A, Mangin L, Charron P, Gavazzi A, Tavazzi L, Arbustini E, Komajda M. Epidemiology of desmin and cardiac actin gene mutations in a european population of dilated cardiomyopathy. Eur Heart J. 2000;21:1872–6.
88. Berko BA, Swift M. X-linked dilated cardiomyopathy. N Engl J Med. 1987;316:1186–91.
89. Ortiz-Lopez R, Li H, Su J, Goytia V, Towbin JA. Evidence for a dystrophin missense mutation as a cause of X-linked dilated cardiomyopathy. Circulation. 1997;95:2434–40.
90. Muntoni F, Cau M, Ganau A, Congiu R, Arvedi G, Mateddu A, Marrosu MG, Cianchetti C, Realdi G, Cao A. Brief report: deletion of the dystrophin muscle-promoter region associated with X-linked dilated cardiomyopathy. N Engl J Med. 1993;329:921–5.
91. Arimura T, Hayashi T, Terada H, Lee SY, Zhou Q, Takahashi M, Ueda K, Nouchi T, Hohda S, Shibutani M, Hirose M, Chen J, Park JE, Yasunami M, Hayashi H, Kimura A. A Cypher/ZASP mutation associated with dilated cardiomyopathy alters the binding affinity to protein kinase C. J Biol Chem. 2004;279:6746–52.
92. Vatta M, Mohapatra B, Jimenez S, Sanchez X, Faulkner G, Perles Z, Sinagra G, Lin JH, Vu TM, Zhou Q, Bowles KR, Di Lenarda A, Schimmenti L, Fox M, Chrisco MA, Murphy RT, McKenna W, Elliott P, Bowles NE, Chen J, Valle G, Towbin JA. Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular non-compaction. J Am Coll Cardiol. 2003;42:2014–27.
93. Theis JL, Martijn Bos J, Bartleson VB, Will ML, Binder J, Vatta M, Towbin JA, Gersh BJ, Ommen SR, Ackerman MJ. Echocardiographic-determined septal morphology in Z-disc hypertrophic cardiomyopathy. Biochem Biophys Res Commun. 2006;351:896–902.
94. Xing Y, Ichida F, Matsuoka T, Isobe T, Ikemoto Y, Higaki T, Tsuji T, Haneda N, Kuwabara A, Chen R, Futatani T, Tsubata S, Watanabe S, Watanabe K, Hirono K, Uese K, Miyawaki T, Bowles KR, Bowles NE, Towbin JA. Genetic analysis in patients with left ventricular noncompaction and evidence for genetic heterogeneity. Mol Genet Metab. 2006;88:71–7.
95. Parks S, Kushner J, Nauman D. Lamin A/C mutation analysis in a cohort of 324 unrelated patients with idiopathic or familial dilated cardiomyopathy. Am Heart J. 2008;156:161–9.
96. Olson TM, Michels VV, Ballew JD, Reyna SP, Karst ML, Herron KJ, Horton SC, Rodeheffer RJ, Anderson JL. Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. JAMA. 2005;293:447–54.
97. Cheng J, Morales A, Siegfried JD, Li D, Norton N, Song J, Gonzalez-Quintana J, Makielski JC, Hershberger RE. SCN5A rare variants in familial dilated cardiomyopathy decrease peak sodium current depending on the common polymorphism H558R and common splice variant Q1077del. Clin Transl Sci. 2010;3:287–94.
98. Laurent G, Saal S, Amarouch MY, Béziau DM, Marsman RFJ, Faivre L, Barc J, Dina C, Bertaux G, Barthez O, Thauvin-Robinet C, Charron P, Fressart V, Maltret A, Villain E, Baron E, Mérot J, Turpault R, Coudière Y, Charpentier F, Schott JJ, Loussouarn G, Wilde AAM, Wolf JE, Baró I, Kyndt F, Probst V. Multifocal ectopic Purkinje-related premature contractions: a new SCN5A-related cardiac channelopathy. J Am Coll Cardiol. 2012;60:144–56.
99. Mann SA, Castro ML, Ohanian M, Guo G, Zodgekar P, Sheu A, Stockhammer K, Thompson T, Playford D, Subbiah R, Kuchar D, Aggarwal A, Vandenberg JI, Fatkin D. R222Q SCN5A mutation is associated with reversible ventricular ectopy and dilated cardiomyopathy. J Am Coll Cardiol. 2012;60:1566–73.
100. Nair K, Pekhletski R, Harris L, Care M, Morel C, Farid T, Backx PH, Szabo E, Nanthakumar K. Escape capture bigeminy: phenotypic marker of cardiac sodium channel voltage sensor mutation R222Q. Heart Rhythm. 2012;9:1681–8.
101. McNair WP, Ku L, Taylor MRG, Fain PR, Dao D, Wolfel E, Mestroni L. SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia. Circulation. 2004;110:2163–7.
102. Bione S, D'Adamo P, Maestrini E, Gadeon AK, Bolhuis PAA, Toniolo D, Gedeon AK. A novel X-linked gene, G4.5 is responsible for Barth syndrome. Nat Genet. 1996;12:385–9.

103. D'Adamo P, Fassone L, Gedeon A, Janssen EA, Bione S, Bolhuis PA, Barth PG, Wilson M, Haan E, Orstavik KH, Patton MA, Green AJ, Zammarchi E, Donati MA, Toniolo D. The X-linked gene G4.5 is responsible for different infantile dilated cardiomyopathies. Am J Hum Genet. 1997;61:862–7.
104. Schmitt JP, Kamisago M, Asahi M, Li GH, Ahmad F, Mende U, Kranias EG, MacLennan DH, Seidman JG, Seidman CE. Dilated cardiomyopathy and heart failure caused by a mutation in phospholamban. Science. 2003;299:1410–3.
105. DeWitt MM, MacLeod HM, Soliven B, McNally EM. Phospholamban R14 deletion results in late-onset, mild, hereditary dilated cardiomyopathy. J Am Coll Cardiol. 2006;48:1396–8.
106. Norgett EE, Hatsell SJ, Carvajal-Huerta L, Cabezas JC, Common J, Purkis PE, Whittock N, Leigh IM, Stevens HP, Kelsell DP. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. Hum Mol Genet. 2000;9:2761–6.
107. Chalabreysse L, Senni F, Bruyère P, Aime B, Ollagnier C, Bozio A, Bouvagnet P. A new hypo/oligodontia syndrome: Carvajal/Naxos syndrome secondary to desmoplakin-dominant mutations. J Dent Res. 2011;90:58–64.
108. Arimura T, Ishikawa T, Nunoda S, Kawai S, Kimura A. Dilated cardiomyopathy-associated BAG3 mutations impair Z-disc assembly and enhance sensitivity to apoptosis in cardiomyocytes. Hum Mutat. 2011;32:1481–91.
109. Norton N, Li D, Rieder MJ, Siegfried JD, Rampersaud E, Züchner S, Mangos S, Gonzalez-Quintana J, Wang L, McGee S, Reiser J, Martin E, Nickerson DA, RE H. Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy. Am J Hum Genet. 2011;88:273–82.
110. Schönberger J, Wang L, Shin JT, Kim SD, Depreux FFS, Zhu H, Zon L, Pizard A, Kim JB, Macrae CA, Mungall AJ, Seidman JG, Seidman CE. Mutation in the transcriptional coactivator EYA4 causes dilated cardiomyopathy and sensorineural hearing loss. Nat Genet. 2005;37:418–22.
111. Li D, Parks SB, Kushner JD, Nauman D, Burgess D, Ludwigsen S, Partain J, Nixon RR, Allen CN, Irwin RP, Jakobs PM, Litt M, Hershberger RE. Mutations of presenilin genes in dilated cardiomyopathy and heart failure. Am J Hum Genet. 2006;79:1030–9.
112. Carballo S, Robinson P, Otway R, Fatkin D, Jongbloed JDH, De Jonge N, Blair E, Van Tintelen JP, Redwood C, Watkins H. Identification and functional characterization of cardiac troponin I as a novel disease gene in autosomal dominant dilated cardiomyopathy. Circ Res. 2009;105:375–82.
113. Hassel D, Dahme T, Erdmann J, Meder B, Huge A, Stoll M, Just S, Hess A, Ehlermann P, Weichenhan D, Grimmler M, Liptau H, Hetzer R, Regitz-Zagrosek V, Fischer C, Nürnberg P, Schunkert H, Katus H, Rottbauer W. Nexilin mutations destabilize cardiac Z-disks and lead to dilated cardiomyopathy. Nat Med. 2009;15:1281–8.
114. Olson TM, Illenberger S, Kishimoto NY, Huttelmaier S, Keating MT, Jockusch BM. Metavinculin mutations alter actin interaction in dilated cardiomyopathy. Circulation. 2002;105:431–7.
115. Murakami T, Hayashi YK, Noguchi S, Ogawa M, Nonaka I, Tanabe Y, Ogino M, Takada F, Eriguchi M, Kotooka N, Campbell KP, Osawa M, Nishino I. Fukutin gene mutations cause dilated cardiomyopathy with minimal muscle weakness. Ann Neurol. 2006;60:597–602.
116. Knöll R, Postel R, Wang J, Krätzner R, Hennecke G, Vacaru AM, Vakeel P, Schubert C, Murthy K, Rana BK, Kube D, Knöll G, Schäfer K, Hayashi T, Holm T, Kimura A, Schork N, Toliat MR, Nürnberg P, Schultheiss HP, Schaper W, Schaper J, Bos E, Den Hertog J, Van Eeden FJM, Peters PJ, Hasenfuss G, Chien KR, Bakkers J. Laminin-α4 and integrin-linked kinase mutations cause human cardiomyopathy via simultaneous defects in cardiomyocytes and endothelial cells. Circulation. 2007;116:515–25.
117. Taylor MRG, Slavov D, Gajewski A, Vlcek S, Ku L, Fain PR, Carniel E, Di Lenarda A, Sinagra G, Boucek MM, Cavanaugh J, Graw SL, Ruegg P, Feiger J, Zhu X, Ferguson DA, Bristow MR, Gotzmann J, Foisner R, Mestroni L. Thymopoietin (lamina-associated polypeptide 2) gene mutation associated with dilated cardiomyopathy. Hum Mutat. 2005;26:566–74.
118. Gotic I, Leschnik M, Kolm U, Markovic M, Haubner BJ, Biadasiewicz K, Metzler B, Stewart CL, Foisner R. Lamina-associated polypeptide 2alpha loss impairs heart function and stress response in mice. Circ Res. 2010;106:346–53.
119. Posch MG, Posch MJ, Geier C, Erdmann B, Mueller W, Richter A, Ruppert V, Pankuweit S, Maisch B, Perrot A, Buttgereit J, Dietz R, Haverkamp W, Ozcelik C. A missense variant in desmoglein-2 predisposes to dilated cardiomyopathy. Mol Genet Metab. 2008;95:74–80.
120. Theis JL, Sharpe KM, Matsumoto ME, Chai HS, Nair AA, Theis JD, De Andrade M, Wieben ED, Michels VV, Olson TM. Homozygosity mapping and exome sequencing reveal GATAD1 mutation in autosomal recessive dilated cardiomyopathy. Circ Cardiovasc Genet. 2011;4:585–94.
121. Burkett EL, Hershberger RE. Clinical and genetic issues in familial dilated cardiomyopathy. J Am Coll Cardiol. 2005;45:969–81.
122. Baig MK, Goldman JH, Caforio ALP, Coonar AS, Keeling PJ, McKenna WJ. Familial dilated cardiomyopathy: cardiac abnormalities are common in asymptomatic relative and may represent early disease. J Am Coll Cardiol. 1998;31:195–201.
123. Mahon NG, Murphy RT, MacRae CA, Caforio ALP, Elliott PM, McKenna WJ. Echocardiographic evaluation in asymptomatic relatives of patients with dilated cardiomyopathy reveals preclinical disease. Ann Intern Med. 2005;143.
124. Charron P, Arad M, Arbustini E, Basso C, Bilinska Z, Elliott P, Helio T, Keren A, McKenna WJ, Monserrat L, Pankuweit S, Perrot A, Rapezzi C, Ristic A, Seggewiss H, van Langen I, Tavazzi L. Genetic counselling and testing in cardiomyopathies: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2010;31:2715–26.
125. Hershberger RE, Lindenfeld J, Mestroni L, Seidman CE, Taylor MRG, Towbin JA. Genetic evaluation of cardiomyopathy-a Heart Failure Society of America practice guideline. J Card Fail. 2009;15:83–97.
126. Liu GS, Morales A, Vafiadaki E, Lam CK, Cai WF, Haghighi K, Adly G, Hershberger RE, Kranias EG. A novel human R25C-phospholamban mutation is associated with super-inhibition of calcium cycling and ventricular arrhythmia. Cardiovasc Res. 2015;107:164–74.
127. Huang W, Liang J, Yuan C-C, Kazmierczak K, Zhou Z, Morales A, McBride KL, Fitzgerald-Butt SM, Hershberger RE, Szczesna-Cordary D. Novel familial dilated cardiomyopathy mutation in MYL2 affects the structure and function of myosin regulatory light chain. FEBS J. 2015;282:2379–93.

Arrhythmogenic Cardiomyopathy

6

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Abstract

Arrhythmogenic cardiomyopathy (ACM) is a progressive heart muscle disease characterized by ventricular fibrofatty replacemen. Patients typically present between the 2nd and 4th decade of life with exercise-induced tachycardia episodes, but sudden death at already young age also occurs.

A large subcategory of ACM is arrhythmogenic right ventricular cardiomyopathy (ARVC) in which the right ventricle is primarily affected. However, the left ventricle is also frequently involved and predominantly LV disease may also occur. The causative genes frequently encode proteins of mechanical cell junctions (JUP, PKP2, DSG2, DSC2, DSP) and account for intercalated disk remodeling. ARVD/C is inherited as an autosomal-dominant trait, with variable expression. The diagnosis is made according to a set of Task Force criteria from 2011, which are based on family history, depolarization and repolarization abnormalities, ventricular arrhythmias with a LBBB morphology, functional and structural alterations of the RV, and fibro-fatty replacement in endomyocardial biopsy. Two-dimensional echocardiography, cineangiography and magnetic resonance are the imaging tools to visualize structural-functional abnormalities. The main differential diagnoses are idiopathic RVOT tachycardia, myocarditis and sarcoidosis. Palliative therapy consists of antiarrhythmic drugs, catheter ablation and ICD. Young age, proband status, overt left ventricular involvement, VT, syncope and previous cardiac arrest are the major risk factors for adverse prognosis.

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Introduction

Arrhythmogenic cardiomyopathy (ACM) is a progressive heart muscle disease, characterized histologically by ventricular fibrofatty replacement and clinically by ventricular arrhythmias already in the early stage, followed by structural and functional abnormalities usually at later stages. On the contrary, in dilated cardiomyopathy (DCM), a reversed sequence pattern with arrhythmias limited to the advanced late disease stage is more typical.

A large subcategory of ACM is arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) with primarily right ventricular (RV) involvement [1–3]. However, the left ventricle (LV) is also frequently involved in

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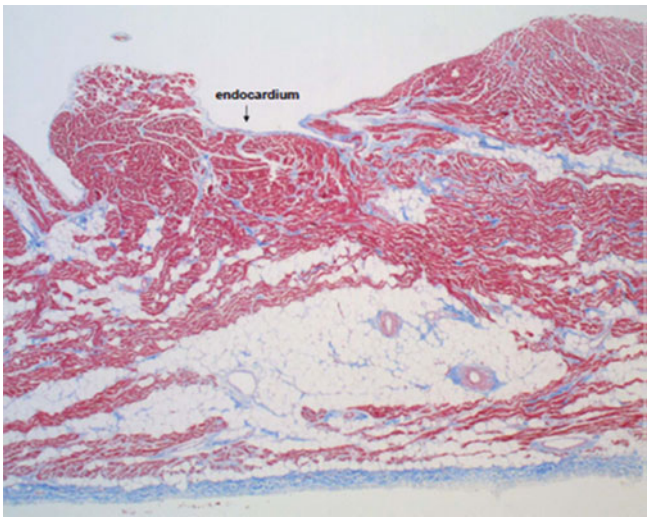

Fig. 6.1 Histology of right ventricle of a 13-year-old girl who died suddenly during exercise. AZAN staining (400×) with cardiac myocytes (red), collagen (blue), and adipocytes (white). Shown is the typical pattern of ACM with strands of fibrosis reaching all the way to the endocardium. Bundles of cardiac myocytes are embedded in between the fibrotic strands, particularly in the subendocardial layers. These interconnecting bundles of myocytes give rise to activation delay and reentrant circuits, the typical electrophysiologic substrate for ventricular arrhythmias in ACM

ARVD/C, and predominantly LV disease may also occur [4]. Recently, these observations favored ACM as more appropriate terminology, although ARVD, ARVC, ARVD/C, and ARVC/D are still used by many authors, particularly to denote the typical forms with apparently predominant RV involvement.

Affected individuals usually present between the second and fourth decade of life with arrhythmias usually originating from the RV. However, ACM can also be the cause of sudden death already in adolescence, mainly in athletes [5]. From autopsy studies, it is known that massive amounts of fibrofatty tissue can replace major parts of normal myocardium even in young teenagers (Fig. 6.1) [3].

The first series of ARVD/C patients was published in 1982, when it was called a disease in which "the right ventricular musculature is partially or totally absent and is replaced by fatty and fibrous tissue" [1]. At that time, the disease was thought to be a defect in RV development, which is why it was classified as a "dysplasia." In the past 30 years, increased insight into the development of the disease as well as the discovery of pathogenic mutations involved, led to the current concept of ACM as a genetically determined "cardiomyopathy" [3, 6].

The molecular genetic era has provided new insights into the understanding that ACM is frequently a desmosomal disease resulting from defective cell adhesion proteins. The first disease-causing mutation in the gene encoding the desmosomal protein plakoglobin (*JUP*) was identified in patients with Naxos disease, an autosomal recessive variant of ACM [7]. Its discovery pointed research in the direction of other desmosomal genes. Until 2004, evidence for mutations underlying the autosomal dominantly inherited ACM had been very limited, with three genes and six loci being identified [8–16]. The desmoplakin gene (*DSP*) was the first desmosomal gene to be associated with the autosomal dominant form of ACM [16]. It was followed by discovery of pathogenic mutations in plakophilin-2 (*PKP2*), desmoglein-2 (*DSG2*), and desmocollin-2 (*DSC2*), also components of the cardiac desmosome [17–19]. Recent studies have shown that altered desmosomal function is associated with trafficking abnormalities and redistribution of other intercalated disc proteins giving rise to primary electrical changes and disruption of the myocardial architecture, both leading to activation delay and thereby life-threatening ventricular arrhythmias [20, 21].

In rare cases, autosomal dominant ACM has been linked to other genes seemingly unrelated to the cell adhesion complex, that is, the genes encoding the cardiac ryanodine receptor (*RyR2*), the transforming growth factor-β3 gene (*TGFβ3*), and transmembrane protein 43 (*TMEM43*) [14, 15, 22]. More recently, a specific nondesmosomal founder mutation in the gene-encoding phospholamban (*PLN*) appeared to be associated with a specific form of biventricular ACM [23]. In addition, mutations in the genes encoding desmin (*DES*), lamins A and C (*LMNA*), titin (*TTN*), and αT-catenin (*CTNNA3*), which binds plakophilins, have been identified in patients with ACM [24–27].

Currently, pathogenic mutations are identified in approximately 60 % of patients with ACM, mainly in desmosomal genes. In the large registries from the Johns Hopkins Hospital, the Netherlands and Switzerland, *PKP2* mutations were the most frequent [28].

Estimations of the prevalence of ACM in the general populations vary from 1:2000 to 1:5000 [29]. The exact prevalence, however, is unknown and is possibly higher because of many nondiagnosed or misdiagnosed cases.

The disease appears to be especially common in adolescents and young adults in Northern Italy, accounting for approximately 11 % of cases of sudden cardiac death (SCD) overall and even 22 % in athletes [30, 31]. In as many as 20 % of SCD occurring in people under 35 years of age, features of ACM were detected at postmortem evaluation [31]. In nearly half of them, no prior symptoms had been reported. In comparison, ACM accounted for only 4 % of SCD cases in young competitive athletes in North America [32]. Founder mutations have been identified, for example, in the Netherlands, Newfoundland, and South Africa, and these could in part explain the difference in prevalence in different geographical areas [33–35].

Molecular and Genetic Background

Desmosomes and Other Intercalated Disc Structures

The functional and structural integrity of cardiac myocytes is enabled by cell adhesion junctions in the intercalated disc. Intercalated discs are located between cardiomyocytes at their longitudinal ends and contain three different kinds of intercellular connections: desmosomes, adherens junctions, and gap junctions. Desmosomes are important for cell–cell adhesion and are predominantly found in tissues that experience mechanical stress, the heart, and epidermis. They couple cytoskeletal elements to the plasma membrane at cell–cell adhesions. Desmosomes also protect the other components of the intercalated disc from mechanical stress and are involved in structural organization of the intercalated disc. Desmosomes consist of multiple proteins, which belong to three different families:

1. Transmembranous cadherins: desmogleins and desmocollins
2. Linker armadillo repeat proteins: plakoglobin and plakophilin
3. Plakins: desmoplakin and plectin

Figure 6.2 schematically represents the organization of the various proteins in the cardiac desmosome.

Within desmosomes, cadherins are connected to armadillo proteins, which for their parts interact with plakins. The plakins anchor the desmosomes to intermediate filaments, mainly desmin. Thereby, they form a three-dimensional scaffold providing mechanical support.

Adherens junctions act as bridges that link the actin filaments within sarcomeres of neighboring cells. These junctions are involved in force transmission and together with desmosomes, these mechanical junctions act as "spot welds" to create membrane domains that are protected from shear stress caused by contraction of the neighboring cells. Furthermore, they facilitate assembly and maintenance of gap junctions, securing intercellular electrical coupling.

Cardiomyocytes are individually bordered by a lipid bilayer, which gives a high degree of electrical insulation. The electrical current that forms the impulse for mechanic contraction can travel from one cell to the other via gap junctions. Gap junctions provide electrical coupling by enabling ion transfer between cells. The number, size, and distribution of gap junctions all influence impulse propagation in cardiac muscle. Consequently, alterations in function or structure of gap junctions can lead to intercellular propagation disturbances and contribute to arrhythmogenesis [37].

In addition to desmosomes, adherens junctions, and gap junctions, the intercalated disc also contains ion channels. In particular, the voltage-gated sodium channel subunit $Na_v1.5$ is critically important for rapid transmission of the myocardial action potential.

Recent studies demonstrates a close interaction between the various components of the intercalated disc, linking mechanical, and electrical phenomena, which has been referred to as the cardiac connexome [20, 38, 39]. In mouse models of ACM, desmosomal mutations were associated

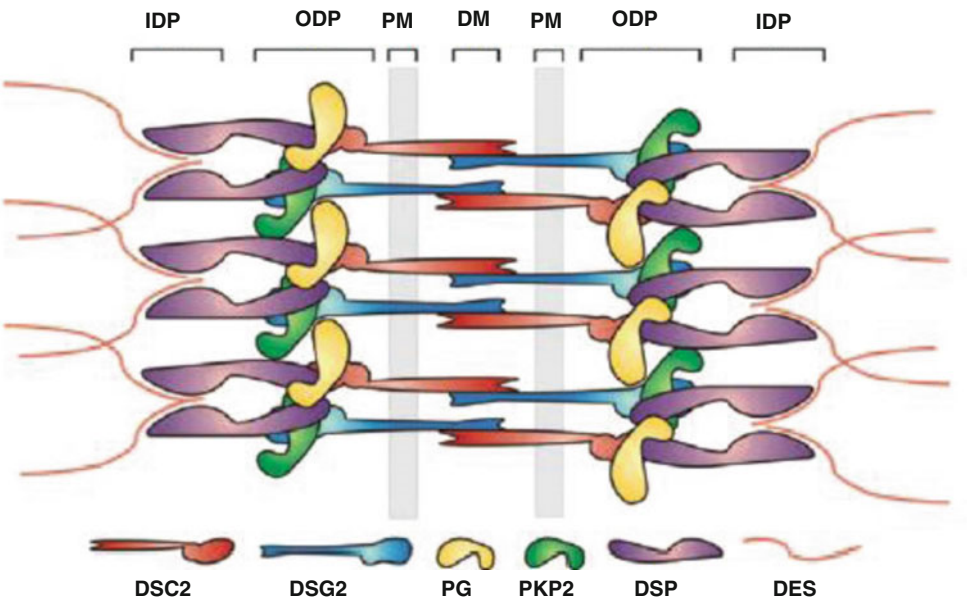

Fig. 6.2 Schematic representation of the molecular organization of cardiac desmosomes. The plasma membrane (*PM*) spanning proteins desmocollin-2 (*DSC2*) and desmoglein-2 (*DSG2*) interact in the extracellular space at the dense midline (*DM*). At the cytoplasmic side, they interact with plakoglobin (*PG*) and plakophilin-2 *(PKP2)* at the outer dense plaque (*ODP*). *PKP2* and *PG* interact also with desmoplakin (*DSP*). At the inner dense plaque (*IDP*), the C-terminus of *DSP* anchors the intermediate filament desmin *(DES).* (Reprint with permission from Van Tintelen et al. Curr Opin Cardiol 2007 [36])

with reduced peak sodium current by impaired $Na_v1.5$ function and inducibility of ventricular fibrillation prior to myocardial fibrofatty infiltration [40]. Intercalated disc abnormalities, reduced Na(+) current density, and conduction slowing in *PKP-2* mutant mice occurred prior to cardiomyopathic changes [38].

Desmosomal Dysfunction and ACM Pathophysiology

Although the functions of different parts of the intercalated disc seem clear, the exact mechanism through which the mutations of desmosomal genes exactly cause disease remains to be elucidated. Various hypotheses, all based on the different functions of desmosomes, have been proposed.

First of all, genetic defects in a desmosomal protein are thought to lead to impairment in mechanical function provoking detachment of myocytes at the intercalated discs, particularly under condition of mechanical stress (like that occurring during competitive sports activity). Such defective mechanical connection followed by mechanical and electrical uncoupling of cardiomyocytes leads to cell death with fibrofatty replacement. A recent study Swiss has shown that proapoptotic and proadipogenic molecules are upregulated in myocardial tissue of patients with ACM independent of the underlying genetic mutation as compared to patients with DCM and healthy controls [41]. Interconnecting bundles of surviving myocardium embedded in the fibrofatty tissue lead to lengthening of conduction pathways (Fig. 6.1), and load mismatch due to instantaneous marked enlargement of the amount of excitable myocardial tissue at pivotal points of these bundles, and at the transition of small bundles into large excitable areas. This results in marked activation delay, which is the key mechanism for reentry and thereby ventricular tachycardia (VT). Previous invasive electrophysiologic studies have, by various mapping techniques, confirmed that sustained VT in patients with ACM is due to reentry circuits in areas of abnormal myocardium [42]. In this structural model, environmental factors, such as exercise or inflammation, from viral infection could aggravate impaired adhesion and accelerate disease progression. The RV might be more vulnerable to disease than the LV because of its thinner walls and its physiological dilatory response to exercise.

Second, recent studies have shown that impairment of cell–cell adhesion due to changes in desmosomal components may affect amount and distribution of other intercalated disc proteins, including connexin43, the major protein forming gap junctions in the ventricular myocardium [20, 43, 44]. This was shown for *DSP* and *JUP* by Western blotting and confocal immunofluorescence techniques, but alterations in other desmosomal components, such as *PKP2*, *DSG2*, and *DSC2*, are thought to have similar effects. Interestingly, using identical staining techniques, abnormalities in the desmosomal components have also been identified in ACM patients with *LMNA*, *DES*, and *PLN* mutations [23–25, 45]. Changes in number and function of gap junctions will diminish intercellular electrical coupling. This may contribute to intraventricular activation delay, and the substrate for reentry.

The third hypothesis involves the canonical Wnt/β--catenin-signaling pathway. Plakoglobin can localize to both the plasma membrane and the nucleus. It was demonstrated that disruption of desmoplakin frees plakoglobin from the plasma membrane allowing it to translocate to the nucleus and suppress canonical Wnt/β-catenin signaling. Wnt signaling can inhibit adipogenesis by preventing mesodermal precursors from differentiating into adipocytes [46]. Suppression of Wnt signaling by plakoglobin nuclear localization could, therefore, promote the differentiation to adipose tissue in the cardiac myocardium in patients with ACM [47].

Finally, redistribution of $Na_v1.5$ due to reduced transportation of the channel toward the intercalated disc cell membrane appeared to be related to reduced *PKP2* [38].

The pathophysiological mechanisms proposed above are not mutually exclusive and could occur at the same time.

Genetic Background

Two patterns of inheritance have been described in ACM. The most common or classical form of ACM (i.e., ARVD/C) is inherited as an autosomal dominant trait. The rare Naxos disease and Carvajal syndrome are usually inherited autosomal recessively, although autosomal dominant forms have been described in these cardiocutaneous syndromes [48] Table 6.1 summarizes the different genes involved in ACM with the corresponding phenotypes.

Autosomal Recessive ACM

In Naxos disease, the affected individuals were found to be homozygous for a two base pair deletion in the *JUP* gene [7]. All patients who are homozygous for this mutation have diffuse palmoplantar keratosis and woolly hair in infancy; children usually have no cardiac symptoms but may have electrocardiographic (ECG) abnormalities and nonsustained ventricular arrhythmias [49]. In an Arab family, an autosomal recessive mutation in the desmoplakin gene caused ACM, also combined with woolly hair, and a pemphigus-like skin disorder [50]. A different autosomal recessive disease, Carvajal syndrome, is associated with a desmoplakin gene mutation, and is manifested by woolly hair, epidermolytic

Table 6.1 Mutated genes with concurrent type of arrhythmogenic cardiomyopathy (*ACM*) (Modified from Van Tintelen et al. Curr Opin Cardiol 2007 [36])

	Gene	Type of disease	Inheritance trait
Desmosomal	*PKP2*	Typical ARVD/C	Autosomal dominant
	DSG2	Typical ARVD/C	Autosomal dominant
	DSC2	Typical ARVD/C	Autosomal dominant
	JUP	Naxos disease	Autosomal recessive
		ARVD/C	Autosomal dominant
	DSP	Carvajal syndrome	Autosomal recessive
		Typical ARVD/C	Autosomal dominant
	CTNNA3	LDAC	Autosomal dominant
		ARVD/C	Autosomal dominant
Nondesmosomal	*RyR2*	CPVT	Autosomal dominant
		ARVD/C	Autosomal dominant
	TGF-β	Typical ARVD/C	Autosomal dominant
	TMEM43	ARVD/C	Autosomal dominant
	PLN	ARVD/C and LDAC	Autosomal dominant
	LMNA	ARVC/D and LDAC	Autosomal dominant
	DES	ARVD/C and LDAC	Autosomal dominant
	TTN	ARVD/C and LDAC	Autosomal dominant

CPVT catecholaminergic polymorphic VT, *LDAC* left dominant arrhythmogenic cardiomyopathy. See text for other abbreviations

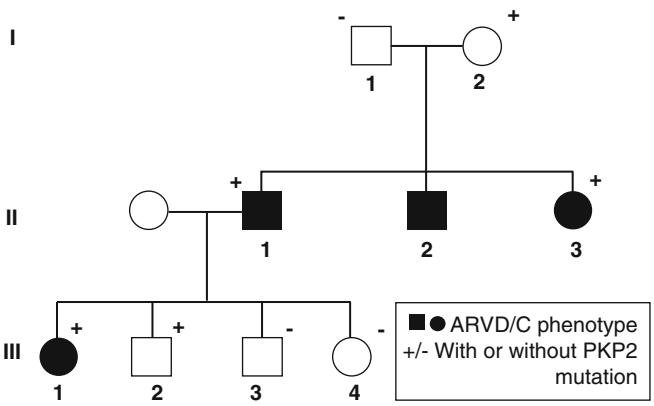

Fig. 6.3 Pedigree of family with arrhythmogenic right ventricular dysplasia/cardiomyopathy (*ARVD/C*) and plakophilin-2 (*PKP2*) mutation. This figure shows the variability in penetrance and clinical expression. Both the 72-year-old grandmother (I:2) and 20-year-old grandson (III:2) are free of any signs of disease, despite carrying the mutation. The proband (II:1) was resuscitated at age 35, his brother (II:2) died suddenly at age 18. Both the proband's sister (II:3) and daughter (III:1) were diagnosed with the disease due to a positive family history, and RV structural abnormalities. The sister (II:3) of the proband has structural and ECG abnormalities, but no arrhythmias

palmoplantar keratoderma, and cardiomyopathy [51]. The cardiomyopathy of Carvajal syndrome was thought to have a predilection for the LV, but subsequent evaluation of a deceased child revealed typical ACM changes in both ventricles [44]. The cardiac phenotype in the Arab family appeared to be classic ARVD/C.

Autosomal Dominant ACM

Overall, mutations in the *PKP2* gene are the most frequently observed in classic ARVD/C. Figure 6.3 shows the pedigree of a family with a *PKP2* mutation, showing an autosomal dominant inheritance pattern with incomplete penetrance and variable expressivity. Incomplete and age-dependent penetrance and clinical variability in ACM is well documented. In five studies from different countries, analyzing 56 to 149 patients with ACM each, *PKP2* mutations were found in 11–51 % of unrelated probands who fulfilled diagnostic task force criteria (TFC) for ARVD/C [17, 52–55]. In a recent large combined study from the USA and the Netherlands, including 1001 ARVD/C probands and family members, pathogenic desmosomal, and nondesmosomal mutations were identified in 63 % of the 439 probands. In 46 % of subjects, a single *PKP2* mutation was found [28]. In addition, multiple mutations were found in 4 % of cases, which in the majority of subjects also included a *PKP2* mutation. In that large cohort, a total of 94 different desmosomal mutations (59 in *PKP2*) were observed. Thus, *PKP2* was mutated in approximately half of the included American and Dutch ACM patients.

Pathogenic mutations in the other desmosomal genes were much less common in the USA–Dutch study: *DSG2* 4 %, *DSC2* 1 %, *JUP* 0.5 %, and *DSP* 3 % [28]. Although *PKP2* mutations are also frequently found in other ACM cohorts from other countries, the distribution of the different genes encoding desmosomal proteins is highly variable [35, 56]. In one Italian study including 134 desmosomal mutation carriers, *DSP* mutations were most frequent (39 %) [57].

Mutations in *DSG2* and *DSC2* encoding transmembranous desmosomal proteins are associated with classic typical ACM [58]. *JUP* is primarily associated with the recessive Naxos disease, although a rare autosomal dominant form has been identified.

Mutations in the gene encoding the intracellular desmosomal component *DSP* lead to "classic ARVD/C" with a clinical presentation of VT, sudden death, and LV involvement as the disease progresses [16, 59, 60]. However, only *DSP* mutations have also been associated with predomi-

nantly left-sided ACM and, as noted earlier, with autosomal recessive disease. A recent study showed that *DSP* mutation carriers are more prone to sudden cardiac death, LV dysfunction and heart failure [61].

Other, Nondesmosomal, Genes

Mutations in the gene encoding the cardiac ryanodine receptor RyR2, which is responsible for calcium release from the sarcoplasmic reticulum, have been described in one Italian ARVD/C family [14]. Affected patients have exercise-induced polymorphic VT [62]. Mutations in *RyR2* have primarily been associated with familial catecholaminergic polymorphic VT without ACM [62]. RyR2 mediates the release of calcium from the sarcoplasmic reticulum that is required for myocardial contraction. The FK506-binding protein (FKBP12.6) stabilizes RyR2, preventing aberrant activation. The mutations in *RyR2* interfere with the interaction with FKBP12.6, increasing channel activity under conditions that simulate exercise [63]. Although the general opinion is that *RyR2* mutations lead to catecholaminergic polymorphic VT, without structural abnormalities, the mutations in ARVD/C have been advocated to act differently from those in familial polymorphic VT without ACM [64–66]. Of note, in a recent Swiss study a patient with definite ARVD/C carried compound heterozygous missense mutations in *RyR2* and *DSG2* [67].

Transforming growth factor-β-3 (TGFβ3) regulates the production of extracellular matrix components and modulates expression of genes encoding desmosomal proteins. Its gene has been mapped to chromosome 14. Initial sequencing studies failed to identify any disease-causing mutations in the exonic regions of *TGFβ3*. This led to screening of the promoter and untranslated regions, where a mutation of the *TGFβ3* gene was found in all clinically affected members of a large family with ACM [15]. The mutation is predicted to produce an amino acid substitution in a short peptide with an inhibitory role in TGFβ3 regulation. The implication of these observations is that regulatory mutations resulting in overexpression of TGFβ3 may contribute to the development of ACM in these families. The TGFβ family of cytokines stimulates production of components of the extracellular matrix. It is therefore possible that enhanced TGFβ activity can lead to myocardial fibrosis. However, genetic analysis of two other families with ACM failed to identify mutations in any of the regions of the *TGFβ3* gene. Recently, a mutation in exon 1 of *TGFβ3* has been described in a patient with ARVD/C from a Chinese population [56].

A missense mutation (p.S358L) in the *TMEM43* gene was found in 15 unrelated ACM families from a genetically isolated population in New Foundland and caused a fully penetrant, sex-influenced (males at increased risk), high-risk form of ARVD/C [22]. The *TMEM43* gene contains the response element for PPAR gamma, an adipogenic transcription factor. The *TMEM43* gene mutation is thought to cause dysregulation of an adipogenic pathway regulated by PPAR gamma, which may explain the fibrofatty replacement of myocardium in patients with ACM. Of interest, Milting et al. have recently demonstrated that this mutation was imported from Europe, increases the stiffness of the cell nucleus, and due to its deleterious clinical phenotype, the authors suggest that this mutation should be checked in any case of ACM [68].

Recently, in the Netherlands, the c.40_42delAGA (p.Arg14del) founder mutation in the phospholamban (*PLN*) gene was identified in a large series of patients diagnosed with either DCM or ARVD/C [23, 69, 70]. This specific *PLN* mutation was found in 5 % of the 439 probands of the combined USA–Dutch ACM cohort and appeared to be the most frequently observed individual gene mutation, after all *PKP2* mutations together [28]. However, nearly all *PLN* mutation carriers were due to a founder effect in the Netherlands, where *PLN* is the most frequently observed non-desmosomal mutation in ACM patients. *PLN* is associated with ACM with frequently more LV manifestation and LV dominant forms than most desmosomal mutations, although the histology of fibrofatty alteration is similar [69]. Onset of overt disease is usually later than in desmosomal mutation carriers. However, *PLN* is much more often associated with severe LV dysfunction and heart failure. These more prominent LV features show similarity with *DSP* mutation carriers and are clearly in favor of ACM as preferred terminology. The pathophysiologic mechanism of the *PLN* mutation underlying ACM and specific phenotypic abnormalities is unknown yet. Intra- and extracellular calcium levels are involved in desmosome assembly and disassembly [71]. Hypotheses of PLN-mediated increased cytosolic calcium levels and thereby desmosome disassembly have been described [23]. In line with these hypotheses, it was recently shown that phospholamban expression is increased in myocardial tissue from patients with ACM independent of the underlying genetic mutation as compared to patients with DCM and healthy controls [41]. The gene-encoding lamins A and C, parts of the nuclear lamina, a protein network supporting the inner nuclear membrane, are believed to be involved in left dominant forms of ACM, although *LMNA* mutations have also been identified in patients fulfilling the ACM task force criteria [25, 72]. The patients and family members also demonstrate clinical features suggestive of a cardiac laminopathy including conduction disease and atrial fibrillation.

Desmin is a large protein connected to the cardiac desmosome through desmoplakin. Mutations in *DES* have been identified in patients diagnosed with ACM including a large multigenerational family from Sweden [24, 73, 74]. ACM's typical histological features were also found in these patients [24]. Some of the patients also depict typical clinical desminopathy-related features like neuromuscular disease or cardiac conduction disease. Interestingly, in different fami-

lies with *DES* mutations, right ventricular involvement has also been described [45, 75].

Titin is another protein that is functionally linked to the desmosome, since the titin filament connects to the transitional junction at the intercalated disc. Mutations in *TTN* have been described in ACM [26]. Because rare *TTN* variants, leading to a truncated protein, can also be identified in control populations, its pathogenicity might be questioned. The *TTN* mutations identified in ACM have so far only been missense mutations. These are even more difficult to interpret than mutations leading to a truncated protein. However, one of the patients belonged to a large multigeneration family with six affected individuals with segregation of the *TTN* missense mutation with the phenotype [26].

Mutations in the gene-encoding alpha-T catenin (*CTNNA3*) have only recently been described [27]. This protein is part of the area composita, a mixed-type junctional structure composed of both desmosomal and adherens junctional proteins. Mutations were identified in two of 76 desmosomal gene-mutation-negative patients studied [27].

Recently, next-generation sequencing showed that sarcomeric genetic mutations/variants are associated with possible or borderline diagnosis of ACM resulting in a disease spectrum, including DCM or phenocopies of ACM [67].

Genetics and Prognosis

ACM has an incomplete penetrance and extremely variable clinical expression. For instance, family screening has identified pathogenic mutation carriers, who had stayed free of any sign of disease up to over 70 years of age (Fig. 6.3).

Although from a genetic point of view, both men and women have to be equally affected, men are more frequently diagnosed with ACM than women. However, as many women as men do show at least some signs of disease, but women more often do not fulfil enough criteria to meet the diagnosis. In a recent combined Dutch–USA study with 577 ACM-associated mutation carriers, sustained arrhythmia and sudden death occurred more often in men compared to women [61]. While female sex was a risk factor for ACM diagnosis among at-risk family members, men with ACM had a higher likelihood of experiencing VT or VF than female patients [76]. This difference in severity of disease expression may be due to more frequent and more vigorous sports activities in men [77]. Also, hormonal factors may play a role.

The effect of genetic background on prognosis is clearly demonstrated by the identification of multiple pathogenic mutations, which has been reported in 4–16 % of the studied ACM populations [57, 61]. Carrying more than a single mutation, either being digenic, homozygous, or compound heterozygous, was associated with worse arrhythmic and hemodynamic outcome in different studies [57, 61]. However, the type of mutation, being premature truncating, splice site, or missense mutation, did not result in a significant difference in outcome in the USA–Dutch study. As shown in sections 5.2.5 and 5.2.6, mutations in the *DSP*, *PLN*, *and TMEM43* genes are associated with a worse outcome.

When interpreting these data, one should bear in mind that epidemiologic differences in the studied populations certainly exist, and different methods to evaluate the pathogenicity of missense variants have been applied, which may account for the variability of the genetic data.

Clinical Presentation

Patients with ACM typically present between the second and fourth decade of life with arrhythmias originating from the RV. However, in a minority of cases, sudden death, frequently at a young age, is the first disease manifestation [61, 78, 79]. Based on clinicopathologic and patient follow-up studies, four different disease phases have been described for the classical form of ACM, that is, primarily affecting the RV (Table 6.2).

1. Early ACM is often described as “concealed” owing to the frequent absence of clinical findings, although minor ventricular arrhythmias such as premature ventricular complexes (PVC) and subtle structural changes may be found. Although patients tend to be asymptomatic, they may nonetheless be at risk of sudden death, mainly during intense exercise.
2. The overt phase follows, in which patients suffer from palpitations, syncope, and ventricular arrhythmias of left bundle branch block morphology, ranging from isolated PVC to sustained monomorphic VT and ventricular fibrillation (VF).
3. The third phase is characterized by RV failure due to progressive loss of myocardium with severe dilatation and systolic dysfunction, in the presence of preserved LV function.

Table 6.2 Different phases of disease severity

Phase	Characteristics
1. Concealed	Asymptomatic patients with possibly only minor ventricular arrhythmia and subtle structural changes However, risk of sudden death
2. Overt	Symptoms due to LBBB VT or multiple premature complexes, with more obvious structural RV abnormalities
3. RV failure	With relatively preserved LV function
4. Biventricular	Significant overt LV involvement

LBBB left bundle branch block, *VT* ventricular tachycardia, *RV* right ventricle, *LV* left ventricle

4. Biventricular failure occurs, due to LV involvement. This phase may mimic DCM and may require cardiac transplantation.

In the initially described classical form of ARVD/C, the RV is primarily affected, with usually in a later stage, overt LV involvement. Two additional patterns of disease have been identified by clinicogenetic characterization of families. These are the left dominant phenotype, with early and predominant LV manifestations (*DSP*, *LMNA*, and *PLN* mutation carriers), and the biventricular phenotype (also *DSP*, *LMNA*, and *PLN* mutation carriers), with equal involvement of both ventricles. Immunohistochemical analysis of human myocardial samples from various mutation and nonmutation carriers demonstrated that on a desmosomal level, both ventricles are affected by the disease [80]. A marked reduction in immunoreactive signal levels for plakoglobin was observed both in the RV and in the LV, independent of genotype. This strengthens the concept that ACM is a biventricular disease. However, histologically and functionally overt manifestations of the disease usually start in the RV. The reason for this is still unclear. The most advocated cause is that the thin-walled RV is less able to withstand volume (over)load when the mechanical junctions have an impaired function [81].

Clinical Diagnosis

Diagnosis of ACM can be very challenging and can only be made when all other diseases causing VT episodes and structural RV/LV abnormalities have been ruled out (see paragraph on differential diagnosis). Although VF and sudden death may be the first manifestations of ACM, symptomatic patients typically present with sustained monomorphic VT with left bundle branch block morphology, thus originating from the RV. Disease manifestation before the age of 11 years is very rare [79]. The occurrence of VT episodes is usually driven by adrenergic stimulation and starts mainly during exercise or in the early recovery phase after exercise, especially during competitive endurance sports [77, 82] ACM is a disease that shows progression over time, and may manifest differently according to the time of patient presentation [83].

The gold standard for ACM diagnosis is the demonstration of fibrous (± fatty infiltration) replacement primarily of RV myocardium, determined at biopsy, surgery, or postmortem. Originally, predilection sites for these structural abnormalities were thought to be in the so-called triangle of dysplasia formed by the RV outflow tract (RVOT), the apex, and the subtricuspid region [1]. However, these observations were made mainly in patients with advanced overt disease. Recently, evidence was obtained that the disease process starts in the subepicardial layers of the subtricuspid area, or in the posterolateral LV wall. The RV apical area was only affected in advanced stages [84]. Access to histologic examination is obtainable with endomyocardial biopsies. However, biopsies have major limitations. Tissue sampling from the affected often thin RV-free wall, directed by imaging techniques or voltage mapping, is rather straightforward but is associated with a risk of perforation. Sampling from the interventricular septum is relatively safe. However, the septum is histopathologically rarely affected in less advanced ACM stages. In addition, even in potentially affected areas, histology may be classified as normal because of the focal nature of the lesions. Finally, since subendocardial layers are usually not affected in an early stage of the disease, histologic diagnosis may be hampered by the nontransmural nature of endomyocardial biopsies. This is particularly relevant since ACM starts in subepicardial and midmyocardial areas. However, septal endomyocardial biopsy guided by contrast-enhanced MRI may be useful to diagnose cardiac sarcoid, a common differential diagnosis of ACM.

Clinical diagnosis has been facilitated by a set of clinically applicable criteria for ACM diagnosis defined by a consensus-based task force in 1994, [85] and revised in 2010 [86]. These last international task force criteria (TFC) included six different groups of clinical criteria on global or regional RV dysfunction and structural alterations, tissue characteristics, repolarization abnormalities, depolarization abnormalities, arrhythmias, and family history/genetics in order to improve the sensitivity of the original 1994 TFC by preserving their specificity. Within these groups, diagnostic criteria were assigned major or minor according to their specificity for the disease. Every major criterion is scored as two points and every minor as one point. In total, four points have to be scored in order to fulfil ACM diagnosis, that is, two major, one major plus two minor, or four minor criteria. From each different group, only one criterion can be counted for diagnosis, even when multiple criteria in one group are being fulfilled. These 2010 TFC are listed in Table 6.3. The *2010 TFC* are the essential standard for classification of individuals suspected of *ACM*. In addition, its universal acceptance contributed importantly to unambiguous interpretation of clinical studies and facilitated comparison of their results. It should be kept in mind that these TFC are of high practical importance in classical ACM with predominant RV disease. In left dominant ACM, however, their applicability may be hampered by the absence of LV imaging and VT morphology criteria.

Specific evaluations are recommended in all patients suspected of ACM. Detailed history and family history, physical examination, 12-lead ECG, signal-averaged ECG (SAECG; when available), 24 h Holter monitoring, exercise testing, and at least one imaging method, including MRI or transthoracic 2D-echocardiography with quantitative wall motion analysis, should be performed in all. Since MRI combined with late gadolinium enhancement allows visualization of tissue alterations, as well as evaluation of morphology and function in a single investigation, this technique is particu-

Table 6.3 Diagnostic task force criteria

2010 task force criteria for ACM diagnosis	
I. Global or regional dysfunction and structural alterations	**Major:** By 2D echo Regional RV akinesia, dyskinesia, or aneurysm And 1 of the following (end diastole): PLAX RVOT ≥32 mm (corrected for body size [PLAX/BSA] ≥19 mm/m^2), PSAX ≥36 mm (corrected for body size [PSAX/BSA] ≥21 mm/m^2, or fractional area change ≤33 % By MRI Regional RV akinesia or dyskinesia or dyssynchronous RV contraction And 1 of the following: ratio of RVEDV to BSA ≥110 mL/m^2 (male) or ≥100 mL/m^2 (female), or RV ejection fraction ≤40 % By RV cine-angiography Regional RV akinesia, dyskinesia, or aneurysm
	Minor: By 2D echo Regional RV akinesia or dyskinesia And 1 of the following (end diastole): PLAX RVOT ≥29mm to <32mm (corrected for body size [PLAX/BSA] ≥16 mm/m^2 to <19 mm/m^2), PSAX ≥32 mm to <36 mm (corrected for body size [PSAX/BSA] ≥18 mm/m^2 to <21 mm/m^2, or fractional area change >33 % to ≤40 % By MRI Regional RV akinesia or dyskinesia or dyssynchronous RV contraction And 1 of the following: ratio of RVEDV to BSA ≥100 mL/m^2 to <110 mL/m^2 (male) or ≥90 mL/m^2 to <100 mL/m^2 (female), or RV ejection fraction >40 % to ≤45 %
II. Tissue characterization of wall	**Major:** Residual myocytes <60 % by morphometric analysis (or <50 % if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy
	Minor: Residual myocytes 60–75 % by morphometric analysis (or 50–65 % if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy
III. Repolarization abnormalities	**Major:** Inverted T waves in right precordial leads (V1, V2, V3) or beyond in individuals >14 years of age
	Minor: Inverted T waves in leads V1 and V2 in individuals >14 years of age or in V4, V5, V6 Inverted T waves in leads V1, V2, V3, and V4 in individuals >14 years of age in the presence of complete right bundle- branch block
IV. Depolarization/ conduction abnormalities	**Major:** Epsilon wave (reproducible low-amplitude signals after the end of the QRS complex to onset of the T wave) in right precordial leads (V1, V2, V3)
	Minor: Late potentials by SAECG in ≥1 of 3 parameters in the absence of a QRS duration of ≥110 ms on the standard ECG Filtered QRS duration (fQRS) ≥114 ms Duration of terminal QRS <40 uV (low-amplitude signal duration) ≥38 ms Root-mean-square voltage of terminal 40 ms ≤20 uV Terminal activation duration ≥55 ms measured from the nadir of the S wave to the end of all depolarization deflections, including R', in V1, V2, or V3 in the absence of complete right bundle branch block
V. Arrhythmias	**Major:** Nonsustained or sustained ventricular tachycardia of left bundle branch block morphology with superior axis (negative or indeterminate QRS in leads II, III, and aVF and positive in lead aVL)
	Minor: Nonsustained or sustained ventricular tachycardia of RVOT configuration, left bundle branch block morphology with inferior axis (positive QRS in II, III, and aVF and negative in aVL) or of unknown axis 500 ventricular extrasystoles per 24 hours (Holter)

(continued)

Table 6.3 (continued)

2010 task force criteria for ACM diagnosis	
VI. Family History	**Major:** ACM confirmed in a first-degree relative who meets current TFC ACM confirmed pathologically at autopsy or surgery in a first-degree relative Identification of a pathogenic mutation categorized as associated or probably associated with ACM in the patient under evaluation
	Minor: History of ACM in a first-degree relative in whom it is not possible or practical to determine whether the family member meets current TFC Premature sudden death (<35 years of age) due to suspected ACM in a first-degree relative ACM confirmed pathologically or by current TFC in second-degree relative

MRI magnetic resonance imaging, *PLAX* parasternal long axis, *PSAX* parasternal short axis, *BSA* body surface area, *RV* right ventricle, *RVEDV* right ventricular enddiastolic volume, *SAECG* signal averaged ECG, *ACM* arrhythmogenic cardiomyopathy, *TFC* task force criteria

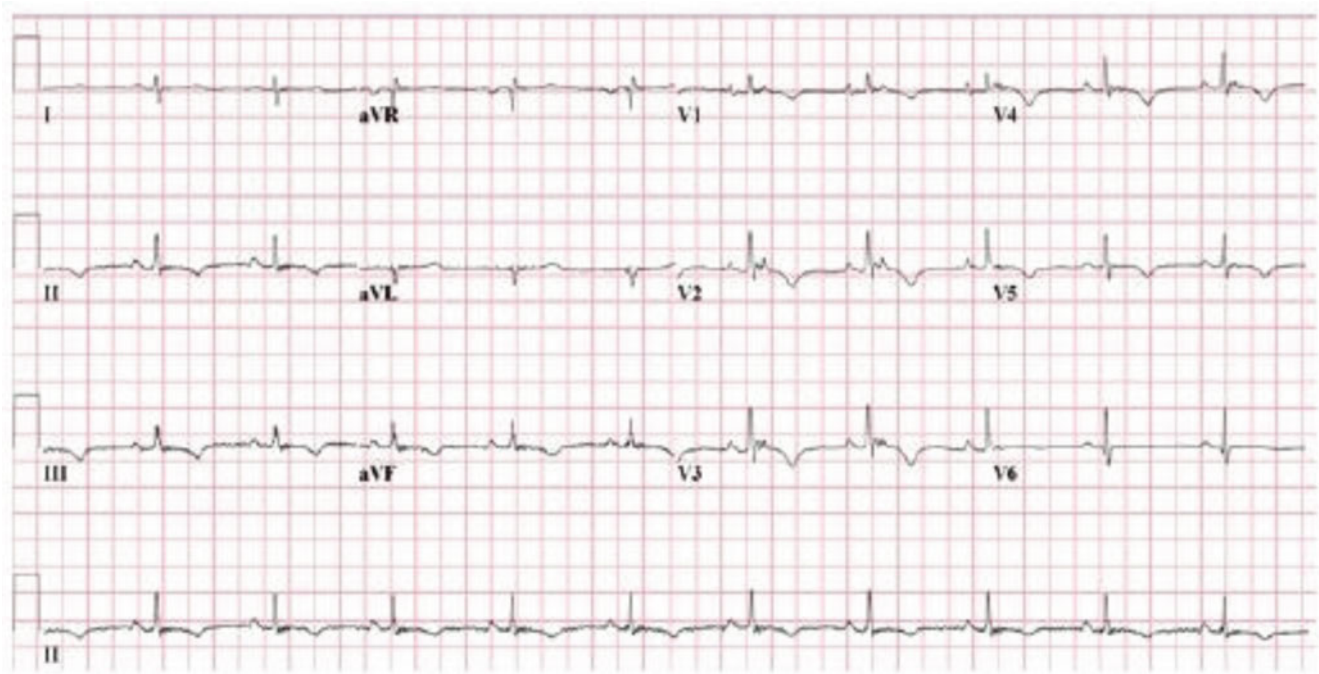

Fig. 6.4 Epsilon waves visible as late positive deflections in between the QRS compex and the T-wave in V1-3, and negative T-waves in V1-5. In addition, the terminal activation duration (TAD) is clearly prolonged

larly useful in ACM workup. In addition, imaging of tissue alteration is particularly useful in predominant LV disease with underestimation of TFC fulfilment. Eventually, invasive tests are also available for diagnostic purposes: endomyocardial biopsy, RV cine-angiography, and electrophysiologic testing. The next paragraphs give detailed information about the various diagnostic aspects summarized in Table 6.3.

ECG Criteria

Criteria on ECG changes have to be determined in sinus rhythm, and while off antiarrhythmic drugs. ECG changes are detected in the large majority of patients with ACM. However, rarely sustained ventricular arrhythmias may occur with a normal ECG during sinus rhythm [87, 88].

Depolarization Abnormalities

As explained earlier, RV activation delay is a hallmark of ACM. This delay is conveyed by the criteria of *epsilon waves* or *prolonged Terminal Activation Duration (TAD)* in V1-3, and late potentials on Signal-Averaged ECG (SAECG).

Epsilon waves are defined as low-amplitude potentials after and clearly separated from the QRS complex, in at least one of V1-3 (Fig. 6.4) [89]. This highly specific major criterion is unfortunately observed in only a small minority of patients [90, 91]. In addition, recording of an epsilon wave is often questionable because of interpretation difficulties related to defining the end of the QRS complex and filter settings [92]. Of note, once epsilon waves are present, typically other (ECG) abnormalities are present and the diagnosis does not depend on epsilon waves by itself. TAD is defined

from the nadir of the S-wave to the end of all depolarization deflections in V1-3, thereby covering all forms of RV activation delay, including epsilon waves. TAD is considered prolonged when ≥55 ms (Fig. 6.5) [93]. Since prolonged TAD is less specific for ACM, it counts as a minor criterion. Since an epsilon wave only occurs with severe activation delay, its recording is usually associated with advanced disease, whereas prolonged TAD is recorded in both minor and major forms of activation delay, and can even be present at early disease stages such as in oligosymptomatic family members carrying the pathogenic mutation of the index patient [28]. Since both criteria are obtained from V1-3, predominantly delay in the RV outflow tract will be recorded. Thus, in the early stage of ACM with exclusively subtricuspid or LV involvement, these criteria may be absent. Finally, a low QRS voltage (<0.5 mV) in the standard extremity leads (not being one of the TFC) suggests advanced disease stage or a *PLN* mutation [94].

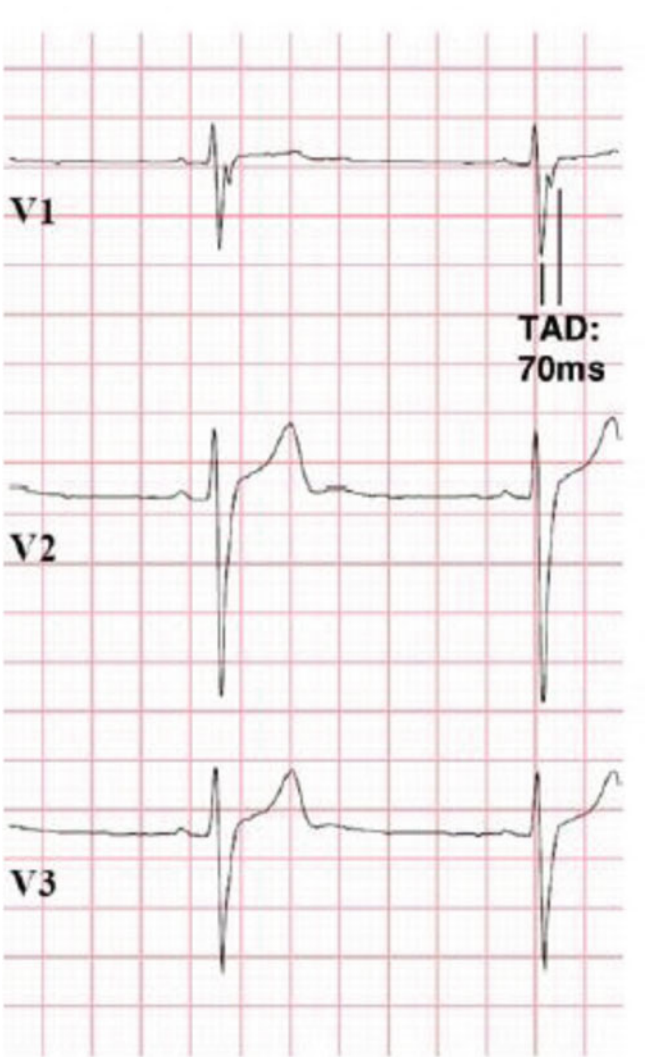

Fig. 6.5 Prolonged terminal activation duration (≥55 ms from nadir of S-wave to end of depolarization)

The detection of *late potentials* on SAECG is the surface counterpart of delayed activation or late potentials detected during mapping in electrophysiologic studies. They are frequently found in patients with documented VT. However, these late potentials can also be observed after myocardial infarction and other structural heart diseases. Due to this lack of specificity, it is considered a minor criterion. For all criteria on depolarization abnormalities, it is apparent that their finding will correlate with disease severity. For instance, a positive correlation has been found between late potentials and the extent of RV fibrosis, reduced RV systolic function, and significant morphological abnormalities on imaging [94–98].

Repolarization Abnormalities

Negative T-waves in leads V1-3 or beyond, in the absence of right bundle branch block (RBBB) are a major ECG criterion on repolarization abnormalities (see Fig. 6.4). They are the most frequently observed criterion. In the initial series reported by Marcus et al., this was detected in over 85 % of cases [1]. Subsequent studies have reported variable prevalences of right precordial T-wave inversion, ranging from 19 % to 94 % [85, 88, 90]. The lower rates are often due to the evaluation of family members, while higher rates are seen in series consisting of unrelated index patients. *T-wave inversion* can be a normal feature of the ECG in children and early adolescence. Therefore, this finding is not considered pathogenic in persons aged 14 years and younger. Negative T-waves only in V1-2 are less specific for ACM and counted as a minor criterion.

Although these negative T-waves were observed consistently in a series of evaluated patients with ACM, T-wave inversions in the right precordial leads can also be observed in 1–3 % of the healthy population aged 19–45 years, and in patients with RV overload, such as major pulmonary embolism and intracardial left to right shunt, or may develop following intracranial hemorrhage as a sign of adrenergic response to the cerebral insult.

In patients with RBBB negative T-waves are common in V1-3. However, the presence of T-wave inversions in V4 or beyond is not physiologic even in the presence of complete RBBB and thus considered as a minor criterion for ACM [99]. To facilitate fulfilment of ACM diagnosis in patients with LV involvement, recording of negative T-waves in V4-6 is also a minor criterion.

Arrhythmias

Ventricular arrhythmias range from PVC to sustained VT and VF, leading to cardiac arrest [93, 99]. Because of the frequent origin in the RV, QRS complexes of ventricular arrhythmias

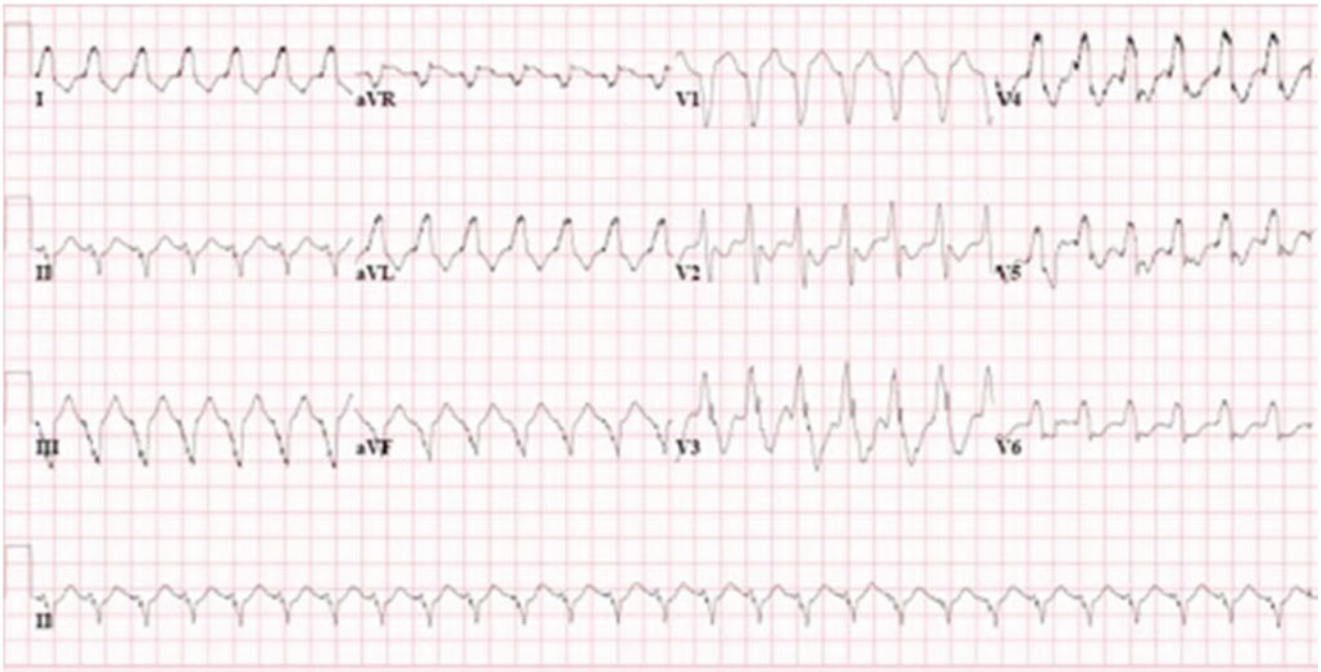

Fig. 6.6 ECG (25 mm/s) from a patient with arrhythmogenic right ventricular dysplasia/cardiomyopathy harboring a plakophilin-2 (*PKP2*) mutation. This ventricular tachycardia has a LBBB morphology and superior axis, thus originates from the inferior aspect of the right ventricle

usually show a left bundle branch block (LBBB) morphology. Moreover, the QRS axis gives an indication of the VT origin, that is, superior axis from the RV inferior wall, frequently the subtricuspid area, and inferior axis from the RV outflow tract (see Fig. 6.6). Since VT originating in the RV outflow tract is often idiopathic and benign, its occurrence in the setting of ACM gives only a minor criterion. On the contrary, a VT with LBBB morphology and superior axis is more specific for ACM and thus a major criterion. Patients with extensively affected RV may show multiple VT morphologies [93].

VF is the mechanism of instantaneous sudden death especially occurring in young people and athletes with ACM, who were often previously asymptomatic. In this subset of patients, VF may occur from deterioration of rapid monomorphic VT, or in a phase of acute disease progression, due to acute myocyte death and reactive inflammation [3]. In a recent study, the median age of presentation with SCD versus monomorphic VT in ACM was only 23 years and 36 years, respectively, suggesting a different arrhythmogenic mechanism [61].

Global and/or Regional Dysfunction and Structural Alterations

Evaluation of RV size and function can be done by various imaging modalities, including echocardiography, MRI, and/or cineangiography. Computed tomography (CT) may be used as well. However, in the absence of clinical studies investigating its value in ACM evaluation, it is not included as diagnostic modality in the 2010 TFC [86]. According to the 2010, TFC regional wall motion abnormalities (akinesia, dyskinesia, or aneurysm) are needed to define major and minor criteria for ACM diagnosis on any imaging modality (see Fig. 6.7). This is combined with functional parameters (RV function and dilatation) for echocardiography and MRI. Gadolinium late enhancement for tissue alteration analysis is not part of the current 2010 TFC [86]. RV cineangiography in at least two perpendicular projections has historically been considered the gold standard to visualize regional RV structural abnormalities, with a high specificity of 90 %, which obviated the necessity of additional functional parameters to score a major criterion [100]. It is important to note that only akinesia (lack of motion); dyskinesia (systolic outward bulging), and aneurysms (both systolic and diastolic bulging) are considered to be diagnostic as wall motion abnormalities. Hypokinesia is not used anymore because of variable interpretation [101].

Echocardiography is noninvasive, widely used, and often serves as the first-line imaging technique in evaluating patients suspected of ACM and screening of their family members. Accurate evaluation of the RV by echocardiography, however, requires considerable expertise, since its complex geometry and difficult appropriate interpretation of the movement of the apical area complicates adequate visualization. This may lead to over as well as underdiagnosis of individuals with subtle structural disease [102]. With new echocardiographic modalities, such as three-dimensional echocardiography, deformation imaging and tissue Doppler, sensitivity and specificity of echocardiography have increased, but these novel modalities are not included in the 2010 TFC yet.

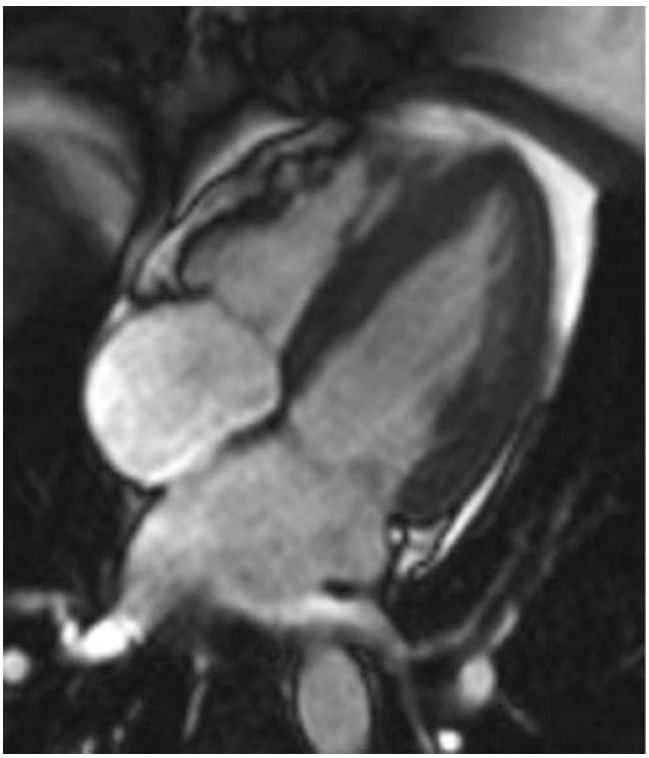

Fig. 6.7 Magnetic resonance imaging in a patient with arrhythmogenic right ventricular dysplasia/cardiomyopathy (*ARVD/C*) at the end of systole. Dyskinetic bulgings are clearly visible in the right ventricle free wall

Cardiac MRI is an interesting technique for ACM evaluation, since it is multiplane, allows for morphologic and functional evaluation, but also has the unique possibility of visualizing the myocardium to characterize tissue composition. Cardiac MRI serves as the gold standard for deriving RV volumes and function. However, MRI is expensive and not widely available and requires great expertise to prevent mis- or overdiagnosis of ACM [103]. Also, in ICD-carrying patients, this technique cannot always be applied. Cardiac MRI appears to be the most common cause of overdiagnosis, and physicians should therefore be very reluctant to diagnose ACM when structural abnormalities are only present on MRI [104, 105]. Furthermore, it is important to note that the presence of fat in the epi- and midmyocardial layers (without fibrosis) is often an unspecific finding, sometimes referred to as cor adiposum and should not be considered diagnostic of ACM.

Tissue Characterization

The gold standard for ACM diagnosis is the demonstration of fibrous replacement ± fatty infiltration. Myocardial tissue for histologic examination is usually obtained by endomyocardial biopsy, autopsy or from explanted hearts. However, for reasons outlined earlier, undirected endomyocardial biopsies are infrequently diagnostic. It had been included as a major criterion by the 2010 TFC, since the finding of fibrofatty replacement was considered to strongly support any findings derived from other clinical investigations. The rather vague terminology of any "fibrofatty replacement of myocardium" has been quantified. Diagnostic values according to the 2010 task force criteria are considered major if histomorphometric analysis of endomyocardial biopsies shows that the number of residual myocytes is below 60 % or below 50 % by estimation, with fibrous replacement of the RV free wall in at least one sample, with or without fatty tissue replacement [106]. If the number of residual myocytes is higher but still below 75 % (morphometric) or below 65 % (estimated) only a minor criterion is fulfilled.

Family History

Already before the discovery of pathogenic mutations underlying the disease, it was recognized that ACM often occurs in family members [1]. Having a family member with proven ACM is considered an increased risk for other family members to be affected. Therefore, having a first-degree relative who meets the 2010 TFC or who has ACM confirmed pathologically at autopsy or during surgery, or identification of a pathogenic mutation in the patient under evaluation, is included as a major diagnostic criterion. If there is a history of a first-degree relative being diagnosed with ACM, but it cannot be verified whether he or she does fulfil the 2010 TFC, only a minor criterion is counted. SCD of a family member under the age of 35 years, presumably but not proven to be due to ACM, is also a minor criterion [86].

Nonclassical ACM Subtypes

Naxos Disease

All patients who homozygously carry the recessive *JUP* mutation for Naxos disease have diffuse palmoplantar keratosis and woolly hair in infancy; children usually have no cardiac symptoms but may have ECG abnormalities and nonsustained ventricular arrhythmias [20, 49]. The cardiac disease is 100 % penetrant by adolescence, being manifested by symptomatic arrhythmias, ECG abnormalities, right ventricular structural alterations, and LV involvement. In one series of 26 patients followed for 10 years, 62 % had structural progression of RV abnormalities, and 27 % developed heart failure due to LV involvement. Almost half of the patients developed symptomatic arrhythmias, and annual cardiac and SCD mortality were 3 % and 2.3 %, respectively, which are higher than seen in autosomal dominant forms of ACM. A minority of heterozygotes has minor ECG and structural changes, but clinically significant disease is not present.

Carvajal Syndrome

Carvajal syndrome is associated with *DSP* mutations and is also a recessive disease manifested by woolly hair, epidermolytic palmoplantar keratoderma, and cardiomyopathy [51]. The cardiomyopathy part of Carvajal syndrome was first thought to be a type of dilated LV cardiomyopathy. A number of patients with Carvajal syndrome suffered from heart failure in their teenage years, resulting in early morbidity. However, further research revealed that it is characterized mainly by ventricular hypertrophy, ventricular dilatation, and discrete focal ventricular aneurysms. In the RV in particular, focal wall thinning and aneurysmal dilatation were identified.

Left Dominant ACM (LDAC)

As previously mentioned, in classic ACM, the histologic process predominantly involves the RV and extends to the LV in more advanced stages [91, 104, 105, 107]. In contrast, patients with left-dominant arrhythmogenic cardiomyopathy (LDAC, also known as left-sided ACM or arrhythmogenic left ventricular cardiomyopathy) have fibrofatty changes that predominantly involve the LV [4, 69]. Clinically, this disease entity is characterized by (infero)lateral T-wave inversion and arrhythmias of LV origin [4].

Patients presented with arrhythmia or chest pain at ages ranging from adolescence to over 80 years. By cardiac MRI, about one third of patients show a LV ejection fraction <50 %. Furthermore, contrast MRI demonstrated late enhancement in the subepicardial/midwall layers of the LV posterolateral wall. Similar to classic ACM, some patients with LDAC have desmosomal mutations, frequently in *DSP*, or in the nondesmosomal *PLN*, *LMNA*, or *DES* genes (see later).

Differential Diagnosis

Although diagnosis in an overt case of ACM is often not difficult, early and occasionally late stages of the disease may show similarities with a few other diseases. Especially differentiation from idiopathic VT originating from the RV outflow tract (RVOT) can be challenging. However, idiopathic *RVOT VT* is a benign nonfamilial condition, in which the ECG shows no depolarization or repolarization abnormalities, and no RV structural changes can be detected. Furthermore, VT episodes have a single morphology (LBBB morphology with inferior axis) and are usually not inducible by premature extrastimuli at programmed stimulation during electrophysiologic studies, since the mechanism of RVOT VT is automaticity or triggered activity [108, 109]. Thus, idiopathic RVOT VT may be inducible by regular burst pacing and isoproterenol infusion. It is important to differentiate idiopathic RVOT VT from ACM for several reasons. First, ACM has a known genetic etiology, which is not the case for idiopathic RVOT VT. Therefore, it has implications with regard to screening of family members. Second, the prognosis of idiopathic RVOT tachycardia is usually excellent with SCD occurring extremely rarely. Finally, in contrast to ACM, catheter ablation is usually a curative procedure in idiopathic RVOT VT.

Another disease mimicking ACM is cardiac *sarcoidosis.* Sarcoidosis is an inflammatory disease with unknown etiology, characterized by the presence of noncaseating granulomas in affected tissues; mainly lungs, but heart, skin, eyes, the reticuloendothelial system, kidneys, and central nervous system (CNS) can also be affected. The prevalence of this condition varies in geographical regions (high prevalence in Japan), and the disease may also be familial and occurring in specific racial subgroups [110]. Clinical symptoms of cardiac involvement are present in approximately 5 % of all patients with sarcoidosis. The clinical manifestations of cardiac sarcoidosis depend on the location and extent of granulomatous inflammation and include conduction abnormalities, ventricular arrhythmias, valvular dysfunction, and congestive heart failure. Myocardial sarcoid granulomas or areas of myocardial scarring are typically present in the LV and septum of patients with this condition, but the RV can sometimes be predominantly affected. A VT associated with right ventricular abnormalities can, therefore, result in diagnostic confusion, especially if there is no systemic evidence of sarcoidosis. Patients can present with clinical features similar to those of ACM including arrhythmias and SCD [111]. A recent study has evaluated parameters that distinguish ACM from sarcoidosis. This manuscript has shown that older age of symptom onset, the presence of cardiovascular comorbidities, nonfamilial pattern of disease, PR interval prolongation, high-grade atrioventricular block, significant left ventricular dysfunction, myocardial delayed enhancement of the septum, and mediastinal lymphadenopathy should raise the suspicion for cardiac sarcoidosis [112]. Cardiac sarcoidosis can only be diagnosed definitively by endomyocardial biopsy, when granulomas are visualized [113]. To strengthen differentiation from ACM, gadolinium-enhanced MRI may be beneficial by detecting located abnormalities in the septum, which is typical for sarcoidosis, but seldomly seen in ACM. Active foci of sarcoidosis can be visualized by positron emission tomography (PET) scan [114]. Therapy with corticosteroids is recommended for patients with a clear diagnosis of cardiac sarcoidosis. Treatment aims to control inflammation and fibrosis in order to maintain cardiac structure and function.

Also, any other form of *myocarditis* has to be excluded before diagnosis of ACM can be made. Myocarditis may arise from viral or other pathogen exposure as well as toxic or immunologic insult. Cardiac MRI (particularly MRI with

T2-weighted imaging) may be useful to visualize tissue edema, which is present in myocarditis but not in ACM. However, in general, endomyocardial biopsy is required to distinguish ACM from myocarditis. Some reports have suggested an overlap between ACM and viral myocarditis, rendering this differential diagnosis extremely difficult to exclude [115].

Especially in more advanced stages of the disease, when LV ejection fraction drops below 50 %, ACM may mimic DCM. Patients with DCM usually present with heart failure or thromboembolic disease, including stroke. Since it is uncommon to have sustained VT or sudden death as the initial presenting symptom of DCM, patients with these symptoms should be first suspected of having ACM. Since in particular the *PLN* c.40_42delAGA (p.Arg14del) founder mutation is associated with heart failure, the disease manifestation is frequently labelled as DCM. However, expression of this mutation may start with ventricular arrhythmias years before hemodynamic deterioration [69].

Molecular Genetic Analysis

It is important to realize that ACM diagnosis is based exclusively on fulfilment of the 2010 TFC [86]. Mutations underlying the disease show incomplete penetrance and variable clinical expression. Some genetically affected patients can have no signs or symptoms whatsoever, whereas no mutations can be identified in nearly half of clinically diagnosed patients. However, DNA analyses of the index patient may identify *DSP*, *PLN*, *TMEM43*, or multiple pathogenic mutations, with a higher risk of sudden death and/or heart failure than in the more common single *PKP2* mutation carriers. DNA analyses are crucial to identify whether family members are predisposed to disease development. In some forms of ACM with a high disease penetrance and elevated risk of SCD such as in carriers of the *TMEM43* Newfoundland mutation, identification of such a mutation may be considered diagnostic even in the absence of TFC.

The strategy for genetic testing in ACM is as follows: index patients (=probands) with a clinical diagnosis of ACM are the first to be tested. Preferably a next-generation sequencing panel encompassing the genes mentioned in this chapter including a specific analysis to identify deletions, as these can be identified in up to 2 % of cases [55, 116]. Because genetic testing also involves more than a cardiac disease and a mutation, genetic counseling by a trained genetic counselor or clinical geneticist is strongly advised. In a genetic couseling session also psychosocial aspects, reproductive options and issues regarding family screening including discussing how to reach out to family members are being discussed. In addition, the patient has to be informed in advance that a gene result is not always unequivocal and that variants of uncertain significance can be found.

The detection of a pathogenic mutation does not make a diagnosis of ACM. In contrast, if no mutation can be identified in a patient diagnosed with ACM, the clinical diagnosis of ACM is still applicable since not all mutations and epigenetic factors causing ACM are known. If (a) pathogenic mutation(s) is (are) identified in the proband, parents, siblings, and children (first-degree family members) of this patient can be subsequently tested for this particular mutation(s). In a further step, second-degree family members can be screened as well. This sequential testing is commonly referred to as cascade screening. When an asymptomatic relative is found to carry a pathogenic mutation, cardiologic screening is required at regular intervals, for example, at least annually, or more frequently depending on patient age, clinical symptoms, and sports participation.

Table 6.1 shows the different genes associated with ACM. Although the yield of genetic testing varies per country, *PKP2* accounts for the large majority of mutations found. Currently, DNA analysis for at least *PKP2*, *DSG2*, *DSC2*, *DSP*, and *JUP* is recommended in all patients with ACM including an evaluation for (large) deletions [55, 116]. But preferably a panel encompassing all ACM-related genes mentioned in this chapter should be tested. Selecting genes by geographical regions alone is insufficient. The reason is that several ACM-related nondesmosomal mutations initially believed to be regional founder mutations, have also been identified in different countries or even continents, like *PLN* p.Arg14del (the Netherlands, Germany, Spain, Greece, USA, Canada), *TMEM43* p.S358L (Newfoundland, Denmark, Germany) [68], *DES* p.S13F (the Netherlands, Singapore, USA) [23, 117–120].

Recently, next-generation sequencing has identified more genes associated with ACM [67]. Rapidly decreasing costs of gene panels facilitate simultaneous analysis of a large number of genes. This has great impact on the yield of DNA variants and may elucidate previously unexpected causative mutations. However, data interpretation is more difficult since many variants are benign and not disease causing [121]. Therefore, this should preferably be a team effort of cardiologists, clinical geneticists/genetic counselors, molecular biologists, and pathologists [122].

Prognosis and Therapy

The prognosis of ACM is usually better than that of patients with sustained VT and left ventricular structural heart disease. However, ACM is a progressive disease and will eventually lead to RV and LV failure, and sudden cardiac death. The death rate for treated index patients with ACM has been estimated at 1.4 % per year, and lower in family members detected through family screening [28, 76]. Retrospective

analysis of clinical and pathologic studies identified several risk factors for sudden death, such as previously aborted SCD, sustained VT, nonsustained VT on 24 h Holter monitoring or exercise testing, ≥1000 premature ventricular complexes in 24 h, male gender, proband status, unexplained syncope, young age at the time of diagnosis, severe dilatation/dysfunction of RV and/or LV, compound and digenic heterozygosity of ACM-associated genes, *DSP* and *PLN* mutations, extent of T-wave inversions, low QRS voltage, fractionated QRS-complex, inducibility by invasive programmed ventricular stimulation, and amount of electroanatomic scar, as recently summarized by the international consensus report by Corrado et al. [61, 123–126]. In addition to symptomatic treatment, prevention of SCD is the most important therapeutic goal in ACM. Bhonsale et al. reported that in asymptomatic patients the combination of ≥2 factors such as VT/VF inducibility, proband status, nonsustained VT, and PVCs ≥1000/24 hours predicts an incremental risk of appropriate implantable defibrillator (ICD) interventions [127]. However, a statistically significant association with life-saving shocks for treatment of rapid VT or VF has not been demonstrated in this study.

Evidence suggests that asymptomatic patients and healthy mutation carriers do not require any prophylactic treatment. A betablocker may be considered even in the absence of symptoms, although currently data are missing on a favorable prognostic effect [124, 125]. They should instead undergo regular cardiologic check-ups including 12-lead ECG, 24 h Holter monitoring, echocardiography, and exercise testing for early identification of disease onset. A recent study has shown that siblings of patients with ACM have the highest likelihood of fulfilling ACM criteria and that fulfillment of TFC independent of family history is superior to conventional TFC for arrhythmic risk stratification of relatives [76]. To all patients diagnosed with ACM, as well as all pathogenic mutation carriers, specific life style advises have to be given, indifferent from which additional therapeutic measures are taken. Sports participation has been shown to increase the risk of SCD in patients with ACM fivefold [128]. Furthermore, excessive mechanical stress, such as during competitive sports may aggravate the underlying myocardial abnormalities and accelerate disease progression [77]. Therefore, patients with ACM, and pathogenic mutation carriers with or without symptoms, should be advised against practicing competitive and endurance sports such as running marathons.

Therapeutic options in patients with ACM include sports restriction, β-blockers, pharmacologic treatment of heart failure such as ACE-inhibitors, antiarrhythmic drugs, catheter ablation, and implantation of a cardioverter defibrillator (ICD). Patients with VT have a favorable outcome when they are treated medically, and therefore, pharmacologic treatment is the first choice. This concerns not only patients who have presented with sustained VT, but also patients and family members with nonsustained VT or >500 ventricular extrasystoles on 24 h Holter monitoring. Since ventricular arrhythmias and cardiac arrest occur frequently during or after physical exercise, or may be triggered by catecholamines, antiadrenergic β-blockers are recommended. Regarding antiarrhythmic drugs, studies have shown that sotalol is particularly effective in patients with ACM. Alternatively, other β-receptor blocking agents, amiodarone and flecainide, have all been reported as useful [129]. *Catheter ablation* is an alternative in patients who are refractory to drug treatment and have frequently recurring VT episodes or ICD discharges. However, all reported results with exclusively endocardial approaches were mediocre, related to the technical difficulties of the procedure, the subepicardial onset of the disease, and disease progression. In recent years, results improved markedly in highly experienced centers by using a combined epicardial and endocardial approach nowadays reaching VT/VF free survival rates of up to 80 % at 2 years [124, 125, 130–132]. Yet, catheter ablation is considered as a palliative and not curative treatment option. Owing to disease progression, new VTs with different morphologies may occur after a certain period of time [133]. Interestingly, recent evidence has indicated that bilateral sympathectomy may serve as an effective treatment method for achieving arrhythmia control, even when all other treatment options including repeated catheter ablation have been exhausted [134]. Although *antiarrhythmic drugs* and *catheter ablation* can significantly reduce VT burden, no prospective trials have been performed to demonstrate that these therapies will also prevent SCD.

ICD implantation is indicated in patients with a moderate (1–10 % per year) to high risk (>10 % risk per year) of SCD. As such, implantation of an ICD is warranted in ACM patients with aborted SCD or sustained VT for secondary prevention, and for primary prevention in those patients with risk factors for SCD as mentioned earlier.

If heart failure progresses toward end-stage disease or intractable ventricular arrhythmias occur despite optimal pharmacologic and ablative treatment, heart transplantation should be considered in this rather young population with 1-year and 5-year survival rates after transplantation of 94 % and 88 %, respectively [135].

Summary

ACM is usually a genetically determined disease characterized by fibrofatty replacement of myocardial tissue. Clinically and histologically, it affects most often the RV, but extension to the LV occurs, especially in more advanced stages of the disease. In addition, biventricular forms and predominant LV forms exist. At the molecular level, both ventricles are equally affected, presumably in all stages of

the disease. Patients typically present between the second and fourth decade of life with exercise-induced ventricular tachycardias originating from the RV. However, it is also a major cause of SCD in the young and athletes. Its prevalence has been estimated to vary from 1:2000 to 1:5000. The causative genes encode proteins of mechanical cell junctions (e.g., plakoglobin, plakophilin2, desmoglein2, desmocollin2, desmoplakin) and account for intercalated disc remodeling. The classical form of ACM is inherited in an autosomal dominant trait, but shows reduced penetrance and variable expression. The rare recessively inherited variants are often associated with palmoplantar keratoderma and woolly hair. Clinical diagnosis is made according to a set of task force criteria, based on family history/genetics, depolarization and repolarization abnormalities, ventricular arrhythmias, functional and structural alterations of the RV, and fibrofatty replacement in endomyocardial biopsy. Two-dimensional echocardiography, cineangiography, and cardiac MRI are the imaging tools for visualizing RV structural/functional abnormalities. The main differential diagnoses are idiopathic RVOT VT, myocarditis, and sarcoidosis. Currently, only palliative therapy is available and consists of sports restriction, betablockers, other antiarrhythmic drugs, pharmacologic treatment of heart failure, catheter ablation, implantable cardioverter defibrillators, and heart transplantation. Previously aborted SCD, sustained VT, arrhythmic syncope, and severe RV/LV dysfunction are major risk factors for adverse prognosis warranting ICD implantation.

Take Home Messages

- ACM is a biventricular disease, with predominant RV (typical or classic ARVD/C) or LV involvement.
- After exclusion of alternative diagnoses, fulfilment of two major, or one major and two minor, or four minor criteria of the in 2010, by international consensus obtained, revised task force criteria (TFC) is required for definite ACM diagnosis.
- Since the 2010 TFC preferentially focus on RV parameters, diagnostic fulfilment is more difficult to obtain in left-dominant ACM. Particularly, in this subgroup, cardiac MRI with late gadolinium enhancement in the LV wall may add to the diagnostic evidence.
- Exclusively pathogenic mutations with accepted causative correlation with the ACM phenotype may count as major molecular genetic criterion for the 2010 TFC. At present, this means a limited number of specific variants in the desmosomal genes *PKP2*, *DSP*, *DSG2*, *DSC2*, *JUP*, and the nondesmosomal genes *TMEM43*, and *PLN*. This listing does not exclude pathogenicity or phenotype modifying effects of other variants, but their causative evidence is still to low to count for ACM diagnosis.
- SCD in ACM frequently occurs in adolescence and in young adults and may occur as first manifestation of ACM.
- Most common mutations are in *PKP2*. However, mutations in *DSP*, *PLN*, *TMEM43*, and multiple mutations are associated with a worse outcome (SCD and/or heart failure).
- Inclusion of patients with ACM and their family members in national and international databases is crucial to enhance genotype–phenotype correlation to improve appropriate counseling.

References

1. Marcus FI, Fontaine GH, Guiraudon G, et al. Right ventricular dysplasia: a report of 24 adult cases. Circulation. 1982;65(2):384–98.
2. Corrado D, Basso C, Thiene G, et al. Spectrum of clinicopathologic manifestations of arrhythmogenic right ventricular cardiomyopathy/dysplasia: a multicenter study. J Am Coll Cardiol. 1997;30:1512–20.
3. Basso C, Thiene G, Corrado D, et al. Arrhythmogenic right ventricular cardiomyopathy: dysplasia, dystrophy, or myocarditis? Circulation. 1996;94:983–91.
4. Sen-Chowdhry S, Syrris P, Prasad SK, et al. Left-dominant arrhythmogenic cardiomyopathy: an underrecognized clinical entity. J Am Coll Cardiol. 2008;52:2175–87.
5. Thiene G, Nava A, Corrado D, Rossi L, Pennelli N. Right ventricular cardiomyopathy and sudden death in young people. N Engl J Med. 1988;318(3):129–33.
6. Richardson P, McKenna W, Bristow M, et al. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. Circulation. 1996;93(5):841–2.
7. McKoy G, Protonotarios N, Crosby A, et al. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). Lancet. 2000;355:2119–24.
8. Rampazzo A, Nava A, Miorin M, et al. ARVD4, a new locus for arrhythmogenic right ventricular cardiomyopathy, maps to chromosome 2 long arm. Genomics. 1997;45:259–63.
9. Ahmad F, Li D, Karibe A, et al. Localization of a gene responsible for arrhythmogenic right ventricular dysplasia to chromosome 3p23. Circulation. 1998;98:2791–5.
10. Li D, Ahmad F, Gardner MJ, et al. The locus of a novel gene responsible for arrhythmogenic right-ventricular dysplasia characterized by early onset and high penetrance maps to chromosome 10p12–p14. Am J Hum Genet. 2000;66:148–56.
11. Melberg A, Oldfors A, Blomstrom-Lundqvist C, et al. Autosomal dominant myofibrillar myopathy with arrhythmogenic right ventricular cardiomyopathy linked to chromosome 10q. Ann Neurol. 1999;46:684–92.
12. Rampazzo A, Nava A, Danieli GA, et al. The gene for arrhythmogenic right ventricular cardiomyopathy maps to chromosome 14q23–q24. Hum Mol Genet. 1994;3:959–62.

13. Severini GM, Krajinovic M, Pinamonti B, et al. A new locus for arrhythmogenic right ventricular dysplasia on the long arm of chromosome 14. Genomics. 1996;31:193–200.
14. Tiso N, Stephan DA, Nava A, et al. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). Hum Mol Genet. 2001;10(3):189–94.
15. Beffagna G, Occhi G, Nava A, et al. Regulatory mutations in transforming growth factor-beta3 gene cause arrhythmogenic right ventricular cardiomyopathy type 1. Cardiovasc Res. 2005;65: 366–73.
16. Rampazzo A, Nava A, Malacrida S, et al. Mutation in human desmoplakin domain binding to plakoglobin causes a dominant form of arrhythmogenic right ventricular cardiomyopathy. Am J Hum Genet. 2002;71:1200–6.
17. Gerull B, Heuser A, Wichter T, et al. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. Nat Genet. 2004;36:1162–4.
18. Pilichou K, Nava A, Basso C, et al. Mutations in desmoglein-2 gene are associated to arrhythmogenic right ventricular cardiomyopathy. Circulation. 2006;113:1171–9.
19. Syrris P, Ward D, Evans A, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in the desmosomal gene desmocollin-2. Am J Hum Genet. 2006;79:978.
20. Kaplan SR, Gard JJ, Protonotarios N, et al. Remodeling of myocyte gap junctions in arrhythmogenic right ventricular cardiomyopathy due to a deletion in plakoglobin (Naxos disease). Heart Rhythm. 2004;1(1):3–11.
21. Sato PY, Musa H. Coombs W, et al. Loss of plakophilin-2 expression leads to decreased sodium current and slower conduction velocity in cultured cardiac myocytes. Circ Res. 2009;105: 523–6.
22. Merner ND, Hodgkinson KA, Haywood AF, et al. Arrhythmogenic right ventricular cardiomyopathy type 5 is a fully penetrant, lethal arrhythmic disorder caused by a missense mutation in the TMEM43 gene. Am J Hum Genet. 2008;82:809–21.
23. van der Zwaag PA, van Rijsingen IA, Asimaki A, et al. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. Eur J Heart Fail. 2012;14:1199–207.
24. van Tintelen JP, van Gelder IC, Asimaki A, et al. Severe cardiac phenotype with right ventricular predominance in a large cohort of patients with a single missense mutation in the DES gene. Heart Rhythm. 2009;6:1574–83.
25. Quarta G, Syrris P, Ashworth M, et al. Mutations in the Lamin A/C gene mimic arrhythmogenic right ventricular cardiomyopathy. Eur Heart J. 2012;33:1128–36.
26. Taylor M, Graw S, Sinagra G, et al. Genetic variation in titin in arrhythmogenic right ventricular cardiomyopathy-overlap syndromes. Circulation. 2011;124:876–85.
27. van Hengel J, Calore M, Bauce B, et al. Mutations in the area composita protein αT-catenin are associated with arrhythmogenic right ventricular cardiomyopathy. Eur Heart J. 2013;34: 201–10.
28. Groeneweg JA, Bhonsale A, James CA, et al. Clinical presentation, long-term follow-up, and outcomes of 1001 arrhythmogenic right ventricular dysplasia/cardiomyopathy patients and family members. Circ Cardiovasc Genet. 2015;8:437–67.
29. Gemayel C, Pelliccia A, Thompson PD. Arrhythmogenic right ventricular cardiomyopathy. J Am Coll Cardiol. 2001;38:1773–81.
30. Corrado D, Pelliccia A, Bjørnstad HH, et al. Cardiovascular preparticipation screening of young competitive athletes for prevention of sudden death: proposal for a common European protocol. Consensus Statement of the Study Group of Sport Cardiology of the Working Group of Cardiac Rehabilitation and Exercise Physiology and the Working Group of Myocardial and Pericardial Diseases of the European Society of Cardiology. Eur Heart J. 2005;26:516–24.
31. Basso C, Corrado D, Thiene G. Cardiovascular causes of sudden death in young individuals including athletes. Cardiol Rev. 1999;7:127–35.
32. Maron BJ, Thompson PD, Ackerman MJ, et al. Recommendations and considerations related to preparticipation screening for cardiovascular abnormalities in competitive athletes: 2007 update: a scientific statement from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: endorsed by the American College of Cardiology Foundation. Circulation. 2007;115: 1643–455.
33. van der Zwaag PA, Cox MG, van der Werf C, et al. Recurrent and founder mutations in the Netherlands: Plakophilin-2 p.Arg79X mutation causing arrhythmogenic right ventricular cardiomyopathy/dysplasia. Neth Hear J. 2010;18:583–91.
34. Hodgkinson KA, Connors SP, Merner N, et al. The natural history of a genetic subtype of arrhythmogenic right ventricular cardiomyopathy caused by a p.S358L mutation in TMEM43. Clin Genet. 2013;83:321–31.
35. Watkins DA, Hendricks N, Shaboodien G, et al. Clinical features, survival experience, and profile of plakophylin-2 gene mutations in participants of the arrhythmogenic right ventricular cardiomyopathy registry of South Africa. Heart Rhythm. 2009;6(11 Suppl): S10–7.
36. van Tintelen JP, Hofstra RM, Wiesfeld AC, et al. Molecular genetics of arrhythmogenic cardiomyopathy: emerging horizon? Curr Opin Cardiol. 2007;22:185–92.
37. Bernstein SA, Morley GE. Gap junctions and propagation of the cardiac action potential. Adv Cardiol. 2006;42:71–85.
38. Cerrone M, Noorman M, Lin X, et al. Sodium current deficit and arrhythmogenesis in a murine model of plakophilin-2 haploinsufficiency. Cardiovasc Res. 2012;95:460–8.
39. Agullo-Pascual E, Cerrone M, Delmar M, et al. Arrhythmogenic cardiomyopathy and Brugada syndrome: diseases of the connexome. FEBS Lett. 2014;588:1322–30.
40. Rizzo S, Lodder EM, Verkerk AO, et al. Intercalated disc abnormalities, reduced Na(+) current density, and conduction slowing in desmoglein-2 mutant mice prior to cardiomyopathic changes. Cardiovasc Res. 2012;95:409–18.
41. Akdis D, Medeiros-Domingo A, Gaertner-Rommel A, et al. Myocardial expression profiles of candidate molecules in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia compared to those with dilated cardiomyopathy and healthy controls. Heart Rhythm. 2016;13:731–41.
42. Ellison KE, Friedman PL, Ganz LI, Stevenson WG. Entrainment mapping and radiofrequency catheter ablation of ventricular tachycardia in right ventricular dysplasia. J Am Coll Cardiol. 1998;32:724–8.
43. Saffitz JE. Dependence of electrical coupling on mechanical coupling in cardiac myocytes: insights gained from cardiomyopathies caused by defects in cell-cell connections. Ann N Y Acad Sci. 2005;1047:336–44.
44. Kaplan SR, Gard JJ, Carvajal-Huerta L, Ruiz-Cabezas JC, Thiene G, Saffitz JE. Structural and molecular pathology of the heart in Carvajal syndrome. Cardiovasc Pathol. 2004;13(1):26–32.
45. Otten E, Asimaki A, Maass A, et al. Desmin mutations as a cause of right ventricular heart failure affect the intercalated disks. Heart Rhythm. 2010;7:1058–64.
46. Ross SE, Hemati N, Longof KA, et al. Inhibition of adipogenesis by Wnt signaling. Science. 2000;289:950–3.
47. Garcia-Gras E, Lombardi R, Giocondo MJ, et al. Suppression of canonical Wnt/beta-catenin signaling by nuclear plakoglobin

recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. J Clin Invest. 2006;116:2012–21.
48. Keller DI, Stepowski D, Balmer C, et al. De novo heterozygous desmoplakin mutations leading to Naxos-Carvajal disease. Swiss Med Wkly. 2012;142:w13670.
49. Protonotarios N, Tsatsopoulou A, Anastasakis A, et al. Genotype-phenotype assessment in autosomal recessive arrhythmogenic right ventricular cardiomyopathy (Naxos disease) caused by a deletion in plakoglobin. J Am Coll Cardiol. 2001;38(5): 1477–84.
50. Alcalai R, Metzger S, Rosenheck S, Meiner V, Chajek-Shaul T. A recessive mutation in desmoplakin causes arrhythmogenic right ventricular dysplasia, skin disorder, and woolly hair. J Am Coll Cardiol. 2003;42:319.
51. Norgett EE, Hatsell SJ, Carvajal-Huerta L, et al. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. Hum Mol Genet. 2000;9:2761–6.
52. Syrris P, Ward D, Asimaki A, et al. Clinical expression of plakophilin-2 mutations in familial arrhythmogenic right ventricular cardiomyopathy. Circulation. 2006;113:356–64.
53. Dalal D, Molin LH, Piccini J, et al. Clinical features of arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in plakophilin-2. Circulation. 2006;113: 1641–9.
54. van Tintelen JP, Entius MM, Bhuiyan ZA, et al. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. Circulation. 2006; 113:1650–8.
55. Cox MG, van der Zwaag PA, van der Werf C, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy: pathogenic desmosome mutations in index-patients predict outcome of family screening: Dutch ARVD/C genotype-phenotype follow-up study. Circulation. 2011;123:2690–700.
56. Bao J, Wang J, Yao Y, et al. Correlation of ventricular arrhythmias with genotype in arrhythmogenic right ventricular cardiomyopathy. Circ Cardiovasc Genet. 2013;6:552–6.
57. Rigato I, Bauce B, Rampazzo A, et al. Compound and digenic heterozygosity predicts lifetime arrhythmic outcome and sudden cardiac death in desmosomal gene-related. Circ Cardiovasc Genet. 2013;6:533–42.
58. Syrris P, Ward D, Asimaki A, et al. Desmoglein-2 mutations in arrhythmogenic right ventricular cardiomyopathy: a genotype-phenotype characterization of familial disease. Eur Heart J. 2007;28:581–8.
59. Bauce B, Basso C, Rampazzo A, et al. Clinical profile of four families with arrhythmogenic right ventricular cardiomyopathy caused by dominant desmoplakin mutations. Eur Heart J. 2005; 26:1666–75.
60. Sen-Chowdhry S, Syrris P, McKenna WJ. Desmoplakin disease in arrhythmogenic right ventricular cardiomyopathy: early genotype-phenotype studies. Eur Heart J. 2005;26:1582–4.
61. Bhonsale A, Groeneweg JA, James CA, et al. Impact of genotype on clinical course in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated mutation carriers. Eur Heart J. 2015; 36:847–55.
62. Rampazzo A, Beffagna G, Nava A, et al. Arrhythmogenic right ventricular cardiomyopathy type 1 (ARVD1): confirmation of locus assignment and mutation screening of four candidate genes. Eur J Hum Genet. 2003;11:69–76.
63. Wehrens XH, Lehnart SE, Huang F, et al. FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. Cell. 2003;113:829–40.
64. Bauce B, Nava A, Rampazzo A, et al. Familial effort polymorphic ventricular arrhythmias in arrhythmogenic right ventricular cardiomyopathy map to chromosome 1q42–43. Am J Cardiol. 2000;85:573–9.
65. Priori SG, Napolitano C, Memmi M, et al. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. Circulation. 2002;106:69–74.
66. Tiso N, Salamon M, Bagattin A, Danieli GA, et al. The binding of the RyR2 calcium channel to its gating protein FKBP12.6 is oppositely affected by ARVD2 and VTSIP mutations. Biochem Biophys Res Commun. 2002;299:59–8.
67. Medeiros-Domingo et al. Arrhythmogenic right ventricular cardiomyopathy: implications of next-generation sequencing in appropriate diagnosis. Europace. 2016; In Press.
68. Milting H, Klauke B, Christensen AH, et al. The TMEM43 Newfoundland mutation p.S358L causing ARVC-5 was imported from Europe and increases the stiffness of the cell nucleus. Eur Heart J. 2015;36:872–81.
69. Groeneweg JA, van der Zwaag PA, Jongbloed JD, et al. Left-dominant arrhythmogenic cardiomyopathy in a large family: associated desmosomal or nondesmosomal phenotype? Heart Rhythm. 2013;10:548–59.
70. Groeneweg JA, van der Zwaag PA, Olde Nordkamp LR, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy according to revised 2010 task force criteria with inclusion of non-desmosomal phospholamban mutation carriers. Am J Cardiol. 2013;112:1197–206.
71. Yin T, Green KJ. Regulation of desmosome assembly and adhesion. Semin Cell Dev Biol. 2004;16:665–77.
72. Forleo LMNA, Forleo C, Carmosino M, Resta N, et al. Clinical and functional characterization of a novel mutation in lamin a/c gene in a multigenerational family with arrhythmogenic cardiac laminopathy. PLoS One. 2015;10:e0121723.
73. Klauke B, Kossmann S, Gaertner A, et al. De novo desmin-mutation N116S is associated with arrhythmogenic right ventricular cardiomyopathy. Hum Mol Genet. 2010;19:4595–607.
74. Hedberg C, Melberg A, Kuhl A, et al. Autosomal dominant myofibrillar myopathy with arrhythmogenic right ventricular cardiomyopathy 7 is caused by a DES mutation. Eur J Hum Genet. 2012;20:984–5.
75. van Spaendonck-Zwarts KY, van Hessem L, Jongbloed JD, et al. Desmin-related myopathy. Clin Genet. 2011;80:354–66.
76. Te Riele AS, James CA, Groeneweg JA, et al. Appproach to family screening in arrhythmogenic right ventricular dysplasia/cardiomyopathy. Eur Heart J. 2016;37:755–62.
77. James CA, Bhonsale A, Tichnell C, et al. Exercise increases age-related penetrance and arrhythmic risk in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated desmosomal mutation carriers. J Am Coll Cardiol. 2013;62:1290–7.
78. Quarta G, Muir A, Pantazis A, et al. Familial evaluation in arrhythmogenic right ventricular cardiomyopathy: impact of genetics and revised task force criteria. Circulation. 2011;123:2701–9.
79. Te Riele AS, James CA, Sawant AC, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy in the pediatric population: clinical characterization and comparison with adult-onset disease. J Am Coll Cardiol EP. 2015;1:551–60.
80. Asimaki A, Tandri H, Huang H, et al. A new diagnostic test for arrhythmogenic right ventricular cardiomyopathy. N Engl J Med. 2009;360:1075–64.
81. Reddy S, Bernstein D. Molecular mechanisms of right ventricular failure. Circulation. 2015;132:1734–42.
82. Ruwald AC, Marcus F, Estes 3rd NA, et al. Association of competitive and recreational sport participation with cardiac events in patients with arrhythmogenic right ventricular cardiomyopathy:

results from the North American multidisciplinary study of arrhythmogenic right ventricular cardiomyopathy. Eur Heart J. 2015;36:1735–43.
83. Saguner AM, Ganahl S, Kraus A, et al. Electrocardiographic features of disease progression in arrhythmogenic right ventricular cardiomyopathy/dysplasia. BMC Cardiovasc Disord. 2015;15:4.
84. Te Riele A, James CA, Philips B, et al. Mutation-positive arrhythmogenic right ventricular dysplasia/cardiomyopathy: the triangle of dysplasia displaced. J Cardiovasc Electrophysiol. 2013;24:1311–20.
85. McKenna WJ, Thiene G, Nava A, et al. Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Task Force of the Working Group Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology. Br Heart J. 1994;71:215–8.
86. Marcus FI, McKenna WJ, Sherrill D, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. Eur Heart J. 2010;31:806–14.
87. Te Riele AS, James CA, Bhonsale A, et al. Malignant arrhythmogenic right ventricular dysplasia/cardiomyopathy with a normal 12-lead electrocardiogram: a rare but underrecognized clinical entity. Heart Rhythm. 2013;10:1484–91.
88. Basso C, Corrado D, Thiene G. Cardiovascular causes of sudden death in young individuals including athletes. Cardiol Rev. 1999;7:127–35.
89. Fontaine G, Umemura J, Di Donna P, Tsezana R, Cannat JJ, Frank R. Duration of QRS complexes in arrhythmogenic right ventricular dysplasia. A new non-invasive diagnostic marker. Ann Cardiol Angeiol (Paris). 1993;42:399–405.
90. Peters S, Trümmel M. Diagnosis of arrhythmogenic right ventricular dysplasia-cardiomyopathy: value of standard ECG revisited. Ann Noninvasive Electrocardiol. 2003;8:238–45.
91. Pinamonti B, Sinagra G, Salvi A, et al. Left ventricular involvement in right ventricular dysplasia. Am Heart J. 1992;123:711–24.
92. Platonov PG, Calkins H, Hauer RN, et al. High interobserver variability in the assessment of epsilon waves: implications for diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia. Heart Rhythm. 2016;13:208–16.
93. Cox MG, Nelen MR, Wilde AA, et al. Activation delay and VT parameters in arrhythmogenic right ventricular dysplasia/cardiomyopathy: toward improvement of diagnostic ECG criteria. J Cardiovasc Electrophysiol. 2008;19:775–81.
94. Saguner AM, Ganahl S, Baldinger SH, et al. Usefulness of electrocardiographic parameters for risk prediction in arrhythmogenic right ventricular dysplasia. Am J Cardiol. 2014;113:1728–34.
95. Nasir K, Rutberg J, Tandri H, Berger R, Tomaselli G, Calkins H. Utility of SAECG in arrhythmogenic right ventricle dysplasia. Ann Noninvasive Electrocardiol. 2003;8:112–20.
96. Oselladore L, Nava A, Buja G, et al. Signal-averaged electrocardiography in familial form of arrhythmogenic right ventricular cardiomyopathy. Am J Cardiol. 1995;75:1038–41.
97. Turrini P, Angelini A, Thiene G, et al. Late potentials and ventricular arrhythmias in arrhythmogenic right ventricular cardiomyopathy. Am J Cardiol. 1999;83:1214–9.
98. Tannawuttiwat, Te Riele AS, Philips B, et al. Electroanatomic correlates of depolarization abnormalities in arrhythmogenic right ventricular dysplasia/cardiomyopathy. J Cardiovasc Electrophysiol. 2016;27:443–52.
99. Jain R, Dalal D, Daly A, et al. Electrocardiographic features of arrhythmogenic right ventricular dysplasia. Circulation. 2009;120:477–87.
100. Zareba W, Piotrowicz K, Turrini P. Electrocardiographic manifestations. In: Marcus FI, Nava A, Thiene G, editors. Arrhythmogenic right ventricular dysplasia/cardiomyopathy, recent advances. Milano: Springer; 2007. p. 121–8.
101. White JB, Razmi R, Nath H, et al. Relative utility of magnetic resonance imaging and right ventricular angiography to diagnose arrhythmogenic right ventricular cardiomyopathy. J Interv Card Electrophysiol. 2004;10:19–26.
102. Teske AJ, Cox MG, Te Riele AS, et al. Early detection of regional functional abnormalities in asymptomatic arrhythmogenic right ventricular dysplasia/cardiomyopathy mutation carriers. J Am Soc Echocardiogr. 2012;25:997–1006.
103. Borgquits R, Haugaa KH, Gilljam T, et al. The diagnostic performance of imaging methods in ARVC using the 2010 task force criteria. Eur Heart J Cardiovasc Imaging. 2014;15:1219–25.
104. Bluemke DA, Krupinski EA, Ovitt T, et al. MR. Imaging of arrhythmogenic right ventricular cardiomyopathy: morphologic findings and interobserver reliability. Cardiology. 2003;99:153–62.
105. Tandri H, Calkins H, Nasir K, et al. Magnetic resonance imaging findings in patients meeting task force criteria for arrhythmogenic right ventricular dysplasia. J Cardiovasc Electrophysiol. 2003;14:476–82.
106. Basso C, Ronco F, Marcus F, et al. Quantitative assessment of endocardial biopsy in arrhythmogenic cardiomyopathy/dysplasia: an in vitro validation of diagnostic criteria. Eur Heart J. 2008;29:2760–71.
107. Nava A, Bauce B, Basso C, et al. Clinical profile and long-term follow-up of 37 families with arrhythmogenic right ventricular cardiomyopathy. J Am Coll Cardiol. 2000;36:2226–33.
108. Lerman BB, Stein KM, Markowitz SM. Idiopathic right ventricular outflow tract tachycardia: a clinical approach. PACE. 1996;19:2120–37.
109. Markowitz SM, Litvak BL, de Arellano EA R, et al. Adenosine-sensitive ventricular tachycardia, right ventricular abnormalities delineated by magnetic resonance imaging. Circulation. 1997;96:1192–200.
110. Thomas KW, Hunninghake GW. Sarcoidosis. JAMA. 2003;289:3300–3.
111. Chapelon C, Piette JC, Uzzan B, et al. The advantages of histological samples in sarcoidosis. Retrospective multicenter analysis of 618 biopsies performed on 416 patients. Rev Med Interne. 1987;8:181–5.
112. Philips B, Madhavan S, James CA, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy and cardiac sarcoidosis distinguishing features when the diagnosis is unclear. Circ Arrhythm Electrophysiol. 2014;7:230–6.
113. Ladyjanskaja GA, Basso C, Hobbelink MG, et al. Sarcoid myocarditis with ventricular tachycardia mimicking ARVD/C. J Cardiovasc Electrophysiol. 2010;21:94–8.
114. Blankstein R, Osborne M, Naya M, et al. Cardiac positron emission tomography enhances prognostic assessment of patients with suspected cardiac sarcoidosis. J Am Coll Cardiol. 2014;63:329–36.
115. Tanawuttiwat T, Sager SJ, Hare JM, Myerburg RJ, et al. Myocarditis and ARVC/D: variants or mimics? Heart Rhythm. 2013;10:1544–8.
116. Roberts JD, Herkert JC, Rutberg J, et al. Detection of genomic deletions of PKP2 in arrhythmogenic right ventricular cardiomyopathy. Clin Genet. 2013;83:452–6.
117. van der Zwaag PA, van Rijsingen IA, de Ruiter, R, et al. Recurrent and founder mutations in the Netherlands-Phospholamban p. Arg14del mutation causes arrhythmogenic cardiomyopathy. Neth Heart J. 201;21:286-293.
118. López-Ayala JM, Boven L, van den Wijngaard A, et al. Phospholamban p.arg14del mutation in a Spanish family with arrhythmogenic cardiomyopathy: evidence for a European founder mutation. Rev Esp Cardiol (Engl Ed). 2015;68:346–9.
119. Van Spaendonck-Zwarts KY, van der Kooi AJ, van den Berg MP, et al. Recurrent and founder mutations in the Netherlands: the cardiac phenotype of DES founder mutations p.S13F and p. N342D. Neth Hear J. 2012;20:219–28.

120. McCormick EM, Kenyon L, Falk MJ. Desmin common mutation is associated with multi-systemic disease manifestations and depletion of mitochondria and mitochondrial DNA. Front Genet. 2015;6:199.
121. Kapplinger JD, Landstrom AP, Salisbury BA, et al. Distinguishing ARVC/D-associated mutations from from background genetic noise. J Am Coll Cardiol. 2011;57:2317–27.
122. Van der Zwaag PA, Jongbloed JD, van Tintelen JP. Genetic diagnosis through whole-exome sequencing. N Engl J Med. 2014; 370:1067.
123. Corrado D, Leoni L, Link MS, et al. Implantable cardioverter-defibrillator therapy for prevention of sudden death in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia. Circulation. 2003;108:3084–91.
124. Corrado D, Wichter T, Link MS, et al. Treatment of arrhythmogenic cardiomyopathy/dysplasia: an international task force consensus statement. Circulation. 2015;132:441–53.
125. Corrado D, Wichter T, Link MS, et al. Treatment of arrhythmogenic cardiomyopathy/dysplasia: an international task force consensus statement. Eur Heart J. 2015;36:3227–37.
126. Saguner AM, Medeiros-Domingo A, Schwyzer MA, et al. Usefulness of inducible ventricular tachycardia to predict long-term adverse outcomes in arrhythmogenic right ventricular cardiomyopathy. Am J Cardiol. 2013;113:1728–34.
127. Bhonsale A, James CA, Tichnell C, et al. Incidence and predictors of implantable cardioverter-defibrillator therapy in patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy undergoing implantable cardioverter-defibrillator implantation for primary prevention. J Am Coll Cardiol. 2011;58: 1485–96.
128. Corrado D, Basso C, Rizzoli G, Schiavon M, Thiene G. Does sports activity enhance the risk of sudden death in adolescents and young adults? J Am Coll Cardiol. 2003;42:1959–63.
129. Wichter T, Paul TM, Eckardt L, et al. Arrhythmogenic right ventricular cardiomyopathy. Antiarrhythmic drugs, catheter ablation, or ICD? Herz. 2005;30:91–101.
130. Garcia FC, Bazan V, Zado ES, et al. Epicardial substrate and outcome of epicardial ablation of ventricular tachycardia in arrhythmogenic right ventricular cardiomyopathy/dysplasia. Circulation. 2009;120:366–75.
131. Philips B, te Riele AS, Sawant A, et al. Outcomes and ventricular recurrence characteristics after epicardial ablation of ventricular tachycardia in arrhythmogenic right ventricular dysplasia/cardiomyopathy. Heart Rhythm. 2015;12(4):716–25.
132. Santangeli P, Zado ES, Supple GE, et al. Long-term outcome with catheter ablation for ventricular tachycardia in patients with arrhythmogenic right ventricular cardiomyopathy. Circ Arrhythm Electrophysiol. 2015;8:1413–21.
133. Dalal D, Jain R, Tandri H, et al. Long-term efficacy of catheter ablation of ventricular tachycardia in patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy. J Am Coll Cardiol. 2007;50:432–40.
134. Te Riele AS, Ajijola OA, Shivkumar K, et al. Role of bilateral sympathectomy in the treatment of refractory ventricular arrhythmias in arrhythmogenic right ventricular dysplasia/cardiomyopathy. Circ Arrhythm Electrophysiol. 2016;9: e003713.
135. Tedford RJ, James C, Judge DP, et al. Cardiac transplantation in arrhythmogenic right ventricular dysplasia/cardiomyopathy. J Am Coll Cardiol. 2012;59:289–90.

Left Ventricular Noncompaction

7

Yvonne M. Hoedemaekers and Sabine Klaassen

Abstract

LVNC is a relatively new, genetically heterogeneous, cardiomyopathy. Clinical presentation and prognosis range from asymptomatic disease with no or slow progression, to severe disabling, rapidly progressive cardiac failure. Initial presentation includes the triad of heart failure (potentially lethal) arrhythmias and/or thrombo-embolism. LVNC may occur at all ages, even prenatally. In childhood, clinical features are often more severe and LVNC is frequently associated with congenital heart defects. In adults, the majority of LVNC is isolated. The echocardiographic diagnostic criteria as proposed by Jenni et al. are currently the most widely applied. General cardiac guidelines for chronic heart failure and ICDs are applicable to the LVNC population. In approximately 40 % of isolated LVNC, molecular testing may yield a genetic (mostly sarcomere) defect, with *MYH7* as the most prevalent disease gene. The nonisolated forms of LVNC are caused by a range of rare genetic defects. Until now, in half of familial isolated LVNC, the genetic defect remains unknown. Genetic defects in a large number of sarcomere and other cardiomyopathy genes and in genes primarily associated with skeletal myopathies indicate that LVNC may result from a wide range of pathophysiologic mechanisms. Shared genetic defects and familial aggregation of LVNC, HCM, and DCM indicates that LVNC may be part of a broad spectrum of cardiomyopathies. The genetic etiology of LVNC requires that patients and their relatives are offered genetic testing and counseling. This may include (predictive) molecular analysis of relatives, when applicable, and/or cardiac evaluation of at-risk relatives, even when they are as yet asymptomatic.

Introduction

Noncompaction of the left ventricle or left ventricular noncompaction (LVNC) is a relatively new clinicopathologic entity, first described by Feldt et al. in 1969 [1]. LVNC is characterized by a prominent trabecular meshwork and deep intertrabecular recesses communicating with the left ventricular (LV) cavity, morphologically reminiscent of early cardiac development, and is therefore thought to be caused by an arrest of normal embryogenesis of the myocardium [2, 3]. Initial presentation includes congestive heart failure, thromboembolic events, and (potentially lethal) arrhythmias, including sudden cardiac death. LVNC may be a part of a more generalized cardiomyopathy, involving both the morphologically normal and the predominantly apical, abnormal LV segments. The cardiologic features of LVNC range from asymptomatic in adults to severe congenital forms [4–6]. LVNC was classified by the American Heart Association (AHA) as a separate primary, genetic cardiomyopathy, based on the predominant myocardial involvement and genetic etiology [7]. The European Society of Cardiology (ESC)

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considers LVNC as unclassified, due to the lack of consensus whether LVNC is a separate individual cardiomyopathy or a nonspecific morphological trait that can be found solitary or in combination with other forms of cardiomyopathy like hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), or with congenital heart disease [8]. The overlap in phenotypes raises the question whether LVNC is in fact a distinct cardiomyopathy or whether it is a morphological expression of different underlying diseases [9]. The majority of LVNC diagnosed in adults is isolated. Nonisolated forms of LVNC are more frequent in childhood and may co-occur with congenital heart malformations, or may be part of a malformation or chromosomal syndrome [6]. The combination of LVNC and neuromuscular disorders is observed in adults as well as in children.

The majority of LVNC, both isolated and nonisolated, is hereditary and LVNC appears to be genetically heterogenous. An important proportion of isolated LVNC in children and adults has been associated with mutations in the same *sarcomere* genes that are involved in HCM, DCM, and restrictive cardiomyopathy (RCM) [10]. Absence of an identifiable genetic defect does not preclude a genetic cause of LVNC. In approximately half of the familial LVNC, the genetic defect remains unknown [10]. Shared sarcomere defects and the occurrence of HCM and DCM in families with LVNC patients indicate that at least some forms of LVNC are part of a broader cardiomyopathy spectrum.

The literature differentially refers to this form of cardiomyopathy as left ventricular noncompaction (LVNC), noncompaction cardiomyopathy (NCCM), noncompaction of the left ventricular myocardium (NCLVM), left ventricular hypertrabeculation (LVHT), spongiform cardiomyopathy, embryonic myocardium, honeycombed myocardium, persisting myocardial sinusoids, myocardial dysgenesis, ventricular dysplasia, or spongy myocardium.

Definition

LVNC is defined by prominent *trabeculations* on the luminal surface of the LV apex, the lateral wall, and rarely the septum in association with deep recesses that extend into the ventricular wall, which do not communicate with the coronary circulation. It is associated with a clinical triad of heart failure, arrhythmias, and/or thromboembolic events [11, 12].

Epidemiology

Estimates of prevalence of LVNC were derived from large retrospective studies of patients referred for echocardiography. Population studies for LVNC have not been performed. In 1997 Ritter et al. identified LVNC in 17 of 37,555 (0.045 %) patients who had an echocardiographic examination [13]. Similarly, in 2006 Aras et al. reported a prevalence of 0.14 % in over 42,000 patients and in 2008 Sandhu identified definite or possible LVNC in 13/4929 (0.26 %) patients referred for echocardiography [14, 15]. Prevalence was much higher (3.7 %) in patients selected for a LV ejection fraction ≤45 % [14]. Depending on the diagnostic criteria applied, even higher prevalence of LVNC (15.8 % by Belanger; 23.6 % by Kohli) was reported recently, indicating that LVNC may be more prevalent than previously indicated [12, 16]. A substantial proportion of individuals is asymptomatic, suggesting that true prevalence of LVNC may be higher, because asymptomatic individuals may go unnoticed in the studies of cardiologic patients [10, 12]. In a large study on childhood cardiomyopathies, LVNC was the most frequent cardiomyopathy after DCM and HCM, with an estimated prevalence of 9 % in pediatric cardiomyopathies [17].

Clinical Aspects

Heart failure is among the most frequent presentations of LVNC, followed by supraventricular and ventricular arrhythmias, including sudden cardiac death, and thromboembolic events. However, as in other cardiomyopathies, there is a great variability in presentation, even within families, ranging from a fully asymptomatic course to severe heart failure necessitating cardiac transplantation. The age of presentation is also highly variable varying from prenatal and neonatal diagnosis to diagnosis at the age of 94 years [5, 18, 19]. Prenatal diagnostic imaging more often detects bilateral ventricular hypertrophy/hypertrabeculations than the typical left ventricular morphologic changes observed postnatally and in adults [20]. The fourth to fifth decade is the median age for diagnosis in adult isolated LVNC, constituting a relatively young population in adult cardiologic practice. Many patients remain asymptomatic and may be detected due to an asymptomatic heart murmur, or by chance by preoperative cardiac evaluation or medical assessment for insurance or jobs or because they participated in cardiologic family screening, after a relative had been diagnosed with LVNC. Symptomatic patients may present clinical symptoms of dyspnea, fatigue (atypical) chest pain, and/or (pre) syncope. LVNC may also present as a peripartum cardiomyopathy [4, 21]. Review of the literature revealed a male to female ratio of almost 2:1 [22]. This gender difference cannot be fully explained by the occurrence of X-linked forms of LVNC.

Different arrhythmias and conduction disorders may occur in LVNC patients (Table 7.1) [23]. None of these arrhythmias is characteristic or pathognomonic for LVNC. Thromboembolic events may include stroke (cerebrovascular event or transient ischemic attack), peripheral embolism, and mesenterial thrombosis.

Clinical Diagnosis

Diagnosis of LVNC is still a challenge and relies on two-dimensional transthoracic echocardiography and/or cardiac magnetic resonance imaging (MRI) (Table 7.2). Improvements in cardiac imaging techniques have led to increased recognition and diagnosis of LVNC. Figure 7.1 displays echocardiographic and cardiac MRI images of two LVNC patients, showing the abnormal segmental trabeculations as the hallmark of this entity.

Table 7.1 Arrhythmia and conduction disorders associated with left ventricular noncompaction (LVNC)

Arrhythmia/conduction disorders associated with LVNC	Reference
Atrial fibrillation	[24–26]
Atrioventricular nodal reentrant tachycardia	[27]
Bigeminy ventricular extra systole	[28]
Complete atrioventricular block	[1, 29–31]
Complete left bundle branch block	[28, 32]
Early repolarization	[33]
Giant P-waves and focal atrial tachycardia	[34]
Long QT syndrome 2	[35]
Narrow QRS complex	[36–38]
Persistent atrial standstill	[39]
Sick sinus syndrome	[40, 41]
Sinus bradycardia	[41–44]
Supraventricular tachyarrhythmia	[6, 25, 28, 45, 46]
Ventricular fibrillation	[29, 36, 47]
Ventricular tachycardia	[6, 32, 36, 42, 48]
Wolff–Parkinson–White syndrome	[2, 6, 25, 28, 42]

Table 7.2 Echocardiographic diagnostic criteria for LVNC

I. Chin et al. [2]
Focusing on trabeculae localized at the LV apex on the parasternal short axis and apical views and on LV free-wall thickness at end-diastole LVNC is defined by a ratio of X/Y ≤ 0.5 with
X = distance from the epicardial surface to the trough of the trabecular recess
Y = distance from the epicardial surface to the peak of the trabeculation
II. Jenni et al. [11]
1. An excessively thickened LV myocardial wall with a two-layered structure consisting of a compact epicardial layer (C) and a noncompacted endocardial layer (NC) of prominent trabeculations and deep intertrabecular recesses
2. A maximal end-systolic NC/C ratio >2, measured at the parasternal short axis
3. Color Doppler evidence of deep perfused intertrabecular recesses
4. Absence of coexisting cardiac anomalies
III. Stollberger et al. [24]
1. More than three trabeculations protruding from the LV wall, apical to the papillary muscles and visible in a single image 2. Perfusion of the intertrabecular spaces from the ventricular cavity visualized on color Doppler imaging

Features of noncompaction observed in cardiologic patients and normal controls still illustrate the necessity of defining criteria in order to accurately differentiate between normal physiological trabecularization and LVNC [16].

In 1990, the first diagnostic criteria for LVNC by Chin et al. were derived from the observations made in eight LVNC patients [2]. These diagnostic criteria defined LVNC by the ratio of the distance from the epicardial surface to the trough of the trabecular recess (X) to the distance from the epicardial surface to the peak of the trabeculations (Y), with ratio X/Y ≤ 0.5.

More than a decade later, Jenni et al. proposed new diagnostic criteria for isolated LVNC, consisting of four echocardiographic features: (1) an excessively thickened LV myocardial wall with a two-layered structure consisting of a compact epicardial layer (C) and a noncompacted endocardial layer (NC) of prominent trabeculations and deep intertrabecular recesses; (2) a maximal end-systolic *NC/C ratio* > 2, measured at the parasternal short axis; (3) color-Doppler evidence of deeply perfused intertrabecular recesses; (4) absence of coexisting cardiac anomalies [11].

In 2002, Stollberger et al. proposed other diagnostic criteria for LVNC, wherein the diagnosis was a function of the number of trabeculations (>3) protruding from the LV wall, apically to the papillary muscles and visible in a single image plane with obligatory perfusion of the intertrabecular spaces from the ventricular cavity visualized on color-Doppler imaging [24].

More recently, MRI criteria for LVNC introduced by Petersen et al. indicated that a noncompacted/compacted ratio (NC/C) of >2.3, measured in end-diastole, can differentiate with sufficient sensitivity between the normal variation of noncompaction of the LV in the population, noncompaction in other cardiovascular disorders, and LVNC; the localization of noncompaction appeared to be more in the apical and lateral segments than in the basal and septal segments [49]. Jacquier et al. measured the trabeculated LV mass by MRI and postulated that a mass above 20 % is specific for the diagnosis of LVNC [50].

Belanger et al. proposed a classification system of LVNC by dividing noncompaction into four categories (none, mild, moderate, and severe) according to noncompaction to compaction ratio and the size of the noncompaction area [12]. This new classification scheme used the following criteria: (1) absence of congenital heart disease, hypertrophic or infiltrative cardiomyopathy, and coronary artery disease; (2) evidence of prominent trabeculations in the apex in any view (noncompacted to compacted ratio does not require to be >2); (3) concentration of the noncompacted area in the apex; (4) blood flow through the area of noncompaction.

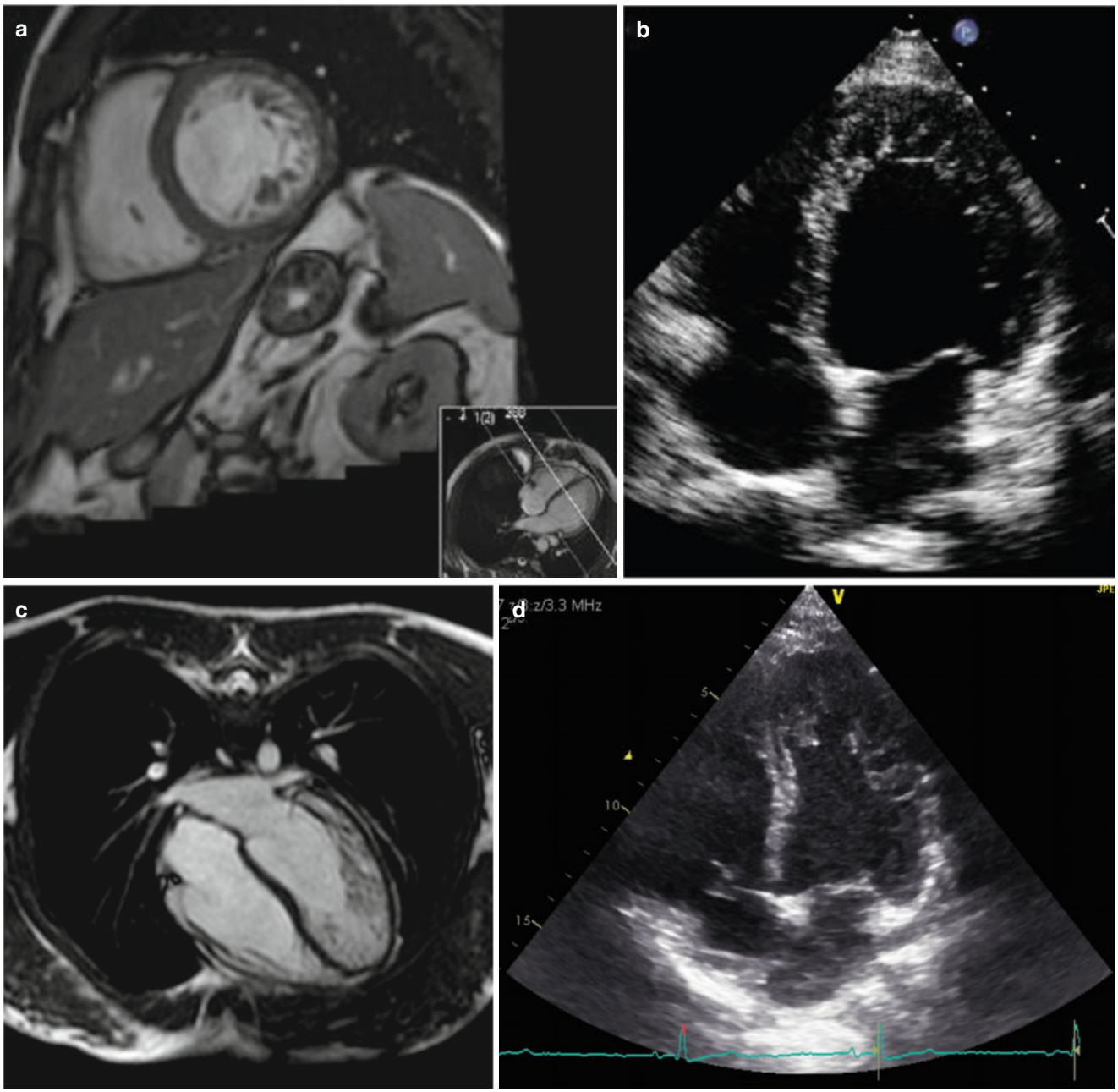


Fig. 7.1 (**a**, **b**) Cardiac MRI and echocardiography of a 43-year-old patient illustrating a two-layered myocardium with prominent intertrabecular recesses. (**c**, **d**) Cardiac MRI and echocardiography, four-chamber view each, of a 15-year-old patient with LVNC

The Jenni echo criteria have been the most convenient to work with in daily clinical practice and have been most widely applied in studies. However, further efforts to reach universal consensus with respect to the diagnosis of LVNC remain needed. A disparity in diagnosis has been observed when comparing the application of three different sets of LVNC criteria (Chin, Jenni, and Stollberger) in a cohort of 199 heart failure patients; 79 % fulfilled the Chin criteria, 64 % fulfilled the Jenni criteria, and 53 % fulfilled the criteria proposed by Stollberger. In only 30 % of patients, there was consensus among the three criteria on the diagnosis. Moreover, 8.3 % of normal controls fulfilled one or more criteria with a higher prevalence in black controls, and overdiagnosis is easily facilitated with the current diagnostic criteria [16, 51].

For now, it is disputable whether any of these diagnostic criteria are sufficiently sensitive to diagnose patients with mild noncompaction, and identify patients who may benefit from careful surveillance. For instance, in LVNC family studies, a substantial proportion of (mostly asymptomatic)

relatives showed mild to moderate features of LVNC [10]. Longitudinal studies of mild forms of LVNC are required to determine whether the current diagnostic criteria are suitable for diagnosis of family members in familial LVNC, or should be adapted in analogy to the criteria proposed for diagnosis of attenuated forms of familial HCM in relatives.

Pathology

Macroscopy

The noncompacted endocardial layer of the myocardium comprises excessively numerous and prominent trabeculations with deep intertrabecular recesses that extend into the compacted myocardial layer. The apical and mid ventricular segments of the LV inferior and lateral wall are predominantly affected [52, 53]. In a pathoanatomical study of LVNC, Burke et al. described the morphology and microscopy of 14 pediatric LVNC cases. The macroscopic appearance varied from anastomosing trabeculae to a relatively smooth endocardial surface, with narrow openings of the recesses to the ventricular cavity. Three types of recess patterns were distinguished: (1) anastomosing broad trabeculae, (2) coarse trabeculae resembling multiple papillary muscles, and (3) interlacing smaller muscle bundles or relatively smooth endocardial surface with compressed invaginations, identified primarily microscopically (Fig. 7.2). In this study, no morphological differences were found between isolated and nonisolated LVNC [52].

Jenni et al. described pathology of seven adult LVNC cases; the pathoanatomical localization of the noncompacted myocardium corresponded to the echocardiographic findings. Two patients also showed involvement of the right ventricular apex [11].

In a review of published pathology of LVNC, Stollberger et al. distinguished three particular morphologic features of LVNC in adults and children: (1) extensive spongiform transformation of the LV, (2) prominent coarse trabeculations and deep recesses, covered with endocardial tissue and not communicating with coronary arteries, and (3) dysplastic thinned myocardium with excessive trabeculations [22]. The first morphology was frequently associated with other cardiac malformations, compared to the second and third.

In 1987, in an autopsy study of 474 normal hearts of all ages, it was found that prominent trabeculations may be observed in as many as 68 % of the hearts, although more than three trabeculations were only identified in 3.4 % [54].

Microscopy

Two patterns of myocardial structure in the superficial noncompacted layer in LVNC have been described by Burke et al.: (1) anastomosing muscle bundles forming irregularly branching endocardial recesses with a staghorn-like appearance and (2) multiple small papillary muscles, resulting in an irregular surface appearance (Fig. 7.3). In most patients, these patterns overlapped. Endocardial fibrosis with prominent elastin deposition was found in all 14 cases, and subendocardial replacement fibrosis, consistent with microscopic ischemic infarcts, was present in 10; right ventricular involvement was identified in 6 cases [52].

Histological examination in another study showed that ventricular endocardium covered the recesses in continuity with the LV cavity and identified ischemic lesions in the thickened endocardium and the prominent trabeculae. Interstitial fibrosis ranged from being absent to severe. No fiber disarray was identified in any of these cases. Signs of chronic inflammation and abnormalities of intramyocardial blood vessels were present in some patients [11].

Freedom et al. proposed two criteria for the pathological diagnosis of LVNC: (1) absence of well-formed LV papillary muscles and (2) histological verification of more than 50 % penetration of invaginated endocardial recesses toward the epicardial surface. The endothelium that covers the recesses extends close to the surface of the compact layer. The recesses neither communicate nor connect with the coronary circulation [55].

Differential Diagnosis

The definitive diagnosis of LVNC relies on the morphological features of the LV myocardium, as defined by an imaging modality, like echocardiography, MRI, CT, or LV angiography. The variability in the extent of physiological trabecularization may complicate distinction of LVNC from normal physiological LV trabeculations. Especially in the area around the base of the papillary muscles of the mitral valve, more trabeculations may be present. However, in the normal heart, there is no excessive segmental thickening (due to hypertrabeculation) like in LVNC and the thickness of these physiological trabeculations does not exceed the thickness of the compact layer. Also, the area of noncompaction is larger in LVNC than in physiological trabeculations [12].

Secondary forms of (acquired) LVNC may be the result of hypertension, chronic volume or pressure overload, ischemic heart disease or extreme physical activity (i.e., athletes), leading to LVNC-like abnormalities. These are referred to as pseudo-left ventricular noncompaction or LVNC look-alike. Hypertensive patients are diagnostically challenging, because of the occurrence of LV hypertrophy due to hypertension. Further studies are needed to confirm whether excessive trabeculation is more prevalent in specific ethnic groups, as suggested by one study [16].

Furthermore, dilated, hypertrophic, and ischemic cardiomyopathy may be mistaken for LVNC or vice versa, due to

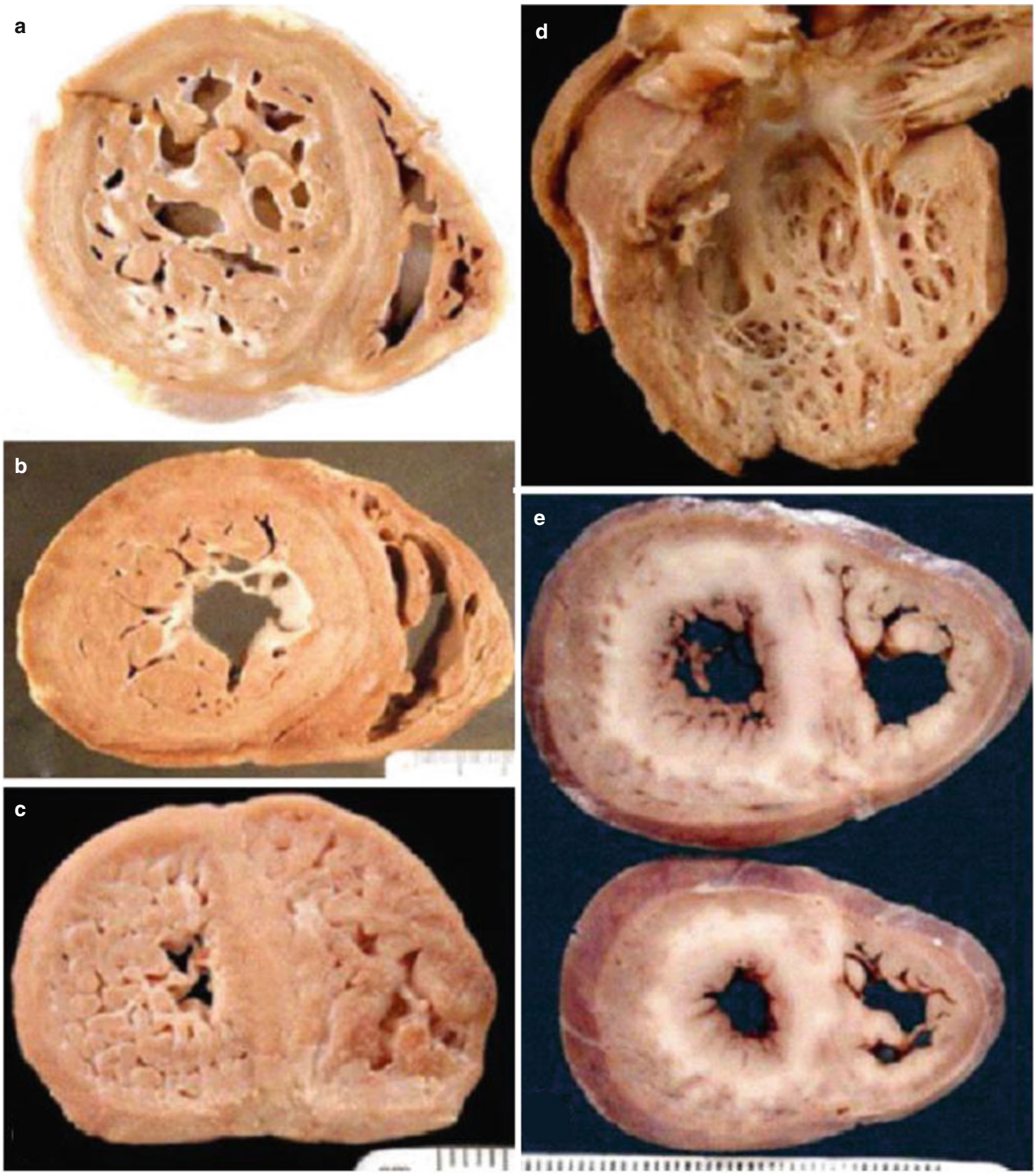


Fig. 7.2 LVNC gross pathology with a variety of LVNC patterns: (**a**) Anastomosing broad trabeculae. (**b**) Coarse trabeculae resembling multiple papillary muscles. (**c**) Interlacing smaller muscle bundles resembling a sponge. (**d**) Trabeculae viewed en face. (**e**) Subtle LVNC on gross section, requires histological confirmation (Reproduced from Burke et al. [52] with permission)

prominent trabeculations or abnormal myocardial thickening. Neuromuscular disorders, syndromes, and chromosomal abnormalities (Tables 7.3, 7.4, and 7.5) should be considered in the differential diagnosis of nonisolated LVNC, especially when LVNC occurs in patients with dysmorphism, growth retardation, or skeletal muscle weakness.

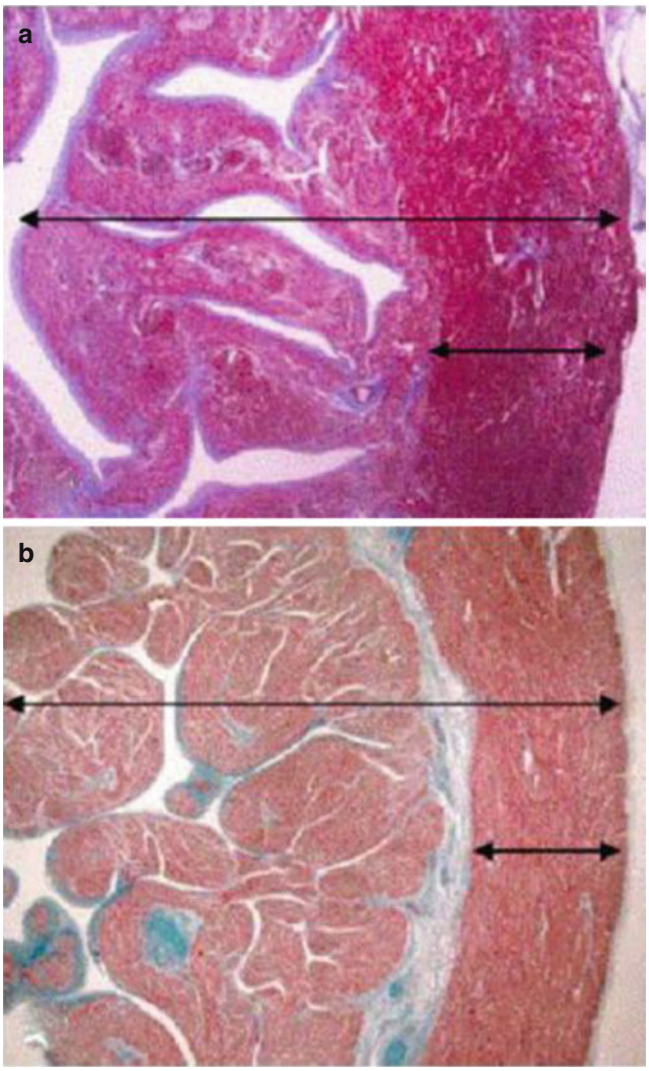


Fig. 7.3 Histological features in LVNC. The ratio of noncompact versus compact myocardium is larger than 2. (**a**) Relatively smooth endocardial surface (*left*) with anastomosing broad trabeculae. (**b**) Polypoid pattern of trabeculae; prominent fibrous band separating the noncompact from the compact myocardium (Reproduced from Burke et al. [52] with permission)

Work-Up, Therapy, Follow-Up, and Prognosis

Work-Up

Work-up of an LVNC patient should focus on identifying the underlying cause, either genetic or other (Table 7.3).

Therapy and Follow-Up

Current guidelines for heart failure, arrhythmias, cardiac resynchronization therapy, and ICD implantation for primary and secondary prevention are applied for LVNC [56–58]. β-Blockers and angiotensin-converting enzyme (ACE) inhibitors are the cornerstones of the treatment in the presence of LV dysfunction and/or arrhythmias. Establishing an expert consensus rapport, similar to HCM [59], based on case reports, small cohorts and clinical registries would be recommended since no randomized trials or studies on management of LVNC have been conducted, and clear-cut evidence-based clinical guidelines for this disorder are therefore missing. An important issue is the use of prophylactic anticoagulants, in view of frequent thromboembolic events. The early case reports and case series emphasized the high risk of thromboembolism and advised routine anticoagulation therapy. However, a review of 22 publications addressing the issue concluded that thromboembolic events are rare in LVNC [60]. Fazio et al. came to the same conclusion [61]. Therefore, anticoagulation therapy is advised only in patients with an ejection fraction less than 40 % (cutoff arbitrary), paroxysmal or persistent atrial fibrillation and/or previous thromboembolic events.

Successful cardiac resynchronization therapy has been described in several LVNC patients, leading to LV reverse remodeling and an increase in LV function [20, 36, 62–64].

Heart transplantation has been performed in some LVNC patients with severe heart failure [23, 65–67]. LV restoration surgery has been reported successful in a single patient [68]. Treatment with an *implantable cardioverter defibrillator* (ICD) will be discussed further on.

The indication for cardiologic follow-up depends on individual symptoms and cardiac abnormalities. In asymptomatic patients with preserved LV function, annual or biannual cardiologic follow-up is recommended, including ECG and echocardiography. If necessary, these could be extended with 24-h-Holter monitoring and exercise-testing. When EF is below 50 %, β-blocker therapy and ACE inhibitors should be prescribed, especially when LVNC is accompanied by hypertension or arrhythmias.

Risk Stratification and Indication for ICD

Patients at the highest risk for sudden death are patients who previously experienced (aborted) cardiac arrest, ventricular fibrillation, and sustained VF. Family history of sudden death, unexplained syncope (especially during exercise), abnormal blood pressure response during exercise tests, frequent premature ventricular beats on the resting ECG, and/or nonsustained ventricular tachycardia on Holter monitoring and significantly impaired LV function may be considered risk factors. The results from longitudinal studies and the understanding of underlying disease mechanisms will hopefully help to gain more insight into

Table 7.3 Proposed diagnostic work-up of a newly identified index patient with LVNC (Modified from the ACCF/AHA guidelines for the diagnosis and management of heart failure in adults [56])

History	Chest pain; palpitations; intake of alcohol, cocaine, medication; chemotherapy; radiation; deficiencies
Family history	Cardiomyopathy; conduction disease, arrhythmia, sudden cardiac or unexplained death; neuromuscular disease
ECG	Conduction disease; arrhythmia; sick sinus syndrome; prolonged QT; Q-waves; hypertrophy (see also Table 7.1)
Echocardiography	Congenital heart disease; Jenni criteria; LV ejection fraction
Laboratory	Complete blood count; serum electrolytes; blood urea nitrogen, serum creatinine, fasting blood glucose, lipids, liver function tests, thyroid function, CRP, iron status, creatine kinase, noradrenaline, cortisol, growth hormone
Viral work-up	Antibodies: Coxsackie-; influenza-; adeno-; echo-; cytomegalo-; human immunodeficiency virus
MRI	Myocardial infarction; infiltrative disease; myocarditis; dilated or hypertrophic cardiomyopathy, late gadolinium enhancement, NC/C ratio
Coronary angiography/ myocardial perfusion scintigraphy	Coronary artery disease
Mitochondrial work-up	When signs of mitochondrial disorder are present (e.g., myopathy; deafness; diabetes; encephalopathy; stroke-like episodes; ophthalmoplegia; retinopathy)
Neurologic examination	When signs of neuromuscular disease are present or when family history is positive for neuromuscular disease
Genetic counseling	Preferably for all cases
Genetic testing	Core panel when available; when unavailable ACTC1, MYBPC3, MYH7, TNNI3, TNNT2 and TPM1 (see also Fig. 7.5)

the risk factors and allow more appropriate risk stratification.

Consensus and guidelines for prophylactic ICD treatment in LVNC patients are also needed. Regular ICD indications include primary and secondary prevention. For secondary prevention, that is, after a previous episode of aborted cardiac death or collapse due to sustained VT or VF, current ICD guidelines advise ICD implantation. In the Rotterdam LVNC cohort of 67 patients, an ICD was indicated in 42 % according to the current ICD guidelines (n = 28; 21 primary and 7 for secondary prevention). After a long-term follow-up, appropriate ICD therapy occurred only in patients with secondary prevention (n = 3). Inappropriate ICD therapy occurred in 33 % of the patients with primary prevention and in 29 % of the patients with secondary prevention [69]. In another study, a follow-up of 12 patients who received an ICD showed overall appropriate therapy in 42 % in primary and secondary prevention combined. In primary prevention, 25 % of ICD therapy was appropriate opposed to 50 % in secondary prevention [45]. This accentuates the need for further research of appropriate risk stratification of sudden cardiac death in patients with LVNC.

Prognosis

Initially, LVNC was reported to have a grave prognosis. However, the application of new imaging techniques allowing diagnosing LVNC in asymptomatic individuals suggests that the first observations were influenced by selection of the most severely affected individuals. In children, age is not a predictor of the outcome [70]. New York Heart Association Class III or higher and presence of cardiovascular complications do seem to be a strong predictor [71]. It has become clear that prognosis of LVNC is as variable as the prognosis in other cardiomyopathies. Even in those with presentation in early childhood, gradual improvement in cardiac function may be observed, although in others evolvement to severe heart failure requiring heart transplantation does occur. Similarly, in some adult patients a rapid deterioration of heart function occurs, whereas in others the disease remains stable up to old age. Malignant arrhythmias leading to sudden cardiac death and heart failure are the main indicators of poor prognosis, also in children [72]. The establishment of appropriate risk stratification will be an important issue in the near future in order to identify patients at risk and to help prevent sudden cardiac death.

Etiology and Molecular Genetics

The etiology of LVNC is rapidly being unraveled as more and more genetic defects in different genes are found, indicating that LVNC is genetically heterogeneous. Currently, genetic defects are identified in approximately 40 % of LVNC patients [10, 63]. Most genetic defects are inherited as autosomal dominant trait (Table 7.4), with exception of rare genetic causes of syndromal LVNC, predominantly diagnosed in children. However, absence of a genetic defect does not exclude a genetic etiology. By performing systematic cardiologic family studies, it was shown that no genetic defect could be found in approximately half of the familial forms of LVNC, indicating that further studies are needed to find additional genetic causes for LVNC [10].

There is evidence that some forms of LVNC are part of a spectrum of cardiomyopathies, including hypertrophic,

Table 7.4 Genes associated with left ventricular noncompaction (LVNC)

Gene	Locus	Protein	Other associated disorders	Reference
ACTC1	15q14	α-Cardiac actin	Hypertrophic and dilated cardiomyopathy	[10, 64, 73]
			Congenital myopathy with fiber-type disproportion	
ACTN2	1q43	α-Actinin	Hypertrophic and dilated cardiomyopathy	[74]
CASQ2	1p13.3-p11	Calsequestrin	Catecholaminergic polymorphic ventricular tachycardia	[10]
			Hypertrophic cardiomyopathy	
DSP	6p24.3	Desmoplakin	Arrhythmogenic cardiomyopathy, dilated cardiomyopathy, epidermolysis bullosa, keratosis palmoplantaris striata, skin fragility–woolly hair syndrome	[65]
DTNA	18q12.1-q12.2	α-Dystrobrevin		[75, 76]
HCN4	15q24.1	Hyperpolarization-activated cyclic nucleotide-gated potassium channel 4	Brugada syndrome, sick sinus syndrome	[41, 44]
KCNH2	7q35-q36	Potassium voltage-gated channel, subfamily H, member 2	Long QT syndrome 2	[35]
			Short QT syndrome	
LDB3[a]	10q22.2-q23.3	LIM-Domain binding protein	Dilated cardiomyopathy	[10, 76–78]
			Late onset distal myopathy	
			Myofibrillar myopathy	
LMNA	1q21.2	Lamin A/C	Dilated cardiomyopathy	[10, 79, 80]
			Emery–Dreifuss muscular dystrophy	
			Lipodystrophy	
			Restrictive dermopathy	
			Werner syndrome	
			Hutchinson–Gilford Progeria	
			Limb girdle muscular dystrophy 1B	
			Charcot-Marie-Tooth 2B1	
MIB1	18q11.2	Mindbomb drosophila homolog 1	Left ventricular noncompaction	[66]
MYBPC3	11p11.2	Cardiac myosin-binding protein C	Hypertrophic and dilated cardiomyopathy	[10, 63]
MYH7	14q12	β-Myosin heavy chain	Hypertrophic, dilated, and restrictive cardiomyopathy	[10, 63, 64, 81, 82]
			Myosin storage myopathy	
			Distal myopathy	
			Scapuloperoneal myopathy	
NKX2.5	5q35.1	NK2 homeobox 5	Hypoplastic left heart syndrome; ventricular septal defect; atrial septal defect; Fallot; congenital hypothyroidism	[83]
PLN	6q22.1	Phospholamban	Hypertrophic and dilated cardiomyopathy	[10]
PRDM16	1p36	PR domain-containing protein 16	Dilated cardiomyopathy Del1p36 syndrome	[84]
SCN5A	3p21	Sodium channel type 5 α-subunit	Long QT syndrome 3	[85]
			Brugada syndrome	
			Sick sinus syndrome	
			Familial heart block	
			Paroxysmal ventricular fibrillation	
			Cardiac conduction defect	
			Dilated cardiomyopathy	
TAZ[b]	Xq28	Taffazin	Barth syndrome	[10, 75–77, 86–93]
			Dilated cardiomyopathy	
TNNI3	19p13.4	Cardiac troponin I	Hypertrophic, dilated, and restrictive cardiomyopathy	[10]
TNNT2	1q32	Cardiac troponin T	Hypertrophic, dilated, and restrictive cardiomyopathy	[10, 63, 64]
TPM1	15q22.1	A-tropomyosin	Hypertrophic and dilated cardiomyopathy	[10, 63]

Except *TAZ* related disorders, all are autosomal dominantly inherited
[a]Cypher/ZASP
[b]G4.5

dilated, and restrictive cardiomyopathy. A shared etiology consisting of genetic defects in the same sarcomere genes, sometimes even with identical mutations, has been found in these types of cardiomyopathy. Co-occurrence of LVNC, HCM, and DCM within families endorses a shared genetic susceptibility to these different forms of cardiomyopathy [10]. The phenotypic variability of cardiomyopathies within families, including variability in age at onset and severity of clinical features, might be explained by additional modifying factors, additional genetic variants or defects, or may depend on yet unidentified exogenous or systemic factors.

Molecular Defects in LVNC

Isolated LVNC has been associated with mutations in 20 different genes (Table 7.4). Defects in sarcomere genes have been identified to be the most prevalent genetic cause occurring in approximately 30 % of all patients with isolated LVNC [10, 63].

Over 40 different mutations in sarcomere genes encoding thick (*MYH7*), intermediate (*MYBPC3*), and thin filaments (*TNNT2*, *TNNI3*, *TPM1*, *ACTC*) have been described. In particular in *MYH7*, the most frequent LVNC-associated gene, accounting for up to 21 % of isolated LVNC [10, 63]. *MYH7* mutations currently associated with LVNC cluster in the ATP-ase active site of the head region in the N-terminal part of MYH7. This is an evolutionary well-conserved region of MYH7. As the ATP-ase active site is required for normal force production, impaired force generation might play a role in the etiology of LVNC. Mutations in this region have been associated with LVNC with or without Ebstein anomaly [64, 81]. Other *MYH7* mutations (30 %) were found in the C-terminal rod region of the MYH7 protein that plays an important role in the formation of the core of the thick filament. Mutations in this region of the gene are more commonly associated with skeletal myopathies. Relatively few cardiomyopathy mutations are situated in this region.

With the availability of targeted cardiomyopathy panels (next generation sequencing), more genes are and will be associated with LVNC, but as these data are still unpublished they are not mentioned here. Complex genotypes will become more common when more genes are analyzed per patient.

Multiple or compound/double heterozygous mutations were identified in 25 % of the children and in 10 % of the adult LVNC patients [10]. In hypertrophic cardiomyopathy, complex genotypes have been described in 7 % [94]. In HCM, double heterozygosity for truncating sarcomere mutations have been previously associated with severe congenital forms mostly inherited in an autosomal recessive mode [95–98]. Nonsarcomere genetic causes for isolated LVNC include mutations in the calcium-handling genes calsequestrin (*CASQ2*) and phospholamban (*PLN*), in taffazin (*TAZ*), α-dystrobrevin (*DTNA*), lamin A/C (*LMNA*) and LIM domain binding 3 (*LDB3*), potassium voltage-gated channel (*KCNH2*), and sodium channel type 5 (*SCN5A*) genes. However, mutations in these genes were only rare causes of LVNC in single families.

The absence of a mutation in approximately half of familial LVNC could be explained by phenotype assignment errors, the involvement of other yet unidentified genes, the presence of mutations in nonanalyzed gene sequences, and incomplete sensitivity of the methods used.

Pathogenesis

Mutations in different genes associated with LVNC affect different mechanisms in the cardiomyocyte leading to changes that may individually cause LVNC or lead to a common cellular disturbance resulting in LVNC. Cellular growth and differentiation signaling pathways are thought to be involved in LVNC pathogenesis [83, 99–101].

Mutations in sarcomere genes may have their effect through defective force generation (either by a dominant negative mechanism where the mutant protein acts as a "poison polypeptide" or by haploinsufficiency resulting in less protein); mutated cytoskeletal proteins may lead to a defective force transmission; myocardial energy deficits may be the result of mutations in ATP-regulatory genes and a fourth possible mechanism is abnormal calcium homeostasis due to either changes in calcium availability or myofibrillar sensitivity for calcium [102]. The development of LVNC features might be a compensatory response to dysfunction in one of these mechanisms.

The variable phenotypic expression of (sarcomere) gene mutations leading to different types of cardiomyopathy has not been explained. The localization of the mutations may partly explain phenotypic diversity. Another theory is "dose-effect"; the extent of the defective mechanism may determine which phenotype develops. Third, there might be independent pathways leading to the different cardiomyopathies. Finding identical mutations in different phenotypes suggests a role for additional factors, either environmental or molecular.

Isolated LVNC

The first hypothesis on the pathogenesis of LVNC stemmed from observations that the morphology of LVNC was reminiscent of the embryonic stages of cardiac development. Consequently, it was postulated that LVNC could be the result from an arrest of compaction of myocardial fibers [103]. Figure 7.4 illustrates the striking resemblance between LVNC and the physiological embryonic noncompaction in the eighth to tenth embryonic week. However, the possible mechanisms causing the arrest remain unclear. Epicardium-

derived cells are thought to play an important role in myocardial architecture and in the development of noncompaction [104, 105]. Mutations in genes involved in myocardial genesis like peroxisome proliferator activator receptor binding protein (*PBP*), jumonji (*JMJ*), FK506 binding protein (*FKBP12*), transcription factor specificity protein (*Sp3*), homeobox factor *NKX2.5*, bone morphogenetic protein 10 (*BMP10*) lead to congenital LVNC in knock out mice [106–111]. However, apart from the NKX2.5 gene, in human LVNC, no mutations in these genes have been described.

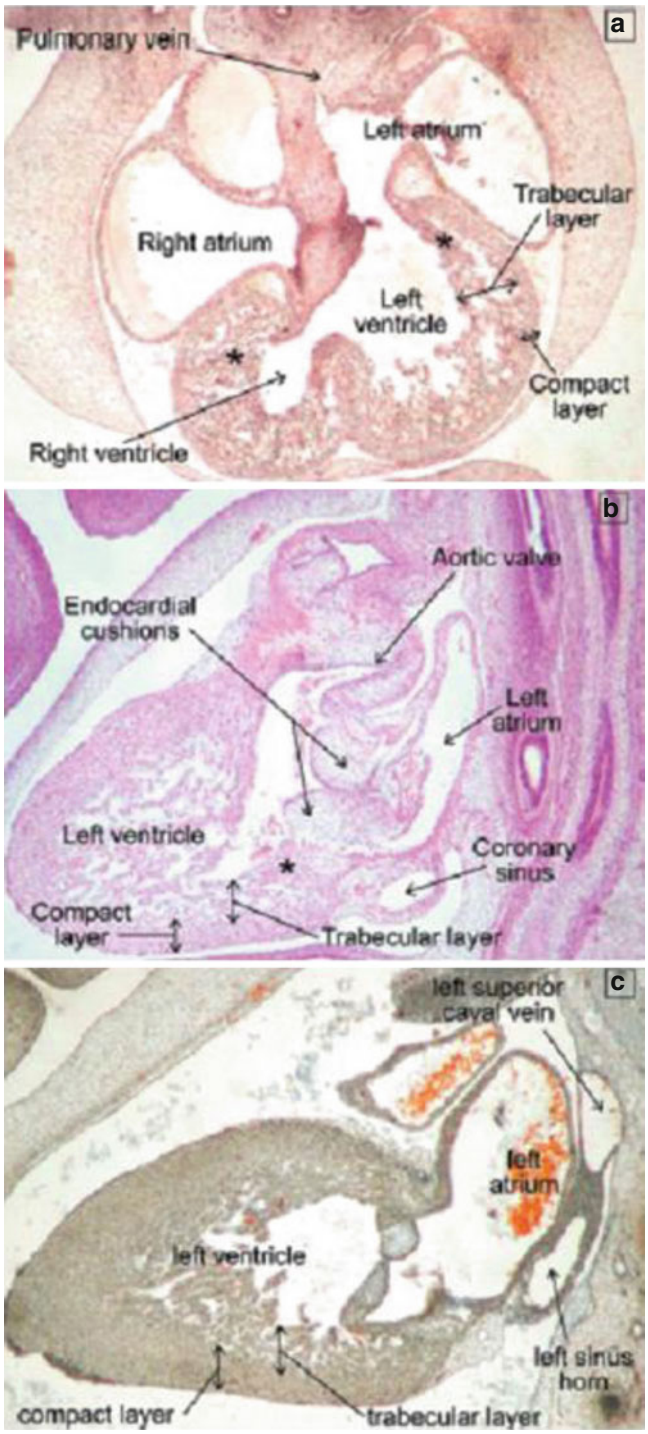


Fig. 7.4 Human embryos at Carnegie stage 16 (**a**), stage 18 (**b**) and after closing of the embryonic interventricular foramen (**c**). During development, there is an extensive trabecular layer forming the greater part of the ventricular wall thickness compared to the extent of the compact layer. The trabecular layer becomes compacted and forms the papillary muscles of the atrioventricular valves (*asterisks*) (Reproduced from Freedom et al. [55] with permission)

Until now, there is very little insight into factors that influence the variability in age at onset and severity of symptoms of LVNC, or any other familial form of cardiomyopathy.

In the majority of patients, LVNC is diagnosed in adulthood, similar to HCM and DCM, which are rarely congenital. Of course, it could be that in LVNC the lesions detected in adult patients were present from birth on, but remained unnoticed until symptoms developed and high-resolution cardiac imaging techniques were applied. However, the detection of sarcomere defects in LVNC patients may suggest otherwise, since mutations in sarcomere genes are known to cause late-onset HCM and DCM. Similarly, sarcomere mutations might lead to late onset LVNC. Longitudinal cardiologic studies of unaffected carriers of pathogenic mutations are necessary to provide insight whether noncompaction may develop later in life. The pathogenetic mechanism(s) of sarcomere defects in cardiomyopathies are not fully understood. It is possible that the pathological myocardial changes in the adult onset sarcomere related cardiomyopathies are caused by a compensatory response to impaired myocyte function resulting from mutations in the sarcomere genes [102, 112].

Nonisolated LVNC

LVNC has been observed in a number of neuromuscular disorders, metabolic and mitochondrial disease, congenital malformations, and chromosomal syndromes.

Some of these disorders may share pathogenetic mechanisms with LVNC. Alternatively, LVNC might be secondary to other cardiac malformations or other malformations or even vice versa. Another possibility is that the co-occurrence is coincidental. Congenital heart malformations, for instance, are relatively frequent (birth prevalence 0.008) and may therefore occasionally coincide with LVNC without a mutual etiology.

Congenital Heart Disease

The co-occurrence of congenital heart disease and noncompaction is predominantly observed in children. Tsai et al. showed that 78 % of 46 children with LVNC had a congenital heart defect [6]. Nevertheless, congenital heart defects and LVNC also co-occur in adults [113]. The large number of structural heart malformations reported in association with noncompaction is presented in Table 7.5, indi-

Table 7.5 Congenital heart disease associated with left ventricular noncompaction (LVNC)

Congenital heart disease in LVNC	Proportion of CHD		References
	In LVNC studies[a]	Case reports	
Aberrant origin of right/left subclavian artery	1/12 (8 %)	1	[28, 114]
Absent aortic valve		1	[115]
Anomalous pulmonary venous return	2/26 (8 %)		[28, 52]
Aortic coarctation	6/204 (3 %)		[6, 10, 25, 28, 116]
Aortico-LV tunnel		1	[117]
Aortic stenosis	2/46 (4 %)	2	[6, 55, 118]
Aortopulmonary window	1/21 (5 %)		[25]
Atrial septal defect	22/135 (16 %)	3	[6, 10, 25, 43, 81, 119]
Atrioventricular diverticulum		1	[120]
Bicuspid aortic valves	3/64 (5 %)	3	[6, 25, 121, 122]
Bicuspid pulmonary valve	1/14 (7 %)		[52]
Cardiac aneurysms		4	[31, 123–125]
Coronary ostial stenosis	1/14 (7 %)		[52]
Cor triatriatum	1/46 (2 %)		[6]
Dextrocardia	2/58 (3 %)	1	[1, 6, 28]
Dextro malposed great arteries	1/12 (8 %)		[28]
Dextroversion		1	[126]
Double inlet left ventricle	1/46 (2 %)		[6]
Double orifice mitral valve		4	[127–129]
Double outlet right ventricle	1/54 (2 %)		[116]
Ebstein's anomaly	11/130 (8 %)	14	[6, 10, 43, 82, 130–135]
Fallot's tetralogy	1/71 (1 %)	1	[10, 114]
Hypoplastic left heart syndrome	3/54 (6 %)		[116]
Hypoplastic right ventricle	3/58 (5 %)		[6, 28]
Isomerism of the left atrial appendage	4/66 (6 %)	8	[28, 55, 116, 136]
Left-sided superior vena cava	1/46 (2 %)		[6]
Mitral valve atresia		1	[115]
Mitral valve cleft	2/54 (4 %)	1	[31, 116]
Mitral valve dysplasia	2/14 (14 %)		[52]
Mitral valve prolaps	1/46 (2 %)		[6]
Patent ductus arteriosus	16/182 (9 %)	1	[6, 10, 43, 116]
Persistent left superior vena cava	1/14 (7 %)	1	[52, 125]
Pulmonary atresia	6/125 (5 %)	1	[10, 43, 116]
Pulmonary valve dysplasia	2/14 (14 %)		[52]
Pulmonary stenosis	4/97 (4 %)	1	[10, 28, 43, 52]
Single ventricle	1/12 (8 %)	1	[28, 137]
Subaortic membrane	2/55 (4 %)		[116]
Transposition of the great arteries	1/46 (2 %)	1	[6, 138]
Tricuspid atresia	2/54 (4 %)		[116]
Tricuspid valve dysplasia	1/14 (7 %)		[52]
Ventricular septal defect	23/218 (11 %)	3	[1, 6, 10, 25, 28, 52, 116, 118, 125]

[a]Cumulative number of LVNC patients with congenital heart defect (CHD) described in one or more LVNC studies

cating that septal defects, patent ductus arteriosus, and Ebstein's anomaly are the most prevalent congenital heart defects in LVNC.

Increasingly, *congenital cardiac malformations* (septal defects, Ebstein anomaly, patent ductus arteriosus, Fallot's tetralogy, aortic coarctation, and aortic aneurysms) are being reported in familial cardiomyopathies (HCM, DCM, and LVNC) linked to sarcomere mutations, suggesting that these specific sarcomere defects may have been involved in cardiac morphogenesis [10, 73, 81, 139–142]. But since there is rarely more than one patient with a congenital heart defect, even in families with multiple cardiomyopathy patients, the association of sarcomere defects and heart defects still demands further exploration.

Neuromuscular Disease

Similar to HCM and DCM, LVNC has been associated with neuromuscular disorders. Stollberger and Finsterer identified LVNC-like morphological features in Duchenne and Becker muscular dystrophy and in myotonic dystrophy (see section, "Neuromuscular Disorders") [143–145]. The gene mutated in Duchenne and Becker muscular dystrophy is a part of the dystrophine complex, a complex of muscle membrane associated proteins, connecting the cytoskeleton to the surrounding extracellular matrix and may also play a role in cell signaling. The dystrophine gene is expressed in skeletal and cardiac myocytes. Other genes previously associated with neuromuscular disorders, like adult onset myofibrillar myopathy (*LDB3* or *Cypher/ZASP*), limb girdle muscular dystrophy (LGMD) (*LMNA*), scapuloperoneal myopathy (*MYH7*), myosin storage distal myopathy (*MYH7*), and Barth syndrome (*TAZ*) have recently been associated with isolated LVNC (Table 7.4). ZASP, lamin A and C, β-myosin heavy chain, and taffazin are all expressed in cardiac and skeletal muscle tissue. ZASP has a function in cytoskeletal assembly. Mutations in ZASP can lead to DCM and to skeletal myopathy. Lamin A and C, proteins situated in the nuclear membrane, play an important role in maintaining nuclear architecture. *LMNA* mutations have been described in three LVNC patients [10, 79, 80]. In one of them, there was familial limb girdle muscular dystrophy (LGMD) as well as DCM [10]. Over 200 mutations have been described in *LMNA*, causing over 20 different phenotypes, including isolated DCM, LGMD, Emery–Dreifuss muscular dystrophy, Hutchinson–Gilford progeria, partial lipodystrophy, and peripheral neuropathy. For many of the phenotypes, there is no clear genotype–phenotype correlation, phenotypes may overlap, and different phenotypes are associated with single mutations. Up to 25 % of patients with an LMNA mutation may remain cardiologically asymptomatic [146].

Syndromes

LVNC can occur as part of a syndrome in combination with dysmorphic features and other congenital malformations. When there are other congenital defects or when there are dysmorphic features in a patient, one of the listed syndromes in Table 7.6 or one of the chromosomal defects in Table 7.7 could be considered in the differential diagnosis.

Table 7.6 Syndromes associated with left ventricular noncompaction (LVNC)/hypertrabeculation

Syndrome	Gene	Inheritance	Features	Reference
Barth syndrome/3-methylglutaconic aciduria	*TAZ*	XR	Growth retardation, dilated cardiomyopathy, skeletal myopathy, intermittent lactic acidemia, granulocytopenia, recurrent infections	[75–77, 86–93, 147]
Branchio-oto-renal syndrome I/ Melnick Fraser syndrome	*EYA1*	AD	Long narrow face; hearing loss (sensory/conductive/ mixed); preauricular pits; microtia; cup-shaped ears; lacrimal duct stenosis; cleft palate; bifid uvula; branchial cleft fistulas/cysts; renal dysplasia/aplasia; polycystic kidneys; vesico-ureteric reflux	[148]
Congenital adrenal hypoplasia	*NR0B1*	XR	Failure to thrive; hypogonadotropic hypogonadism; cryptorchidism; hyperpigmentation; primary adrenocortical failure; adrenal insufficiency; gluco-mineralocorticoid insufficiency; salt-wasting; delayed puberty	[149]
Contractural arachnodactyly/ Beals syndrome	*FBN2*	AD	Marfanoid habitus; micrognathia; frontal bossing; crumpled ear helices; ectopia lentis; high-arched palate; septal defects; bicuspid aortic valve; mitral valve prolapse; patent ductus arteriosus; aortic root dilatation; pectus carinatum; kyphoscoliosis; hip/knee/elbow contractures; arachnodactyly; ulnar deviation of fingers; talipes equinovarus; hypoplastic calf muscles; motor development delay	[150]
Cornelia de Lange syndrome I	*NIPBL*	AD	Short stature; microcephaly; long philtrum; micrognathia; low-set ears; sensorineural hearing loss; synophrys; myopia; long curly eyelashes; ptosis; anteverted nostrils; depressed nasal bridge; cleft lip/ palate; thin upper lip; widely spaced teeth; congenital heart defect; pyloric stenosis; hypoplastic male genitalia; structural renal anomalies; phocomelia; oligodactyly; syndactyly of second and third toes; single transverse palmar crease; cutis marmorata; hirsutism; low posterior hair line; mental retardation; language delay; automutilation	[25]

(continued)

Table 7.6 (continued)

Syndrome	Gene	Inheritance	Features	Reference
Leopard syndrome	*PTPN11* *RAF1*	AD	Short stature; triangular face; low-set ears; sensorineural hearing loss; hypertelorism; ptosis; epicanthal folds; broad flat nose; cleft palate; short neck; pulmonic stenosis; HCM; subaortic stenosis; complete heart block; bundle branch block; winged scapulae; hypospadia; absent/hypoplastic ovary; unilateral renal agenesis; spina bifida occulta; dark lentigines (mostly neck and trunk); café-au-lait spots	[151]
Melnick Needles osteodysplasty	*FLNA*	XD	Short stature; micrognathia; large ears; hypertelorism; exophthalmos; cleft palate; misaligned teeth; long neck; mitral/tricuspid valve prolapse; LVNC; pulmonary hypertension; pectus excavatum; omphalocele; hydronephrosis; tall vertebrae; bowing of humerus/radius/ulna/tibia; short distal phalanges of the fingers; pes planus; coarse hair; delayed motor development; hoarse voice	[152]
Nail Patella syndrome	*LMX1B*	AD	Short stature; sensorineural hearing loss; ptosis; cataract; cleft lip/palate; malformed sternum; hypoplasia of first ribs; glomerulonephritis; renal failure; kyphoscoliosis; elbow deformities; hypoplastic or absent patella; clinodactyly; talipes equinovarus; longitudinal ridging nails; slow nail growth; koilonychias; anonychia; aplasia pectoralis minor/biceps/triceps/quadriceps	[153]
Noonan syndrome	*PTPN11*[a] *KRAS* *SOS1* *RAF1*	AD	Short stature; triangular face; low-set ears; hypertelorism; downslanting palpebral fissures; epicanthal folds; myopia; micrognathia; high arched palate; low posterior hairline; webbed neck; septal defects; pulmonic stenosis; patent ductus arteriosus; pectus carinatum superiorly/pectus excavatum inferiorly; cryptorchidism; clinodactyly; woolly hair; mental retardation (mild); bleeding tendency; malignant schwannoma	[154]
Roifman syndrome		XR	Short-trunk dwarfism; long philtrum; strabismus; narrow and downslanting palpebral fissures; long eyelashes; retinal dystrophy; narrow upturned nose; LVNC; hepatosplenomegaly; spondyloepiphyseal dysplasia; eczema; hyperconvex nails; hypotonia; (mild) mental retardation; hypogonadotropic hypogonadism; recurrent infections; antibody deficiency	[155]
Syndromic microphthalmia/MIDAS syndrome (MIcrophthalmia, Dermal Aplasia, Sclerocornea)	*HCCS*	XD	Short stature; microcephaly; hearing loss; microphthalmia; sclerocornea; cataract; iris coloboma; retinopathy; septal defects; cardiac conduction defects; cardiomyopathy; overriding aorta; anteriorly placed anus; hypospadia; linear skin defects; corpus callosum agenesis; hydrocephalus; mental retardation; seizures	[156, 157]

AD autosomal dominant, *XD* X-linked dominant, *XR* X-linked recessive
[a]Most frequently involved genes

Mitochondrial

Mitochondrial disorders often lead to multiorgan disease, including the central and peripheral nervous system, eyes, heart, kidney, and endocrine organs. One of the cardiac features observed in mitochondrial disease is LVNC. Cardiac features may be the first or only feature in patients suffering from a mitochondrial disorder. In a study of 113 pediatric patients with mitochondrial disease, LVNC was identified in 13 % [171]. Pignatelli et al. showed that 5 of the 36 pediatric LVNC patients who underwent a skeletal muscular biopsy had morphologic and biochemical evidence for a mitochondrial defect, including a partial deficiency of complex I-III of the mitochondrial respiratory chain [149]. Mutations in mitochondrial DNA (mtDNA) and in nuclear DNA have been identified in the mitochondrial disorders associated with LVNC [172, 173].

Table 7.7 Chromosomal defects associated with left ventricular noncompaction (LVNC)

Chromosomal defects	Features	Reference
Deletion		
1p36	Microcephaly; sensorineural hearing loss; deep-set eyes; flat nose; cleft lip/palate; cardiomyopathy; septal defects; patent ductus arteriosus; dilated aortic root; feeding problems; gastro-esophageal reflux; short fifth finger and clinodactyly; mental retardation (severe); seizures; hypotonia	[84, 158–162]
1q43-q43	Microcephaly; upslanting palpebral fissures; epicanthus, broad nasal bridge, micrognathia; low set ears; bow-shaped upper lip; widely spaced teeth; short webbed neck; congenital heart defects; mental retardation (severe); speech impairment; seizures; corpus callosum agenesis	[163]
5q35.1q35.3	Facial hirsutism; synophrys; downslanting palpebral fissures; atrial septal defect and patent ductus arteriosus; LVNC with sick sinus syndrome and second degree heart block; feeding problems; gastro-esophageal reflux; joint hypermobility	[164]
7p14.3p14.1	Ventricular septal defect, atrial septal defect, aortic valve dysplasia, mental retardation, sacral fistula, growth retardation, microcephaly, facial dysmorphism	[162]
18p subtelomeric deletion	Esophageal atresia, otodysplasia, short stature, deafness, mental retardation, facial dysmorphism	[162]
22q11.2	Velo-cardio-facial syndrome: short stature; microcephaly; retrognathia; narrow palpebral fissures; square nasal root; prominent tubular nose; cleft palate; velopharyngeal insufficiency; congenital heart defect (85 %): ventricular septal defect; Fallot's tetralogy; inguinal/umbilical hernia; slender hands and digits; learning disability; mental retardation; schizophrenia; bipolar disorder	[149, 162]
Numeric		
4q trisomy/1q monosomy	Senile-like appearance; narrow palpebral fissures; telecanthus; epicanthus; broad nasal bridge; low-set ears; long philtrum; dimple below lower lip; anteriorly displaced anus; rocker-bottom feet; mental retardation; hypotonia, hypoplastic corpus callosum	[165]
Trisomy 13	Microcephaly; hypotelorism; cleft lip/palate; coloboma; low-set ears; septal defects; patent ductus arteriosus	[166]
	Polydactyly; overlapping fingers; mental retardation (severe); hypotonia; seizures	
Trisomy 21	Short stature; brachycephaly; flat facial profile; conductive hearing loss; epicanthal folds; upslant; iris brushfield spots; protruding tongue; congenital heart malformation; duodenal atresia; Hirschsprung disease; joint laxicity; single transverse palmar crease; excess nuchal skin; mental retardation; hypothyroidism; leukemia	[10, 116]
Mosaic trisomy 22	Microcephaly; hypertelorism; preauricular pits/tags; low-set ears; micrognathia, long philtrum; septal defects; double aortic arch; clinodactyly; hypoplastic nails; hemiatrophy; mental retardation	[167]
45,X0 (including mosaics)	Turner syndrome: short stature; short webbed neck; low hair line; broad nasal bridge; low-set ears; congenital heart defects: aortic coarctation; bicuspid aortic valves; aortic dilatation; lymphedema of hands and feet; renal abnormalities: single horseshoe kidney; renal vascular abnormalities; delayed puberty; amenorrhea; infertility; hypothyroidism	[162, 168, 169]
Translocation		
Robertsonian t13;14	Ventricular septal defect, mental retardation, linear cutaneous acromic lesions, growth retardation, toe syndactyly (II–III), facial dysmorphism	[162]
Loci		
6p24.3-21.1	LVNC; bradycardia; pulmonary valve stenosis; atrial septal defect; left bronchial isomerism; azygous continuation of the inferior vena cava; polysplenia; intestinal malrotation	[43]
11p15	LVNC; mild pulmonary stenosis; mild mitral valve prolapse; atrial septal defect	[170]

Cardiogenetic Aspects

Molecular and Cardiologic Family Screening

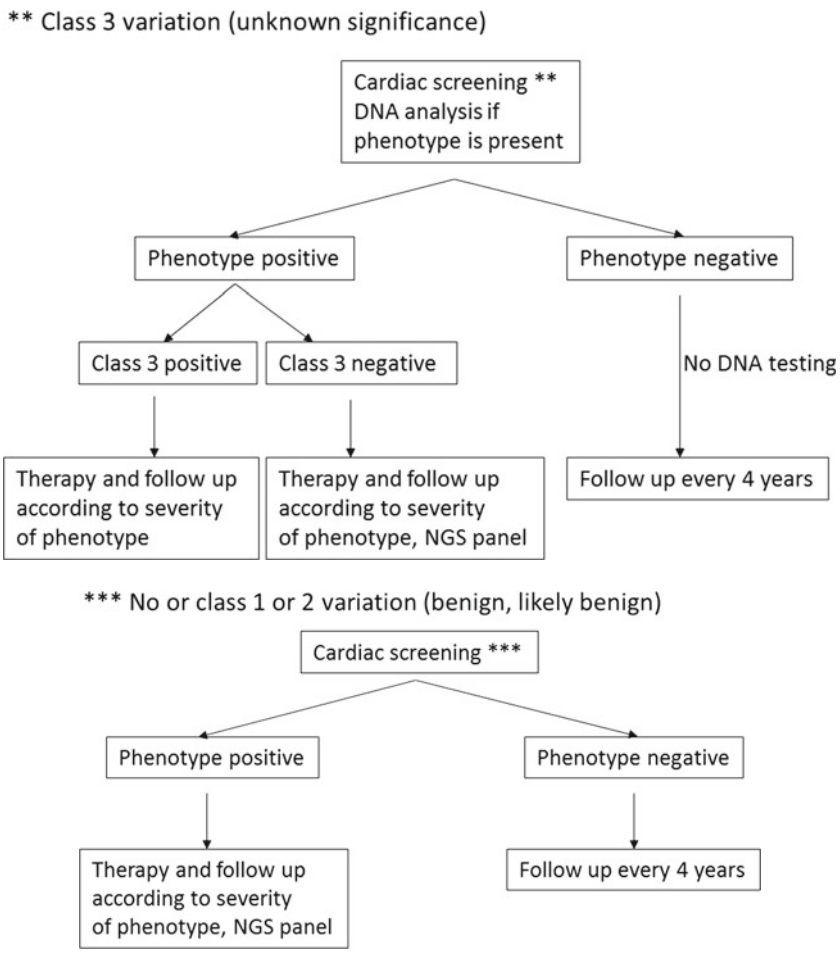
Familial LVNC has been estimated to occur in 18–71 % of adults with isolated LVNC, mostly consistent with an autosomal dominant mode of inheritance, indicating the importance of informing and examining relatives of patients with isolated LVNC [2, 10, 15, 149, 174–177]. Since extensive family studies showed that the majority of affected relatives are asymptomatic, cardiologic evaluation should include all adult relatives irrespective of medical history. Obviously, taking a family history is by itself insufficient to identify familial disease, given the high frequency of asymptomatic

disease in families [10]. In families where a pathogenic mutation has been identified, relatives can be offered predictive DNA analysis. In families without a pathogenic mutation, cardiac family screening remains the method of choice to identify relatives at risk of developing symptomatic cardiomyopathy, who may benefit from early treatment. In families where a variant (class 3 or 4) is identified, DNA analysis and cardiologic screening are advised as depicted in Fig. 7.5.

Apart from LVNC, other cardiomyopathies may co-occur within families, like hypertrophic and dilated cardiomyopa-

Fig. 7.5 Flowchart for family screening in LVNC including *likely pathogenic variants (class 4), **variants of unknown significance (class 3) and ***no variants or class 1 or 2 variants; # if clinically indicated; @ core panel: *ACTC1, ACTN2, ANKRD1, BAG3, CALR3, CAV3, CRYAB, CSRP3, CTNNA3, DES, DSC2, DSG2, DSP, EMD, FHL1, GLA, JPH2, JUP, LAMA4, LAMP2, LMNA, LDB3, MIB1, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOZ2, MYPN, NEXN, PKP2, PLN, PRDM16, PRKAG2, RBM20, SCN5A, TAZ, TCAP, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR, VCL*

Fig. 7.5 (continued)



thy, so cardiac screening should aim at identifying all cardiomyopathies. Cardiac screening of relatives may show minor abnormalities not fulfilling LVNC criteria, which may be difficult to differentiate from normal physiologic trabecularization. Hypothetically, these minor abnormalities might develop into LVNC eventually. Longitudinal studies of patients with mild LVNC features are needed to investigate the natural history of these forms of noncompaction.

Genotype–Phenotype Correlations

Molecular studies of LVNC have thus far shown that there are few recurrent mutations. Therefore, it is difficult to establish genotype–phenotype correlations. Additionally, intrafamilial phenotypic variability complicates predictions based on an identified mutation. Te presence of multiple (truncating) sarcomere mutations in an individual appears to result in a more severe phenotype with childhood onset [10, 97]. Multiple mutations identified in adults mostly also comprise involvement of a nonsarcomere gene. Adult patients with multiple mutations seem to have more symptoms than adults with a single mutation [10].

The proposed strategies for the molecular and cardiologic evaluation of LVNC are depicted in the flowchart in Fig. 7.5. Extensive genetic screening, preferably with a targeted cardiomyopathy gene panel, may lead to the identification of a molecular defect in over 40 % of isolated LVNC patients and in half of these patients an *MYH7* mutation is found [10].

When no targeted panel is available, *MYH7* gene sequencing should be considered as an initial approach, being the most prevalent cause for LVNC in adults and children. Further molecular analyses of the other genes within the LVNC spectrum, which quantitatively have a relatively modest contribution to LVNC morbidity, may be considered when no mutation in *MYH7* can be identified. Sarcomere gene analysis is also warranted in pediatric patients, given the high percentage of sarcomere mutations in this group. When an adult or pediatric patient is severely affected, screening for a second molecular defect is advised, given the high frequency of multiple mutations in LVNC.

Summary

LVNC is a relatively new, genetically heterogeneous, cardiomyopathy. Clinical presentation and prognosis range from asymptomatic disease with no or slow progression to severe disabling, rapidly progressive cardiac failure. Initial presentation includes the triad of heart failure (potentially lethal)

arrhythmias and/or thromboembolism. In adults, the majority of LVNC is isolated.

The first clinical presentation of LVNC may occur at all ages, even prenatally. In childhood, clinical features are often more severe and LVNC is frequently associated with congenital heart defects. The echocardiographic diagnostic criteria as proposed by Jenni et al. are convenient in daily practice and currently the most widely applied. The general cardiac guidelines for chronic heart failure and ICDs are suitable and applicable to the LVNC population.

In as much as 40 % of isolated LVNC, molecular testing may yield a genetic defect, mostly in sarcomere genes. The *MYH7* gene is the most prevalent disease gene. The nonisolated forms of LVNC are caused by a range of different (rare) genetic defects. Until now, in half of familial isolated LVNC, the genetic defect remains unknown. Genetic defects in a large number of sarcomere and other cardiomyopathy genes and in genes primarily associated with skeletal myopathies indicate that LVNC may result from a wide range of pathophysiologic mechanisms.

Shared genetic defects and familial aggregation of LVNC, HCM, and DCM indicate that LVNC may be part of a broad spectrum of cardiomyopathies.

The genetic etiology of LVNC requires that patients and their relatives are offered genetic testing and counseling. This may include (predictive) molecular analysis of relatives, when applicable, and/or cardiac evaluation of at-risk relatives, even when they are as yet asymptomatic.

Take Home Messages

- LVNC is a difficult (clinical) diagnosis and is genetic/hereditary in the majority of cases.
- Sarcomere gene defects (especially in *MYH7*) are the most frequent cause of genetic isolated LVNC.
- Treatment consists of standard heart failure care and prevention of arrhythmia.
- Prognosis is highly variable, even within families.
- Relatives at risk may be asymptomatic, warranting active screening and a follow-up of first-degree relatives.

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References

1. Feldt RH, Rahimtoola SH, Davis GD, Swan HJ, Titus JL. Anomalous ventricular myocardial patterns in a child with complex congenital heart disease. Am J Cardiol. 1969;23(5):732–4.
2. Chin TK, Perloff JK, Williams RG, Jue K, Mohrmann R. Isolated noncompaction of left ventricular myocardium. A study of eight cases. Circulation. 1990;82(2):507–13.
3. Oechslin EN, Attenhofer Jost CH, Rojas JR, Kaufmann PA, Jenni R. Long-term follow-up of 34 adults with isolated left ventricular noncompaction: a distinct cardiomyopathy with poor prognosis. J Am Coll Cardiol. 2000;36(2):493–500.
4. Hoedemaekers YM, Caliskan K, Majoor-Krakauer D, van de Laar I, Michels M, Witsenburg M, et al. Cardiac {beta}-myosin heavy chain defects in two families with non-compaction cardiomyopathy: linking non-compaction to hypertrophic, restrictive, and dilated cardiomyopathies. Eur Heart J. 2007;28(22):2732–7.
5. Moura C, Hillion Y, Daikha-Dahmane F, Eydoux P, Fallet C, Oury JF, et al. Isolated non-compaction of the myocardium diagnosed in the fetus: two sporadic and two familial cases. Cardiol Young. 2002;12(3):278–83.
6. Tsai SF, Ebenroth ES, Hurwitz RA, Cordes TM, Schamberger MS, Batra AS. Is left ventricular noncompaction in children truly an isolated lesion? Pediat Cardiol. 2009.
7. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation. 2006;113(14):1807–16.
8. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, et al. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2008;29(2):270–6.
9. Oechslin E, Jenni R. Left ventricular non-compaction revisited: a distinct phenotype with genetic heterogeneity? Eur Heart J. 2011;32(12):1446–56. Epub 2011/02/03.
10. Hoedemaekers YM, Caliskan K, Michels M, Frohn-Mulder I, van der Smagt JJ, Phefferkorn JE, et al. The importance of genetic counseling, DNA diagnostics, and cardiologic family screening in left ventricular noncompaction cardiomyopathy. Circ Cardiovasc Genet. 2010;3(3):232–9. Epub 2010/06/10.
11. Jenni R, Oechslin E, Schneider J, Attenhofer Jost C, Kaufmann PA. Echocardiographic and pathoanatomical characteristics of isolated left ventricular non-compaction: a step towards classification as a distinct cardiomyopathy. Heart. 2001;86(6):666–71.
12. Belanger AR, Miller MA, Donthireddi UR, Najovits AJ, Goldman ME. New classification scheme of left ventricular noncompaction and correlation with ventricular performance. Am J Cardiol. 2008;102(1):92–6.
13. Ritter M, Oechslin E, Sutsch G, Attenhofer C, Schneider J, Jenni R. Isolated noncompaction of the myocardium in adults. Mayo Clin Proc. 1997;72(1):26–31.
14. Sandhu R, Finkelhor RS, Gunawardena DR, Bahler RC. Prevalence and characteristics of left ventricular noncompaction in a community hospital cohort of patients with systolic dysfunction. Echocardiography (Mount Kisco, NY). 2008;25(1):8–12.
15. Aras D, Tufekcioglu O, Ergun K, Ozeke O, Yildiz A, Topaloglu S, et al. Clinical features of isolated ventricular noncompaction in adults long-term clinical course, echocardiographic properties, and predictors of left ventricular failure. J Card Fail. 2006;12(9):726–33.
16. Kohli SK, Pantazis AA, Shah JS, Adeyemi B, Jackson G, McKenna WJ, et al. Diagnosis of left-ventricular non-compaction in patients with left-ventricular systolic dysfunction: time for a reappraisal of diagnostic criteria? Eur Heart J. 2008;29(1):89–95.
17. Nugent AW, Daubeney PE, Chondros P, Carlin JB, Cheung M, Wilkinson LC, et al. The epidemiology of childhood cardiomyopathy in Australia. N Engl J Med. 2003;348(17):1639–46.

18. Ozkutlu S, Bostan O, Karagoz T, Deren O, Tekinalp G. Prenatal diagnosis of isolated non-compaction of the ventricular myocardium: study of six cases. Pediatr Int. 2007;49(2):172–6.
19. Sato Y, Matsumoto N, Matsuo S, Yoda S, Iida K, Kunimasa T, et al. Isolated noncompaction of the ventricular myocardium in a 94-year-old patient: depiction at echocardiography and magnetic resonance imaging. Int J Cardiol. 2007;119(1):e32–4.
20. Hoedemaekers YM, Cohen-Overbeek TE, Frohn-Mulder IM, Dooijes D, Majoor-Krakauer DF. Prenatal ultrasound diagnosis of MYH7 non-compaction cardiomyopathy. Ultrasound Obstet Gynecol. 2013;41(3):336–9. Epub 2012/08/04.
21. Bahl A, Swamy A, Sharma Y, Kumar N. Isolated noncompaction of left ventricle presenting as peripartum cardiomyopathy. Int J Cardiol. 2006;109(3):422–3.
22. Stollberger C, Finsterer J. Left ventricular hypertrabeculation/noncompaction. J Am Soc Echocardiogr. 2004;17(1):91–100.
23. Steffel J, Duru F. Rhythm disorders in isolated left ventricular noncompaction. Ann Med. 2012;44(2):101–8. Epub 2011/06/07.
24. Stollberger C, Finsterer J, Blazek G. Left ventricular hypertrabeculation/noncompaction and association with additional cardiac abnormalities and neuromuscular disorders. Am J Cardiol. 2002;90(8):899–902.
25. Fazio G, Pipitone S, Iacona MA, Marchi S, Mongiovi M, Zito R, et al. The noncompaction of the left ventricular myocardium: our paediatric experience. J Cardiovasc Med (Hagerstown, MD). 2007;8(11):904–8.
26. Sajeev CG, Francis J, Shanker V, Vasudev B, Abdul Khader S, Venugopal K. Young male with isolated noncompaction of the ventricular myocardium presenting with atrial fibrillation and complete heart block. Int J Cardiol. 2006;107(1):142–3.
27. Enriquez SG, Entem FR, Cobo M, Olalla JJ. Uncommon etiology of syncope in a patient with isolated ventricular noncompaction. Pacing Clin Electrophysiol. 2007;30(4):577–9.
28. Ozkutlu S, Ayabakan C, Celiker A, Elshershari H. Noncompaction of ventricular myocardium: a study of twelve patients. J Am Soc Echocardiogr. 2002;15(12):1523–8.
29. Celiker A, Kafali G, Dogan R. Cardioverter defibrillator implantation in a child with isolated noncompaction of the ventricular myocardium and ventricular fibrillation. Pacing Clin Electrophysiol. 2004;27(1):104–8.
30. Taniguchi M, Hioka T, Maekawa K, Takagagi K, Shoji K, Yoshida K. Adult case of isolated ventricular noncompaction discovered by complete atrioventricular block. Circ J. 2004;68(9):873–5.
31. Dagdeviren B, Eren M, Oguz E. Noncompaction of ventricular myocardium, complete atrioventricular block and minor congenital heart abnormalities: case report of an unusual coexistence. Acta Cardiol. 2002;57(3):221–4.
32. Okubo K, Sato Y, Matsumoto N, Kunimasa T, Kasama S, Sano Y, et al. Cardiac resynchronization and cardioverter defibrillation therapy in a patient with isolated noncompaction of the ventricular myocardium. Int J Cardiol. 2009;136(3):e66–8.
33. Caliskan K, Ujvari B, Bauernfeind T, Theuns DA, Van Domburg RT, Akca F, et al. The prevalence of early repolarization in patients with noncompaction cardiomyopathy presenting with malignant ventricular arrhythmias. J Cardiovasc Electrophysiol. 2012;23(9):938–44. Epub 2012/05/17.
34. Zhou Y, Zhang P, Zhou Q, Guo J, Xu Y, Li X. Giant P waves and focal atrial tachycardia in a patient with ventricular noncompaction. Int J Cardiol. 2008;123(2):210–2.
35. Ogawa K, Nakamura Y, Terano K, Ando T, Hishitani T, Hoshino K. Isolated non-compaction of the ventricular myocardium associated with Long QT Syndrome. Circ J. 2009.
36. Oginosawa Y, Nogami A, Soejima K, Aonuma K, Kubota S, Sato T, et al. Effect of cardiac resynchronization therapy in isolated ventricular noncompaction in adults: follow-up of four cases. J Cardiovasc Electrophysiol. 2008;19(9):935–8.
37. Saito K, Ibuki K, Yoshimura N, Hirono K, Watanabe S, Watanabe K, et al. Successful cardiac resynchronization therapy in a 3-year-old girl with isolated left ventricular non-compaction and narrow QRS complex. Circ J. 2009.
38. Kubota S, Nogami A, Sugiyasu A, Kasuya K. Cardiac resynchronization therapy in a patient with isolated noncompaction of the left ventricle and a narrow QRS complex. Heart Rhythm. 2006;3(5):619–20.
39. El Menyar AA, Gendi SM. Persistent atrial standstill in noncompaction cardiomyopathy. Pediatr Cardiol. 2006;27(3):364–6. Epub 2006/03/28.
40. Ozkutlu S, Onderoglu L, Karagoz T, Celiker A, Sahiner UM. Isolated noncompaction of left ventricular myocardium with fetal sustained bradycardia due to sick sinus syndrome. Turk J Pediatr. 2006;48(4):383–6.
41. Schweizer PA, Schroter J, Greiner S, Haas J, Yampolsky P, Mereles D, et al. The symptom complex of familial sinus node dysfunction and myocardial noncompaction is associated with mutations in the HCN4 channel. J Am Coll Cardiol. 2014;64(8):757–67. Epub 2014/08/26.
42. Celiker A, Ozkutlu S, Dilber E, Karagoz T. Rhythm abnormalities in children with isolated ventricular noncompaction. Pacing Clin Electrophysiol. 2005;28(11):1198–202.
43. Wessels MW, De Graaf BM, Cohen-Overbeek TE, Spitaels SE, de Groot-de Laat LE, Ten Cate FJ, et al. A new syndrome with noncompaction cardiomyopathy, bradycardia, pulmonary stenosis, atrial septal defect and heterotaxy with suggestive linkage to chromosome 6p. Hum genet. 2008;122(6):595–603.
44. Milano A, Vermeer AM, Lodder EM, Barc J, Verkerk AO, Postma AV, et al. HCN4 mutations in multiple families with bradycardia and left ventricular noncompaction cardiomyopathy. J Am Coll Cardiol. 2014;64(8):745–56. Epub 2014/08/26.
45. Kobza R, Jenni R, Erne P, Oechslin E, Duru F. Implantable cardioverter-defibrillators in patients with left ventricular noncompaction. Pacing Clin Electrophysiol. 2008;31(4):461–7.
46. Fazio G, Corrado G, Pizzuto C, Zachara E, Rapezzi C, Sulafa AK, et al. Supraventricular arrhythmias in noncompaction of left ventricle: is this a frequent complication? Int J Cardiol. 2008;127(2):255–6.
47. Sato Y, Matsumoto N, Takahashi H, Imai S, Yoda S, Kasamaki Y, et al. Cardioverter defibrillator implantation in an adult with isolated noncompaction of the ventricular myocardium. Int J Cardiol. 2006;110(3):417–9.
48. Sato Y, Matsumoto N, Matsuo S, Imai S, Yoda S, Tani S, et al. Subendomyocardial perfusion abnormality and necrosis detected by magnetic resonance imaging in a patient with isolated noncompaction of the ventricular myocardium associated with ventricular tachycardia. Cardiovasc Revasc Med. 2009;10(1):66–8.
49. Petersen SE, Selvanayagam JB, Wiesmann F, Robson MD, Francis JM, Anderson RH, et al. Left ventricular non-compaction: insights from cardiovascular magnetic resonance imaging. J Am Coll Cardiol. 2005;46(1):101–5.
50. Jacquier A, Thuny F, Jop B, Giorgi R, Cohen F, Gaubert JY, et al. Measurement of trabeculated left ventricular mass using cardiac magnetic resonance imaging in the diagnosis of left ventricular noncompaction. Eur Heart J. 2010;31(9):1098–4. Epub 2010/01/22.
51. Niemann M, Stork S, Weidemann F. Left ventricular noncompaction cardiomyopathy: an overdiagnosed disease. Circulation. 2012;126(16):e240–3. Epub 2012/10/17.
52. Burke A, Mont E, Kutys R, Virmani R. Left ventricular noncompaction: a pathological study of 14 cases. Hum Pathol. 2005;36(4):403–11.
53. Hughes SE, McKenna WJ. New insights into the pathology of inherited cardiomyopathy. Heart. 2005;91(2):257–64.
54. Boyd MT, Seward JB, Tajik AJ, Edwards WD. Frequency and location of prominent left ventricular trabeculations at autopsy in 474 normal human hearts: implications for evaluation of mural

thrombi by two-dimensional echocardiography. J Am Coll Cardiol. 1987;9(2):323–6.
55. Freedom RM, Yoo SJ, Perrin D, Taylor G, Petersen S, Anderson RH. The morphological spectrum of ventricular noncompaction. Cardiol Young. 2005;15(4):345–64.
56. Hunt SA. ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). J Am Coll Cardiol. 2005;46(6):e1–82.
57. Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldman AM, Francis GS, et al. ACC/AHA Guidelines for the Evaluation and Management of Chronic Heart Failure in the Adult: Executive Summary A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1995 Guidelines for the Evaluation and Management of Heart Failure): Developed in Collaboration With the International Society for Heart and Lung Transplantation; Endorsed by the Heart Failure Society of America. Circulation. 2001;104(24):2996–3007.
58. Nieminen MS, Bohm M, Cowie MR, Drexler H, Filippatos GS, Jondeau G, et al. Executive summary of the guidelines on the diagnosis and treatment of acute heart failure: the Task Force on Acute Heart Failure of the European Society of Cardiology. Eur Heart J. 2005;26(4):384–416.
59. Maron BJ, McKenna WJ, Danielson GK, Kappenberger LJ, Kuhn HJ, Seidman CE, et al. American College of Cardiology/European Society of Cardiology clinical expert consensus document on hypertrophic cardiomyopathy. A report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and the European Society of Cardiology Committee for Practice Guidelines. J Am Coll Cardiol. 2003;42(9):1687–713.
60. Stollberger C, Finsterer J. Thrombi in left ventricular hypertrabeculation/noncompaction--review of the literature. Acta Cardiol. 2004;59(3):341–4.
61. Fazio G, Corrado G, Zachara E, Rapezzi C, Sulafa AK, Sutera L, et al. Anticoagulant drugs in noncompaction: a mandatory therapy? J Cardiovasc Med (Hagerstown, MD). 2008;9(11):1095–7.
62. Battaglia A. Del 1p36 syndrome: a newly emerging clinical entity. Brain Dev. 2005;27(5):358–61. Epub 2005/07/19.
63. Probst S, Oechslin E, Schuler P, Greutmann M, Boye P, Knirsch W, et al. Sarcomere gene mutations in isolated left ventricular noncompaction cardiomyopathy do not predict clinical phenotype. Circ Cardiovasc Genet. 2011;4(4):367–74. Epub 2011/05/10.
64. Klaassen S, Probst S, Oechslin E, Gerull B, Krings G, Schuler P, et al. Mutations in sarcomere protein genes in left ventricular noncompaction. Circulation. 2008;117(22):2893–901.
65. Williams T, Machann W, Kuhler L, Hamm H, Muller-Hocker J, Zimmer M, et al. Novel desmoplakin mutation: juvenile biventricular cardiomyopathy with left ventricular non-compaction and acantholytic palmoplantar keratoderma. Clin Res Cardiol. 2011;100(12):1087–93. Epub 2011/07/27.
66. Luxan G, Casanova JC, Martinez-Poveda B, Prados B, D'Amato G, MacGrogan D, et al. Mutations in the NOTCH pathway regulator MIB1 cause left ventricular noncompaction cardiomyopathy. Nat Med. 2013;19(2):193–201. Epub 2013/01/15.
67. Kovacevic-Preradovic T, Jenni R, Oechslin EN, Noll G, Seifert B, Attenhofer Jost CH. Isolated left ventricular noncompaction as a cause for heart failure and heart transplantation: a single center experience. Cardiology. 2009;112(2):158–64.
68. Shimamoto T, Marui A, Yamanaka K, Shikata N, Tambara K, Ikeda T, et al. Left ventricular restoration surgery for isolated left ventricular noncompaction: report of the first successful case. J Thorac Cardiovasc Surg. 2007;134(1):246–7.
69. Caliskan KTD, Hoedemaekers YM, Ten Cate FJ, Jordaens L, Szili TT. Implantable cardioverter-defibrillators for primary and secondary prevention in patients with noncompaction cardiomyopathy. J Am Coll Card. 2009;53(10, supplement 1):):A136.
70. Zuckerman WA, Richmond ME, Singh RK, Carroll SJ, Starc TJ, Addonizio LJ. Left-ventricular noncompaction in a pediatric population: predictors of survival. Pediatr Cardiol. 2011;32(4):406–12. Epub 2010/12/29.
71. Greutmann M, Mah ML, Silversides CK, Klaassen S, Attenhofer Jost CH, Jenni R, et al. Predictors of adverse outcome in adolescents and adults with isolated left ventricular noncompaction. Am J Cardiol. 2012;109(2):276–81. Epub 2011/11/01.
72. Brescia ST, Rossano JW, Pignatelli R, Jefferies JL, Price JF, Decker JA, et al. Mortality and sudden death in pediatric left ventricular noncompaction in a tertiary referral center. Circulation. 2013;127(22):2202–8. Epub 2013/05/02.
73. Monserrat L, Hermida-Prieto M, Fernandez X, Rodriguez I, Dumont C, Cazon L, et al. Mutation in the alpha-cardiac actin gene associated with apical hypertrophic cardiomyopathy, left ventricular non-compaction, and septal defects. Eur Heart J. 2007;28(16):1953–61.
74. Bagnall RD, Molloy LK, Kalman JM, Semsarian C. Exome sequencing identifies a mutation in the ACTN2 gene in a family with idiopathic ventricular fibrillation, left ventricular noncompaction, and sudden death. BMC Med Genet. 2014;15:99. Epub 2014/09/17.
75. Ichida F, Tsubata S, Bowles KR, Haneda N, Uese K, Miyawaki T, et al. Novel gene mutations in patients with left ventricular noncompaction or Barth syndrome. Circulation. 2001;103(9):1256–63.
76. Xing Y, Ichida F, Matsuoka T, Isobe T, Ikemoto Y, Higaki T, et al. Genetic analysis in patients with left ventricular noncompaction and evidence for genetic heterogeneity. Mol Genet Metab. 2006;88(1):71–7.
77. Marziliano N, Mannarino S, Nespoli L, Diegoli M, Pasotti M, Malattia C, et al. Barth syndrome associated with compound hemizygosity and heterozygosity of the TAZ and LDB3 genes. Am J Med Genet. 2007;143(9):907–15.
78. Vatta M, Mohapatra B, Jimenez S, Sanchez X, Faulkner G, Perles Z, et al. Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular non-compaction. J Am Coll Cardiol. 2003;42(11):2014–27.
79. Hermida-Prieto MML, Castro-Beiras A, et al. Familial dilated cardiomyopathy and isolated left ventricular noncompaction associated with Lamin A/C gene mutations. Am J Cardiol. 2004;94:50–4.
80. Rankin J, Auer-Grumbach M, Bagg W, Colclough K, Nguyen TD, Fenton-May J, et al. Extreme phenotypic diversity and nonpenetrance in families with the LMNA gene mutation R644C. Am J Med Genet. 2008;146A(12):1530–42.
81. Budde BS, Binner P, Waldmuller S, Hohne W, Blankenfeldt W, Hassfeld S, et al. Noncompaction of the ventricular myocardium is associated with a de novo mutation in the beta-myosin heavy chain gene. PLoS One. 2007;2(12):e1362.
82. Postma AV, van Engelen K, van de Meerakker J, Rahman T, Probst S, Baars MJ, et al. Mutations in the sarcomere gene MYH7 in Ebstein anomaly. Circ Cardiovasc Genet. 2011;4(1):43–50. Epub 2010/12/04.
83. Zhang W, Chen H, Qu X, Chang CP, Shou W. Molecular mechanism of ventricular trabeculation/compaction and the pathogenesis of the left ventricular noncompaction cardiomyopathy (LVNC). Am J Med Genet C: Semin Med Genet. 2013;163C(3):144–56. Epub 2013/07/12.
84. Arndt AK, Schafer S, Drenckhahn JD, Sabeh MK, Plovie ER, Caliebe A, et al. Fine mapping of the 1p36 deletion syndrome identifies mutation of PRDM16 as a cause of cardiomyopathy. Am J Hum Genet. 2013;93(1):67–77. Epub 2013/06/19.

85. Shan L, Makita N, Xing Y, Watanabe S, Futatani T, Ye F, et al. SCN5A variants in Japanese patients with left ventricular noncompaction and arrhythmia. Mol Genet Metab. 2008;93(4):468–74.
86. Bione S, D'Adamo P, Maestrini E, Gedeon AK, Bolhuis PA, Toniolo D. A novel X-linked gene, G4.5. is responsible for Barth syndrome. Nat Genet. 1996;12(4):385–9.
87. Bleyl SB, Mumford BR, Brown-Harrison MC, Pagotto LT, Carey JC, Pysher TJ, et al. Xq28-linked noncompaction of the left ventricular myocardium: prenatal diagnosis and pathologic analysis of affected individuals. Am J Med Genet. 1997;72(3):257–65.
88. Bleyl SB, Mumford BR, Thompson V, Carey JC, Pysher TJ, Chin TK, et al. Neonatal, lethal noncompaction of the left ventricular myocardium is allelic with Barth syndrome. Am J Hum Genet. 1997;61(4):868–72.
89. Brady AN, Shehata BM, Fernhoff PM. X-linked fetal cardiomyopathy caused by a novel mutation in the TAZ gene. Prenat Diagn. 2006;26(5):462–5.
90. Chen R, Tsuji T, Ichida F, Bowles KR, Yu X, Watanabe S, et al. Mutation analysis of the G4.5 gene in patients with isolated left ventricular noncompaction. Mol Genet Metab. 2002;77(4):319–25.
91. Cortez-Dias N, Varela MG, Sargento L, Brito D, Almeida A, Cerqueira R, et al. Left ventricular non-compaction: a new mutation predisposing to reverse remodeling? Rev Port Cardiol. 2009;28(2):185–94.
92. Kenton AB, Sanchez X, Coveler KJ, Makar KA, Jimenez S, Ichida F, et al. Isolated left ventricular noncompaction is rarely caused by mutations in G4.5, alpha-dystrobrevin and FK Binding Protein-12. Mol Genet Metab. 2004;82(2):162–6.
93. Yen TY, Hwu WL, Chien YH, Wu MH, Lin MT, Tsao LY, et al. Acute metabolic decompensation and sudden death in Barth syndrome: report of a family and a literature review. Eur J Pediatr. 2008;167(8):941–4.
94. Morita H, Rehm HL, Menesses A, McDonough B, Roberts AE, Kucherlapati R, et al. Shared genetic causes of cardiac hypertrophy in children and adults. N Engl J Med. 2008;358(18):1899–908.
95. Lekanne Deprez RH, Muurling-Vlietman JJ, Hruda J, Baars MJ, Wijnaendts LC, Stolte-Dijkstra I, et al. Two cases of severe neonatal hypertrophic cardiomyopathy caused by compound heterozygous mutations in the MYBPC3 gene. J Med Genet. 2006.
96. Van Driest SL, Vasile VC, Ommen SR, Will ML, Tajik AJ, Gersh BJ, et al. Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. J Am Coll Cardiol. 2004;44(9):1903–10.
97. Wessels MW, Herkert JC, Frohn-Mulder IM, Dalinghaus M, van den Wijngaard A, de Krijger RR, et al. Compound heterozygous or homozygous truncating MYBPC3 mutations cause lethal cardiomyopathy with features of noncompaction and septal defects. Eur J Hum Genet. 2015;23(7):922–8. Epub 2014/10/23.
98. Zahka K, Kalidas K, Simpson MA, Cross H, Keller BB, Galambos C, et al. Homozygous mutation of MYBPC3 associated with severe infantile hypertrophic cardiomyopathy at high frequency among the Amish. Heart. 2008;94(10):1326–30.
99. Chen H, Zhang W, Li D, Cordes TM, Mark Payne R, Shou W. Analysis of ventricular hypertrabeculation and noncompaction using genetically engineered mouse models. Pediatr Cardiol. 2009;30(5):626–34. Epub 2009/04/28.
100. Yang J, Bucker S, Jungblut B, Bottger T, Cinnamon Y, Tchorz J, et al. Inhibition of Notch2 by Numb/Numblike controls myocardial compaction in the heart. Cardiovasc Res. 2012;96(2):276–85. Epub 2012/08/07.
101. Chen H, Zhang W, Sun X, Yoshimoto M, Chen Z, Zhu W, et al. Fkbp1a controls ventricular myocardium trabeculation and compaction by regulating endocardial Notch1 activity. Development. 2013;140(9):1946–57. Epub 2013/04/11.
102. Fatkin D, Graham RM. Molecular mechanisms of inherited cardiomyopathies. Physiol Rev. 2002;82(4):945–80.
103. Sedmera D, Pexieder T, Vuillemin M, Thompson RP, Anderson RH. Developmental patterning of the myocardium. Anat Rec. 2000;258(4):319–37.
104. Lie-Venema H. The role of epicardium-derived cells (EPDCs) in the development of non-compaction cardiomyopathy. Florence International Course on Advances in Cardiomyopathies – 5th meeting of the European Myocardial and Pericardial Disease WG of the ESC; 22/24 May 2008; Florence Italy 2008.
105. Lie-Venema H, van den Akker NM, Bax NA, Winter EM, Maas S, Kekarainen T, et al. Origin, fate, and function of epicardium-derived cells (EPDCs) in normal and abnormal cardiac development. TheScientificWorldJOURNAL. 2007;7:1777–98.
106. Breckenridge RA, Anderson RH, Elliott PM. Isolated left ventricular non-compaction: the case for abnormal myocardial development. Cardiol Young. 2007;17(2):124–9.
107. Crawford SE, Qi C, Misra P, Stellmach V, Rao MS, Engel JD, et al. Defects of the heart, eye, and megakaryocytes in peroxisome proliferator activator receptor-binding protein (PBP) null embryos implicate GATA family of transcription factors. J Biol Chem. 2002;277(5):3585–92.
108. Lee Y, Song AJ, Baker R, Micales B, Conway SJ, Lyons GE. Jumonji, a nuclear protein that is necessary for normal heart development. Circ Res. 2000;86(9):932–8.
109. Shou W, Aghdasi B, Armstrong DL, Guo Q, Bao S, Charng MJ, et al. Cardiac defects and altered ryanodine receptor function in mice lacking FKBP12. Nature. 1998;391(6666):489–92.
110. van Loo PF, Mahtab EA, Wisse LJ, Hou J, Grosveld F, Suske G, et al. Transcription factor Sp3 knockout mice display serious cardiac malformations. Mol Cell Biol. 2007;27(24):8571–82.
111. Tian T, Liu Y, Gao L, Wang J, Sun K, Zou Y, et al. Isolated left ventricular noncompaction: clinical profile and prognosis in 106 adult patients. Heart Vessel. 2014;29(5):645–52. Epub 2013/10/03.
112. Lombardi R, Betocchi S. Aetiology and pathogenesis of hypertrophic cardiomyopathy. Acta Paediatr Suppl. 2002;91(439):10–4.
113. Stahli BE, Gebhard C, Biaggi P, Klaassen S, Valsangiacomo Buechel E, Attenhofer Jost CH, et al. Left ventricular noncompaction: prevalence in congenital heart disease. Int J Cardiol. 2013;167(6):2477–81. Epub 2012/06/19.
114. Tunaoglu FS, Kula S, Olgunturk R, Ozturk G. Noncompaction with arcus aorta anomalies. Turk J Pediatr. 2003;45(4):363–6.
115. Niwa K, Ikeda F, Miyamoto H, Nakajima H, Ando M. Absent aortic valve with normally related great arteries. Heart Vessel. 1987;3(2):104–7.
116. Ali SK. Unique features of non-compaction of the ventricular myocardium in Arab and African patients. Cardiovasc J Afr. 2008;19(5):241–5.
117. Vijayalakshmi IB, Chitra N, Prabhu Deva AN. Use of an Amplatzer duct occluder for closing an aortico-left ventricular tunnel in a case of noncompaction of the left ventricle. Pediatr Cardiol. 2004;25(1):77–9.
118. Tatu-Chitoiu A, Bradisteanu S. A rare case of biventricular noncompaction associated with ventricular septal defect and descendent aortic stenosis in an young man. Eur J Echocardiogr. 2006.
119. Song ZZ. A combination of right ventricular hypertrabeculation/noncompaction and atrial septal defect. Int J Cardiol. 2009.
120. Salazar Gonzalez JJ, Rite Montanes S, Asso Abadia A, Pueo Crespo E, Salazar Gonzalez E, Placer Peralta LJ. [Isolated noncompaction of the ventricular myocardium] Miocardio ventricular no compacto aislado. Anales espanoles de pediatria. 2002;57(6):570-573.
121. Cavusoglu Y, Ata N, Timuralp B, Gorenek B, Goktekin O, Kudaiberdieva G, et al. Noncompaction of the ventricular myocardium: report of two cases with bicuspid aortic valve demonstrating

poor prognosis and with prominent right ventricular involvement. Echocardiography (Mount Kisco NY). 2003;20(4):379–83.
122. Cavusoglu Y, Aslan R, Birdane A, Ozbabalik D, Ata N. Noncompaction of the ventricular myocardium with bicuspid aortic valve. Anadolu Kardiyol Derg. 2007;7(1):88–90.
123. Sato Y, Matsumoto N, Yoda S, Inoue F, Kunimoto S, Fukamizu S, et al. Left ventricular aneurysm associated with isolated noncompaction of the ventricular myocardium. Heart Vessel. 2006;21(3): 192–4.
124. Cavusoglu Y, Tunerir B, Birdane A, Timuralp B, Ata N, Gorenek B, et al. Transesophageal echocardiographic diagnosis of ventricular noncompaction associated with an atrial septal aneurysm in a patient with dilated cardiomyopathy of unknown etiology. Can J Cardiol. 2005;21(8):705–7.
125. Unlu M, Ozeke O, Kara M, Yesillik S. Ruptured sinus of Valsalva aneurysm associated with noncompaction of the ventricular myocardium. Eur J Echocardiogr. 2008;9(2):311–3.
126. Friedman MA, Wiseman S, Haramati L, Gordon GM, Spevack DM. Noncompaction of the left ventricle in a patient with dextroversion. Eur J Echocardiogr. 2007;8(1):70–3.
127. Gorgulu S, Celik S, Eksik A, Tezel T. Double-orifice mitral valve associated with nonisolated left ventricular noncompaction--a case report. Angiology. 2004;55(6):707–10.
128. Sugiyama H, Hoshiai M, Toda T, Nakazawa S. Double-orifice mitral valve associated with noncompaction of left ventricular myocardium. Pediatr Cardiol. 2006;27(6):746–9.
129. Wang XX, Song ZZ. A combination of left ventricular noncompaction and double orifice mitral valve. Cardiovasc Ultrasound. 2009;7:11.
130. Betrian Blasco P, Gallardo Agromayor E. Ebstein's anomaly and left ventricular noncompaction association. Int J Cardiol. 2007; 119(2):264–5.
131. Ilercil A, Barack J, Malone MA, Barold SS, Herweg B. Association of noncompaction of left ventricular myocardium with Ebstein's anomaly. Echocardiography (Mount Kisco, NY). 2006;23(5):432–3.
132. Sinkovec M, Kozelj M, Podnar T. Familial biventricular myocardial noncompaction associated with Ebstein's malformation. Int J Cardiol. 2005;102(2):297–302.
133. Arslan S, Gurlertop HY, Gundogdu F, Senocak H. Left ventricular noncompaction and mid-caviter narrowing associated with Ebstein's anomaly: three-dimensional transthoracic echocardiographic image. Int J Cardiol. 2007;115(1):e52–5.
134. Attenhofer Jost CH, Connolly HM, Warnes CA, O'Leary P, Tajik AJ, Pellikka PA, et al. Noncompacted myocardium in Ebstein's anomaly: initial description in three patients. J Am Soc Echocardiogr. 2004;17(6):677–80.
135. Bagur RH, Lederlin M, Montaudon M, Latrabe V, Corneloup O, Iriart X, et al. Images in cardiovascular medicine. Ebstein anomaly associated with left ventricular noncompaction. Circulation. 2008;118(16):e662–4.
136. Friedberg MK, Ursell PC, Silverman NH. Isomerism of the left atrial appendage associated with ventricular noncompaction. Am J Cardiol. 2005;96(7):985–90.
137. Vanpraagh R, Ongley PA, Swan HJ. Anatomic types of single or common ventricle in man. Morphologic and geometric aspects of 60 necropsied cases. Am J Cardiol. 1964;13:367–86.
138. Dogan R, Dogan OF, Oc M, Duman U, Ozkutlu S, Celiker A. Noncompaction of ventricular myocardium in a patient with congenitally corrected transposition of the great arteries treated surgically: case report. Heart Surg Forum. 2005;8(2): E110–3.
139. Guo DC, Pannu H, Tran-Fadulu V, Papke CL, Yu RK, Avidan N, et al. Mutations in smooth muscle alpha-actin (ACTA2) lead to thoracic aortic aneurysms and dissections. Nat Genet. 2007;39(12): 1488–93.
140. Wessels MW, Willems PJ. Mutations in sarcomeric protein genes not only lead to cardiomyopathy but also to congenital cardiovascular malformations. Clin Genet. 2008;74(1):16–9.
141. Xin B, Puffenberger E, Tumbush J, Bockoven JR, Wang H. Homozygosity for a novel splice site mutation in the cardiac myosin-binding protein C gene causes severe neonatal hypertrophic cardiomyopathy. Am J Med Genet. 2007;143A(22):2662–7.
142. Zhu L, Vranckx R, Khau Van Kien P, Lalande A, Boisset N, Mathieu F, et al. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. Nat Genet. 2006;38(3):343–9.
143. Finsterer J, Stollberger C. Spontaneous left ventricular hypertrabeculation in dystrophin duplication based Becker's muscular dystrophy. Herz. 2001;26(7):477–81.
144. Finsterer J, Stollberger C, Feichtinger H. Noncompaction in Duchenne muscular dystrophy: frustrated attempt to create a compensatory left ventricle? Cardiology. 2006;105(4):223–5.
145. Finsterer J, Stollberger C, Wegmann R, Jarius C, Janssen B. Left ventricular hypertrabeculation in myotonic dystrophy type 1. Herz. 2001;26(4):287–90.
146. Malhotra R, Mason PK. Lamin A/C deficiency as a cause of familial dilated cardiomyopathy. Curr Opin Cardiol. 2009;24(3): 203–8.
147. D'Adamo P, Fassone L, Gedeon A, Janssen EA, Bione S, Bolhuis PA, et al. The X-linked gene G4.5 is responsible for different infantile dilated cardiomyopathies. Am J Hum Genet. 1997;61(4):862–7.
148. Stollberger C, Finsterer J. Noncompaction in Melnick Fraser syndrome. Pacing Clin Electrophysiol. 2007;30(8):1047 .author reply 8
149. Pignatelli RH, McMahon CJ, Dreyer WJ, Denfield SW, Price J, Belmont JW, et al. Clinical characterization of left ventricular noncompaction in children: a relatively common form of cardiomyopathy. Circulation. 2003;108(21):2672–8.
150. Matsumoto T, Watanabe A, Migita M, Gocho Y, Hayakawa J, Ogawa S, et al. Transient cardiomyopathy in a patient with congenital contractural arachnodactyly (Beals syndrome). J Nippon Med Sch = Nihon Ika Daigaku zasshi 2006;73(5):285-288.
151. Limongelli G, Pacileo G, Marino B, Digilio MC, Sarkozy A, Elliott P, et al. Prevalence and clinical significance of cardiovascular abnormalities in patients with the LEOPARD syndrome. Am J Cardiol. 2007;100(4):736–41.
152. Wong JA, Bofinger MK. Noncompaction of the ventricular myocardium in Melnick-Needles syndrome. Am J Med Genet. 1997; 71(1):72–5.
153. Finsterer J, Stollberger C, Kopsa W. Noncompaction on cardiac MRI in a patient with nail-patella syndrome and mitochondriopathy. Cardiology. 2003;100(1):48–9.
154. Amann G, Sherman FS. Myocardial dysgenesis with persistent sinusoids in a neonate with Noonan's phenotype. Pediatr Pathol. 1992;12(1):83–92.
155. Mandel K, Grunebaum E, Benson L. Noncompaction of the myocardium associated with Roifman syndrome. Cardiol Young. 2001;11(2):240–3.
156. Happle R, Daniels O, Koopman RJ. MIDAS syndrome (microphthalmia, dermal aplasia, and sclerocornea): an X-linked phenotype distinct from Goltz syndrome. Am J Med Genet. 1993;47(5): 710–3.
157. Kherbaoui-Redouani L, Eschard C, Bednarek N, Morville P. [Cutaneous aplasia, non compaction of the left ventricle and severe cardiac arrhythmia: a new case of MLS syndrome (microphtalmia with linear skin defects)]. Arch Pediatr. 2003;10(3):224-226. Aplasie cutanee congenitale, defaut de compaction du ventricule gauche et troubles du rythme cardiaque graves : un nouveau cas de syndrome MLS (microphtalmia with linear skin defects).

158. Battaglia A, Hoyme HE, Dallapiccola B, Zackai E, Hudgins L, McDonald-McGinn D, et al. Further delineation of deletion 1p36 syndrome in 60 patients: a recognizable phenotype and common cause of developmental delay and mental retardation. Pediatrics. 2008;121(2):404–10.
159. Cremer K, Ludecke HJ, Ruhr F, Wieczorek D. Left-ventricular non-compaction (LVNC): a clinical feature more often observed in terminal deletion 1p36 than previously expected. Eur J Med Genet. 2008;51(6):685–8.
160. Saito S, Kawamura R, Kosho T, Shimizu T, Aoyama K, Koike K, et al. Bilateral perisylvian polymicrogyria, periventricular nodular heterotopia, and left ventricular noncompaction in a girl with 10.5–11.1 Mb terminal deletion of 1p36. Am J Med Genet. 2008;146A(22):2891–7.
161. Thienpont B, Mertens L, Buyse G, Vermeesch JR, Devriendt K. Left-ventricular non-compaction in a patient with monosomy 1p36. Eur J Med Genet. 2007;50(3):233–6.
162. Digilio MC, Bernardini L, Gagliardi MG, Versacci P, Baban A, Capolino R, et al. Syndromic non-compaction of the left ventricle: associated chromosomal anomalies. Clin Genet. 2013;84(4):362–7. Epub 2012/12/06.
163. Kanemoto N, Horigome H, Nakayama J, Ichida F, Xing Y, Buonadonna AL, et al. Interstitial 1q43-q43 deletion with left ventricular noncompaction myocardium. Eur J Med Genet. 2006;49(3):247–53.
164. Pauli RM, Scheib-Wixted S, Cripe L, Izumo S, Sekhon GS. Ventricular noncompaction and distal chromosome 5q deletion. Am J Med Genet. 1999;85(4):419–23.
165. De Rosa G, Pardeo M, Bria S, Caresta E, Vasta I, Zampino G, et al. Isolated myocardial non-compaction in an infant with distal 4q trisomy and distal 1q monosomy. Eur J Pediatr. 2005;164(4):255–6.
166. McMahon CJ, Chang AC, Pignatelli RH, Miller-Hance WC, Eble BK, Towbin JA, et al. Left ventricular noncompaction cardiomyopathy in association with trisomy 13. Pediatr Cardiol. 2005;26(4):477–9.
167. Wang JC, Dang L, Mondal TK, Khan A. Prenatally diagnosed mosaic trisomy 22 in a fetus with left ventricular non-compaction cardiomyopathy. Am J Med Genet. 2007;143A(22):2744–6.
168. Altenberger H, Stollberger C, Finsterer J. Isolated left ventricular hypertrabeculation/noncompaction in a Turner mosaic with male phenotype. Acta Cardiol. 2009;64(1):99–103.
169. van Heerde M, Hruda J, Hazekamp MG. Severe pulmonary hypertension secondary to a parachute-like mitral valve, with the left superior caval vein draining into the coronary sinus, in a girl with Turner's syndrome. Cardiol Young. 2003;13(4):364–6.
170. Sasse-Klaassen S, Probst S, Gerull B, Oechslin E, Nurnberg P, Heuser A, et al. Novel gene locus for autosomal dominant left ventricular noncompaction maps to chromosome 11p15. Circulation. 2004;109(22):2720–3.
171. Scaglia F, Towbin JA, Craigen WJ, Belmont JW, Smith EO, Neish SR, et al. Clinical spectrum, morbidity, and mortality in 113 pediatric patients with mitochondrial disease. Pediatrics. 2004;114(4):925–31.
172. Finsterer J, Bittner R, Bodingbauer M, Eichberger H, Stollberger C, Blazek G. Complex mitochondriopathy associated with 4 mtDNA transitions. Eur Neurol. 2000;44(1):37–41.
173. Finsterer J, Stollberger C, Schubert B. Acquired left ventricular hypertrabeculation/noncompaction in mitochondriopathy. Cardiology. 2004;102(4):228–30.
174. Espinola-Zavaleta N, Soto ME, Castellanos LM, Jativa-Chavez S, Keirns C. Non-compacted cardiomyopathy: clinical-echocardiographic study. Cardiovasc Ultrasound. 2006;4:35.
175. Ichida F, Hamamichi Y, Miyawaki T, Ono Y, Kamiya T, Akagi T, et al. Clinical features of isolated noncompaction of the ventricular myocardium: long-term clinical course, hemodynamic properties, and genetic background. J Am Coll Cardiol. 1999;34(1):233–40.
176. Lofiego C, Biagini E, Pasquale F, Ferlito M, Rocchi G, Perugini E, et al. Wide spectrum of presentation and variable outcomes of isolated left ventricular non-compaction. Heart. 2007;93(1):65–71.
177. Murphy RT, Thaman R, Blanes JG, Ward D, Sevdalis E, Papra E, et al. Natural history and familial characteristics of isolated left ventricular non-compaction. Eur Heart J. 2005;26(2):187–92.

Restrictive Cardiomyopathy

8

J.H. Kirkels and N. de Jonge

Abstract

Restrictive cardiomyopathy (RCM) is a rare disease, characterized by increased stiffness of the ventricular walls, which causes heart failure because of impaired diastolic filling. In the early stages, systolic function may be normal, but when the disease progresses, systolic function usually declines as well. There is an overlap with other types of cardiomyopathy, such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and left ventricular noncompaction. Indeed, an autosomal dominantly segregating cardiomyopathy has been described where a single sarcomere gene mutation caused idiopathic RCM in some and HCM in other family members [1].

Introduction

Restrictive cardiomyopathy (RCM) is a rare disease, characterized by increased stiffness of the ventricular walls, which causes heart failure because of impaired diastolic filling. In the early stages, systolic function may be normal, but when the disease progresses, systolic function usually declines as well. There is an overlap with other types of cardiomyopathy, such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and left ventricular noncompaction. Indeed, an autosomal dominantly segregating cardiomyopathy has been described where a single sarcomere gene mutation caused idiopathic RCM in some and HCM in other family members [1].

According to the most recent AHA classification of cardiomyopathies [2], RCM is defined by restrictive ventricular physiology associated with normal or reduced diastolic volumes (of one or both ventricles), normal or near-normal systolic function, and normal or only mildly increased ventricular wall thickness. Several studies indicate that RCM is not a single entity; it is a heterogeneous group of disorders that can present with a spectrum of cardiac phenotypes [3].

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Classification of RCM is based on the underlying pathophysiological process: noninfiltrative, infiltrative, storage diseases, and endomyocardial (Table 8.1). Approximately 50 % of cases are caused by a specific clinical disorder, the majority in western countries being amyloidosis, whereas the remainder represents an "idiopathic" or "primary" process. RCM may also be associated with neuromuscular disorders, both congenital and acquired forms [4]. Hypertrophic cardiomyopathy may be particularly difficult to distinguish, since HCM in a late phase may start to dilate and wall thickness may appear normal or even reduced. Conversely, thickening of ventricular walls in cardiac infiltration or storage disease may resemble HCM. RCM must also be clinically distinguished from constrictive pericarditis, which is also characterized by abnormal ventricular filling with (near) normal systolic function.

Hereditary forms of RCM can be found in all subgroups, with both autosomal dominant and recessive genetic properties. Family history and investigation of first-degree relatives may therefore be important.

Molecular Background

Several inherited and acquired disorders may cause RCM, but many cases remain idiopathic. Familial RCM has been reported, but it remains uncertain whether this is a distinct genetic entity.

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RCM may be due to myocardial fibrosis, hypertrophy, or infiltration of varying compounds, like amyloid or storage of, for example, glycogen. The terms hypertrophic and RCM do not refer to specific diseases, but are instead purely descriptive terms used to characterize myocardial disease associated with a broad spectrum of genetic syndromes or systemic diseases.

Cardiomyocyte contraction is dependent on intracellular calcium concentration and regulated by the troponin complex. *In vitro* studies have shown that RCM-causing mutations in TNNI3 show a greater increase in Ca^{2+} sensitivity than HCM-causing mutations, resulting in more severe diastolic impairment and potentially accounting for the RCM phenotype in humans [5].

The molecular background of different forms of RCM is highly variable, depending on the underlying cause, and will be discussed in more detail in specific clinical entities.

Clinical Aspects

Inability of the ventricles to fill limits cardiac output and raises filling pressures, leading to exercise intolerance and dyspnea. In most patients, venous pressure is elevated, which may lead to edema, ascites, and liver enlargement. Palpitations are often seen, with a relatively high occurrence of atrial fibrillation, which in turn may lead to rapid clinical deterioration due to high ventricular rates with short diastolic filling times. Third and fourth heart sounds may be present on physical examination.

Table 8.1 Classification of restrictive cardiomyopathy

			Genetic	Primary cardiac presentation	Common primary site or presentation
Myocardial	*Noninfiltrative*				
		Idiopathic restrictive cardiomyopathy	+	+	N.a.
		Scleroderma	±	–	Skin, joints, Raynaud, GI-tract, lungs
		Pseudoxanthoma elasticum	+		Skin, vascular wall (GI-tract)
		Diabetic cardiomyopathy		–	
	Infiltrative				
		Amyloidosis	±(AL)/+(AA)	+	AL: bone marrow, kidneys
					AA: peripheral neuropathy
		Sarcoidosis		±	Lungs
		Gaucher disease	+	–	Spleen, liver, bone marrow, bone
		Hurler disease	+	–	Bone, liver, spleen, brain
	Storage disease				
		Hemochromatosis	+		Liver, skin pigmentation, diabetes mellitus, arthropathy, impotence in male
		Fabry disease	+	+	Neuropathy, skin, kidney, stroke
		Glycogen storage disease	+	(+)/–	Hypoglycemia, muscle weakness, fatigability
Endomyocardial					
		Endomyocardial fibrosis	?	+	N.a.
		Hypereosinophilic syndrome	?	+	Systemic thromboemboli, neuropathy, GI-tract inflammation, lungs, bone marrow
		Carcinoid heart disease		–	Flushing, diarrhea, bronchospasm
		Metastatic cancers		–	N.a.
		Radiation		(+)[a]	N.a.
		Anthracycline toxicity		(+)[a]	N.a.
		Fibrous endocarditis caused by drugs (serotonin, methysergide, ergotamine, mercurial agents, busulfan)		+	N.a.

[a]Primary cardiac presentation after treatment of previous malignancy

Diagnosis

In typical cases, echocardiography, cardiac CT, or MRI will reveal normal or concentric thickened ventricles with normal or reduced intraventricular volumes. In contrast to hypertrophic cardiomyopathy, macroscopic hypertrophy and reduction of intraventricular volume are not pronounced. The atria are usually enlarged, sometimes exceeding ventricular volume. Systolic function may be normal or slightly reduced; diastolic function is reduced, with high E-wave, shortened deceleration time (<150 ms), and an E/A ratio of >2 on transmitral Doppler echocardiography (Fig. 8.1). Especially in infiltrative cardiomyopathies, the ECG may show low-voltage and nonspecific ST segment or T-wave abnormalities (Fig. 8.2). Cardiac catheterization shows a reduced cardiac output and elevation of left and right ventricular end-diastolic pres-

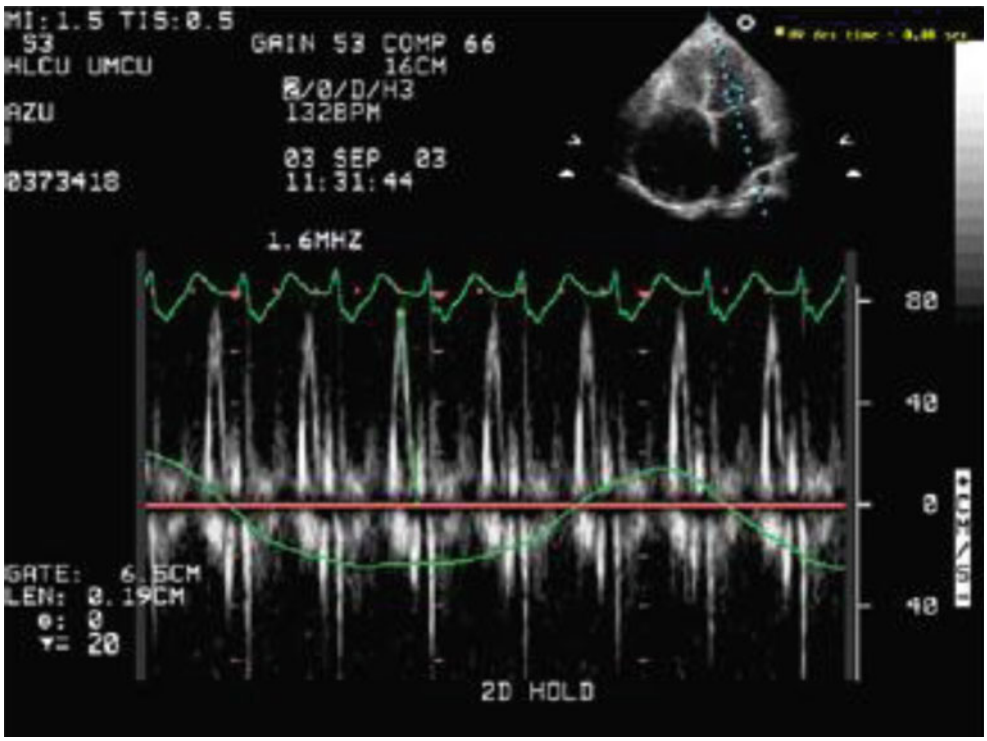

Fig. 8.1 2D-echocardiogram and transmitral Doppler signals in restrictive cardiomyopathy. In the upper part the 2D echocardiogram (apical four-chamber view) is shown, with normal sized ventricles (*top*) and huge atria below. In the main panel, the transmitral Doppler recording is shown in relation to the ECG, indicating E-waves with short deceleration time (*green line*) and almost absent A-waves (high E/A ratio)

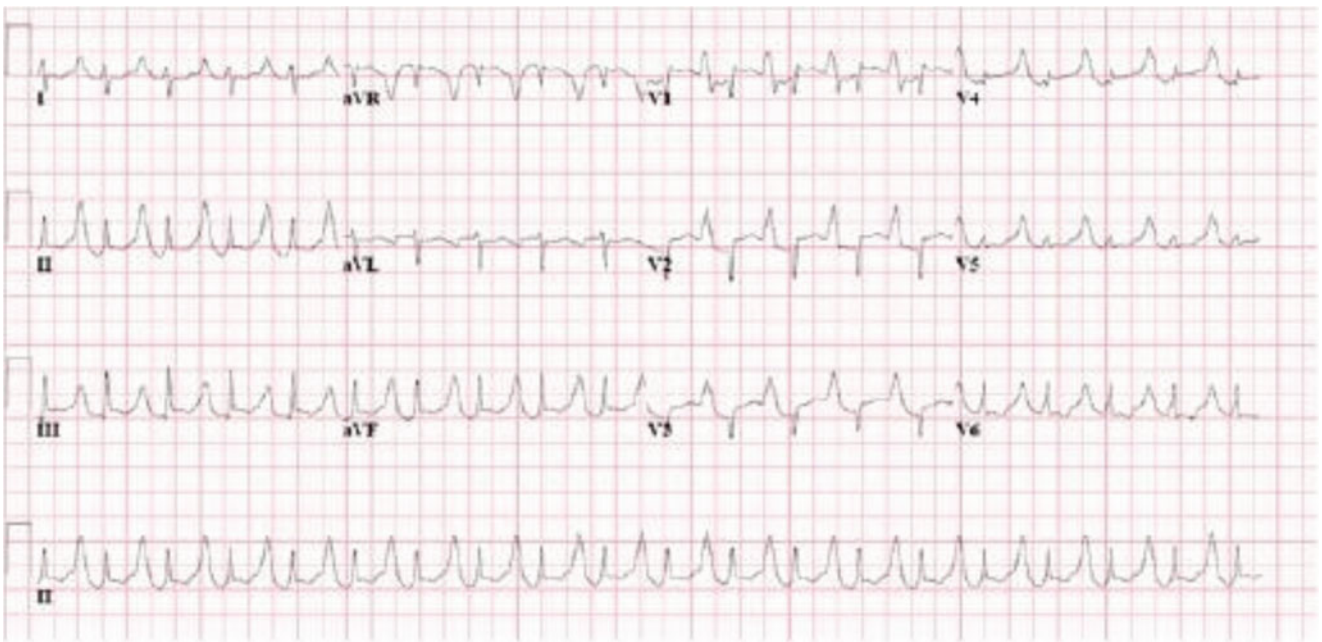

Fig. 8.2 ECG in restrictive cardiomyopathy. Low voltage abnormal QRS-complexes, preceded by huge P-waves in a 16-year-old girl with restrictive cardiomyopathy

sures with a dip-plateau representing an abrupt termination of filling in the first one third to one half of diastole. This configuration may resemble constrictive pericarditis; however, in constrictive pericarditis, there usually is a thickened pericardium, best seen on CT or MRI. In addition, interventricular dependence and respiratory variation of transmitral inflow on Doppler examination will be more pronounced in constrictive pericarditis; in difficult cases, volume challenge and simultaneous LV and RV pressure recording in relation to respiratory activity may be of help. Recently, tissue Doppler imaging was shown to reliably discriminate between the two conditions, with a cutoff value of >5 cm/s mean annular velocity (averaged from four walls) ruling out RCM [6]. Surprisingly, BNP values showed a large overlap between the two conditions.

The mainstay of diagnosis is endomyocardial biopsy, revealing fibrosis or the underlying specific infiltration or storage.

Clinical Approach and Differential Diagnosis

Since RCM often occurs in the setting of a systemic disease, in many cases the primary underlying disease is already known, like in Gaucher disease (GD), where noncardiac manifestations usually precede cardiac involvement. In these cases the clinical question may not be making the right diagnosis, but proving or excluding cardiac involvement. This may have consequences for the work-up; for instance, in case of a patient with known hemochromatosis, it may be best to start with cardiac MRI in order to find cardiac iron overload, whereas in suspected amyloidosis it may be best to start with endomyocardial biopsy.

Clinical history taking and clinical examination should be directed at symptoms indicative of underlying disease [4]. Ophthalmologic, otologic, dermatologic, gastroenterologic, nephrologic, hematologic, and neurologic examination may be necessary to help establishing a possibly treatable cause of RCM before the disease becomes intractable.

In apparently idiopathic RCM, it may be necessary to clinically exclude other causes of restriction, like hypertension, and to exclude the presence of specific infiltration or storage in an endomyocardial biopsy. In addition, taking an extensive family history including other phenotypes of cardiomyopathy and performing a genetic evaluation may be of help [7].

Treatment

In many cases, treatment is disappointing since myocardial damage is progressive and irreversible, with a possible exception for hemochromatosis and Fabry's disease (see below). In case of amyloidosis, aggressive anticancer treatment and/or bone marrow transplantation may slow progression of the disease, but this does not remove the already existing deposits of amyloid. In general, there is no specific medication for diastolic heart failure, other than diuretics to treat pulmonary or systemic congestion. The balance between pulmonary congestion due to fluid overload on the one hand, and forward failure due to too low filling pressures on the other hand, often is very delicate. Controlling heart rate with betablockers to allow adequate filling time is important; however, when restriction progresses, ventricular filling may no longer improve with longer diastole. In end-stage disease, a higher heart rate may even be the only way to compensate for a very low stroke volume. Atrial fibrillation occurs very often in RCM as a result of chronically elevated filling pressures and dilated atria, warranting oral anticoagulation to prevent stroke or embolism and adequate rate control when rhythm control is not possible anymore. Like in mitral stenosis and sinus rhythm, there is no consensus on the preventive use of anticoagulants in RCM and sinus rhythm. The only exception may be endomyocardial fibrosis (EMB) and hypereosinophilic syndrome, where endocavitary thrombosis and fibrosis with apical filling are thought to occur.

Heart transplantation may be an option in carefully selected cases, but due to the malignant nature or the multiorgan involvement of many underlying diseases, heart transplantation is often contraindicated. In idiopathic RCM, heart transplantation may offer good survival.

Prognosis

Prognosis is very much dependent on the underlying disease. In a study of 94 patients with idiopathic RCM after 68 months 50 % had died [8]. The causes of death were heart failure (47 %), sudden death (17 %), cancer (13 %), infection (13 %), and arrhythmias (11 %).

Idiopathic and Familial RCM

Idiopathic RCM is characterized by myocyte hypertrophy and interstitial fibrosis, with a restrictive hemodynamic pattern of the ventricles with reduced diastolic volumes, in the presence of normal or near-normal wall thickness and systolic function. By definition, there is no known underlying or related disease to explain cardiac involvement. In childhood, RCM is very rare, accounting for 2–5 % of pediatric cardiomyopathies [3]. About 30 % of children with RCM have a family history of cardiomyopathy and prognosis is poor (2-year mortality >50 %) [3]. In their study of 12 children, in one third, a mutation in genes coding for sarcomeric proteins was found; in the other two thirds, it was speculated that some might have been caused by mutations in genes encoding cytoskeletal or nuclear envelope proteins, more commonly

associated with DCM. Others might have been associated to – as yet unknown – inborn errors of metabolism or storage disorders with predominant cardiac involvement [3].

Familial RCM is an autosomal dominant cardiomyopathy with incomplete penetrance [9], generally considered in the absence of specific genetic abnormalities known to cause hypertrophic cardiomyopathy (HCM). However, some have suggested that RCM is part of the clinical expression of cardiac troponin I mutations [3]. A bundle branch block leading to complete heart block usually develops in the third or fourth decade [10]. Those who survive the fifth decade may develop a progressive myopathy [3, 11], although there are also reports of families with multiple affected individuals without skeletal myopathy [10]. Mogensen et al. [1] described a large family in which individuals were affected by either idiopathic RCM or HCM. Linkage analysis to selected sarcomeric contractile protein genes identified cardiac troponin I (TNNI3) as the likely disease gene. Several mutations were found, which also appeared to be present in six of nine unrelated RCM patients. They conclude that the restrictive phenotype is part of the spectrum of hereditary sarcomeric contractile protein disease. Changes in actin-binding affinity, affinity to troponin C, and the ability to inhibit thin filaments during diastole, caused by certain TNNI3 mutations, may lead to an altered interaction within the actin–troponin–tropomyosin complex, and thus may cause either severe diastolic dysfunction and RCM, or myocardial hypertrophy [12]. Myofilament hypersensitivity to cytoplasmic Ca^{2+} is a common feature that RCM-causing mutations share with HCM-causing mutations, with even more pronounced Ca^{2+} hypersensitivity in RCM [13].

Genetic engineering of adult cardiac myocytes [14] was used to identify effects of mutant cardiac troponin I (cTnI). The p.R193H mutant cTnI was associated with incomplete relaxation and acute remodeling to a contracted state as a direct correlate of the stiff heart characteristic of RCM in vivo. This occurred independently of Ca^{2+} concentration or sensitivity. Transgenic mice, expressing R193H cTnI in the heart, showed gradual changes in 12 months from impaired relaxation to diastolic dysfunction and eventually a phenotype similar to human RCM [15]. Treating RCM mice (caused by p.R193H mutant cTnI) with catechin was shown to cause myofibril desensitization and restoration of diastolic function [16]. These results demonstrate a critical role of the COOH-terminal domain of cTnI in the development of RCM. On the other hand, Cubero et al. [10] present a family of RCM patients with autosomal dominant inheritance, without signs of skeletal myopathy and no troponin I mutations.

Familial RCM may also occur as autosomal recessive or X-linked disease, and mutations in genes encoding *MYH7*, *TNNT2*, *ACTC1*, *MYPN*, and *TTN* have been described as rare causes of RCM [13, 17]. More recently, familial RCM could be related to a *FLNC* mutation; filamins are actin-cross-linking proteins, and filaminopathies can primarily affect the heart, apart from skeletal muscle disorders [17]. To make it even more complex, a recent study [15] described a unique family with autosomal dominant heart disease variably expressed as RCM, HCM, and dilated cardiomyopathy. They showed that a cardiac troponin T (*TNNT2*) mutation cosegregated with the disease phenotype. A missense mutation resulting in a p.I79N substitution was found in all nine affected family members, but none of the six unaffected relatives. Segregation analyses excluded a primary pathogenic role for eight other sarcomeric protein genes; however, this does not exclude a potential modifying effect of variants within these or other genes on cardiac phenotype [18].

RCM as Part of Specific Clinical Conditions with Known or Suspected Genetic Background (Selected Subjects)

Noninfiltrative Restrictive Cardiomyopathy

Scleroderma/Systemic Sclerosis

Apart from cardiac complications due to systemic or pulmonary hypertension, primary cardiac involvement can also occur in systemic sclerosis (SSc). Patchy myocardial fibrosis as a result of recurrent vasospasm of small vessels may lead to the clinical picture of RCM. Extensive fibrosis may be seen in patients with a long history of Raynaud phenomenon [19]. Familial clustering and ethnic influence have been demonstrated. Polymorphisms in genes coding for extracellular matrix proteins and cell-signaling molecules implicate non-MHC areas in SSc pathogenesis [20]. There are associations of polymorphisms in several genes with susceptibility and severity of SSc. All patients showed genetically predisposed high TGFbeta1 production, with polymorphisms at codons 10 and 25 of the TGFbeta1 gene [21]. Current data suggest that SSc is a multigenic complex disorder.

Pseudoxanthoma Elasticum

Pseudoxanthoma elasticum (PXE) is an inherited disorder that is associated with accumulation of mineralized and fragmented elastic fibers in the skin, vascular walls, and Bruch's membrane in the eye. It may lead to peripheral and coronary arterial occlusive disease as well as gastrointestinal bleedings. There is yet no definitive therapy. Recent studies suggest that PXE is inherited almost exclusively as an autosomal recessive trait. Its prevalence has been estimated to be 1:25,000–100,000. Very recently, the *ABCC6* gene on chromosome 16p13.1 was found to be associated with the disease. Mutations within *ABCC6* cause reduced or absent transmembraneous transport that leads to accumulation of extracellular material. Presumably, this mechanism causes calcification of elastic fibers.

In a study of 19 patients, it was found that systolic function was normal, but diastolic parameters were abnormal in seven patients [22]. Explanations for these abnormalities could be silent myocardial ischemia due to early coronary involvement and/or the direct consequences of ultrastructural defects of the elastic tissue of the heart.

Diabetic Cardiomyopathy

In diabetes mellitus, alterations in cardiac structure or function in the absence of ischemic heart disease, hypertension, or other cardiac pathologies is termed diabetic cardiomyopathy.

Structural changes include myocardial hypertrophy, fibrosis, and fat droplet deposition, initially leading to abnormal diastolic function. This phenotype is more prevalent in obese type 2 diabetic patients [23]. Advanced glycation endproducts (AGEs) are thought to be important in the pathophysiology of diabetic cardiomyopathy. Irreversible modification of proteins by glucose results in the formation of AGEs, a heterogeneous family of biologically and chemically reactive compounds with cross-linking properties. This process of protein modification is magnified by the high ambient glucose concentration present in diabetes [24].

The genetic background of diabetes is beyond the scope of this chapter. However, there are some very interesting studies pointing to a genetic link between diabetes and cardiac damage. Oxidative stress is known to be enhanced with diabetes, and oxygen toxicity may alter cardiac progenitor cell (CPC) function resulting in defects in CPC growth and myocyte formation, which may favor premature myocardial aging and heart failure. Ablation of the p66shc gene in a mouse model [25] prevented these negative effects, pointing at a possible genetic link between diabetes, reactive oxygen species, and the development of heart failure.

Infiltrative

Cardiac Amyloidosis

Amyloidosis comprises a group of diseases characterized by extracellular deposition of insoluble fibrillar proteins with concomitant destruction of normal tissue structure and function [26]. This results in stiffening and thickening of the myocardial walls, which can be easily demonstrated by echocardiography and often has a granular sparkling appearance. Absence of high ECG voltages further strengthens the suspicion of amyloidosis. Cardiac clinical manifestations include diastolic and systolic dysfunction, arrhythmias and conduction disturbances, orthostatic hypotension, coronary insufficiency, valvular dysfunction, and pericardial effusion.

Endomyocardial biopsy is the method of choice to diagnose cardiac amyloidosis and also allows characterization of the amyloid protein [27].

About 30 different proteins are known to form amyloid fibrils *in vivo*, of which only 11 have been identified that involve the heart [26]. The nomenclature is based on these proteins [28]. In clinical practice, however, amyloidosis is often classified as primary, secondary, hereditary, and age related.

Primary amyloidosis or *systemic AL amyloidosis* is the result of monoclonal immunoglobulin light chains secreted by clonal plasma cells (multiple myeloma) and predominantly deposited in the heart, kidney, and nerves. Congestive heart failure and conduction disturbances are frequent cardiovascular complications and often result in early death of the patients.

Although multiple myeloma is not considered a genetic disease, there are reports of around 130 families with two or more cases of multiple myeloma, MGUS or Waldenström's macroglobulinemia [29].

Secondary or systemic AA amyloidosis is associated with chronic diseases and manifested mainly in the kidney, liver and spleen, and, only rarely, in the heart. Proteinuria and renal failure are paramount.

Hereditary systemic amyloidosis is predominantly caused by deposition of amyloid fibrils derived from genetic variants of transthyretin (TTR), a transport protein synthesized mainly by the liver. More than 100 mutations are known already, of which the Val122I variant is the most common, occurring in 3 to 4 % of black Americans [30, 31]. Val122I reduces the stability of TTR tetramers, causing cardiac deposition of misfolded monomers and resulting in a cardiomyopathy typically during or after the sixth decade. Inheritance is often autosomal dominant with varying degree of penetrance [32, 33].Clinical syndromes include cardiomyopathy, nephropathy, and neuropathy. The presenting symptom often is the peripheral ascending neuropathy; cardiac involvement often is the final cause of death. On the other hand, the overall prognosis in Val122I carriers was not significantly different from noncarriers, as studied in a large community study by Quarta et al. [31]. The risk of heart failure was increased among carriers, suggesting that amyloidosis associated with the Val122I TTR variant may be more benign than previously thought.

Senile systemic amyloidosis is caused by the deposition of amyloid fibrils from normal nonmutant TTR, especially in the heart. It is age related, with male predominance and rare in patients younger than 60 years of age. Clinically it manifests as congestive heart failure, relatively frequently accompanied by carpal tunnel syndrome [30]. Progression of this disease is much slower than in AL amyloidosis, despite the more severe hypertrophy present in the senile form. Autopsy studies suggest that in up to 25 % of individuals over the age of 80 years, this type of TTR-derived amyloid can be found in the heart [33].

Sarcoidosis

Myocardial sarcoidosis generally occurs in association with other manifestations of the systemic disease, but primary cardiac symptomatology does occur. Cardiac infiltration by sarcoid granulomas may result in increased stiffness of the heart, with overt features of RCM. In addition, systolic dysfunction, conduction abnormalities, and arrhythmias may be seen. Treatment is empirically with glucocorticoids.

A genetic predisposition is likely, based on increased familial occurrence and different disease modes in different ethnic groups [34]. The strongest genetic associations are found within the human leukocyte antigen (HLA) antigens and functional polymorphisms within the butyrophilin-like 2 (BTNL2) gene [35].

Gaucher Disease

Although Gaucher disease (GD) is the most common lysosomal storage disease, it very rarely affects the heart (only subtype 3, occurring 1 in 200,000). It is caused by deficiency of glucocerebrosidase, which results in abnormal accumulation of glycolipids within cellular lysosomes. GD is one of the few inherited metabolic disorders that can be treated by enzyme replacement therapy with recombinant enzyme; early identification is crucial to improving ultimate outcome.

GD is inherited as an autosomal recessive disorder. The glucocerebrosidase gene is located on chromosome 1q21, and more than 180 distinct mutations are known. However, three mutant alleles account for most cases: p.N370S, p.L444P, and 84GG. The prevalence of these alleles varies with ethnicity. P.N370S is exclusively present in Ashkenazi Jews and non-Jewish Europeans, whereas p.L444P is common in northern Sweden. The diagnosis of GD is confirmed by the finding of reduced glucocerebrosidase activity in peripheral leukocytes. Diagnosis can also be confirmed by mutation analysis, which is an effective method for patient classification and carrier diagnosis.

Hurler disease

The mucopolysaccharidoses (MPS) are lysosomal storage disorders caused by the deficiency of enzymes required for the stepwise breakdown of glycosaminoglycans (GAGs), previously known as mucopolysaccharides. Fragments of partially degraded GAGs accumulate in the lysosomes, resulting in cellular dysfunction and clinical abnormalities. These are rare conditions, with an estimated total incidence of all types of MPS of approximately one in 20,000 live births. Hurler syndrome is the severe form of MPS I and is characterized by a broad spectrum of clinical problems including skeletal abnormalities, hepatosplenomegaly, and severe mental retardation. The incidence is approximately one in 100,000 births.

Cardiac abnormalities become apparent between birth and 5 years of age. These include cardiomyopathy, endocardial fibroelastosis, and valvular regurgitation, which on itself or combined may lead to heart failure. GAG storage within blood vessels causes irregular and diffuse narrowing of the coronary arteries and irregular lesions of the aorta. Coronary artery disease is often unrecognized until autopsy examination; it should be considered in affected patients with cardiac problems.

Mucopolysaccharidosis II (Hunter syndrome) is caused by a deficiency of iduronate 2-sulfatase (IDS), which results in storage of heparan and dermatan sulfate. MPS II is caused by mutations in the gene encoding for IDS, which is located on chromosome Xq28. Although the disorder is X-linked, cases in females have been reported on rare occasions.

Storage Diseases

Hemochromatosis

Iron-overload cardiomyopathy is often the result of multiple transfusions or a hemoglobinopathy, most frequently B-thalassemia. If cardiomyopathy occurs in the presence of diabetes, hepatic cirrhosis, and increased skin pigmentation, it may also result from familial hemochromatosis, an autosomal recessive disorder that arises from a mutation in the HFE gene, which codes for a transmembrane protein that is responsible for regulating iron uptake in the intestine and liver. The HFE gene is tightly linked to the HLA-A locus on chromosome 6p. The most common mutation is Cys282Tyr (C282Y), identified in 85–90 % of hemochromatosis patients in Northern Europe [36]. A second, relatively common HFE mutation (p.H63D) is not associated with clinically relevant iron overload but in case of compound heterozygosity with p.C282Y, iron overload can occur.

Cardiac involvement causes a mixture of systolic and diastolic dysfunction, often with arrhythmias. Cardiac dysfunction is due to direct toxicity from free iron and to adverse effects caused by myocardial cell infiltration, preferentially in the sarcoplasmic reticulum. The ventricles are more affected than the atria and the conduction system is often involved. Loss of myocytes occurs with replacement fibrosis. Macroscopically, the heart may be dilated or nondilated with thickened ventricular walls.

On cardiac MRI, a reduced T2* signal will be seen with increasing cardiac iron storage.

Phlebotomy and iron chelators like desferoxamine may reduce cardiac and other iron stores and result in clinical improvement.

Fabry Disease

This is an X-linked lysosomal storage disorder, caused by deficiency of lysosomal α-galactosidase A (GLA), leading to

the accumulation of glycosphingolipids in tissues like the heart. The ensuing ventricular hypertrophy is often classified as a RCM, although it may also resemble HCM. It is the second most prevalent lysosomal storage disease after Gaucher disease. The gene is located on the long arm (Xq22.1 region) of the X chromosome. Several hundred mutations in the gene have been identified.

The prevalence of Fabry disease is estimated to range from 1:17,000 to 1:117,000 males in Caucasians. Clinical manifestations are usually evident by the age of ten, often starting with neuropathy (burning pains of the palms and soles) and skin lesions (angiokeratomas). At higher age, cardiac and renal disease and stroke become more important. Cardiac involvement may lead to (symmetrical) ventricular hypertrophy, conduction defects, coronary artery disease, valve insufficiencies, and aortic root dilatation [37]. In general, cardiac involvement will be accompanied by other signs of Fabry disease, although these may be missed. Sometimes, the disease is limited to the myocardium. Therefore, screening for Fabry disease is advised in patients with otherwise unexplained LVH. Tissue Doppler may provide a preclinical diagnosis of cardiac involvement, even in patients without LVH. Echocardiographic appearance of Fabry disease may be distinguished from other forms of LVH based on a thickened, hyperechogenic layer in the endocardium and subendocardial myocardium, caused by local intracellular glycolipid deposition. This is paralleled by a hypoechogenic layer, representing the mildly affected midwall myocardium. A definitive diagnosis can be made based on a low plasma α-galactosidase A level in males or by endomyocardial biopsy, showing concentric lamellar bodies in the sarcoplasm of heart cells on electron microscopy. In females the diagnosis can be made by analysis of the GLA gene.

Although the disease is generally considered X-linked recessive, a better name would be X-linked semidominant. LVH may occur in heterozygous females in up to 64 %; end-stage renal disease and stroke may also develop and the overall negative effect on life span may be as much as 15 years.

Enzyme replacement therapy is available, albeit very expensive. Recombinant agalsidase-β may partly clear microvascular endothelial deposits in the heart and kidneys. Therapy can reduce LVH and enhance myocardial function.

Glycogen Storage Disease

Disorders of glycogen metabolism most often affect the liver and skeletal muscle, where glycogen is most abundant. To date, 12 subforms of glycogen storage disease (GSD) have been identified. The physiologic importance of a given enzyme determines the clinical manifestations of the disease. In general, hypoglycemia, hepatomegaly, and skeletal muscle weakness and easy fatigability are the predominant clinical features. In GSD type II (Pompe disease) and IIa (Danon disease), cardiac involvement may occur. The classic infantile form is characterized by cardiomyopathy and severe generalized muscular hypotonia [38]. The tongue may be enlarged. Hepatomegaly also may be present and is usually due to heart failure. Pompe disease is an autosomal recessive disorder with considerable allelic heterogeneity. It is caused by mutations in the gene encoding lysosomal alpha-1,4-glucosidase (GAA) located at 17q25.2-q25. More than 200 mutations have been reported [39].

Endomyocardial Causes of Restrictive Cardiomyopathy

Endomyocardial Fibrosis

Endomyocardial fibrosis (EMB) is an obliterative cardiomyopathy characterized by fibrotic thickening and obliteration of left, right, or both ventricles, with a predilection to selectively involve the apices and inflow region and spare the outflow tract. The fibrotic process does not affect the valve leaflets, the atria, or the great vessels, and extracardiac involvement is not known. There is a peculiar distribution of the disease in very specific areas within some countries around the equator [40]. In an epidemiological study of 214 families in Mozambique, 99 had no cases of EMB, 63 had one case, and 52 had more than one case [41]. The familial occurrence could be caused by genetic factors or susceptibility; however, this has not yet been elucidated. It may also rely on environmental factors, like the abundance of thorium and cerium in the soil, accompanied by magnesium deficiency. It has also been related to filariasis and altered immunological response to streptococcal infection in individuals whose immune status had been altered by parasitic infections

Hypereosinophilic Syndrome

Hypereosinophilic syndrome (HES) is a heterogeneous group of disorders characterized by unexplained persistent primary eosinophilia causing end-organ damage.

In the acute necrotic stage, there is endocardial damage, myocardial infiltration with eosinophils and lymphocytes, eosinophil degranulation, and myocardial necrosis. This phase may be clinically silent without abnormalities on echocardiography. However, serum troponin levels may be raised and contrast-enhanced MRI may detect myocardial inflammation. In the second stage, thrombus formation occurs along areas of damaged endocardium. This may lead to systemic embolization. In the third phase, progressive scar formation produces endomyocardial fibrosis and finally a RCM.

Apart from cardiac manifestations and thromboembolic (cerebral) complications, encephalopathy and peripheral neuropathy may occur.

One HES variant, myeloproliferative, is actually chronic eosinophilic leukemia, which has a unique genetic marker,

FIP1L1-PDGFRA, with consequences for the treatment [42]. Loeffler endocarditis, eosinophilic endomyocardial disease, or fibroplastic endocarditis appears to be a subcategory of the hypereosinophilic syndrome in which the heart is predominantly involved.

Autosomal dominant transmission of marked eosinophilia has been reported. In one family, the gene has been mapped to chromosome 5q31-33 [43].

MRI may be helpful in cases of RCM with luminal obliteration to differentiate perfused and enhancing myocardium from poorly vascularized and hypoenhancing thrombus or eosinophilic infiltrate [44].

Summary

RCM is a rare disease, often presenting with fatigue, exercise intolerance, or dyspnea. In many cases, RCM occurs as part of a multiorgan disease or malignancy, where cardiac involvement may occur early or late in time. Therefore, depending on clinical suspicion or other, noncardiac symptoms and findings, additional investigations are necessary before a definitive diagnosis can be made. The diagnosis of idiopathic RCM can only be made by exclusion. Idiopathic RCM sometimes presents as a familial or genetic form, related to mutations in the cardiac troponin I genes. There may also be overlap, both clinically and genetically, with family members with hypertrophic or dilated cardiomyopathy. Prognosis is often poor and treatment options are scarce: symptomatic therapy with diuretics and/or betablockers and occasionally specific therapy for the underlying disease.

References

1. Mogensen J, Kubo T, Duque M, Uribe W, Shaw A, Murphy R, Gimeno JR, Elliott P, McKenna WJ. Idiopathic restrictive cardiomyopathy is part of the clinical expression of cardiac troponin I mutations. J Clin Invest. 2003;111:209–16.
2. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, Moss AJ, Seidman CE, Young JB. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation. 2006;113:1807–16.
3. Kaski JP, Syrris P, Burch M, Tomé-Esteban MT, Fenton M, Christiansen M, Andersen PS, Sebire N, Ashworth M, Deanfield JE, McKenna WJ, Elliott PM. Idiopathic restrictive cardiomyopathy in children is caused by mutations in cardiac sarcomere protein genes. Heart. 2008;94:1478–84.
4. Stöllberger C, Finsterer J. Extracardiac medical and neuromuscular implications in restrictive cardiomyopathy. Clin Cardiol. 2007;30:375–80.
5. Gomes AV, Liang J, Potter JD. Mutations in human cardiac troponin I that are associated with restrictive cardiomyopathy affect basal ATPase activity and the calcium sensitivity of force development. J Biol Chem. 2005;280:30909–15.
6. Sengupta PP, Krishnamoorthy VK, Abhayaratna WP, Korinek J, Belohlavek M, Sundt 3rd TM, Chandrasekaran K, Seward JB, Tajik AJ, Khandheria BK. Comparison of usefulness of tissue Doppler imaging versus brain natriuretic peptide for differentiation of constrictive pericardial disease from restrictive cardiomyopathy. Am J Cardiol. 2008;102:357–62.
7. Hershberger RE, Lindenfeld J, Mestroni L, Seidman CE, Taylor MR, Towbin JA. Genetic evaluation of cardiomyopathy – a Heart Failure Society of America practice guideline. J Card Fail. 2009;15:83–97.
8. Ammash NM, Seward JB, Bailey KR, Edwards WD, Tajik AJ. Clinical profile and outcome of idiopatic restrictive cardiomyopathy. Circulation. 2000;101:2490–6.
9. Katritsis D, Wilmshurst PT, Wendon JA, Davies MJ, Webb-Peploe MM. Primary restrictive cardiomyopathy: clinical and pathologic characteristics. J Am Coll Cardiol. 1991;18:1230–5.
10. Cubero GI, Larraya GL, Reguero JR. Familial restrictive cardiomyopathy with atrioventricular block without skeletal myopathy. Exp Clin Cardiol. 2007;12:54–5.
11. Fitzpatrick AP, Shapiro LM, Rickards AF, Poole-Wilson PA. Familial restrictive cardiomyopathy wieth atrioventricular block and skeletal myopathy. Br Heart J. 1990;63:114–8.
12. Kostareva A, Gudkova A, Sjöberg G, Mörner S, Semernin E, Krutikov A, Shlyakhto E, Sejersen T. Deletion in TNNI3 gene is associated with restrictive cardiomyopathy. Int J Cardiol. 2009;131:410–2.
13. Nonaka M, Morimoto S. Experimental models of inherited cardiomyopathy and its therapeutics. World J Cardiol. 2014;6:1245–51.
14. Davis J, Wen H, Edwards T, Metzger JM. Thin filament disinhibition by restrictive cardiomyopathy mutant R193H troponin I induces Ca2+-independent mechanical tone and acute myocyte remodeling. Circ Res. 2007;100(10):1494–502.
15. Du J, Liu J, Feng HZ, Hossain MM, Gobara N, Zhang C, Li Y, Jean-Charles PY, Jin JP, Huang XP. Impaired relaxation is the main manifestation in transgenic mice expressing a restrictive cardiomyopathy mutation, R193H, in cardiac TnI. Am J Physiol Heart Circ Physiol. 2008;294:H2604–13.
16. Zhang L, Nan C, Chen Y, Tian J, Jean-Charles PY, Getfield C, Wang X, Huang X. Calcium desensitizer catechin reverses diastolic dysfunction in mice with restrictive cardiomyopathy. Arch Biochem Biophys. 2015;573:69–76.
17. Brodehl A, Ferrier RA, Hamilton SJ, Greenway SC, Brundler MA, Yu W, Gibson WT, McKinnon ML, McGillivray B, Alvarez N, Giuffre M, Schwartzentruber J, Gerull B, FORGE Canada Consortium. Mutations in FLNC are associated with familial restrictive cardiomyopathy. Hum Mutat. 2016;37:269–79.
18. Menon SC, Michels VV, Pellikka PA, Ballew JD, Karst ML, Herron KJ, Nelson SM, Rodeheffer RJ, Olson TM. Cardiac troponin T mutation in familial cardiomyopathy with variable remodeling and restrictive physiology. Clin Genet. 2008;74:445–54.
19. Tzelepis GE, Kelekis NL, Plastiras SC, Mitseas P, Economopoulos N, Kampolis C, Gialafos EJ, Moyssakis I, Moutsopoulos HM. Pattern and distribution of myocardial fibrosis in systemic sclerosis: a delayed enhanced magnetic resonance imaging study. Arthritis Rheum. 2007;56:3827–36.
20. Johnson RW, Tew MB, Arnett FC. The genetics of systemic sclerosis. Curr Rheumatol Rep. 2002;4:99–107.
21. Crilly A, Hamilton J, Clark CJ, Jardine A, Madhok R. Analysis of transforming growth factor beta1 gene polymorphisms in patients with systemic sclerosis. Ann Rheum Dis. 2002;61:678–81.
22. Nguyen LD, Terbah M, Daudon P, Martin L. Left ventricular systolic and diastolic function by echocardiogram in pseudoxanthoma elasticum. Am J Cardiol. 2006;97:1535–7.

23. Seferović PM, Paulus WJ. Clinical diabetic cardiomyopathy: a two-faced disease with restrictive and dilated phenotypes. Eur Heart J. 2015;36:1718–27.
24. Brownlee M (1994 Jun) Lilly Lecture 1993. Glycation and diabetic complications. Diabetes 43(6):836–841
25. Rota M, LeCapitaine N, Hosoda T, Boni A, De Angelis A, Padin-Iruegas ME, Esposito G, Vitale S, Urbanek K, Casarsa C, Giorgio M, Lüscher TF, Pelicci PG, Anversa P, Leri A, Kajstura J. Diabetes promotes cardiac stem cell aging and heart failure, which are prevented by deletion of the p66shc gene. Circ Res. 2006;99:42–52.
26. Maleszewski JJ. Cardiac amyloidosis: pathology, nomenclature, and typing. Cardiovasc Pathol. 2015;24:343–50.
27. Kholova I, Niessen HWM. Amyloid in the cardiovascular system: a review. J Clin Pathol. 2005;58:125–33.
28. Selvanayagam JB, Hawkins PN, Paul B, Myerson G, Neubauer S. Evaluation and management of cardiac amyloidosis. J Am Coll Cardiol. 2007;50:2101–10.
29. Ogmundsdóttir HM, Einarsdóttir HK, Steingrímsdóttir H, Haraldsdóttir V. Familial predisposition to monoclonal gammopathy of unknown significance, Waldenström's macroglobulinemia, and multiple myeloma. Clin Lymphoma Myeloma. 2009;9:27–9.
30. Rapezzi C, Merlini G, Quarta CC, Riva L, Longhi S, Leone O, et al. Systemic cardiac amyloidosis. Disease profiles and clinical courses of the 3 main types. Circulation. 2009;120:1203–12.
31. Quarta CC, Buxbaum JN, Shah AM, Falk RH, Claggett B, Kitzman DW, Mosley TH, Butler KR, Boerwinkle E, Solomon SD. The amyloidogenic V122I transthyretin variant in elderly black Americans. N Engl J Med. 2015;372:21–9.
32. Hesse A, Altland K, Linke RP, Almeida MR, Saraiva MJ, Steinmetz A, Maisch B. Cardiac amyloidosis: a review and report of a new transthyretin (prealbumin) variant. Br Heart J. 1993;70:111–5.
33. Ruberg FL, Judge DP, Maurer MS. Familial amyloid cardiomyopathy due to TTR mutations: an underground cause of restrictive cardiomyopathy. Letter to the editor.J Card Fail. 2009;15:464.
34. Grunewald J. Genetics of sarcoidosis. Curr Opin Pulm Med. 2008;14:434–9.
35. Spagnolo P, Sato H, Grutters JC, Renzoni EA, Marshall SE, Ruven HJ, Wells AU, Tzouvelekis A, van Moorsel CH, van den Bosch JM, du Bois RM, Welsh KI. Analysis of BTNL2 genetic polymorphisms in British and Dutch patients with sarcoidosis. Tissue Antigens. 2007;70:219–27.
36. Pietrangelo A. Hereditary hemochromatosis – a new look at an old disease. N Engl J Med. 2004;350:2383–97.
37. Mehta A, Ricci R, Widmer U, Dehout F, Garcia de Lorenzo A, Kampmann C, Linhart A, Sunder-Plassmann G, Ries M, Beck M. Fabry disease defined: baseline clinical manifestations of 366 patients in the Fabry Outcome Survey. Eur J Clin Investig. 2004;34:236–42.
38. Howell RR, Byrne B, Darras BT, Kishnani P, Nicolino M, van der Ploeg A. Diagnostic challenges for Pompe disease: an under-recognized cause of floppy baby syndrome. Genitourin Med. 2006;8:289–96.
39. Kroos M, Pomponio RJ, van Vliet L, Palmer RE, Phipps M, Van der Helm R, Halley D, Reuser A. Update of the Pompe disease mutation database with 107 sequence variants and a format for severity rating. Hum Mutat. 2008;29:E13–26.
40. Sivasankaran S. Restrictive cardiomyopathy in India: the story of a vanishing mystery. Heart. 2009;95:9–14.
41. Mocumbi AO, Ferreira MB, Sidi D, Yacoub MH. A population study of endomyocardial fibrosis in a rural area of Mozambique. N Engl J Med. 2008;359:43–9.
42. Gleich GJ, Leiferman KM. Hypereosinophilic syndromes: current concepts and treatments. Br J Haematol. 2009;145:271–85.
43. Rioux JD, Stone VA, Daly MJ, Cargill M, Green T, Nguyen H, Nutman T, Zimmerman PA, Tucker MA, Hudson T, Goldstein AM, Lander E, Lin AY. Familial eosinophilia maps to the cytokine gene cluster on human chromosomal region 5q31-q33. Am J Hum Genet. 1998;63:1086–94.
44. Kleinfeldt T, Ince H, Nienaber CA. Hypereosinophilic syndrome: a rare case of Loeffler's endocarditis documented in cardiac MRI. Int J Cardiol. 2009;15.

Mitochondrial Cardiomyopathy

9

N. de Jonge and J.H. Kirkels

Abstract

The mitochondrial diseases are a heterogeneous group of rare disorders which can affect virtually all organ systems, like the heart, the brain and the skeletal muscles. Most of the mitochondrial diseases are caused by mutations in the nuclear DNA, but approximately 15% are caused by mutations in the mitochondrial DNA, making genetic counseling difficult. The combination of cardiomyopathy, deafness, diabetes, encephalopathy and myopathy suggests mitochondrial disease.

Cardiomyopathy, however, may be the first and only symptom. MELAS syndrome with hypertrophic cardiomyopathy and Kearns-Sayre syndrome with progressive conduction disorders are two examples of mitochondrial disease.

Introduction

Mitochondria are the major sites of energy production in the cell as they harbor the process of oxidative phosphorylation (OXPHOS). The OXPHOS system consists of five enzyme complexes, of which complexes I–IV give rise to the mitochondrial respiratory chain, located at the inner mitochondrial membrane. Energy produced by this respiratory chain drives the production of adenosine-tri-phosphate (ATP) from adenosine-di-phosphate (ADP) by complex V.

As the heart is an energy-dependent tissue, mitochondria constitute 20–40 % of the cellular volume of cardiomyocytes (Fig. 9.1). The mitochondrial energy production is under the genetic control of both nuclear (99 %) and mitochondrial genes (mtDNA) (1 %) [1]. Mutations within these genes may cause defects in oxidative phosphorylation and have severe consequences for those organs that are heavily dependent on energy production like the heart, the brain, and skeletal muscle. Because myopathy is often one of the main presenting symptoms, patients with mitochondrial diseases tend to be seen primarily by neurologists and pediatricians. However, the importance of mitochondrial disease in cardiology is being more and more recognized, as cardiomyopathy may be the first and only manifestation of mitochondrial disease.

Mitochondrial DNA (mtDNA) is a circular double-stranded genome of 16.5 kilobases, encoding 13 polypeptides of the respiratory chain subunits, 28 ribosomal RNAs, and 22 transfer RNAs (tRNAs). All these mitochondrial gene

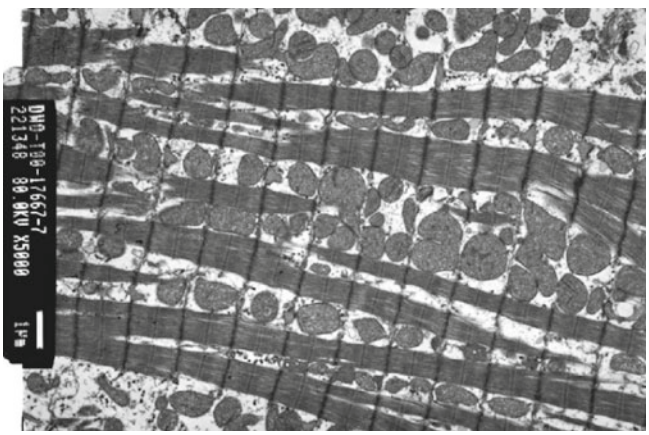

Fig. 9.1 A cardiomyocyte demonstrating the high numbers of mitochondria in between the contractile filaments

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products are involved in mitochondrial energy production, but as stated before, many other components of the respiratory chain and regulatory mitochondrial proteins are coded by nuclear genes. Mitochondrial diseases may thus be caused by mutations in mtDNA, but most are caused, however, by mutations in nuclear DNA.

Therefore, different modes of inheritance may be observed in mitochondrial disease, as mtDNA is exclusively maternally inherited, while nuclear DNA follows Mendelian inheritance. The maternal inheritance of mtDNA is due to the fact that the mammalian egg contains about 100,000 mitochondria and mtDNA, whereas the sperm contains only in the order of 100 mtDNA [2].

Mammalian mtDNA has a very high mutation rate in comparison to nuclear DNA. Each cell contains hundreds to thousands of mitochondria and each mitochondrion contains many copies of mtDNA. Mutations in mtDNA therefore result in *heteroplasmy*: the presence of two or more different genomes (with and without a mutation) in one cell, the proportion of which may change over time as the mitochondria multiply and are randomly distributed over daughter cells during cell division. Due to this process, the proportion of mutant mtDNA varies considerably between organ systems and even within a specific tissue, resulting in different phenotypes and marked variability in severity and symptom patterns. The heart, central nervous system (CNS), and the skeletal muscles are particularly vulnerable to defects in energy metabolism, and therefore are often involved in mitochondrial disease.

Phenotype–genotype correlation in mitochondrial disease is complex: patients with the same clinical syndrome do not always show the same mutation in the mtDNA and, conversely, a single mutation can be associated with different clinical syndromes [3].

Many mutations in mtDNA may lead to *cardiomyopathy*, mostly *hypertrophic*, but *dilating cardiomyopathy* and *left ventricular noncompaction* are also possible [4]. A list of known mutations reported in patients with cardiomyopathy is reviewed elsewhere and some are shown in Table 9.1 [5].

Besides mutations in the mtDNA, many mutations in nuclear genes encoding mitochondrial proteins may also cause cardiomyopathy. Some examples include mutations in the mitochondrial transport protein *frataxin* leading to *Friedreich's ataxia*, an autosomal recessive neurodegenerative disorder characterized by progressive ataxia, dysarthria, and hypertrophic cardiomyopathy, and mutations in the gene encoding the protein *tafazzin*, resulting in *Barth syndrome*, an X-linked neonatal disorder characterized by dilating cardiomyopathy, skeletal myopathy, cyclic neutropenia, and growth retardation. A comprehensive list of mitochondrial diseases associated with cardiomyopathy is reviewed elsewhere [1].

The most frequently reported cardiac manifestations in mitochondrial disease are mentioned in the Table 9.2.

Table 9.1 Specific mitochondrial DNA (mtDNA) point mutations in cardiac disease (Adapted from Marin-Garcia [5])

Gene	Site	Cardiac phenotype
tRNA mutations		
Leu	3243 A- > G	DCM
Leu	3260 A- > G	Tachycardia, adult onset
Leu	3303 C- > T	Fatal infantile CM
Leu	3254 C- > G	HCM
Leu	12997 T- > C	DCM
Ile	4300 A- > G	HCM, adult onset
Ile	4317 A- > G	Fatal infantile CM
Ile	4320 C- > T	Fatal infantile CM
Ile	4269 A- > G	CF at 18 year, adult onset
Ile	4295 A- > G	HCM
Ile	4284 G- > A	CM
Lys	8363 G- > A	HCM
Lys	8334 A- > G	HCM
Lys	8269 A- > G	HCM
Lys	8348 A- > G	HCM
Gly	9997 T- > C	Ventricular arrhythmia, HCM
Cys	5814 A- > G	HCM
Ala	5587 T- > C	DCM
Arg	10415 T- > C	DCM
Arg	10424 T- > C	Fatal DCM
rRNA mutations		
12s	1555 A- > G	CM
16s	3093 C- > G	CM
Gene	Site	Cardiac phenotype
Structural gene mutations		
Cytb	14927 A- > G	HCM
Cytb	15236 A- > G	DCM
Cytb	15508 C- > G	DCM
Cytb	15509 A- > C	Fatal postpartum CM
Cytb	15498 G- > A	Histiocytoid CM
COI	6860 A- > C	DCM
COII	7923 A- > G	DCM
COIII	9216 A- > G	DCM
ND5	14069 C- > T	DCM
ATPase6	8993 T- > G	Leigh syndrome/HCM

DCM dilated cardiomyopathy, *HCM* hypertrophic cardiomyopathy, *CF* cardiac failure

Many mitochondrial disorders become apparent in the first years of life. The frequency of cardiomyopathy in mitochondrial disease has been reported to be from 17 % to 40 % and the incidence of mitochondrial cardiomyopathy in children and young adults is estimated to be at least 1/50,000 [4, 6]. Children with mitochondrial cardiomyopathy generally have an earlier onset, more severe morbidity, and increased mortality compared with children who have mitochondrial disorders without cardiac involvement [7]. One study showed that of the patients with cardiomyopathy 71 % died or underwent heart

Table 9.2 Cardiac manifestations of mitochondrial disease

Hypertrophic (nonobstructive) cardiomyopathy
Dilated cardiomyopathy
Left ventricular noncompaction
Left ventricular hypertrophy
WPW-syndrome
Long QT-syndrome
Ventricular tachycardia
Left anterior hemiblock
Right bundle branch block
Total AV block
Mitral valve prolapse

transplantation, in contrast to 26 % in patients with mitochondrial disease without cardiomyopathy [6]. Risk factors for major adverse cardiac events in patients with mitochondrial disease were intraventricular conduction block left bundle branch block (LBBB), right bundle branch block (RBBB), left anterior fascicular block (LAFB), left posterior fascicular block (LPFB), diabetes, premature ventricular complexes (>15 PVC/Hr) and left ventricular hypertrophy [8].

As mentioned before, cardiac involvement in mitochondrial disease is usually part of multisystem manifestations of the disorders in oxidative phosphorylation. It is important to realize, however, that seemingly isolated cardiac pathology may be the presenting symptom in mitochondrial disease. In one study this was the case in approximately 10% of patients [9]. Furthermore, a novel mutation in mtDNA (*m.8528 T > C*) was described in four young patients presenting with an isolated hypertrophic cardiomyopathy, further underlining OXPHOS defects as a potential cause of isolated cardiomyopathy [7].

In this Chapter, two syndromes will be described in more detail: mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (*MELAS)* and *Kearns–Sayre syndrome*.

MELAS Syndrome

This is a multisystem clinical syndrome manifested by mitochondrial myopathy, *encephalopathy*, *lactic acidosis*, and *recurrent stroke-like episodes* [10]. The most commonly described gene mutation causing MELAS syndrome is a mitochondrial adenine-to-guanine transition at nucleotide pair 3243 (*m.3243A > G*) encoding the mitochondrial $tRNA^{(Leu)}$ [11]. At least 29 other specific point mutations have been associated with the MELAS syndrome [12]. These mutations lead to impaired oxidative phosphorylation, resulting in the inability of the mitochondria to produce sufficient ATP to meet the energy needs of the cell. This causes a shift to lactate production, which can be systemically noticed as lactate acidosis.

Due to the variability in severity and symptoms and the problems confirming the diagnosis, the incidence of MELAS syndrome is difficult to assess. It is estimated to be as common as neuromuscular diseases like Duchenne muscular dystrophy (frequency 18 per 100,000) [12].

The clinical features of MELAS syndrome vary widely, but almost all patients are diagnosed with stroke-like episodes before 40 years of age, encephalopathy characterized by seizures, dementia, or both, and lactic acidosis. Although age at onset may be high in some patients, most patients, however, present with initial symptoms between 2 and 20 years of age [10]. Other symptoms related to MELAS syndrome are hearing loss, migraine headaches, peripheral neuropathy, depression, learning disabilities, growth failure, diabetes mellitus, gastrointestinal symptoms, renal involvement, and myopathy.

Cardiac involvement in MELAS syndrome is reported to be as high as 18–100 % [13–15]. The most common pathology is *nonobstructive concentric hypertrophy*, although dilatation is also reported and might be seen as progression of the initial hypertrophic cardiomyopathy. Left ventricular hypertrophy (LVH) appears to correlate positively with LV dilatation and negatively with systolic function [16]. In children, cardiomyopathy may actually be the first manifestation of MELAS syndrome. Wolff–Parkinson–White (WPW) syndrome has also been reported in MELAS syndrome in up to 17 % of patients [13, 17].

The clinical suspicion for mitochondrial disease is based on the combination of symptoms related to different organ systems. On the other hand, especially in young children, the presence of a cardiomyopathy may be the only manifestation of a mitochondrial disorder.

Laboratory examination will show lactic acidosis in almost all patients. Magnetic resonance imaging (MRI) of the brain in MELAS syndrome will typically show asymmetric lesions of the occipital and parietal lobes, mimicking ischemia, although not restricted to one specific vascular region.

ECGs may demonstrate specific abnormalities suggestive of cardiomyopathy, like LVH, negative T-waves in the precordial leads, a left-oriented electrical axis, and prolonged QT_c [6]. Echocardiographic examination is mandatory in demonstrating cardiac involvement in mitochondrial disease. In addition to LVH, diastolic and systolic dysfunction may be present.

Muscular biopsy in most patients will show *ragged red fibers*: deposits of mitochondrial material beneath the sarcolemma, visualized by Gomori trichrome staining or succinate dehydrogenase [12, 14].

Ultrastructural analysis of the heart demonstrates abnormal and markedly enlarged mitochondria.

Molecular diagnosis of mtDNA mutations is complicated by the variability in heteroplasmy depending on the specific tissue sampled. A detectable mutation in muscle cells is not necessarily detectable in leucocytes, cells regularly used for DNA analysis. Urine sediment cells and

cheek mucosa appear to be a better alternative for DNA analysis [18].

No specific treatment is available for mitochondrial cardiomyopathies, although there are some suggestions that the use of l-*arginine* and *coenzyme Q10* in addition to vitamin supplementation might be advantageous [12]. As in other cardiomyopathies, regular heart failure therapy is indicated, consisting of diuretics, angiotensin-converting enzyme (ACE) inhibitors, and β-blockers. In cases of refractory heart failure, despite optimal medical therapy, heart transplantation can be considered in selected patients [19]. This requires extensive evaluation of extracardiac involvement, especially with regard to potential contraindications such as recurrent strokes, dementia, and muscle wasting.

Furthermore, heart transplantation and other operations are generally accompanied by a significantly increased perioperative risk, in particular due to stroke, coma, seizures, respiratory failure, and cardiac arrhythmias [20]. *Perioperative management* includes generous hydration, loading with intravenous glucose, and careful control of body temperature and pH. Ringer's solution should be avoided because of the lactate load. *Anesthetic agents* in these patients may increase the susceptibility to reactive oxygen species (ROS) and apoptosis, resulting in neurotoxicity. In general, an increased sensitivity to anesthetics is reported, requiring adjustment of dosing and careful management during surgery, including optimal oxygenation [20].

In summary, given the high incidence of cardiac involvement, all patients with MELAS syndrome should undergo regular cardiac examination because of the therapeutic and prognostic consequences of cardiac involvement. On the other hand, patients with hypertrophic cardiomyopathy at a younger age should be considered having mitochondrial disease, especially when they also suffer from short stature, seizures, hemiparesis, hemianopsia, or cortical blindness. MELAS syndrome is maternally inherited, but genotype–phenotype correlation is complex, which hampers the role of genetic counseling in this syndrome.

Kearns–Sayre Syndrome (KSS)

Clinically, this mitochondrial disease is characterized by progressive *external ophthalmoplegia* resulting in ptosis, and *pigmentary retinopathy*. Other manifestations of KSS are short stature, cerebellar signs, hearing loss, mental retardation, vestibular system dysfunction, delayed puberty, and high cerebrospinal fluid protein content. Typical onset is before the age of 20. Progression of the disease can be accompanied by proximal myopathy [21].

Cardiac pathology consists of conduction defects caused by *infra-His block*, resulting in *total AV-block*, *right bundle branch* block, or *left anterior hemiblock* [22, 23]. These conduction defects may be rapidly progressive and result in *acute cardiac death*. Transition of a normal electrocardiogram into total AV-block has been reported within the course of 10 months [24]. Complete heart block may also be the presenting symptom of KSS in some patients [25]. It seems plausible that early pacemaker implantation improves survival, but criteria for prophylactic implantation have not been clearly defined. Third-degree and advanced second-degree AV block associated with neuromuscular disease like KSS, with or without symptoms constitute a class I indication for permanent pacemaker implantation [26]. Given the rapid progression to complete AV block, the presence of a fascicular block in KSS has been suggested to warrant prophylactic implantation of a pacemaker [27]. In patients with a normal ECG, regular ECG follow-up, at least every year, is advisable.

Although conduction defects constitute the main cardiac problems in KSS, cardiomyopathy has been reported in a minority of cases [28]. The incidence of cardiomyopathy in KSS, may increase in the future due to the prolonged longevity in patients treated by early pacemaker implantation [27].

In contrast to the MELAS syndrome, which is caused by a point mutation in the mtDNA and is maternally inherited, genetic analysis in KSS typically shows a large *deletion of mtDNA* and most cases are sporadic [23, 29]. Early mortality in this syndrome is often related to sudden cardiac death due to AV-block, which may be prevented by timely pacemaker implantation.

Conclusion

The mitochondrial diseases are a heterogeneous group of disorders that can affect virtually all organ systems, not only in infancy, but also during the early-to-mid adult years. Most of the mitochondrial diseases are caused by mutations in the nuclear DNA, of which several have been identified thus far. Approximately 15 % are caused by mutations in the mitochondrial DNA. Some examples are reviewed elsewhere and a list of genes is regularly updated at MitoMap (www.mitomap.org/MITOMAP) [1].

Mitochondrial diseases should be included in the differential diagnosis whenever a patient presents with progressive multisystem involvement that does not clearly fit with an established pattern of disease. The combination of cardiomyopathy, deafness, diabetes, together with encephalopathy and myopathy are highly susceptible of mitochondrial disease [21]. Diagnostic algorithms for mitochondrial disease have been recently suggested [1, 30].

Cardiomyopathy may be the presenting and predominant clinical expression of MELAS syndrome and is one of the causes of death in this disease, underlining the importance of this condition to the cardiologist. The same holds for the progressive conduction disorders in Kearns–Sayre syndrome, which may require pacemaker implantation to prevent sudden death.

Apart from these rather well-delineated disorders, many others exist and the phenotypes frequently overlap, complicating things even further.

Given these facts, genetic counseling in mitochondrial disease is difficult. There is only a very small chance that males with mtDNA mutations will transmit the disease. The risk in females is depending on the level of heteroplasmy, but it remains difficult to give advice in the clinical routine.

Diagnosis of mitochondrial diseases is notoriously difficult and relies on a high level of suspicion, but is important, given the potential management implications, not only with respect to cardiac disease, but also more in general like decreased anesthetic requirement during surgical procedures.

Take Home Message

- Mitochondrial diseases are rare and can be caused by mutations in nuclear DNA as well as mitochondrial DNA, making genetic counseling difficult.
- The combination of cardiomyopathy, deafness, diabetes, encephalopathy, and myopathy suggests mitochondrial disease.
- Cardiomyopathy may be the first and only symptom.
- MELAS syndrome with hypertrophic cardiomyopathy and Kearns–Sayre syndrome with progressive conduction disorders are two examples of mitochondrial disease.

References

1. Brunel-Guitton C, Levtova A, Sasarman F. Mitochondrial diseases and cardiomyopathies. Can J Cardiol. 2015;31:1360–76.
2. Wallace DC. Mitochondrial defects in cardiomyopathy and neuromuscular disease. Am Heart J. 2000;139:S70–85.
3. Ashizawa T. What is Kearns-Sayre syndrome after all? Arch Neurol. 2001;58:1053–4.
4. Scaglia F, Towbin JA, Craigen WJ, et al. Clinical spectrum, morbidity, and mortality in 113 pediatric patients with mitochondrial disease. Pediatrics. 2004;114:925–31.
5. Garcia J-M, Goldenthal MJ. Understanding the impact of mitochondrial defects in cardiovascular disease: a review. J Card Fail. 2002;8:347–61.
6. Holmgren D, Wahlander H, Eriksson BO, Oldfors A, Holme E, Tulinius M. Cardiomyopathy in children with mitochondrial disease. Eur Heart J. 2003;24:280–8.
7. Ware SM, El-Hassan N, Kahler SG, et al. Infantile cardiomyopathy caused by a mutation in the overlapping region of mitochondrial ATPase 6 and 8 genes. J Med Genet. 2009;46:308–14.
8. Wahbi K, Bougouin W, Béhin A, et al. Long-term cardiac prognosis and risk stratification in 260 adults presenting with mitochondrial diseases. Eur Heart J. 2015;36:2886–93.
9. Yaplito-Lee J, Weintraub R, Jamsen K, et al. Cardiac manifestations in oxidative phosphorylation disorders of childhood. J Pediatr. 2007;150:407–11.
10. Pavlakis SG, Phillips PC, DiMauro S, De Vivo DC, Rowland LP. Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes: a distinctive clinical syndrome. Ann Neurol. 1984;16:481–8.
11. Goto Y, Nonaka I, Horai S. A mutation in the tRNA (Leu) (UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature. 1990;348:651–3.
12. Sproule D, Kaufman P. Mitochondrial encephalopathy, lactic acidosis and strokelike episodes. Basic concepts, clinical phenotype, and therapeutic management of MELAS syndrome. Ann N Y Acad Sci. 2008;1142:133–58.
13. Hirano M, Pavlakis SG. Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS) current concepts. J Child Neurol. 1994;9:4–13.
14. Vydt TCG, de Coo RFM, Soliman OII, ten Cate FJ, van Geuns RJM, et al. Cardiac involvement in adults with m.3242A>G MELAS gene mutation. Am J Cardiol. 2007;99:264–9.
15. Wortmann SB, Rodenburg RJ, Backx AP, Schmitt E, Smeitink JAM, Morava E. Early cardiac involvement in children carrying the A3243G mtDNA mutation. Acta Paediatr. 2007;96:450–1.
16. Okajima Y, Tanabe Y, Takayanagi M, Aotsuka H. A follow up study of myocardial involvement in patients with mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS). Heart. 1998;80:292–5.
17. Sproule DM, Kaufmann P, Engelstad K, et al. Wolff-Parkinson-White syndrome in patients with MELAS. Arch Neurol. 2007;64:1625–7.
18. Shanske S, Pancrudo J, Kaufmann P, et al. Varying loads of mitochondrial DNA A3243G mutation in different tissues: implications for diagnosis. Am J Med Genet. 2004;130A:134–7.
19. Bhati RS, Sheridan BC, Mill MR, Selzman CH. Heart transplantation for progressive cardiomyopathy as a manifestation of MELAS syndrome. J Heart Lung Transplant. 2005;24:2286–9.
20. Muravchick S. Clinical implications of mitochondrial disease. Adv Drug Deliv Rev. 2008;60:1553–60.
21. Finsterer J. Mitochondriopathies. Eur J Neurol. 2004;11:163–86.
22. Roberts NK, Perloff JK, Kark RAP. Cardiac conduction in the Kearns-Sayre Syndrome (a neuromuscular disorder associated with progressive external ophthalmoplegia and pigmentary retinopathy). Am J Cardiol. 1979;44:1396–400.
23. Anan R, Nakagawa M, Miyata M, Higuchi I, Nakao S, et al. Cardiac involvement in mitochondrial diseases. A study on 17 patients with documented mitochondrial DNA defects. Circulation. 1995;91:955–61.
24. Welzing L, von Kleist-Retzow JC, Kribs A, et al. Rapid development of life-threatening complete atrioventricular block in Kearns-Sayre syndrome. Eur J Pediatr. 2009;168:757–9.
25. Chawla S, Coku J, Forbes T, Kannan S. Kearns-Sayre syndrome presenting as complete heart block. Pediatr Cardiol. 2008;29:659–62.
26. Epstein AE, DiMarco JP, Ellenbogen KA, et al. ACC/AHA/HRS 2008 guidelines for device based therapy of cardiac rhythm abnormalities. J Am Coll Cardiol. 2008;51:e1–e62.
27. Charles R, Holt S, Kay JM, et al. Myocardial ultrastucture and the development of atrioventricular block in Kearns-Sayre syndrome. Circulation. 1981;63:214–9.
28. Channer KS, Channer JL, Campbell MJ, Russel RJ. Cardiomyopaty in the Kearns-Sayre syndrome. Br Heart J. 1988;59:486–90.
29. Limongelli G, Tome-Esteban M, Dejthevaporn C, et al. Prevalence and natural history of heart disease in adults with primary mitochondrial respiratory chain disease. Eur J Heart Fail. 2010;12:114–21.
30. Bates MGD, Bourke JP, Giordano C, et al. Cardiac involvement in mitochondrial DNA disease: clinical spectrum, diagnosis and management. Eur Heart J. 2012;33:3023–33.

Part III

Hereditary Arrhythmia Syndromes

Long QT Syndrome

10

Yanushi D. Wijeyeratne and Elijah R. Behr

Abstract

The so-called congenital long QT syndrome (LQTS) is an inherited arrhythmia syndrome predisposing to life-threatening ventricular arrhythmias and sudden death. It is caused by prolongation of the repolarization phase of the cardiac action potential, which may manifest as lengthening of the heart rate-corrected QT interval (QTc) on the surface electrocardiogram (ECG). This chapter reviews the clinical presentation of LQTS, its diagnosis, and principles of management in the context of recent clinical advances and molecular genetics, with a focus on the most common forms of LQTS – LQT1, LQT2, and LQT3.

Introduction

The so-called congenital long QT syndrome (LQTS) is an inherited arrhythmia syndrome predisposing to life-threatening ventricular arrhythmias and sudden death. It is caused by prolongation of the repolarization phase of the cardiac action potential, which may manifest as lengthening of the heart rate-corrected QT interval (QTc) on the surface electrocardiogram (ECG).

LQTS was first described as an autosomal recessive form with sensorineural deafness, the Jervell and Lange-Nielsen syndrome, and the autosomal dominant form, the Romano-Ward syndrome [1–3].

It was not until the 1990s that the molecular basis of the condition began to unravel through linkage studies and the subsequent identification of mutations in specific genes that encode cardiac ion channels [4–8].

The prevalence of LQTS among Caucasians is estimated to be 1:2000–1:2500 based upon a large study of neonatal ECGs and targeted genetic testing in nearly 45,000 Italian infants [9]. The clinically diagnosed prevalence in the general population is, however, likely to be less than 1:2000. This is because of incomplete penetrance with a large proportion of mutation carriers remaining asymptomatic throughout life and escaping detection. In others, the first presentation may be a fatal cardiac arrhythmia and the diagnosis made postmortem. Indeed, LQTS is one of the most common causes of autopsy-negative sudden death, also known as sudden arrhythmic death syndrome (SADS), accounting for up to 20 % of cases [10–12]. Patients with LQTS have been identified worldwide, although there is a paucity of cases reported among black ethnic groups [13].

This chapter reviews the clinical presentation of LQTS, its diagnosis, and principles of management in the context of recent clinical advances and molecular genetics, with a focus on the most common forms of LQTS – LQT1, LQT2, and LQT3.

Pathophysiology

LQTS is most frequently caused by loss of function mutations of genes encoding cardiac potassium channels responsible for I_{Ks} and I_{Kr}, the slow- and rapid-rectifying currents, resulting in reduced outward potassium currents and longer action potential duration (APD). The ion channel dysfunction is secondary to two distinct biophysical mechanisms. One is the failure of assembly and trafficking of the channel

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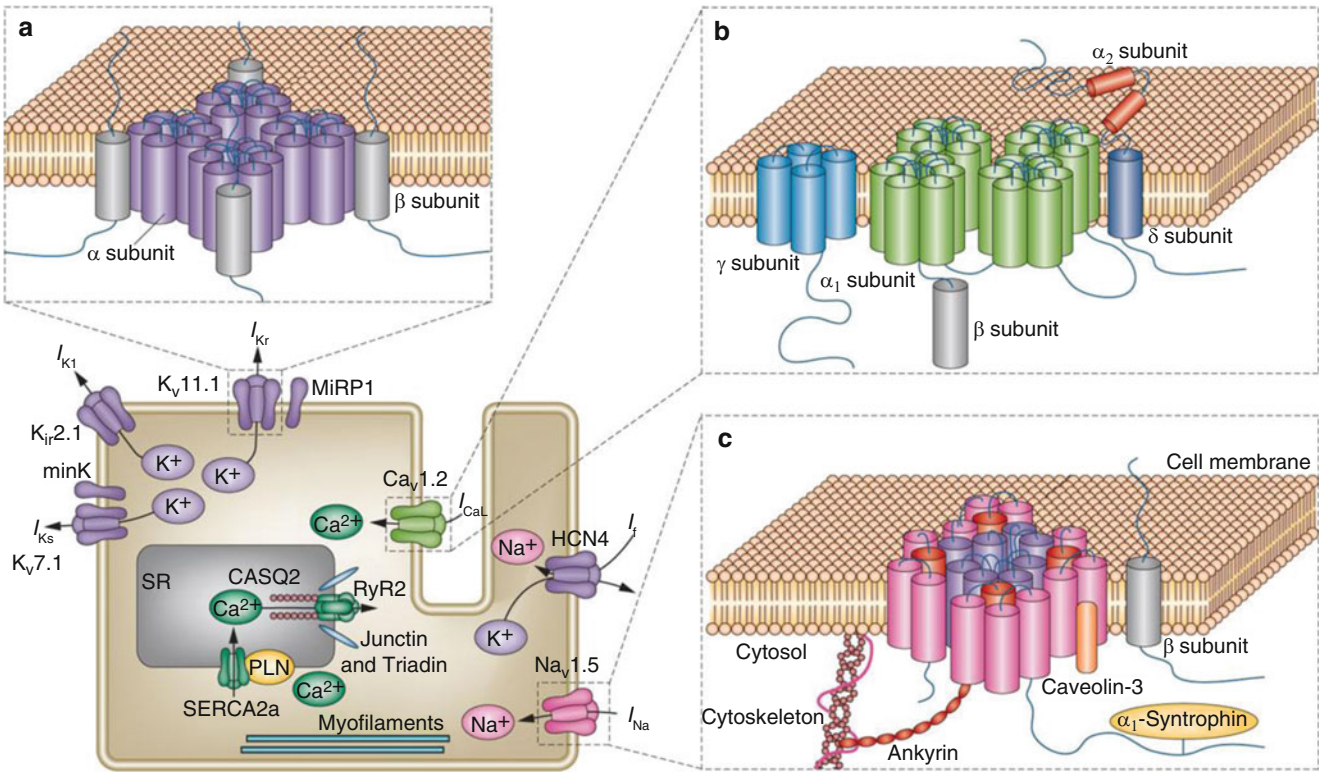

Fig. 10.1 Mutations in the encoding genes lead to aberrant function of the respective proteins and to different cardiac channelopathies. (**a**) Potassium (I_{Kr}), (**b**) calcium (I_{CaL}), and (**c**) sodium (I_{Na}) channel structures and subunits are shown. Abbreviations: *CASQ2* calsequentrin-2; *PLN* cardiac phospholamban; *RyR2* ryanodine receptor 2; *SERCA2a* sarcoplasmic/endoplasmic reticulum calcium ATPase 2a; *SR* sarcoplasmic reticulum (Reproduced with permission from Wilde and Behr [14])

to the cell membrane resulting in haploinsufficiency. The other is successful trafficking to the membrane of defective channels with a dominant-negative effect (where channel α-subunits are organized in tetramers and mutant protein acts antagonistically to the wild-type protein). Gain of function mutations affecting the cardiac sodium channel and its interacting proteins may lengthen the plateau phase of the APD by increasing the late inward sodium current (late I_{Na}) while gain of function mutations affecting the calcium channel (I_{CaL}) may also occasionally be associated. Figure 10.1 depicts some of the cardiac ion channels and other proteins involved in different inherited arrhythmia syndromes, though not all of these are implicated in LQTS [14].

Molecular Genetics

Advances in molecular genetics over the past 20 years have led to the discovery of mutations in a total of 15 genes causing LQTS and have provided insights into underlying molecular mechanisms. A mutation is identified in 80–85 % of definite cases, but 15–20 % of LQTS remains genetically elusive (no pathogenic variant identified despite standard clinical genetic testing).

LQT1, LQT2, and LQT3, caused by mutations in *KCNQ1*, *KCNH2*, and *SCN5A,* respectively, account for over 90 % of genetically confirmed LQTS. Genes encoding β-subunits, membrane scaffolding proteins, or proteins interacting with cardiac ion channels have been implicated in some rarer types of LQTS and have only been identified in a few families or isolated individuals [14, 15].

More than 70 % of cases of LQTS are caused by missense mutations, while the remainder comprises frameshift (10 %) and splice-site mutations and in-frame insertions and deletions [16, 17]. Most reported mutations are exonic (in coding regions of the gene), but noncoding mutations that affect allele expression, and larger genomic rearrangements, have been described [18].

Table 10.1 summarizes the genes known to cause LQTS.

Mechanisms of Arrhythmia in LQTS

Polymorphic ventricular tachycardia (polymorphic VT), or Torsades de Pointes (TdP), is the classical ventricular arrhythmia associated with LQTS. TdP can be self-limiting causing syncope but it can also rapidly deteriorate into ventricular fibrillation (VF) and cardiac arrest.

TdP is typically triggered by a ventricular extrasystole. Accentuated spatial dispersion of repolarization within the ventricular myocardium can lead to a unidirectional

Table 10.1 The genes implicated in LQTS

LQTS subtype	Gene	Locus	Frequency (%)	Affected protein	Physiological role	Predominantly affected current	Functional effect
LQT1	*KCNQ1*	11p15.5-p15.4	40–55	Kv7.1	α-subunit	I_{Ks}	Loss of function
LQT2	*KCNH2*	7q36.1	30–45	Kv11.1	α-subunit	I_{Kr}	Loss of function
LQT3	*SCN5A*	3p22.2	5–10	Nav1.5	α-subunit	I_{Na}	Gain of function
LQT4	*ANK2*	4q25-q26	<1	ANK2	Scaffolding	I_{Na} and others	Loss of function
LQT5	*KCNE1*	21q22.12	<1	MinK	β-subunit	I_{Ks}	Loss of function
LQT6	*KCNE2*	21q22.11	<1	MIRP1	β-subunit	I_{Kr}	Loss of function
LQT7	*KCNJ2*	17q24.3	<1	Kir2.1	α-subunit	I_{K1}	Loss of function
LQT8	*CACNA1C*	12p13.33	<1	CaV1.2	α-subunit	I_{Ca}	Gain of function
LQT9[a]	*CAV3*	3p25.3	<1	Caveolin 3	Scaffolding	I_{Na}	Gain of function?
LQT10	*SCN4B*	11q23.3	<1	Navβ4	β-subunit	I_{Na}	Loss of function
LQT11	*AKAP9*	7q21.2	<1	Yotiao	ChIP	I_{Ks}	Loss of function
LQT12	*SNTA1*	20q11.21	<1	Syntrophin α1	ChIP	I_{Na}	Loss of function
LQT13	*KCNJ5*	11q24.3	<1	Kir3.4	Transmembrane domain	I_{KACh}	Loss of function
LQT14	*CALM1*	14q32.11	<1	Calmodulin	Ca^{2+} binding protein	–	Loss of function
LQT15	*CALM2*	2p21	<1	Calmodulin	Ca^{2+} binding protein	–	Loss of function

[a]Indicates conflicting data [19]

block in conduction. This can set the stage for reentry, which forms the arrhythmic substrate. The ventricular extrasystole initiating the reentry may either be pause-dependent, or it may be triggered in the absence of a preceding pause [20, 21].

Pause-dependent TdP typically occurs in LQT2 but can also occur in LQT3 [21]. It is initiated by "early afterdepolarizations" (EADs) that develop under conditions of prolonged cellular repolarization [22, 23]. Acceleration of an initially slow heart rate or a short-long-short sequence of preceding R-R intervals may then trigger EADs [21, 24]. The prolonged plateau phase of the action potential, together with the enhancement of the sodium-calcium exchange current, allows for the reactivation of the L-type calcium current before repolarization is complete, thereby generating the EAD [22]. In contrast, early animal models suggested that TdP in LQT1 may be triggered by "delayed afterdepolarizations" (DADs) secondary to intracellular calcium overload at higher heart rates, as a result of blockade of the slowly activating delayed rectifier potassium current (I_{Ks}). Data from a canine-wedge preparation also showed that in the presence of I_{Ks} block, β-adrenoceptor stimulation accentuates transmural dispersion of the APD [20]. More recent data from yet another animal model indicate that TdP in LQT1 may be initiated by focal excitations in the right ventricular wall, which may be triggered by either DADs or EADs, and maintained by complex biventricular excitation dynamics [25].

Atrial arrhythmias, particularly atrial fibrillation, are more frequent at younger ages in LQTS compared to the general population. Abnormal atrial repolarization and refractoriness may contribute to the pathogenesis of atrial fibrillation [26–28].

Clinical Presentation

The principal clinical presentations of LQTS constitute arrhythmic events and distinct ECG features, which may be detected incidentally or as a consequence of family screening. Symptomatic individuals may present with syncope (unexplained loss of consciousness without warning or preceding neurological symptoms), documented ventricular

arrhythmias (VT/VF), and/or cardiac arrest. On evaluation, every effort must be made to distinguish true cardiac syncope from other likely etiologies not relevant to a diagnosis of LQTS, although making such a distinction may often be challenging.

Genotype-Phenotype Correlation

The typical phenotypic features of LQTS may vary depending on the genotype.

LQT1 patients have a high risk of developing ventricular arrhythmias with physical exertion (particularly swimming or diving), or emotional stress. The I_{Ks} current is normally activated by adrenergic stress, thereby shortening ventricular repolarization during fast heart rates. Reduced I_{Ks} in LQT1 leads to an abnormal response to adrenergic stimulation with inadequate action potential shortening at higher heart rates. This is manifest as a progressive prolongation of the QTc during exercise and early recovery [21, 24]. The increase in risk with swimming and diving may be related to changes in autonomic function at the onset of activity known as the "dive reflex" [29].

Arrhythmic events in LQT2 are particularly associated with auditory stimuli, particularly sudden loud noises such as alarm clocks. The I_{Kr} current is modulated by α- and β-adrenergic stimulation, leading to the increased incidence of arrhythmias during sudden stress or auditory stimuli [30].

In LQT3, increased late inward sodium current (late I_{Na}) during the plateau phase of the action potential results in prolongation of repolarization that is particularly marked at slow heart rates. The highest risk of arrhythmias in LQT3 patients is during sleep, and their corrected QTc intervals typically shorten with exercise. It has been proposed that as a result of the persistence of the late inward sodium current (I_{Na}) in LQT3, Na^+ accumulates in the cardiomyocyte at faster heart rates, lowering the Na+ gradient and thereby I_{Na}. The effect of this reduction would be most apparent in the plateau phase of the action potential, leading to a shortening of APD at faster heart rates [31]. In addition, the normal I_{Ks} current in LQT2 and LQT3 may provide protection against ventricular arrhythmias during physical exercise [32, 33]. A number of LQT3 mutations may also cause an overlap phenotype with Brugada syndrome and progressive cardiac conduction defect due to accompanying reduced current density and/or peak current [34, 35]. Table 10.2 summarizes some of the unique features of LQT1-3.

Incomplete penetrance is common in LQTS with concealed mutation-positive carriers who have neither clinical symptoms nor ECG features of LQTS [13]. Such genotype-phenotype mismatch may be due to age- and gender-related penetrance, genetic modifiers, or environmental factors. More recently, it has been proposed that differences in autonomic function may also contribute to variation in phenotype between carriers of the same mutation [36].

Table 10.2 Typical features of LQT1, LQT, and LQT3

	LQT1	LQT2	LQT3
Typical circumstances of cardiac events	Physical exertion/ swimming	Sudden loud noise	Sleep
Events in childhood	++	+	Rare
Events <40 years	+++	++	++
Typical T-wave morphology	Broad-based and prolonged	Low amplitude, wide, notched and/or bifid	Long isoelectric segment with late-appearing T-wave
QTc shortening with exercise	Reduced	Normal	Accentuated
Efficacy of beta-blockers	+++	++	++

Natural History

The occurrence of the initial cardiac event appears to be related to age, sex, and the severity of QT prolongation. In particular, male children and female adults are at greater risk. Early data suggested that genotype may also predict risk. For example, early registry data indicated a higher incidence of lethal events in LQT3 despite therapy, especially at the first episode [37]. Cardiac events occur earlier in LQT1 males than in females, and LQT1 patients typically experience cardiac events at a younger age, with the majority experiencing their first event (syncope, resuscitated cardiac arrest or sudden death) by the age of 20 years [33]. Other data, however, have suggested that LQT2 and LQT3 patients have the highest incidence of first cardiac event under the age of 40 years (42–45 %) compared to LQT1 (30 %) [38]. The most recent data from the long QT registry suggest, however, that while the gene affected in an individual may influence the incidence of cardiac events, it plays less of a role in the overall arrhythmic mortality than the above main factors. The exception is the female LQT2 carrier who is at higher risk than other genotypes [39, 40].

The overall 10-year mortality of untreated, symptomatic LQTS patients is estimated at nearly 50 % [41].

Diagnosis

Figure 10.2 summarizes the current Heart Rhythm Society/ European Heart Rhythm Association/Asia Pacific Heart Rhythm Society (HRS/EHRA/APHRS) consensus recommendations for diagnosis of LQTS. European Society of Cardiology (ESC) guidelines that were published in 2015 recommended less stringent clinical diagnostic criteria (LQTS risk score of >3; QTc of ≥480 ms on repeated ECGs,

Expert Consensus Recommendations on LQTS Diagnosis

1. LQTS is diagnosed:
   a) in the presence of an LQTS risk score ≥3.5 (Table 10.3) in the absence of a secondary cause for QT prolongation and/or
   b) in the presence of an unequivocally pathogenic mutation in one of the LQTS genes or
   c) in the presence of a QT interval corrected for heart rate using Bazett's formula (QTc) ≥500 ms in repeated 12-lead electrocardiogram (ECG) and in the absence of a secondary cause for QT prolongation.
2. LQTS can be diagnosed in the presence of a QTc between 480–499 ms in repeated 12-lead ECGs in a patient with unexplained syncope in the absence of a secondary cause for QT prolongation and in the absence of a pathogenic mutation.

Fig. 10.2 Current HRS/EHRA/APHRS expert consensus recommendations on LQTS diagnosis (Reproduced with permission from Priori et al. [13])

Table 10.3 Schwartz LQTS risk score [43]

			Points
Electrocardiographic findings[a]			
A	QTc[b]	≥480 ms	3
		460–479 ms	2
		450–459 ms (male)	1
B	QTc[b] 4th minute of recovery from exercise stress test ≥480 ms		1
C	Torsades de pointes[c]		2
D	T-wave alternans		1
E	Notched T-wave in three leads		1
F	Low heart rate for age[d]		0.5
Clinical history			
A	Syncope	With stress	2
		Without stress	1
B	Congenital deafness		0.5
Family history			
A	Family members with definite LQTS[e]		1
B	Unexplained sudden cardiac death below age 30 among immediate family members[e]		0.5

SCORE: ≤1 point: low probability of LQTS. 1.5–3 points: intermediate probability of LQTS. ≥3.5 points high probability.
[a]In the absence of medications or disorders known to affect these electrocardiographic features.
[b]QTc calculated by Bazett's formula where QTc = QT/$\sqrt{RR}$.
[c]Mutually exclusive.
[d]Resting heart rate below the second percentile for age.
[e]The same family member cannot be counted in A and B.
Reproduced with permission from Schwartz and Ackerman [44].

or QTc of ≥460 ms on repeated ECGs with unexplained syncope) [42]. The level of evidence for these recommendations is Level Ic-IIa. The presence of an unequivocally pathogenic LQTS mutation is sufficient to make the diagnosis according to both guidelines (Table 10.3).

Differential Diagnosis

When making the diagnosis of LQTS, structural heart disease must be excluded using transthoracic echocardiography, and if necessary cardiac magnetic resonance imaging (MRI). Several hereditary and acquired cardiomyopathies can cause intrinsic prolongation of QTc [45–47].

Other conditions that need to be considered, particularly in symptomatic patients with a borderline QTc interval, include vasovagal syncope, catecholaminergic polymorphic VT (CPVT), and epilepsy. In particular, increased ventricular ectopy on exercise in a patient with normal or borderline QTc should raise suspicion of CPVT.

Syncope with seizure activity can be misdiagnosed as epilepsy, while in other cases absence secondary to epilepsy can be misdiagnosed as cardiac syncope. Therefore if unclear, a neurological opinion can be helpful. Other differential diagnoses for a syncopal episode include vasovagal syncope that may be preceded by postural changes, or associated with other physiological circumstances such as micturition, emotion, dehydration, or environmental factors such as external stressors or heat. Taking a thorough history of the symptoms and the circumstances, including collateral history, examination (e.g., looking for postural changes in blood pressure), and undertaking a tilt test if required, can help ascertain if the reported event should be considered as a cardiac event, that is, whether the patient is having symptomatic LQTS.

A careful history should be taken to exclude acquired LQTS secondary to QT-prolonging drugs or electrolyte abnormalities secondary to intercurrent illness, metabolic disorders, or eating disorders. Drugs that cause QT prolongation are numerous including cardiac and noncardiac agents. In approximately 10 % of cases of drug-induced TdP, the congenital LQTS may be uncovered [48, 49]. Electrolyte abnormalities that will cause QTc prolongation include hypokalemia, hypomagnesemia, and hypocalcemia. Other situations causing prolonged QTc to be aware of include hypothermia and hypothyroidism.

Multisystem Disorders

Several multisystem disorders have been described with LQTS. Jervell and Lange-Nielson syndrome is associated with congenital deafness and may be caused by autosomal recessive mutations in *KCNQ1* or *KCNE1*. Affected

individuals may be either homozygotes (same mutation in both alleles) or compound heterozygotes (two different mutated alleles), and usually have a more malignant phenotype [50–52]. Mutations in *KCNJ2* (LQT7) cause Andersen-Tawil syndrome, which is associated with neurological and musculoskeletal abnormalities as well as QT prolongation with marked U-waves. Its main extracardiac features include minor facial abnormalities and periodic hypokalemic paralysis [53, 54]. Mutations in *CACNA1C* (LQT8) are responsible for Timothy syndrome that results in multisystem defects in addition to prolonged cardiac repolarization. These include developmental delay, autism, congenital heart defects, cutaneous syndactyly, and distinctive facial features [55]. Timothy syndrome is extremely rare: fewer than 20 individuals have been reported worldwide, and survival beyond childhood is unusual.

ECG Features of LQTS

Prolongation of the heart rate-QTc interval is the hallmark of LQTS, but the QTc will vary and patients with prolonged QTc can intermittently be within normal limits. Other features to look for on the ECG include T-wave morphology, T-wave alternans, and evidence of sinus node dysfunction.

QT Interval

The QT interval is defined as the time between the onset of the QRS complex and the end of the T-wave. The end of the T-wave has classically been defined as the intersection of a tangent to the steepest slope of the last limb of the T-wave and the baseline, in lead II or V5/V6 [56]. However, this method is not without its shortcomings and it is easy to overestimate or underestimate the slope of the tangent. An alternative method is to use the visual end of the T-wave. With either method, the end of the T-wave can be difficult to determine in the presence of certain T-wave morphologies, or if U-waves are present and merge with the terminal part of the T-wave. Generally if the U-wave is of low amplitude compared to the T-wave, it is unlikely to be part of the T-wave and should not be measured. If both waves are of similar amplitude with a biphasic or notched appearance, then the U-wave can be included in the measurement [57]. The only exception is Anderson-Tawil syndrome where the QT/U--wave is routinely measured [58].

The measured QT interval is then corrected for heart rate: the QTc interval. QTc is most commonly calculated using Bazett's formula (QT interval/$\sqrt{}$(R-R interval)) or occasionally Fredericia's (QT interval/$\sqrt[3]{}$(R-R interval)), Framingham or Hodges formulae [59]. Ideally, the QT and RR intervals are measured on at least three separate beats and the mean values taken to minimize error due to sinus arrhythmia. Atrial fibrillation further complicates the time correction due to rhythm irregularity. Automated QTc measurements can be taken as a guide, but it is important that the QT interval is measured manually by a clinician with suitable expertise. Bazett's formula leads to an underestimation of the QTc at slow heart rates, while at fast heart rates there is under-correction of the QT interval leading to an overestimated QTc.

Furthermore, interventricular conduction delay and bundle-branch block may lead to overestimation of the QTc interval. In left bundle-branch block (LBBB), it has been proposed that a "modified QTc" can be calculated to correct for the QRS prolongation, but this was based on a small study where 60 patients with sinus rhythm and narrow QRS underwent electrophysiological study with ventricular pacing at the right ventricular apex and right ventricular outflow tract to simulate LBBB [60]. It is important that the physician is aware of these considerations when calculating the QTc interval [61–65].

There is considerable overlap of QTc intervals between unaffected individuals and LQTS patients in families and the general population. Many genetically confirmed LQTS patients may have QTc within the normal range due to variable penetrance: 30–40 % of genotype-positive LQT1 patients, approximately 20 % of LQT2 patients, and 10 % of LQT3 patients do not have overt QT prolongation on the baseline ECG [38]. As a result, QTc on the surface ECG alone is not sufficient to diagnose LQTS in the vast majority of patients. An exception is if QTc is <400 ms, which has a negative-predictive value of nearly 100 %, and QTc >480 ms has a high positive-predictive value. In primary care, LQTS should be suspected if QTc is >460 ms in a child under the age of 15 years, QTc >450 ms in an adult male, or if QTc >470 ms in an adult female, and the patient should be referred for further evaluation [66].

T-Wave Morphology

In addition to QTc prolongation, there are changes in T-wave morphology in LQTS, with some morphologies being characteristically associated with mutations in certain genes. LQT1 (*KCNQ1*) is characterized by broad-based, often peaked T-waves; LQT2 (*KCNH2*) by low amplitude, sometimes notched, T-waves, and LQT3 (*SCN5A*) by late-onset T-waves of normal duration and amplitude with a long isoelectric ST segment [67]. These characteristic morphologies, however, may not always be present and can even overlap [68]. Examples of LQT1, LQT2, and LQT3 ECGs are shown in Fig. 10.3

T-Wave Alternans

T-wave alternans is a beat-to-beat variation in the T-wave morphology and ST segment secondary to regional heterogeneity of repolarization. Macroscopic T-wave alternans is one of the diagnostic criteria for LQTS and is a marker of high cardiac electrical instability. T-wave alternans may be present at rest, but more commonly appears during periods of emotional stress or physical exertion [69]. It is associated with a propensity to life-threatening ventricular arrhythmias and high risk of cardiac events as it can precede

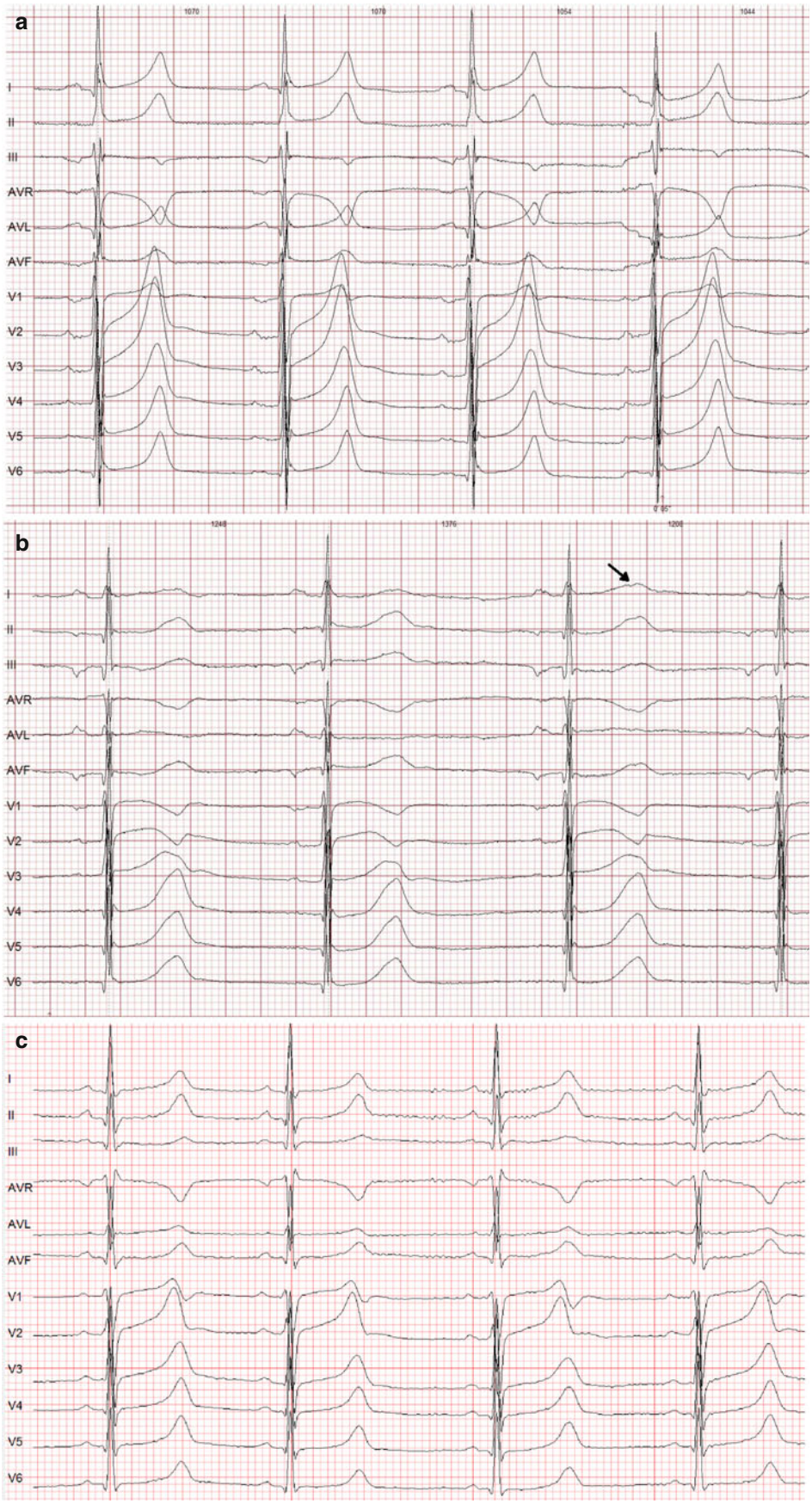

Fig. 10.3 Typical LQTS ECGs. (**a**) LQT1 (**b**) LQT2 (the arrow points to a notched T-wave) (**c**) LQT3. The authors gratefully acknowledge Velislav N Batchvarov for the assistance in preparing this figure

TdP [70]. More recently, microvolt T-wave alternans, calculated from 24-h continuous 12-lead ECG recordings, was shown to be prevalent in LQTS patients and a marker of arrhythmia risk [71].

Sinus Node Dysfunction

Long sinus pauses or inappropriate sinus bradycardia indicative of sinus node dysfunction may be seen in patients with LQT3 [34, 72]. Incomplete penetrance of sinus node dysfunction in LQT3 may be manifest as exaggerated sinus arrhythmia, heart rates slower than would be expected for age in infants and children, or inappropriate sinus bradycardia.

Clinical Evaluation

The standard evaluation includes a thorough clinical history, ECG, Echo, and 12-lead Holter, ideally by a cardiologist with expertise. Provocative tests for QT prolongation such as QT measurement during change from a supine to standing position, exercise testing, or during an epinephrine infusion have been proposed, but the clinical validity of these tests is yet to be fully determined [13]. The recovery phase of exercise does appears to offer the best additional value and has been included in the LQTS risk score [43, 44, 73, 74]. The exercise test thus should be a standard part of clinical evaluation.

LQTS can be overdiagnosed by physicians with less experience in the condition. In one study, diagnostic concordance was present in less than one third of patients diagnosed with LQTS and subsequently referred to a tertiary center for second opinion [57]. Comprehensive evaluation by a cardiologist with expertise in electrophysiology and inherited cardiac conditions working in close association with a clinical geneticist and genetic counselor is recommended. Such a setting would provide the optimal facilities for accurate diagnosis and holistic management of patients suspected to have LQTS, as well as their families.

Clinical History and Examination

A thorough history of relevant symptoms must be taken, with emphasis on distinguishing the aetiology of any syncopal episode by eliciting details of any unexplained collapses or loss of consciousness at all ages. Personal or family history of unexplained road traffic accidents or drowning incidents should raise suspicion of syncope. Furthermore, note should be made of any family history of epilepsy or sudden infant death. If there have been any sudden deaths in the family, every effort must be taken to (1) find out the circumstances of the death, (2) obtain any antemortem ECGs, (3) the postmortem report, and (4) find out if a specialist cardiac autopsy was carried out to confirm if it was a SADS death [75]. A physical examination should be carried out to look for any features described in section, "Natural History".

Electrocardiogram

The baseline 12-lead ECG must be carefully evaluated as described in section ECG Features of LQTS, and a 12-lead ECG should be repeated at each follow-up appointment for dynamic evaluation.

Cardiac Imaging

Cardiac structural abnormalities are excluded using transthoracic echocardiography, and if there is any doubt, cardiac MRI.

Holter

Ambulatory ECG, or 12-lead Holter, monitoring over a 24- or 48-hour period can provide information on QT intervals over a 24- or 48-hour period. However, there are insufficient data on the normal range for maximum QTc on a Holter and QTc intervals detected on Holter alone are insufficient to diagnose LQTS. Supportive diagnostic features on Holter monitoring include T-wave morphology, as well as features of electrical instability such as T-wave alternans that can indicate higher risk [76, 77]. Holter recordings have shown that LQT1 patients have more frequent QTc prolongation during the day compared to nighttime, but this variation is much less marked in LQT2 [78]. In LQT3, the QT prolongation is often more pronounced at nighttime [79].

Lying/Standing ECG

Patients with LQTS tend to have inadequate shortening of QTc in response to the sudden heart rate accelerations provoked by standing, resulting in QTc prolongation that can persist even after the heart rate returns to baseline. This test can be carried out conveniently using continuous ECG recordings to measure the QTc in the supine position, on standing quickly, and for 5 min afterward. The normal values for this test have, however, not been agreed and its diagnostic value is still unclear [80–82].

Exercise ECG

Exercise testing can be useful to measure exercise-induced QT prolongation during exercise and in early recovery, particularly in LQT1 patients who will have a relative QTc prolongation with exercise. The QTc prolongation would be most marked in early recovery and measurement of QTc in the 4th min of recovery can be used for the diagnosis of LQTS among asymptomatic relatives of affected individuals [43, 73, 74].

LQT1 is also associated with a diminished chronotropic response to exercise. In LQT2, there is normal QT shortening with exercise, and a normal chronotropic response. Conversely, in LQT3 QT shortening with exercise is often

exaggerated [31]. Exercise-induced ventricular ectopy is uncommon in LQTS and should raise the suspicion of CPVT, particularly if there is exercise-induced bigeminy [83].

Epinephrine Challenge

This provocation test can be useful to unmask QTc prolongation in concealed LQT1 [84]. The characteristic T-wave morphology in LQT2 (notched T-wave) may also appear with epinephrine challenge, potentially unmasking concealed LQT2 [85]. There are two widely used protocols for this challenge [86, 87]. Induction of TdP or VF is unusual. The utility of epinephrine challenge in routine clinical practice is debatable, however, as noncarriers can also show QTc prolongation, and in our experience false positives outside of familial testing are common [88].

Risk Stratification

A critical aspect of management in LQTS is the prevention of sudden death. Accurate risk stratification can be challenging; however, there are certain clinical and genetic markers that can facilitate risk assessment.

Age and Gender

In LQT1, male gender is independently associated with a higher risk of cardiac events before the age of 15 years, but beyond this age, gender risk reversal has been observed with adult females being at a higher risk. In LQT2 too, females aged 16 years and older are at a higher risk of cardiac events than males [89, 90].

LQT1 males who are asymptomatic at a young age are at low risk of becoming symptomatic later in life, whereas females, especially those with LQT2, remain at risk of having their first cardiac event even after the age of 40 years. In females aged 40–60 years, the LQTS-related risk of sudden death remains higher than in unaffected females but the LQTS-related risk in males of this age group is lower.

The exact mechanisms of gender differences are unclear, but may be due to environmental or hormonal factors [13, 39, 91, 92]. Recent data suggest that estradiol plays an important role in I_{Kr} trafficking [93].

Physiological States

Effects of exercise, emotion, and rest in different LQTS genotypes are described in section, "Genotype-Phenotype Correlation"

Pregnancy also appears to affect the risk of cardiac events in LQTS although data are limited. The risk of arrhythmias is reduced during pregnancy, possibly secondary to hormonal factors. However, the risk is increased 10–20 % during the postpartum period in LQT2 patients and is most marked in the first 9 months postpartum, with approximately one in ten female LQT2 probands experiencing their first cardiac event in the postpartum period. Apart from hormonal factors, environmental factors including sleep deprivation, emotional stress, and noise (crying of the baby) may contribute to risk [94–96]. Close cardiac follow-up and adherence to medical therapy in the postpartum period is important, particularly if there is evidence of QTc prolongation compared to prepregnancy, or if QTc is >500 ms.

Family History

Early data indicated that the severity of a proband's presentation did not influence risk in first-degree relatives [97]. A family history of multiple cardiac arrests can be indicative of higher risk; however, sudden death in one sibling does not predict the risk of cardiac arrest [98]. In view of these data, sudden death in one first-degree relative is not, by itself, an indication for implantable cardioverter defibrillator (ICD) implantation in a surviving affected family member unless there are other features of risk [13].

Symptoms

Data from the International LQTS registry indicate that a history of syncope over time is a powerful predictor of risk with different implications depending on age. The risk of recurrent cardiac events is higher in children with syncope or a cardiac arrest before the age of 7 years, even if they are on appropriate medical therapy. Furthermore, infants who have had a cardiac event before the age of 1 year are at especially high risk of further lethal arrhythmias [99]. Adolescents who have had one syncopal event over the preceding 24 months have a 12-fold increased risk of recurrence compared to asymptomatic teenagers with the condition. Two or more syncopal events over the preceding 24 months are associated with an 18-fold increased risk. If the event is more remote, then the increase in risk is threefold. In adults, a previous syncopal event before the age of 18 years is not associated with increased risk, provided there have not been any further events. Syncope after the age of 18 years is then associated with a fivefold increase in risk [39, 89]. Patients who have recurrent cardiac events despite preventative lifestyle measures and appropriate medical therapy are at increased risk regardless of age [13].

QTc Interval

There is often marked variability in the QTc interval when serial ECGs are measured over time. It has been suggested that the maximum QTc measured on follow-up ECGs at any one time provides incremental prognostic information beyond the baseline measurement [100]. However, this needs to be further validated. Nonetheless, the more prolonged a resting QTc interval is, the greater the arrhythmic risk is. Patients exceeding a QTc of 500 ms have a 3.3-fold increased risk of cardiac events, and the risk increases to 6.3-fold when

QTc is >550 ms [39]. In adolescents, QTc >530 ms is associated with a particular increase in risk with an adjusted hazard ratio of 2.3 [89]. Concealed mutation-positive carriers who are asymptomatic and have normal QTc intervals have a relatively low risk of arrhythmic events although this is still greater than noncarriers [13, 101].

Genotype

Certain subtypes of LQTS, such as the Jervell and Lange-Nielson syndrome (homozygous or compound heterozygous LQT1 and LQT5), and the Timothy syndrome (LQT8), have a high risk of malignant ventricular arrhythmias at an early age and have a poor prognosis. Other compound and multiple mutation carriers also seem to be at an elevated risk [51, 102, 103]. Initial evidence had suggested that LQT2 and LQT3 are associated with a higher risk of sudden death than LQT1 [38]. Subsequent larger studies have indicated, however, that genotype locus is of less utility other than LQT2 females being of moderately higher risk [39, 40].

Studies have since focused on the biophysical consequences of specific mutations. In LQT1, mutations in the pore region of the potassium channel Kv7.1 are associated with a higher frequency of cardiac events, as opposed to mutations in the C-terminal region that are associated with a milder phenotype [17]. Furthermore, missense and dominant-negative mutations are also associated with an increase in risk. The dominant-negative *KCNQ1 A341V* mutation causes a particularly severe phenotype [13, 104]. In LQT2, mutations in the pore-forming region are associated with a more severe phenotype [105]. In addition, evidence is growing that additional genetic variants can modify the clinical phenotype of known pathogenic mutations [106–109]. Common variants known to modulate the QT interval in the general population have been associated with longer QT intervals and clinical severity [110, 111]. These newer genetic risk stratifiers have not been incorporated into clinical practice as yet, pending further confirmation in larger populations with longitudinal data.

Management

There is a paucity of randomized controlled trials on the management of LQTS owing to the low prevalence of the condition and variable penetrance. Current guidelines are based on data from large registries and tertiary center experience.

Determination of the appropriate management strategy is dependent on the risk assessment – patients with a higher lifetime risk of life-threatening arrhythmic events should be managed more aggressively than those who are considered to be at a lower risk. Figure 10.4 summarizes the current consensus guidelines on the management of LQTS.

Lifestyle

Patients should be advised of lifestyle changes that can minimize risk. LQT2 patients can minimize exposure to arrhythmogenic triggers such as abrupt loud noises by removing telephones and clocks from their bedrooms [33]. Prompt identification, prevention, and treatment of conditions associated with electrolyte disturbances and hypokalemia (e.g., diarrheal illness) can mitigate arrhythmic risk. Patient education and good communication with other medical professionals involved in their care (primary-care physicians as well as other specialists) are critical.

Drugs to Avoid

Patients and all health-care professionals involved in their care need to be aware of drugs to avoid in LQTS. These include antiarrhythmic agents such as amiodarone, disopyramide, and sotalol, antimicrobials such as erythromycin and ciprofloxacin, and psychotropic drugs. A complete list of drugs that prolong the QTc interval is beyond the scope of this chapter and is available on crediblemeds.org [49].

Competitive Sports

The 2005 and 2008 Bethesda Conference guidelines and the ESC consensus recommendations first addressed the risk of sudden cardiac death during sport for athletes with LQTS. The Bethesda guidelines recommended that regardless of QTc or underlying genotype, all competitive sport should be restricted in LQTS patients who have previously experienced a cardiac event that could have been attributed to LQTS. Furthermore, asymptomatic patients with baseline QTc prolongation were advised to restrict low-intensity sport. If they were an LQT3 carrier, they were then allowed to participate in more intensive sport. Asymptomatic patients with concealed LQTS (i.e., genotype-positive/phenotype-negative) were allowed to participate in competitive sport, with the exception of competitive swimming for concealed *KCNQ1* mutation carriers (LQT1). Patients with an ICD or permanent pacemaker were advised to avoid contact sport to prevent traumatic damage to the implanted device system [113].

The ESC guidelines were much more restrictive and recommended that anyone with a definitive diagnosis of LQTS after comprehensive evaluation should be restricted from all competitive sport, regardless of the presence/absence of any arrhythmic events. The recommended QTc cutoff values for using as a trigger for further evaluation were also much lower in the 2005 ESC guidelines [114, 115].

Expert Consensus Recommendations on management of LQTS

Class I Recommendations:

1. The following lifestyle changes are recommended in all patients with a diagnosis of LQTS:
   a) Avoidance of QT-prolonging drugs (www.crediblemeds.org/new-drug-list/)
   b) Identification and prompt correction of dehydration/electrolyte abnormalities (hypokalaemia, hypomagnesaemia, hypocalcaemia) that may occur during diarrhoea, vomiting, metabolic conditions, imbalanced diets for weight loss or eating disorders.
   c) Avoidance and prompt treatment of hyperthermia, including training-related heat exhaustion in athletes
   d) Avoidance of genotype-specific triggers for arrhythmias (strenuous swimming, especially in LQT1, and exposure to loud noises in LQT2 patients).
2. Beta-blockers are recommended for patients with a clinical diagnosis of LQTS
3. ICD implantation with the use of beta blockers is recommended for patients with a diagnosis of LQTS who are survivors of a cardiac arrest.
4. Left cardiac sympathetic denervation (LCSD) is recommended for high-risk patients with a diagnosis of LQTS in whom:
   a) Implantable cardioverter defibrillator (ICD) therapy is contraindicated or refused and/or
   b) Beta-blockers are either not effective in preventing syncope/arrhythmias, not tolerated, not accepted or contraindicated.
5. All LQTS patients who wish to engage in competitive sports should be referred to a clinical expert for evaluation of risk.

Class IIa Recommendations:

6. Beta-blockers should be considered in carriers of a causative LQTS mutation and normal QT interval
7. ICD implantation in addition to beta-blockers should be considered in patients with a diagnosis of LQTS who experience recurrent syncope and/or VT while on adequate beta-blocker therapy.
8. LCSD can be useful in patients with a diagnosis of LQTS who experience breakthrough events while on therapy with beta-blockers/ICD.

Class IIb recommendations

9. Sodium channel blockers can be useful, as add-on therapy, for LQT3 patients with a QTc >500 ms who shorten their QTc by >40 ms following an acute oral drug test with one of these compounds.
10. ICD implantation may be considered in addition to beta-blocker therapy in asymptomatic carriers of a pathogenic mutation in KCNH2 or SCN5A

Fig. 10.4 Amalgamation of current expert consensus guidelines on the management of LQTS (Adapted from Priori et al. 2013 and Priori et al. 2015 [13, 42, 112])

In 2012, a single-center retrospective study of over 350 athletes who chose to continue competitive sport against guideline recommendations demonstrated a low rate of LQTS-triggered cardiac events during sport. The mean follow-up was 5 years (650 athlete-years) and the overall rate of events per athlete-year in this study was 0.003 (1 event in 331 athlete-years). Limitations of this study, apart from being a retrospective analysis, include the short follow-up period and it is unclear how generalizable the results are [116, 117].

The most recent American Heart Association/American College of Cardiology (AHA/ACC) guidelines are driven by these recent data and concur with the 2013 HRS/EHRA/APHRS guidelines. Both recommend that all athletes with suspected LQTS who wish to pursue competitive sports must be referred for comprehensive evaluation by an electrophysiologist or a cardiologist with expertise in managing LQTS patients. The AHA/ACC guidelines go further, however, in liberalizing the approach to managing any patient with suspected or diagnosed with LQTS. Initially, these athletes should be advised to abstain from all competitive sport until

1. They have had appropriate comprehensive evaluation of their risk by a suitable expert.
2. The patient and family have received appropriate counseling and education on minimizing risk.
3. The patient has been asymptomatic on therapy for at least 3 months [112].

Unlike earlier guidelines, it is now recommended that asymptomatic patients with concealed LQTS (i.e., genotype-positive/phenotype-negative) may participate in competitive sport once they have had comprehensive cardiac evaluation, as long as they follow appropriate precautionary measures (Fig. 10.1: Lifestyle changes). Facilities that need to be in place before such participation include (1) availability of an

automatic external defibrillator at the sports venue and (2) establishment of an emergency action plan with the patient's school/club/team officials [112]. Asymptomatic patients with borderline QTc prolongation but no other high-risk features may also participate in competitive sport after thorough expert evaluation if appropriate precautionary measures as described above are in place [13]. There is clearly conflict with current European guidelines and a global consensus is still required.

Drug Therapies

It is recommended that all affected patients with LQTS are treated with beta-blockers unless there is a contraindication to beta-blocker use. Mutation carriers with concealed LQTS should be offered beta-blocker therapy although evidence of efficacy is limited. Beta-blockers are particularly effective in LQT1, and provided that compliance is good, can be sufficient to provide adequate protection against arrhythmic risk in LQT1 [118, 119]. Beta-blockers are thought to act by reducing intracellular calcium overload, thereby minimizing the arrhythmogenic substrate for reentry.

Long-acting nonselective drugs such as nadolol or sustained-release propranolol are preferred for ease of administration (once or twice daily) and to avoid wide fluctuations in serum drug concentrations. Beta-blockers that could potentially increase sinus node recovery time and prolong sinus cycle length, such as bisoprolol or metoprolol, are less preferred, particularly in LQT3 patients who have evidence of sinus node dysfunction [120]. It is recommended that patients are maintained on the maximum tolerated dose for age and weight. Abrupt discontinuation of beta-blockers must be avoided if at all possible due to upregulation of beta receptors while on treatment. In principle, beta-blockers should be continued during and after pregnancy [13]. While beta-blockers may cause intrauterine growth restriction, neonatal hypoglycemia, and bradycardia, the decision to continue treatment during pregnancy must take into account arrhythmic risk. Detailed discussion with the patient is essential but the general experience has been reassuring especially if the fetus and neonate are closely monitored for side effects of beta-blockade.

The sodium-channel blocker mexiletine reduces the late I_{Na} in LQT3 and can be particularly effective to reduce the QTc interval and arrhythmic risk in some LQT3 mutations [121–123]. The late I_{Na} blocker ranolazine has also been shown to be useful in LQT3, but at present its therapeutic role is less certain [124, 125].

Patient-specific models of disease in the form of induced pluripotent stem cell-derived cardiomyocytes raise the possibility of tailoring therapies and novel agents to suit the phenotype [126, 127].

Device Therapies

The ICD implantation is appropriate for selected patients who are at a high risk of sudden cardiac death. ICD implantation is, however, an invasive treatment strategy and the lifetime risk of complications is not insignificant, especially when implanted in younger patients. Careful consideration of benefits versus risks and appropriate counseling, taking into account overall risk and patient preference, are essential. TdP is often self-limiting but an ICD discharge in a conscious patient may trigger further arrhythmias due to the adrenergic surge, leading to an "electrical storm" (recurrent episodes of VT/VF in a 24-h period). Programming should therefore be directed at minimizing the chances of shocks to conscious patients by extending time to therapy as well as avoiding inappropriate shocks. These can otherwise leave patients with devastating psychological consequences [13, 128, 129].

ICD implantation is indicated for secondary prevention in any LQTS patient who has been resuscitated from a cardiac arrest. Very high-risk patients, such as those carrying two or more LQTS mutations (compound heterozygotes) or homozygotes, including those with Jervell and Lange-Nielson syndrome, should be considered for prophylactic ICD implantation at an early age. ICD implantation should also be strongly considered in any LQTS patient with cardiac syncope while on appropriate beta-blocker therapy, but there should be a very high threshold to implant ICD in an asymptomatic patient [13].

The addition of an atrial lead to an ICD system provides several potential benefits. In LQT2, where the onset of TdP is preceded by a pause, a pacemaker with appropriate pause-preventing algorithms can prevent the onset of TdP and minimize the need for ICD therapy. Dual-chamber devices can provide atrial pacing to LQT3 patients with sinus node dysfunction. If a pacemaker is required LQTS, implanting an ICD with appropriate pacing modes may be the most appropriate strategy to prevent the need to have a further procedure if a device upgrade is needed in the future. This needs to be balanced with the potential for a higher risk of defibrillator lead failure. Beta-blocker therapy should be continued following device implantation [13, 129].

Surgical Options

Left cardiac sympathetic denervation (LCSD) involves the removal of the first four thoracic ganglia and has a significant antiarrhythmic effect in patients with LQTS [130]. The precise mechanism of the therapeutic effect is not fully understood. Some patients may have paradoxically increased QTc post-LCSD, but these effects are often transient and not associated with risk of arrhythmia [131, 132]. The procedure may be undertaken through a thoracotomy, but is increasingly performed as a video-assisted minimally invasive procedure.

LCSD is useful in reducing arrhythmic risk in patients who are intolerant of beta-blockers or if they have refractory symptoms on beta-blockers. This procedure can also be useful in high-risk infants or children where it would be desirable to avoid ICD implantation for as long as possible due to the physical size of the patient. The most common complication is Horner's syndrome.

Follow-Up

The frequency of follow-up should be guided by the initial risk assessment and age. High-risk patients need to be followed up closely, while low-risk patients can be followed up less frequently, for example, every other year. Children should be followed up more closely during development and puberty, especially girls, as there is age-dependent evolution of risk and beta-blocker therapy will need to be up-titrated with increasing weight. Changes in genetic technology and family findings may indicate the need for review of relatives as required.

Molecular Diagnostics

Clinical assessment by a cardiologist with expertise in assessing the likelihood of LQTS, and genetic counseling are essential prerequisites to genetic testing. Implications of genetic testing for the patient as well as the family, and the option of not testing, must be discussed. Other issues that should be covered during genetic counseling include implications on lifestyle, job prospects, and health insurance. The possibility of finding unclassified variants or variants of uncertain significance, and the resultant psychological consequences need to be discussed. The patient should understand that a negative genetic test does not rule out the diagnosis.

Genetic testing can be in the form of either cascade screening of a single-known pathogenic variant that was found in the proband, or a targeted gene panel to investigate the proband. This may consist of a limited panel focusing upon the most well-understood genes accounting for LQT1-3 and LQT5-6 or an all-inclusive panel covering all known associated genes (see below) enabled by the increased availability of next-generation sequencing (NGS). While this has resulted in more detailed genetic testing over a short timescale, it has also led to the discovery of many novel variants that are "private" to that family meaning that they have not been identified elsewhere and therefore their diagnostic value may be limited. These are then labeled as "unclassified variants" or "variants of unknown significance" until pathogenicity can be established (see Section Unclassified Variants). "Founder" mutations have also been identified that are specific to distinct populations where a mutation has occurred in a single ancestor and has survived to be passed down to subsequent generations clustered in a geographical region [35, 110, 133].

Whole-genome sequencing (WGS) is becoming faster, cheaper, and more accessible and may eventually replace other NGS methods such as whole-exome sequencing (WES) due to the greater range of analyses possible. The extent of genetic testing to be carried out, however, should only be decided after thorough discussion with the patient and appropriate genetic counseling. An additional point of consideration is the opportunity to test for appropriately selected non-LQTS genes. This can even lead to the correct diagnosis in selected cases. For example, in one study, over 5 % of patients with negative LQTS genetic testing who were tested for RyR2 mutations were found to be mutation positive and diagnosed subsequently with CPVT [134].

Unclassified Variants

Establishing the pathogenicity of variants is crucial as genetic screening has become an important tool in family screening. Identification of a variant of uncertain significance (VUS) leads to diagnostic uncertainty with potential adverse psychological and economic consequences to patients and families. Many studies implicating genetic variants with pathogenicity have relied on small control samples data without necessarily showing genotype-phenotype cosegregation [135, 136]. A study of the exome variant server in 2012, however, identified a much higher prevalence of previously LQTS-associated variants than expected in exome data from population studies, suggesting that caution must be exercised when interpreting the pathogenicity of variants in individuals and families [137]. In a further Danish population study, 33 genetic variants that were previously reported in LQTS patients were identified in 243 individuals from a sample of 7000 individuals from the general population. Ten variants were identified in eight or more individuals included in this study. There were no associations with QTc prolongation, history of syncope, or mortality. Interestingly, the authors report that half of the identified variants also had functional studies supporting pathogenicity. The 33 variants called into question in this study only comprise <2.5 % of all variants previously associated with LQTS; however, these studies raise important questions about the true pathogenicity of some variants previously thought to be disease-causing [135].

The classification of LQT9 thought to be caused by mutations in CAV3 has been called into question. Mutations in CAV3 causing LQTS are rare, and one of the variants that was identified in 2006 that led to the discovery of LQT9, CAV3 p.T78M, did not associate with either prolonged QTc

intervals or abnormal T-wave morphology in a subsequent study [19, 138]. Incidentally, the variant in question was also the most frequently identified variant in the Danish population study described above [135].

Thus, given the potential uncertainties that can arise with genetic testing, it is essential that these services are provided in expert centers where cardiologists with expertise in inherited heart disease, genetic counselors, and clinical geneticists, preferably with cardiac expertise, work closely as a team. Where an unclassified variant is found, it will not add to the diagnosis. Further analysis of the variant must be made by testing more family members with and without the phenotype to establish linkage. If further resources are available, in vitro studies can help understand the functional significance of a particular variant and add to the knowledge base.

Genetic Modifiers

Genetic loci, tagged by single nucleotide polymorphisms (SNPs), both in LQTS-associated genes and in other genes, have been shown to be associated with QTc in the general population [139–142]. These are thought to influence variable penetrance and incomplete expression in some pedigrees. For example, variants in the NOS1AP gene in chromosome 1 have been shown to be associated with an increased risk of sudden death in a South African population harboring a founder mutation in *KCNQ1* [110]. More recently, NOS1AP SNPs were shown to be strongly associated with QTc interval in patients with LQT2. There was also a trend for effect on the risk of cardiac events in these patients. Furthermore, common genetic variation at *KCNQ1* was shown to be associated with the risk of LQTS [143]. At this time, these markers have not entered into routine clinical guidelines.

Family Screening

Genetic Screening

Identification of a pathogenic mutation in the index patient (proband) enables cascade genetic screening of other family members, starting with first-degree relatives. Cascade genetic screening enables the identification of affected family members with a single test, including very young children. Thereby, asymptomatic mutation carriers can be easily identified and preventative measures can be started in a timely manner. Unaffected family members can be reassured and safely discharged from further follow-up. However, this is only possible when a clear pathogenic variant has been identified.

Clinical Screening

Upon making the diagnosis of LQTS in a proband, immediate steps must be taken to arrange clinical screening of the family. This should ideally be undertaken alongside genetic screening if a pathogenic mutation has been identified in the proband. The assessment should be carried out by, or at least discussed with, an appropriately experienced specialist in inherited cardiac conditions. There should be systematic evaluation of first-degree blood relatives, with a focus on symptomatic relatives and obligate carriers. A focused but thorough medical history should be obtained from each relative and a physical examination performed. Resting 12-lead and exercise ECGs are a fundamental part of screening. Following this, a transthoracic echocardiogram may be performed to confirm a structurally normal heart [144]. If any suspicions of structural abnormalities are raised on echocardiography, cardiac MRI could be considered. If, after the above, there is no suspicion of LQTS (including negative genetic test), the individual can be safely discharged from further follow-up. If, however, clinical screening is negative but it is not possible to carry out cascade genetic screening due to the absence of a clearly pathogenic mutation in the proband, reassessment for age-related penetrance at periodic intervals is important. This is particularly relevant to children.

A multidisciplinary approach involving a cardiologist with expertise in LQTS, a pediatric cardiologist for managing the screening of children, a clinical geneticist, a genetic counselor, together with facilities for appropriate psychosocial support, is critical for the holistic management of the affected patient and their family.

Summary

The congenital LQTS is the first described arrhythmia syndrome and its understanding has evolved remarkably as genetic technology has developed. Its diagnosis and management can be challenging and expert evaluation is recommended if the diagnosis is suspected. Nonetheless, guidelines for diagnosis, risk stratification, and the prevention of sudden death are increasingly well defined and genetic information is playing an ever more important role. Expert physicians are progressively more comfortable with their patients undertaking normal lifestyle activities including sports. While ICD therapy is a crucial component of management, improved utilization of beta-blockers, LSCD, and unconventional medications can minimize its usage. The future promises the potential for mutation-specific and genetic modifier-guided risk assessment. Patient-specific models of disease in the form of induced pluripotent stem cell-derived cardiomyocytes raise the possibility of tailoring therapies

and novel agents to suit the phenotype. Of all the cardiac genetic disorders, the era of personalized medicine for LQTS patients is closest to hand.

Take Home Messages

- LQT1, LQT2, and LQT3, caused by mutations in *KCNQ1*, *KCNH2*, and *SCN5A*, account for over 90 % of genetically confirmed LQTS.
- Diagnosis of LQTS can be challenging – refer for expert evaluation if the diagnosis is suspected.
- The hallmark of management constitutes accurate risk assessment, prevention of cardiac events, and timely screening of family members at risk.

References

1. Jervell A, Lange-Nielsen F. Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death. Am Heart J. 1957;54(1):59–68.
2. Romano C, Gemme G, Pongiglione R. Rare cardiac arrhythmias of the pediatric age. II. Syncopal attacks due to paroxysmal ventricular fibrillation (presentation of 1st case in Italian Pediatric literature). Clin Pediatr (Bologna). 1963;45:656–83.
3. Ward OC. A new familial cardiac syndrome in children. J Ir Med Assoc. 1964;54:103–6.
4. Keating M, Atkinson D, Dunn C, Timothy K, Vincent GM, Leppert M. Linkage of a cardiac arrhythmia, the long QT syndrome, and the Harvey ras-1 gene. Science. 1991;252(5006):704–6.
5. Jiang C, Atkinson D, Towbin JA, et al. Two long QT syndrome loci map to chromosomes 3 and 7 with evidence for further heterogeneity. Nat Genet. 1994;8(2):141–7. doi:10.1038/ng1094-141.
6. Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell. 1995;80(5):795–803.
7. Wang Q, Shen J, Splawski I, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell. 1995;80(5):805–11.
8. Wang Q, Curran ME, Splawski I, et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat Genet. 1996;12(1):17–23. doi:10.1038/ng0196-17.
9. Schwartz PJ, Stramba-Badiale M, Crotti L, et al. Prevalence of the congenital long-QT syndrome. Circulation. 2009;120(18):1761–7. doi:10.1161/CIRCULATIONAHA.109.863209.
10. Tester DJ, Ackerman MJ. Postmortem long QT syndrome genetic testing for sudden unexplained death in the young. J Am Coll Cardiol. 2007;49(2):240–6. doi:10.1016/j.jacc.2006.10.010.
11. Behr ER, Dalageorgou C, Christiansen M, et al. Sudden arrhythmic death syndrome: familial evaluation identifies inheritable heart disease in the majority of families. Eur Heart J. 2008;29(13):1670–80. doi:10.1093/eurheartj/ehn219.
12. Papadakis M, Raju H, Behr ER, et al. Sudden cardiac death with autopsy findings of uncertain significance: potential for erroneous interpretation. Circ Arrhythm Electrophysiol. 2013;6(3):588–96. doi:10.1161/CIRCEP.113.000111.
13. Priori SG, Wilde AA, Horie M, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. Heart Rhythm. 2013;10(12):1932–63. doi:10.1016/j.hrthm.2013.05.014.
14. Wilde AAM, Behr ER. Genetic testing for inherited cardiac disease. Nat Rev Cardiol. 2013;10(10):571–83. doi:10.1038/nrcardio.2013.108.
15. Ackerman MJ, Priori SG, Willems S, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Heart Rhythm. 2011;8(8):1308–39. doi:10.1016/j.hrthm.2011.05.020.
16. Splawski I, Shen J, Timothy KW, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation. 2000;102(10):1178–85.
17. Moss AJ, Shimizu W, Wilde AAM, et al. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. Circulation. 2007;115(19):2481–9. doi:10.1161/CIRCULATIONAHA.106.665406.
18. Tester DJ, Benton AJ, Train L, Deal B, Baudhuin LM, Ackerman MJ. Prevalence and spectrum of large deletions or duplications in the major long QT syndrome-susceptibility genes and implications for long QT syndrome genetic testing. Am J Cardiol. 2010;106(8):1124–8. doi:10.1016/j.amjcard.2010.06.022.
19. Hedley PL, Kanters JK, Dembic M, et al. The role of CAV3 in long-QT syndrome: clinical and functional assessment of a caveolin-3/Kv11.1 double heterozygote versus caveolin-3 single heterozygote. Circ Cardiovasc Genet. 2013;6(5):452–61. doi:10.1161/CIRCGENETICS.113.000137.
20. Burashnikov A, Antzelevitch C. Block of I(Ks) does not induce early afterdepolarization activity but promotes beta-adrenergic agonist-induced delayed afterdepolarization activity. J Cardiovasc Electrophysiol. 2000;11(4):458–65.
21. Tan HL, Bardai A, Shimizu W, et al. Genotype-specific onset of arrhythmias in congenital long-QT syndrome: possible therapy implications. Circulation. 2006;114(20):2096–103. doi:10.1161/CIRCULATIONAHA.106.642694.
22. Viswanathan PC, Rudy Y. Pause induced early afterdepolarizations in the long QT syndrome: a simulation study. Cardiovasc Res. 1999;42(2):530–42.
23. Antzelevitch C. M cells in the human heart. Circ Res. 2010;106(5):815–7. doi:10.1161/CIRCRESAHA.109.216226.
24. Burashnikov A, Antzelevitch C. Acceleration-induced action potential prolongation and early afterdepolarizations. J Cardiovasc Electrophysiol. 1998;9(9):934–48.
25. Kim TY, Kunitomo Y, Pfeiffer Z, et al. Complex excitation dynamics underlie polymorphic ventricular tachycardia in a transgenic rabbit model of long QT syndrome type 1. Heart Rhythm. 2015;12(1):220–8. doi:10.1016/j.hrthm.2014.10.003.
26. Johnson JN, Tester DJ, Perry J, Salisbury BA, Reed CR, Ackerman MJ. Prevalence of early-onset atrial fibrillation in congenital long QT syndrome. Heart Rhythm. 2008;5(5):704–9. doi:10.1016/j.hrthm.2008.02.007.
27. Zellerhoff S, Pistulli R, Mönnig G, et al. Atrial Arrhythmias in long-QT syndrome under daily life conditions: a nested case control study. J Cardiovasc Electrophysiol. 2009;20(4):401–7. doi:10.1111/j.1540-8167.2008.01339.x.
28. Mandyam MC, Soliman EZ, Alonso A, et al. The QT interval and risk of incident atrial fibrillation. Heart Rhythm. 2013;10(10):1562–8. doi:10.1016/j.hrthm.2013.07.023.
29. Batra AS, Silka MJ. Mechanism of sudden cardiac arrest while swimming in a child with the prolonged QT syndrome. J Pediatr. 2002;141(2):283–4. doi:10.1067/mpd.2002.126924.

30. Thomas D, Kiehn J, Katus HA, Karle CA. Adrenergic regulation of the rapid component of the cardiac delayed rectifier potassium current, I(Kr), and the underlying hERG ion channel. Basic Res Cardiol. 2004;99(4):279–87. doi:10.1007/s00395-004-0474-7.
31. Schwartz PJ, Priori SG, Locati EH, et al. Long QT syndrome patients with mutations of the SCN5A and HERG genes have differential responses to Na+ channel blockade and to increases in heart rate. Implications for gene-specific therapy. Circulation. 1995;92(12):3381–6.
32. Priori SG, Barhanin J, Hauer RN, et al. Genetic and molecular basis of cardiac arrhythmias; impact on clinical management. Study group on molecular basis of arrhythmias of the working group on arrhythmias of the european society of cardiology. Eur Heart J. 1999;20(3):174–95.
33. Schwartz PJ, Priori SG, Spazzolini C, et al. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. Circulation. 2001;103(1):89–95.
34. Makita N, Behr E, Shimizu W, et al. The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. J Clin Invest. 2008;118(6):2219–29. doi:10.1172/JCI34057.
35. Postema PG, Van den Berg M, Van Tintelen JP, et al. Founder mutations in the Netherlands: SCN5a 1795insD, the first described arrhythmia overlap syndrome and one of the largest and best characterised families worldwide. Neth Heart J. 2009;17(11):422–8.
36. Porta A, Girardengo G, Bari V, et al. Autonomic control of heart rate and QT interval variability influences arrhythmic risk in long QT syndrome type 1. J Am Coll Cardiol. 2015;65(4):367–74. doi:10.1016/j.jacc.2014.11.015.
37. Zareba W, Moss AJ, Schwartz PJ, et al. Influence of genotype on the clinical course of the long-QT syndrome. International long-QT syndrome registry research group. N Engl J Med. 1998;339(14):960–5. doi:10.1056/NEJM199810013391404.
38. Priori SG, Schwartz PJ, Napolitano C, et al. Risk stratification in the long-QT syndrome. N Engl J Med. 2003;348(19):1866–74. doi:10.1056/NEJMoa022147.
39. Sauer AJ, Moss AJ, McNitt S, et al. Long QT syndrome in adults. J Am Coll Cardiol. 2007;49(3):329–37. doi:10.1016/j.jacc.2006.08.057.
40. Migdalovich D, Moss AJ, Lopes CM, et al. Mutation and gender-specific risk in type 2 long QT syndrome: implications for risk stratification for life-threatening cardiac events in patients with long QT syndrome. Heart Rhythm. 2011;8(10):1537–43. doi:10.1016/j.hrthm.2011.03.049.
41. Chiang CE, Roden DM. The long QT syndromes: genetic basis and clinical implications. J Am Coll Cardiol. 2000;36(1):1–12.
42. Priori SG, Blomström-Lundqvist C, Mazzanti A, et al. 2015 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: the task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the Europe. Eur Heart J. 2015;36(41):2793–867. doi:10.1093/eurheartj/ehv316.
43. Schwartz PJ, Crotti L. QTc behavior during exercise and genetic testing for the long-QT syndrome. Circulation. 2011;124(20):2181–4. doi:10.1161/CIRCULATIONAHA.111.062182.
44. Schwartz PJ, Ackerman MJ. The long QT syndrome: a transatlantic clinical approach to diagnosis and therapy. Eur Heart J. 2013;34(40):3109–16. doi:10.1093/eurheartj/eht089.
45. Johnson JN, Grifoni C, Bos JM, et al. Prevalence and clinical correlates of QT prolongation in patients with hypertrophic cardiomyopathy. Eur Heart J. 2011;32(9):1114–20. doi:10.1093/eurheartj/ehr021.
46. Madias C, Fitzgibbons TP, Alsheikh-Ali AA, et al. Acquired long QT syndrome from stress cardiomyopathy is associated with ventricular arrhythmias and torsades de pointes. Heart Rhythm. 2011;8(4):555–61. doi:10.1016/j.hrthm.2010.12.012.
47. Gray B, Ingles J, Medi C, Semsarian C. Prolongation of the QTc interval predicts appropriate implantable cardioverter-defibrillator therapies in hypertrophic cardiomyopathy. JACC Heart Fail. 2013;1(2):149–55. doi:10.1016/j.jchf.2013.01.004.
48. Behr ER, Roden D. Drug-induced arrhythmia: pharmacogenomic prescribing? Eur Heart J. 2013;34(2):89–95. doi:10.1093/eurheartj/ehs351.
49. Arizona Center for Education and Research on Therapeutics (AZCERT). 2016. crediblemeds.org
50. Neyroud N, Tesson F, Denjoy I, et al. A novel mutation in the potassium channel gene KVLQT1 causes the Jervell and Lange-Nielsen cardioauditory syndrome. Nat Genet. 1997;15(2):186–9. doi:10.1038/ng0297-186.
51. Westenskow P, Splawski I, Timothy KW, Keating MT, Sanguinetti MC. Compound mutations: a common cause of severe long-QT syndrome. Circulation. 2004;109(15):1834–41. doi:10.1161/01.CIR.0000125524.34234.13.
52. Goldenberg I, Moss AJ, Zareba W, et al. Clinical course and risk stratification of patients affected with the Jervell and Lange-Nielsen syndrome. J Cardiovasc Electrophysiol. 2006;17(11):1161–8. doi:10.1111/j.1540-8167.2006.00587.x.
53. Tawil R, Ptacek LJ, Pavlakis SG, et al. Andersen's syndrome: potassium-sensitive periodic paralysis, ventricular ectopy, and dysmorphic features. Ann Neurol. 1994;35(3):326–30. doi:10.1002/ana.410350313.
54. Sansone V, Griggs RC, Meola G, et al. Andersen's syndrome: a distinct periodic paralysis. Ann Neurol. 1997;42(3):305–12. doi:10.1002/ana.410420306.
55. Splawski I, Timothy KW, Sharpe LM, et al. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. Cell. 2004;119(1):19–31. doi:10.1016/j.cell.2004.09.011.
56. Lepeschkin E, Surawicz B. The measurement of the Q-T interval of the electrocardiogram. Circulation. 1952;6(3):378–88.
57. Taggart NW, Haglund CM, Tester DJ, Ackerman MJ. Diagnostic miscues in congenital long-QT syndrome. Circulation. 2007;115(20):2613–20. doi:10.1161/CIRCULATIONAHA.106.661082.
58. Kukla P, Biernacka EK, Baranchuk A, Jastrzebski M, Jagodzinska M. Electrocardiogram in Andersen-Tawil syndrome. New electrocardiographic criteria for diagnosis of type-1 Andersen-Tawil syndrome. Curr Cardiol Rev. 2014;10(3):222–8.
59. Luo S, Michler K, Johnston P, Macfarlane PW. A comparison of commonly used QT correction formulae: the effect of heart rate on the QTc of normal ECGs. J Electrocardiol. 2004;37(Suppl):81–90.
60. Bogossian H, Frommeyer G, Ninios I, et al. New formula for evaluation of the QT interval in patients with left bundle branch block. Heart Rhythm. 2014;11(12):2273–7. doi:10.1016/j.hrthm.2014.08.026.
61. Viskin S, Rosovski U, Sands AJ, et al. Inaccurate electrocardiographic interpretation of long QT: the majority of physicians cannot recognize a long QT when they see one. Heart Rhythm. 2005;2(6):569–74. doi:10.1016/j.hrthm.2005.02.011.
62. Postema PG, De Jong JSSG, Van der Bilt IAC, Wilde AAM. Accurate electrocardiographic assessment of the QT interval: teach the tangent. Heart Rhythm. 2008;5(7):1015–8. doi:10.1016/j.hrthm.2008.03.037.
63. Hibino S, Ueda N, Horiba M, et al. Detection of QT prolongation through approximation of the T wave on Gaussian mixture modeling. Circ J. 2013;77(11):2728–35.
64. Chiladakis J, Kalogeropoulos A, Koutsogiannis N, et al. Optimal QT/JT interval assessment in patients with complete bundle branch block. Ann Noninvasive Electrocardiol. 2012;17(3):268–76. doi:10.1111/j.1542-474X.2012.00528.x.
65. Cobos MA. Assessment of QT and JT intervals in bundle branch block: a new formula. Circulation. 2013;128:A176.
66. Goldenberg I, Moss AJ, Zareba W. QT interval: how to measure it and what is "normal". J Cardiovasc Electrophysiol. 2006;17(3):333–6. doi:10.1111/j.1540-8167.2006.00408.x.

67. Moss AJ, Zareba W, Benhorin J, et al. ECG T-wave patterns in genetically distinct forms of the hereditary long QT syndrome. Circulation. 1995;92(10):2929–34.
68. Zhang L, Timothy KW, Vincent GM, et al. Spectrum of ST-T-wave patterns and repolarization parameters in congenital long-QT syndrome: ECG findings identify genotypes. Circulation. 2000;102(23):2849–55.
69. Kop WJ, Krantz DS, Nearing BD, et al. Effects of acute mental stress and exercise on T-wave alternans in patients with implantable cardioverter defibrillators and controls. Circulation. 2004;109(15):1864–9.doi:10.1161/01.CIR.0000124726.72615.60.
70. Zareba W, Moss AJ, le Cessie S, Hall WJ. T wave alternans in idiopathic long QT syndrome. J Am Coll Cardiol. 1994;23(7):1541–6.
71. Takasugi N, Goto H, Takasugi M, et al. Prevalence of microvolt T-wave alternans in patients with long QT syndrome and its association with Torsade de Pointes. Circ Arrhythm Electrophysiol. 2016;9(2):e003206. doi:10.1161/CIRCEP.115.003206.
72. Veldkamp MW, Wilders R, Baartscheer A, Zegers JG, Bezzina CR, Wilde AAM. Contribution of sodium channel mutations to bradycardia and sinus node dysfunction in LQT3 families. Circ Res. 2003;92(9):976–83. doi:10.1161/01.RES.0000069689.09869.A8.
73. Horner JM, Horner MM, Ackerman MJ. The diagnostic utility of recovery phase QTc during treadmill exercise stress testing in the evaluation of long QT syndrome. Heart Rhythm. 2011;8(11):1698–704. doi:10.1016/j.hrthm.2011.05.018.
74. Sy RW, van der Werf C, Chattha IS, et al. Derivation and validation of a simple exercise-based algorithm for prediction of genetic testing in relatives of LQTS probands. Circulation. 2011;124(20):2187–94. doi:10.1161/CIRCULATIONAHA.111.028258.
75. de Noronha SV, Behr ER, Papadakis M, et al. The importance of specialist cardiac histopathological examination in the investigation of young sudden cardiac deaths. Europace. 2014;16(6):899–907. doi:10.1093/europace/eut329.
76. Lupoglazoff JM, Denjoy I, Berthet M, et al. Notched T waves on Holter recordings enhance detection of patients with LQt2 (HERG) mutations. Circulation. 2001;103(8):1095–101.
77. Seethala S, Singh P, Shusterman V, Ribe M, Haugaa KH, Němec J. QT adaptation and intrinsic QT variability in congenital long QT syndrome. J Am Heart Assoc. 2015;4(12). doi:10.1161/JAHA.115.002395.
78. Page A, Aktas MK, Soyata T, Zareba W, Couderc J-P. "QT clock" to improve detection of QT prolongation in long QT syndrome patients. Heart Rhythm. 2016;13(1):190–8. doi:10.1016/j.hrthm.2015.08.037.
79. van den Berg MP, Haaksma J, Veeger NJGM, Wilde AAM. Diurnal variation of ventricular repolarization in a large family with LQT3-Brugada syndrome characterized by nocturnal sudden death. Heart Rhythm. 2006;3(3):290–5. doi:10.1016/j.hrthm.2005.11.023.
80. Viskin S, Postema PG, Bhuiyan ZA, et al. The response of the QT interval to the brief tachycardia provoked by standing: a bedside test for diagnosing long QT syndrome. J Am Coll Cardiol. 2010;55(18):1955–61. doi:10.1016/j.jacc.2009.12.015.
81. Aziz PF, Wieand TS, Ganley J, et al. Genotype- and mutation site-specific QT adaptation during exercise, recovery, and postural changes in children with long-QT syndrome. Circ Arrhythm Electrophysiol.2011;4(6):867–73.doi:10.1161/CIRCEP.111.963330.
82. Adler A, van der Werf C, Postema PG, et al. The phenomenon of "QT stunning": the abnormal QT prolongation provoked by standing persists even as the heart rate returns to normal in patients with long QT syndrome. Heart Rhythm. 2012;9(6):901–8. doi:10.1016/j.hrthm.2012.01.026.
83. Horner JM, Ackerman MJ. Ventricular ectopy during treadmill exercise stress testing in the evaluation of long QT syndrome. Heart Rhythm. 2008;5(12):1690–4. doi:10.1016/j.hrthm.2008.08.038.
84. Vyas H, Hejlik J, Ackerman MJ. Epinephrine QT stress testing in the evaluation of congenital long-QT syndrome: diagnostic accuracy of the paradoxical QT response. Circulation. 2006;113(11):1385–92. doi:10.1161/CIRCULATIONAHA.105.600445.
85. Khositseth A, Hejlik J, Shen W-K, Ackerman MJ. Epinephrine-induced T-wave notching in congenital long QT syndrome. Heart Rhythm. 2005;2(2):141–6. doi:10.1016/j.hrthm.2004.11.008.
86. Ackerman MJ, Khositseth A, Tester DJ, Hejlik JB, Shen W-K, Porter CJ. Epinephrine-induced QT interval prolongation: a gene-specific paradoxical response in congenital long QT syndrome. Mayo Clin Proc. 2002;77(5):413–21. doi:10.4065/77.5.413.
87. Shimizu W, Noda T, Takaki H, et al. Epinephrine unmasks latent mutation carriers with LQT1 form of congenital long-QT syndrome. J Am Coll Cardiol. 2003;41(4):633–42.
88. Krahn AD, Healey JS, Chauhan VS, et al. Epinephrine infusion in the evaluation of unexplained cardiac arrest and familial sudden death: from the cardiac arrest survivors with preserved ejection fraction registry. Circ Arrhythm Electrophysiol. 2012;5(5):933–40. doi:10.1161/CIRCEP.112.973230.
89. Hobbs JB, Peterson DR, Moss AJ, et al. Risk of aborted cardiac arrest or sudden cardiac death during adolescence in the long-QT syndrome. JAMA. 2006;296(10):1249–54. doi:10.1001/jama.296.10.1249.
90. Goldenberg I, Moss AJ, Peterson DR, et al. Risk factors for aborted cardiac arrest and sudden cardiac death in children with the congenital long-QT syndrome. Circulation. 2008;117(17):2184–91. doi:10.1161/CIRCULATIONAHA.107.701243.
91. Locati EH, Zareba W, Moss AJ, et al. Age- and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome: findings from the International LQTS Registry. Circulation. 1998;97(22):2237–44.
92. Goldenberg I, Moss AJ, Bradley J, et al. Long-QT syndrome after age 40. Circulation. 2008;117(17):2192–201. doi:10.1161/CIRCULATIONAHA.107.729368.
93. Anneken L, Baumann S, Vigneault P, et al. Estradiol regulates human QT-interval: acceleration of cardiac repolarization by enhanced KCNH2 membrane trafficking. Eur Heart J. 2016;37(7):640–50. doi:10.1093/eurheartj/ehv371.
94. Khositseth A, Tester DJ, Will ML, Bell CM, Ackerman MJ. Identification of a common genetic substrate underlying postpartum cardiac events in congenital long QT syndrome. Heart Rhythm. 2004;1(1):60–4. doi:10.1016/j.hrthm.2004.01.006.
95. Seth R, Moss AJ, McNitt S, et al. Long QT syndrome and pregnancy. J Am Coll Cardiol. 2007;49(10):1092–8. doi:10.1016/j.jacc.2006.09.054.
96. Meregalli PG, Westendorp ICD, Tan HL, Elsman P, Kok WEM, Wilde AAM. Pregnancy and the risk of torsades de pointes in congenital long-QT syndrome. Neth Heart J. 2008;16(12):422–5.
97. Kimbrough J, Moss AJ, Zareba W, et al. Clinical implications for affected parents and siblings of probands with long-QT syndrome. Circulation. 2001;104(5):557–62.
98. Kaufman ES, McNitt S, Moss AJ, et al. Risk of death in the long QT syndrome when a sibling has died. Heart Rhythm. 2008;5(6):831–6. doi:10.1016/j.hrthm.2008.02.029.
99. Spazzolini C, Mullally J, Moss AJ, et al. Clinical implications for patients with long QT syndrome who experience a cardiac event during infancy. J Am Coll Cardiol. 2009;54(9):832–7. doi:10.1016/j.jacc.2009.05.029.
100. Goldenberg I, Mathew J, Moss AJ, et al. Corrected QT variability in serial electrocardiograms in long QT syndrome: the importance of the maximum corrected QT for risk stratification. J Am Coll Cardiol. 2006;48(5):1047–52. doi:10.1016/j.jacc.2006.06.033.
101. Goldenberg I, Horr S, Moss AJ, et al. Risk for life-threatening cardiac events in patients with genotype-confirmed long-QT syndrome and normal-range corrected QT intervals. J Am Coll Cardiol. 2011;57(1):51–9. doi:10.1016/j.jacc.2010.07.038.
102. Itoh H, Shimizu W, Hayashi K, et al. Long QT syndrome with compound mutations is associated with a more severe phenotype:

a Japanese multicenter study. Heart Rhythm. 2010;7(10):1411–8. doi:10.1016/j.hrthm.2010.06.013.
103. Mullally J, Goldenberg I, Moss AJ, et al. Risk of life-threatening cardiac events among patients with long QT syndrome and multiple mutations. Heart Rhythm. 2013;10(3):378–82. doi:10.1016/j.hrthm.2012.11.006.
104. Crotti L, Spazzolini C, Schwartz PJ, et al. The common long-QT syndrome mutation KCNQ1/A341V causes unusually severe clinical manifestations in patients with different ethnic backgrounds: toward a mutation-specific risk stratification. Circulation. 2007;116(21):2366–75. doi:10.1161/CIRCULATIONAHA.107.726950.
105. Moss AJ, Zareba W, Kaufman ES, et al. Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go-related gene potassium channel. Circulation. 2002;105(7):794–9.
106. Crotti L, Lundquist AL, Insolia R, et al. KCNH2-K897T is a genetic modifier of latent congenital long-QT syndrome. Circulation. 2005;112(9):1251–8.doi:10.1161/CIRCULATIONAHA.105.549071.
107. Duchatelet S, Crotti L, Peat RA, et al. Identification of a KCNQ1 polymorphism acting as a protective modifier against arrhythmic risk in long-QT syndrome. Circ Cardiovasc Genet. 2013;6(4):354–61. doi:10.1161/CIRCGENETICS.113.000023.
108. de Villiers CP, van der Merwe L, Crotti L, et al. AKAP9 is a genetic modifier of congenital long-QT syndrome type 1. Circ Cardiovasc Genet. 2014;7(5):599–606. doi:10.1161/CIRCGENETICS.113.000580.
109. Earle N, Yeo Han D, Pilbrow A, et al. Single nucleotide polymorphisms in arrhythmia genes modify the risk of cardiac events and sudden death in long QT syndrome. Heart Rhythm. 2014;11(1):76–82. doi:10.1016/j.hrthm.2013.10.005.
110. Crotti L, Monti MC, Insolia R, et al. NOS1AP is a genetic modifier of the long-QT syndrome. Circulation. 2009;120(17):1657–63. doi:10.1161/CIRCULATIONAHA.109.879643.
111. Tomás M, Napolitano C, De Giuli L, et al. Polymorphisms in the NOS1AP gene modulate QT interval duration and risk of arrhythmias in the long QT syndrome. J Am Coll Cardiol. 2010;55(24):2745–52. doi:10.1016/j.jacc.2009.12.065.
112. Ackerman MJ, Zipes DP, Kovacs RJ, Maron BJ. Eligibility and disqualification recommendations for competitive athletes with cardiovascular abnormalities: task force 10: the cardiac channelopathies: a scientific statement from the American Heart Association and American College of Cardiology. J Am Coll Cardiol. 2015;66(21):2424–8. doi:10.1016/j.jacc.2015.09.042.
113. Maron BJ, Zipes DP. Introduction: eligibility recommendations for competitive athletes with cardiovascular abnormalities-general considerations. J Am Coll Cardiol. 2005;45(8):1318–21. doi:10.1016/j.jacc.2005.02.006.
114. Pelliccia A, Fagard R, Bjørnstad HH, et al. Recommendations for competitive sports participation in athletes with cardiovascular disease: a consensus document from the study group of sports cardiology of the working group of cardiac rehabilitation and exercise physiology and the working group of My. Eur Heart J. 2005;26(14):1422–45. doi:10.1093/eurheartj/ehi325.
115. Pelliccia A, Zipes DP, Maron BJ. Bethesda conference #36 and the European Society of Cardiology consensus recommendations revisited a comparison of U.S. and European criteria for eligibility and disqualification of competitive athletes with cardiovascular abnormalities. J Am Coll Cardiol. 2008;52(24):1990–6. doi:10.1016/j.jacc.2008.08.055.
116. Johnson JN, Ackerman MJ. Competitive sports participation in athletes with congenital long QT syndrome. JAMA. 2012;308(8):764–5. doi:10.1001/jama.2012.9334.
117. Johnson JN, Ackerman MJ. Return to play? Athletes with congenital long QT syndrome. Br J Sports Med. 2013;47(1):28–33. doi:10.1136/bjsports-2012-091751.
118. Vincent GM, Schwartz PJ, Denjoy I, et al. High efficacy of beta-blockers in long-QT syndrome type 1: contribution of noncompliance and QT-prolonging drugs to the occurrence of beta-blocker treatment "failures". Circulation. 2009;119(2):215–21. doi:10.1161/CIRCULATIONAHA.108.772533.
119. Goldenberg I, Bradley J, Moss A, et al. Beta-blocker efficacy in high-risk patients with the congenital long-QT syndrome types 1 and 2: implications for patient management. J Cardiovasc Electrophysiol. 2010;21(8):893–901. doi:10.1111/j.1540-8167.2010.01737.x.
120. Wilde AAM, Ackerman MJ. Beta-blockers in the treatment of congenital long QT syndrome: is one beta-blocker superior to another? J Am Coll Cardiol. 2014;64(13):1359–61. doi:10.1016/j.jacc.2014.06.1192.
121. Ruan Y, Liu N, Napolitano C, Priori SG. Therapeutic strategies for long-QT syndrome: does the molecular substrate matter? Circ Arrhythm Electrophysiol. 2008;1(4):290–7. doi:10.1161/CIRCEP.108.795617.
122. Funasako M, Aiba T, Ishibashi K, et al. Pronounced shortening of QT interval with mexiletine infusion test in patients with type 3 congenital long QT syndrome. Circ J. 2016;80(2):340–5. doi:10.1253/circj.CJ-15-0984.
123. Mazzanti A, Maragna R, Faragli A, et al. Gene-specific therapy with mexiletine reduces arrhythmic events in patients with long QT syndrome type 3. J Am Coll Cardiol. 2016;67(9):1053–8. doi:10.1016/j.jacc.2015.12.033.
124. Moss AJ, Zareba W, Schwarz KQ, Rosero S, McNitt S, Robinson JL. Ranolazine shortens repolarization in patients with sustained inward sodium current due to type-3 long-QT syndrome. J Cardiovasc Electrophysiol. 2008;19(12):1289–93. doi:10.1111/j.1540-8167.2008.01246.x.
125. Huang H, Priori SG, Napolitano C, O'Leary ME, Chahine M. Y1767C, a novel SCN5A mutation, induces a persistent Na+ current and potentiates ranolazine inhibition of Nav1.5 channels. Am J Physiol Heart Circ Physiol. 2011;300(1):H288–99. doi:10.1152/ajpheart.00539.2010.
126. Egashira T, Yuasa S, Suzuki T, et al. Disease characterization using LQTS-specific induced pluripotent stem cells. Cardiovasc Res. 2012;95(4):419–29. doi:10.1093/cvr/cvs206.
127. Wang Y, Liang P, Lan F, et al. Genome editing of isogenic human induced pluripotent stem cells recapitulates long QT phenotype for drug testing. J Am Coll Cardiol. 2014;64(5):451–9. doi:10.1016/j.jacc.2014.04.057.
128. Schwartz PJ, Spazzolini C, Priori SG, et al. Who are the long-QT syndrome patients who receive an implantable cardioverter-defibrillator and what happens to them?: data from the European Long-QT Syndrome Implantable Cardioverter-Defibrillator (LQTS ICD) Registry. Circulation. 2010;122(13):1272–82. doi:10.1161/CIRCULATIONAHA.110.950147.
129. Kusumoto FM, Calkins H, Boehmer J, et al. HRS/ACC/AHA expert consensus statement on the use of implantable cardioverter-defibrillator therapy in patients who are not included or not well represented in clinical trials. J Am Coll Cardiol. 2014;64(11):1143–77. doi:10.1016/j.jacc.2014.04.008.
130. Schwartz PJ, Priori SG, Cerrone M, et al. Left cardiac sympathetic denervation in the management of high-risk patients affected by the long-QT syndrome. Circulation. 2004;109(15):1826–33. doi:10.1161/01.CIR.0000125523.14403.1E.
131. Desimone CV, Bos JM, Bos KM, et al. Effects on repolarization using dynamic QT interval monitoring in long-QT patients following left cardiac sympathetic denervation. J Cardiovasc Electrophysiol. 2015;26(4):434–9. doi:10.1111/jce.12609.
132. Schneider AE, Bos JM, Ackerman MJ. Effect of left cardiac sympathetic denervation on the electromechanical window in patients with either type 1 or type 2 long QT syndrome: A pilot study. Congenit Heart Dis. 2016. doi:10.1111/chd.12332.
133. Takahashi K, Shimizu W, Miyake A, Nabeshima T, Nakayashiro M, Ganaha H. High prevalence of the SCN5A E1784K mutation in school children with long QT syndrome living on the Okinawa islands. Circ J. 2014;78(8):1974–9.

134. Tester DJ, Kopplin LJ, Will ML, Ackerman MJ. Spectrum and prevalence of cardiac ryanodine receptor (RyR2) mutations in a cohort of unrelated patients referred explicitly for long QT syndrome genetic testing. Heart Rhythm. 2005;2(10):1099–105. doi:10.1016/j.hrthm.2005.07.012.
135. Ghouse J, Have CT, Weeke P, et al. Rare genetic variants previously associated with congenital forms of long QT syndrome have little or no effect on the QT interval. Eur Heart J. 2015;36(37):2523–9. doi:10.1093/eurheartj/ehv297.
136. Mogensen J, van Tintelen JP, Fokstuen S, et al. The current role of next-generation DNA sequencing in routine care of patients with hereditary cardiovascular conditions: a viewpoint paper of the European Society of Cardiology working group on myocardial and pericardial diseases and members of the Europ. Eur Heart J. 2015;36(22):1367–70. doi:10.1093/eurheartj/ehv122.
137. Refsgaard L, Holst AG, Sadjadieh G, Haunsø S, Nielsen JB, Olesen MS. High prevalence of genetic variants previously associated with LQT syndrome in new exome data. Eur J Hum Genet. 2012;20(8):905–8. doi:10.1038/ejhg.2012.23.
138. Vatta M, Ackerman MJ, Ye B, et al. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. Circulation. 2006;114(20):2104–12. doi:10.1161/CIRCULATIONAHA.106.635268.
139. Nolte IM, Wallace C, Newhouse SJ, et al. Common genetic variation near the phospholamban gene is associated with cardiac repolarisation: meta-analysis of three genome-wide association studies. PLoS One. 2009;4(7):e6138. doi:10.1371/journal.pone.0006138.
140. Arking DE, Pfeufer A, Post W, et al. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. Nat Genet. 2006;38(6):644–51. doi:10.1038/ng1790.
141. Pfeufer A, Sanna S, Arking DE, et al. Common variants at ten loci modulate the QT interval duration in the QTSCD Study. Nat Genet. 2009;41(4):407–14. doi:10.1038/ng.362.
142. Newton-Cheh C, Eijgelsheim M, Rice KM, et al. Common variants at ten loci influence QT interval duration in the QTGEN Study. Nat Genet. 2009;41(4):399–406. doi:10.1038/ng.364.
143. Kolder ICRM, Tanck MWT, Postema PG, et al. Analysis for genetic modifiers of disease severity in patients with long-QT syndrome type 2. Circ Cardiovasc Genet. 2015;8(3):447–56. doi:10.1161/CIRCGENETICS.114.000785.
144. Mellor G, Behr ER. Sudden unexplained death – treating the family. Arrhythmia Electrophysiol Rev. 2014;3(3):156–60. doi:10.15420/aer.2014.3.3.156.

Brugada Syndrome

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Abstract

In 1992, the manuscript "*Right bundle branch block, persistent ST segment elevation and sudden cardiac death: A distinct clinical and electrocardiographic syndrome*" was published in the Journal of the American College of Cardiology [1]. This publication described eight individuals with a common phenotype: resuscitated from sudden cardiac death (SCD) caused by documented ventricular fibrillation (VF), showing a characteristic ST-segment elevation in the right precordial leads (Fig. 11.1) in a structurally normal heart. Nowadays, this entity is known as Brugada Syndrome (BrS). In the last 20 years, major advances in clinical and mechanistic knowledge have provided very valuable information about the disease, but remaining questions still propel a large research activity on the subject. In this chapter, we review the present knowledge, on clinical, genetic, and molecular features of BrS.

Introduction

In 1992, the manuscript "*Right bundle branch block, persistent ST segment elevation and sudden cardiac death: A distinct clinical and electrocardiographic syndrome*" was published in the Journal of the American College of Cardiology [1]. This publication described eight individuals with a common phenotype: resuscitated from sudden cardiac death (SCD) caused by documented ventricular fibrillation (VF), showing a characteristic ST-segment elevation in the right precordial leads (Fig. 11.1) in a structurally normal heart. Nowadays, this entity is known as Brugada Syndrome (BrS). In the last 20 years, major advances in clinical and mechanistic knowledge have provided very valuable information about the disease, but remaining questions still propel a large research activity on the subject. In this chapter, we review the present knowledge, on clinical, genetic, and molecular features of BrS.

The prevalence of the BrS has been estimated 5/10,000 inhabitants, although this rate should be interpreted with caution, first, because many patients present with concealed forms of the disease, the prevalence is likely to be underestimated, and, second, because significant ethnic and geographic differences have been described [2]. For example, a type 1 electrocardiogram (ECG) pattern was observed in

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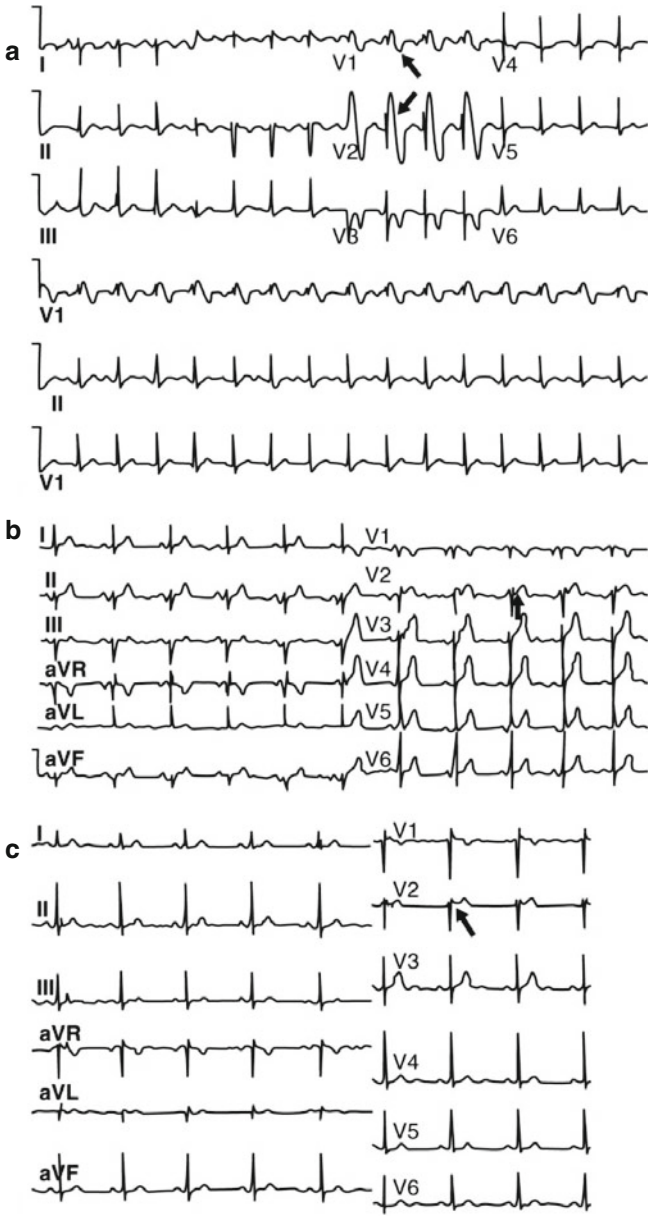

Fig. 11.1 Brugada electrocardiogram (ECG) patterns. (**a**) A diagnostic coved-type (type I) Brugada ECG pattern documented in a 9-year-old girl who presented with syncope and positive family history of BrS. Note the pattern resembling a right bundle-branch block (arrows) in leads V_1 and V_2, with typical ST elevation. (**b**) Baseline ECG of a 58-year-old asymptomatic man with positive family history of BrS. An example of a type II saddleback Brugada ECG pattern. Genetic analysis revealed a pathogenic variant in the *SCN5A* gene. Note the saddleback-shaped patterns, with a high initial augmentation followed by an ST elevation greater than 2 mm in lead V_2. (**c**) An example of a baseline type III saddleback Brugada ECG pattern (arrow) documented in a 61-year-old asymptomatic man who was diagnosed on the basis of a positive result on class IC antiarrhythmic drug testing

12/10,000 Japanese inhabitants [3], whereas the few available data on North American and European populations point to a much lower prevalence [4, 5]. In fact, the syndrome is considered to be endemic in certain Southeast Asian areas, where it has been long recognized as the sudden unexplained death syndrome (SUDS), also named *bangungot* (Philippines), *pokkuri* (Japan), or *lai tai* (Thailand), all of them known to be phenotypically, genetically, and functionally identical disorders as BrS [6].

Clinical Presentation and Diagnosis

Certain ambiguities appeared in the years following the initial description of the syndrome, basically concerning the characteristic ECG hallmark and the specific diagnostic criteria. The hallmark of BrS is the transient or persistent appearance of typical ECG changes in the right precordial leads. The second BrS Consensus Report of 2005 (endorsed by the Heart Rhythm Society and the European Heart Rhythm Association) [2] stated the current recommendations regarding the diagnostic criteria. Three different ECG patterns (Fig. 11.1), all featuring ST-segment elevation in the right precordial leads, have been recognized: Type I is the only pattern that is diagnostic for BrS. It consists of a coved-type ST-segment elevation greater than 2 mm, followed by a descending negative T-wave in at least one right precordial lead (V_1–V_3). Type II and type III are saddleback-shaped patterns, with a high initial augmentation followed by an ST elevation of 2 mm for type II and less than 2 mm for type III. Both patterns are suggestive of but not diagnostic for BrS. BrS should be diagnosed if the ECG pattern occurs in a patient with no structural heart disease, a family history of SD or type I Brugada ECG pattern, a genetic defect known to cause BrS, or symptoms such as syncope or aborted SD.

Whenever a large number of baseline ECGs were available during follow-up, the diagnostic pattern could be documented in only approximately 25 % of the tracings. Almost every individual with a type I ECG will show normalization of the ECG during follow-up. Because the presence of a spontaneous coved-type (type I) ECG pattern is a useful predictor of future arrhythmic events in asymptomatic patients, this variation of the ECG pattern is of great clinical importance. The class IC antiarrhythmic drug (AAD) test provides a sensitive tool to unmask these concealed forms. Intravenous administration of ajmaline, flecainide, or procainamide was able to elicit the diagnostic coved-type BrS ECG pattern. On the basis of the results of comparative studies, and clinical experience, ajmaline, in a dose of 1 mg/kg, was found to be the most sensitive drug in unmasking BrS. The full stomach test was proposed as an alternative tool in diagnosing BrS [7]. Here, the ST-segment changes appear to be provoked by an enhanced vagal tone. Adrenergic stimulation decreases the ST-segment elevation, whereas vagal stimulation increases it. Obviously, it is important to exclude other causes of ST-segment elevation before making the diagnosis of BrS (Table 11.1).

Table 11.1 Acquired BrS: Differential diagnosis of ST-segment elevation in electrocardiogram leads V_1 and V_2

Drugs	Antiarrhythmic drugs	Class 1C sodium-channel blockers (e.g., flecainide, pilsicainide, propafenone)
		Class 1 A sodium-channel blockers (e.g., procainamide, disopyramide, cibenzoline)
		Verapamil (L-type calcium-channel blocker)
		Beta-blockers (inhibit $I_{Ca,L}$)
	Antianginal drugs	Nitrates
		Calcium-channel blockers (e.g., nifedipine, diltiazem)
	Psychotropic agents	Tricyclic antidepressants (e.g., amitriptiline, desipramine, clomipramine, nortriptyline)
		Tetracyclic antidepressants (e.g., maprotiline)
		Phenothiazines (e.g., perphenazine, cyamemazine)
		Selective serotonine uptake inhibitors (e.g., fluoxetine)
		Cocaine intoxication
	Antiallergic agents	Histamine H1 antihistaminics. First-generation (dimenhydrinate)
Acute ischemia in RVOT		
Electrolyte disturbances		Hyperkalemia
		Hypercalcemia
Hyperthermia and hypothermia		
Elevated insulin level		
Mechanical compression of RVOT		

RVOT right ventricular outflow tract

The BrS is included among the so-called channelopathies, that is, primary electrical disorders produced by the dysfunction of a cardiac channel participating in the action potential (AP), the electrical change favoring the development of arrhythmias. Characteristically, no underlying structural heart disease exists concomitantly. In fact, the BrS is thought to be responsible for 4–12 % of all SD and for up to 20 % of SD in subjects with structurally normal heart [8]. However, some studies have been published [9–11], supporting the presence of structural alterations in BrS patients. The finding of morphologic changes has been supported by recent identification of BrS patients carrying pathogenic variants in the *PKP2* gene [12, 13]. *PKP2* is the primary gene responsible for arrhythmogenic cardiomyopathy (ACM), a desmosomal disease characterized by fibro-fatty replacement of myocardium leading to SCD in young men, mainly during exercise. Hence, in a recent publication, Nademanee et al. performed genetic and immunohistological analyses of six forensic samples from family members who were affected with BrS [14]. They reported an increase in epicardial collagen and fibrosis and a decrease in gap junction Connexin43 expression, especially in the right ventricular outflow tract (RVOT) area. Despite these contributions, the clinical phenotype of BrS concomitant with cardiac fibrosis remains a matter of debate and a point to be clarified in future investigations.

Treatment

Implantable Cardioverter Defibrillator

To date, the only proven effective therapeutic strategy for the prevention of SCD in BrS patients is the implantable cardioverter defibrillator (ICD) (Fig. 11.2). The precise indications for ICD as primary prophylaxis are a spontaneous type I ECG and inducible ventricular arrhythmias in the electrophysiological study (EPS) in asymptomatic patients. This fact is supported by a recent study of Conte et al., treating potentially lethal arrhythmias in 17 % of patients during a follow-up period of nearly 85 months. Appropriate shocks were significantly associated with the presence of aborted SCD [15]. It is important to remark that ICDs are not free from several disadvantages, especially in this group of patients, sharing a particular profile: active young individuals, facing a long-lasting coexistence with the device and multiple device replacements, long-life expectation (especially after the device implantation). Some series have reported low rates of appropriate shocks (8–15 %, median follow-up 45 months) and a high rate of complications, mainly consisting of inappropriate shocks (20–36 % at 21–47 months follow-up) [16–18]. A recent study reports that T-wave oversensing is a potential reason of inappropriate shocks in patients with BrS carrying ICDs. In the vast majority, the problem can be solved by reprogramming. However, in some patients these complications require invasive intervention. Importantly, the reported incidence of complications is significantly lower using an integrated bipolar lead system when compared with a dedicated bipolar lead system and hence the former should be routinely used in BrS cases [19].

Pharmacological Treatment

With the aim of rebalancing the ionic currents affected in BrS during the cardiac AP, drugs that inhibit the transient outward currentor increase the Na^+ and Ca^{+2} currents have been tested in BrS:

– Isoproterenol (which increases the ICa L current) has proved to be useful for the treatment of electrical storms in BrS [20].

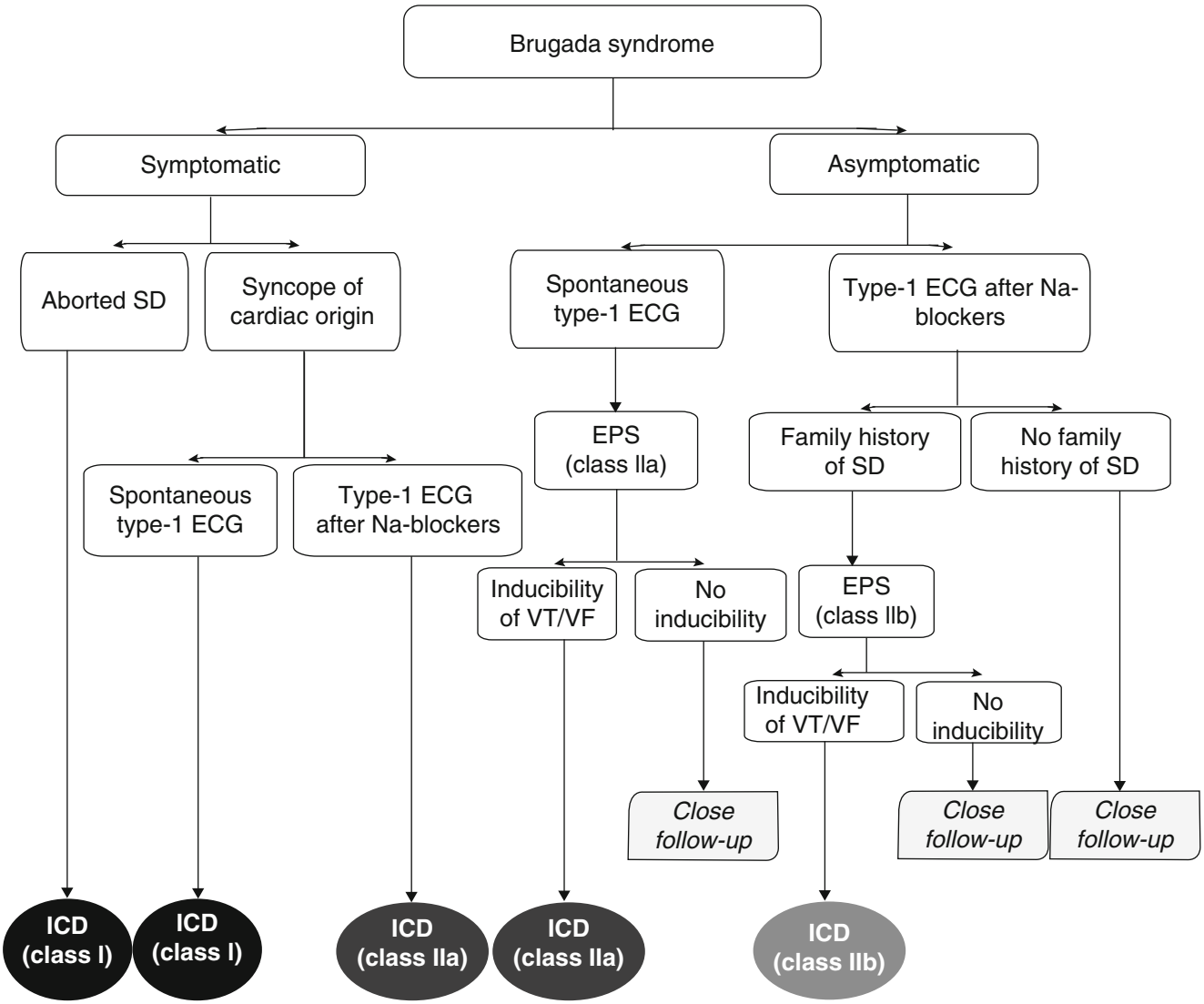

Fig. 11.2 Indications for implantable cardioverter defibrillator (ICD) implantation in patients with Brugada syndrome. Class I designation indicates clear evidence that the procedure or treatment is useful or effective; Class II, conflicting evidence about usefulness or efficacy; Class IIa, weight of evidence in favor of usefulness or efficacy; Class IIb, usefulness or efficacy less well established

– Quinidine, a class Ia AADs with I_{to}- and I_{Kr}-blocker effects, has shown to prevent induction of VF and suppress spontaneous ventricular arrhythmias in a clinical setting, being currently used in patients with ICD and multiple shocks, cases in which ICD implantation is contraindicated, or for the treatment of supraventricular arrhythmias. It has been suggested that it could be also useful in children with BrS, as a bridge to ICD or as an alternative to it [21].

Radiofrequency Catheter Ablation

After the demonstration that VF events were triggered by similar ventricular ectopy, radiofrequency catheter ablation (RFCA) of ventricular ectopy has been postulated as a therapeutical approach in BrS patients. Few anecdotic cases in high-risk BrS implanted with an ICD have shown no short-term recurrence of VF, syncope, or SCD [22, 23]. Nademanee presented the first series showing that electrical disconnection of the RVOT can prevent VF inducibility in a high-risk population [24]. In 2014, a BrS case of an ablation of the epicardial arrhythmogenic substrate in the RVOT was published [25]. In 2015, Forkmann et al. reported a BrS case in which epicardial ventricular tachycardia (VT) ablation was performed and noninducibility of any VT during programmed ventricular stimulation (PVS) was shown. During 9 months of follow-up, device readout showed no recurrence of any VT episodes [26]. Recently, an interesting study

focused on epicardial ablation in a cohort of patients suffering from BrS has been published. The authors demonstrate that ablation of the arrhythmogenic electrical substrate identified in the presence of Flecainide can eliminate the BrS phenotype [27]. Epicardial areas variable in extension and distribution mainly located in the RV free wall and RVOT unmasked by Flecainide testing are responsible for both BrS ECG pattern and VT/VF development. Elimination of these abnormal arrhythmogenic electrical areas by epicardial RFCA or cryotherapy was able to eliminate both BrS ECG pattern and VF inducibility without complications. Although larger studies with longer follow-up are required, these results might have physiopathologic and clinical implications as they provide new information leading to a potential definitive treatment of the phenotypic manifestations of BrS.

Molecular Mechanisms

Transmembrane ionic fluxes generate an AP. In the ventricular myocyte, a rapid inward Na^+ current, I_{Na}, depolarizes the cell membrane, produces the phase 0 of the AP, and subsequently activates other ion currents. Activation of the transient outward K^+ current, I_{to}, will overwhelm the late phase of the inward I_{Na} initiating the repolarization phase, which will be followed by the activation of L-type Ca^{2+} current, rapid- and slow-delayed rectifier K^+ currents, I_{Kr} and I_{Ks}, and the inward rectifier K^+ current, I_{K1} to finally return to the resting negative potential (Figs. 11.3 and 11.4).

The BrS is a channelopathy which induces an electric dysfunction in those channels which participate in the generation of the cardiac AP. Experimental and clinical data

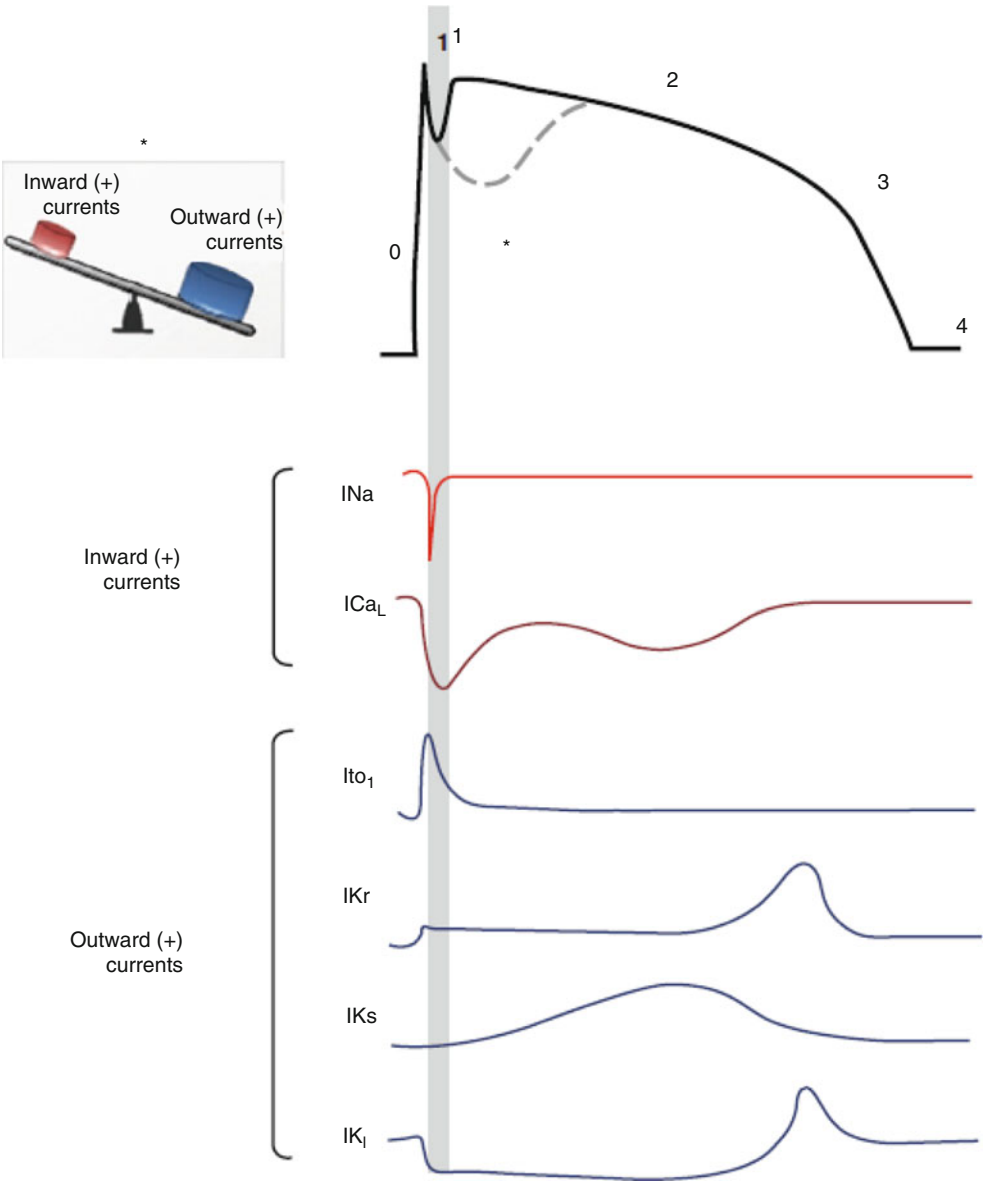

Fig. 11.3 Ventricular myocyte action potential and main underlying ionic currents. The *shaded area* highlights phase 1, mostly determined by the balance between I_{Na}, I_{Ca}, and I_{to}. When positive inward currents are impaired with respect to positive outward currents (*), the cell achieves greater degree of repolarization, and the normal dome of the action potential is lost, leading to the development of a particular notch at the end of phase 1 (*dashed line*). This is the mechanism that is thought to underlie the Brugada syndrome. I_{Ca} inward calcium current, I_{Na} inward sodium current, I_{to} transient outward potassium current

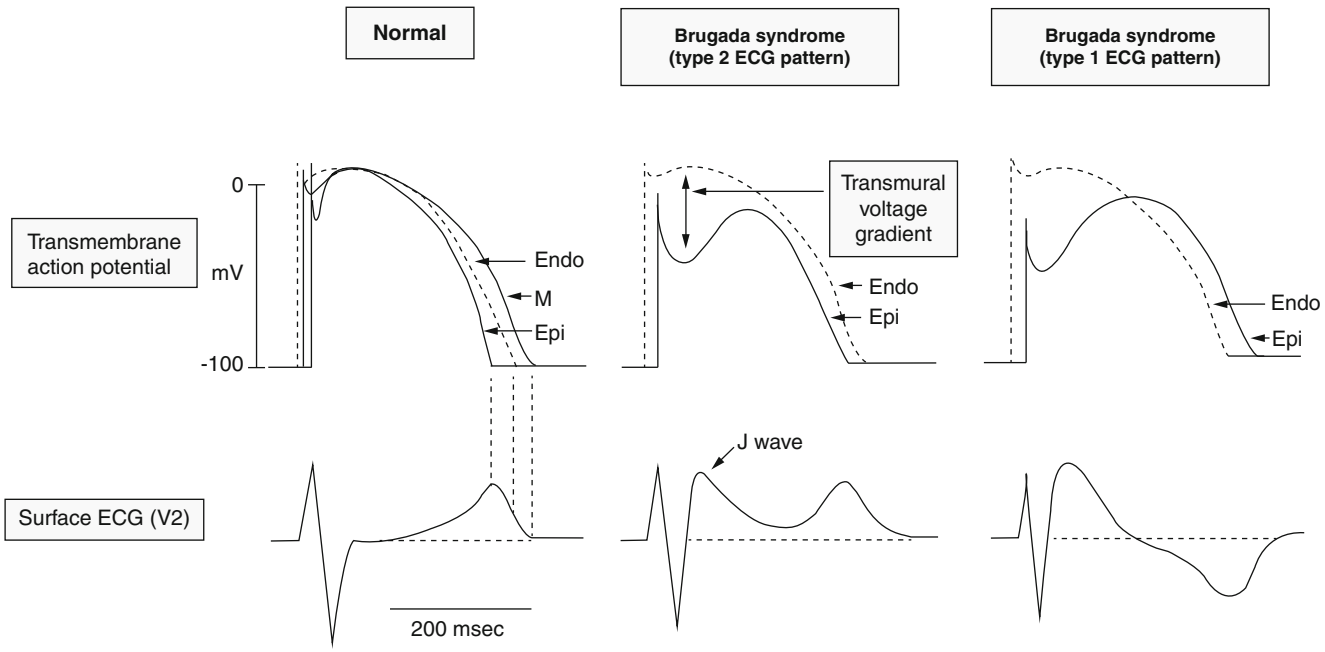

Fig. 11.4 Proposed mechanism that underlies ST-segment elevation in Brugada syndrome. The accentuated notch present in epicardium but not in endocardium gives rise to transmural voltage gradient and J-point elevation (Brugada saddleback). Further accentuation of the notch may be accompanied by a prolongation of the action potential in epicardium, which becomes longer than in endocardium, thus leading to the development of negative T-waves in addition to the ST-segment elevation (Brugada coved-type)

have provided insight into the cellular and molecular basis for the ECG morphology and arrhythmogenesis of BrS. [28]. Two proposed mechanisms are believed to potentially explain the ST-segment elevation in the right precordial leads: (1) a disequilibrium between I_{na} and I_{to}, which preferentially affects the right ventricular myocardium, generating transmural dispersion of repolarization and the substrate for arrhythmias. It has been suggested that the abnormal migration of neural crest cells to the right ventricle might predispose to arrhythmias in adulthood [29]. The cellular basis of this phenomenon is thought to be the result of a loss-of-function Na^+ channel that differentially alters the AP morphology in epicardial versus endocardial cells. The fast transient outward K^+ current I_{to} is most prominent in epicardial cells of the right ventricle. This K^+ current is quickly activated by membrane depolarization. It opposes and exceeds the depolarizing effect of the Na^+ inward flux during the early phase of the AP plateau resulting in a pronounced AP notch and, in combination with depolarizing Ca^{2+} currents, in a *"spike-and-dome"* morphology. Consequently, Na^+ current reduction leads to an outward shift of the net transmembrane current in epicardial cells finally resulting in premature repolarization and in a significant AP shortening. In contrast, endocardial cells display a much smaller I_{to}, and consequently Na^+ current reduction would not affect significantly AP morphology and duration. The transmural in homogeneity of the cellular membrane voltage finally causes ST-segment elevation [30] and (2) Conduction slowing in the RVOT, leading to ST-segment elevation in right precordial leads [31].

Genetics

The BrS is a familial disease with an autosomal dominant pattern of inheritance. To date, nearly 300 pathogenic variants in 22 genes have been published (Table 11.2). The first gene associated with BrS was *SCN5A* which encodes alpha subunit of the cardiac sodium channel [32]. The SCN5A gene is responsible for the phase 0 of the cardiac AP. Pathogenic variants in SCN5A found in BrS result in loss of function of the sodium channel. In around 20–25 % of patients with BrS, a pathogenic variant in the SCN5A gene is found, classified as BrS type 1 [33]. Recently, an extensive deletion of the *SCN5A* gene was found in an individual diagnosed with BrS and concomitant conduction system disease [34]. Despite the ongoing developments in understanding the genetic causes of BrS, only 30–35 % of clinically diagnosed cases are genetically diagnosed, and most of these (25–30 %) result from pathogenic alterations in *SCN5A* [33]. Therefore, current guidelines recommend only the genetic test of *SCN5A*. However, next-generation sequencing (NGS) technology allows a genetic analysis of several genes in a cost-effective way. Hence, we believe that a comprehensive genetic analysis using NGS technology should be performed in BrS families. One of the main problems of NGS technology

Table 11.2 Brugada Syndrome (BrS) types

Inheritance	Locus	Gene	Protein	Percentage
(Sodium)	3p21–p24	*SCN5A*	Nav1.5	25–30
Autosomic dominant	3p22.3	*GPD1-L*	Glycerol-3-P-DH-1	<1
	19q13.1	*SCN1B*	Navβ1	<1
	11q24.1	*SCN3B*	Navβ3	<1
	11q23.3	*SCN2B*	Navβ2	<1
	3p22.2	*SCN10A*	Nav1.8	<1
	6q21	*HEY2*	Hes-Related Family BHLH Transcription Factor YRPW Motif 2	<1
	17p13.1	*RANGRF*	RAN-G-release factor (MOG1)	<1
	3p14.3	*SLMAP*	Sarcolemma-associated protein	<1
	3q28	*FGF12*	Fibroblast Growth Factor 12	<1
	12p11.21	*PKP2*	Plakofilin-2	<1
(Potassium)	12p12.1	*ABCC9*	Adenosine triphosphate (ATP)-sensitive	<1
Autosomic dominant	11q13–q14	*KCNE3*	MiRP2	<1
Chromosome X	12p12.1	*KCNJ8*	Kv6.1 Kir6.1	<1
	15q24.1	*HCN4*	Hyperpolarization cyclic nucleotide-gated 4	<1
	1p13.2	*KCND3*	Kv4.3 Kir4.3	<1
	7p12.1	*SEMA3A*	Semaphorin III	<1
	Xq22.3	*KCNE5*	Potassium voltage-gated channel subfamily E member 1	<1
(Calcium)	2p13.3	*CACNA1C*	Cav1.2	<1
Autosomic dominant	10p12.33	*CACNB2B*	Voltage-dependent β-2	<1
	7q21–q22	*CACNA2D1*	Voltage-dependent α2/δ1	<1
	19q13.33	*TRPM4*	Transient receptor potential M4	<1

is the amount of data obtained and interpretation before clinical translation. Familial testing helps to clarify the pathogenic role of rare variants identified using NGS technology but also allows the identification of relatives carrying potential genetic variants and, in consequence, at risk of SCD, despite asymptomatic.

Copy number variation (CNV) is the only rearrangement identified as a cause of the disease to date. In a recent study, a comprehensive genetic evaluation of main BrS-susceptibility genes and CNV in a Spanish BrS cohort was published. Selga et al. report that the mean pathogenic variation detection yield was higher than that described for other European BrS cohorts (32.7 % vs. 20–25 %, respectively), and was even higher for patients in the 30–50 years age range [35]. In addition, pathogenic variants in SCN1B (sodium-channel beta-1 subunit) [36], *SCN2B* (sodium-channel beta-2 subunit), and SCN3B (sodium-channel beta-3 subunit) [37] modify the function of the channel (increasing or decreasing I_{Na}) [36–38]. Recently, the *SCN10A* gene (neuronal sodium-channel $Na_v1.8$) has been shown to modulate *SCN5A* expression and the electrical function of the heart [39]. In this study of Bezzina et al., the transcriptional factor HEY2 was also identified as associated with BrS [40]. Another gene reported as responsible for BrS was GPD1-L. Pathogenic variants in GPD1-L reduce both the surface membrane expression and the inward sodium current [41]. Recently, Kattygnarath et al. published a study supporting that RANGRF can impair the trafficking of Nav1.5 to the membrane, leading to I_{Na} reduction and clinical manifestation of BrS [42]. In 2012, Ishikawa et al. reported pathogenic variations in the sarcolemmal membrane-associated protein (*SLMAP*) gene, a gene of unknown function that is found at T-tubules and the sarcoplasmic reticulum. SLMAP causes BrS by modulating the intracellular trafficking of the $Na_v1.5$ channel [43]. In 2013, a pathogenic variant in the *FGF12* gene was reported as a susceptibility gene associated with BrS due to reduction of I_{Na} [44–46]. Recently, pathogenic variations in the plakophilin-2 (*PKP2)* gene were reported to be associated with BrS [12, 13]. Correlation between the loss of expression of PKP2 and reduced I_{Na} has been identified in BrS patients. Apart from sodium channels, several potassium channels have also been related to BrS. The first one described was KCNE3, which codifies MiRP2 protein (β-subunit that regulates the potassium channel I_{to}), and that modulates some potassium currents in the heart [47]. Another gene associated to BrS is KCNJ8, also previously related to early repolarization syndrome (ERS) [48]. The KCNJ8 was described as a novel J-wave syndrome susceptibility gene and based on a gain of function in the cardiac $K_{(ATP)}$ Kir6.1 channel [49]. In 2011, Giudicessi et al. provided the first molecular and functional evidence implicating novel KCND3 gain-of-function pathogenic variants (Kir4.3 protein) in the pathogenesis and phenotypic expression of BrS, with enhanced I_{to} current gradient within the right ventricle where KCND*3* gene expression is the highest [50]. In 2014, Boczek et al. identified that mutations in the *SEMA3A* gene disrupted SEMA3A's ability to inhibit Kv4.3 channels, resulting in a significant gain of Kv4.3 current. Semaphorin 3 A (SEMA3A)-encoded semaphorin is a chemorepellent that disrupts neural patterning in the nervous and cardiac systems. This study is the first to demonstrate SEMA3A as a naturally occurring protein that selectively

inhibits Kv4.3 and SEMA3A as a possible BrS susceptibility gene through a Kv4.3 gain-of-function mechanism [51]. In 2011, novel variants in KCNE*5* appeared to cause gain-of-function effects on I_{to}. The KCNE5 gene is located in the X chromosome and encodes an auxiliary β-subunit for K channels [52]. Similar is the role of sulfonylurea receptor subunit 2 A (SUR2A), encoded by the ATP-binding cassette, subfamily C member 9 (*ABCC9*) gene [53]. Gain-of-function pathogenic variants in *ABCC9* induce changes in ATP-sensitive potassium (K-ATP) channels, and, when coupled with loss-of-function pathogenic variants in *SCN5A*, these pathogenic variants may underlie a severe arrhythmic phenotype of BrS. The BrS was also associated to HCN4, which encodes for the HCN4 channel protein (or If channel, hyperpolarization-activated cyclic nucleotide-gated potassium channel) [4]. Pathogenic variants in this gene also predispose to inherited sick sinus syndrome (SSS), and Long QT syndrome (LQTS) associated with bradycardia [54]. Calcium channels have also been associated with BrS. Pathogenic variants in the CACNA1C gene are responsible for a defective unit of the type-L calcium channel. Pathogenic variant of the CACNB2B gene leads to a defect in the L-type calcium channel. Both induce a loss of channel function [55]. In 2010 was reported *CACNA2D1* gene as responsible for BrS. The alpha-2/delta subunit of voltage-dependent calcium channels regulates current density and activation/inactivation kinetics of the calcium channel [56]. Finally, pathogenic variations have also been reported in the transient receptor potential melastatin protein number 4 (*TRPM4*) gene, a calcium-activated nonselective cation channel that is a member of a large family of transient receptor potential genes [57]. This gene is involved in conduction blocks, and the consequences of pathogenic variations are diverse. Thus, reduction or increase in TRPM4 channel function may reduce the availability of the sodium channel and lead to BrS.

Genetic Modulators

Recent studies reported several genetic and environmental modulators of the phenotype. It is well known that environmental factors may play a role in the predisposition to arrhythmias in patients with BrS (see below: environment section). The incomplete penetrance of the disease, as well as the variable expression, has brought into question the role of additional genetic factors in the final phenotype. Single nucleotide polymorphisms (SNPs) became one of the first players to be studied in defining this variability. The *SCN5A* polymorphism p.H558R is present in 20 % of the population. This polymorphism has been shown to partially restore the sodium current impaired by other co-occurring BrS causing pathogenic mutations in *SCN5A* [58]. Thus, this common variant is a genetic modifier of BrS among carriers of an *SCN5A* mutation, in whom the presence of this less common allele results in a less severe BrS phenotype [59]. Genetic variants in the *SCN5A* promoter region may also play a pathophysiologic role in BrS. One haplotype containing six polymorphisms in the *SCN5A* gene promoter has been identified and functionally linked to a reduced expression of the sodium current in the Japanese population [60]. As mentioned before, the *SCN10A* gene (neuronal sodium-channel $Na_v1.8$) has been shown to modulate *SCN5A* expression and the electrical function of the heart [39]. In the study, the transcriptional factor HEY2 was also identified as associated with BrS [40]. Other studies have shown the role of double mutations in causing a more severe phenotype [61, 62]. The role of these genetic modifiers variant in risk stratification has yet to be clearly defined. Recent data proposed the type of genetic pathogenic variant as a tool for risk stratification in BrS. In this study, patients and relatives with a truncated protein had a more severe phenotype and more severe conduction disorders. Despite that this is the proof of concept that some of the pathogenic variants appear to confer a worse prognosis, the use of these data in the clinical setting is not yet sufficient to take clinical decisions [63]. Recently, Stocchi et al. reported a study focused on potential association between mtDNA mutation/s and BrS. Their results show that a specific mtDNA variation responsible for BrS can be excluded. However, the authors found a high substitution rate in the mtDNA of BrS patients, suggesting this could be an important cofactor for a BrS phenotype modifier [64].

Brugada Syndrome and Overlapping Syndromes

Several of the families studied show several phenotypes among its members. These so-called "*overlapping syndromes*" represent a tremendous challenge to physicians for diagnosis and risk stratification. In this group, the ERS can be included [65]. The ERS and BrS share cellular, ionic, and ECG similarities (appearance of J-waves), representing parts of a phenotypic spectrum called "J-wave syndromes," although the degree to which ERS and BrS may overlap remains undetermined [66–68]. A recent study of Maeda et al. investigated the seasonal, weekly, and circadian distribution of VF in patients with BrS and ERS. In patients with ERS, episodes of VF mainly occurred during the winter months, in contrast with BrS patients whose episodes were most frequent in spring months. In patients with ERS, the frequencies of VF events by month negatively correlated with the monthly mean external air temperatures. In addition, VF occurred more frequently on weekends in patients with ERS and on weekdays in patients with BrS. Both ERS and BrS patients displayed a peak of VF event in nighttime

[69]. Other related entity is the Lev-Lenègre syndrome (also called progressive cardiac conduction disease (PCCD)). The presence of PCCD in the BrS families is not uncommon, as they both result from a reduction in the sodium current, and it has been described as a different expression of the same genetic phenotype [70, 71]. Another syndrome is the SSS, associated with dysfunction of the sinoatrial node (SAN). The course of SSS can be intermittent and unpredictable, related to the severity of the underlying heart disease [72–74]. Atrial fibrillation (AF) is reported to be the most common atrial arrhythmia found in BrS [75]. Approximately 15–20 % of patients with BrS develop supraventricular arrhythmias [76]. Some studies reported prolongation of atrial-His and His-ventricular (HV) conduction interval; these changes occur principally in patients with *SCN5A* pathogenic variants [77], and are consistent with a decreased excitability in the conduction system secondary to the loss of function of sodium-channel activity [78]. Another inherited arrhythmogenic disease is the LQTS, characterized by prolongation of the QT interval and susceptibility to ventricular tachyarrhythmias [79, 80]. The overlap between the LQTS type 3 and BrS phenotypes was also reported in families carrying pathogenic variants in *SCN5A* [81]. However, it is still unclear whether development of the BrS phenotype in a patient with LQTS type 3 is solely determined by the biophysical properties of the mutant channel, or by co-inherited genetic variations, gender, ethnicity, or other environmental factors [82]. It is also increasingly demonstrated that the etiologies of BrS and epilepsy may partly overlap. It has been reported that *SCN5A* pathogenic variants may confer susceptibility for recurrent seizure activity, supporting the emerging concept of a genetically determined cardiocerebral channelopathy [83, 84]. Currently, except for nonspecific cardiac arrhythmias described in two *SCN4A*-associated case reports [85, 86], no overlapping phenotypes between muscular and cardiac sodium channelopathies have been reported. In a recent study, Bissay et al. reported a high number of patients with coexisting BrS and sodium-channel myotonia, suggesting a possible impact of *SCN4A* variants on the pathophysiological mechanism underlying the development of a type 1 ECG pattern and of malignant arrhythmia symptoms in some patients with BrS [87].

Risk of Sudden Death

After the diagnosis of BrS is made, the next step is risk stratification, the main objective of which is the accurate identification and treatment of those individuals at high risk for SCD. To date, some markers of high risk in BrS patients have been clearly identified and accepted by all groups, but the issue of risk stratification of asymptomatic BrS patients remains controversial [88]. The reported annual rate of events has decreased from the first patients initially reported to the most recently published series, the change probably reflects the inherent referral bias during the first years following the description of a novel disease, in which particularly severe forms of the disease are most likely to be diagnosed [89].

A recent study of Sieira et al. shows that arrhythmic events in asymptomatic BrS patients are not insignificant (0.5 % annual incidence rate). In this cohort, inducibility of ventricular arrhythmias, spontaneous type I ECG, and the presence of sinus node dysfunction might be considered as risk factors and used to drive long-term management [90]. A meta-analysis also recently published showed that asymptomatic subjects with either spontaneous diagnostic ECG pattern or inducible ventricular arrhythmias at PVS are at an increased risk [91]. Several clinical variables have been demonstrated to predict a worse outcome in patients with BrS. In almost all the analysis, the presence of symptoms (e.g., syncope) before diagnosis, a spontaneous type 1 ECG at baseline and male sex have consistently shown to be related to the occurrence of cardiac events in follow-up [92, 93]. Little controversy exists on the value of a previous cardiac arrest as a risk marker for future events (between 17% and 62 % of patients will have a new arrhythmic event within 48 and 84 months of follow-up). Similarly, the presence of syncope identifies patients with a high risk for events (6–19 % at 24–39 months follow-up); thus, there is general agreement that these patients should be protected with an ICD irrespective to the presence of other risk factors. Spontaneous ECG type 1 has been identified as an independent predictor of ventricular arrhythmias in multivariate analysis of the largest cohort of BrS patients published to date [94] (hazard ratio (HR) = 1.8; 95 % confidence interval (CI) 1.03–3.33; $p = 0.04$) and in the majority of series by others.

Male sex has consistently shown a trend to present more arrhythmic events in all the studies, and even has been defined as an independent predictor for a worse outcome in a meta-analysis [95]. The BrS, besides ventricular tachycardia and VF, can also be complicated by nonventricular arrhythmias [96]. Hence, spontaneous AF, which can appear in 10–53 % of cases, has been shown to have prognostic significance and spontaneous AF was associated with a higher incidence of syncopal episodes (60.0 % vs. 22.2 %, $p < 0.03$) and documented VF (40.0 % vs. 14.3 %, $p < 0.05$) [97]. The risk of lethal or near-lethal arrhythmic episodes among previously asymptomatic patients with BrS varies according to the series: 8 % recurrence rate at 33 ± 39 months of follow-up reported by Brugada et al. [98], 6 % recurrence rate at 34 ± 44 months by Priori et al. [99], 1 % recurrence rate after 40 ± 50 months and 30 ± 21 months of follow-up, respectively, by Eckardt et al. [100] and Giustetto et al. [101], and finally, Probst et al. reported a 1.5 % recurrence rate at 31 months of follow-up [94].

Although large registries agree that EPS inducibility is greatest among BrS patients with previous SD or syncope [98], there is no consensus on the value of the EPS in predicting outcome in asymptomatic BrS patients. The results published by Brugada et al. [98] indicate that inducibility during EP study is an independent predictor of cardiac events, and Giustetto et al. [101] stressed the negative-predictive value (none of the patients with a negative EPS developed arrhythmic events vs. 15 % of patients with a positive EPS result during 30 ± 21 months of follow-up), while the rest of the registries failed to demonstrate this [94]. The largest series of BrS patients published so far found that inducibility of sustained ventricular arrhythmias was significantly associated with a shorter time to first arrhythmic event in the univariate analysis but when performing the multivariable analysis, inducibility did not predict arrhythmic events [94], results confirmed in a recent prospective study in previously asymptomatic patients. In 2015, a single-center study has been recently published, showing results in a cohort of 96 BrS patients with various clinical presentations and who have inducible VF using an aggressive PVS protocol. The authors reported an excellent protective effect of class 1 AAD (mainly quinidine) during EP testing and an excellent clinical outcome in drug-treated patients [102]. In addition, Sieira et al. published a series of 403 BrS cases. The authors conclude that PVS of the heart is a good predictor of outcome in individuals with BrS. It might be of special value guide further management when performed in asymptomatic individuals. The overall accuracy of the test makes it a suitable screening tool to reassure noninducible asymptomatic individuals [103].

A family history of SD or the presence of an *SCN5A* pathogenic variant has not been proven to be risk markers in any of the large studies conducted thus far [95]. However, recent data suggest that other genetic findings, such as the presence of pathogenic variants leading to a truncated protein, or the presence of common polymorphisms located in *SCN5A* may modulate the effect of pathogenic variants resulting in a counterbalance of its deleterious consequences resulting in a milder BrS phenotype, and suggests the possibility of polymorphisms as a useful tool in risk stratification. In addition, these polymorphisms may be possible targets for therapeutical interventions. A recent publication by Meregalli of 147 BrS patients with *SCN5A* identified pathogenic variants showed a significantly higher rate of syncope among patients carrying *SCN5A* truncation pathogenic variants (caused by a premature stop codon) and those with *SCN5A* missense pathogenic variants resulting in a decrease of more than 90 % of the I_{Na} (nonfunctional Na^+ channels), compared to patients with *SCN5A* missense pathogenic variants that produce a decrease of Na current (≤90 %). They could not demonstrate a higher rate of more serious arrhythmic events (SCD or VF) in those patients with pathogenic variants encoding nonfunctional Na^+ channels. The first two groups of patients also presented longer PR intervals in the basal ECG, and showed a greater increase of PR and QRS intervals after the class I AAD test. This is the first study that proposed the use of genetics in risk stratification for BrS. The recent finding that common polymorphisms located in *SCN5A* may modulate the effect of pathogenic variants causing, resulting in a counterbalance of its deleterious consequences resulting in a milder BrS phenotype, suggests the possibility of polymorphisms as a useful tool in risk stratification. In addition, these polymorphisms may be possible targets for therapeutical interventions.

In summary, several things are clear from the risk stratification data (Fig. 11.5): symptomatic patients are at a higher risk than asymptomatic ones; sudden death survivors are at a higher risk than patients with syncope, males are at a higher risk than females, patients with type I ECG at baseline have a higher risk than those who require class I antiarrhythmics, and asymptomatic patients may also die suddenly. This latter statement is based on the fact that the vast majority of symptomatic patients with BrS have remained asymptomatic for decades. Thus, at present the biggest challenge is the detection of these few asymptomatic patients who will develop symptoms.

Postulated Noninvasive Markers of Arrhythmic Risk in Brugada Syndrome

In an effort to solve the complex issue of risk stratification in BrS, several noninvasive methods have been postulated as markers of arrhythmic events among these patients: A decreased nocturnal standard deviation of the 5- minutes averaged NN intervals (SDANN) measured in Holter recordings; an S-wave width in V1 ≥ 80 mseg and ST-segment elevation ≥0.18 mV in V2; spontaneous changes in ST-segment, a corrected QT interval (QTc) higher than 460 ms in V2, prolonged T peak-T end (Tp-e) interval, and T p-e dispersion; the "aVR sign" (R-wave ≥0.3 mV or R/q ≥ 0.75 in lead aVR); prolonged QRS duration in precordial leads (r-J interval in V2 ≥ 90 ms and QRS ≥ 90 ms in V6; QRS ≥ 120 ms in V2); even an indicator of interventricular mechanical dyssynchrony has been recently found to be associated with a high risk of fatal or near-fatal arrhythmias in BrS. The usefulness of late potentials (LPs) assessed by signal-averaged ECG (SAECG) as a marker of high risk has been extensively studied by various groups, and a recent prospective study showed that positive LP was an independent marker of high risk in BS patients, with an HR of 10.9 (95 % CI: 1.1–104.3, $p = 0.038$), sensitivity of 95.7 %, specificity of 65 %, positive-predictive value of

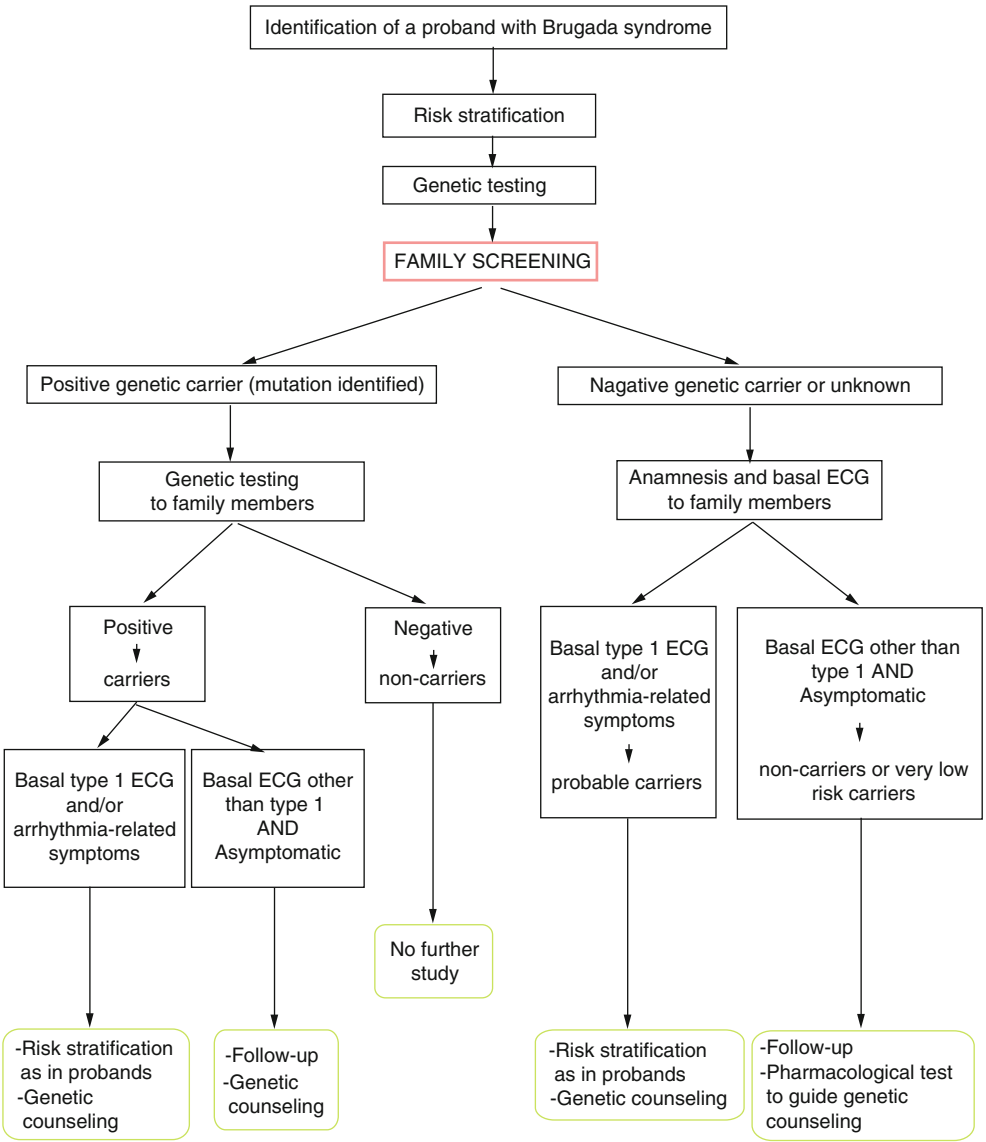

Fig. 11.5 Proposed algorithm for family screening after identification of a proband with confirmed Brugada syndrome

75.9 %, negative-predictive value of 92.9 %, and predictive accuracy of 81.4 %. Before including LP as a marker for risk, there is the need of more prospective studies, including more patients and with a longer follow-up, evaluating the value of different noninvasive markers of risk in BrS.

In conclusion, risk stratification is really the most controversial issue so far. Looking at the literature, there are two types of series: ones with almost no events at all during follow-up and in which obviously the lack of events brings to a negative value of any studied factor. Others have a reasonable number of events during follow-up and different factors have been studied and some of them have shown a value for stratification. The discussion is not which factor is more efficient to stratify patients but why there is this big and so far unexplained difference among series. Clearly, international consensus will have to be reached, on how to establish the diagnosis BrS based on the recent and updated studies that have been published.

Environment Modulators

It is important to underline that ECG patterns typically fluctuate over time in BrS patients and thus can change between the three BrS patterns or even may be transiently normal [104]. On the basis of this, it seems that repeated ECG recordings may be mandatory in patients with (suspected) BrS. It is worth noting that some factors can account for an ECG abnormality that can closely resemble the Brugada ECG (Table 11.1).

Importantly, some of them are conditions different from the syndrome and should be carefully excluded during

the differential diagnosis, whereas others may induce ST-segment elevation in the presence of a genetic predisposition (e.g., fever).

Modulating factors play a major role in the dynamic nature of the ECG and also may be responsible for the ST-segment elevation in genetically predisposed patients. Sympathovagal balance, hormones, metabolic factors, and pharmacologic agents, by means of specific effects on transmembrane ionic currents, are thought to not only modulate the ECG morphology but also explain the development of ventricular arrhythmias under certain conditions. Indeed, bradycardia and vagal tone may contribute to ST-segment elevation and arrhythmia initiation by decreasing calcium currents [105]. This explains the greater ST-segment elevation recorded in vagal settings [106], and the notorious incidence of cardiac arrhythmias and SD at night in patients with BrS [107].

The role of sex hormones is currently being established. Published data suggest that they might also play a role in the phenotypic manifestations of BrS [108]. For example, regression of the typical ECG features has been reported in castrated men [109], and levels of testosterone seem to be higher in Brugada male patients as compared with controls [110]. Two main hypotheses have been proposed for the sex distinction, perhaps interacting with each other: the sex-related intrinsic differences in ionic currents and the hormonal influence. Since children of both sexes have low levels of testosterone, the observations indicating that boys and girls with BrS do not show differences in phenotypic presentation are in line with the hormonal hypothesis [111]. Temperature may be an important modulator in some patients with BrS. Premature inactivation of the sodium channel has been shown to be accentuated at higher temperatures in some *SCN5A* pathogenic variants, suggesting that febrile states may unmask certain BrS patients or temporarily increase the risk of arrhythmias [112]. It seems that fever would be a particularly important trigger factor among the pediatric population [111]. Thus, temperature control is crucial in BrS patients.

Brugada Syndrome and Pregnancy

The sex-related differences in the phenotypic expression of BrS have been widely reported, but the basis for this distinction is not yet fully understood [113]. During pregnancy, autonomic and hemodynamic alterations occur, and estrogen and progesterone blood levels are reduced at peripartum. The largest study of pregnant women with BrS has been recently published by Rodríguez-Mañero et al. [114]. This study describes a relatively benign course of pregnancy and peripartum period among women with BrS. In addition, only a few cases exhibiting syncope were found, and the presence of syncope during pregnancy did not seem to be related to a worse outcome of the disease, neither in postpartum nor in peripartum periods. Nevertheless, the management of pregnant women affected by BrS should be very strict and multidisciplinary in cooperation with a cardiologist and an anesthesiologist [115]. Further clinical assessment and follow-up during the pregnant, postpartum, and peripartum periods should be performed, taking into account the favorable maternal and fetal outcome of disease.

Brugada Syndrome in Children

SCD accounts for approximately 20 % of sudden deaths on pediatric age group. Inherited arrhythmias are increasingly known as recognized as responsible for these deaths. The prevalence of BrS in children is variable among different studies, accounting up to 0.0098 % in Japanese series [116]. Despite massive progress in characterizing BrS, little is known about this disease in the pediatric population. In the initial description of the disease, three out of eight patients were children [1]. Since then, several authors have reported isolated cases [117, 118]. Finally, in 2007 Probst et al. published a study with 30 affected individuals under 16 years of age from 13 European institutions [111], the largest series in pediatric BrS patients. A Brugada ECG pattern in children, including its transiency, is similar as in adults. Moreover, there are no standardized data for optimal positioning of the right precordial leads in children and the shape of the chest in a growing body can lead into confusion. With all these characteristics, symptoms of syncope associated with typical ECG pattern should alert to the possibility of BrS. From asymptomatic patients (mainly discovered in routine ECG screening or familial screening) to sudden death, in children as in adults the whole spectrum of clinical presentations is possible. In contrast to adults, no male predominance in symptomatic patients is found. This could be related to lower levels of testosterone in prepubertal children [111]. Several case reports have demonstrated the importance of fever as a precipitating factor for ventricular arrhythmias in children, probably not because of special predisposition of children. Interestingly, as febrile state can unmask Brugada ECG pattern, it is recommended to perform a 12-lead ECG test during fever. Moreover, as febrile convulsions are a relatively common occurrence in childhood, we wonder if ECG should be part of the diagnostic routine when a febrile seizure occurs [111].

Regarding the drug challenge test, sodium-channel blockers test (ajmaline 1 mg/kg or flecainide 2 mg/kg over 10 minutes) [119] should be restricted to children with normal baseline ECG and typical symptoms with positive family history. As in adults, spontaneous type I ECG pattern is enough to establish diagnose and performing the drug

challenge can be dangerous. The existence of an age-dependent response to ajmaline challenge is an intriguing recent finding and might have relevant clinical implications [120]. In a recent study, Conte et al. showed that a repeated ajmaline challenge after puberty unmasked BrS in 23 % of relatives with a previously negative drug test performed during childhood. The ECG phenotype does not appear during childhood in most cases, but may develop later in response to hormonal, autonomic, or genetic factors [121]. However, repeating ajmaline challenge after puberty in patients with an initial negative drug test remains controversial and should be further investigated. Thus, controversy exists whether to practice drug challenge for asymptomatic normal-ECG children of BrS patients and when it should be performed. Moreover, taking into account that false-negative result can be seen in up to 30 %, depending on the drug used for provocation testing, the question remains whether a second test should be performed some years later.

If controversy exists whether performing EPS testing or not should be performed in adult population, this is even more so in the discussion whether children should undergo programmed extrastimulation techniques to test malignant arrhythmias inducibility [98]. When indicated, the protocol remains the same as in the adult population. As discussed in other parts of this chapter, BrS can overlap with other entities as LQTS type 3 or Lev-Lenègre syndrome. Bradyarrhythmias can be a cause of death in these patients, thus pacemaker implantation is mandatory in certain cases [111]. Hydroquinidine has shown to be a good alternative for ICD implantation at a short follow-up in children who are at risk of developing malignant arrhythmias, but further studies are required [111]. Patients presenting with aborted SD and syncope with spontaneous type I ECG are clearly at a high risk of malignant arrhythmias, thus ICD should be considered, irrespective of age. Special approaches for ICD implantation have been described for small children when needed. Finally, concerning family screening, first-degree relatives of all BrS patients should be screened by clinical examination, interrogation, and performance of a 12-lead ECG (basal and upper intercostal space recording).

Genetic testing should be offered to index cases and, when a pathogenic variant is identified, DNA testing should be performed in children, regardless of their age, in order to follow recommendation on fever control and avoidance of listed drugs (www.brugadadrugs.org). Pathogenic-variant carriers should be annually screened for ECG when asymptomatic or in the event of any potentially cardiac symptom. Genetic testing is not perfect, only 30 % of families are genetically identified. In the event that genetic testing is negative in proband, ajmaline testing is not recommended in asymptomatic children with a normal ECG. Data prove that these individuals are at low risk. In the era of personalized medicine using high-throughput tools NGS is the best cost-effective approach to identify the cause of the disease. The main problems in using NGS technologies are the large amount of data provided and the insufficient experience to translate this information into clinical practice [122, 123]. One of the crucial elements for the correct interpretation of pathogenicity is the genotype–phenotype correlation in families. This leads to the need for each family to be studied separately, analyzing the variations in each relative, and correlating clinical-genetic information. Final decisions should be made by a group consensus based on the experience of each of the members of the working group in each institution dedicated to this purpose.

Brugada Syndrome in Older Individuals

The fourth decade of life is the mean age of clinical manifestations of BrS, mainly in men. The clinical course and prognosis of BrS in older individuals remains largely unknown. Recently, Conte at al. published a systematic analysis of BrS in the aging population, reporting a benign prognosis and lower-risk category of BrS patients in comparison to younger patients. Consequently, older patients presented less ventricular arrhythmias and less family history of SCD. However, two main topics remain controversial: use of drug-induced tests and device-guided management. Thus, despite Conte et al. reporting in the same abovementioned study that "*BrS was diagnosed after ajmaline challenge in 86 % of elderly patients,*" the value of unmasking a type I ECG as well as its safety has not been systematically assessed [124]. Regarding the use of an ICD, a consensus conference reported that older BrS patients with syncope should undergo ICD implantation if life expectancy is at least 6 months [8]. Recently, Kamakura et al. reported that long-term follow-up of high-risk BrS patients with ICD showed a low incidence of VF in those aged >70 years. Considering the increasing risk of inappropriate shocks because of the relatively late onset of supraventricular tachycardia and lead failures, avoidance of ICD implantation or replacement may be considered in elderly BrS patients who remain free from VF until 70 years of age [125]. However, clinical decisions regarding both controversies should be analyzed on a case-by-case basis.

Summary

The BrS is a familial cardiac disease leading to ventricular arrhythmias and SCD. It is characterized by a typical ECG morphology and an increased susceptibility to present ventricular arrhythmias and sudden death in the absence of structural heart disease. The characteristic ECG pattern, known as coved-type or type 1, consists of a persistent ST-segment elevation in right precordial leads followed by

negative T-waves, and must be distinguished from other conditions that also present with right ST-segment elevation. To date, 19 genes have been associated with the disease, being *SCN5A* the most common gene. However, only 30–40 % of diagnosed cases are attributable to pathogenic variants in known genes, emphasizing the need for further genetic studies. Mechanistic processes responsible for variable expressivity and incomplete penetrance remain to be clarified, impeding proper clinical diagnosis, risk stratification, and management. The ICD is the only proven effective therapy for patients at high risk so far, despite several pharmacological approaches that are also currently being used. Combined preclinical, clinical, and comprehensive genetic studies in large cohorts will be indispensable for improving the current guidelines to diagnose BrS, stratify the risk of SCD, and prevent lethal episodes in families.

Take-Home Message

- The BrS is a rare inherited ion channel caused by an alteration of the ionic currents leading to "*right bundle branch block, ST segment elevation and sudden death syndrome,*" with incomplete penetrance and variable expressivity.
- The diagnosis of BrS occurs in patients with ECG pattern type 1 and any of the following clinical features: ventricular fibrillation documented, polymorphic ventricular tachycardia (PVT), inducibility of PVT with programmed electrical stimulation, family history of SCD under 45 years, type 1 ECG in family members, unexplained syncope or nocturnal agonal respiration, and no structural heart alterations.
- Ajmaline is the drug with better results unmasking the BrS type I pattern.
- The main current challenge is the early detection of asymptomatic patients who are at risk of developing symptoms and even SCD, sometimes as the first manifestation of the disease.
- The ECG should be always performed as part of a diagnosis in BrS when a febrile episode occurs, especially in children.
- The ICD is the most effective strategy for the prevention of SCD in patients suffering from BrS.

References

1. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. J Am Coll Cardiol. 1992;20(6):1391–6.
2. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, et al. Brugada syndrome: report of the second consensus conference. Heart Rhythm. 2005;2(4):429–40.
3. Miyasaka Y, Tsuji H, Yamada K, Tokunaga S, Saito D, Imuro Y, et al. Prevalence and mortality of the Brugada-type electrocardiogram in one city in Japan. J Am Coll Cardiol. 2001;38(3):771–4.
4. Hermida JS, Lemoine JL, Aoun FB, Jarry G, Rey JL, Quiret JC. Prevalence of the brugada syndrome in an apparently healthy population. Am J Cardiol. 2000;86(1):91–4.
5. Donohue D, Tehrani F, Jamehdor R, Lam C, Movahed MR. The prevalence of Brugada ECG in adult patients in a large university hospital in the western United States. Am Heart Hosp J. 2008; 6(1):48–50.
6. Vatta M, Dumaine R, Varghese G, Richard TA, Shimizu W, Aihara N, et al. Genetic and biophysical basis of sudden unexplained nocturnal death syndrome (SUNDS), a disease allelic to Brugada syndrome. Hum Mol Genet. 2002;11(3):337–45.
7. Ikeda T, Abe A, Yusu S, Nakamura K, Ishiguro H, Mera H, et al. The full stomach test as a novel diagnostic technique for identifying patients at risk of Brugada syndrome. J Cardiovasc Electrophysiol. 2006;17(6):602–7.
8. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, et al. Brugada syndrome: report of the second consensus conference: endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. Circulation. 2005;111(5):659–70.
9. Frustaci A, Priori SG, Pieroni M, Chimenti C, Napolitano C, Rivolta I, et al. Cardiac histological substrate in patients with clinical phenotype of Brugada syndrome. Circulation. 2005;112(24):3680–7.
10. Frustaci A, Russo MA, Chimenti C. Structural myocardial abnormalities in asymptomatic family members with Brugada syndrome and SCN5A gene mutation. Eur Heart J. 2009;30(14):1763.
11. Frigo G, Rampazzo A, Bauce B, Pilichou K, Beffagna G, Danieli GA, et al. Homozygous SCN5A mutation in Brugada syndrome with monomorphic ventricular tachycardia and structural heart abnormalities. Europace. 2007;9(6):391–7 Epub 2007/04/20.
12. Cerrone M, Delmar M. Desmosomes and the sodium channel complex: implications for arrhythmogenic cardiomyopathy and Brugada syndrome. Trends Cardiovasc Med. 2014;24(5):184–90 Epub 2014/03/25.
13. Cerrone M, Lin X, Zhang M, Agullo-Pascual E, Pfenniger A, Chkourko Gusky H, et al. Missense mutations in plakophilin-2 cause sodium current deficit and associate with a Brugada syndrome phenotype. Circulation. 2014;129(10):1092–103 Epub 2013/12/20.
14. Nademanee A. Fibrosis, connexin-43, and conduction abnormalities in the Brugada syndrome. J Am Coll Cardiol. 2015;66: 1976–86.
15. Conte G, Sieira J, Ciconte G, de Asmundis C, Chierchia GB, Baltogiannis G, et al. Implantable cardioverter-defibrillator therapy in Brugada syndrome: a 20-year single-center experience. J Am Coll Cardiol. 2015;65(9):879–88 Epub 2015/03/07.
16. Sarkozy A, Boussy T, Kourgiannides G, Chierchia GB, Richter S, De Potter T, et al. Long-term follow-up of primary prophylactic implantable cardioverter-defibrillator therapy in Brugada syndrome. Eur Heart J. 2007;28(3):334–44.
17. Rosso R, Glick A, Glikson M, Wagshal A, Swissa M, Rosenhek S, et al. Outcome after implantation of cardioverter defibrillator [corrected] in patients with Brugada syndrome: a multicenter Israeli study (ISRABRU). Isr Med Assoc J. 2008;10(6):435–9.
18. Zipes DP, Camm AJ, Borggrefe M, Buxton AE, Chaitman B, Fromer M, et al. [Guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. Executive summary]. Rev Esp Cardiol. 2006;59(12):1328 .Guias de Practica Clinica del ACC/AHA/ESC 2006 sobre el manejo de pacientes con arritmias ventriculares y la prevencion de la muerte cardiaca subita. Version resumida
19. Rodriguez-Manero M, de Asmundis C, Sacher F, Arbelo E, Probst V, Castro-Hevia J, et al. T-wave oversensing in patients with Brugada syndrome: true bipolar versus integrated bipolar implantable cardioverter defibrillator leads: multicenter retro-

spective study. Circ Arrhythm Electrophysiol. 2015;8(4):792–8 Epub 2015/06/05.
20. Maury P, Hocini M, Haissaguerre M. Electrical storms in Brugada syndrome: review of pharmacologic and ablative therapeutic options. Indian Pacing Electrophysiol J. 2005;5(1):25–34.
21. Schweizer PA, Becker R, Katus HA, Thomas D. Successful acute and long-term management of electrical storm in Brugada syndrome using orciprenaline and quinine/quinidine. Clin Res Cardiol. 2010;99(7):467–70.
22. Nakagawa E, Takagi M, Tatsumi H, Yoshiyama M. Successful radiofrequency catheter ablation for electrical storm of ventricular fibrillation in a patient with Brugada syndrome. Circ J. 2008;72(6): 1025–9.
23. Morita H, Zipes DP, Morita ST, Lopshire JC, Wu J. Epicardial ablation eliminates ventricular arrhythmias in an experimental model of Brugada syndrome. Heart Rhythm. 2009;6(5): 665–71.
24. Nademanee K, Veerakul G, Chandanamattha P, Chaothawee L, Ariyachaipanich A, Jirasirirojanakorn K, et al. Prevention of ventricular fibrillation episodes in brugada syndrome by catheter ablation over the anterior right ventricular outflow tract epicardium. Circulation. 2011;123(12):1270–9.
25. Szeplaki G, Ozcan EE, Osztheimer I, Tahin T, Merkely B, Geller L. Ablation of the epicardial substrate in the right ventricular outflow tract in a patient with brugada syndrome refusing implantable cardioverter defibrillator therapy. Can J Cardiol. 2014;30(10):1249 e9–e11 Epub 2014/09/30.
26. Forkmann M, Tomala J, Huo Y, Mayer J, Christoph M, Wunderlich C, et al. Epicardial Ventricular Tachycardia Ablation in a Patient With Brugada ECG Pattern and Mutation of PKP2 and DSP Genes. Circ Arrhythm Electrophysiol. 2015;8(2):505–7 Epub 2015/04/23.
27. Brugada J, Pappone C, Berruezo A, Vicedomini G, Manguso F, Ciconte G, et al. Brugada syndrome phenotype elimination by epicardial substrate ablation. Circ Arrhythm Electrophysiol. 2015. Epub 2015/08/21.
28. Morita H, Zipes DP, Wu J. Brugada syndrome: insights of ST elevation, arrhythmogenicity, and risk stratification from experimental observations. Heart Rhythm. 2009;6(11 Suppl):S34–43.
29. Elizari MV, Levi R, Acunzo RS, Chiale PA, Civetta MM, Ferreiro M, et al. Abnormal expression of cardiac neural crest cells in heart development: a different hypothesis for the etiopathogenesis of Brugada syndrome. Heart Rhythm. 2007;4(3):359–65.
30. Antzelevitch C. Brugada syndrome. Pacing Clin Electrophysiol. 2006;29(10):1130–59.
31. Meregalli PG, Wilde AA, Tan HL. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder, or more? Cardiovasc Res. 2005;67(3):367–78.
32. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature. 1998;392(6673):293–6.
33. Kapplinger JD, Tester DJ, Alders M, Benito B, Berthet M, Brugada J, et al. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. Heart Rhythm. 2010;7(1):33–46.
34. Eastaugh LJ, James PA, Phelan DG, Davis AM. Brugada syndrome caused by a large deletion in SCN5A only detected by multiplex ligation-dependent probe amplification. J Cardiovasc Electrophysiol. 2011;22(9):1073–6 Epub 2011/02/04.
35. Selga E, Campuzano O, Pinsach-Abuin ML, Perez-Serra A, Mademont-Soler I, Riuro H, et al. Comprehensive Genetic Characterization of a Spanish Brugada Syndrome Cohort. PloS one. 2015;10(7):e0132888 .Epub 2015/07/15
36. Watanabe H, Koopmann TT, Le Scouarnec S, Yang T, Ingram CR, Schott JJ, et al. Sodium channel beta1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. J Clin Invest. 2008;118(6):2260–8.
37. Hu D, Barajas-Martinez H, Burashnikov E, Springer M, Wu Y, Varro A, et al. A mutation in the beta 3 subunit of the cardiac sodium channel associated with Brugada ECG phenotype. Circ Cardiovasc Genet. 2009;2(3):270–8.
38. Riuro H, Beltran-Alvarez P, Tarradas A, Selga E, Campuzano O, Verges M, et al. A missense mutation in the sodium channel beta2 subunit reveals SCN2B as a new candidate gene for Brugada syndrome. Hum Mutat. 2013;34(7):961–6 Epub 2013/04/06.
39. Hu D, Barajas-Martinez H, Pfeiffer R, Dezi F, Pfeiffer J, Buch T, et al. Mutations in SCN10A are responsible for a large fraction of cases of Brugada syndrome. J Am Coll Cardiol. 2014;64(1):66–79 Epub 2014/07/07.
40. Bezzina CR, Barc J, Mizusawa Y, Remme CA, Gourraud JB, Simonet F, et al. Common variants at SCN5A-SCN10A and HEY2 are associated with Brugada syndrome, a rare disease with high risk of sudden cardiac death. Nat Genet. 2013;45(9):1044–9 Epub 2013/07/23.
41. London B, Michalec M, Mehdi H, Zhu X, Kerchner L, Sanyal S, et al. Mutation in glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) decreases cardiac Na + current and causes inherited arrhythmias. Circulation. 2007;116(20):2260–8.
42. Kattygnarath D, Maugenre S, Neyroud N, Balse E, Ichai C, Denjoy I, et al. MOG1: a new susceptibility gene for Brugada syndrome. Circ Cardiovasc Genet. 2011;4(3):261–8.
43. Ishikawa T, Sato A, Marcou CA, Tester DJ, Ackerman MJ, Crotti L, et al. A novel disease gene for Brugada syndrome: sarcolemmal membrane-associated protein gene mutations impair intracellular trafficking of hNav1.5. Circ Arrhythm Electrophysiol. 2012;5(6):1098–107 Epub 2012/10/16.
44. Hennessey JA, Marcou CA, Wang C, Wei EQ, Tester DJ, Torchio M, et al. FGF12 is a candidate Brugada syndrome locus. Heart Rhythm. 2013;10(12):1886–94 Epub 2013/10/08.
45. Hennessey JA, Wei EQ, Pitt GS. Fibroblast growth factor homologous factors modulate cardiac calcium channels. Circ Res. 2013;113(4):381–8 Epub 2013/06/28.
46. Wang C, Hennessey JA, Kirkton RD, Graham V, Puranam RS, Rosenberg PB, et al. Fibroblast growth factor homologous factor 13 regulates Na + channels and conduction velocity in murine hearts. Circ Res. 2011;109(7):775–82 Epub 2011/08/06.
47. Delpon E, Cordeiro JM, Nunez L, Thomsen PE, Guerchicoff A, Pollevick GD, et al. Functional effects of KCNE3 mutation and its role in the development of Brugada syndrome. Circ Arrhythm Electrophysiol. 2008;1(3):209–18.
48. Haissaguerre M, Chatel S, Sacher F, Weerasooriya R, Probst V, Loussouarn G, et al. Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/KATP channel. J Cardiovasc Electrophysiol. 2009;20(1):93–8.
49. Medeiros-Domingo A, Tan BH, Crotti L, Tester DJ, Eckhardt L, Cuoretti A, et al. Gain-of-function mutation S422 L in the KCNJ8-encoded cardiac K(ATP) channel Kir6.1 as a pathogenic substrate for J-wave syndromes. Heart Rhythm. 2010;7(10):1466–71.
50. Giudicessi JR, Ye D, Tester DJ, Crotti L, Mugione A, Nesterenko VV, et al. Transient outward current (I(to)) gain-of-function mutations in the KCND3-encoded Kv4.3 potassium channel and Brugada syndrome. Heart Rhythm. 2011;8(7):1024–32.
51. Boczek NJ, Ye D, Johnson EK, Wang W, Crotti L, Tester DJ, et al. Characterization of SEMA3A-encoded semaphorin as a naturally occurring Kv4.3 protein inhibitor and its contribution to Brugada syndrome. Circ Res. 2014;115(4):460–9 Epub 2014/06/26.
52. Ohno S, Zankov DP, Ding WG, Itoh H, Makiyama T, Doi T, et al KCNE5 (KCNE1L) variants are novel modulator of Brugada syndrome and idiopathic ventricular fibrillation. Circ Arrhythm Electrophysiol. 2011.
53. Hu D, Barajas-Martinez H, Terzic A, Park S, Pfeiffer R, Burashnikov E, et al. ABCC9 is a novel Brugada and early repolarization syndrome susceptibility gene. Int J Cardiol. 2014;171(3):431–42 Epub 2014/01/21.

54. Ueda K, Hirano Y, Higashiuesato Y, Aizawa Y, Hayashi T, Inagaki N, et al. Role of HCN4 channel in preventing ventricular arrhythmia. J Hum Genet. 2009;54(2):115–21.
55. Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, et al. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation. 2007;115(4):442–9.
56. Burashnikov E, Pfeiffer R, Barajas-Martinez H, Delpon E, Hu D, Desai M, et al. Mutations in the cardiac L-type calcium channel associated with inherited J-wave syndromes and sudden cardiac death. Heart Rhythm. 2010;7(12):1872–82.
57. Stallmeyer B, Zumhagen S, Denjoy I, Duthoit G, Hebert JL, Ferrer X, et al. Mutational spectrum in the Ca(2+)--activated cation channel gene TRPM4 in patients with cardiac conductance disturbances. Hum Mutat. 2012;33(1):109–17 Epub 2011/09/03.
58. Poelzing S, Forleo C, Samodell M, Dudash L, Sorrentino S, Anaclerio M, et al. SCN5A polymorphism restores trafficking of a Brugada syndrome mutation on a separate gene. Circulation. 2006;114(5):368–76.
59. Lizotte E, Junttila MJ, Dube MP, Hong K, Benito B, Dez M, et al. Genetic modulation of brugada syndrome by a common polymorphism. J Cardiovasc Electrophysiol. 2009;20(10):1137–41.
60. Bezzina CR, Shimizu W, Yang P, Koopmann TT, Tanck MW, Miyamoto Y, et al. Common sodium channel promoter haplotype in asian subjects underlies variability in cardiac conduction. Circulation. 2006;113(3):338–44.
61. Makita N, Mochizuki N, Tsutsui H. Absence of a trafficking defect in R1232W/T1620 M, a double SCN5A mutant responsible for Brugada syndrome. Circ J. 2008;72(6):1018–9.
62. Cordeiro JM, Barajas-Martinez H, Hong K, Burashnikov E, Pfeiffer R, Orsino AM, et al. Compound heterozygous mutations P336L and I1660V in the human cardiac sodium channel associated with the Brugada syndrome. Circulation. 2006;114(19):2026–33.
63. Meregalli PG, Tan HL, Probst V, Koopmann TT, Tanck MW, Bhuiyan ZA, et al. Type of SCN5A mutation determines clinical severity and degree of conduction slowing in loss-of-function sodium channelopathies. Heart Rhythm. 2009;6(3):341–8.
64. Stocchi L, Polidori E, Potenza L, Rocchi MB, Calcabrini C, Busacca P, et al. Mutational analysis of mitochondrial DNA in Brugada syndrome. Cardiovasc Pathol. 2015; Epub 2015/11/10. Cardiovasc Pathol. 2016 Jan–Feb;25(1):47–54. doi: 10.1016/j.carpath.2015.10.001. Epub 2015 Oct 22.
65. Miyazaki S, Shah AJ, Haissaguerre M. Early repolarization syndrome – a new electrical disorder associated with sudden cardiac death. Circ J. 2010;74(10):2039–44.
66. Antzelevitch C, Yan GX. J wave syndromes. Heart Rhythm. 2010;7(4):549–58.
67. McIntyre WF, Perez-Riera AR, Femenia F, Baranchuk A. Coexisting early repolarization pattern and Brugada syndrome: recognition of potentially overlapping entities. J Electrocardiol. 2011;45(3):195–8.
68. Antzelevitch C, Yan GX. J-wave syndromes. from cell to bedside. J Electrocardiol. 2011;44(6):656–61.
69. Maeda S, Takahashi Y, Nogami A, Yamauchi Y, Osaka Y, Shirai Y, et al. Seasonal, weekly, and circadian distribution of ventricular fibrillation in patients with J-wave syndrome from the J-PREVENT registry. J Arrhythm. 2015;31(5):268–73 Epub 2015/11/10.
70. Schott JJ, Alshinawi C, Kyndt F, Probst V, Hoorntje TM, Hulsbeek M, et al. Cardiac conduction defects associate with mutations in SCN5A. Nat Genet. 1999;23(1):20–1.
71. Wang DW, Viswanathan PC, Balser JR, George Jr AL, Benson DW. Clinical, genetic, and biophysical characterization of SCN5A mutations associated with atrioventricular conduction block. Circulation. 2002;105(3):341–6.
72. Brignole M. Sick sinus syndrome. Clin Geriatr Med. 2002;18(2):211–27.
73. Benson DW, Wang DW, Dyment M, Knilans TK, Fish FA, Strieper MJ, et al. Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (SCN5A). J Clin Invest. 2003;112(7):1019–28.
74. Smits JP, Koopmann TT, Wilders R, Veldkamp MW, Opthof T, Bhuiyan ZA, et al. A mutation in the human cardiac sodium channel (E161K) contributes to sick sinus syndrome, conduction disease and Brugada syndrome in two families. J Mol Cell Cardiol. 2005;38(6):969–81.
75. Yamada T, Watanabe I, Okumura Y, Takagi Y, Okubo K, Hashimoto K, et al. Atrial electrophysiological abnormality in patients with Brugada syndrome assessed by P-wave signal-averaged ECG and programmed atrial stimulation. Circ J. 2006;70(12):1574–9.
76. Letsas KP, Sideris A, Efremidis M, Pappas LK, Gavrielatos G, Filippatos GS, et al. Prevalence of paroxysmal atrial fibrillation in Brugada syndrome: a case series and a review of the literature. J Cardiovasc Med (Hagerstown). 2007;8(10):803–6.
77. Smits JP, Eckardt L, Probst V, Bezzina CR, Schott JJ, Remme CA, et al. Genotype-phenotype relationship in Brugada syndrome: electrocardiographic features differentiate SCN5A-related patients from non-SCN5A-related patients. J Am Coll Cardiol. 2002;40(2):350–6.
78. Francis J, Antzelevitch C. Atrial fibrillation and Brugada syndrome. J Am Coll Cardiol. 2008;51(12):1149–53.
79. Kramer DB, Zimetbaum PJ. Long-QT syndrome. Cardiol Rev. 2011;19(5):217–25.
80. Bezzina C, Veldkamp MW, van Den Berg MP, Postma AV, Rook MB, Viersma JW, et al. A single Na(+) channel mutation causing both long-QT and Brugada syndromes. Circ Res. 1999;85(12):1206–13.
81. Grant AO, Carboni MP, Neplioueva V, Starmer CF, Memmi M, Napolitano C, et al. Long QT syndrome, Brugada syndrome, and conduction system disease are linked to a single sodium channel mutation. J Clin Invest. 2002;110(8):1201–9.
82. Makita N. Phenotypic overlap of cardiac sodium channelopathies: individual-specific or mutation-specific? Circ J. 2009;73(5):810–7.
83. Parisi P, Oliva A, Coll Vidal M, Partemi S, Campuzano O, Iglesias A, et al. Coexistence of epilepsy and Brugada syndrome in a family with SCN5A mutation. Epilepsy Research. 2013;105(3):415–8 Epub 2013/03/30.
84. Camacho Velasquez JL, Rivero Sanz E, Velazquez Benito A, Mauri Llerda JA. Epilepsy and Brugada syndrome. Neurologia. 2015. Epub 2015/06/04. Epilepsia y sindrome de Brugada.
85. Pereon Y, Lande G, Demolombe S, Nguyen-The-Tich S, Sternberg D, Le Marec H, et al. Paramyotonia congenita with an SCN4A mutation affecting cardiac repolarization. Neurology. 2003;60(2):340–2 Epub 2003/01/29.
86. Maffe S, Signorotti F, Perucca A, Bielli M, Hladnik U, Ragazzoni E, et al. Atypical arrhythmic complications in familial hypokalemic periodic paralysis. J Cardiovasc Med (Hagerstown). 2009;10(1):68–71 Epub 2009/08/27.
87. Bissay V, Van Malderen SC, Keymolen K, Lissens W, Peeters U, Daneels D, et al. SCN4A variants and Brugada syndrome: phenotypic and genotypic overlap between cardiac and skeletal muscle sodium channelopathies. Eur J Hum Genet. 2015; Epub 2015/06/04.
88. Adler A, Rosso R, Chorin E, Havakuk O, Antzelevitch C, Viskin S. Risk stratification in Brugada syndrome: clinical characteristics, electrocardiographic parameters, and auxiliary testing. Heart Rhythm. 2015; Epub 2015/09/06.
89. Kamakura S, Ohe T, Nakazawa K, Aizawa Y, Shimizu A, Horie M, et al. Long-term prognosis of probands with Brugada-pattern ST-elevation in leads V1-V3. Circ Arrhythm Electrophysiol. 2009;2(5):495–503.
90. Sieira J, Ciconte G, Conte G, Chierchia GB, de Asmundis C, Baltogiannis G, et al. Asymptomatic Brugada Syndrome: clinical characterization and long-term prognosis. Circ Arrhythm Electrophysiol. 2015;8(5):1144–50 Epub 2015/07/29.

91. Letsas KP, Liu T, Shao Q, Korantzopoulos P, Giannopoulos G, Vlachos K, et al. Meta-Analysis on Risk Stratification of Asymptomatic Individuals With the Brugada Phenotype. Am J Cardiol. 2015;116(1):98–103 Epub 2015/05/03.
92. Brugada J, Brugada R, Brugada P. Right bundle-branch block and ST-segment elevation in leads V1 through V3: a marker for sudden death in patients without demonstrable structural heart disease. Circulation. 1998;97(5):457–60.
93. Benito B, Sarkozy A, Mont L, Henkens S, Berruezo A, Tamborero D, et al. Gender differences in clinical manifestations of Brugada syndrome. J Am Coll Cardiol. 2008;52(19):1567–73.
94. Probst V, Veltmann C, Eckardt L, Meregalli PG, Gaita F, Tan HL, et al. Long-term prognosis of patients diagnosed with Brugada syndrome: Results from the FINGER Brugada Syndrome Registry. Circulation. 2010;121(5):635–43.
95. Gehi AK, Duong TD, Metz LD, Gomes JA, Mehta D. Risk stratification of individuals with the Brugada electrocardiogram: a meta-analysis. J Cardiovasc Electrophysiol. 2006;17(6):577–83.
96. Liu B, Guo C, Fang D, Guo J. Clinical observations of supraventricular arrhythmias in patients with brugada syndrome. Inter J Clin Exp. 2015;8(8):14520–6 Epub 2015/11/10.
97. Kusano KF, Taniyama M, Nakamura K, Miura D, Banba K, Nagase S, et al. Atrial fibrillation in patients with Brugada syndrome relationships of gene mutation, electrophysiology, and clinical backgrounds. J Am Coll Cardiol. 2008;51(12):1169–75.
98. Brugada J, Brugada R, Brugada P. Determinants of sudden cardiac death in individuals with the electrocardiographic pattern of Brugada syndrome and no previous cardiac arrest. Circulation. 2003;108(25):3092–6.
99. Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Giordano U, et al. Natural history of Brugada syndrome: insights for risk stratification and management. Circulation. 2002;105(11):1342–7.
100. Eckardt L, Probst V, Smits JP, Bahr ES, Wolpert C, Schimpf R, et al. Long-term prognosis of individuals with right precordial ST-segment-elevation Brugada syndrome. Circulation. 2005;111(3):257–63.
101. Giustetto C, Drago S, Demarchi PG, Dalmasso P, Bianchi F, Masi AS, et al. Risk stratification of the patients with Brugada type electrocardiogram: a community-based prospective study. Europace. 2009;11(4):507–13.
102. Belhassen B, Rahkovich M, Michowitz Y, Glick A, Viskin S. Management of Brugada syndrome: A 33-year experience using electrophysiologically-guided therapy with class 1 a antiarrhythmic drugs. Circ Arrhythm Electrophysiol. 2015; Epub 2015/09/12.
103. Sieira J, Conte G, Ciconte G, de Asmundis C, Chierchia GB, Baltogiannis G, et al. Prognostic value of programmed electrical stimulation in Brugada syndrome: 20 years experience. Circ Arrhythm Electrophysiol. 2015;8(4):777–84 Epub 2015/04/24.
104. Veltmann C, Schimpf R, Echternach C, Eckardt L, Kuschyk J, Streitner F, et al. A prospective study on spontaneous fluctuations between diagnostic and non-diagnostic ECGs in Brugada syndrome: implications for correct phenotyping and risk stratification. Eur Heart J. 2006;27(21):2544–52.
105. Yan GX, Antzelevitch C. Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST-segment elevation. Circulation. 1999;100(15):1660–6.
106. Tatsumi H, Takagi M, Nakagawa E, Yamashita H, Yoshiyama M. Risk stratification in patients with Brugada syndrome: analysis of daily fluctuations in 12-lead electrocardiogram (ECG) and signal-averaged electrocardiogram (SAECG). J Cardiovasc Electrophysiol. 2006;17(7):705–11.
107. Matsuo K, Kurita T, Inagaki M, Kakishita M, Aihara N, Shimizu W, et al. The circadian pattern of the development of ventricular fibrillation in patients with Brugada syndrome. Eur Heart J. 1999;20(6):465–70.
108. Benito B, Brugada R, Brugada J, Brugada P. Brugada syndrome. Prog Cardiovasc Dis. 2008;51(1):1–22.
109. Matsuo K, Akahoshi M, Seto S, Yano K. Disappearance of the Brugada-type electrocardiogram after surgical castration: a role for testosterone and an explanation for the male preponderance. Pacing Clin Electrophysiol. 2003;26(7 Pt 1):1551–3.
110. Shimizu W, Matsuo K, Kokubo Y, Satomi K, Kurita T, Noda T, et al. Sex hormone and gender difference--role of testosterone on male predominance in Brugada syndrome. J Cardiovasc Electrophysiol. 2007;18(4):415–21.
111. Probst V, Denjoy I, Meregalli PG, Amirault JC, Sacher F, Mansourati J, et al. Clinical aspects and prognosis of Brugada syndrome in children. Circulation. 2007;115(15):2042–8.
112. Dumaine R, Towbin JA, Brugada P, Vatta M, Nesterenko DV, Nesterenko VV, et al. Ionic mechanisms responsible for the electrocardiographic phenotype of the Brugada syndrome are temperature dependent. Circ Res. 1999;85(9):803–9.
113. Tadros R, Ton AT, Fiset C, Nattel S. Sex differences in cardiac electrophysiology and clinical arrhythmias: epidemiology, therapeutics, and mechanisms. Can J Cardiol. 2014;30(7):783–92 Epub 2014/06/28.
114. Rodriguez-Manero M, Casado-Arroyo R, Sarkozy A, Leysen E, Sieira JA, Namdar M, et al. The clinical significance of pregnancy in Brugada syndrome. Rev Esp Cardiol (Engl Ed). 2014;67(3):176–80 Epub 2014/04/30.
115. Giambanco L, Incandela D, Maiorana A, Alio W, Alio L. Brugada syndrome and pregnancy: highlights on antenatal and prenatal management. Case Reports in Obstetrics and Gynecology. 2014;2014:531648 .Epub 2014/06/26
116. Yamakawa Y, Ishikawa T, Uchino K, Mochida Y, Ebina T, Sumita S, et al. Prevalence of right bundle-branch block and right precordial ST-segment elevation (Brugada-type electrocardiogram) in Japanese children. Circ J. 2004;68(4):275–9.
117. Probst V, Allouis M, Sacher F, Pattier S, Babuty D, Mabo P, et al. Progressive cardiac conduction defect is the prevailing phenotype in carriers of a Brugada syndrome SCN5A mutation. J Cardiovasc Electrophysiol. 2006;17(3):270–5.
118. Probst V, Evain S, Gournay V, Marie A, Schott JJ, Boisseau P, et al. Monomorphic ventricular tachycardia due to Brugada syndrome successfully treated by hydroquinidine therapy in a 3-year-old child. J Cardiovasc Electrophysiol. 2006;17(1):97–100.
119. Sorgente A, Sarkozy A, De Asmundis C, Chierchia GB, Capulzini L, Paparella G, et al. Ajmaline challenge in young individuals with suspected Brugada syndrome. Pacing Clin Electrophysiol. 2011;34(6):736–41.
120. Conte G, Brugada P. The challenges of performing ajmaline challenge in children with suspected Brugada syndrome. Open Heart. 2014;1(1):e000031.Epub 2014/10/22
121. Conte G, de Asmundis C, Ciconte G, Julia J, Sieira J, Chierchia GB, et al. Follow-up from childhood to adulthood of individuals with family history of Brugada syndrome and normal electrocardiograms. Jama. 2014;312(19):2039–41 Epub 2014/11/17.
122. Allegue C, Coll M, Mates J, Campuzano O, Iglesias A, Sobrino B, et al. Genetic analysis of Arrhythmogenic diseases in the era of NGS: The complexity of clinical decision-making in Brugada syndrome. PloS one. 2015;10(7):e0133037 .Epub 2015/08/01
123. Di Resta C, Pietrelli A, Sala S, Della Bella P, De Bellis G, Ferrari M, et al. High-throughput genetic characterization of a cohort of Brugada syndrome patients. Hum Mol Genet. 2015;24(20):5828–35 Epub 2015/07/30.
124. Conte G, Dea C, Sieira J, Levinstein M, Chierchia GB, Dig G, et al. Clinical characteristics, management, and prognosis of elderly patients with Brugada syndrome. J Cardiovasc Electrophysiol. 2014;25(5):514–9 Epub 2014/01/10.
125. Kamakura T, Wada M, Nakajima I, Ishibashi K, Miyamoto K, Okamura H, et al. Evaluation of the necessity for cardioverter-defibrillator implantation in elderly patients with Brugada syndrome. Circ Arrhythm Electrophysiol. 2015;8(4):785–91 Epub 2015/06/13.

Catecholaminergic Polymorphic Ventricular Tachycardia

12

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Abstract

Catecholaminergic polymorphic ventricular tachycardia is a rare yet severe inherited arrhythmia syndrome characterized by polymorphic ventricular tachyarrhythmias during exercise or emotion. It is caused by mutations in genes involved in intracellular calcium cycling. β-Blockers and lifestyle advices are the cornerstone of therapy.

Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmia syndrome with polymorphic ventricular tachyarrhythmias during exercise or emotion as its key feature [1]. In some patients, the classic bidirectional ventricular tachycardia, characterized by a beat-to-beat 180-degree alternating QRS axis, can be observed (Fig. 12.1).

The prevalence of CPVT is frequently estimated at 1 in 10,000, but is in fact completely unknown. The first descriptions of patients with clinical characteristics of CPVT were published in 1960 [2] and 1975 [3]. Thereafter, two important case series were published by the Paris group of Philippe Coumel in 1978 [4] and 1995 [5], resulting in the definite recognition of CPVT as a distinct inherited arrhythmia syndrome. The genetic background of CPVT was discovered in 2001, when mutations in the cardiac ryanodine receptor gene (*RYR2*) [6] and cardiac calsequestrin (*CASQ2*) [7] were found to underlie the common autosomal-dominant and rare autosomal-recessive forms of CPVT, respectively.

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Clinical Presentation

Patients with CPVT typically present at young age with exercise- or emotion-induced syncope, aborted cardiac arrest or sudden cardiac death. The mean age of onset of symptoms is approximately 10–12 years [8, 9]. The syncopal events may resemble epileptic seizures and lead to an incorrect diagnosis of epilepsy, causing an important delay until CPVT is diagnosed [9]. Family history of the patients will often include relatives with syncope, aborted cardiac arrest, sudden cardiac death, or "epilepsy" under similar conditions.

Today, however, large differences in the clinical presentation and natural course of CPVT have been recognized. For example, putative pathogenic *RYR2* mutations have been identified in victims of sudden infant death syndrome [10], suggesting a very malignant phenotype in some cases. On the other hand, some patients are symptomatic in the third or fourth decade of life [11]. In addition, within families with CPVT large differences exist in the phenotype displayed by carriers of the same familial mutation, including mutation carriers who remain completely asymptomatic during life [12].

In one study, the natural course of CPVT was studied by comparing the mortality of past generations of a large Dutch family carrying the *RYR2* p.R420W founder mutation with the mortality of the general population [13]. Overall, mortality between family members and the general population was similar. However, in the 20–30-year age-group excess mortality was observed among family members. These data, however, may only reflect the natural course of this specific

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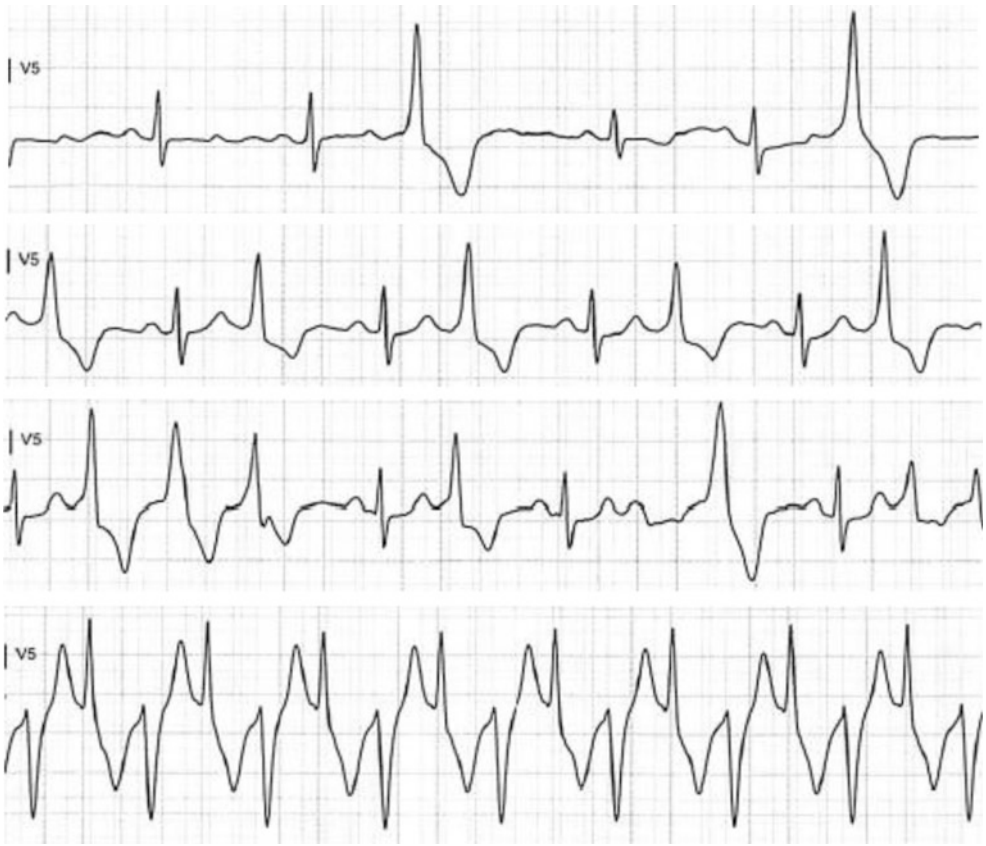

Fig. 12.1 Electrocardiographic manifestations of CPVT (different phases of an exercise test in a patient with CPVT. The *upper panel* shows the appearance of isolated ventricular premature beats (VPBs), which become bigeminal in the *second panel*. The *third panel* shows a triplet and bigeminal VPBs. The *fourth panel* shows a bidirectional ventricular tachycardia

mutation and may be different in other patients and families with CPVT.

In general and based on observations in a large series of young patients with CPVT, including 10–33 % who experiences an aborted cardiac arrest before CPVT was diagnosed and a significant number of patients with a family history of sudden cardiac death <40 years of age [8, 9], the odds of an adverse outcome in untreated patients are considered high. However, contemporary data on the prognosis of untreated patients do not exist, because nearly all patients receive some form of therapy.

Clinical Diagnosis

Cardiological Evaluation

A definite clinical diagnosis of CPVT requires the presence of reproducible unexplained exercise- or emotion-induced polymorphic or bidirectional ventricular tachycardia in the absence of structural heart disease and resting electrocardiogram (ECG) abnormalities [14, 15]. In individuals over 40 years of age, the exclusion of (significant) coronary artery disease is required [14]. In addition, CPVT is diagnosed in individuals who carry a pathogenic CPVT-associated mutation in *RYR2* or *CASQ2* with or without clinical signs of the disease [14, 15].

According to the HRS/EHRA/APHRS expert consensus recommendations CPVT can also be diagnosed in patients with adrenergically mediated polymorphic or bidirectional VPBs, although the minimally required ventricular arrhythmia burden is not further detailed [14]. In patients with possible CPVT, that is, not meeting the aforementioned clinical diagnostic criteria, genetic testing is critical to make a definite diagnosis of CPVT.

CPVT patients typically have a normal 12-lead resting ECG, including a normal QTc interval. However, sinus bradycardia and prominent U-waves can be observed [12].

Provocative testing, preferably by use of exercise testing, is the gold standard to diagnose CPVT. Typically, a gradual increase of ventricular arrhythmia burden and complexity is observed. First, isolated late-coupled VPBs and with a predominant left bundle branch inferior axis or right bundle branch block superior axis morphology appear at a heart rate of approximately 110–130 beats per minute [11, 16]. Ventricular arrhythmia threshold heart rate and VPB morphology are usually accurately reproducible in an individual patient, unless important therapeutic modifications are made. Later, bigeminal VPBs and polymorphic couplets or nonsustained ventricular tachycardia, including bidirectional ventricular tachycardia, usually appear. When exercise testing is terminated, the ventricular arrhythmias usually rapidly recede, and VPBs recorded more than 1 min into the recov-

ery phase are uncommon [16]. In some patients who reach a high maximum heart rate, the ventricular arrhythmias are paradoxically suppressed at maximum heart rates [17], although the exercise test might be stopped before this point is reached in case of severe ventricular arrhythmias.

Other provocative tests to diagnose CPVT are adrenaline infusion and Holter monitoring. Adrenaline infusion is initiated at a dose of 0.05 μg per kg per minute and then titrated at 4 or 5-min intervals to a maximum dose of 0.2–0.4 μg per kg per minute. One study which compared the diagnostic accuracy of adrenaline infusion and exercise testing in 36 CPVT patients and 45 unaffected relatives showed a low sensitivity of adrenaline infusion, probably because the maximum heart rate achieved upon adrenaline challenge was markedly lower compared with exercise testing [18]. Only 7 of 25 CPVT patients with a positive exercise test had a positive adrenaline test, yielding a poor sensitivity of 28 %. The specificity of adrenaline infusion was 98 %.

Holter monitoring, during which a patient should be encouraged to perform exercise, can be used in selected patients, such as very young or old patients who are unable to undergo exercise testing, but has a lower diagnostic yield as compared with other provocative tests. Holter monitoring may also help to identify supraventricular dysrhythmias (including intermittent ectopic atrial rhythm and tachyarrhythmias), which have been reported in 16–26 % of patients with CPVT [11, 12].

Cardiac imaging is mandatory in every patient suspected of CPVT to rule out structural heart disease. Structural heart disease is, by definition, absent in patients with CPVT. However, mutations in *RYR2* have been linked to fibrofatty myocardial replacement in the right ventricle, mimicking arrhythmogenic cardiomyopathy [19], (left ventricular) noncompaction cardiomyopathy [20], and a complex phenotype including sinoatrial node and atrioventricular node dysfunction, atrial fibrillation, atrial standstill, and left ventricular dysfunction and dilatation [21], in addition to the classic CPVT phenotype.

Differential Diagnosis

The differential diagnosis of CPVT often includes congenital long-QT syndrome, Andersen–Tawil syndrome, and concealed structural heart disease.

In patients with a nondiagnostic resting ECG, exercise testing is helpful in distinguishing CPVT and congenital long-QT syndrome. QTc-interval prolongation in the recovery phase of the exercise test may unmask congenital long-QT syndrome patients with a normal or borderline QTc-interval at rest [22]. The presence of exercise-induced ventricular ectopy beyond isolated VPBs points toward a diagnosis of CPVT [23].

Andersen–Tawil syndrome is characterized by the classic triad of ventricular arrhythmias, periodic paralysis, and facial and limb dysmorphism. Mutations in the gene encoding potassium inwardly rectifying channel Kir2.1 (*KCNJ2*) are reported in approximately 60 % of cases. Common cardiac manifestations include mild QTc prolongation, prominent U-waves, and ventricular arrhythmias, which include bidirectional or polymorphic ventricular tachycardia. In patients lacking the classic triad of Andersen–Tawil syndrome, the phenotype may very much mimic CPVT. For example, in a series of 24 *KCNJ2* mutation carriers, two individuals (8 %) displayed a CPVT phenotype [24]. Genetic testing may distinguish Andersen–Tawil syndrome from CPVT, which is important, because the prognosis in Andersen–Tawil syndrome is more benign.

Initially concealed structural heart disease that may cause exercise-induced ventricular arrhythmias include arrhythmogenic or hypertrophic cardiomyopathy, mitral valve prolapse, or ischemic heart disease. Advanced cardiac imaging and genetic testing may help making a specific diagnosis, although in some cases the underlying condition may reveal only during follow-up.

Clinical Therapy

Risk Stratification

Current guidelines recommend β-blocker therapy in all patients with CPVT, including mutation carriers with no ventricular arrhythmias during provocative testing [14, 15]. This is because very little clinical and genetic risk factors for the occurrence of arrhythmic events in CPVT have been identified. In one large CPVT series, a young age at diagnosis and a history of aborted cardiac arrest were associated with future arrhythmic events [8]. Among asymptomatic *RYR2* or *CASQ2* mutation-carrying relatives who are identified by cascade screening, the presence of exercise-induced ventricular arrhythmias seems to increase the risk of arrhythmic events [25]. In one series, relatives with a *RYR2* mutation in the C-terminal channel-forming domain had an increased odds of nonsustained ventricular tachycardia compared with those carrying a *RYR2* N-terminal mutation [12], but whether this translates into an increased risk of arrhythmic events is unknown. In summary, studies including larger patient populations and longer follow-up durations are needed to develop a more detailed risk stratification model.

Lifestyle

All patients with CPVT are advised to limit or avoid competitive sports, strenuous exercise, and exposure to stressful

environments (class I recommendation) [14, 15]. However, very recent recommendations state that genotype-positive phenotype-negative athletes may participate in all competitive sports with appropriate precautionary measures, including acquisition of a personal automatic external defibrillator and establishment of an emergency action plan [26].

Importantly, patients should thoroughly be educated about the importance of medication adherence. In our experience, a significant number of arrhythmic events in patients on β-blockers and/or flecainide can be attributed to nonadherence.

β-Blockers

β-Blockers are the mainstay of therapy in CPVT. β-Blockers are recommended in all patients with a clinical diagnosis of CPVT (class I recommendation) and should be considered in genotype-positive phenotype-negative individuals (class IIa recommendation) [14, 15].

β-Blockers significantly reduce the risk of arrhythmic events, and nadolol seems superior to other β-blockers [8, 27]. β-Blocker should be titrated to the highest tolerable dose. In a meta-analysis on the efficacy of β-blockers including 11 CPVT patient series, the estimated overall 4- and 8-year arrhythmic event rates were 18.0 % [95 % confidence interval (CI): 7.7–28.9] and 35.9 % (95 % CI: 15.3–56.5), respectively [28]. Four- and 8-year fatal or near-fatal arrhythmic event rates were 7.2 % (95 % CI: 3.1–11.3 %) and 14.3 % (95 % CI: 6.1–22.5), respectively.

In a recent large series of 211 children with CPVT, arrhythmic events occurred in 25 % of patients on β-blockers [9]. However, nonoptimal dosing and poor adherence contributed to 40 and 48 % of all events, respectively. Among 98 *RYR2* mutation-carrying relatives, only two asymptomatic relatives experienced exercise-induced syncope during a median follow-up of 4.7 years (range: 0.3–19 years), while no other arrhythmic events occurred [12].

Thus, β-blocker therapy seems sufficiently protective in the majority of CPVT patients, in particular relatives who are identified through cascade screening and display a mild phenotype. The presence of side effects (reported in approximately a quarter of CPVT patients) [12] should be seriously addressed, as these may hamper medication adherence.

Flecainide

Flecainide (2–3 mg per kg per day) in addition to β-blockers is recommended in patients who experience an arrhythmic event while on β-blockers (class IIa recommendation) [8, 27]. In addition, flecainide should be considered in addition to β-blockers and carriers of an implantable cardioverter-defibrillator (ICD) to reduce the risk of appropriate ICD shocks (class IIa recommendation) [15]. We also add flecainide to β-blockers when patients display couplets or nonsustained ventricular tachycardia during exercise testing while on β-blockers.

Flecainide has a possible direct RYR2 blocking effect in a CPVT mouse model [29], although this has been disputed by others [30]. The efficacy of flecainide was shown in a study including 33 severely affected patients, in whom flecainide (1.5–4.5 mg per kg body weight) partially or completely suppressed exercise-induced ventricular arrhythmias in 76 % of patients [31]. Arrhythmic events were prevented during a median follow-up of 20 months (range: 12–40), except for one patient who received appropriate ICD shocks after nonadherence. A similar efficacy of flecainide was observed in a series of 12 patients with genotype-negative CPVT [32], and 10 insufficiently controlled CPVT patients carrying *CASQ2* mutations [33, 9]. However, seven events occurred on a suboptimal dose and six were probably related to nonadherence. A small case series reported favorable ventricular arrhythmia-suppressing effects of flecainide monotherapy, [34] but at present this is only recommended in patients who are intolerant to β-blocker therapy.

Left Cardiac Sympathetic Denervation

Left cardiac sympathetic denervation (LCSD), usually by use of video-assisted thoracoscopic surgery, may be considered when ventricular arrhythmias cannot be controlled by medication (class IIb recommendation) [8, 27]. During LCSD, the lower half of the left stellate ganglion and thoracic ganglia T2–T4 is removed, thereby inhibiting largely preventing norepinephrine release in the heart. In the largest series on LCSD in CPVT, the 2-year cumulative event-free survival rate in 63 severely symptomatic patients who underwent LCSD was 81 % [35]. Patients with an incomplete LCSD were more likely to experience major arrhythmic events. The quality of life of CPVT patients who underwent LCSD was well, despite minor side effects that were reported by the majority of patients [36, 37].

Implantable Cardioverter-Defibrillator

ICD implantation is indicated in patients with previous aborted cardiac arrest and in patients with arrhythmic events or polymorphic or bidirectional ventricular tachycardia despite optimal medical therapy (class 1 recommendation) [14, 15].

Two early case reports pointed toward the possible proarrhythmic effect of ICD therapy in CPVT by describing patients in whom appropriate or inappropriate ICD shocks

and its subsequent catecholamine release initiated fatal ventricular arrhythmia storms [38, 39]. Two recent studies demonstrated that ICD shocks delivered to terminate ventricular tachycardia were often unsuccessful, whereas ventricular fibrillation was frequently terminated [40, 41]. In addition, one study reported induction of more malignant ventricular arrhythmias by ICD therapy in 36 % of patients, including electrical storm in 29 %, and 8.5 % of total shocks [40]. In 94 children with CPVT, appropriate and inappropriate shocks occurred in 46 % and 22 % of cases, respectively [9]. Electrical storm occurred in 18 % and ICD-related complications in 23 %. In a meta-analysis on ICD harm in young patients with inherited cardiac diseases, CPVT patients had the highest annual rate of inappropriate shocks (7.6 %) and other ICD-related complications (21.2 %) [42].

Thus, among patients who did not have an aborted cardiac arrest, ICD implantation should in our opinion be restricted to CPVT patients who do not sufficiently respond to an aggressive therapeutic strategy including β-blockers, flecainide and LCSD. If an ICD is implanted, additional therapy with β-blockers, flecainide and sometimes LCSD should be considered to lower the risk of appropriate and inappropriate ICD shocks. Careful ICD programming, that is, with one ventricular fibrillation zone with a detection interval of 240 beats per minute and (exceptionally) long detection intervals, is crucial.

Molecular Diagnostics

In CPVT, genetic testing has a diagnostic value. The results of genetic testing may help confirm the diagnosis in patients with a possible clinical diagnosis of CPVT. Current guidelines recommend comprehensive CPVT genetic testing in probands with a suspicion for CPVT based on examination of the patient's clinical history, family history, and expressed electrocardiographic phenotype during provocative stress testing with cycle, treadmill, or catecholamine infusion [43]. In addition, genetic testing may also be considered in cases of adrenergically mediated idiopathic ventricular fibrillation, which may justify genetic testing in such instances [43]. This recommendation is based on several case reports that described patients with idiopathic ventricular fibrillation with no CPVT phenotype in whom *RYR2* mutations were identified [44].

Molecular Genetics

CPVT Genes

Mutations in *RYR2* show an autosomal-dominant inheritance [6] and are identified in approximately 60 % of patients with CPVT [45, 46]. RYR2 regulates the release of calcium from the sarcoplasmic reticulum, which initiates cardiac muscle contraction. Today, over 170 unique, mostly missense mutations in *RYR2* have been identified [47]. Approximately 20 % of the *RYR2* mutations are de novo in origin and in one study multiple *RYR2* mutations were identified in 5.5 % of patients [45]. Mutations in *RYR2* tend to cluster in three hotspots: the N-terminal domain (codons 44–466; ~16 % of mutations), the central domain (codons 2246–2534; ~20 % of mutations), and the C-terminal channel-forming domain (codons 3778–4959; ~50 % of mutations). Several *RYR2* founder mutations have been identified, including the p.G357S mutation in approximately 180 family members from the Canary Islands [48] and the p.R420W mutation in over 60 family members from the Netherlands [12].

In one study, rare missense mutations in *RYR2* were, however, also identified in 3 % of control populations [45]. Another study reported a prevalence of previously reported CPVT-associated *RYR2* variants of 6.7 % in control subjects, yielding a prevalence of up to 1:150, which is much higher than the estimated prevalence of clinically diagnosed CPVT [47]. It is therefore likely that a proportion of the *RYR2* variants identified are not the major or monogenic cause of CPVT. Therefore extreme caution needs to be taken before classifying a novel *RYR2* variant as pathogenic, in particular when the variant resides outside of the three regional hot spots.

Mutations in *CASQ2* cause a malignant autosomal-recessive inherited form of CPVT [7] and are identified in less than 5 % of CPVT index cases. CASQ2 is located within the sarcoplasmic reticulum and also plays an important role in calcium homeostasis. *CASQ2* mutations are typically identified in consanguineous families, but compound heterozygosity in nonconsanguineous families have also been observed.

Mutations in the gene encoding triadin (*TRDN)* have been identified in autosomal recessively inherited cases of CPVT [49]. In the first report, three *TRDN* mutations were identified in 2 out of 97 genotype-negative CPVT probands (2 %) [49]. Next, three related children carrying two heterozygous *TRDN* mutations and displaying significant ventricular arrhythmias during isoproterenol infusion testing were reported [50]. A heterozygous missense mutation in *CALM1* (encoding calmodulin) was identified in a large family with a classic CPVT phenotype [51]. Subsequently, another *CALM1* missense mutation was identified in 63 *RYR2* mutation-negative individuals [51]. Triadin and calmodulin are also components of the cardiac calcium release complex. Another autosomal-recessive form of CPVT was previously mapped to a 25-Mb interval on chromosome 7p14–p22 in a report including four children from an inbred Arabic family [52]. The causal gene, *TECRL*, was recently identified (unpublished data).

CPVT Phenocopies

Patients with mutations in other genes may cause adrenergically mediated ventricular arrhythmias, making these CPVT phenocopies (Table 12.1).

Loss-of-function mutations in the membrane adaptor protein ankyrin-B (*ANK2*) are associated with type 4 congenital long-QT syndrome. However, some patients have exercise-induced ventricular arrhythmias in the absence of QTc-interval prolongation [53].

Mutations in *KCNJ2* are generally associated with Andersen–Tawil syndrome, but may also cause a CPVT phenocopy including the classic bidirectional VT (see section, "Differential Diagnosis").

A phenotype with polymorphic ventricular ectopy in carriers of gain-of-function mutations in gene encoding the pore-forming subunit of the cardiac sodium channel, Nav 1.5 (*SCN5A*) has been reported [54, 55], including families in which these ventricular arrhythmias were exercise-induced [54].

Table 12.1 Genes associated with CPVT and CPVT phenocopies

Gene	Protein	Prevalence
CPVT		
RYR2	Cardiac ryanodine receptor	60 %
CASQ2	Cardiac calsequestrin	<5 %
TRDN	Triadin	<1 %
CALM1	Calmodulin	Unknown
TECRL	Trans-2,3-enoyl-CoA reductase like	Unknown
CPVT phenocopies		
ANK2	Ankyrin-B	Unknown
KCNJ2	Kir 2.1	Unknown
SCN5A	Nav 1.5	Unknown

Family Screening

When a likely pathogenic mutation is identified in an index patient with CPVT, genetic testing of all first-degree relatives is indicated (Fig. 12.2) [43]. Genetic testing is recommended at young age, possibly even at birth, because of the young age of manifestation of CPVT and its association with sudden infant death syndrome [43]. Among *RYR2* mutation-carrying relatives identified by cascade screening, approximately 50 % have exercise-induced ventricular arrhythmias [12]. Relatives who are noncarrier of the familial CPVT-causing mutation can be dismissed from further cardiologic evaluation.

First-degree relatives of a mutation-negative index patient should be screened clinically, in particular with exercise testing (Fig. 12.2). Children and young adults should be followed up regularly, even if the initial clinical screening is normal.

Summary

- CPVT is a rare yet severe inherited arrhythmia syndrome characterized by polymorphic ventricular tachyarrhythmias during exercise or emotion.
- CPVT must be considered in anyone with adrenergically mediated ventricular arrhythmias or cardiac symptoms, such as syncope or cardiac arrest, or a positive family history for such events.
- CPVT is caused by mutations in genes involved in intracellular calcium cycling, in particular the cardiac ryanodine receptor (*RYR2*) in the majority of cases.

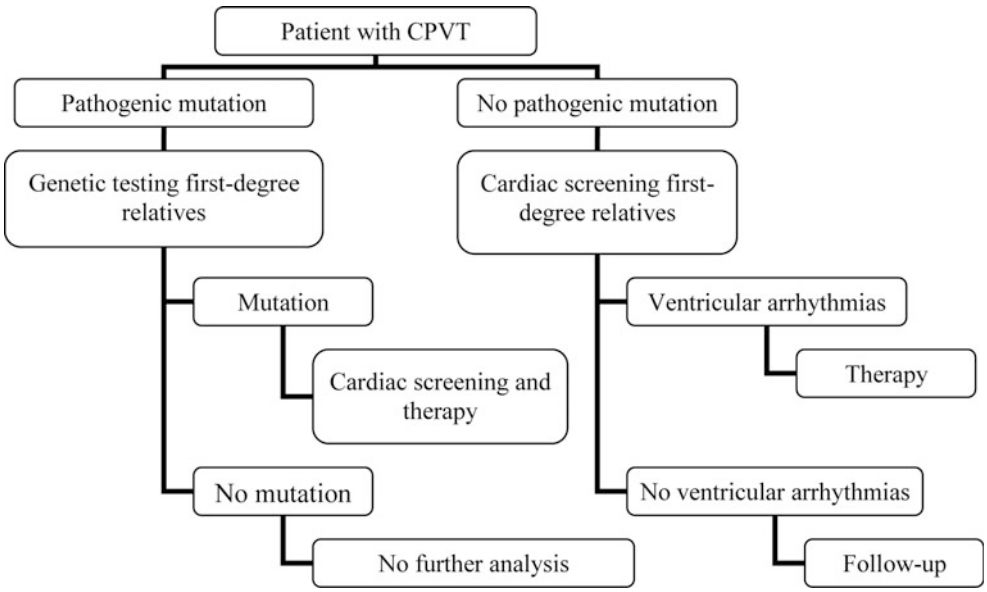

Fig. 12.2 Family screening in CPVT

- β-Blockers and lifestyle advices are the cornerstone of therapy, whereas flecainide, LCSD, and ICD implantation may be indicated in severely affected patients.

References

1. Leenhardt A, Denjoy I, Guicheney P. Catecholaminergic polymorphic ventricular tachycardia. Circ Arrhythm Electrophysiol. 2012;5(5):1044–52.
2. Berg K. Multifocal ventricular extrasystoles with Adams-Stokes syndrome in children. Am Heart J. 1960;60(6):965–70.
3. Reid DS, Tynan M, Braidwood L, Fitzgerald GR. Bidirectional tachycardia in a child. A study using His bundle electrography. Br Heart J. 1975;37(3):339–44.
4. Coumel P, Fidelle J, Lucet V, Attuel P, Bouvrain Y. Catecholamine-induced severe ventricular arrhythmias with Adam-Stokes in children: report of four cases. Br Heart J. 1978;40(supplement):28–37.
5. Leenhardt A, Lucet V, Denjoy I, Grau F, Ngoc DD, Coumel P. Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. Circulation. 1995;91(5):1512–9.
6. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. Circulation. 2001;103(2):196–200.
7. Lahat H, Pras E, Olender T, Avidan N, Ben-Asher E, Man O, et al. A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. Am J Hum Genet. 2001;69(6):1378–84.
8. Hayashi M, Denjoy I, Extramiana F, Maltret A, Buisson NR, Lupoglazoff J-M, et al. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. Circulation. 2009;119(18):2426–34.
9. Roston TM, Vinocur JM, Maginot KR, Mohammed S, Salerno JC, Etheridge SP, et al. Catecholaminergic Polymorphic Ventricular Tachycardia in Children: An Analysis of Therapeutic Strategies and Outcomes from an International Multicenter Registry. Circ Arrhythm Electrophysiol. 2015;8(3):633–42.
10. Tester DJ, Medeiros-Domingo A, Will ML, Haglund CM, Ackerman MJ. Cardiac channel molecular autopsy: insights from 173 consecutive cases of autopsy-negative sudden unexplained death referred for postmortem genetic testing. Mayo Clin Proc. 2012;87(6):524–39.
11. Sy RW, Gollob MH, Klein GJ, Yee R, Skanes AC, Gula LJ, et al. Arrhythmia characterization and long-term outcomes in catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm. 2011;8(6):864–71.
12. van der Werf C, Nederend I, Hofman N, van Geloven N, Ebink C, Frohn-Mulder IME, et al. Familial evaluation in catecholaminergic polymorphic ventricular tachycardia: disease penetrance and expression in cardiac ryanodine receptor mutation-carrying relatives. Circ Arrhythm Electrophysiol. 2012;5(4):748–56.
13. Nannenberg EA, Sijbrands EJG, Dijksman LM, Alders M, van Tintelen JP, Birnie M, et al. Mortality of inherited arrhythmia syndromes: insight into their natural history. Circ Cardiovasc Genet. 2012;5(2):183–9.
14. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. Heart Rhythm. 2013;10(12):1932–63.
15. Authors/Task Force Members, Priori SG, Blomström-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, et al. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: the task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European society of cardiology (ESC) Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). Eur Heart J. 2015;36(41):2793–867.
16. Blich M, Marai I, Suleiman M, Lorber A, Gepstein L, Boulous M, et al. Electrocardiographic comparison of ventricular premature complexes during exercise test in patients with CPVT and healthy subjects. Pacing Clin Electrophysiol. 2015;38(3):398–402.
17. Faggioni M, Hwang HS, van der Werf C, Nederend I, Kannankeril PJ, Wilde AAM, et al. Accelerated sinus rhythm prevents catecholaminergic polymorphic ventricular tachycardia in mice and in patients. Circ Res. 2013;112(4):689–97.
18. Marjamaa A, Hiippala A, Arrhenius B, Lahtinen AM, Kontula K, Toivonen L, et al. Intravenous epinephrine infusion test in diagnosis of catecholaminergic polymorphic ventricular tachycardia. J Cardiovasc Electrophysiol. 2012;23(2):194–9.
19. Tiso N, Stephan DA, Nava A, Bagattin A, Devaney JM, Stanchi F, et al. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). Hum Mol Genet. 2001;10(3):189–94.
20. Ohno S, Omura M, Kawamura M, Kimura H, Itoh H, Makiyama T, et al. Exon 3 deletion of RYR2 encoding cardiac ryanodine receptor is associated with left ventricular non-compaction. Europace. 2014;16(11):1646–54.
21. Bhuiyan ZA, van den Berg MP, van Tintelen JP, Bink-Boelkens MTE, Wiesfeld ACP, Alders M, et al. Expanding spectrum of human RYR2-related disease: new electrocardiographic, structural, and genetic features. Circulation. 2007;116(14):1569–76.
22. Sy RW, van der Werf C, Chattha IS, Chockalingam P, Adler A, Healey JS, et al. Derivation and validation of a simple exercise-based algorithm for prediction of genetic testing in relatives of LQTS probands. Circulation. 2011;124(20):2187–94.
23. Horner JM, Ackerman MJ. Ventricular ectopy during treadmill exercise stress testing in the evaluation of long QT syndrome. Heart Rhythm. 2008;5(12):1690–4.
24. Kimura H, Zhou J, Kawamura M, Itoh H, Mizusawa Y, Ding W-G, et al. Phenotype variability in patients carrying KCNJ2 mutations. Circ Cardiovasc Genet. 2012;5(3):344–53.
25. Hayashi M, Denjoy I, Hayashi M, Extramiana F, Maltret A, Roux-Buisson N, et al. The role of stress test for predicting genetic mutations and future cardiac events in asymptomatic relatives of catecholaminergic polymorphic ventricular tachycardia probands. Europace. 2012;14(9):1344–51.
26. Ackerman MJ, Zipes DP, Kovacs RJ, Maron BJ. Eligibility and Disqualification Recommendations for Competitive Athletes With Cardiovascular Abnormalities: Task Force 10: The Cardiac Channelopathies: A Scientific Statement From the American Heart Association and American College of Cardiology. J Am Coll Cardiol. 2015;66(21):2424–8.
27. Leren IS, Saberniak J, Majid E, Haland TF, Edvardsen T, Haugaa KH. Nadolol decreases the incidence and severity of ventricular arrhythmias during exercise stress testing compared with β1-selective β-blockers in patients with catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm. 2015;13(2):433–40.
28. van der Werf C, Zwinderman AH, Wilde AAM. Therapeutic approach for patients with catecholaminergic polymorphic ventricular tachycardia: state of the art and future developments. Europace. 2012;14(2):175–83.
29. Watanabe H, Chopra N, Laver D, Hwang HS, Davies SS, Roach DE, et al. Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. Nat Med. 2009;15(4):380–3.

30. Bannister ML, Thomas NL, Sikkel MB, Mukherjee S, Maxwell C, MacLeod KT, et al. The mechanism of flecainide action in CPVT does not involve a direct effect on RyR2. Circ Res. 2015;116(8): 1324–35.
31. van der Werf C, Kannankeril PJ, Sacher F, Krahn AD, Viskin S, Leenhardt A, et al. Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. J Am Coll Cardiol. 2011;57(22): 2244–54.
32. Watanabe H, van der Werf C, Roses-Noguer F, Adler A, Sumitomo N, Veltmann C, et al. Effects of flecainide on exercise-induced ventricular arrhythmias and recurrences in genotype-negative patients with catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm. 2013;10(4):542–7.
33. Khoury A, Marai I, Suleiman M, Blich M, Lorber A, Gepstein L, et al. Flecainide therapy suppresses exercise-induced ventricular arrhythmias in patients with CASQ2-associated catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm. 2013;10(11): 1671–5.
34. Padfield GJ, AlAhmari L, Lieve KVV, AlAhmari T, Roston TM, Wilde AA, et al. Flecainide monotherapy is an option for selected patients with catecholaminergic polymorphic ventricular tachycardia intolerant of β-blockade. Heart Rhythm. 2015;13(2):609–13.
35. De Ferrari GM, Dusi V, Spazzolini C, Bos JM, Abrams DJ, Berul CI, et al. Clinical Management of Catecholaminergic Polymorphic Ventricular Tachycardia: The Role of Left Cardiac Sympathetic Denervation. Circulation. 2015;131(25):2185–93.
36. Waddell-Smith KE, Ertresvaag KN, Li J, Chaudhuri K, Crawford JR, Hamill JK, et al. Physical and Psychological Consequences of Left Cardiac Sympathetic Denervation in Long-QT Syndrome and Catecholaminergic Polymorphic Ventricular Tachycardia. Circ Arrhythm Electrophysiol. 2015;8(5):1151–8.
37. Antiel RM, Bos JM, Joyce DD, Owen HJ, Roskos PL, Moir C, et al. Quality of life after videoscopic left cardiac sympathetic denervation in patients with potentially life-threatening cardiac channelopathies/cardiomyopathies. Heart Rhythm. 2015;13(1):62–9.
38. Pizzale S, Gollob MH, Gow R, Birnie DH. Sudden death in a young man with catecholaminergic polymorphic ventricular tachycardia and paroxysmal atrial fibrillation. J Cardiovasc Electrophysiol. 2008;19(12):1319–21.
39. Mohamed U, Gollob MH, Gow RM, Krahn AD. Sudden cardiac death despite an implantable cardioverter-defibrillator in a young female with catecholaminergic ventricular tachycardia. Heart Rhythm. 2006;3(12):1486–9.
40. Roses-Noguer F, Jarman JWE, Clague JR, Till J. Outcomes of defibrillator therapy in catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm. 2014;11(1):58–66.
41. Miyake CY, Webster G, Czosek RJ, Kantoch MJ, Dubin AM, Avasarala K, et al. Efficacy of implantable cardioverter defibrillators in young patients with catecholaminergic polymorphic ventricular tachycardia: success depends on substrate. Circ Arrhythm Electrophysiol. 2013;6(3):579–87.
42. Olde Nordkamp LRA, Postema PG, Knops RE, van Dijk N, Limpens J, Wilde AAM, et al. Implantable cardioverter-defibrillator harm in young patients with inherited arrhythmia syndromes: A systematic review and meta-analysis of inappropriate shocks and complications. Heart Rhythm. 2015;13(2):442–54.
43. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Heart Rhythm. 2011;8(8):1308–39.
44. Paech C, Gebauer RA, Karstedt J, Marschall C, Bollmann A, Husser D. Ryanodine receptor mutations presenting as idiopathic ventricular fibrillation: a report on two novel familial compound mutations, c.6224 T > C and c.13781 A > G, with the clinical presentation of idiopathic ventricular fibrillation. Pediatr Cardiol. 2014;35(8):1437–41.
45. Medeiros-Domingo A, Bhuiyan ZA, Tester DJ, Hofman N, Bikker H, van Tintelen JP, et al. The RYR2-encoded ryanodine receptor/calcium release channel in patients diagnosed previously with either catecholaminergic polymorphic ventricular tachycardia or genotype negative, exercise-induced long QT syndrome: a comprehensive open reading frame mutational analysis. J Am Coll Cardiol. 2009;54(22):2065–74.
46. Bai R, Napolitano C, Bloise R, Monteforte N, Priori SG. Yield of genetic screening in inherited cardiac channelopathies: how to prioritize access to genetic testing. Circ Arrhythm Electrophysiol. 2009;2(1):6–15.
47. Jabbari J, Jabbari R, Nielsen MW, Holst AG, Nielsen JB, Haunsø S, et al. New exome data question the pathogenicity of genetic variants previously associated with catecholaminergic polymorphic ventricular tachycardia. Circ Cardiovasc Genet. 2013;6(5): 481–9.
48. Wangüemert F, Bosch Calero C, Pérez C, Campuzano O, Beltran-Alvarez P, Scornik FS, et al. Clinical and molecular characterization of a cardiac ryanodine receptor founder mutation causing catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm. 2015;12(7):1636–43.
49. Roux-Buisson N, Cacheux M, Fourest-Lieuvin A, Fauconnier J, Brocard J, Denjoy I, et al. Absence of triadin, a protein of the calcium release complex, is responsible for cardiac arrhythmia with sudden death in human. Hum Mol Genet. 2012;21(12):2759–67.
50. Rooryck C, Kyndt F, Bozon D, Roux-Buisson N, Sacher F, Probst V, et al. New Family With Catecholaminergic Polymorphic Ventricular Tachycardia Linked to the Triadin Gene. J Cardiovasc Electrophysiol. 2015;26(10):1146–50.
51. Nyegaard M, Overgaard MT, Søndergaard MT, Vranas M, Behr ER, Hildebrandt LL, et al. Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. Am J Hum Genet. 2012;91(4):703–12.
52. Bhuiyan ZA, Hamdan MA, Shamsi ETA, Postma AV, Mannens MMAM, Wilde AAM, et al. A novel early onset lethal form of catecholaminergic polymorphic ventricular tachycardia maps to chromosome 7p14–p22. J Cardiovasc Electrophysiol. 2007;18(10): 1060–6.
53. Mohler PJ, Splawski I, Napolitano C, Bottelli G, Sharpe L, Timothy K, et al. A cardiac arrhythmia syndrome caused by loss of ankyrin-B function. Proc Natl Acad Sci U S A. 2004;101(24):9137–42.
54. Swan H, Amarouch MY, Leinonen J, Marjamaa A, Kucera JP, Laitinen-Forsblom PJ, et al. Gain-of-function mutation of the SCN5A gene causes exercise-induced polymorphic ventricular arrhythmias. Circ Cardiovasc Genet. 2014;7(6):771–81.
55. Laurent G, Saal S, Amarouch MY, Béziau DM, Marsman RFJ, Faivre L, et al. Multifocal ectopic Purkinje-related premature contractions: a new SCN5A-related cardiac channelopathy. J Am Coll Cardiol. 2012;60(2):144–56.

Short QT Syndrome

13

Christian Wolpert and Norman Rüb

Abstract

In 2000, Gussak et al. first described an idiopathic short QT interval associated with atrial fibrillation (AF) in one family and a sudden death in an unrelated individual (Gussak et al. Cardiology 94:99–102, 2000). Three years later, in 2003, Gaita et al. reported the association of a short QT interval and sudden cardiac death in two unrelated European families (Gaita et al. Circulation 108:965–70, 2003). Within the following years, a variety of mutations in different genes most likely causative for the short QT interval were identified. The initially reported mutations either caused a gain of function of cardiac potassium channels I_{Kr}, I_{Ks}, and I_{K1}, or a loss of function in the cardiac L-type calcium channel (I_{Ca}) (Bellocq et al. Circulation 109:2394–7, 2004; Priori et al. Circ Res 96:800–7, 2005; Antzelevitch et al. Circulation 115:442–9, 2007; Hong et al. Cardiovasc Res 68:433–40, 2005; Brugada et al. Circulation 109:30–5, 2004; El Harchi et al. PLoS One 7:e52451, 2012; Moreno et al. Cardiovasc Res 107:613–23, 2015; Giustetto et al. Eur Heart J 27:2440–7; 2006).

Introduction

In 2000, Gussak et al. first described an idiopathic short QT interval associated with atrial fibrillation (AF) in one family and a sudden death in an unrelated individual [1]. Three years later, in 2003, Gaita et al. reported the association of a short QT interval and sudden cardiac death in two unrelated European families [2]. Within the following years, a variety of mutations in different genes most likely causative for the short QT interval were identified. The initially reported mutations either caused a gain of function of cardiac potassium channels I_{Kr}, I_{Ks}, and I_{K1}, or a loss of function in the cardiac L-type calcium channel (I_{Ca}) [3–10]. Meanwhile, new mutations have been reported recently resulting in different alterations of ion channel activity.

The scope of this chapter is to provide a comprehensive description of the short QT syndrome (SQTS) and an update on this still quite young disease, including the clinical, genetic, and pathophysiologic aspects as well as therapeutic consequences and treatment options.

Clinical Presentation

The clinical presentation of patients with SQTS is heterogeneous. The first comprehensive data were presented by Giustetto et al. from the EUROSHORT registry [10]. A total of 29 patients (21 m, 8 f) were studied. Eighteen of 29 patients were symptomatic at the time of enrollment. Nine of the patients had a history of cardiac arrest, six had suffered syncope, and seven had documented atrial flutter or AF [10]. The onset of symptoms was highly variable ranging from the age of 4 months up to the age of 62 years, and it was distributed over all decades of life. Sudden deaths occurred in the youngest patient at the age of 4 months. Thus, SQTS represented also a new potential cause for the sudden infant death syndrome (SIDS).

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Mazzanti and coworkers studied a population of 47 probands who were referred to the database for cardiac arrest (n = 19), syncope (n = 9), family history of sudden death (n = 2), or an incidentally found short QTc interval [11]. Twelve subjects had a family history of sudden death in the young and four had multiple victims in their family (2.5 ± 0.6). The QTc interval among the asymptomatic individuals, the sudden death victims, and patients with syncope did not differ significantly. The age at the time of syncope or sudden death was also comparable with 21 ± 11 versus 25 ± 13 years. Of note, the QTc interval in those, in whom a mutation was identified, was significantly shorter (300 vs. 335 ms). There was no difference in the likelihood of sudden death between mutation positive and mutation negative probands.

Villafane and coworkers published an international series of 21 pediatric short QT patients [12, 13]. The median age was 15 years. Fifty-six percent (84 % males) of the patients were symptomatic for syncope (n = 4) or sudden death (n = 6). Sixteen patients had either a personal or family history of sudden death. The rate of AF was high for this young cohort with 4/21 patients.

A gene mutation was identified in only 24 %. Eleven of 21 patients received an implantable cardioverter-defibrillator (ICD) and 2 patients received an appropriate shock and 64 % inappropriate shocks. The authors applied the Gollob score and observed that asymptomatic individuals with a Gollob score of <5 were asymptomatic for VT/VF or sudden death and syncope over a 6-year follow-up. In a series of five Japanese unrelated families, symptoms were AF in two, ventricular fibrillation (VF) in two, sudden death in three patients, and severe bradycardia in one newborn. The QTc in this series was between 280 and 340 ms, that is, somewhat longer than in patients with SQT1 [14, 15].

Triggers of VF

There is only little reported about the circumstances of symptoms and sudden death in patients with a SQTS. In the first series published by Giustetto et al., there were three symptoms during sleep, three at rest, three during definite effort, and the remainder was either unknown or in two during normal daily activity. This means that there is no common trigger such as in catecholaminergic polymorphic ventricular tachycardia during exercise or like in long QT syndrome during stress or exercise [10].

Clinical Diagnosis of Short QT Syndrome and Prevalence

To date, there is no clearcut definition of what a SQTS is. It can nevertheless be stated here that a short QT interval is not necessarily the same as a short QT syndrome.

What Is a Normal QT Interval?

In the general population, QTc intervals follow a Gaussian normal distribution [16–23]. Normal QT intervals were proposed as QTc intervals within two standard deviations from the mean. Thus, 95 % of the QTc intervals of the general population are "normal." QTc lesser than the 2.5th percentile were defined as "short." Following this calculation, QTc of <350 ms for men and QTc of <360 ms for women are considered short. In large population-based studies, the prevalence of a short QT interval was analyzed. Within an Italian predominantly male cohort, the prevalence of a QTc of <360 ms was 0.5 %. Anttonen et al. analyzed a population of 10,822 subjects and found short QTc intervals of <340 ms in 0.4 % of the subjects [16]. Very short QTc intervals <320 ms were seen in 0.1 % of the cases. Both patients with a short and a very short QTc interval had no cardiac events [8]. In a Japanese cohort of 12,149 subjects, 0.01 % exhibited a QTc interval within the 2.5th percentile (men QTc <354 ms; females QTc <364 ms) and only 3 male subjects a QTc of <300 ms [17]. In another recent analysis of 19,153 subjects undergoing biannual health examinations in the follow-up program in Hiroshima and Nagasaki since 1958, the prevalence for a short QT interval (QTc <350 ms) was 0.01 % [13]. Kobza et al. found a similar low prevalence of 0.01 % of QTc intervals <320 ms in 41,767 male army conscripts [19].

A recent report on an electrocardiogram (ECG) population sample among 1.7 million persons yielded a QTc of <300 ms in 2.7 subjects in 100.000 individuals. The risk of dying in a period of 8.3 years of follow-up was increased 2.6-fold [21].

What Is a Too Short QT Interval and What Is Found in Short QT Syndrome?

The hallmark of diagnosis is a short QT-interval on baseline ECG. QTc intervals of <350 ms for males and <360 ms for females should gain attention and warrant further clinical work-up. In co-incidence with clinical symptoms such as AF, sudden cardiac death, family history of SQTS, or sudden cardiac death the diagnosis of SQTS is established.

The ECG of the first patients identified with a SQTS (SQT1) showed very short QT intervals and in addition short QT intervals corrected for heart rate (QTc <300 ms) (Figs. 13.1 and 13.2). The patients identified as SQT2–SQT5 exhibited QTc of up to 360 ms. The ECG in SQT1–3 reveals tall, symmetrical, and asymmetrical peaked T wave especially in the precordial leads (Fig. 13.2). In SQT3, the T wave has a less steep ascending part and a steep downslope. In most cases, a ST segment is absent with the T wave originating directly form the S wave. Another finding in SQTS is a prolonged T_{peak}–T_{end} interval. Recently, Anttonen et al. compared the J_{point}–T_{peak} interval in

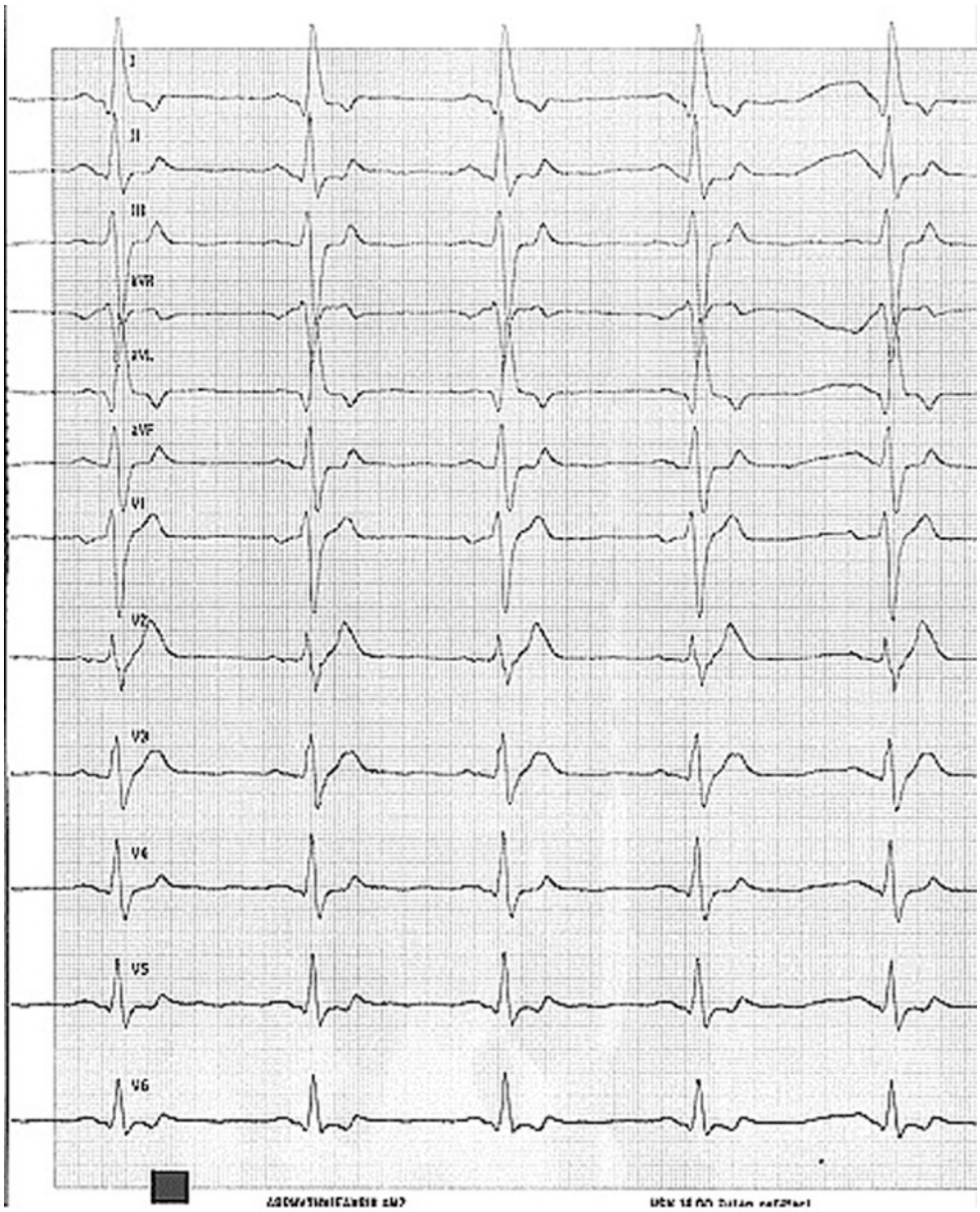

Fig. 13.1 This figure depicts the chest leads of an ECG of a patient with SQT1

symptomatic patients with SQTS, probands with a short QT interval, and a control group of subjects with normal QT interval [24]. Symptomatic patients with SQTS had significantly shorter J_{point}–T_{peak} intervals and higher corrected Tpeak-Tend/QTc ratio compared to asymptomatic probands with a short QT interval and subjects with a normal QT interval. Patients diagnosed with SQT4 and SQT5 on the basis of a mutation in the cardiac calcium channel exhibit shorter than normal QT intervals of 330–360 ms, which is relatively longer than in SQT1–SQT3. These patients additionally show ST segment elevation diagnostic of Brugada syndrome either spontaneously or after the administration of intravenous ajmaline [5].

Villafane et al. reported the data on pediatric patients with a SQTS. The QTc ranged here from 194 to 355 ms (mean 312 ms) [12, 13].

QT Adaptation to Heart Rate

Another important finding in the initially reported SQT1 patients was the inappropriate adaptation of the QT interval to heart rate. In the first patients with the *KCNH2* mutation, the QT interval did not shorten adequately compared to normal controls. Quinidine was able to restore the QTc/heart rate ratio toward the normal range. This lack of adaptation of QT interval with heart rate seemed to be one additional criterion for the diagnosis of SQTS [10, 25].

Giustetto and coworkers further studied the usefulness of exercise testing in the diagnosis of SQTS in the largest series of patients with different mutations in order to see if QT behavior during exercise helps to differentiate between short QT patients and individuals with a shorter than normal QT interval. They looked at 21 patients with a SQTS including

the patients from the first report and matched controls. Rest and peak exercise heart rates did not differ between the groups. The baseline QT intervals at rest were 276 versus 364 ms and at peak exercise 228 ± 27 versus 245 ± 26 ms with a mean variation from rest to peak exercise of 48 ± 14 versus 120 ± 20 ms. The QT/HR slope never exceeded 0.9 ms/bpm. The mean was −0.53 versus −1.29 ms/bpm.

Other Findings: PQ-Depression

There have been other interesting observations such as PQ-depression and echocardiographic findings, which, however, are not fully understood yet and therefore cannot be considered diagnostic or pathognomonic for SQTS. The first, PQ-depression was analyzed and first described by Tülümen et al. [26]. In their series of patients, the segment between the P wave and the QRS complex was depressed below the isoelectric point in 81 % of the 64 patients. The authors speculate that a heterogeneous abbreviation of atrial repolarisation could lead to the PQ segment depression. They compared the patients with a SQTS to a control group of 117 healthy matched pairs.

Echocardiographic Findings

Two groups have investigated echocardiographic findings in SQTS.

The first investigation correlated the surface ECG to the mechanics in SQTS and described a certain electromechanical dissociation of specific phases of relaxation and the time course of repolarization [27].

The second, more recent report analyzed the myocardial performance index, mechanical dispersion, and global longitudinal strain. They found that in SQT patients myocardial function was slightly reduced and mechanical dispersion was increased [28].

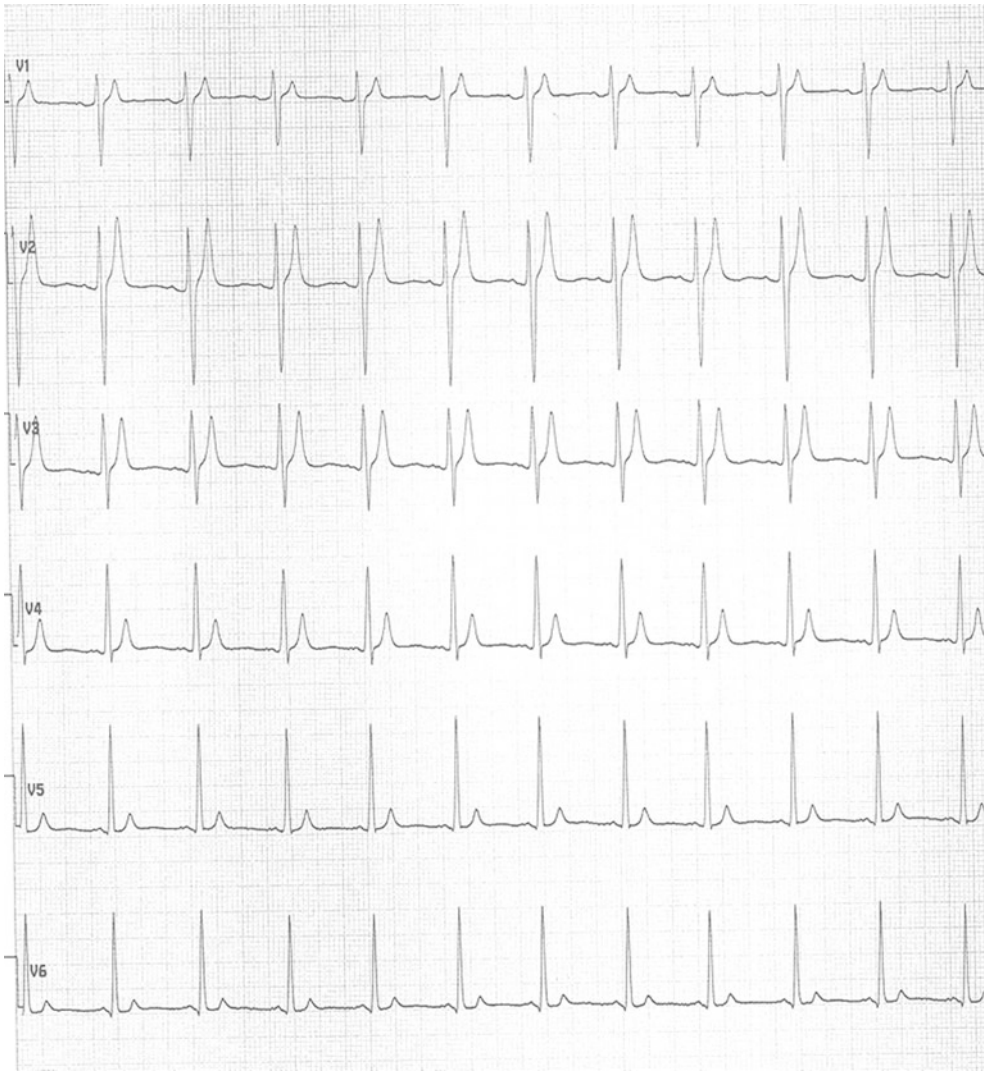

Fig. 13.2 ECG of a patient with SQT syndrome type 1 and a *KCNH2* mutation

However, these are only preliminary findings and have to be confirmed in larger populations.

Electrophysiological Studies

A further diagnostic tool tested in SQTS is the electrophysiological study. Atrial and ventricular effective refractory periods are significantly shortened especially in SQT1 (*KCNH2*) [29]. An atrial refractory period of 140 ms and a ventricular effective refractory period of 150 ms or less are highly suspicious criteria of the SQTS. Another finding is the high inducibility of VF during programmed ventricular stimulation in patients with SQTS 1 (Fig. 13.3) [16]. The current consensus recommendations and the European Society of Cardiology (ESC) guidelines, however, do not anymore recommend programmed ventricular stimulation for diagnostic purposes or risk stratification.

Scores and Guideline Recommendation

The QTc intervals of these patients are ranging from <300 ms up to <360 ms. In summary, a short QT interval on the 12-lead ECG does not predict a risk for life-threatening tachyarrhythmias per se. However, the rare finding of a short QT interval should initiate a diagnostic work-up including family members. In the case of a short QT interval together with episodes of AF, sustained palpitation, unexplained syncope, VF, and/or a positive family history for premature sudden cardiac death, SQTS should be suspected [30, 31].

Gollob et al proposed a SQTS Diagnostic criteria score analogous to the Schwartz score for long QT syndrome in which a high probability of SQTS was reached when more or equal to four points were given. In his criteria, a QTc of <370 ms was one point, <350 ms two points, and <330 ms equal to three points [31].

The 2015 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death provide a class I recommendation for a diagnosis of a SQTS, when the QTc is <340 ms. It should be considered, if the QTc is <360 ms and one or more of the following conditions exists: (a) a confirmed pathogenic mutation, (b) a family history of SQTS, (c) a family history of sudden death at age <40 years, or (d) survival from a VT/VF episode in the absence of heart disease. The guidelines discourage with a class III indication the EP study for risk stratification [30].

Differential Diagnosis

The heritable SQTS should be differentiated from the acquired or secondary forms of QT shortening. Documentation of a short QT interval on the ECG should lead therefore to the

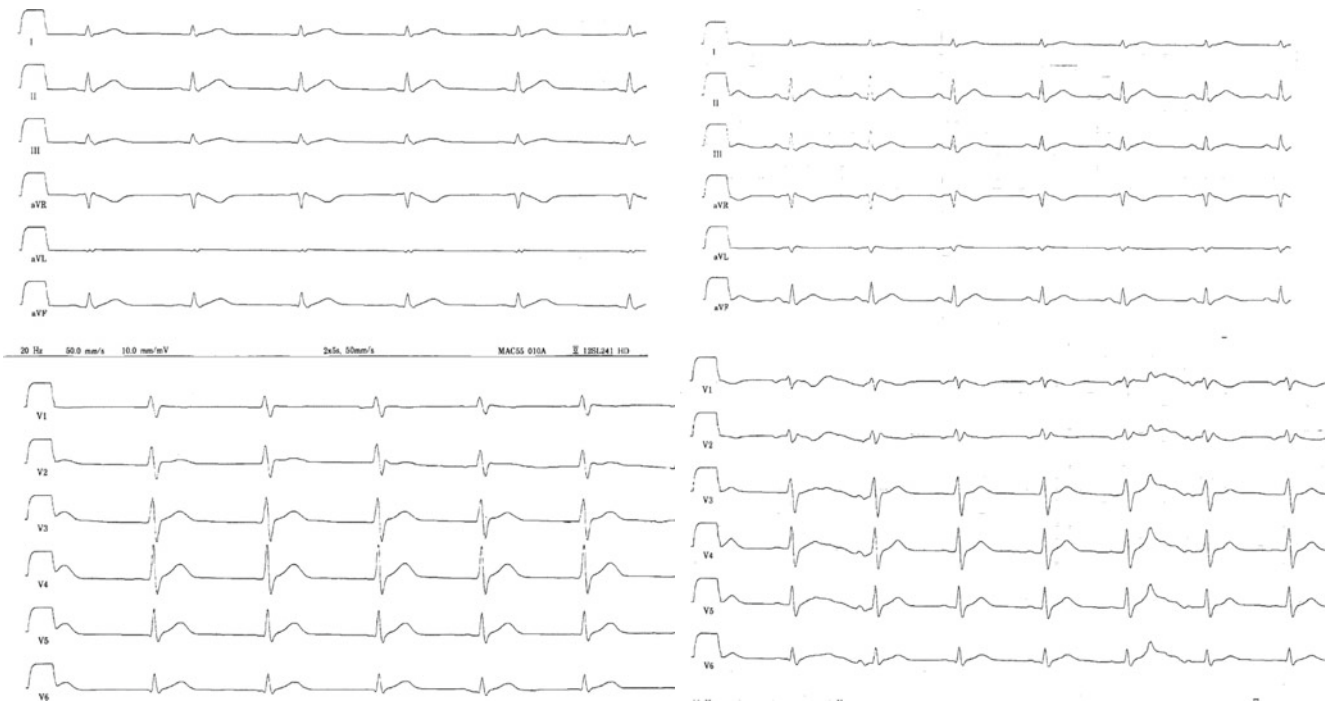

Fig. 13.3 ECG of a patient with a mutation in the L-type calcium channel during oral quinidine treatment. Without quinidine the patient had recurrent atrial flutter and fibrillation and intermittent type II Brugada-ECG changes

exclusion of structural heart diseases and underlying conditions such as hyperkalemia, hypercalcemia, hyperthermia, the period immediately following VF, acidosis, and/or digitalis overdose. Furthermore, structural heart disease, especially dilated cardiomyopathy, should be ruled out.

Molecular and Genetic Background

The SQTS is a genetically heterogeneous disease just like the congenital long QT syndrome. Since the first edition of this textbook, new mutations have been added to the initially reported in different genes. The mutations are located on different chromosomes, for example, 7, 10, 11, 12, and 17 and encode for different cardiac ion channels. According to the chronology of their first description, the mutations are termed SQT1–SQT6 (Table 13.1).

The first mutation identified to be causative for the SQTS (SQT1) was a gain-of-function mutation leading to an increase of the rapid component of the delayed rectifier potassium current (I_{Kr}) [7]. Two different missense mutations were identified resulting in the same amino acid change in HERG (*KCNH2*). These mutations at nucleotide 1764 in the *KCNH2* gene substitute the asparagine at codon 588 for a positively charged lysine (p. N588K). The residue is located in the S5-P loop region of HERG at the outer mouth of the channel. The p.N588K mutation causes a loss of the normal rectification of the current at plateau voltages, which results in a significant increase of I_{Kr} during phases 2 and 3 of the action potential leading to abbreviation of the action potential and both atrial and ventricular refractoriness. Bellocq et al. shortly after reported on a mutation in a single sporadic case of a 70-year-old patient with SQTS (QTc 302 ms) and sudden cardiac arrest. They identified a gain-of-function mutation (p. V307L) in the *KCNQ1* gene which encodes the slow component of the delayed rectifier potassium channel (I_{Ks}) (SQT2). The mutation caused a −20 mV shift of the half-activation potential and acceleration of the activation kinetics and activation of the mutant channels at more negative potentials. This resulted in a gain of function of I_{Ks} and abbreviation of the action potential. A further missense mutation in the same gene (p. V141M) was identified in a baby with bradycardia and AF in utero [6]. The ECG revealed a shortened QT interval and episodes of AF.

Priori and coworkers identified in two relatives without sudden cardiac arrest a gain of function in *KCNJ2*, encoding the inward rectifier potassium channel (I_{K1}) causing abbreviation of the QT interval and asymmetrical T waves with a rapid terminal downslope [4].

Later our own group together with Antzelevitch and coworkers further described novel mutations of the cardiac L-type calcium channel genes responsible for shortening of the QT interval in families characterized by sudden cardiac death, AF, and a Brugada type I ECG pattern (Fig. 13.3). These patients displayed a mixed phenotype [5]. Functional analyses revealed loss-of-function missense mutations of the *CACNA1C* (p. A39V and p. G490R) and *CACNB2b* (p. S481L) genes encoding the pore forming of $Ca_v1.2$ α1 – and β2b-subunits of the cardiac L-type calcium channel. The decreased net current of the cardiac L-type calcium channels led to an abbreviation of the plateau phase of the action potential and thus to a short QT interval.

In the last 5 years since the last edition of this book, a number of new patients and families have been added to the literature who suffered from mutations in *KCNH2* and *KCNQ1*. A Japanese group identified a novel mutation in KCNH2-p.I560T that resulted in a 2.5-fold increase of peak current density in COS-7 cells and a mutation in *KCNH2*-p.T618L [14]. This identical mutation had already been described by a Chinese group. The Japanese authors also found a mutation in *KCNQ1*-p. V141M. Moreno et al presented a male individual with a family history of sudden death with a QTc of 356 ms who had a mutation in the S5 segment of the KCNQ1 that impaired its association with KCNE1 [9]. Suzuki and coworkers published a case of an asymptomatic 10-year-old boy who displayed a QTc interval of 260 ms. In molecular genetic screening, they found a mutation in the *KCNH2*-p. N588K, identical to the one identified in the first two unrelated families in 2000 and 2003 [32].

Deo et al. described a mutation in *KCNJ2* – p.A896T that resulted in a strongly enhanced IK1 outward current leading to a phenotype of an extremely abbreviated QT interval and AF. A mutation in this gene had already been identified by Priori et al. in 2004 [33].

Finally, a report from a French group presents a family with inherited L-carnitine deficiency, in which a short QT interval was observed in all affected members. After substitution of carnitine, the QT interval was significantly prolonged toward the normal range. To confirm the role of carnitine in the evolution of a short QT interval, the authors used a mouse model, in which they could demonstrate that carnitine deficiency induced a short QT interval and a predisposition to inducible VF [34].

Table 13.1 Short QT subtypes

SQT	Gene	Channel
SQT1	*KCNH2*	IKr
SQT2	*KCNQ1*	IKs
SQT3	*KCNJ2*	IK1
SQT4	*CACNA1C*	ICa
SQT5	*CACNB2b*	ICa

Pathophysiology

After the identification of the underlying mutations and affected cardiac ion channels, the cellular basis and arrhythmogenesis in SQTS were examined.

The first experiments in transmural left ventricular wedge preparations and Langendorff heart preparations were performed using pinacidil, an activator of $I_{K\text{-}ATP}$ as no specific I_{Kr}, I_{Ks}, or I_{K1} agonists were available [35, 36]. Under pinacidil, the QT interval was shortened and transmural dispersion of repolarization increased. The action potential was abbreviated heterogeneously among different cell types spanning the ventricular wall and thus open the window for the genesis for polymorphic ventricular tachycardia (phase-2-reentry). Transmural dispersion of repolarization was associated with the inducibility of ventricular tachyarrhythmias. Quinidine application was able to reduce monophasic action potential duration and dispersion of repolarization [29].

In the clinical setting a number of VF or PVT onsets could be recorded either from monitoring or from ICD data storage. The coupling interval of the initiating PVC to the previous normal beat was extremely short favoring this hypothesis for arrhythmogenicity in SQTS. Mazzanti, Schimpf, and Giustetto reported on coupling intervals ranging from 190 to 320 ms, but predominantly around 230–250 ms [11, 37, 38].

There are some new experiments also on modeling currents in hiPSC-CMs, in which in silico currents are injected to study loss and gain of function of KCNJ2. Meijer van Putten and others have nicely demonstrated that effects of specific mutations can be simulated by this new technique [39].

Molecular Genetics and Specific Consequences of the Genotype for Therapy, Follow-Up, and Prognosis

Pharmacologic Therapy of Short QT Syndrome

After the identification of the genetic background and the cellular mechanisms of the SQTS, clinical and experimental studies have been conducted with respect to the pharmacologic treatment. However, data on the pharmacologic treatment of patients with SQTS are still limited with respect to the clinical use and long-term outcome because of the low number of patients diagnosed with SQTS at the moment.

Most of the experiences in vitro and in vivo are available for SQT1. Studies on heterogeneous expression exhibited that the p. N588K mutation increased the density of I_{Kr} and reduced the affinity of I_{Kr} blockers like d-sotalol 20-fold [7]. Thus, in vitro experiments could prove the failure of d-sotalol restoring QT interval in vivo. McPate et al. could demonstrate that the effect of E-4031, a specific I_{Kr} blocker, was also significantly attenuated by the p.N588K mutation, whereas quinidine was less and disopyramide the least affected by p. N588K-HERG [40]. Cordeiro et al. could nicely show that these findings are based on the +90 mV shift in the voltage dependence of inactivation of the HERG channels. Most I_{Kr}-blockers interact with the HERG channels in the inactivated state. Thus, a failure of inactivation of the HERG channel leads to the inefficacy of the specific I_{Kr} blockers [41]. Recently, McPate et al. could demonstrate that besides disopyramide and quinidine also propafenone and amiodarone were only slightly inhibited by the mutant p. N588K [41]. Thus, these drugs may serve as an additional option in the pharamacologic treatment of SQT1. For SQT3 El, Harchi et al. could identify in in vitro experiments that chloroquine is an effective pharmacologic inhibitor of the SQT3 p.D172N mutant Kir2.1 [7].

In the clinical setting, several class I and III antiarrhythmic drugs have been tested in patients with the gain-of-function mutation in HERG (SQT1). For class III antiarrhythmics, neither d-sotalol nor ibutilide was able to prolong QT interval in the first STQ-1 patients. Flecainide, a Na^+-channel blocker, which has in addition a blocking effect on I_{Kr} and on the transient outward potassium current (I_{to}), led to an increase in ventricular effective refractory periods. However, acute administration of flecainide did cause only a mild prolongation of refractoriness and only slight prolongation of the QT interval [42]. In contrast, the class I antiarrhythmic agent, quinidine, was able to normalize the QT interval and to prolong the ventricular effective refractory period in patients with a SQT1 [29]. Additionally, quinidine restored the heart rate dependence of the QT interval toward the normal range and rendered ventricular tachyarrhythmias non-inducible in patients in whom baseline electrophysiological studies demonstrated reproducible inducibility of VF. Following the positive effects of disopyramide in in vitro experiments, disopyramide has also been shown to be effective in a pilot study in patients with a SQT1.

The most frequently used drug in the past was quinidine. No patient on quinidine therapy suffered from VF or a recurrence of AF during the mid-term follow-up in the short QT registry [10, 38]. A subset from the SQT-1 family published by Bjerregaard et al. treated with propafenone is free of recurrences of AF without prolongation of the QT interval (personal communication). Whether quinidine, propafenone, or disopyramide represent an alternative to ICD therapy in prevention of sudden cardiac death cannot be finally answered. Drugs may be an alternative in patients refusing ICD implantation or for those who are not eligible for ICD therapy. In addition, drugs can be given to ICD-bearing patients who experience recurrent electrical shocks.

Whether the effects of the investigated class I and III drugs can be translated to SQT2–SQT5 is not clear. However, in a patient with SQT4 quinidine was able to prolong QT interval and suppress paroxysms of AF.

Due to the electrophysiological and genetic heterogeneity of the SQTS, therapy may have very different effects depending on the type of mutation and the affected channel. Further studies of pharmacologic therapy are warranted to elucidate the potential long-term benefit of pharmacologic treatment.

The 2015 ESC guidelines recommend quinidine or sotalol when patients refuse an ICD or have a contraindication and in asymptomatic patients and a family history with a class IIb indication. Finally, however, some drug combinations have been successfully used in patients, which are not mentioned in the guidelines and to some extent the treatment of this very heterogeneous patients will remain individual [30].

ICD Therapy

By now the only reliable treatment to prevent patients from sudden cardiac death is the implantation of an ICD. In symptomatic patients with SQTS, the ICD is the therapy of choice, while antiarrhythmic drug therapy may represent an adjunct or an alternative therapy in children or in newborns, where ICD implantation is technically challenging and often associated with high morbidity. The risk for inappropriate ICD discharges due to T wave oversensing is increased in patients with SQTS compared to other conditions with ICD implanted, since intra-cardiac T waves are high and closely coupled to the preceding R wave. This issue can be solved by individual ICD programming of the sensing parameters and selection of specific devices. Additionally, quinidine therapy helped to avoid T wave oversensing by increasing the QT interval [43]. The urgent need to program the ICD very diligently and carefully select device and leads is underlined by the high reported incidence of inappropriate shocks, especially in the pediatric cohort.

Risk Stratification and Indication for ICD

Risk stratification in SQTS is based on the QTc-interval, family history of sudden death <40 years of age in a relative, and symptoms. An ICD is indicated following the 2015 ESC guidelines for survivors of sudden death or patients with a spontaneous documented VT.

Unfortunately, there is no mentioning of patients with a history of syncope and a short QTc, for example, a positive family history, in whom an ICD may be helpful in individual cases as we know from our own experience.

Cardiogenetic Aspects

Due to the high genetic heterogeneity in SQTS and the low number of patients, a general genotype–phenotype correlation cannot be established. Currently, the yield of genetic testing is still quite low in patients with a clinical SQTS. This suggests that other, unknown, genetic defects may be involved. For scientific reasons after the identification of a patient with SQTS, genetic analysis should be attempted and family screening initiated depending on the relatives' phenotype. As far as the published series of patients are concerned, the percentage of mutation positive probands was 5/21 children in the Villafane report, 5/22 index patients in the Euro Short registry, 5/45 for potassium channel screening, and 1/35 for calcium channel screening in the Mazzanti series. Segregation studies suggested in the last series that the mutation was inherited in three and most likely de novo in two probands. The penetrance, which the authors defined as QTc <360 ms was 100 % in the pedigrees with multiple carriers ($n = 3$ probands and $n = 4$ family members).When they compared QTc data among patients with complete genetic screening, they observed significantly shorter QTc intervals in mutation carriers versus non-carriers. Giustetto et al. found a statistically significant difference in QT, QTc, JT-peak when comparing HERG and non-HERG-mutation carriers. The QTc was 297/–29 ms (HERG) versus 319 ± 17 ms (non-HERG). Interestingly, the QTc prolongation following hydroquinidine was significantly longer in HERG versus Non-HERG patients with 105 ± 14 versus 49 ± 9 ms.

The yield of genetic testing is lower than, for example, in Brugada syndrome or long QT syndrome. This may change in the future with more known genes and better techniques.

Currently, it seems recommendable to perform genetic testing for the known genes involved and also new genes in the proband and then, if proven to be a mutation carrier, in relatives, who could be at risk.

Summary

The SQTS is one of the primary electrical diseases of the heart with a high incidence of syncope and sudden cardiac death. The hallmark for the diagnosis is a short QT interval on the baseline ECG. Up to now, approximately 150 patients are identified worldwide. Due to the limited number of patients and the genetic heterogeneity of the disease, a strong genotype–phenotype correlation and a conclusive risk stratification are not yet available.

Patients with SQTS should be referred for genetic counseling, molecular genetic analysis, and initiation of family screening.

Take Home Message

- SQTS is a very rare but potentially highly malignant disease.
- SQTS should be considered in anyone with a QT <350 ms without potential other causes.
- One must always think about SQTS in the following special cases:
  - aborted cardiac arrest or sudden cardiac death of unknown origin
- AF at young age

References

1. Gussak I, Brugada P, Brugada J, Wright RS, Kopecky SL, Chaitman BR, et al. Idiopathic short QT interval: a new clinical syndrome? Cardiology. 2000;94:99–102.
2. Gaita F, Giustetto C, Bianchi F, Wolpert C, Schimpf R, Riccardi R, et al. Short QT syndrome: a familial cause of sudden death. Circulation. 2003;108:965–70.
3. Bellocq C, van Ginneken AC, Bezzina CR, Alders M, Escande D, Mannens MM, et al. Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. Circulation. 2004;109:2394–7.
4. Priori SG, Pandit SV, Rivolta I, Berenfeld O, Ronchetti E, Dhamoon A, et al. A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. Circ Res. 2005;96:800–7.
5. Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, et al. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation. 2007;115:442–9.
6. Hong K, Piper DR, Diaz-Valdecantos A, Brugada J, Oliva A, Burashnikov E, et al. De novo KCNQ1 mutation responsible for atrial fibrillation and short QT syndrome in utero. Cardiovasc Res. 2005;68:433–40.
7. Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, et al. Sudden death associated with short-QT syndrome linked to mutations in HERG. Circulation. 2004;109:30–5.
8. El Harchi A, Melgari D, Zhang YH, Zhang H, Hancox C. Action potential clamp and pharmacology of the variant 1 short QT syndrome T618I hERG K+ Channel. PLoS One. 2012;7:e52451.
9. Moreno C, Olivera A, de la Cruz A, Bartolucci C, Mun C, Salar E, et al. A new KCNQ1 mutation at the S5 segment that impairs its association with KCNE1 is responsible for short QT syndrome. Cardiovasc Res. 2015;107:613–23.
10. Giustetto C, Di Monte F, Wolpert C, Borggrefe M, Schimpf R, Sbragia P, et al. Short QT syndrome: clinical findings and diagnostic-therapeutic implications. Eur Heart J. 2006;27:2440–7.
11. Mazzanti A, Kanthan A, Monteforte N, Memmi M, Bloise R, Novelli V, et al. Novel insight into the natural history of short QT syndrome. J Am Coll Cardiol. 2014;63:1300–8.
12. Villafane J, Atalla J, Gollob M, Maury P, Wolpert C, Gebauer R, et al. Long-term follow-up of a pediatric cohort with short QT syndrome. J Am Coll Cardiol. 2013;61:1183–91.
13. Villafane J, Fischbach P, Gebauer R. Short QT syndrome manifesting with neonatal atrial fibrillation and bradycardia. Cardiology. 2014;128:236–40.
14. Harrell DT, Ashihara T, Ishikawa T, Tominaga I, Mazzanti A, Takahashi K, et al. Genotype-dependent differences in age of manifestation and arrhythmia complications in short QT syndrome. Int J Cardiol. 2015;190:393–402.
15. Fukuyama M, Ohno S, Wang W, Kimura H, Makivama T, Itoh H, et al. L-Type calcium channel mutations in Japanese patients. With inherited arrhythmias. Circ J. 2013;77:1799–806.
16. Anttonen O, Junttila MJ, Rissanen H, Reunanen A, Viitasalo M, Huikuri HV. Prevalence and prognostic significance of short QT interval in a middle-aged Finnish population. Circulation. 2007;116:714–20.
17. Funada A, Hayashi K, Ino H, Fujino N, Uchiyama K, Sakata K, et al. Assessment of QT intervals and prevalence of short QT syndrome in Japan. Clin Cardiol. 2008;31:270–4.
18. Gallagher MM, Magliano G, Yap YG, Padula M, Morgia V, Postorino C, et al. Distribution and prognostic significance of QT intervals in the lowest half centile in 12,012 apparently healthy persons. Am J Cardiol. 2006;98:933–5.
19. Kobza R, Roos M, Niggli B, Abacherli R, Lupi GA, Frey F, et al. Prevalence of long and short QT in a young population of 41,767 predominantly male Swiss conscripts. Heart Rhythm. 2009;6:652–7.
20. Viskin S. The QT interval: too long, too short or just right. Heart Rhythm. 2009;6:711–5.
21. Iribarren C, Round AD, Peng J, Lu M, Klatzsky AL, Zaroff JG, et al. Short QT in a cohort of 1.7 million persons: prevalence, correlates, and prognosis. Ann Noninvasive Electrocardiol. 2014;19:490–500.
22. Portugal G, Olivera MM, Cunha P, Ferreira F, Lousinha A, Fiarresga A, et al. Short QTsyndrome presenting as syncope: how short is too short? Rev Port Cardiol. 2014;33:649.e1–6.
23. Postema PG, Wilde AMM. The measurement of the QT interval. Curr Cardiol Rev. 2014;10:287–94.
24. Anttonen O, Junttila MJ, Maury P, Schimpf R, Wolpert C, Borggrefe M, et al. Differences in twelve-lead electrocardiogram between symptomatic and asymptomatic subjects with short QT interval. Heart Rhythm. 2009;6:267–71.
25. Giustetto C, Scorocco C, Schimpf R, Maury P, Mazzanti A, Levetto M, et al. Usefulness of exercise test in the diagnosis of shortQT syndrome. Europace. 2015;17:628–34.
26. Tülümen E, Giustetto C, Wolpert C, Maury P, Anttonen O, Probst V, et al. PQ segment depression in patients with short QT syndrome: a novel marker for diagnosing short QT syndrome? Heart Rhythm. 2014;11:1024–30.
27. Schimpf Echo
28. Frea S, Giustetto C, Capriolo M, Scrocco C, Fornengo C, Benedetto S, et al. New echocardiographic insights in short QT syndrome: more than a channelopathy? Heart Rhythm. 2015;12:2096–105.
29. Wolpert C, Schimpf R, Giustetto C, Antzelevitch C, Cordeiro J, Dumaine R, et al. Further insights into the effect of quinidine in short QT syndrome caused by a mutation in HERG. J Cardiovasc Electrophysiol. 2005;16:54–8.
30. Priori SG, Blomström-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, et al. 2015 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. Eur Heart J. 2015;36:2793–867.
31. Gollob MH, Redpath CJ, Roberts JD. The short QT syndrome. JACC. 2011;57:802–12.
32. Suzuki H, Hoshina S, Ozawa J, Sato A, Minamino T, Aizawas Y. Short QT syndrome in a boy diagnosed on screening for heart disease. Pediatr Int. 2014;56:774–6.
33. Deo M, Ruan Y, Pandit SV, Shah K, Berenfeld O, Blaufox A, Cerrone M, et al. KCNJ2 mutation in short QT syndrome 3 results in atrial fibrillation and ventricular proarrhythmia. Proc Natl Acad Sci U S A. 2013;110:4291–6.
34. Roussel J, Labarthe F, Thireau J, Ferro F, Farah C, Roy J, et al. Carnitine deficiency induces a short QT syndrome. Heart Rhythm. 2016;13:165–74.

35. Extramiana F, Antzelevitch C. Amplified transmural dispersion of repolarization as the basis for arrhythmogenesis in a canine ventricular-wedge model of short-QT syndrome. Circulation. 2004;110:3661–6.
36. Milberg P, Tegelkamp R, Osada N, Schimpf R, Wolpert C, Breithardt G, et al. Reduction of dispersion of repolarization and prolongation of postrepolarization refractoriness explain the antiarrhythmic effects of quinidine in a model of short QT syndrome. J Cardiovasc Electrophysiol. 2007;18:658–64.
37. Schimpf R, Bauersfeld U, Gaita F, Wolpert C. Short QT syndrome: successful prevention of sudden cardiac death in an adolescent by implantable cardioverter-defibrillator treatment for primary prophylaxis. Heart Rhythm. 2005;2:416–7.
38. Giustetto C, Schimpf R, Mazzanti A, Scorocco S, Maury P, Anttonen O, et al. Long-term follow-up of patients with short QT syndrome. J Am Coll Cardiol. 2011;58:587–95.
39. Meijer van Putten R, Mengarelli I, Guan K, Zegers JG, van Ginneken ACG, Verkerk AO. Ion channelopathies in human induced pluripotent stem cell derived cardiomyocytes: a dynamic clamp study with virtual IK1. Front Physiol. 2015;6:1–18.
40. McPate MJ, Zhang H, Adeniran I, Cordeiro JM, Witchel HJ, Hancox JC. Comparative effects of the short QT N588K mutation at 37 degrees C on hERG K+ channel current during ventricular, Purkinje fibre and atrial action potentials: an action potential clamp study. J Physiol Pharmacol. 2009;60:23–41.
41. Cordeiro JM, Brugada R, Wu YS, Hong K, Dumaine R. Modulation of I(Kr) inactivation by mutation N588K in KCNH2: a link to arrhythmogenesis in short QT syndrome. Cardiovasc Res. 2005;67:498–509.
42. Gaita F, Giustetto C, Bianchi F, Schimpf R, Haissaguerre M, Calo L, et al. Short QT syndrome: pharmacological treatment. J Am Coll Cardiol. 2004;43:1494–9.
43. Schimpf R, Wolpert C, Bianchi F, Giustetto C, Gaita F, Bauersfeld U, et al. Congenital short QT syndrome and implantable cardioverter defibrillator treatment: inherent risk for inappropriate shock delivery. J Cardiovasc Electrophysiol. 2003;14:1273–7.

Idiopathic Ventricular Fibrillation

14

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Abstract

Idiopathic ventricular fibrillation (IVF) is a rare condition in which patients without major structural heart diseases develop VF and often suffer from sudden cardiac death. To date, the IVF was defined excluding other primary electrical disorders. IVF may not be composed of a single entity but contain multiple forms by clinical manifestations and possible pathogenetic backgrounds. In this chapter, IVF can be described in two groups: the early repolarization syndrome (ERS), and IVF in a narrow sense excluding ERS. ERS is characterized by elevation of J-point in inferior or lateral leads, accompanied with notch or slur in the terminal portion of QRS complex. Although early repolarization pattern is not rate in healthy subjects, some of them suffer from lethal ventricular tachyarrhythmias. The intramural discordant repolarization property may explain the pathogenesis of ERS partly, but other confounding factors also contribute to the ventricular tachyarrhythmias. The IVF excluding ERS is a very rate, and may include several different types of electrocardiographic and clinical manifestation. Several electrical abnormalities are involved in the pathogenesis of the IVF, including the abnormality in conduction of His-Purkinje system, short-coupled premature contraction, and conduction disturbance in ventricle.

Idiopathic Ventricular Fibrillation

Idiopathic ventricular fibrillation (IVF) is a rare condition in which patients without major structural heart diseases develop VF and often suffer from sudden cardiac death (SCD). While sporadic case reports of VF developing in patients without pathological conditions of the heart have been presented in the literature, the collected case studies under the terminology of IVF appeared as several publications during late 1980s to early 1990s [1–3]. Several conditions that are now categorized as primary electrical diseases also develop VF without major cardiac structural abnormalities. These are generally excluded from IVF as a different disease entity due to its unique clinical manifestations and special pathogenic mechanism. Further, IVF may not be composed of a single entity but rather contain multiple forms with different electrocardiographic manifestations and probable causative genes.

In this chapter, IVF is divided into two groups: (A) the early repolarization syndrome (ERS) and (B) IVF in a narrow sense without ERS. Early repolarization (ER) pattern in ECG indicates the elevation of the QRS and ST-segment junction (J-point), notch and slur at the terminal portion of QRS complex. IVF excluding ERS shows no ER pattern on the electrocardiography (ECG) and develops VF events. Clinical presentations of both groups of IVF develop as a sudden onset of VF and/or aborted SCD in subjects without structural heart diseases and any known conditions that causes fatal arrhythmic events. In some cases, there is a family history of ER pattern on ECG and/or SCD. Diagnosis

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of ERS can be made in patients after the resuscitation from VF/aborted SCD with an exclusion of structural heart diseases and any other reason for fatal arrhythmic events. ECG during sinus rhythm shows ER pattern in the inferior and/or lateral leads. The prevention of SCD by antiarrhythmic drugs is not sufficiently achieved and implantable cardioverter defibrillator (ICD) implantation is the only available therapy for the SCD prevention. The mechanism of ERS is assumed to be explained by the repolarization theory based on experimental studies, but clinical and genetic proof of this concept has not fully been clarified. Other types of IVF include several different types of clinical manifestation with mostly unknown diagnostic criteria and underlying mechanisms. Some forms of IVF have a familiar inheritance and genetic backgrounds have been identified as a possible pathogenesis in the limited cases.

Early Repolarization Syndrome (ERS)

Introduction

Early repolarization syndrome (ERS) is a type of IVF, in which patients with ER pattern in ECG develop sudden attacks of VF in the absence of structural heart disease and any known causes of fatal arrhythmias. ER pattern in ECG has for a long time been considered as a benign ECG sign except for the "Osborn wave" by hypothermia [4, 5].

In 1992, Brugada and Brugada reported a unique form of IVF, now known as Brugada syndrome, where patients with J-point and ST-segment elevation (ER pattern) in V1–V3 without structural heart disease are prone to develop VF and SCD [6]. Introduction of Brugada syndrome has brought about a strong interest for ER having possible correlation or trigger for the development of fatal arrhythmias. Actually, several studies of IVF with ER pattern excluding Brugada syndrome were published as reports of a single or collected cases, suggesting a possible arrhythmogeneity of ER [7–11]. The hypothesis was challenged by the experimental study dealing with canine wedge preparations for models of ER, which were shown to be capable of developing rapid polymorphic ventricular tachycardia (PVT) [12, 13].

Clinical results supporting this hypothesis were then provided with the seminal work by Haissaguerre et al. They demonstrated a high prevalence of ER pattern in patients with IVF [14]. In their study, 206 IVF and 412 control cases were explored, and ER pattern was more frequently observed in IVF patients compared with the controls (31 % vs. 5 %, respectively). The odds ratio (OR) for the presence of ER in the IVF patients with the control was 10.9 (95 % confidence interval (CI): 6.3–18.9) after adjusting for age, sex, race and level of physical activity. Similar observations were confirmed in IVF patients with case–control studies [15, 16]. The ratio of ER pattern in patients with IVF was 42–60 %, which was significantly higher than the controls (3.3–13 %, $p < 0.05$). Figure 14.1 presents representative ECGs from one IVF patients with and without ER. Subsequently, Tikkanen et al. studied the prognostic significance of ER pattern in the general population [17]. An ER pattern in inferior leads was associated with an increased risk of cardiac death in middle-aged population.

The term "early repolarization" (ER) in 12-lead ECG has been used in cardiology for many years, but its exact definition varies widely depending on the investigators. Because of such variations, the prevalence of ER in normal population varied between 2 % and 31 % [18]. In 1976, Kambara and Phillips [19] proposed the following definition of ER: (1) end-QRS notching or slurring; (2) elevation of the ST-segment; and (3) an upward-sloping ST-segment followed by a tall, symmetrical T wave. In the clinical practice, many physicians regarded the presence of J-point and ST-segment elevation merged with positive T wave, as ER being a benign ECG sign.

Therefore, definition of ER and its terminology has not yet achieved a general consensus. Recently, Macfarlene et al., have proposed a unified definition of ER to assist future studies [20]. According to their consensus paper, ER is present if all of the following criteria are met:

(i) There is an end-QRS notch or slur on the down-slope of a prominent R-wave. A notch should lie entirely above the baseline. The onset of a slur must also be above the baseline. (A notch and slur should occur on the final 50 % segment of the QRS complex).
(ii) The peak of J point is ≥ 0.1 mV in two or more contiguous leads of the 12-lead ECG, excluding lead V1 to V3.
(iii) QRS duration is <120 ms.

If the ST-segment is upward sloping and followed by an upright T wave, the pattern should be described as "ER with ascending ST segment".

If the ST-segment is horizontal or downward sloping, the pattern should be described as "ER with a horizontal or descending ST segment".

Clinical Presentation of ERS

ERS refers to the patients with IVF showing ER pattern on the ECG in inferior and/or lateral leads. It is important to recognize that ERS and ER patterns in ECGs should be separated from each other, since an ER pattern itself is mostly a benign ECG finding. Clinical diagnosis of ERS can be made in specific patients who were resuscitated from cardiac arrest due to VF, or PVT, and with a 12-lead ECG demonstrating ER pattern during sinus rhythm. At the same time, it is absolutely necessary to exclude structural heart diseases and other primary electrical disorders including

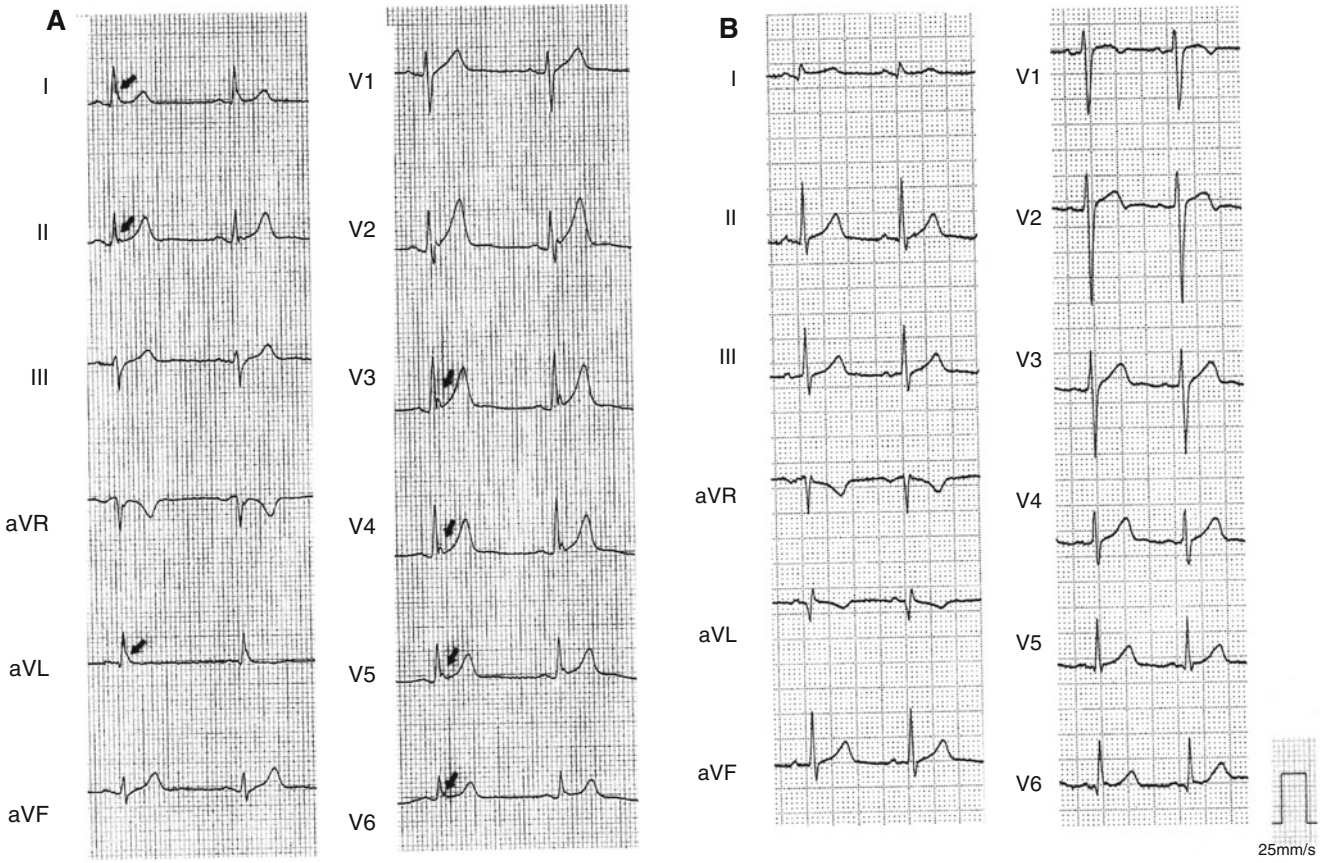

Fig. 14.1 Representative electrocardiograms (ECGs) of the IVF patients with ER (panel A) and without ER (panel B) (Panel A: ERs were recorded in both lateral and inferior leads. Panel B: ER was not observed in any lead (Reproduced by permission from Sekuguchi et al. [60]))

long QT syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, and short QT syndrome (see the details in the individual sections of this book).

The most difficult diagnostic dilemma is to differentiate the malignant ER from benign ER in subjects showing this particular ECG sign in the inferior and/or lateral leads. The prevalence of the ER pattern in the inferior and/or lateral leads has been reported to be in a range of 3–24 % in the general population [21, 22]. The prevalence varies considerably depending on age, sex, race, and physical activity. Most of these subjects are asymptomatic without developing VF events. While clinical implications of ER pattern in asymptomatic subjects are not clear, it is assumed that the presence of ER pattern triples the risk of VF. Despite of this increase, the overall risk is still negligible because IVF itself is a rare disorder. Adler et al. have estimated that the risk of developing IVF in an individual younger than 45 years is 3:100,000. The risk increases to 11:100,000 when a J-point elevation is present [23]. A meta-analysis estimated absolute difference of subjects with ER pattern by seven cases of arrhythmic death per 100,000 subjects per year [24]. Although the presence of an ER pattern increases relative risk of VF, the absolute risk is still very low.

Clinical Diagnosis and Differential Diagnosis

ERS usually develops with sudden and unexpected onset of syncope and/or aborted SCD due to life-threatening ventricular arrhythmias, VF/PVT. A diagnosis of ERS can be confirmed in resuscitated patients after VF who show an ER pattern in their ECGs, while other causes of arrhythmic events are excluded (Figs. 14.2 and 14.3). Heart Rhythm Society (HRS)/European Heart Rhythm Association (EHRA)/ Asia Pacific Heart Rhythm Society (APHRS) expert consensus statement has provided recommendations for ERS diagnosis [25] (Table 14.1). A diagnostic definition is stated as "ERS is diagnosed in the presence of J-point elevation ≥1 mm in ≥2 contiguous inferior and/or lateral leads of a standard 12-lead ECG in a patient resuscitated from otherwise unexplained VF/PVT". A highly possible case and definition of ER pattern are also described.

Other features of clinical manifestations have been presented in various reports on an observational basis with only limited numbers of cases. Thus, definite diagnostic criteria for ERS and risk stratification has not been achieved in general consensus at present time, but physicians should follow the HRS/EHRA/APHRS expert consensus recommendations to make a diagnosis.

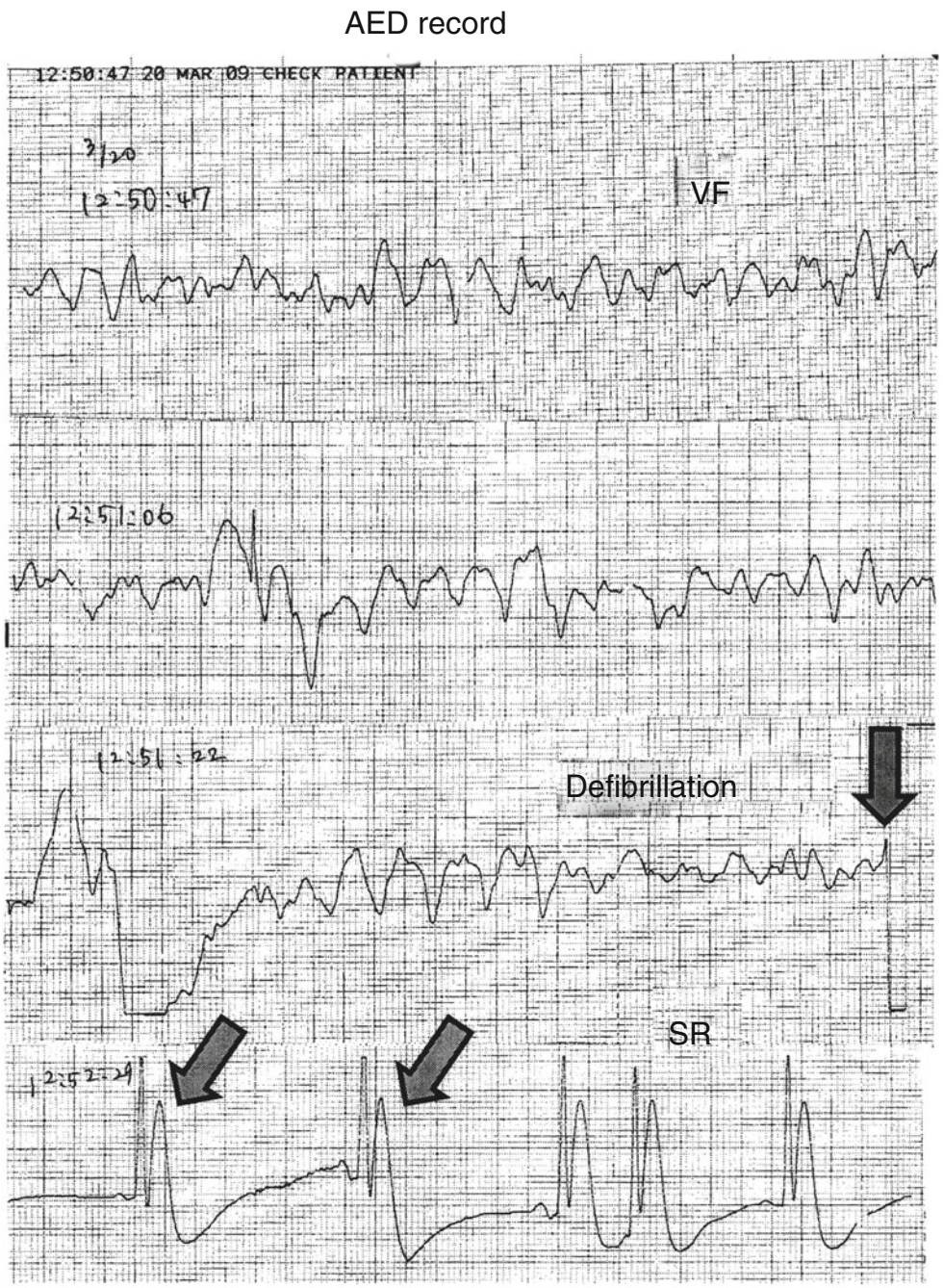

Fig. 14.2 VF and giant J-wave in sinus rhythm after defibrillation (ECG recorded by automated external defibrillator (AED) from 37 years old man with aborted sudden cardiac death. AED detected VF and delivered electric shock (the right corner of the third row) restored sinus rhythm with giant J-wave (the bottom row). He was shown to have no structural heart disease by various cardiac examinations including echocardiography, coronary angiography, and pilsicainide provocation test for Brugada syndrome)

In addition, the following findings may help with the diagnosis in suspected cases or an atypical manifestation in clinical practice:

ERS has a male predominance. The mean age of the first VF episode is 35–43 years [14–16, 26, 27]. VF attacks are more likely to develop during sleep than during physical activity [14]. In patients with ERS, the amplitude of a J-point elevation increases with slow heart rate and after a pause [27] (Fig. 14.4). VF is usually triggered by short-long-short sequence and a short-coupled extrasystole initiates the arrhythmic events [28]. Circadian variation of the J-point elevation often occurs in association with the vagal tone [26]. Increased vagal tone augments and sympathetic stimulation by isoproterenol attenuates the amplitude of the J-point elevation [27]. Sodium channel blockers also attenuate the J-point elevation [27, 29–31]. The amplitude of ER increases prominently just before VF episodes [11, 14, 16, 28]. This is recognized as a hallmark of the disease. A global appearance of a J-point elevation on 12-lead ECG was suggested to develop within 30 min of VF storms [16]. Such J-point elevation may completely disappear within weeks after VF events [27]. Dynamic manifestation of the J-point elevation in

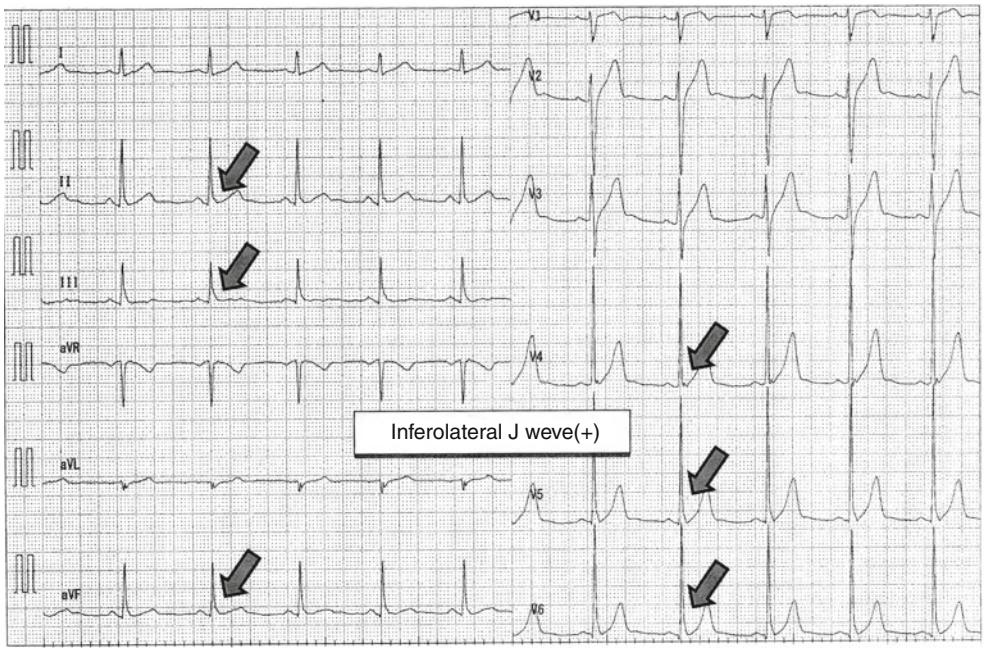

Fig. 14.3 Twelve-lead ECG with inferolateral ER (ECG was recorded from the same patient in Fig. 14.2 on admission. ERs (arrows) were observed in the inferior (II, III, aVF) and lateral (V3–V6) leads)

Table 14.1 Early repolarization diagnosis

1.	ER syndrome is diagnosed in the presence of J-point elevation ≥1 mm ≥2 contiguous inferior and/or lateral leads of a standard 12-lead ECG in a patient resuscitated from otherwise unexplained VF/polymorphic VT
2.	ER syndrome can be diagnosed in an SCD victim with a negative autopsy and medical chart review with a previous ECG demonstrating J-point elevation ≥1 mm in ≥2 contiguous inferior and/or lateral leads of a standard 12-lead ECG
3.	ER pattern can be diagnosed in the presence of J-point elevation ≥1 mm in ≥2 contiguous inferior and/or lateral leads of a standard 12-lead ECG

ECG electrocardiogram, ER early repolarization, VF ventricular fibrillation, SCD sudden cardiac death
(Reproduced by permission from Priori et al. [25])

malignant cases of ERS might be in contrast to rather stable expression of ER pattern in healthy individuals [17, 32, 33]. The magnitude of the J-point elevation may have some prognostic significance. Either slurred or notched J-point elevations ≥0.2 mV is relatively rare in the general population and appears to be associated with an increased risk [17]. A horizontal or descending ST segment following J-point elevation is associated with a worse outcome in the general population [32]. This ECG pattern also gives information in distinguishing IVF patients from matched controls and is probably a key sign to differentiate malignant form from benign ER patterns [33].

Signal averaged ECG demonstrated that late potentials were frequently positive and were concordant with the time of VF events in ERS patients, but no such correlation was found in IVF cases without ER or controls [26]. The repolarization parameters (T-wave alternans and QT dispersion) were not different between IVF patients with and without an ER pattern. Electrophysiologic study (EPS) was not an effective method to assess the risk of ERS patients. Inducibility of VF was low (34 %) in patients with a history of VF and was not different in IVF patients with or without ER pattern [14]. The results of a multicenter study to determine the role of EPS in risk stratification of ERS patients with a recent history of VF showed a low (22 %) inducibility and a low prediction rate (33 %) for VF recurrence in EPS-positive cases [34]. Therefore, the current programmed stimulation protocol does not enhance risk stratification in patients with ERS.

Differential diagnosis is to exclude any form of structural heart diseases to develop life-threatening ventricular arrhythmia and electrolyte imbalance, especially hypokalemia. Primary electrical diseases should be excluded by unique clinical manifestations depending on each disorder and genetic screening (Refer to each section).

Clinical Therapy

Table 14.2 presents the therapeutic interventions for ERS patients according to the HRS/EHRA/APHRS expert consensus recommendations [25]. The table indicates medical treatment and indication for ICD implantation. It also stresses that ICD implantation is not recommended for asymptomatic patients with an isolated ER pattern on ECG.

Electrical storm is relatively common after ICD implantation in patients with ERS. The acute use of isoproterenol was effective for the suppression of recurrent VF and VF storms.

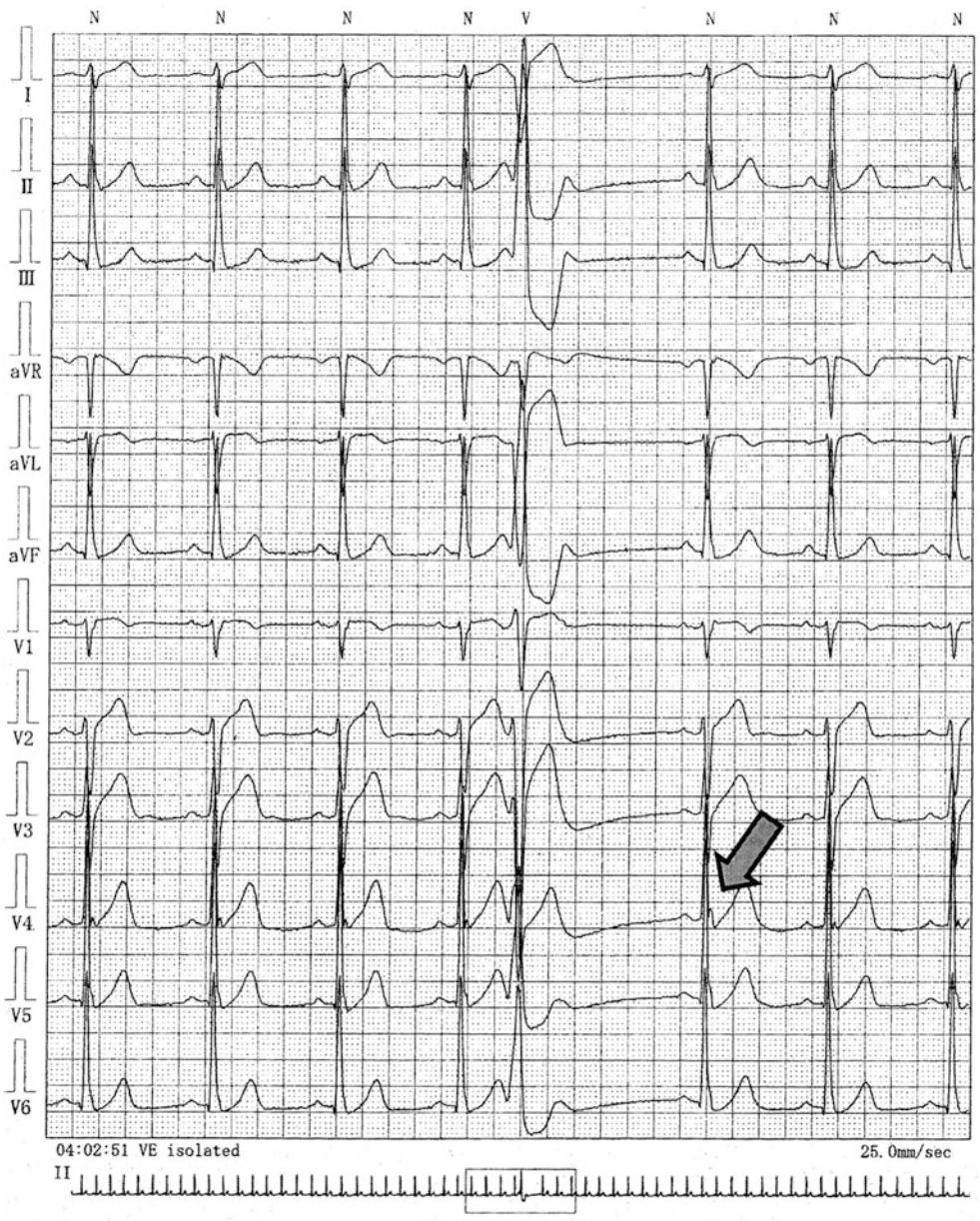

Fig. 14.4 Pause-dependent augmentation of ER (ER was augmented in a beat (arrow) following a long pause after premature ventricular contraction (recorded from the same patient shown in Fig. 14.2))

Table 14.2 Early repolarization therapeutic interventions

Class I	1. ICD implantation is recommended in patients with a diagnosis of ER syndrome who have survived a cardiac arrest
Class IIa	2. Isoproterenol infusion can be useful in suppressing electrical storms in patients with a diagnosis of ER syndrome
	3. Quinidine in addition to an ICD can be useful for secondary prevention of VF in patients with a diagnosis of ER syndrome
Class IIb	4. ICD implantation may be considered in symptomatic family members of ER syndrome patients with a history of syncope in the presence of ST-segment elevation >1 mm in two or more inferior or lateral leads
	5. ICD implantation may be considered in asymptomatic individuals who demonstrate a high-risk ER ECG pattern (high J-wave amplitude, horizontal/descending ST segment) in the presence of a strong family history of juvenile unexplained sudden death with or without pathogenic mutation
Class III	6. ICD implantation is not recommended asymptomatic patients with an isolated ER ECG pattern

ICD implantable cardioverter defibrillator
(Reproduced by permission from Priori et al. [25])

Isoproterenol is typically initiated at 1.0 μg/min, targeting a 20 % increase in heart rate or an absolute heart rate >90 bpm, titrated to hemodynamic response and suppression of recurrent ventricular arrhythmia [25]. Quinidine together with ICD implantation was suggested for long-term suppression of VF recurrences during the chronic phase [16, 29]. A small series of case study demonstrated that the combination of cilostazol and bepridil was shown to suppress VF recurrences and to attenuate the amplitude of the J-wave in patients with ICD implantation [35].

The clinical implications for asymptomatic subjects with ER pattern in ECG are not clear. While the presence of ER pattern is associated with times the risk of developing VF, the absolute risk is still negligible in the general population [24, 36]. Based on these population studies and clinical observations, middle-aged subjects with an ER pattern on the ECG, especially those with a high amplitude (≥0.2 mV) J-point elevation and horizontal/descending ST segment, should pay attention to risk reduction for long-term basis, especially on the occasions of acute coronary events [37].

The Mechanism of ER Pattern and Early Repolarization Syndrome

The genesis of J-wave or ER pattern on the ECG was proposed by the group of Antzelevitch et al. based on animal experiments using the canine ventricular wedge preparations [12, 13]. The proposed mechanism explains the genesis of a J-wave which is formed by the transmural voltage gradient during the early repolarization phase due to different action potential configurations between the epicardium and endocardium. The action potential of the epicardial cells exhibits a prominent notch at the early phase of repolarization and that of endocardial cells lacks such notch. The voltage gradient caused by the presence and absence of the notch between the epicardial and endocardial action potentials produces the J-wave configuration on ECG. The differences in action potential configurations are brought by the membrane current distribution: epicardial cells are rich in the transient outward potassium current (I_{to}), but the endocardial cells have least development of I_{to} [38]. Conditions that augment or reduce I_{to} could modify the manifestation of the J-wave on ECG. When I_{to} was augmented or changed the current kinetics by exposure to hypothermia, slow heart rate, application of the calcium and sodium channel blockers, or I_{to} agonist such as NS5806, epicardial action potential notch, and J-wave were augmented. Reduction of I_{to} by the application of I_{to} blockers such as 4-aminopyridine, quinidine, or premature stimulation caused parallel changes of decrease in the notch and J-wave [12, 39]. With further increase in I_{to}-mediated notch, some area of epicardial action potentials become markedly abbreviated while those of other area and endocardium are not much shortened, which provide the development of "phase-2 reentry" and initiate PVT/VF.

Antzelevitch and Yan [40] proposed the terminology of "J-wave syndrome". This concept is based on several lines of observations suggesting that arrhythmias associated with ER pattern in the infero-lateral leads, like Brugada syndrome, hypothermia, and acute ST-segment elevation myocardial infarction are mechanistically linked to abnormalities in the manifestation of It_o-mediated J-wave. Although ERS and Brugada syndrome differ with respect of the lead location and the magnitude of abnormal J-wave manifestation, they can be considered to represent a continuous spectrum of phenotypic expression that the authors propose the term "J-wave syndrome". They divide J-wave syndrome into three types: type 1, displaying an ER pattern predominantly in the lateral leads, is prevalent among healthy male athletes and rarely seen in VF survivors; type 2, displaying an ER pattern predominantly in the inferior or inferolateral leads, is associated with a higher level of risk; type 3, displaying an ER pattern globally in the inferior, lateral, and right precordial leads, is associated with the highest level of risk for the development of malignant arrhythmias and is often associated with VF storms. This terminology may not be widely accepted since it includes both benign and malignant forms.

The concept of the repolarization theory can explain the experimental results and support some clinical observations. There are, however, several problems left un-clarified. For example, contiguous myocardial cells exhibit fairly good electrical couplings among individual cells so that different action potential configurations are prone to be averaged and a steep voltage gradient is not likely to exist between the epicardium and endocardium, or among adjacent cells in the epicardial regions [41]. There may be an additional factor necessary to create the observed conditions in the limited region of the inferior or lateral wall of the ventricle, such as myocardial fibrosis. Second, the I_{to} current is composed of different genetic subunits that exhibit fast and slow current kinetics: the fast component is formed by *Kv4.2 (KCND2) + Kv4.3 (KCND3)* and the slow one by *Kv1.5 (KCNA4)*. Their expression and the combination differ in different cardiac regions and species [42]. It is not known whether the candidate genes for I_{to} current in human heart, especially their distribution at the inferolateral wall, is similar to canine ventricle or not. Clinically, if the heterogeneity of repolarization caused by I_{to} is the mechanism for developing VF in patients with ERS, it is still difficult to explain why the risk of arrhythmic death is so low (7 cases per 100,000 subjects per year), while an ER pattern in the general population is common (3–24 %) [21–24]. Electrophysiologic, genetic, and clinical documentations to prove the repolarization theory as an actual mechanism for ERS await further study.

Molecular Diagnostics and Molecular Genetics

Familiar ER pattern in ECG has been reported to have an autosomal dominant inheritance with incomplete penetrance.

Population-based studies also suggested some degree of inheritance of ER pattern in the general population [43, 44]. Genetic background to ER has been suggested by observations in subjects of a common family history of SCD with ER and IVF [14, 45], but the familiar inheritance of malignant ER pattern has not clearly been demonstrated.

A 14-year-old girl with IVF-showing ER pattern on the ECG and frequent episodes of VF was found to have a mutation in the *KCNJ8* gene, encoding *Kir6.1*, – the pore-forming subunit of the cardiac ATP-sensitive potassium (K.ATP) channel. The mutation identified in this girl, which was not found in 382 healthy controls, had the substitution of highly conserved serine residue at amino acid position 422 of the channel by leucine, *Kir6.1*-S422L [46]. Subsequently, *Kir6.1*-S422L was defined as a rare variant rather than mutation, and it was also found in sporadic cases of ERS. Electrophysiologic studies of the *Kir6.1*-S422L-mutant coexpressed with the *SUR2A* subunit in COS cells demonstrated an increase in the K.ATP channel current [47, 48]. No familiar inheritance of ERS was documented in these sporadic cases. Furthermore, a case of a homozygote mutation of *Kir6.1*-S422L was found in a Ashkenazi Jewish boy, who had no significant ECG abnormalities and no clinical symptoms, while the heterozygous father presented with a subtle J-point elevation. The study also demonstrated high frequency (~4 %) of *Kir6.1*-S422L in Ashkenazi Jews without ER pattern or ERS as compared to European, Middle Eastern non-Jewish, and non-Ashkenazi Jews (<0.25 %) [49]. The results suggest that this rare variant may not represent a sole pathogenic mechanism but require an additional modifier for the clinical manifestation of ERS.

Mutations in the L-type Ca channel genes, including *CACNA1C, CACNB2B,* and *CACNA2D1* [50] as well as loss-of-function mutations in *SCN5A* [51] have also been reported in patients with ERS, but inheritance is not clearly identified. Because of a high prevalence of ER pattern in the general population, ER may be caused by polygenic basis influenced by non-genomic factors as well. A recent genomewide association meta-analysis in three independent populations of European ancestry found eight loci associated with ER, the strongest association being found with SNPs located at the *KCND3* genes, which encode I_{to} channel (*Kv4.3*) coding gene [52]. These observations need further confirmations in other populations.

Family Screening and Follow-up in Relatives

There are currently no recommendations to screen the families of individuals with asymptomatic ER pattern on the ECG. No provocation tests are available to diagnose concealed ER in family members of ERS patients. Therapeutic recommendation by the HRS/EHRA/APHRS consensus statement uses the term "strong family history" [25]. There is no clear definition of this term, but it is typically chosen when more than one family member is affected, deaths occur at an early age and a first-degree relative is affected.

Summary

ERS is a specific type of IVF, which is a very rare but highly malignant disease. Diagnosis can be made in resuscitated patients after having VF showing a J-point elevation in the inferior and/or lateral leads during sinus rhythm, after exclusion of major structural heart diseases or primary electrical disorders. Treatment should be directed to protect recurrence of VF and SCD by implantation of an ICD. Isoproterenol infusion is effective for suppressing VF events during acute phase and VF storms. Quinidine may be useful in patients with ICD implantation for preventing recurrent VF events. The mechanism and genetic background of ERS have not been fully clarified.

Take Home Message

- ERS is a very rare but potentially highly malignant disease.
- ERS should be considered in anyone resuscitated from VF showing ER pattern in inferior and/or lateral leads of a standard 12-lead ECG during sinus rhythm and a strong family history of juvenile unexpected sudden death, without potential other causes.
- One always must think about ERS in the following:
  - Aborted cardiac arrest or SCD of unknown origin.
  - The presence of J-point elevation ≥1 mm in ≥2 contiguous inferior and/or lateral leads of a standard 12-lead ECG during sinus rhythm.
  - A strong family history of juvenile unexplained sudden death.
- No recommendation to screen the families of individuals with asymptomatic ER pattern on the ECG.

Idiopathic Ventricular Fibrillation Without ER Pattern

Idiopathic ventricular fibrillation (IVF) without ER pattern on the ECG is characterized by spontaneous VF in the absence of structural heart disease and in the absence of known electrical disorders. IVF cases are diagnosed and studied only after the resuscitation from the cardiac arrest with exclusion of structural heart diseases and primary electrical disorders. While incidences of IVF without ER are quite rare, scattered descriptions of small numbers of IVF patients have been presented in the literature without achieving a definite and uniform clarification of clinical characterization, genetic background, and diagnostic criteria/risk stratification of arrhythmic events. There are several clinical

forms of IVF, which may be caused by different pathogenic mechanisms.

IVF Related with His-Purkinje Conduction Disturbances

Recently, several reports described that the conduction disturbances in the His-Purkinje system were involved in the mechanism of IVF. In the latter part of this chapter, we focus on His-Purkinje conduction disturbance and its involvement in IVF.

Deletion of Irx3 in Mouse Model

The Iroquois homeobox (Irx) family is an iroquios homeoboxtranscription factor, which contains a highly conserved DNA-binding homeodomain and Iro motif in Drosophila and vertebrates. The *IRX* gene family has six subtypes forming two clusters (*IRX* 1, 2, and 4 on chromosome 13 and *IRX* 3, 5, and 6 on chromosome 16 in human) [53]. The *Irx* family genes were expressed in heart during mouse development [54–56]. Among them, it was reported that *Irx3* was selectively expressed in subendocardial layer in the ventricles and plays a critical role in generating the His-Purkinje system. Zhang et al., reported that a genetic deletion of *Irx3* in mice resulted in the disruption of the fast conducting system through the His-Purkinje network [57]. The Purkinje cells express Connexin-40 (Cx40) rather than Connexin-43 (Cx43), dominantly expressed in working ventricular myocytes. The conductance of Cx40 is larger than Cx43, which explains the faster conduction through the Purkinje network. The deletion of *Irx3* resulted in the reduced expression of Cx40 in the His-Purkinje system.

Following study utilizing *Irx3* knockout mouse identified that the deletion of *Irx3* showed not only ventricular conduction disturbance but also ventricular tachyarrhythmias [58]. Ventricular tachyarrhythmias were not seen in a baseline condition, but evoked by physical exercise, sympathetic activation, or acute myocardial infarction. *Ex vivo* electrophysiological study using optical mapping showed that delayed conduction at the right ventricular outflow tract (RVOT) at baseline, and the administration of isoproterenol-induced atrioventricular block, followed by nonsustained VT (Fig. 14.5). The findings suggested that these stimulations increased the discrepancy in conduction between impaired His-Purkinje system and intact myocardium.

Irx3 Mutation-related with IVF

Koizumi et al. further performed genetic screening in *IRX3* from 130 IVF cases including Brugada syndrome, short QT syndrome, and ERS and found two novel point mutations in VF cases (R421P and P485T) [58]. For the functional analysis of these mutations, they generated identical mutations

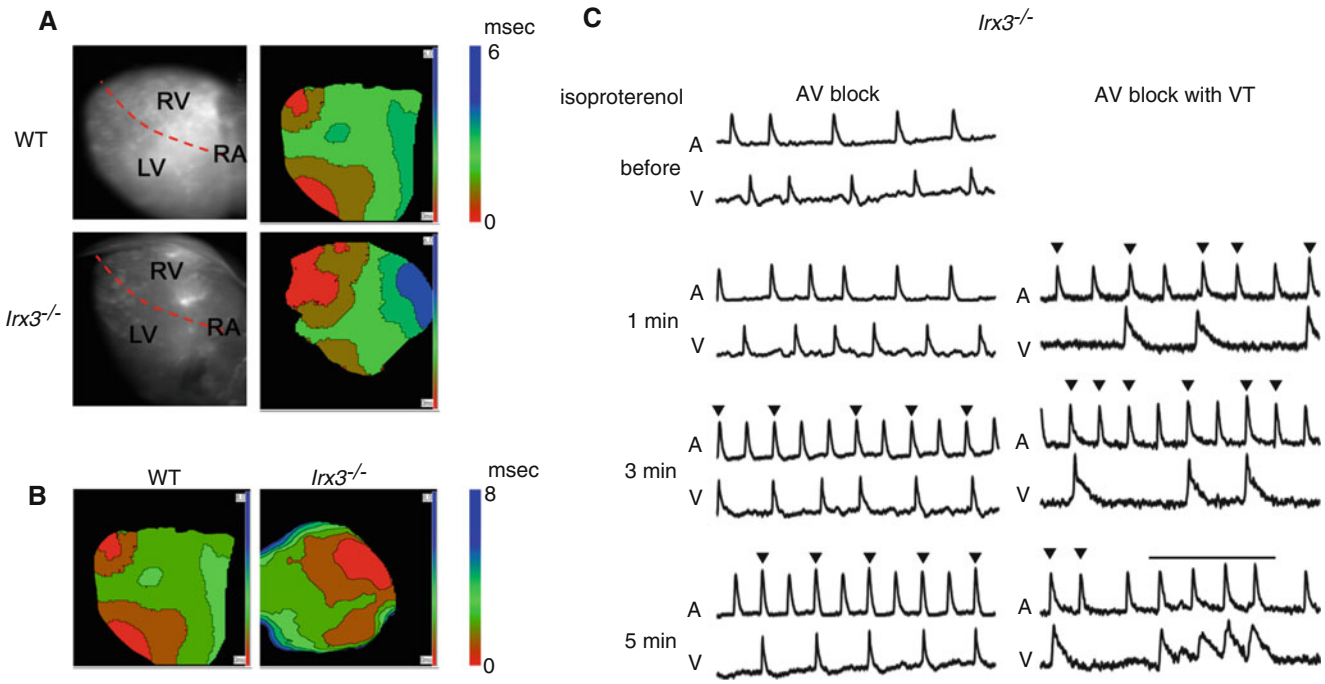

Fig. 14.5 *Ex vivo* optical epicardial mapping and arrhythmia development in Irx3$^{-/-}$ mice (Panel A: Representative optical epicardial mapping in WT and Irx3$^{-/-}$ mice in basal condition. Panel B: Representative optical epicardial mapping in WT and Irx3$^{-/-}$ mice after isoproterenol application. In Irx3$^{-/-}$ mice, epicardial breakthrough occurs from the base of the right ventricle and propagates to the apex; the propagation of depolarization became markedly slow. Panel C, AV block and AV block with nonsustained VT occurred after administration of isoproterenol. Reverse triangles indicate atrial action potential without following ventricular action potential. Solid bar indicates nonsustained VT (Reproduced by permission from Koizumi et al. [58])

using murine *Irx3* and transfected wild-type and mutated *Irx3* into HL-1 murine atrial cells or neonatal mouse ventricular myocytes. Transfection of wild-type *Irx3* increased the expression of *Cx40* and *SCN5A*, but the transfection of these two mutated *Irx3* showed significantly reduced expression of *Cx40* and *SCN5A*. Thus, these mutations contributed to the conduction disturbance in the His-Purkinje system.

The intriguing feature of these cases is that they suffered VF during physical activity. One case with the *Irx*-R421P mutation had a Brugada-type ECG but suffered syncope while he was ice-skating. Another case had syncope during commuting. These findings indicate that IVF associated with *Irx3* mutation had a different clinical manifestation than Brugada syndrome.

Although the physiological phenotype of *Irx3* knockout mouse was evident, the precise molecular mechanism linking *Irx3* mutation and conduction disturbance to IVF has not been fully elucidated. Since *Irx3* did not directly bind to the promotor region of *Cx40*, other unknown molecules should be involved in the regulation of *Cx40* expression. In addition, two mutations found in IVF cases were not in the already known functional domains (TALE-homeobox domain and Iro domain). Thus, the mechanism explaining reduced expression of *Cx40* in these two mutations is still under investigation.

Other IVF-related with Conduction Disturbance

Accumulation of the several observational studies has indicated an additional possibility of the involvement of the His-Purkinje system in IVF. One phenotype is the association of right bundle branch block (RBBB). Aizawa et al. investigated 87 cases diagnosed as IVF, excluding Brugada syndrome and catecholaminergic polymorphic VT, and found 10 of 87 patients (11.5 %) had complete RBBB and the incidence was much higher than in the age and sex comparable controls (1.37 %) [59]. There were no differences in ECG parameters except QRS duration.

The Japanese Idiopathic Ventricular Fibrillation Study (J-IVFS) summarized 64 IVF cases excluding Brugada syndrome [60]. Out of 64 cases, 24 cases had ER pattern. In remaining 40 patients, nine cases (14 %) had an abnormal axis deviation and/or RBBB, indicating conduction disturbance (CD). They classified IVF cases into ER(+) group, ER(−)CD(−) group, and ER(−)CD(+) group. The ER(−)CD(+) group consisted of five males and four females – lower proportion of male than ER(+) group. The ER(−)CD(+) group also had longer PR interval and QRS duration than the groups of ER(−)CD(−) and ER(+) CD(−). Two studies indicated about 10 % of IVF patients showing ventricular conduction disturbances, but no specific ECG parameters were noted in the rest of IVF cases. VF was initiated with short-coupled premature ventricular contractions (PVCs) in some cases. Inducibility of VF by EPS was low in both types. There were no indications for familiar pattern of IVF with or without RBBB in two studies. No risk stratification was available so far and drug treatment for the prevention of SCD was not effective. Isoproterenol infusion was proven effective for the treatment of VF storms in some cases. ICD implantation was the only therapeutic option for the prevention of SCD.

Haissaguirre et al., described short-coupled PVCs originating from the distal Purkinje fibers as the main triggering factor for VF in IVF patients [61]. The PVCs had different morphologies and were mapped in several locations of the Purkinje system, including the anterior right ventricular region and large areas of the lower half of the left ventricular septum. The PVC origins were eliminated by catheter ablation and 89 % of patients were free of VF events during the follow-up of 24 ± 28 months.

In contrast to the IVF cases with *IrxX3* mutation, both reports indicated the prevalence of exercise-induced ventricular tachy-arrhythmias was low, which suggested that the IVF related with RBBB might have a different mechanism compared with the *Irx3* deficiency.

Short-coupled Variant of Torsade de Pointes

Another type of IVF probably having association with the Purkinje system is the PVT initiated by short-coupled PVT. Leenhardt et al., described a unique from of idiopathic ventricular tachyarrhythmias in young adults and called "short-coupled variant of torsade de pintes (TdP)" [62]. The unique feature of this arrhythmia was the development of TdP under normal QT interval, in contrast to frequent association with QT prolongation in congenital and acquired forms of long QT syndrome. The TdP was initiated by PVCs of short coupling interval (245 ± 28 ms), and one-third of these patients had a family history of sudden death. Approximately 70 % of patients with short-coupled variant of TdP degenerated into VF. In the following year, similar characteristics of patients were reported [63] and the term "Short-coupled variant of TdP" could be well recognized as a specific form of PVT/VF. This form of arrhythmias was prevalent mainly in females. The initial clinical presentation of the patients was often syncope, and the type of arrhythmia was not inducible by EPS. The arrhythmia was partially suppressed by verapamil, but the drug could not prevent SCD and ICD implantation was the only option for the prevention of SCD. Recent several studies were motivated to treat the triggering PVCs from the Purkinje system in patients with PVT/VF by catheter ablation, and the ablation achieved a freedom from VF recurrences during the follow-up of short- and long-term basis [61, 64, 65].

The underlying molecular mechanism of short-coupled variant of Tdp has not been elucidated yet. A recent case report, however, indicated that point mutation in ryanodine receptor 2 (*RyR2*-H29D) was related with PVT [66]. In contrast to the

RyR2 mutation associated with catecholaminergic polymorphic VT, this case had short-coupled PVCs and PVT at rest. The *RyR2*-H29D mutation converted *RyR2* to a leaky channel. This may explain some part or the principal mechanism of short-coupled variant of TdP.

IVF Related to Over-expression of DPP6

Studies dealing with a large cohort of familiar IVF in the Netherlands were conducted to clarify a pathogenic mechanism and risk stratification for asymptomatic patients in the affected family members. Alders et al., performed a genome-wide haplotype-sharing analysis for the identification of the responsible gene in three Dutch families in which multiple individuals died suddenly or were resuscitated from cardiac arrest at young age [67]. They identified a haplotype, on chromosome 7q36, that was conserved in these three families and was also shared by 7 of 42 independent IVF patients. The shared chromosomal segment harbors part of the dipeptidyl-aminopeptidase-like protein 6 (DPP6) gene, which encodes a putative component of the I_{to} in the heart [68]. Clinical evaluation of 84 risk-haplotype carriers and 71 noncarriers revealed no ECG or structural parameters indicative of cardiac disease. Penetrance of IVF was high; 50 % of risk-haplotype carriers experienced aborted SCD before the age of 58 years. Their study also demonstrated a 20-fold increase in *DPP6* mRNA levels in the myocardium of carriers as compared to controls. From these results, they propose *DPP6* as a gene for IVF and increased *DPP6* expression as the likely pathogenic mechanism. Despite of the finding of an association between familial IVF and a risk haplotype on chromosome 7q36, identification of asymptomatic patients at risk of IVF remains challenging, and no clinical parameters to guide treatment have been defined [69–71].

Further study by Xiao et al., explored the link between the overexpression of *DPP6* and the pathogenesis of IVF [72]. According to the results, baseline ECG was normal in *DPP6* risk-haplotype carriers. Ventricular arrhythmias manifested as short-coupled PVCs that sometimes initiated PVT. PVCs consistently displayed LBBB morphology with superior/left axis, suggesting a lower RV origin. The short-coupling intervals of PVCs under normal QT interval along with relatively narrow QRS complexes suggested an origin in the Purkinje system, as observed by Haissaguerre et al., [59] in 25 % of their IVF patients. In one patient undergoing ablation for repeated VF storms after ICD implantation, RV pace mapping produced a morphology similar to that of PVCs. Radiofrequency ablation was applied at a site with early diastolic Purkinje fiber potentials in the anterior lower RV. Neither VF nor typical morphology of PVCs recurred during the 43-month follow-up.

While I_{to} density was similar in Purkinje fiber and ventricular muscle, their tetraethylammonium (TEA) sensitivity and slow recovery from inactivation were different between the I_{to} in two tissues [73, 74]. In nondiseased human heart, the expressions of *DPP6* and neuronal calcium sensor-1 (NCS-1) were rich in Purkinje fiber, while K^+ channel interacting protein type 2 (KChIP2) was rich in ventricular muscle, which indicated different β-subunit compositions of the I_{to} channel in two tissues. Heterologous expression of *Kv4.3* in Chinese hamster ovary cells demonstrated that coexpression of *DPP6* and *NCS-1* (to mimic Purkinje I_{to} composition) enhanced I_{to} compared to *Kv4.3/NCS-1* and recapitulated kinetic/pharmacologic properties of Purkinje I_{to}. Overexpression of *DPP6*-enhanced and knockdown of *DPP6*-suppressed native Purkinje fiber I_{to}. A mathematical model of cardiac Purkinje fiber action potentials showed that I_{to} enhancement can greatly accelerate the repolarization of Purkinje fiber action potential. From these results, the authors suggest that a *DPP6*-mediated Purkinje fiber early repolarization might be a novel molecular mechanism for some forms of IVF. While the suggested mechanism is related to the ER of the Purkinje fibers, further clarification is mandatory whether it represents a subset of ERS limited to the conduction system, or other additional mechanism that may be involved in the clinical manifestation of IVF.

Possible Gene Mutations for Other Types of IVF Without ER Pattern

Genetic screening of Japanese IVF patients disclosed a mutation in the human cardiac sodium channel α-subunit gene (*SCN5A*) in a symptomatic IVF patient who did not exhibit typical Brugada ECG and showed rate-dependent RBBB [75]. A novel *SCN5A* missense mutation, S1710L, was identified and its channel function studied by heterologous expression system revealed markedly reduced current due to accelerated current decay, negative shift of steady state inactivation, and positive shift of activation (Fig. 14.6). Genetic screening of his family members was refused, and therefore, cosegregation studies could not be performed.

Valdivia et al., reported loss-of-function mutation of the *SCN3B*-encoded sodium channel β3 subunit [76]. A 20-year-old healthy male suddenly lost consciousness while playing basketball and the emergency team found him in VF. After resuscitation from VF, his ECG showed epsilon wave in the right precordial leads without inverted T wave. Cardiac examinations, including echocardiography and cardiac CT scan, did not reveal any structural abnormality of the heart, and hence, he was diagnosed as IVF. Mutation analysis disclosed a missense mutation V54G in *SCN3B*, which was absent in 800 references alleles. His mother was an asymptomatic gene-mutation carrier and exhibited a J-point elevation in her ECG. Functional analysis of HEK293 cells expressing *SCN5A* coexpressed with *Navβ3*-V54G revealed markedly decreased peak sodium current density, with positive shift of activation and negative shift in inactivation compared to wild type, resulting in loss-of-function

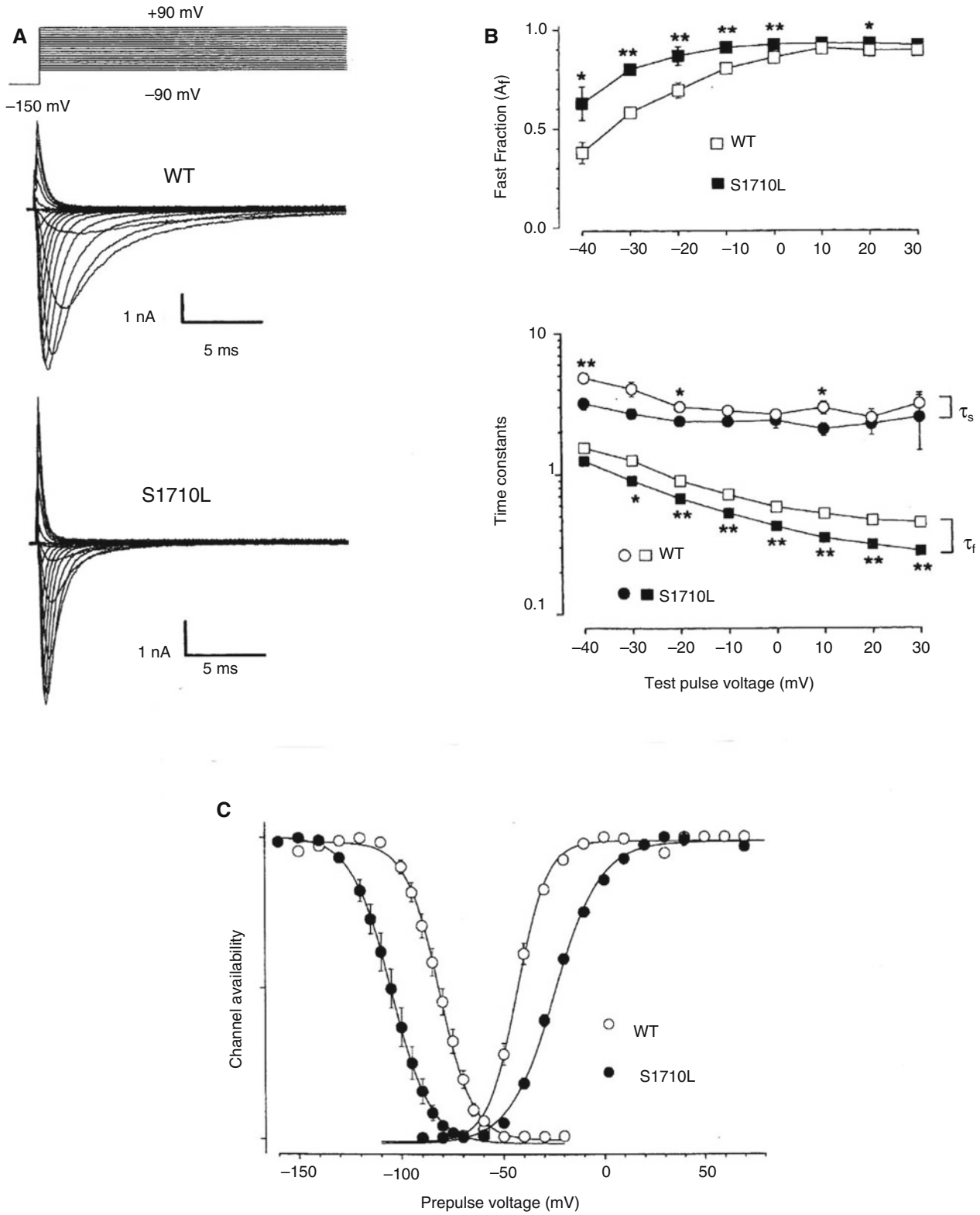

Fig. 14.6 Whole-cell current and its analysis obtained from HEK-hβ1 cells transfected with either WT or S1710L sodium channel (A: Whole-cell current records obtained from HEK cells transfected with either WT or S1710L sodium channel. Current was recorded from a holding potential of −150 mV stepped from –90 mV to +90 mV for 20 ms in 10 mV increments. Current decay is faster in S1710L than WT. B: The time course of inactivation was fit with a two exponential function. Af at the upper panel indicates the fraction of fast inactivation component. τf and τs at the lower panel indicate fast and slow the time constant of fast and slow inactivation components, respectively. C: Voltage dependence of steady-state inactivation and activation. S1710L current shows negative shift of inactivation and positive shift of activation compared to WT (Reproduced by permission from Akai et al. [75]))

by *Navβ3*-V54G. Immunocytochemistry and confocal microscopy demonstrated that *Navβ3*-V54G caused an *SCN5A*-trafficking defect. The results of the two reports may indicate that dysfunction of cardiac sodium channel due to gene mutations of main and/or auxiliary subunit is responsible for the pathogenesis in some forms of IVF.

Marsman et al., sought the genetic defect in a family with IVF manifesting in childhood and adolescence [77]. They characterized a family with a history of VF and SCD without electrocardiographic and echocardiographic abnormalities at rest. Two siblings died suddenly at ages of 9 and 10 years, and another two were resuscitated from cardiac arrest with documented VF at ages 10 and 16 years. Exome sequencing identified a missense mutation, F90L in the *CALM1* gene-encoding calmodulin in two resuscitated cases and one SCD victim. The functional analysis of this mutation was not available. The mutation was found in the mother and another sibling both being asymptomatic. Exome sequencing may be a strong tool to identify the genetic defect in families with a small numbers of affected individuals.

Summary

IVF without ER pattern in ECG may include several different types of electrocardiographic and clinical manifestation. They show either no specific ECG sign, ventricular conduction disturbance, or short-coupled variant of TdP with normal QT interval. Ventricular tachyarrythmias in most of these cases are initiated by short-coupled PVCs without QT prolongation. Diagnosis of IVF can be made only after resuscitated from VF events excluding structural heart disease and primary electrical disorder. No risk stratification is available at present time and ICD implantation is the only option to prevent SCD.

Take Home Message

- IVF without ER pattern in ECG is a very rare but potentially highly malignant disease.
- IVF without ER pattern in ECG should be considered in anyone resuscitated from VF showing no specific ECG pattern, RBBB, or short-coupled variant of TdP with normal QT interval in a standard 12-lead ECG, without potential other cause.
- Family history of juvenile unexpected sudden death
- One always must think about IVF without ER pattern in ECG in the following case:
  - Aborted cardiac arrest or SCD of unknown origin.
  - Short-coupled PVCs with normal QT interval precede the initiation of PVT.
  - A strong family history of juvenile unexplained sudden death.

References

1. Belhassen B, Shapira I, Shoshani D, Paredes A, Miller H, Laniado S. Idiopathic ventricular fibrillation: inducibility and beneficial effects of class I antiarrhythmic agents. Circulation. 1987;75:809–16.
2. Martini B, Nava A, Thiene G, Buja GF, Canciani B, Scognamiglio R, et al. Ventricular fibrillation without apparent heart disease: description of six cases. Am Heart J. 1989;118:1203–9.
3. Viskin S, Belhassen B. Idiopathic ventricular fibrillation. Am Heart J. 1990;120:661–71.
4. Wasserburger RH, Alt WJ. The normal RS-T segment elevation variant. Am J Cardiol. 1961;8:184–92.
5. Clements Jr SD, Hurst JW. Diagnostic value of electrocardiographic abnormalities observed in subjects accidentally exposed to cold. Am J Cardiol. 1972;29:729–34.
6. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome: a multicenter report. J Am Coll Cardiol. 1992;20:1391–6.
7. Aizawa Y, Tamura M, Chinushi M, Naitoh N, Uchiyama H, Kusano Y, et al. Idiopathic ventricular fibrillation and bradycardia-dependent intraventricular block. Am Heart J. 1993;126:1473–4.
8. Garg A, Finneran W, Fred GK. Familiar sudden cardiac death associated with a terminal QRS abnormality on surface 12-lead electrocardiogram in the index case. J Cardiovasc Electrophysiol. 1998;9:642–7.
9. Takagi M, Aihara N, Takaki H, Taguchi A, Shimizu W, Kurita T, et al. Clinical characteristics of patients with spontaneous or inducible ventricular fibrillation without apparent heart disease presenting with J wave and ST segment elevation in inferior leads. J Cardiovasc Electrophysiol. 2000;11:844–8.
10. Kalla H, Yan GX, Marinchak R. Ventricular fibrillation in a patient with prominent J (Osborn) wave and ST segment elevations in the inferior electrocardiographic leads: a Brugada syndrome variant. J Cardiovasc Electrophysiol. 2000;11:95–8.
11. Shinohara T, Takahashi N, Saikawa T, Yoshimura H. Characterization of J wave in a patient with idiopathic ventricular fibrillation. Heart Rhythm. 2006;3:1082–4.
12. Yan GX, Antzelevitch C. Cellular basis for the electrocardiographic J wave. Circulation. 1996;93:372–9.
13. Gussak I, Antzelevitch C. Early repolarization syndrome: clinical characteristics and possible cellular and ionic mechanisms. J Electrocardiol. 2000;33:299–309.
14. Haissaguerre M, Derval N, Sacher F, et al. Sudden cardiac arrest associated with early repolarization. N Engl J Med. 2008;358:2016–23.
15. Rosso R, Kogan E, Belhassen B, Rozovski U, Sheinman M, et al. J-point elevation in survivors of primary ventricular fibrillation and matched control subjects: incidence and clinical significance. J Am Coll Cardiol. 2008;52:1231–8.
16. Nam GB, Kim YH, Antzelevitch C. Augmentation of J waves and electrical storms in patients with early repolarization. New Eng J Med. 2008;358:2078–9.
17. Tikkanen JT, Anttonen O, Junttila MJ, Aro AL, Kerola T, Rissanen HA, et al. Long-term outcome associated with early repolarization on electrocardiography. New Eng J Med. 2009;361:2529–37.
18. Maury P, Rollin A. Prevalence of early repolarization/J wave pattern in the normal population. J Electrocardiol. 2013;46:411–6.
19. Kambara H, Phillips J. Long-term evaluation of early repolarization syndrome (normal variant RST-T segment elevation). Am J Cardiol. 1976;38:157–61.
20. Macfarlene PW, Antzelevitch C, Haissaguerre M, Huikuri HV, Potse M, Rosso R, et al. The early repolarization. A consensus paper. J Am Coll Cardiol. 2015;66:470–7.
21. Junttila MJ, Sager SJ, Tikkanen JT, Anttonen O, Huikuri HV, Myerburg RJ. Clinical significance of variants of J-points and J-waves: early repolarization patterns and risk. Eur Heart J. 2012;33:2639–43.

22. Huikuri HV, Marcus F, Krahn AD. Early repolarization: an epidemiologist's and a clinician's view. J Electrocardiol. 2013;46:466–9.
23. Adler A, Rosso R, Viskin D, Halkin A, Viskin S. What do we know about the "malignant form" of early repolarization. J Am Coll Cardiol. 2013;62:863–8.
24. Wu S-H, Lin X-X, Cheng Y-J, Qiang C-C, Zhang J. Early repolarization pattern and risk for arrhythmia death. A meta-analysis. J Am Coll Cardiol. 2013;61:645–50.
25. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmic syndromes. Heart Rhythm. 2013;10:1932–63.
26. Abe A, Ikeda T, Tsukada T, Ishiguro H, Miwa Y, Miyakoshi M, et al. Circadian variation of late potentials in idiopathic ventricular fibrillation associated with J wave: insights into alternative pathophysiology and risk stratification. Heart Rhythm. 2010;7:675–82.
27. Aizawa Y, Sato A, Watanabe H, Chinushi M, Furushima H, Horie M, et al. Dynamicity of the J-wave in idiopathic ventricular fibrillation with a special reference to pause-dependent augmentation of the J-wave. J Am Coll Cardiol. 2012;59:1948–53.
28. Nam GB, Ko KH, Kim J, Park KM, Rhee KS, Choi KJ, Kim YH, Antzelevitch C. Mode of onset of ventricular fibrillation in patients with early repolarization pattern vs Brugada syndrome. Eur Heart J. 2010;31:330–9.
29. Haissaguerre M, Sacher F, Nogami A, Komiya N, Bernard A, Probst V, et al. Characteristics of recurrent ventricular fibrillation associated with inferolateral early repolarization: role of drug therapy. J Am Coll Cardiol. 2009;53:612–9.
30. Kawata H, Noda T, Yamada Y, Okamura H, Satomi K, Aiba T, et al. Effect of sodium-channel blockade on early repolarization in infero/lateral leads in patients with idiopathic ventricular fibrillation and Brugada syndrome. Heart Rhythm. 2012;9:77–83.
31. Roten L, Derval N, Sacher F, Pascale P, Wilton SB, Scherr D, et al. Ajmalin attenuates electrocardiogram characteristics of inferolateral early repolarization. Heart Rhythm. 2012;9:232–9.
32. Tikkanen JT, Junttila MJ, Anttonen O, Aro AL, Luttinen S, Kerola T, et al. Early repolarization: electrocardiographic phenotypes associated with favorable long-term outcome. Circulation. 2011;123:2666–73.
33. Rosso R, Glikson E, Belhassen B, Katz A, Halkin A, Steinvil A, et al. Distinguishing "benign" from " malignant early repolarization": the value of the ST-segment morphology. Heart Rhythm. 2012;9:225–9.
34. Mahida S, Derval N, Sacher F, Leenhardt A, Deisenhofer I, Babuty D, et al. Role of electrophysiologic studies in predicting risk of ventricular arrhythmia in early repolarization syndrome. J Am Coll Cardiol. 2015;65:151–9.
35. Shinohara T, Ebata Y, Ayabe R, Fukui A, Okada N, Yufu K, et al. Combination therapy of cilostazol and bepridil suppresses recurrent ventricular fibrillation related to J-wave syndrome. Heart Rhythm. 2014;11:1441–5.
36. Rosso R, Adler A, Halkin A, Viskin S. Risk of sudden death among young individuals with J wave and early repolarization: putting the evidence into perspective. Heart Rhythm. 2011;8:923–9.
37. Naruse Y, Tada H, Harimura Y, Hayashi M, Noguchi Y, Sato A, et al. Early repolarization is an independent predictor of occurrences of ventricular fibrillation in the very early phase of acute myocardial infarction. Circ Arrhythm Electrophysiol. 2012;5:506–13.
38. Litovsky SH, Antzelevitch C. Transient outward current prominent in canine ventricular epicardium but not endocardium. Circ Res. 1988;62:116–26.
39. Antzelevitch C, Yan GX. J wave syndrome: Brugada and early repolarization syndromes. Heart Rhythm. 2015;12:1852–66.
40. Antzelevitch C, Yan GX. J wave syndrome. Heart Rhythm. 2010;7:549–58.
41. Kleber AG, Rudy Y. Basic mechanisms of cardiac impulse propagation and associated arrhythmia. Physiol Rev. 2004;84:431–88.
42. Niwa N, Nelbonne JM. Molecular determinants of cardiac transient outward potassium current (Ito) expression and regulation. J Mol Cell Cardiol. 2010;48:12–25.
43. Noseworthy PA, Tikkanen JT, Porthan K, Oikarinen L, Pietiila A, Harald K, et al. The early repolarization pattern in the general population: clinical correlates and heritability. J Am Coll Cardiol. 2011;57:2284–9.
44. Reinhard W, Kaess BM, Debiec R, Nelson CP, Stark K, Tobin MD, et al. Heritability of early repolarization: a population-based study. Circulation Cardiovasc genetics. 2011;4:134–8.
45. Nunn LM, Bhar-Amato J, Lowe MD, Macfarlane PW, Rogers P, McKenna WJ, et al. Prevalence of J-point elevation in sudden arrhythmic death syndrome families. J Am Coll Cardiol. 2011;58:286–90.
46. Haissaguerre M, Chatel S, Sacher F, Weerasooriya R, Probst V, Roussouran G, et al. Ventricular fibrillation with prominent early repolarization associated with a rare variant of *KCNJ8/KATP* channel. J Cardiovasc Electrophysiol. 2009;20:93–8.
47. Medeiros-Domingo A, Tan BH, Crotti L, Tester D, Eckhardt L, Cuoretti A, et al. Gain-of-function mutation S422L in the *KCNJ8*-encoded cardiac KATP channel Kir6.1 as a pathologic substrate for J-wave syndromes. Heart Rhythm. 2010;7:1466–71.
48. Barajas-Martinez H, Hu D, Ferrer T, Onetti CG, Wu Y, Burashinikov E, et al. Molecullar genetic and functional association of Brugada and early repolarization syndromes with S422L missense mutation in KCNJ8. Heart Rhythm. 2012;9:548–55.
49. Veeramah KR, Karafet TM, Wolf D, Samson RA, Hammer MF. The *KCNJ8-S422L* variant previously associated with J-wave syndromes is found at an increased frequency in Ashkenazi Jews. Eur J Human Genetics. 2014;22:94–8.
50. Burashnikov E, Pfeiffer R, Barajas-Martinez H, Delpon E, Hu D, Desai M, et al. Mutations in the cardiac L-type calcium channel associated with inherited J-wave syndromes and sudden cardiac death. Heart Rhythm. 2010;7:1872–82.
51. Watanabe H, Nogami A, Ohkubo K, Kawata H, Hayashi Y, Ishikawa T, et al. Electrocardiographic characteristics and SCN5A mutations in idiopathic ventricular fibrillation associated with early repolarization. Circ Arrhythm Electrophysiol. 2011;4:874–81.
52. Sinner MF, Porthan K, Noseworthy PA, Havulinna AS, Tikkanen JT, Muller-Narasyid M, et al. A meta-analysis of genome-wide association studies of the electrocardiographic early repolarization pattern. Heart Rhythm. 2012;9:1627–34.
53. Gómez-Skarmeta JL, Modolell J. Iroquois genes: genomic organization and function in vertebrate neural development. Curr Opin Genet Dev. 2002;12(4):403–8.
54. Bruneau BG, Bao Z-Z, Fatkin D, Xavier-Neto J, Georgakopoulos D, Maguire CT, et al. Cardiomyopathy in Irx4-deficient mice is preceded by abnormal ventricular gene expression. Mol Cell Biol. 2001;21(5):1730–6.
55. Christoffels VM, Keijser AGM, Houweling AC, Clout DEW, Moorman AFM. Patterning the Embryonic Heart: Identification of Five Mouse Iroquois Homeobox Genes in the Developing Heart. Dev Biol. 2000;224(2):263–74.
56. Costantini DL, Arruda EP, Agarwal P, Kim K-H, Zhu Y, Zhu W, et al. The homeodomain transcription factor Irx5 establishes the mouse cardiac ventricular repolarization gradient. Cell. 2005;123(2):347–58.
57. Zhang S-S, Kim K-H, Rosen A, Smyth JW, Sakuma R, Delgado-Olguín P, et al. Iroquois homeobox gene 3 establishes fast conduction in the cardiac His–Purkinje network. Proceedings of the National Academy of Sciences. 2011;108(33):13576–81.
58. Koizumi A, Sasano T, Kimura W, Miyamoto Y, Aiba T, Ishikawa T, et al. Genetic defects in a His-Purkinje system transcription

factor, IRX3, cause lethal cardiac arrhythmias. Eur Heart J. 2016;37:1469–75.
59. Aizawa Y, Takatsuki S, Kimura T, Nishiyama N, Fukumoto K, Tanimoto Y, et al. Ventricular fibrillation associated with complete right bundle branch block. Heart Rhythm. 2013;10:1028–35.
60. Sekiguchi Y, Aonuma K, Takagi M, Aihara N, Yokoyama Y, Hiraoka M. New clinical and electrocardiographic classification in patients with idiopathic ventricular fibrillation. J Cardiovasc Electrophysiol. 2013;24:902–8.
61. Haissaguerre M, Shoda M, Jais P, Nogami A, Shah DC, Kautzner J, et al. Mapping and ablation of idiopathic ventricular fibrillation. Circulation. 2002;106:962–7.
62. Leenhardt A, Glaser E, Burguera M, Numberg M, Maison-Blanche P, Coumel P. Short-coupled variant of torsade de pointes. A new electrocardiographic entity in the spectrum of idiopathic ventricular tachyarrhythmias. Circulation. 1994;89:206–15.
63. Eisenberg SJ, Scheinman MM, Dullet NK, Finkbeiner WE, Griffin JC, Eldar M, et al. Suddencardiac death and polymorphous ventricular tachycardia in patients with normal QT intervals and normal systolic function. Am J Cardiol. 1995;75:687–92.
64. Nogami A, Sugiyasu A, Kubota S, Kato K. Mapping and ablation of idiopathic ventricular fibrillation from the Purkinje system. Heart Rhythm. 2004;2:646–9.
65. Knecht S, Sacher F, Wright M, Hocini M, Nogami A, Arentz T, et al. Long-term follow-up of idiopathic ventricular fibrillation ablation: a multicenter study. J Am Coll Cardiol. 2009;54:522–8.
66. Cheung JW, Meli AC, Xie W, Mittal S, Reiken S, Wronska A, et al. Short-coupled polymorphic ventricular tachycardia at rest linked to a novel ryanodine receptor (RyR2) mutation: leaky RyR2 channels under non-stress conditions. Int J Cardiol. 2015;189:228–36.
67. Alders M, Koopmann TT, Christiaan I, Postema PG, Beekman L, Tanck MW, et al. Haplotype-sharing analysis implicates chromosome 7q36 harboring DPP6 in familiar idiopathic ventricular fibrillation. Am J Hum Genet. 2009;84:468–76.
68. Radicke S, Cottella D, Graf EM, Ravens U, Wettwer E. Expression and function of dipeptidyl-aminopeptidase-like protein 6 as a putative beta-subunit of human cardiac transient outward current encoded by Kv4.3. J Physiol. 2005;565:751–6.
69. van der Warf C, Hofman N, Tan HL, et al. Diagnostic yield in sudden unexplained death and aborted cardiac arrest in the young: the experience of a tertiary referral center in The Netherlands. Heart Rhythm. 2010;7:1383–9.
70. Postema PG, Christiaans I, Hofman N, Alders M, Koopmann TT, Bezzina CR, et al. Founder mutations in the Netherlands: familial idiopathic ventricular fibrillation and DPP6. Neth Heart J. 2011; 19:290–6.
71. ten Sande JN, Postema PG, Boekholdt SM, Tan HL, van der Heijden JF, de Groot NMS, et al. Detailed characterization of familiar idiopathic ventricular fibrillation linked to the DPP6 locus. heart Rhythm. 2016;13:905–12.
72. Xiao L, Koopmann T, Ordog B, Postema PG, Verkerk AO, Iyer V, et al. Unique cardiac Purkinje fiber transient outward current β-subunit composition. A potential molecular link to idiopathic ventricular fibrillation. Circ Res. 2013;112:1310–22.
73. Han W, Wang Z, Nattel SA. A comparison of transient outward currents in cardiac Purkinje cells and ventricular myocytes. Am J Physiol Heart Circ Physiol. 2000;279:H466–74.
74. Han W, Xhang L, Schram G, Nattel SA. Properties of potassium currents in Purkinje cells of failing human hearts. Am J Physiol heart Circ Physiol. 2002;282:H2495–503.
75. Akai J, Makita N, Sakurada H, Shirai N, Ueda K, Kitabatake A, et al. A novel SCN5A mutation associated with idiopathic ventricular fibrillation without typical ECG findings of Brugada syndrome. FEBS Lett. 2000;479:29–34.
76. Valdivia CR, Medeiros-Domingo A, Ye B, Shen W-K, Algiers TJ, Ackerman MJ, Makielski JC. Loss-of-function mutation of the SCN3B-encoded sodium channel β3 subunit associated with a case of idiopathic ventricular fibrillation. Cardiovasc Res. 2010;86:392–400.
77. Marsman RF, Barc J, Beekman L, Alders M, Dooijes D, van der Wijngaard A, et al. A mutation in CALM1 encoding calmodulin in familiar idiopathic ventricular fibrillation in childhood and adolescence. J Am Coll Cardiol. 2014;63:259–66.

A Molecular Genetic Perspective on Atrial Fibrillation

15

Jason D. Roberts and Michael H. Gollob

Abstract

Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, is a source of significant morbidity and mortality. Despite its prevalence and clinical significance, its pathophysiology remains incompletely understood and treatment strategies remain relatively ineffective. In recent years, the importance of *genetics* in predisposing to AF has been clearly recognized, and in a remarkably short time period there have been a flurry of landmark discoveries. Insight into the molecular genetics of this condition promises to lead to more effective forms of therapy that will help reduce the burden currently carried by patients and health care systems.

Introduction

Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, is a source of significant morbidity and mortality predominantly through its sequelae of heart failure and stroke. Despite its prevalence and clinical significance, its pathophysiology remains incompletely understood and treatment strategies remain relatively ineffective. In recent years, the importance of *genetics* in predisposing to AF has been clearly recognized, and in a remarkably short time period there have been a flurry of landmark discoveries. Insight into the molecular genetics of this condition promises to lead to more effective forms of therapy that will help reduce the burden currently carried by patients and health care systems (Table 15.1).

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Epidemiology

AF represents the most common cardiac arrhythmia and affects well over two million Americans [1]. Its prevalence increases with age and ranges from less than 0.5 % in those aged less than 55 to 9 % in octogenarians [2]. Because of the advancing age of Western populations, its incidence is expected to increase over the coming years and has been projected to affect as many as 16 million Americans by 2050 [3]. AF independently increases the risk of mortality with an age-adjusted odds ratio for death of 1.9 in females and 1.5 in males; however, its sequelae of stroke and heart failure represent the greatest sources of morbidity and mortality [4]. Accounting for a large proportion of strokes in the elderly, AF has been estimated to cost the American health care system over US $1 billion annually [1, 5]. Its burden on the global population, in terms of both health and health care dollars, will likely only increase in the coming years as populations age. This is further exacerbated by the current lack of highly effective therapies for this exceedingly common condition.

In addition to advancing age, structural heart disease also represents a major risk factor for AF [6]. However, approximately 10–20 % of cases of AF occurs in the absence of known risk factors and have been termed *lone AF* [7]. Without obvious contributing factors, genetics have been hypothesized to play an important role in the develop-

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ment of this form of the arrhythmia. Indeed, a family with lone AF transmitted with an autosomal dominant pattern of inheritance was first documented by Wolff in 1943 [8]. A recent study of siblings has found that brothers and sisters of patients with lone AF have a 70-fold and 34-fold increased risk of developing the arrhythmia relative to the general population, respectively [9]. Previously felt to be rare, contemporary work have begun to suggest that familial AF may be much more common than previously expected. Defining familial lone AF as being the presence of the arrhythmia in both the proband and at least one first-degree relative, it has been shown that it accounts for 15 % of all cases of lone AF [10].

Other studies have found evidence for a genetic component in the more common form of AF associated with structural heart disease. A prospective cohort analysis from the Framingham Heart Study involving 2243 subjects found that parental AF conferred an increased risk for development of the arrhythmia in their offspring (odds ratio of 1.85) [11]. A similar study from Iceland involving 5269 patients identified a 1.77-fold increased risk of developing the arrhythmia in first-degree relatives [12]. Subsequent work from the Framingham Heart Study has reiterated the increased risk of the AF among individuals with affected first-degree relatives [13]. These data emphasize that a genetic predisposition is likely important in the development of all forms of AF.

Table 15.1 The genes associated with atrial fibrillation (AF) and the putative mechanisms leading to the arrhythmia

Gene	Study method	Mode of inheritance	Protein and function	Functional effect of mutation	Mechanism for AF
Potassium channels					
KCNQ1	Linkage analysis	Autosomal dominant	α-subunit of I_{Ks}	Gain-of-function	Reduced atrial ERP/APD
KCNH2[a]	Candidate gene approach	Autosomal dominant	α-subunit of I_{Kr}	Gain-of-function	Reduced atrial ERP/APD
KCNE2	Candidate gene approach	Autosomal dominant	β-subunit of background potassium current	Gain-of-function	Reduced atrial ERP/APD
KCNJ2	Candidate gene approach	Autosomal dominant	$K_{ir}2.1$ responsible for I_{K1}	Gain-of-function	Reduced atrial ERP/APD
KCNA5	Candidate gene approach	Autosomal dominant	$K_v1.5$ responsible for I_{Kur}	Loss-of-function	Prolonged atrial ERP/APD
Connexins					
GJA5	Candidate gene approach	Sporadic and autosomal dominant	Connexin 40 responsible for cell coupling	Loss-of-function	Conduction velocity dispersion
Sodium channels					
SCN5A	Candidate gene approach	Autosomal dominant	$Na_v1.5$ responsible for I_{Na}	Loss-of-function	Prolonged atrial ERP/APD
		Autosomal dominant		Gain-of-function	Cellular hyperexcitability
Circulating hormones					
NPPA	Linkage analysis	Autosomal dominant	Atrial natriuretic peptide	Unknown	Unknown
Atrial sarcomeric proteins					
MYL4	Exome sequencing	Autosomal dominant	Myosin light chain 4	Loss-of-function	Atrial myopathy
Unknown loci					
10q22–24	Linkage analysis	Autosomal dominant	Unknown	Unknown	Unknown
6q14–16	Linkage analysis	Autosomal dominant	Unknown	Unknown	Unknown
10p11–q21	Linkage analysis	Autosomal dominant	Unknown	Unknown	Unknown
5p15	Linkage analysis	Autosomal dominant	Unknown	Unknown	Unknown

ERP effective refractory period, *APD* action potential duration
[a]Identified as a familial cause of AF in the context of short-QT syndrome

Molecular Background

AF reflects a disturbance of the electrical activity within the top chambers of the heart. Transmission of electrical impulses within the heart occurs through *ion channels*, pore-forming proteins present within the plasma membranes of cardiomyocytes [14]. There are a variety of different types of cardiac ion channels, each of which contributes to the cardiac action potential. The two major types of cardiomyocytes within the heart include pacemaker cells and cardiac muscle cells, each with its own distinct *action potential* [14]. The action potential for the cardiac muscle cell is designed to allow for rapid conduction of electrical impulses. In contrast, cardiac pacemaker cells are endowed with a property termed intrinsic automaticity, which allows these cells to function as pacemakers within the heart. The action potential of the cardiac muscle cell will be the main focus of this discussion.

Figure 15.1 depicts the action potential of the cardiac muscle cell, which is divided into five phases. Phase 0 reflects the rapid depolarizing upstroke that occurs secondary to rapid sodium influx into cells and is referred to as I_{Na}. It is mediated by a *voltage-gated sodium channel*, termed $Na_v1.5$, which is the protein product of the *SCN5A* gene [15]. The rapid upstroke, reflective of the rapid flow of current, endows these cells with an ability to transmit electrical impulses with a high conduction velocity. Phase 1 involves a transient current of *repolarization*, termed I_{to}, that occurs secondary to an efflux of potassium ions from the cell through the $K_v4.3$ *voltage-gated potassium channel* encoded by *KCND3* [16]. Phase 2, also referred to as the plateau or dome phase, reflects a balance of inward calcium current and outward potassium current [14]. Flow of calcium occurs through voltage-gated L-type calcium channels. The calcium influx during Phase 2 not only plays a critical role in the

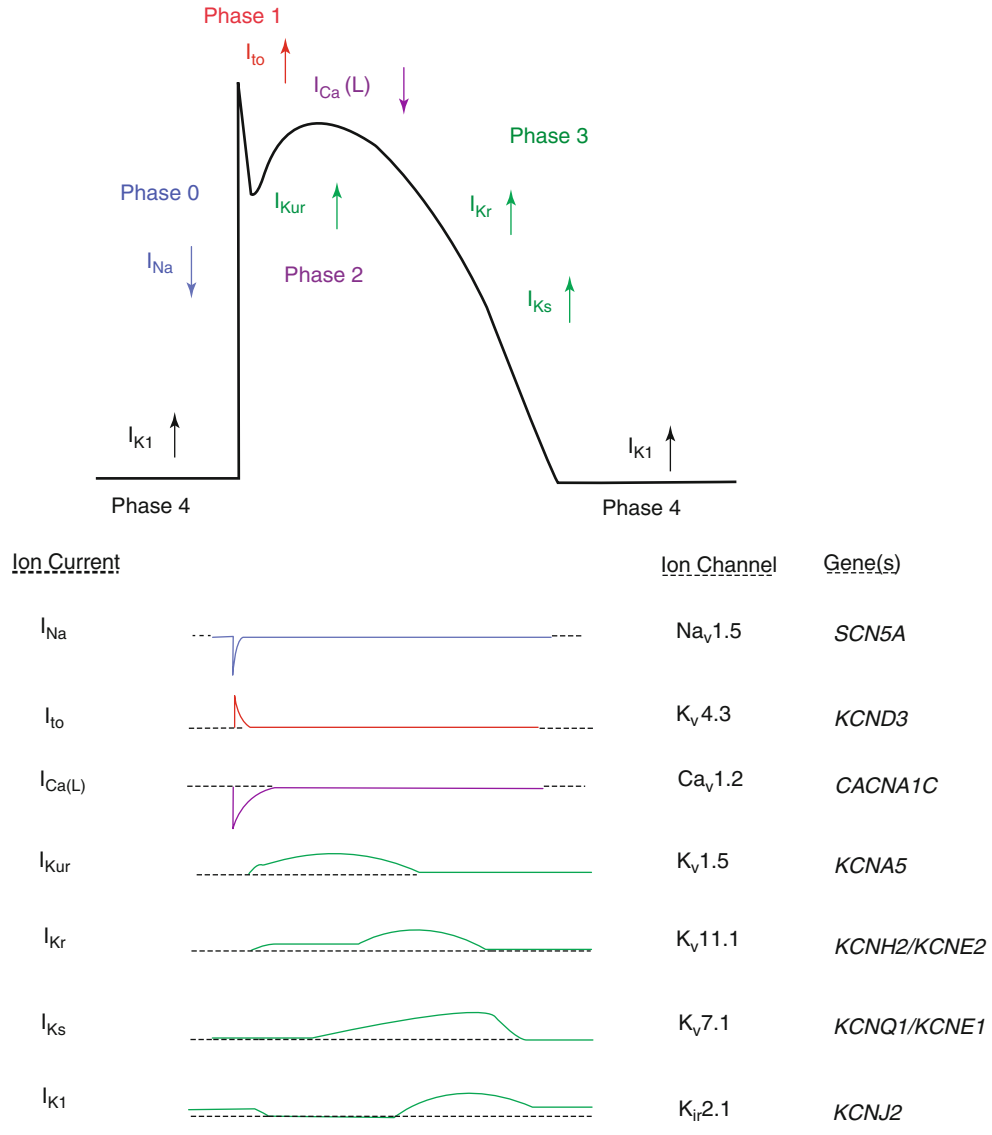

Fig. 15.1 The atrial action potential. The action potential is divided into four phases mediated by distinct ionic currents. These currents are driven by voltage-gated ion channels that allow specific ions to pass across the cardiac sarcolemma. The identity of the currents, the voltage-gated ion channels, and their encoding genes is provided (Adapted from Marban [18])

kinetics of the cardiac action potential but is also important in excitation–contraction coupling [17].

Repolarization is mediated by a current that arises secondary to the flow of potassium ions out of the cell. Referred to as the delayed rectifier potassium current, it is divided into three different components on the basis of timing. The first, termed the *ultrarapid component of the delayed rectifier potassium current* (I_{Kur}), is unique to the atria and is felt to be mediated by Kv1.5, a voltage-gated potassium channel encoded by *KCNA5* [19, 20]. Following I_{Kur} is the *rapid component of the delayed rectifier potassium current* (I_{Kr}) and involves the gene products of both *KCNH2* (HERG) and *KCNE2* [21]. Lastly, there is the *slow component of the delayed rectifier potassium current* (I_{Ks}), which involves the gene products of both *KCNQ1* and *KCNE1* [22]. Although I_{Kur} only occurs within the atria, I_{Kr} and I_{Ks} occur in both the atria and the ventricles [23].

Phase 4 reflects a resting phase whose properties are in part modulated by the cardiac *inward rectifier potassium current*, or I_{K1}, mediated by Kir2.1 and encoded by the *KCNJ2* gene [24]. Although I_{K1} is voltage-gated, its activity differs markedly relative to the previously mentioned voltage-gated potassium channels involved in Phase 3. While the delayed rectifier potassium current is triggered by cellular repolarization, I_{K1} activity predominates when the cell is hyperpolarized or near resting potential [23]. The efflux of potassium ions mediated by I_{K1} during Phase 4 serves as an important contributor to the resting membrane potential of the cell and in this context has the potential to influence cellular excitability [25]. Upon depolarization, the magnitude of I_{K1} is dramatically reduced; a property that may be mediated by intracellular magnesium ions and polyamines interfering with the flow of potassium ions through the channel [26]. This reduced current persists until the terminal portion of Phase 3 when I_{K1} increases and exerts influence on cellular repolarization and action potential duration [23].

Another important current within cardiac atria is the *muscarinic receptor activated potassium current*, $I_{KACh,}$ which mediates the flow of potassium ions across the membrane in response to a vagal stimulus [27]. The cardiac muscarinic receptor, M2, is a G protein-coupled receptor that, upon binding of a cholinergic agonist (acetylcholine), permits the $G_{\beta\gamma}$ subunit to dissociate and subsequently bind and activate I_{KACh} [28]. The constituents of I_{KACh} include Kir3.1 and Kir3.4 encoded by *KCNJ3* and *KCNJ5*, respectively [29]. I_{KACh}, similar to I_{Kur}, is considered to be exclusive to the atria, although mRNA of both subunits has been detected in the ventricles [30]. Activation of I_{KACh} while the cell is depolarized results in a further efflux of potassium ions, which has the potential to shorten the action potential duration and, as will be discussed in subsequent sections, may influence the development of AF [31].

Two major concepts that can be derived from knowledge of the cardiac action potential and its associated currents include the electrical properties of *conduction velocity* and *refractory period*. Conduction velocity reflects the velocity at which an electrical impulse is transmitted through myocardial tissue [14]. Two of the major determinants of conduction velocity include sodium channels and gap junctions. As discussed above, the kinetics of the $Na_v1.5$ channel allow for rapid conduction within the heart [15]. Gap junctions represent intercellular pores that allow electrical current to flow between cells [32]. It is this intercellular coupling, along with the rapid conduction along the cell membrane mediated by $Na_v1.5$ that results in coordinated activity between individual cells of the heart and an ensuing functional electrical syncytium.

The second concept involves the refractory period and refers to the length of time following excitation that a cell requires before it can be re-excited [14]. An electrical impulse that encounters refractory tissue dies out. The length of the refractory period is dependent upon the rate at which a cell is able to repolarize to a potential compatible with re-excitation and therefore phase 3 of the action potential plays a critical role. The mediators of Phase 3, namely, the potassium channels, are therefore important contributors to the refractory period of the cell. Inhibition of potassium channels, as achieved with potassium channel blockers, results in a prolonged repolarization time manifested on the electrocardiogram as a prolonged QT interval in the case of ventricular repolarization.

Heterogeneity of refractory periods and conduction velocities within the heart, also referred to as *dispersion*, results in a substrate that is capable of sustaining *reentry* [33, 34]. Reentrant circuits represent a major mechanism for tachyarrhythmias and are particularly important in the pathophysiology of AF, as will be discussed. Dispersion arises secondary to heterogeneous distributions of ion channels within the heart and occurs in normal individuals due to the different current magnitudes intrinsic to specific cardiac layers such as the endocardium and epicardium [35]. However, the degree of dispersion can be exacerbated from birth secondary to genetic variations altering the function of key protein mediators, or can occur over time as a result of asymmetric cardiac electrical and structural remodeling processes. An example of dispersion on the 12-lead ECG is that of QT dispersion reflecting regional heterogeneity of ventricular repolarization.

An understanding of these concepts is necessary in order to properly address the pathophysiology of AF and its associated molecular genetics.

Pathophysiology

AF reflects disorganized and chaotic activity of the atria with impulses firing at a rate of approximately 400–600 times per minute. The mechanisms underlying the development and maintenance of AF remain incompletely understood, and

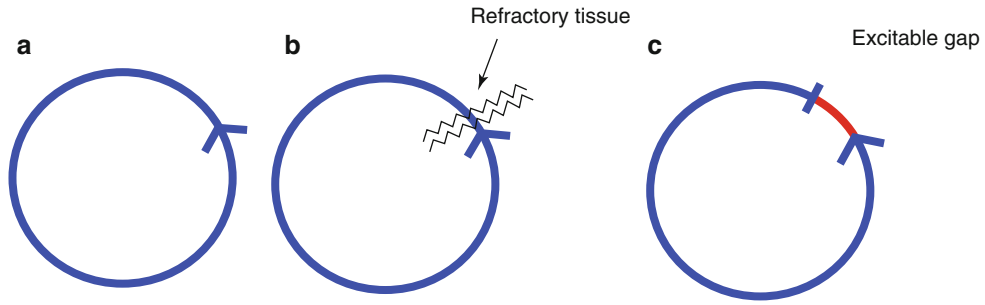

Fig. 15.2 Micro-reentrant circuits in atrial fibrillation (AF). (**a**) A circulating wavelet, whose wavelength is equivalent to its pathlength, exhibiting circus activity. (**b**) An increase in the refractory period of the micro-reentrant circuit has resulted in a wavelength that exceeds the pathlength. The depolarizing current encounters refractory tissue and the circuit is terminated. (**c**) A reduction in the refractory period of the circulating wavelet from A generates a wavelength that is shorter than the corresponding pathlength resulting in the introduction of an excitable gap (Adapted from Nattel [36])

there continues to be a variety of competing theories [36]. The dominant conceptual model of AF over the past 50 years, the *multiple wavelet hypothesis*, is derived from the work of Gordon Moe and involves multiple circuit reentry excitation [37]. In this model, which has been confirmed by high resolution electrical mapping during AF, irregular atrial activity arises from multiple self-perpetuating micro-reentrant circuits that exhibit spatial and temporal variability [38, 39]. The second model implicates rapidly discharging atrial ectopic foci, a concept that has been strengthened following the recognition that ectopic beats originating from pulmonary veins frequently initiate AF [40–42]. This had led to the use of radiofrequency catheter ablation techniques, in which the pulmonary veins are electrically isolated from the surrounding atria, in order to treat AF [43].

The multiple wavelet hypothesis suggests that increasing numbers of reentrant wavelets within the atria favor the maintenance of AF. A wavelet is a small wave of depolarizing current that may circle back upon itself to form a micro-reentrant circuit. In order to appreciate the conditions governing the number of wavelets that can be established, it is important to have an understanding of the *wavelength of reentry* concept. The wavelength of a circulating electrical impulse is defined as the distance traveled within one refractory period and can be calculated as the product of conduction velocity and refractory period [44]. In contrast, the pathlength represents the distance traveled by an electrical impulse during one complete circuit. As denoted in Fig. 15.2b, a wavelength that is greater in size than its pathlength will result in the circulating impulse encountering refractory tissue and the circuit will be terminated [44]. However, a pathlength traveled, that is greater in size than the circulating wavelength, will introduce an excitable gap that will permit ensuing circus movement allowing the reentrant circuit to perpetuate (Fig. 15.2c) [45]. In accordance with the leading circle hypothesis, a circulating wavelet automatically establishes itself within a pathlength equivalent to its wavelength (Fig. 15.2a) [44]. On this basis, coupled with wavelength being the product of conduction velocity and refractory period, the number of wavelets that can be supported by atria of a given size is inversely proportional to both conduction velocity and refractory period.

The notion of an increased number of wavelets promoting the maintenance of AF is supported by increased atrial size serving as a risk factor for the arrhythmia [46]. In a similar fashion, an increase in circulating wavelets through a reduction in refractory period and an ensuing reduction in wavelength theoretically promotes the maintenance of AF. The theory that a shorter wavelength, through a reduced refractory period, predisposes to AF serves as the rationale for using potassium channel blockers to terminate AF. Potassium channel blockers prolong atrial repolarization and result in an increased refractory period, thereby reducing the potential number of circulating wavelets that can be supported by atria of a given size. Of note, the use of sodium channel blockers in AF is not supported by the wavelength of reentry concept. These medications decrease conduction velocity and, given the associated reduction in wavelength, would increase the number of circulating atrial wavelets. Their efficacy provides support for mechanistic heterogeneity within AF, a concept that will become evident as the genetic heterogeneity of the arrhythmia is further explored. Sodium channel blockers may be effective in treating a form of AF characterized by focal firing, a concept that will be addressed in the discussion surrounding *gain-of-function* sodium channel mutations.

The aforementioned atrial electrical properties, conduction velocity, and refractory period are often viewed as contributing to the substrate for arrhythmogenesis. The development of a reentry circuit is dependent upon both substrate and trigger, examples being an early afterdepolarization or enhanced automaticity resulting in a premature beat. It is important to note that the physiology responsible for both trigger and substrate is not static but dynamic secondary to modulation by the autonomic nervous system. As such, the *autonomic nervous system* has been recognized as a critical component of arrhythmogenesis. In the setting of

lone AF, the sentinel observations of the eminent electrophysiologist Phillipe Coumel implicated the parasympathetic nervous system as the major culprit [47]. He noted that the arrhythmia tended to be triggered during periods of high vagal tone such as sleep and postprandially. The mechanism through which the parasympathetic nervous system mediates lone AF appears to be in part dependent upon I_{KAch} [23]. As previously discussed, activation of I_{KAch} when the cell is depolarized triggers an efflux of potassium ions shortening the atrial action potential duration and the corresponding refractory period. The heterogeneous vagal innervation of the atria has the potential to result in regional variation of refractory periods [31]. The resultant dispersion in cellular refractoriness throughout the atria has the potential to serve as an ideal substrate for reentry and arrhythmogenesis.

As evidenced by this discussion, there are multiple variables within atrial electrical physiology that can help contribute to the development of AF. The pathophysiological heterogeneity of this disorder is further supported by the varied genetics that characterize the condition. Effective treatment is likely dependent upon targeted therapy that addresses the specific aberrant pathway, which triggers the arrhythmia development in an individual. As a result, a detailed understanding of the molecular mechanisms underlying AF is warranted.

Molecular Genetics

A genetic contribution to AF was supported by informal observations of familial clustering of the arrhythmia and previous reports of an inheritance pattern consistent with autosomal dominant transmission [8]. However, the first form of definitive evidence did not come until 1997 when a genetic locus was found to segregate with the arrhythmia in a Spanish family that suffered from an autosomal dominant form of lone AF [48]. Linkage analysis localized the culprit locus to the long arm of chromosome 10 (10q22–24). Despite isolation of the locus to a relatively small genomic region, a culprit gene could not be identified. Candidate genes in that region included the β-adrenergic receptor (*ADRB1*), the α-adrenergic receptor (*ADRA2*), and a G-protein-coupled receptor kinase (*GPRK5*). Sequencing of these genes, however, did not reveal a mutation that segregated with disease. Over 15 years later, the culprit gene within this locus remains unknown.

Potassium Channels: Gain-of-Function Mutations

KCNQ1

The first causative gene for familial AF was not found until 6 years following identification of the 10q22–24 locus. The discovery came from the study of a four-generation Chinese family that also exhibited an autosomal dominant pattern of inheritance for lone AF [49]. Linkage analysis mapped the culprit locus to the short arm of chromosome 11 (11p15.5), a region distinct from that found in the Spanish family. This on its own was a significant finding given that it demonstrated that AF was a genetically heterogeneous disorder that could be caused by more than one gene, a finding corroborated by a separate group in the same year [10]. Review of the genetic contents of the 11p15.5 region found that it contained the *KCNQ1* gene whose protein product encodes the pore-forming α-subunit of I_{Ks} (*KCNQ1/KCNE1*). *Loss-of-function* mutations within *KCNQ1* had been previously implicated with congenital long QT syndrome type 1, and therefore its previous association with arrhythmia made it an ideal candidate gene [50]. Sequencing of *KCNQ1* found a Ser140Gly mutation that was present in all affected family members and absent from all but one of the unaffected members. The finding that the Ser140Gly mutation appeared to segregate with disease was further strengthened by its absence in 188 healthy control individuals coupled with it being a highly conserved residue across different species.

Following identification of the putative culprit mutation, functional studies were undertaken in an effort to elucidate the mechanism through which it resulted in a phenotype of AF. Coexpression of mutant *KCNQ1* with *KCNE1* in COS-7 cells resulted in a markedly increased current density relative to the wild-type gene at all voltages, consistent with a gain-of-function mutation. Given that *KCNQ1* contributes to the slow component of the delayed rectifier potassium current (I_{Ks}) and is responsible for repolarization of cardiomyocytes, a gain-of-function mutation could result in more rapid repolarization and reduce the effective refractory period of cells (Fig. 15.3). As discussed previously, this would create a substrate ideal for multiple circuit reentry and promote maintenance of the arrhythmia in a manner consistent with the multiple wavelet hypothesis. This notion is supported by the observation that up to 30 % of patients with short-QT syndrome, a condition characterized by enhanced ventricular repolarization and malignant ventricular arrhythmias, suffer from AF [51, 52].

Although this theory fits nicely, it is worth noting that 9 of the 16 patients with the Ser140Gly mutation were actually found to have a prolonged QT interval on 12-lead electrocardiography. A prolonged QT interval, being consistent with a slower rate of repolarization within the ventricles, is in contrast with the in vitro functional data. It is conceivable that the mutation may have different effects on the atria and ventricles as a result of the different electrical and structural properties of these chambers. This theory was recently hypothesized by Lundby et al. who identified a Gln147Arg *KCNQ1* substitution in a patient with lone AF and a prolonged QT interval [53]. When mutant *KCNQ1* was coexpressed with *KCNE1* in *Xenopus laevis* oocytes, a loss of function was observed; however, coexpression with *KCNE2* resulted in a gain of function. Although these

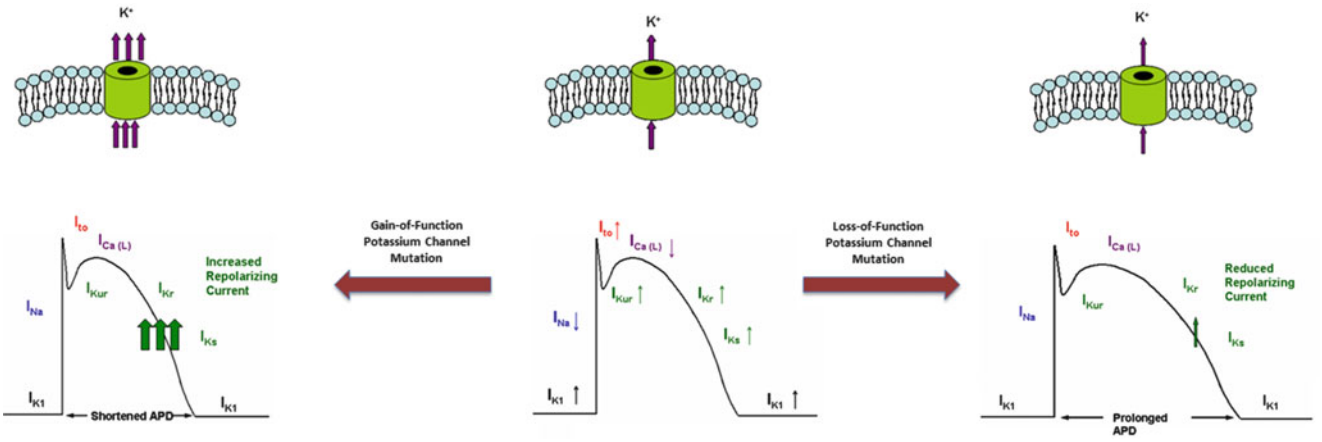

Fig. 15.3 The effect of potassium channel gene mutations on atrial action potential duration. A gain-of-function mutation in a voltage-gated potassium channel increases the efflux of potassium ions during Phase 3 resulting in a shortening of the action potential duration. Conversely, the decrease in Phase 3 current secondary to a loss-of-function mutation in a voltage-gated potassium channel causes prolongation of the atrial action potential

findings are intriguing, it is difficult to arrive at firm conclusions as the relative distributions of *KCNE1* and *KCNE2* within the atria and ventricles are largely unknown [53]. An alternative explanation for the seemingly discordant in vitro and electrocardiographic findings may be secondary to single cell in vitro studies not accurately recapitulating the complex physiology of the heart. It is conceivable that the Ser140Gly substitution within *KCNQ1* actually results in a prolonged action potential duration and effective refractory period within the atria of the intact heart. This could result in AF through an alternative mechanism, which will be further addressed when the *KCNA5* gene is discussed.

KCNE2 and *KCNJ2*

The discovery that a mutation within a potassium channel gene caused an autosomal dominant form of AF alluded to the possibility that other potassium channel genes may contribute to the arrhythmia. Given that linkage analysis studies are frequently limited by small pedigree size, subsequent studies employed a candidate gene approach in which multiple potassium channel genes were screened for mutations in families with AF. This approach led to the identification of two additional potassium channel genes, namely *KCNE2* and *KCNJ2*, as being causal for familial forms of lone AF in two separate studies performed by the same group. A mutation within *KCNE2*, commonly known to encode the β-subunit of I_{Kr}, was discovered following screening of 28 unrelated Chinese kindreds with familial AF for mutations within eight different potassium channels genes (*KCNQ1*, *KCNH2*, *KCNE1-5*, and *KCNJ2*) [54]. Two of the 28 probands were found to carry an Arg27Cys mutation that was subsequently found in affected members of the two kindreds and was absent in 462 healthy controls. It is important to note that each family only had two affected members, while multiple unaffected members carried the Arg27Cys mutation. This may potentially be accounted for by the mutation carrying a low degree of penetrance and necessitating additional genetic and environmental factors in order for the phenotype of AF to be expressed. Additionally, although *KCNE2* is generally considered to serve as the β-subunit of I_{Kr}, coexpression of Arg27Cys *KCNE2* with *KCNH2* did not result in a change in current relative to wild-type. However, there was an increase in current noted when it was coexpressed with *KCNQ1*. Previous work with COS cells has demonstrated that the protein products of *KCNE2* and *KCNQ1* are capable of interacting to generate a background current that is not voltage dependent [55]. It is conceivable that the mutant *KCNE2* may predispose to AF through a background current that may affect cellular repolarization.

KCNJ2 was identified using a similar approach in which 30 Chinese AF kindreds were screened for mutations in ten ion channel or transporter-related genes (*KCNQ1, KCNH2, SCN5A, ANK-B, KCNJ2,* and *KCNE1-5*) [56]. *KCNJ2* encodes Kir2.1, which is responsible for the cardiac inward rectifier potassium current I_{K1}. As discussed, this channel mediates a background potassium current that contributes to the resting membrane potential of the cell and influences cellular excitability and repolarization within the heart. It is also the causative gene for congenital long QT syndrome type 7, also referred to as Andersen–Tawil Syndrome [57]. The proband and the other four affected family members were all found to carry a Val93Ile mutation within *KCNJ2*, a mutation not found in 420 healthy individuals. In this instance, two unaffected family members were found to carry the mutation; however, their unaffected status may have been secondary to their relatively young ages (33 and 42 years old). Functional analysis of the mutant protein revealed increased current density at potentials ranging from −140 to −80 mV

and from −60 to −40 mV consistent with a gain-of-function effect. The putative predisposing mechanism of Val93Ile *KCNJ2* for AF involves enhanced repolarization and a reduction in the refractory period, as hypothesized with *KCNQ1*.

KCNH2

The short-QT syndrome is a rare inherited arrhythmia syndrome characterized by, as its name suggests, a short-QT interval on surface ECG [52, 58]. Affected patients suffer from an increased risk of sudden cardiac death secondary to malignant ventricular arrhythmias, including polymorphic ventricular tachycardia and ventricular fibrillation [51]. In addition to ventricular arrhythmias, short-QT syndrome is also characterized by an increased likelihood of developing AF [59]. To date, a total of six genes have been implicated in the condition [60–64]. The first three genes encode voltage-gated potassium channels and have also been identified as culprits in the long-QT syndrome. In contrast to the long-QT syndrome, which develops secondary to loss-of-function mutations in potassium channels, the pathogenic potassium channel mutations in short-QT syndrome result in a gain of function. The first gene identified in short-QT syndrome was *KCNH2*, which encodes the pore forming α-subunit of I_{Kr}. In the original study evaluating for genetic culprits in this condition, investigators identified an identical *KCNH2* N588 K mutation among two of the three families evaluated [60]. Both of these families had affected members who suffered from paroxysmal AF. Functional evaluation of the *KCNH2* N588 K mutation through its expression in tsA201 cells in the presence and absence of the KCNE2 β-subunit revealed an abbreviation of the cardiac action potential secondary to an increased I_{Kr}. These findings were consistent with the short-QT interval observed on surface ECG and are likely operative in both the atria and ventricles given the clinical features of patients, coupled with the known expression of *KCNH2* in both chambers. Similar to the aforementioned genetic culprits with gain-of-function mutations, *KCNH2* gain-of-function mutations have been presumed to predispose to a mechanistic sub-phenotype of AF reflective of the multiple wavelet hypothesis. More recently, a mutation within *KCNJ2* (E299V) has also been linked to AF in the setting of short-QT syndrome [65].

Potassium Channels: Loss-of-Function Mutations

KCNA5

Up until this point, all of the potassium channel genes implicated in the development of lone AF had been shown to exhibit gain-of-function effects on in vitro functional analysis. As discussed, the purported mechanism involved a reduction in effective refractory period and reentrant wavelength, which in accordance with the multiple wavelet hypothesis would promote and maintain AF. However, was it conceivable that a loss-of-function effect in a potassium channel could result in AF?

Using the candidate gene approach, 154 patients with lone AF were screened for mutations within the *KCNA5* gene, encoding the atrial-specific voltage-gated potassium channel $K_v1.5$ responsible for I_{Kur} [66]. A unique sequence variant was identified in a patient with a family history of the arrhythmia. The patient carried a nonsense mutation (E375X) that resulted in the production of a truncated protein that lacked the S4–S6 voltage sensor, the pore region, and the C-terminus. Because of a lack of available DNA, stringent genetic support for the mutation segregating with the arrhythmia was not possible. Subsequent functional studies revealed that expression of mutant E375X *KCNA5* within HEK293 cells failed to generate current. This was consistent with a loss-of-function effect and not unexpected given the drastic effect of the nonsense mutation on the mature protein. In addition, when coexpressed with wild-type *KCNA5*, cells exhibited a significant reduction in current density compatible with a dominant negative effect, which accounted for the autosomal dominant pattern of inheritance in the setting of a loss-of-function mutation.

Given that loss of function within a voltage-gated potassium channel involved in repolarization presumably results in a prolonged refractory period, the mechanism through which mutant E375X *KCNA5* predisposes to AF would have to be different from the previously described gain-of-function potassium channel mutations (Fig. 15.3). In vitro studies using human atrial myocytes and in vivo studies with a murine model found that administration of 4-aminopyridine, a known blocker of I_{Kur}, dramatically increased the incidence of early afterdepolarizations. The authors hypothesized that increased early afterdepolarizations, in combination with a prolonged atrial action potential duration, could result in disorganized atrial activity akin to that seen in torsade de pointes within the ventricles. Early afterdepolarizations serving as a trigger for AF had previously been suggested in an animal model whereby injection of cesium chloride, a potassium channel blocker, into the sinus node artery of dogs resulted in a polymorphic atrial tachycardia that subsequently degenerated into AF, leading the investigators to coin the term "atrial torsade." [67]

It is important to note that the mechanisms underlying a form of AF caused by a gain-of-function voltage-gated potassium channel are dramatically different, and essentially opposing to a form of the arrhythmia driven by a loss of function in a similar channel. The distinct triggers and substrates in these phenotypically identical forms of the

arrhythmia serve to emphasize the marked heterogeneity that likely underlies the pathophysiology of AF and provides insight into the variable efficacies of many therapies.

A Potassium Channel Variant and "Secondary Hit" Hypothesis

Although genetics play an important role in the development of AF, the critical role of the environment is emphasized by the notion that the arrhythmia rarely develops in childhood and becomes increasingly common with advancing age. The interaction of genetics with environmental influences was eloquently illustrated in a family with autosomal dominant AF who also suffered from a high incidence of hypertension [68]. Mutation screening in four genes (*KCNQ1, KCNE1-3*) identified a novel missense mutation within *KCNQ1*, namely Arg14Cys. Analysis of the family pedigree suggested that the mutation segregated with the arrhythmia, while it was absent in 100 control patients. The interesting findings came following in vitro functional studies in which the mutant Arg14Cys *KCNQ1* was coexpressed with *KCNE1* in CHO cells. The mutant potassium channel initially behaved identically to wild-type; however, following treatment with a hypotonic solution to simulate the cell swelling and stretching consistent with the atrial milieu in a hypertensive patient, the mutant channels exhibited a marked increase in current and a leftward shift in the voltage dependence of activation consistent with a gain-of-function effect. Wild-type channel properties were unaffected by exposure to the hypotonic media. The authors hypothesized that the inherited ion channel defect represented the "first hit"; however, a "second hit" mediated by environmental factors such as hypertension was necessary for development of the arrhythmia. This phenomenon would help to account for the increasing prevalence of AF with aging.

Are Potassium Channel Mutations Common?

Although many of the genes responsible for familial AF encode potassium channel proteins, an obvious question is whether potassium channel mutations are a common cause of AF. Furthermore, most of the genetic studies discussed involved Chinese kindreds, and it was therefore uncertain if these results were applicable to other ethnicities. In order to address this question, two separate studies from the same group screened over 200 patients with lone AF and AF with hypertension for mutations within potassium channel genes (*KCNQ1*, *KCNJ2*, and *KCNE1-5*) [69, 70]. The studies involved predominantly patients of Western European ancestry. Although a number of common polymorphisms were detected, no disease-causing mutations were discovered. It was, therefore, concluded that potassium channel gene mutations represent a rare cause of AF in patients of Western European descent.

Cardiac Gap Junctions

Gap junctions are specialized channels that directly connect cytoplasmic compartments of adjacent cells, allowing for passage of charged ions and coordinated propagation of cardiac action potentials [32]. The molecular constituents of gap junction channels are connexin proteins, which oligomerize into hexameric structures known as connexons or hemichannels (Fig. 15.4). Adjacent cells each contribute a hemichannel to form a functional gap junction channel (Fig. 15.4) [71]. There are multiple different isoforms of connexin proteins; however, the two most highly expressed isoforms within the heart are *connexin* 40 and 43 [72]. Connexin 40 is of particular interest in AF, since it is expressed in atrial myocytes and is absent from ventricular cells [72]. The importance of connexins to AF has been well established in animal studies. In knockout mice lacking the connexin 40 gene, atrial tachyarrhythmias could be induced by burst atrial pacing, whereas this was not possible in wild-type mice [73]. A goat model of persistent AF revealed that connexin 40 distribution within the atria was markedly heterogeneous, a phenomenon also seen in

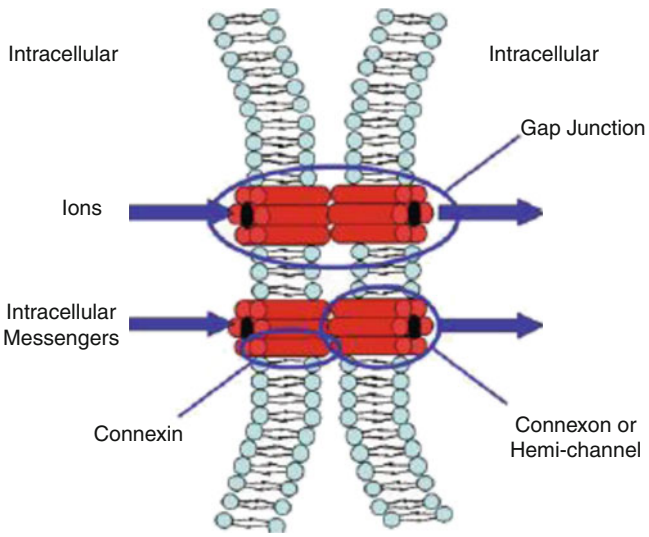

Fig. 15.4 Micro-circuit reentry, secondary to conduction velocity heterogeneity. An ectopic impulse within the atria initiates depolarizing currents in different directions. Depolarizing wavefront A encounters refractory tissue and terminates. Wavefront B travels a different course and encounters a region of slow conduction velocity. The resultant delay allows time for the refractory tissue originally encountered by A to repolarize and conduct allowing a reentry circuit to be established. The region of slow conduction velocity encountered by B may be secondary to reduced connexin activity

humans with AF [74, 75]. Although the latter finding does not establish causality, it does suggest that heterogeneous distribution of connexins within the heart may form an ideal substrate for AF.

In the light of the atrial-specific expression of Cx40 and the vulnerability of Cx40 knockout mice to AF, our group subsequently screened a group of 15 patients with sporadic, lone AF for mutations within connexin 40 (*GJA5*) and 43 (*GJA1*) [76, 77]. We initially identified an Ala96Ser mutation within the highly conserved transmembrane-spanning domain of the connexin 40 protein. Functional studies of the mutant Cx40 Ala96Ser protein were performed in a gap junction-deficient cell line, N2 A cells. Cells expressing the Ala96Ser mutation displayed appropriate trafficking; however, functional electrical cell-to-cell coupling through these channels was significantly reduced. The mutation also demonstrated a dominant negative effect on wild-type Cx40, as well as a trans-dominant negative effect on wild-type Cx43. This latter finding provides strong support for the concept of heteromeric interaction of Cx40 and Cx43 in hemichannel formation. Subsequent work has further validated the role of connexin mutations in AF based on findings from multiple patients and families [78–83]. In addition, a dedicated transgenic murine model possessing the Ala96Ser mutation revealed significantly reduced atrial conduction and prolonged episodes of AF following burst atrial pacing [84]. Collectively, these findings provide strong evidence to support a role for connexins in the pathogenesis of AF.

Sodium Channels: Loss-of-Function Mutations

SCN5A encodes the sodium channel, $Na_v1.5$, responsible for the rapid depolarization upstroke of the cardiac action potential. It has been associated with numerous arrhythmic disorders including the Brugada Syndrome, congenital long QT syndrome type 3, and sick sinus syndrome [85–87]. Given its obvious importance with the electrical properties of the heart, multiple groups employed a candidate gene approach screening patients with AF for mutations within *SCN5A*. The first study involved 157 patients with lone AF; screening did not identify any novel mutations felt to be causative for AF [88]. The H558R single nucleotide polymorphism (SNP), of which approximately one third of the population is heterozygous, was also examined in these patients along with 314 matched controls. The R558 allele, which had previously been shown to alter $Na_v1.5$ function by reducing depolarizing sodium current, was found to confer an increased risk of developing AF (odds ratio: 1.6) [88, 89]. However, the sample size in this study was rather small and the data has yet to be duplicated in larger cohorts.

A second study involving 375 patients with AF (118 had lone AF while 257 had AF associated with heart disease) and 360 well-matched controls identified eight novel mutations in ten separate AF patients [90]. None of the variants were found in controls and all involved highly conserved residues within $Na_v1.5$. Six of the patients appeared to represent familial cases and in each case the variant appeared to segregate with the disease. Functional studies were not performed, and therefore the mechanism through which these variants cause AF is unknown. These findings suggest, in contrast to the previous study, that mutations within *SCN5A* represent a relatively common cause of AF in patients with and without heart disease.

A third group screened *SCN5A* in 57 patients with lone AF or AF with hypertension and a confirmed family history of the arrhythmia [91]. A single novel mutation was found, Asn1986Lys, which was not found in 300 ethnically matched controls. The father of the proband, who also suffered from AF, was found to be a carrier of the mutation, while the unaffected mother did not have any sequence variants within *SCN5A*. Unfortunately, further genetic profiling of the family was not possible due to unwillingness to participate in the study. Expression of the mutant gene within *Xenopus laevis* oocytes suggested a loss-of-function effect as evidenced by a significant hyperpolarizing shift in the midpoint of steady-state inactivation. This alteration was predicted to prolong the atrial action potential duration and, therefore, Asn1986Lys-SCN5A presumably triggers AF through a manner akin to the aforementioned atrial torsade. These findings, which confirmed the association of *SCN5A* with AF, however, suggested that it is not a frequent cause of the condition.

Sodium Channels: Gain-of-Function Mutations

Although previous studies had implicated loss-of-function *SCN5A* mutations in association with AF, subsequent work indicated that gain-of-function mutations within *SCN5A* are also part of the genetic spectrum responsible for AF. Prior to these studies, the only disease related to an *SCN5A* gain-of-function effect was long QT syndrome type 3, which is mediated by a persistent late sodium current [86].

In a four-generation Japanese family with an autosomal dominant form of AF, a novel Met1875Thr mutation was identified within *SCN5A* [92]. The proband was reported to have increased right atrial excitability during radiofrequency catheter ablation for AF. All affected family members were found to carry the mutation, while the mutation was absent in all unaffected family members and 210 ethnically matched controls. Functional analysis of Met1875Thr revealed a

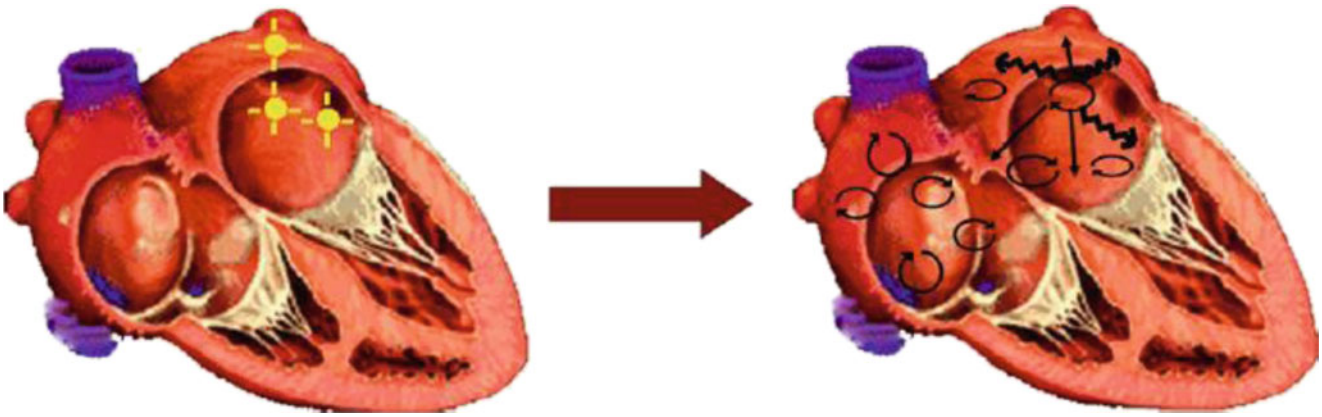

Fig. 15.5 Cellular hyperexcitability triggering AF. Ectopic foci originating from the pulmonary veins contribute to the development of a self-perpetuating micro-reentrant circuit. Rapid, heterogeneous conduction from the reentrant circuit to the surrounding atria results in electrical activity consistent with AF

pronounced depolarizing shift in the midpoint of steady-state inactivation consistent with a gain-of-function effect. No persistent sodium current was observed, consistent with the observation that affected individuals had QT intervals within the normal range.

A second study from our group involving a mother and son with lone AF identified a Lys1493Arg mutation involving a highly conserved residue within the DIII-IV linker located six amino acids downstream from the fast inactivation motif of sodium channels [93]. Biophysical studies demonstrated a significant positive shift in the voltage dependence of inactivation and a large ramp current near resting membrane potential, consistent with a gain-of-function. When expressed in HL-1 atrial cardiomyocytes, enhanced cellular excitability was observed in the form of spontaneous action potential depolarizations and a lower threshold for action potential firing as compared to wild-type cells. Collectively, these studies suggest that both gain- and loss-of-function mutations within *SCN5A* are associated with AF.

The existing evidence suggests that *SCN5A* gain-of-function mutations predispose to AF by enhancing cellular hyperexcitability. The depolarizing shift in steady-state inactivation increases the probability that the channel will be in the open conformation and capable of conducting current [93]. This alteration in the gating of the Na_v1-5 mediated current will presumably result in a predisposition for cells to reach threshold potential and fire, consistent with enhanced automaticity. This increase in focal discharges has the potential to serve as the trigger for AF (Fig. 15.5). In addition, $Na_v1.5$ channels have recently been identified in the autonomic ganglia that surround the pulmonary veins [94]. Mutations within *SCN5A* may therefore result in neuronal hyperexcitability that may trigger AF through a parasympathetic pathway and contribute to the rapidly firing ectopic foci observed in the region of the pulmonary veins in some patients with the arrhythmia.

Atrial Natriuretic Peptide

In contrast to the previous culprits, which all involved cardiac ion channels, the subsequent genetic culprit implicated in the arrhythmia was a circulating hormone, the *atrial natriuretic peptide* (ANP). Although known to be important in cardiac physiology, ANP had been largely viewed as cardioprotective in the setting of heart failure [95]. It was known, however, to be capable of modulating the electrical activities of the heart and there were reports of its effects on specific ion channels [96–98]. However, little work had been done on ANP in the context of AF, and previous studies that had examined for a possible relationship had been negative [99].

Linkage analysis of a Caucasian family of northern European ancestry with autosomal dominant AF mapped the causative locus to the small arm of chromosome 1 (1p36–35) [100]. Review of the genes within this region revealed the presence of *NPPA*, the gene encoding ANP, and subsequent sequencing revealed a two base pair deletion in exon 3 that resulted in a frame shift associated with loss of the stop codon. Extension of the reading frame results in an elongated peptide that is 40 amino acids in length relative to the 28 amino acid length of the wild type. The deletion was present in all of the affected family members and absent in unaffected family members and 560 control patients. Functional studies involving an isolated rat whole-heart model suggested that the mutant ANP resulted in a reduced effective refractory period; however, the mechanism was not entirely clear. ANP mediates its effects on cells through binding to

natriuretic peptide receptors that possess intracellular guanylate cyclase activity [101]. Previous work has suggested that ANP molecules with an elongated C-terminus may be more resistant to degradation and therefore may circulate at higher levels [102]. Therefore, the authors hypothesized that increased circulating ANP may produce increased intracellular levels of cGMP that may in turn, through an unknown mechanism, reduce the effective refractory period.

Atrial Myosin Light Chain 4

Individuals that suffer from genetic forms of ventricular cardiomyopathies, including familial dilated cardiomyopathy and hypertrophic cardiomyopathy, have markedly increased risks of developing AF. The presumed mechanism for this association has generally been assumed to be to increased atrial pressure arising secondary to ventricular systolic and/or diastolic dysfunction. Notably, the genetic culprits responsible for these forms of ventricular cardiomyopathy are also expressed within the atria giving rise to the possibility that AF in these conditions may actually occur secondary to a concomitant atrial myopathy [103].

Our group identified a family with an autosomal dominant form of AF characterized by an early age of onset in the setting of normal biventricular function [104]. Affected family members derived minimal benefit from both antiarrhythmic drugs and catheter ablation for maintenance of sinus rhythm. Notably, while in sinus rhythm, affected individuals having low amplitude p-waves on surface ECG and on echocardiography had markedly dilated atria and severely reduced atrial function. Collectively, these findings, coupled with evidence of large regions of electrical silence observed at the time of catheter ablation, were suggestive of a subphenotype of AF that developed secondary to an atrial myopathy.

In an effort to identify a genetic culprit, we performed exome sequencing on affected and unaffected family members and identified an *MYL4* missense mutation, Glu11Lys, that tracked with the affected phenotype. The *MYL4* gene encodes the atrial-specific myosin light chain, a sarcomeric constituent whose expression is restricted to the atrium. This differential cardiac chamber expression was felt to account for the phenotype in our family consisting of a myopathy restricted to the atria. Subsequent functional work using a transgenic zebrafish model revealed that fish carrying the mutation had markedly dilated atria and prolonged p-wave durations reflective of slow intra-atrial conduction. Electron microscopy revealed evidence of sarcomeric disarray by abnormal organization of H-zones and Z-disks (Fig. 15.6). Collectively, these findings were consistent with an atrial myopathy and mirrored the clinical phenotype exhibited by affected family members. In addition to introducing a novel genetic culprit for AF, our findings serve to validate a novel sub-phenotype of AF characterized by an underlying atrial myopathy.

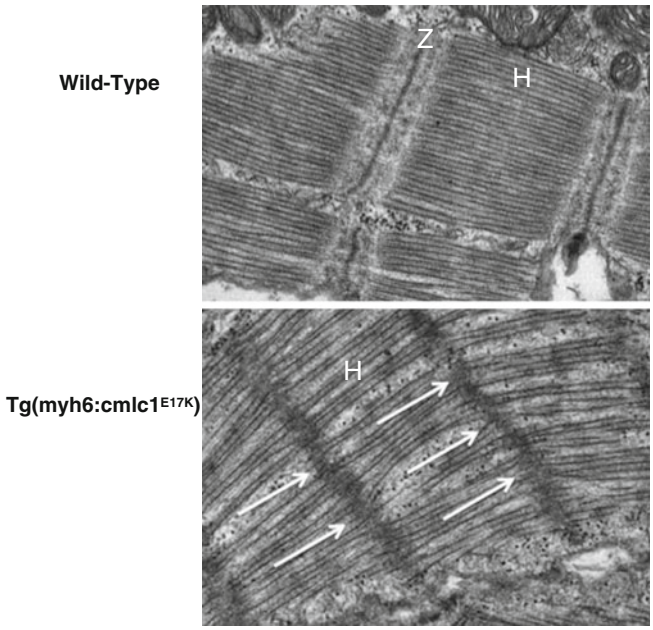

Fig. 15.6 Atrial sarcomeric structure in wild-type and transgenic Glu17Lys zebrafish. Electron microscopy reveals normal H-zones and Z-disks in wild-type, but absent Z-disks (*arrows*) in the transgenic atrium. *H* H-zone, *Z* Z-disk

Unknown Loci

Finally, in addition to 10q22–24, there are multiple other loci that have been identified through linkage analysis in kindreds with autosomal dominant forms of lone AF. These loci include 6q14–16, 10p11–q21, and 5p15; the specific causative genes remain unknown [105–107].

Genome Wide Association Studies

The availability of DNA microarrays containing hundreds of thousands of SNPs has resulted in the opportunity to screen the entire genome for regions that may confer an increased risk for disease. This robust mechanism is proving to be an invaluable tool in unraveling the complex genetics underlying many common diseases including coronary artery disease and AF.

A *genome wide association study* using a DNA microarray containing 316,515 SNPs was performed on 550 patients with AF or flutter in combination with 4476 control patients from Iceland [108]. They discovered a strong

association with SNPs on chromosome 4q25, the most significant being rs2200733 with an odds ratio of 1.84 (95 % confidence intervals: 1.54–2.21). Replication studies using additional samples from Iceland (2251 cases and 13,238 controls), Sweden (143 cases and 738 controls), the USA (636 cases and 804 controls), and China (333 cases and 2836 controls) further reinforced the association with rs2200733. The odds ratio for the combined European population was 1.72 (95 % confidence interval: 1.59–1.86), while that for the Chinese cohort was 1.42 (95 % confidence interval: 1.16–1.73). The haplotype block corresponding to the associated SNPs does not contain a known gene and, therefore, the mechanism for this association is currently unknown. Candidate genes present within the adjacent haplotype block include *PITX2*, which encodes a protein involved in cardiac development, and *ENPEP*, whose protein product is involved in angiotensin II breakdown [90, 91]. Research to delineate the genetic factors at the 4q25 locus responsible for the increased risk for the development of AF is ongoing.

Following identification of the 4q25 locus, two subsequent genome wide association studies were performed with larger numbers of cases and controls in order to improve power and identify previously undetected loci associated with AF. Both groups independently identified two separate SNPs, rs7193343 and rs2106261, which localized to an intronic region within the *ZFHX3* gene on chromosome 16q22 [109, 110]. *ZFHX3* encodes a transcription factor, AT motif-binding factor 1, whose function in the heart is currently unclear. The *ZFHX3* gene has recently been implicated in a vasculitis involving the coronary arteries (Kawasaki disease) [111]. The association of 16q22 with AF was not as strong as for 4q25, with an odds ratio of about 1.2 in most European populations. Furthermore, it was not significantly associated with AF in the Chinese population [110]. Lastly, it is important to note that although the 16q22 SNPs did localize to a gene, it does not necessarily implicate *ZFHX3* in the pathogenesis of AF. These SNPs may appear to associate with AF due to linkage disequilibrium with the true causal variants in surrounding regions. As with the 4q25 locus, further work is necessary in order to better appreciate the apparent relationship between 16q22 and AF.

Subsequent genome wide association studies have identified multiple additional SNPs resulting in a total of 14 common genetic variants associated with AF (Table 15.2) [112–114]. The precise mechanism through which each of these SNPs predisposes to the arrhythmia remains unclear. Experts have hypothesized that these SNPs reside in enhancer or repressor regions and influence expression of nearby genes; however, further work will be necessary to definitively clarify their role in the development of the arrhythmia.

Table 15.2 Single nucleotide polymorphisms identified through genome wide association studies to be associated with the risk of developing AF

SNP	Genetic locus	Nearest gene	Impact of minor allele on AF risk
rs2200733	4q25	*PITX2*	Increased
rs2106261	16q22	*ZFHX3*	Increased
rs6666258	1q21	*KCNN3*	Increased
rs3903239	1q24	*PRRX1*	Increased
rs3807989	7q31	*CAV1*	Protective
rs10821415	9q22	*C9orf3*	Increased
rs10824026	10q22	*SYNPO2L*	Protective
rs1152591	14q23	*SYNE2*	Increased
rs7164883	15q24	*HCN4*	Increased
rs12415501	10q24	*NEURL*	Increased
rs10507248	12q24	*TBX5*	Increased
rs4642101	3p25	*CAND2*	Increased
rs13216675	6q22	*GJA1*	Increased
rs6490029	12q24	*CUX2*	Increased

Other Gene Associations

ACE

The renin–angiotensin system, a pathway of importance in cardiovascular disease, was first implicated with AF following evidence that it was activated in the atria of humans and dogs with the arrhythmia [95, 96]. Multiple studies have since suggested that treatment with angiotensin-converting enzyme (ACE) inhibitors may be protective against the development of AF, and has triggered multicenter randomized controlled trials [97, 98]. The possibility that common polymorphisms within genes of the RAS pathway may contribute to AF was examined in a case control study involving 250 patients with documented nonfamilial structural AF and 250 well-matched controls [99]. Polymorphisms within the genes for ACE, angiotensinogen, and the angiotensin II type 1 receptors were compared between cases and controls. Single locus analysis found that SNPs from the angiotensinogen gene, namely, M235T, G-6A, and G-217A, were significantly associated with AF. However, as with any small study, validation in a much larger cohort is required prior to reaching any definitive conclusions of such an association. Further, recent data indicate that pharmacologic targeting of the renin–angiotensin system pathway had no effect on minimizing recurrence rates of AF [100].

GNB3, Enos, MMP-2, IL-10

The *GNB3* gene encodes the β_3-subunit of a heterotrimeric G protein, which, in broad terms, is important in coordinating

the cellular response to extracellular receptor stimulation via signal transduction [101]. A C825T polymorphism within exon 10 of *GNB3* results in an alternative splicing pattern, such that the 825 T allele generates a modified β_3-subunit that is 41 amino acids shorter and more active than the wild-type form [102]. Previous work in humans had demonstrated that the TT genotype was associated with an increase in I_{K1} but a decrease in I_{Kur} [103]. This triggered an association study involving 291 patients with AF and possible structural heart disease along with 292 control patients without the arrhythmia [104]. The prevalence of the *GNB3* TT genotype was found to be significantly lower in patients with AF relative to the control group, suggesting that the TT genotype is protective against the development of the arrhythmia. Functional studies were not performed.

SNPs within a variety of other genes including endothelial nitric oxide synthase (eNOS), matrix metalloprotease-2 (MMP-2), and interleukin-10 (IL-10) have also been implicated with the development of AF [105, 106]. These studies are intriguing; however, similar to many of the previously described association studies they are limited by relatively small numbers and a frequent lack of functional data corroborating the apparent associations.

Lack of Replication of SNPs Implicated in Atrial Fibrillation

Although multiple SNPs have been implicated in AF beyond the primary genome wide association studies, none have been consistently replicated in subsequent larger studies. A report from two consortia involved in the larger genomewide association studies evaluated 21 SNPs that had been previously implicated in the arrhythmia through smaller studies, and none were found to replicate in these larger independent study populations [115]. These findings highlight the potential for chance associations to be observed and emphasize the need for stringent statistical methods, including transparent adjustment for multiple hypothesis testing, coupled with the need to invariably replicate initial promising findings in subsequent cohorts.

The Autonomic Nervous System

Clinical observations suggest that the autonomic nervous system plays a critical role in the pathogenesis of AF, and this has been supported in different animal models. AF can be readily triggered in structurally normal hearts through exposure to a cholinergic agonist such as carbachol followed by burst pacing. In a canine model, atrial vagal denervation through radiofrequency catheter ablation prevented subsequent induction of the arrhythmia through burst pacing and vagal stimulation [107]. In an effort to investigate the molecular mechanisms underlying this phenomenon, knockout mice that lacked Kir3.4 (previously referred to as GIRK4) were developed [29]. As discussed previously, Kir3.1 and Kir3.4 encode the protein products responsible for I_{KACh} and the absence of either results in the complete loss of I_{KACh}. Unlike wild-type mice, burst pacing in the presence of carbachol was unable to induce AF in the I_{KACh}-deficient knockout mice. These data serve to implicate I_{KACh} in the pathogenesis of AF and suggest that blockers of I_{KACh} may potentially serve as an effective treatment for the arrhythmia. This is an especially attractive treatment option given that I_{KACh}, like I_{Kur}, appears to be localized predominantly to the atria.

Although there are relatively robust data supporting the involvement of the cholinergic system in the pathogenesis of AF, genetic mutations in genes encoding the molecular mediators of the cholinergic response in the heart have not been reported.

Clinical Aspects: Genetic Diagnosis and Targeted Therapy

The contribution of genetics to AF has only recently begun to be appreciated and has not yet been incorporated into routine clinical practice [116]. At the present time, two unresolved issues preclude the routine use of clinical genetic testing for AF cases. Firstly, although a number of genetic etiologies have now been identified, each currently known gene accounts for a very small percentage of cases. Secondly, specific types of AF (e.g., vagal, postoperative) do not reliably predict genotype, and thus phenotype–genotype correlations are not yet apparent. The goal of further research is to identify more common genetic etiologies of AF and to establish phenotype–genotype correlations, with the ultimate achievement of targeted therapy based on the established genotype.

The current status of clinical genetics in AF is in contrast to other known inherited arrhythmia diseases, such as long-QT syndrome and Brugada syndrome. Under these conditions, and the yield for clinical genetic testing in excess of 50 % and 20 %, respectively, is reported. The use of genetic testing in these scenarios is most useful for screening of asymptomatic, phenotype negative family members. In families where a preponderance of AF exists, clinical screening for AF in symptomatic individuals remains the only practical tool available. Routine clinical screening for asymptomatic individuals is not warranted beyond annual routine primary care physical examinations.

A detailed understanding of the genetics contributing to the pathophysiology of AF will likely allow for the development of a pharmacogenomic strategy that improves treatment efficacy and reduces adverse events [117]. AF is a heterogeneous disorder from the perspective of both genetics

and pathophysiology. In one instance, it may arise secondary to a gain-of-function effect within a potassium channel, whereas in another case, it may result from a loss-of-function effect in the same channel. Although phenotypically they may be indistinguishable on electrocardiography, the most efficacious treatment choice is likely to be markedly different in the light of differing electrophysiologic triggers. Given their identical phenotype, genetic characterization will likely be necessary in order to identify the particular AF subtype. In the first example given above, the arrhythmia has likely developed secondary to a shortened atrial refractory period, which has resulted in an ideal substrate for multiple reentrant wavelets within the atria. Effective treatment with a potassium channel blocker that restores the atrial effective refractory period to its normal length, thereby disrupting the reentrant wavelets, may be the optimal agent to restore and maintain sinus rhythm. However, the same treatment in the second case would likely exacerbate the arrhythmia, given that it is secondary to a prolonged atrial refractory period, which may have resulted in "atrial torsade." The genetic and pathophysiologic heterogeneity underlying the arrhythmia is likely responsible for the variable treatment response observed in cases of lone AF [117].

In addition to the above example with potassium channel mutations, a similar approach can be extended to the other genes that have been implicated in AF. For example, forms of AF that develop secondary to cellular hyperexcitability as a result of gain-of-function mutations within sodium channels may benefit from sodium channel blockers. In the context of an AF subtype characterized by conduction velocity heterogeneity that arose secondary to a loss-of-function connexin mutation, such a patient may benefit from a form of therapy that keeps gap junctions in their open state. Although not currently available, there are emerging gap junction pharmacophores that may serve this purpose [108]. Targeted therapy, consistent with a pharmacogenomic approach, should be a goal that is strived for in the coming years. Along with being more efficacious, this strategy should also reduce the unwanted proarrhythmic effects seen with antiarrhythmic drugs.

Summary

An understanding of the genetic factors that lead to the development of AF holds great promise for the development of effective therapies against this exceedingly common arrhythmia. The ability to identify the specific electrophysiologic mechanisms, on the basis of genetic discovery, should lead to more effective forms of targeted therapy that carry minimal risk. This era of pharmacogenomics has yet to arrive for AF; however, it is slowly becoming within reach.

References

1. Feinberg WM, Blackshear JL, Laupacis A, Kronmal R, Hart RG. Prevalence, age distribution, and gender of patients with atrial fibrillation. Analysis and implications. Arch Intern Med. 1995;155:469–73.
2. Go AS, Hylek EM, Phillips KA, Chang Y, Henault LE, Selby JV, Singer DE. Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. JAMA. 2001;285:2370–5.
3. Miyasaka Y, Barnes ME, Gersh BJ, Cha SS, Bailey KR, Abhayaratna WP, Seward JB, Tsang TSM. Secular trends in incidence of atrial fibrillation in Olmsted County, Minnesota, 1980 to 2000, and implications on the projections for future prevalence. Circulation. 2006;114:119–25.
4. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. Circulation. 1998;98:946–52.
5. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation: a major contributor to stroke in the elderly. The Framingham Study. Arch Intern Med. 1987;147:1561–4.
6. Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. JAMA. 1994;271:840–4.
7. Kopecky SL, Gersh BJ, McGoon MD, Whisnant JP, Holmes DR, Ilstrup DM, Frye RL. The natural history of lone atrial fibrillation. A population-based study over three decades. N Engl J Med. 1987;317:669–74.
8. Wolff L. Familial auricular fibrillation. N Engl J Med. 1943;229:396–8.
9. Ellinor PT, Yoerger DM, Ruskin JN, MacRae CA. Familial aggregation in lone atrial fibrillation. Hum Genet. 2005;118:179–84.
10. Darbar D, Herron KJ, Ballew JD, Jahangir A, Gersh BJ, Shen W-K, Hammill SC, Packer DL, Olson TM. Familial atrial fibrillation is a genetically heterogeneous disorder. J Am Coll Cardiol. 2003;41:2185–92.
11. Fox CS, Parise H, D'Agostino RB, Lloyd-Jones DM, Vasan RS, Wang TJ, Levy D, Wolf PA, Benjamin EJ. Parental atrial fibrillation as a risk factor for atrial fibrillation in offspring. JAMA. 2004;291:2851–5.
12. Arnar DO, Thorvaldsson S, Manolio TA, Thorgeirsson G, Kristjansson K, Hakonarson H, Stefansson K. Familial aggregation of atrial fibrillation in Iceland. Eur Heart J. 2006;27:708–12.
13. Lubitz SA, Yin X, Fontes JD, Magnani JW, Rienstra M, Pai M, Villalon ML, Vasan RS, Pencina MJ, Levy D, Larson MG, Ellinor PT, Benjamin EJ. Association between familial atrial fibrillation and risk of new-onset atrial fibrillation. JAMA. 2010;304:2263–9.
14. Katz AM. Cardiac ion channels. N Engl J Med. 1993;328:1244–51.
15. Abriel H. Cardiac sodium channel Nav1.5 and its associated proteins. Arch Mal Coeur Vaiss. 2007;100:787–93.
16. Kääb S, Dixon J, Duc J, Ashen D, Näbauer M, Beuckelmann DJ, Steinbeck G, McKinnon D, Tomaselli GF. Molecular basis of transient outward potassium current downregulation in human heart failure: a decrease in Kv4.3 mRNA correlates with a reduction in current density. Circulation. 1998;98:1383–93.
17. Bers DM. Cardiac excitation-contraction coupling. Nature. 2002;415:198–205.
18. Marbán E. Cardiac channelopathies. Nature. 2002;415:213–8.
19. Tamkun MM, Knoth KM, Walbridge JA, Kroemer H, Roden DM, Glover DM. Molecular cloning and characterization of two voltage-gated K+ channel cDNAs from human ventricle. FASEB J. 1991;5:331–7.

20. Wang Z, Fermini B, Nattel S. Sustained depolarization-induced outward current in human atrial myocytes. Evidence for a novel delayed rectifier K+ current similar to Kv1.5 cloned channel currents. Circ Res. 1993;73:1061–76.
21. Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, Keating MT, Goldstein SA. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. Cell. 1999;97:175–87.
22. Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MT. Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. Nature. 1996;384:80–3.
23. Tamargo J, Caballero R, Gómez R, Valenzuela C, Delpón E. Pharmacology of cardiac potassium channels. Cardiovasc Res. 2004;62:9–33.
24. Kubo Y, Baldwin TJ, Jan YN, Jan LY. Primary structure and functional expression of a mouse inward rectifier potassium channel. Nature. 1993;362:127–33.
25. Dhamoon AS, Jalife J. The inward rectifier current (IK1) controls cardiac excitability and is involved in arrhythmogenesis. Heart Rhythm. 2005;2:316–24.
26. Lu Z. Mechanism of rectification in inward-rectifier K+ channels. Annu Rev Physiol. 2004;66:103–29.
27. Sakmann B, Noma A, Trautwein W. Acetylcholine activation of single muscarinic K+ channels in isolated pacemaker cells of the mammalian heart. Nature. 1983;303:250–3.
28. Pfaffinger PJ, Martin JM, Hunter DD, Nathanson NM, Hille B. GTP-binding proteins couple cardiac muscarinic receptors to a K channel. Nature. 1985;317:536–8.
29. Corey S, Krapivinsky G, Krapivinsky L, Clapham DE. Number and stoichiometry of subunits in the native atrial G-protein-gated K+ channel, IKACh. J Biol Chem. 1998;273:5271–8.
30. Kovoor P, Wickman K, Maguire CT, Pu W, Gehrmann J, Berul CI, Clapham DE. Evaluation of the role of I(KACh) in atrial fibrillation using a mouse knockout model. J Am Coll Cardiol. 2001;37:2136–43.
31. Liu L, Nattel S. Differing sympathetic and vagal effects on atrial fibrillation in dogs: role of refractoriness heterogeneity. Am J Physiol. 1997;273:H805–16.
32. Rohr S. Role of gap junctions in the propagation of the cardiac action potential. Cardiovasc Res. 2004;62:309–22.
33. Brachmann J, Karolyi L, Kübler W. Atrial dispersion of refractoriness. J Cardiovasc Electrophysiol. 1998;9:S35–9.
34. Weiss JN, Qu Z, Chen P-S, Lin S-F, Karagueuzian HS, Hayashi H, Garfinkel A, Karma A. The dynamics of cardiac fibrillation. Circulation. 2005;112:1232–40.
35. Antzelevitch C. Modulation of transmural repolarization. Ann N Y Acad Sci. 2005;1047:314–23.
36. Nattel S. New ideas about atrial fibrillation 50 years on. Nature. 2002;415:219–26.
37. Moe GK, Rheinboldt WC, Abildskov JA. A computer model of atrial fibrillation. Am Heart J. 1964;67:200–20.
38. Konings KT, Kirchhof CJ, Smeets JR, Wellens HJ, Penn OC, Allessie MA. High-density mapping of electrically induced atrial fibrillation in humans. Circulation. 1994;89:1665–80.
39. Cox JL, Canavan TE, Schuessler RB, Cain ME, Lindsay BD, Stone C, Smith PK, Corr PB, Boineau JP. The surgical treatment of atrial fibrillation. II. Intraoperative electrophysiologic mapping and description of the electrophysiologic basis of atrial flutter and atrial fibrillation. J Thorac Cardiovasc Surg. 1991;101:406–26.
40. Mandapati R, Skanes A, Chen J, Berenfeld O, Jalife J. Stable microreentrant sources as a mechanism of atrial fibrillation in the isolated sheep heart. Circulation. 2000;101:194–9.
41. Jalife J, Berenfeld O, Mansour M. Mother rotors and fibrillatory conduction: a mechanism of atrial fibrillation. Cardiovasc Res. 2002;54:204–16.
42. Haïssaguerre M, Jaïs P, Shah DC, Takahashi A, Hocini M, Quiniou G, Garrigue S, Le Mouroux A, Le Métayer P, Clémenty J. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med. 1998;339:659–66.
43. Pappone C, Rosanio S, Oreto G, Tocchi M, Gugliotta F, Vicedomini G, Salvati A, Dicandia C, Mazzone P, Santinelli V, Gulletta S, Chierchia S. Circumferential radiofrequency ablation of pulmonary vein ostia: a new anatomic approach for curing atrial fibrillation. Circulation. 2000;102:2619–28.
44. Allessie MA, Bonke FI, Schopman FJ. Circus movement in rabbit atrial muscle as a mechanism of tachycardia. III. The "leading circle" concept: a new model of circus movement in cardiac tissue without the involvement of an anatomical obstacle. Circ Res. 1977;41:9–18.
45. Rensma PL, Allessie MA, Lammers WJ, Bonke FI, Schalij MJ. Length of excitation wave and susceptibility to reentrant atrial arrhythmias in normal conscious dogs. Circ Res. 1988;62:395–410.
46. Psaty BM, Manolio TA, Kuller LH, Kronmal RA, Cushman M, Fried LP, White R, Furberg CD, Rautaharju PM. Incidence of and risk factors for atrial fibrillation in older adults. Circulation. 1997;96:2455–61.
47. Coumel P, Attuel P, Lavallée J, Flammang D, Leclercq JF, Slama R. The atrial arrhythmia syndrome of vagal origin. Arch Mal Coeur Vaiss. 1978;71:645–56.
48. Brugada R, Tapscott T, Czernuszewicz GZ, Marian AJ, Iglesias A, Mont L, Brugada J, Girona J, Domingo A, Bachinski LL, Roberts R. Identification of a genetic locus for familial atrial fibrillation. N Engl J Med. 1997;336:905–11.
49. Chen Y-H, Xu S-J, Bendahhou S, Wang X-L, Wang Y, Xu W-Y, Jin H-W, Sun H, Su X-Y, Zhuang Q-N, Yang Y-Q, Li Y-B, Liu Y, Xu H-J, Li X-F, Ma N, Mou C-P, Chen Z, Barhanin J, Huang W. KCNQ1 gain-of-function mutation in familial atrial fibrillation. Science. 2003;299:251–4.
50. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, Shen J, Timothy KW, Vincent GM, de Jager T, Schwartz PJ, Toubin JA, Moss AJ, Atkinson DL, Landes GM, Connors TD, Keating MT. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat Genet. 1996;12:17–23.
51. Giustetto C, Di Monte F, Wolpert C, Borggrefe M, Schimpf R, Sbragia P, Leone G, Maury P, Anttonen O, Haissaguerre M, Gaita F. Short QT syndrome: clinical findings and diagnostic-therapeutic implications. Eur Heart J. 2006;27:2440–7.
52. Gollob MH, Redpath CJ, Roberts JD. The short QT syndrome: proposed diagnostic criteria. J Am Coll Cardiol. 2011;57:802–12.
53. Lundby A, Ravn LS, Svendsen JH, Olesen S-P, Schmitt N. KCNQ1 mutation Q147R is associated with atrial fibrillation and prolonged QT interval. Heart Rhythm Off J Heart Rhythm Soc. 2007;4:1532–41.
54. Yang Y, Xia M, Jin Q, Bendahhou S, Shi J, Chen Y, Liang B, Lin J, Liu Y, Liu B, Zhou Q, Zhang D, Wang R, Ma N, Su X, Niu K, Pei Y, Xu W, Chen Z, Wan H, Cui J, Barhanin J, Chen Y. Identification of a KCNE2 gain-of-function mutation in patients with familial atrial fibrillation. Am J Hum Genet. 2004;75:899–905.
55. Tinel N, Diochot S, Borsotto M, Lazdunski M, Barhanin J. KCNE2 confers background current characteristics to the cardiac KCNQ1 potassium channel. EMBO J. 2000;19:6326–30.
56. Xia M, Jin Q, Bendahhou S, He Y, Larroque M-M, Chen Y, Zhou Q, Yang Y, Liu Y, Liu B, Zhu Q, Zhou Y, Lin J, Liang B, Li L, Dong X, Pan Z, Wang R, Wan H, Qiu W, Xu W, Eurlings P, Barhanin J, Chen Y. A Kir2.1 gain-of-function mutation underlies familial atrial fibrillation. Biochem Biophys Res Commun. 2005;332:1012–9.
57. Plaster NM, Tawil R, Tristani-Firouzi M, Canún S, Bendahhou S, Tsunoda A, Donaldson MR, Iannaccone ST, Brunt E, Barohn R,

Clark J, Deymeer F, George AL, Fish FA, Hahn A, Nitu A, Ozdemir C, Serdaroglu P, Subramony SH, Wolfe G, Fu YH, Ptácek LJ. Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. Cell. 2001;105:511–9.
58. Gaita F, Giustetto C, Bianchi F, Wolpert C, Schimpf R, Riccardi R, Grossi S, Richiardi E, Borggrefe M. Short QT Syndrome A Familial Cause of Sudden Death. Circulation. 2003;108:965–70.
59. Hong K, Bjerregaard P, Gussak I, Brugada R. Short QT syndrome and atrial fibrillation caused by mutation in KCNH2. J Cardiovasc Electrophysiol. 2005;16:394–6.
60. Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, Menendez TM, Brugada J, Pollevick GD, Wolpert C, Burashnikov E, Matsuo K, Wu YS, Guerchicoff A, Bianchi F, Giustetto C, Schimpf R, Brugada P, Antzelevitch C. Sudden death associated with short-QT syndrome linked to mutations in HERG. Circulation. 2004;109:30–5.
61. Bellocq C, van Ginneken ACG, Bezzina CR, Alders M, Escande D, Mannens MMAM, Baró I, Wilde AAM. Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. Circulation. 2004;109:2394–7.
62. Priori SG, Pandit SV, Rivolta I, Berenfeld O, Ronchetti E, Dhamoon A, Napolitano C, Anumonwo J, di Barletta MR, Gudapakkam S, Bosi G, Stramba-Badiale M, Jalife J. A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. Circ Res. 2005;96:800–7.
63. Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, Guerchicoff A, Pfeiffer R, Oliva A, Wollnik B, Gelber P, Bonaros EP, Burashnikov E, Wu Y, Sargent JD, Schickel S, Oberheiden R, Bhatia A, Hsu L-F, Haïssaguerre M, Schimpf R, Borggrefe M, Wolpert C. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation. 2007;115:442–9.
64. Templin C, Ghadri J-R, Rougier J-S, Baumer A, Kaplan V, Albesa M, Sticht H, Rauch A, Puleo C, Hu D, Barajas-Martinez H, Antzelevitch C, Lüscher TF, Abriel H, Duru F. Identification of a novel loss-of-function calcium channel gene mutation in short QT syndrome (SQTS6). Eur Heart J. 2011;32:1077–88.
65. Deo M, Ruan Y, Pandit SV, Shah K, Berenfeld O, Blaufox A, Cerrone M, Noujaim SF, Denegri M, Jalife J, Priori SG. KCNJ2 mutation in short QT syndrome 3 results in atrial fibrillation and ventricular proarrhythmia. Proc Natl Acad Sci U S A. 2013;110:4291–6.
66. Olson TM, Alekseev AE, Liu XK, Park S, Zingman LV, Bienengraeber M, Sattiraju S, Ballew JD, Jahangir A, Terzic A. Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation. Hum Mol Genet. 2006;15:2185–91.
67. Satoh T, Zipes DP. Cesium-induced atrial tachycardia degenerating into atrial fibrillation in dogs: atrial torsades de pointes? J Cardiovasc Electrophysiol. 1998;9:970–5.
68. Otway R, Vandenberg JI, Guo G, Varghese A, Castro ML, Liu J, Zhao J, Bursill JA, Wyse KR, Crotty H, Baddeley O, Walker B, Kuchar D, Thorburn C, Fatkin D. Stretch-sensitive KCNQ1 mutation A link between genetic and environmental factors in the pathogenesis of atrial fibrillation? J Am Coll Cardiol. 2007;49:578–86.
69. Ellinor PT, Moore RK, Patton KK, Ruskin JN, Pollak MR, Macrae CA. Mutations in the long QT gene, KCNQ1, are an uncommon cause of atrial fibrillation. Heart Br Card Soc. 2004;90:1487–8.
70. Ellinor PT, Petrov-Kondratov VI, Zakharova E, Nam EG, MacRae CA. Potassium channel gene mutations rarely cause atrial fibrillation. BMC Med Genet. 2006;7:70.
71. Hervé J-C, Bourmeyster N, Sarrouilhe D, Duffy HS. Gap junctional complexes: from partners to functions. Prog Biophys Mol Biol. 2007;94:29–65.
72. Vozzi C, Dupont E, Coppen SR, Yeh HI, Severs NJ. Chamber-related differences in connexin expression in the human heart. J Mol Cell Cardiol. 1999;31:991–1003.
73. Hagendorff A, Schumacher B, Kirchhoff S, Lüderitz B, Willecke K. Conduction disturbances and increased atrial vulnerability in Connexin40-deficient mice analyzed by transesophageal stimulation. Circulation. 1999;99:1508–15.
74. van der Velden HM, Ausma J, Rook MB, Hellemons AJ, van Veen TA, Allessie MA, Jongsma HJ. Gap junctional remodeling in relation to stabilization of atrial fibrillation in the goat. Cardiovasc Res. 2000;46:476–86.
75. Wilhelm M, Kirste W, Kuly S, Amann K, Neuhuber W, Weyand M, Daniel WG, Garlichs C. Atrial distribution of connexin 40 and 43 in patients with intermittent, persistent, and postoperative atrial fibrillation. Heart Lung Circ. 2006;15:30–7.
76. Gollob MH, Jones DL, Krahn AD, Danis L, Gong X-Q, Shao Q, Liu X, Veinot JP, Tang ASL, Stewart AFR, Tesson F, Klein GJ, Yee R, Skanes AC, Guiraudon GM, Ebihara L, Bai D. Somatic mutations in the connexin 40 gene (GJA5) in atrial fibrillation. N Engl J Med. 2006;354:2677–88.
77. Thibodeau IL, Xu J, Li Q, Liu G, Lam K, Veinot JP, Birnie DH, Jones DL, Krahn AD, Lemery R, Nicholson BJ, Gollob MH. Paradigm of genetic mosaicism and lone atrial fibrillation: physiological characterization of a connexin 43-deletion mutant identified from atrial tissue. Circulation. 2010;122:236–44.
78. Yang Y-Q, Liu X, Zhang X-L, Wang X-H, Tan H-W, Shi H-F, Jiang W-F, Fang W-Y. Novel connexin40 missense mutations in patients with familial atrial fibrillation. Europace. 2010;12:1421–7.
79. Yang Y-Q, Zhang X-L, Wang X-H, Tan H-W, Shi H-F, Jiang W-F, Fang W-Y, Liu X. Connexin40 nonsense mutation in familial atrial fibrillation. Int J Mol Med. 2010;26:605–10.
80. Christophersen IE, Holmegard HN, Jabbari J, Sajadieh A, Haunsø S, Tveit A, Svendsen JH, Olesen MS. Rare variants in GJA5 are associated with early-onset lone atrial fibrillation. Can J Cardiol. 2013;29:111–6.
81. Shi H-F, Yang J-F, Wang Q, Li R-G, Xu Y-J, Qu X-K, Fang W-Y, Liu X, Yang Y-Q. Prevalence and spectrum of GJA5 mutations associated with lone atrial fibrillation. Mol Med Rep. 2013;7:767–74.
82. Sun Y, Yang Y-Q, Gong X-Q, Wang X-H, Li R-G, Tan H-W, Liu X, Fang W-Y, Bai D. Novel germline GJA5/connexin40 mutations associated with lone atrial fibrillation impair gap junctional intercellular communication. Hum Mutat. 2013;34:603–9.
83. Sun Y, Tong X, Chen H, Huang T, Shao Q, Huang W, Laird DW, Bai D. An atrial-fibrillation-linked connexin40 mutant is retained in the endoplasmic reticulum and impairs the function of atrial gap-junction channels. Dis Model Mech. 2014;7:561–9.
84. Lübkemeier I, Andrié R, Lickfett L, Bosen F, Stöckigt F, Dobrowolski R, Draffehn AM, Fregeac J, Schultze JL, Bukauskas FF, Schrickel JW, Willecke K. The Connexin40A96S mutation from a patient with atrial fibrillation causes decreased atrial conduction velocities and sustained episodes of induced atrial fibrillation in mice. J Mol Cell Cardiol. 2013;65:19–32.
85. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, Potenza D, Moya A, Borggrefe M, Breithardt G, Ortiz-Lopez R, Wang Z, Antzelevitch C, O'Brien RE, Schulze-Bahr E, Keating MT, Towbin JA, Wang Q. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature. 1998;392:293–6.
86. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AJ, Towbin JA, Keating MT. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell. 1995;80:805–11.
87. Benson DW, Wang DW, Dyment M, Knilans TK, Fish FA, Strieper MJ, Rhodes TH, George AL. Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (SCN5A). J Clin Invest. 2003;112:1019–28.

88. Chen LY, Ballew JD, Herron KJ, Rodeheffer RJ, Olson TM. A common polymorphism in SCN5A is associated with lone atrial fibrillation. Clin Pharmacol Ther. 2007;81:35–41.
89. Makielski JC, Ye B, Valdivia CR, Pagel MD, Pu J, Tester DJ, Ackerman MJ. A ubiquitous splice variant and a common polymorphism affect heterologous expression of recombinant human SCN5A heart sodium channels. Circ Res. 2003;93:821–8.
90. Darbar D, Kannankeril PJ, Donahue BS, Kucera G, Stubblefield T, Haines JL, George AL, Roden DM. Cardiac sodium channel (SCN5A) variants associated with atrial fibrillation. Circulation. 2008;117:1927–35.
91. Ellinor PT, Nam EG, Shea MA, Milan DJ, Ruskin JN, MacRae CA. Cardiac sodium channel mutation in atrial fibrillation. Heart Rhythm. 2008;5:99–105.
92. Makiyama T, Akao M, Shizuta S, Doi T, Nishiyama K, Oka Y, Ohno S, Nishio Y, Tsuji K, Itoh H, Kimura T, Kita T, Horie M. A novel SCN5A gain-of-function mutation M1875T associated with familial atrial fibrillation. J Am Coll Cardiol. 2008;52:1326–34.
93. Li Q, Huang H, Liu G, Lam K, Rutberg J, Green MS, Birnie DH, Lemery R, Chahine M, Gollob MH. Gain-of-function mutation of Nav1.5 in atrial fibrillation enhances cellular excitability and lowers the threshold for action potential firing. Biochem Biophys Res Commun. 2009;380:132–7.
94. Scornik FS, Desai M, Brugada R, Guerchicoff A, Pollevick GD, Antzelevitch C, Pérez GJ. Functional expression of "cardiac-type" Nav1.5 sodium channel in canine intracardiac ganglia. Heart Rhythm. 2006;3:842–50.
95. Rubattu S, Sciarretta S, Valenti V, Stanzione R, Volpe M. Natriuretic peptides: an update on bioactivity, potential therapeutic use, and implication in cardiovascular diseases. Am J Hypertens. 2008;21:733–41.
96. Crozier I, Richards AM, Foy SG, Ikram H. Electrophysiological effects of atrial natriuretic peptide on the cardiac conduction system in man. Pacing Clin Electrophysiol. 1993;16:738–42.
97. Sorbera LA, Morad M. Atrionatriuretic peptide transforms cardiac sodium channels into calcium-conducting channels. Science. 1990;247:969–73.
98. Le Grand B, Deroubaix E, Couétil JP, Coraboeuf E. Effects of atrionatriuretic factor on Ca2+ current and Cai-independent transient outward K+ current in human atrial cells. Pflüg Arch Eur J Physiol. 1992;421:486–91.
99. Ellinor PT, Low AF, Patton KK, Shea MA, Macrae CA. Discordant atrial natriuretic peptide and brain natriuretic peptide levels in lone atrial fibrillation. J Am Coll Cardiol. 2005;45:82–6.
100. Hodgson-Zingman DM, Karst ML, Zingman LV, Heublein DM, Darbar D, Herron KJ, Ballew JD, de Andrade M, Burnett JC, Olson TM. Atrial natriuretic peptide frameshift mutation in familial atrial fibrillation. N Engl J Med. 2008;359:158–65.
101. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. N Engl J Med. 1998;339:321–8.
102. Chen HH, Lainchbury JG, Burnett JC. Natriuretic peptide receptors and neutral endopeptidase in mediating the renal actions of a new therapeutic synthetic natriuretic peptide dendroaspis natriuretic peptide. J Am Coll Cardiol. 2002;40:1186–91.
103. McNally EM, Barefield DY, Puckelwartz MJ. The genetic landscape of cardiomyopathy and its role in heart failure. Cell Metab. 2015;21:174–82.
104. Orr N, Arnaout R, Gula LJ, Spears DA, Leong-Sit P, Li Q, Tarhuni W, Reischauer S, Chauhan VS, Borkovich M, Uppal S, Adler A, Coughlin SR, Stainier DYR, Gollob MH. A mutation in the atrial-specific myosin light chain gene (MYL4) causes familial atrial fibrillation. Nat Commun. 2016;7:11303.
105. Ellinor PT, Shin JT, Moore RK, Yoerger DM, MacRae CA. Locus for atrial fibrillation maps to chromosome 6q14-16. Circulation. 2003;107:2880–3.
106. Volders PGA, Zhu Q, Timmermans C, Eurlings PMH, Su X, Arens YH, Li L, Jongbloed RJ, Xia M, Rodriguez L-M, Chen YH. Mapping a novel locus for familial atrial fibrillation on chromosome 10p11-q21. Heart Rhythm. 2007;4:469–75.
107. Darbar D, Hardy A, Haines JL, Roden DM. Prolonged signal-averaged P-wave duration as an intermediate phenotype for familial atrial fibrillation. J Am Coll Cardiol. 2008;51:1083–9.
108. Gudbjartsson DF, Arnar DO, Helgadottir A, Gretarsdottir S, Holm H, Sigurdsson A, Jonasdottir A, Baker A, Thorleifsson G, Kristjansson K, Palsson A, Blondal T, Sulem P, Backman VM, Hardarson GA, Palsdottir E, Helgason A, Sigurjonsdottir R, Sverrisson JT, Kostulas K, Ng MCY, Baum L, So WY, Wong KS, Chan JCN, Furie KL, Greenberg SM, Sale M, Kelly P, MacRae CA, Smith EE, Rosand J, Hillert J, Ma RCW, Ellinor PT, Thorgeirsson G, Gulcher JR, Kong A, Thorsteinsdottir U, Stefansson K. Variants conferring risk of atrial fibrillation on chromosome 4q25. Nature. 2007;448:353–7.
109. Benjamin EJ, Rice KM, Arking DE, Pfeufer A, van Noord C, Smith AV, Schnabel RB, Bis JC, Boerwinkle E, Sinner MF, Dehghan A, Lubitz SA, D'Agostino RB, Lumley T, Ehret GB, Heeringa J, Aspelund T, Newton-Cheh C, Larson MG, Marciante KD, Soliman EZ, Rivadeneira F, Wang TJ, Eiríksdottir G, Levy D, Psaty BM, Li M, Chamberlain AM, Hofman A, Vasan RS, Harris TB, Rotter JI, Kao WHL, Agarwal SK, Stricker BHC, Wang K, Launer LJ, Smith NL, Chakravarti A, Uitterlinden AG, Wolf PA, Sotoodehnia N, Köttgen A, van Duijn CM, Meitinger T, Mueller M, Perz S, Steinbeck G, Wichmann H-E, Lunetta KL, Heckbert SR, Gudnason V, Alonso A, Kääb S, Ellinor PT, Witteman JCM. Variants in ZFHX3 are associated with atrial fibrillation in individuals of European ancestry. Nat Genet. 2009;41:879–81.
110. Gudbjartsson DF, Holm H, Gretarsdottir S, Thorleifsson G, Walters GB, Thorgeirsson G, Gulcher J, Mathiesen EB, Njølstad I, Nyrnes A, Wilsgaard T, Hald EM, Hveem K, Stoltenberg C, Kucera G, Stubblefield T, Carter S, Roden D, Ng MCY, Baum L, So WY, Wong KS, Chan JCN, Gieger C, Wichmann H-E, Gschwendtner A, Dichgans M, Kuhlenbäumer G, Berger K, Ringelstein EB, Bevan S, Markus HS, Kostulas K, Hillert J, Sveinbjörnsdóttir S, Valdimarsson EM, Løchen M-L, Ma RCW, Darbar D, Kong A, Arnar DO, Thorsteinsdottir U, Stefansson K. A sequence variant in ZFHX3 on 16q22 associates with atrial fibrillation and ischemic stroke. Nat Genet. 2009;41:876–8.
111. Burgner D, Davila S, Breunis WB, Ng SB, Li Y, Bonnard C, Ling L, Wright VJ, Thalamuthu A, Odam M, Shimizu C, Burns JC, Levin M, Kuijpers TW, Hibberd ML, International Kawasaki Disease Genetics Consortium. A genome-wide association study identifies novel and functionally related susceptibility Loci for Kawasaki disease. PLoS Genet. 2009;5:e1000319.
112. Ellinor PT, Lunetta KL, Glazer NL, Pfeufer A, Alonso A, Chung MK, Sinner MF, de Bakker PIW, Mueller M, Lubitz SA, Fox E, Darbar D, Smith NL, Smith JD, Schnabel RB, Soliman EZ, Rice KM, Van Wagoner DR, Beckmann B-M, van Noord C, Wang K, Ehret GB, Rotter JI, Hazen SL, Steinbeck G, Smith AV, Launer LJ, Harris TB, Makino S, Nelis M, Milan DJ, Perz S, Esko T, Köttgen A, Moebus S, Newton-Cheh C, Li M, Möhlenkamp S, Wang TJ, Kao WHL, Vasan RS, Nöthen MM, MacRae CA, Stricker BHC, Hofman A, Uitterlinden AG, Levy D, Boerwinkle E, Metspalu A, Topol EJ, Chakravarti A, Gudnason V, Psaty BM, Roden DM, Meitinger T, Wichmann H-E, Witteman JCM, Barnard J, Arking DE, Benjamin EJ, Heckbert SR, Kääb S. Common variants in KCNN3 are associated with lone atrial fibrillation. Nat Genet. 2010;42:240–4.
113. Ellinor PT, Lunetta KL, Albert CM, Glazer NL, Ritchie MD, Smith AV, Arking DE, Müller-Nurasyid M, Krijthe BP, Lubitz SA, Bis JC, Chung MK, Dörr M, Ozaki K, Roberts JD, Smith JG, Pfeufer A, Sinner MF, Lohman K, Ding J, Smith NL, Smith JD, Rienstra M, Rice KM, Van Wagoner DR, Magnani JW, Wakili R,

Clauss S, Rotter JI, Steinbeck G, Launer LJ, Davies RW, Borkovich M, Harris TB, Lin H, Völker U, Völzke H, Milan DJ, Hofman A, Boerwinkle E, Chen LY, Soliman EZ, Voight BF, Li G, Chakravarti A, Kubo M, Tedrow UB, Rose LM, Ridker PM, Conen D, Tsunoda T, Furukawa T, Sotoodehnia N, Xu S, Kamatani N, Levy D, Nakamura Y, Parvez B, Mahida S, Furie KL, Rosand J, Muhammad R, Psaty BM, Meitinger T, Perz S, Wichmann H-E, Witteman JCM, Kao WHL, Kathiresan S, Roden DM, Uitterlinden AG, Rivadeneira F, McKnight B, Sjögren M, Newman AB, Liu Y, Gollob MH, Melander O, Tanaka T, Stricker BHC, Felix SB, Alonso A, Darbar D, Barnard J, Chasman DI, Heckbert SR, Benjamin EJ, Gudnason V, Kääb S. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. Nat Genet. 2012; 44:670–5.

114. Sinner MF, Tucker NR, Lunetta KL, Ozaki K, Smith JG, Trompet S, Bis JC, Lin H, Chung MK, Nielsen JB, Lubitz SA, Krijthe BP, Magnani JW, Ye J, Gollob MH, Tsunoda T, Müller-Nurasyid M, Lichtner P, Peters A, Dolmatova E, Kubo M, Smith JD, Psaty BM, Smith NL, Jukema JW, Chasman DI, Albert CM, Ebana Y, Furukawa T, Macfarlane PW, Harris TB, Darbar D, Dörr M, Holst AG, Svendsen JH, Hofman A, Uitterlinden AG, Gudnason V, Isobe M, Malik R, Dichgans M, Rosand J, Van Wagoner DR, METASTROKE Consortium, AFGen Consortium, Benjamin EJ, Milan DJ, Melander O, Heckbert SR, Ford I, Liu Y, Barnard J, Olesen MS, Stricker BHC, Tanaka T, Kääb S, Ellinor PT. Integrating genetic, transcriptional, and functional analyses to identify 5 novel genes for atrial fibrillation. Circulation. 2014;130: 1225–35.
115. Sinner MF, Lubitz SA, Pfeufer A, Makino S, Beckmann B-M, Lunetta KL, Steinbeck G, Perz S, Rahman R, Sonni A, Greenberg SM, Furie KL, Wichmann H-E, Meitinger T, Peters A, Benjamin EJ, Rosand J, Ellinor PT, Kääb S. Lack of replication in polymorphisms reported to be associated with atrial fibrillation. Heart Rhythm. 2011;8:403–9.
116. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, Camm AJ, Ellinor PT, Gollob M, Hamilton R, Hershberger RE, Judge DP, Le Marec H, McKenna WJ, Schulze-Bahr E, Semsarian C, Towbin JA, Watkins H, Wilde A, Wolpert C, Zipes DP. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Heart Rhythm. 2011;8:1308–39.
117. Roberts JD, Gollob MH. Impact of genetic discoveries on the classification of lone atrial fibrillation. J Am Coll Cardiol. 2010;55:705–12.

Hereditary Cardiac Conduction Diseases

16

Rafik Tadros and Julia Cadrin-Tourigny

Abstract

Cardiac conduction diseases (CCD) are a group of electrical defects of the heart with multiple hereditary and non-hereditary etiologies. The clinical spectrum of CCD includes asymptomatic patients with incidental electrocardiographic abnormalities, as well as patients presenting with syncope and cardiac arrest. CCD can be associated with other hereditary syndromes including Brugada syndrome, cardiomyopathy and neuromuscular diseases. A comprehensive clinical evaluation of patients with CCD including a detailed family history as well as molecular diagnostics in select cases are key to establishing the correct etiology, guiding patient management and directing family screening. In this chapter, we discuss the differential diagnosis of CCD, guiding principles in cardiogenetic evaluation as well as specific genotype-phenotype correlations.

Introduction

Cardiac conduction disease (CCD) is very common in clinical practice. Its description dates back to more than a century ago. Although the co-occurrence of fainting episodes and severe bradycardia was reported by Morgagni in the eighteenth century and again later by Stokes and Adams (to whom the term Stokes-Adams attacks was dedicated) [21, 37], the first documentation of atrioventricular block (AVB) on surface electrocardiography (ECG) was achieved later [75]. Familial CCD has been first described in 1901 by Morquio, and by many others thereafter [33, 45, 46, 52].

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Reflecting its numerous etiologies and electrical manifestations, the definition of CCD is highly variable. We here define it as *any persistent defect in the formation or propagation of the cardiac impulse at any level of the specialized cardiac electrical system in the absence of drug or metabolic disturbance known to affect cardiac conduction.* Unlike most cardiovascular conditions discussed in this book, CCD is primarily caused by nongenetic etiologies (Table 16.1). In many cases, CCD is *secondary* to structural heart disease (e.g., ischemic heart disease, cardiomyopathy), to a cardiac intervention (e.g., aortic valve replacement, arrhythmia ablation) or to an autoimmune process (e.g., neonatal lupus syndrome). CCD can also be *primary*, most often in the context of senile degeneration of the conduction system in an elderly patient. Primary CCD in a young patient should raise the suspicion for an inherited etiology, especially if there is a positive family history. The mechanism of CCD is either functional (decreased depolarizing currents or impaired cellular coupling) and/or associated with premature conduction system degeneration (referred to as Lenègre's disease). This chapter is focused on primary CCD in the young, which could have an inherited etiology. We review the subject in a clinically oriented manner, in a similar fashion as other chapters. A general discussion on the clinical evaluation and management

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Table 16.1 Etiologies of CCD

Etiology	Definition and suggestive clinical clues
Primary CCD	
Senile progressive CCD	Progressive sinus node dysfunction, atrial arrhythmias and/or atrioventricular conduction defects typically with fascicular blocks (wide QRS). Late onset (e.g., >50 years old) Can be associated with aortic valve calcification. Can be associated with aortic valve calcification.
Hereditary (see Table 16.2)	As above but with a premature onset (e.g., <50 years old). Family history of CCD, SCD, DCM, CHD, and/or the presence of a pathogenic mutation in susceptibility genes
Idiopathic	Unexplained CCD
Secondary CCD	
Ischemic heart disease	Known coronary artery disease or the presence of risk factors for atherosclerosis. Presence of Q-waves on the ECG and the presence of wall motion abnormalities and/or scar on cardiac imaging (typically involving the septum)
Cardiomyopathy	Diagnosed with cardiac imaging. Any cardiomyopathy (most often DCM) can progressively affect the conduction system in proportion with myocardial involvement. When CCD is out of proportion with the severity of cardiomyopathy, a primary CCD should be suspected (e.g., *LMNA* mutations, see Table 16.2)
Cardiac sarcoidosis and myocarditis	Presents with CCD, ventricular arrhythmia, and/or heart failure. The presence of inflammation or scar on cardiac magnetic resonance or positron emission tomography. Diagnosis may require cardiac or extracardiac biopsy. Sarcoidosis should be suspected in all young patients with unexplained severe CCD
Neonatal lupus syndrome	Maternal lupus with transplacental passage of anti-Ro/SSA and anti-La/SSB antibodies resulting in congenital nonprogressive AVB at the level of the AV node (narrow QRS). Recurrence in siblings could mimic a hereditary etiology
Congenital heart disease	CCD commonly seen with certain CHD such as ccTGA and partial or complete AVSD. ccTGA can first present with CCD in adult life. AVSD is often associated with Down syndrome. Diagnosis requires cardiac imaging. Co-occurrence of CCD and CHD also observed in certain hereditary conditions (e.g., *NKX2-5* and *TBX5* mutations, see Table 16.2)
Iatrogenic CCD	CCD resulting from a surgical or transcatheter procedure near the conduction system. Typical examples: valvular procedures (most commonly aortic valve replacement), closure of septal defects, arrhythmia ablation, septal reduction therapy in hypertrophic cardiomyopathy
Other rare causes	Infiltrative malignancies and cardiac tumors, trauma, rheumatological disorders
Causes of transient/reversible cardiac conduction defects	
Increased vagal tone	Often seen in well-trained endurance athletes. Can also be triggered by emotion or posture in susceptible individuals (neurocardiogenic/vasovagal syncope). Presents with sinus bradycardia and different degrees of AVB with a narrow QRS
Metabolic disturbances	Examples include hyperkalemia, hypothermia, thyroid dysfunction
Drugs	Drugs affecting autonomic cardiac modulation or ion channel function. Examples include beta-blockers, calcium-channel blockers, sodium-channel blockers, digitalis, amiodarone

AV, atrioventricular; *AVB,* atrioventricular block; *AVSD,* atrioventricular septal defect; *CCD,* cardiac conduction disease; *ccTGA,* congenitally corrected transposition of the great arteries; *CHD,* congenital heart disease; *DCM,* dilated cardiomyopathy; *SCD,* sudden cardiac death

of suspected hereditary CCD will be followed by a review of the molecular genetics of the disease (section "Molecular Genetics") highlighting gene-specific aspects.

Clinical Presentation

Primary CCD is a progressive disease with variable age of onset and clinical course. During early stages, patients are generally asymptomatic, and ECG abnormalities are detected incidentally or during family screening. With the progression of disease, symptoms occur either because of *severe bradycardia* or *chronotropic incompetence* (incapacity to increase heart rate during exercise). In the *former*, patients may present with pre-syncope or syncope occurring during periods of ventricular asystole (typically >4 s), due to sinus pauses or high degrees of AVB. In the *latter*, patients could present with exercise intolerance and/or dyspnea. Rarely, patients can be symptomatic in the absence of severe bradycardia and chronotropic incompetence. This may occur because of hemodynamic consequences of electrical dyssynchrony (e.g., excessive PR prolongation causing atrioventricular (AV) dyssynchrony) or due to reentrant arrhythmia secondary to excessively slowed conduction (e.g., bundle-branch reentrant ventricular tachycardia).

Hereditary forms of CCD can be associated with other cardiac electrical or structural diseases as well as neurological disease (Table 16.2). As such, the first manifestation of disease might not be related to CCD but to the associated disease. For instance, patients with loss of function mutations in *SCN5A* can have both Brugada syndrome (BS) and CCD. In such cases, the first manifestation of disease can be

Table 16.2 Clinical characteristics of the major subtypes of hereditary CCD

Gene/disease (mutation)	Transmission	Relative frequency in CCD	Other arrhythmias	Cardiomyopathy	CHD	Risk of SCD	Typical ECG	Extracardiac features/ diseases	Management particularities
SCN5A (missense and truncating – loss of function)	AD	++	BS, LQTS, AF	+/–(mild)	–	+	Prolonged PR and QRS. Left axis deviation	–	Avoid sodium-channel blockers Treat fever
TRPM4 (missense – gain-of-function)	AD	++	-	–	–	+	RBBB, fascicular blocks	–	Usual CCD management
LMNA (missense, truncating, large deletion)	AD	++ (in CCD with DCM)	Atrial arrhythmias	++	–	++	Low voltage P-wave, prolonged PR, narrow QRS (initially)	Cardio-embolic stroke *LMNA* also linked to muscular dystrophies (EDMD and LGMD) and other diseases	ICD to be considered in presence of left ventricular dysfunction, ventricular arrhythmia or severe CCD
NKX2-5 (missense, truncating)	AD	+	AF	+/–	++	+	Various degrees of AVB, narrow QRS	–	Usual CCD management
TBX5 (missense, truncating, large deletion)	AD	+/–	AF	–	+	+/–	Various degrees of AVB, narrow QRS	Upper limb skeletal anomalies (HOS)	Usual CCD management
MD (repeat expansions in ***DMPK*** [type 1] or ***CNBP*** [type 2])	AD	++ (in CCD with muscular dystrophy	Atrial and ventricular arrhythmias	+/–	–	++	Progressive PR prolongation and fascicular blocks	Myotonia, muscle pain, muscle weakness, cataracts, GI complaints. Mild CK elevation	Low threshold for pacemaker or ICD. Consider invasive EPS. Optimal approach yet to be developed
EDMD (***EMD***, ***FHL1***, ***LMNA***)	XR AD AR	+/–	Atrial arrhythmias	+	–	+	Sinus bradycardia, atrial standstill, AVB	Contractures, humeroperoneal muscle weakness, cardio-embolic stroke. Moderate CK elevation	Low threshold for pacemaker and anticoagulation

(continued)

Table 16.2 (continued)

Gene/disease (mutation)	Transmission	Relative frequency in CCD	Other arrhythmias	Cardiomyopathy	CHD	Risk of SCD	Typical ECG	Extracardiac features/ diseases	Management particularities
LGMD type 1B (*LMNA*)	AD	+/−	Atrial arrhythmias	+	−	+	Sinus bradycardia, atrial standstill, AVB	Progressive weakness and atrophy of shoulder and pelvic girdle. Overlap with EDMD. Moderate CK elevation	Low threshold for pacemaker? Little available data
DES	AD	+/−	Atrial and ventricular arrhythmias	+	−	+	AVB, fascicular blocks	Proximal and distal muscular weakness. Mild CK elevation	Similar to *LMNA*? Little available data
HCN4 (missense, truncating)	AD	+	AF	LVNC	−	+	Sinus bradycardia	–	Usual CCD management CCD without LVNC has good prognosis
PRKAG2 (missense, truncating)	AD	+/−	WPW, AF	HCM	−	+	Ventricular preexcitation	–	Usual CCD/HCM/WPW management

AD autosomal dominant, *AF* atrial fibrillation, *AR* autosomal recessive, *AVB* atrioventricular block, *BS* Brugada syndrome, *CCD* cardiac conduction disease, *CHD* congenital heart disease, *CK* creatine kinase, *DCM* dilated cardiomyopathy, *EDMD* Emery-Dreifuss muscular dystrophy, *EPS* electrophysiological study, *GI* gastrointestinal, *HCM* hypertrophic cardiomyopathy, *HOS* Holt-Oram syndrome, *ICD* implantable cardioverter defibrillator, *LGMD* limb-girdle muscular dystrophy, *LQTS* long QT syndrome, *LVNC* left ventricular noncompaction cardiomyopathy, *MD* myotonic dystrophy, *RBBB* right bundle-branch block, *SCD* sudden cardiac death, *WPW* Wolff-Parkinson-White syndrome, *XR* X-linked recessive

ventricular arrhythmias in the context of BS. Patients with mutations in *NKX2-5* also present with atrial septal defects (ASDs), while patients with *LMNA* mutation typically develop CCD in association with dilated cardiomyopathy (DCM) in a later stage of disease development. Other genotype-phenotype correlations are summarized in Table 16.2 and further discussed below. A detailed review of symptoms, physical examination, and cardiac imaging is thus crucial in establishing a correct diagnosis and guide molecular genetic testing.

Systematic longitudinal data on the course and natural history of hereditary CCD are limited. When available, such data will be discussed below along with the specific genetic defects (section "Molecular Genetics"). By contrast, the clinical course and prognosis of conduction defects in the general population without known structural heart disease has been abundantly studied. For instance, unexplained sinus bradycardia (<50 beats/min) in healthy volunteers is not associated with adverse events during a mean follow-up of 5.4 years [74]. In a Finnish populational study of 10,685 individuals aged 30–59 years old and followed up for 30 ± 11 years, isolated first-degree AVB (2.1 % of patients) was not associated with an increased risk of adverse events [6]. Mobitz type I second-degree AVB in individuals without underlying heart disease, as often seen in athletes due to increased vagal tone, is often caused by block in the atrioventricular node and also has a benign prognosis [72]. By contrast, Mobitz type II second-degree AVB is caused by conduction block below the AV node and is associated with a bad prognosis, with a high risk of progression to complete AVB, syncope, and sudden cardiac death (SCD) [25]. The association of right bundle-branch block (RBBB) with mortality in the general population is controversial, with a recent meta-analysis showing an increased risk of mortality during follow-up (HR 1.17; 95 % confidence interval (CI): 1.03–1.33) [86]. The prognosis of left bundle-branch block (LBBB) in asymptomatic healthy individuals appears to be age-dependent. Earlier large cohort studies including patients with a mean age below 55 and a mean follow-up less than 10 years do not detect an increased adverse event rate associated with LBBB [29, 61]. By contrast, more recent studies including older patients and longer follow-up show a significant increase in high-degree AVB, cardiovascular, as well as total mortality [7, 28, 87]. Taken together, these data suggest that conduction defects in the young asymptomatic patient have a good prognosis, with the exception of Mobitz II second-degree AVB. Whether these data can be extrapolated to hereditary CCD is debatable. Since the mechanism of hereditary CCD is in part an accelerated degeneration of the conduction system, conduction defects in these patients likely occur at a higher rate than in an unselected CCD population.

Diagnosis and Differential Diagnosis

The clinical diagnosis of CCD requires the presence of a conduction abnormality on the ECG, at any single or multiple levels (Fig. 16.1). Secondary causes of CCD should be excluded (Table 16.1). An echocardiogram is indicated in all cases to assess the presence of structural heart disease, either causing or associated with CCD. Other diagnostic tests can be performed depending on patient's age, comorbidities, and clinical findings. Accumulating evidence from independent groups suggests that cardiac sarcoidosis and giant-cell myocarditis are common causes of unexplained high-degree AVB in young patients (<55–60 years) [41, 53]. In such patients, advanced cardiac imaging with cardiac magnetic resonance (CMR) or fluorodeoxyglucose (^{18}FDG) cardiac positron emission tomography should be considered in the diagnostic strategy. In endemic regions, Lyme disease, an infectious disease caused by the spirochete *Borrelia burgdorferi* should also be suspected in young patients with unexplained AVB [70]. In the presence of a clinical presentation compatible with the disease, the diagnosis is established by serologic testing. It is important to recognize this etiology in order to start an appropriate antibiotic treatment. Because AVB is most often reversible, permanent pacemaker placement is most often unnecessary.

A three-generation family history is recommended when evaluating a young patient with an unexplained cardiac conduction defect. Family history taking is detailed in Chap. 2. In the context of CCD, one should specifically assess for the presence of family history of CCD, SCD, arrhythmia, pacemaker or defibrillator implantation, heart failure or cardiomyopathy, cardiac transplantation, congenital heart disease, as well as neuromuscular disease. When assessing the inheritance pattern, one should note that CCD often accompanies many acquired diseases. For instance, ischemic or valvular heart diseases are common causes of CCD. Age is also an important factor in interpreting family history. The prevalence of CCD greatly increases with age. In a prospective cohort study of randomly selected 855 males born in 1913 [27], the prevalence of bundle-branch block increased from 1 % at the age of 50 to 17 % at 80 years old. Likewise, populational studies from the United States and Australia estimate the prevalence of permanent pacemaker therapy to be less than 0.5 % in patients below 65 years old but higher than 2 % in patients over 75 years old [16, 65]. This age-dependent prevalence is important to keep in mind when assessing the etiology of CCD in family members: In young patients, CCD is rare and more likely to be inherited, while CCD in older patients is common and more likely to be caused by senile degeneration of the conduction system. In sum, a detailed clinical review of family members with suspected CCD, including age at onset and comorbidities, is important to understand the presence and pattern of heritability and identify phenocopies.

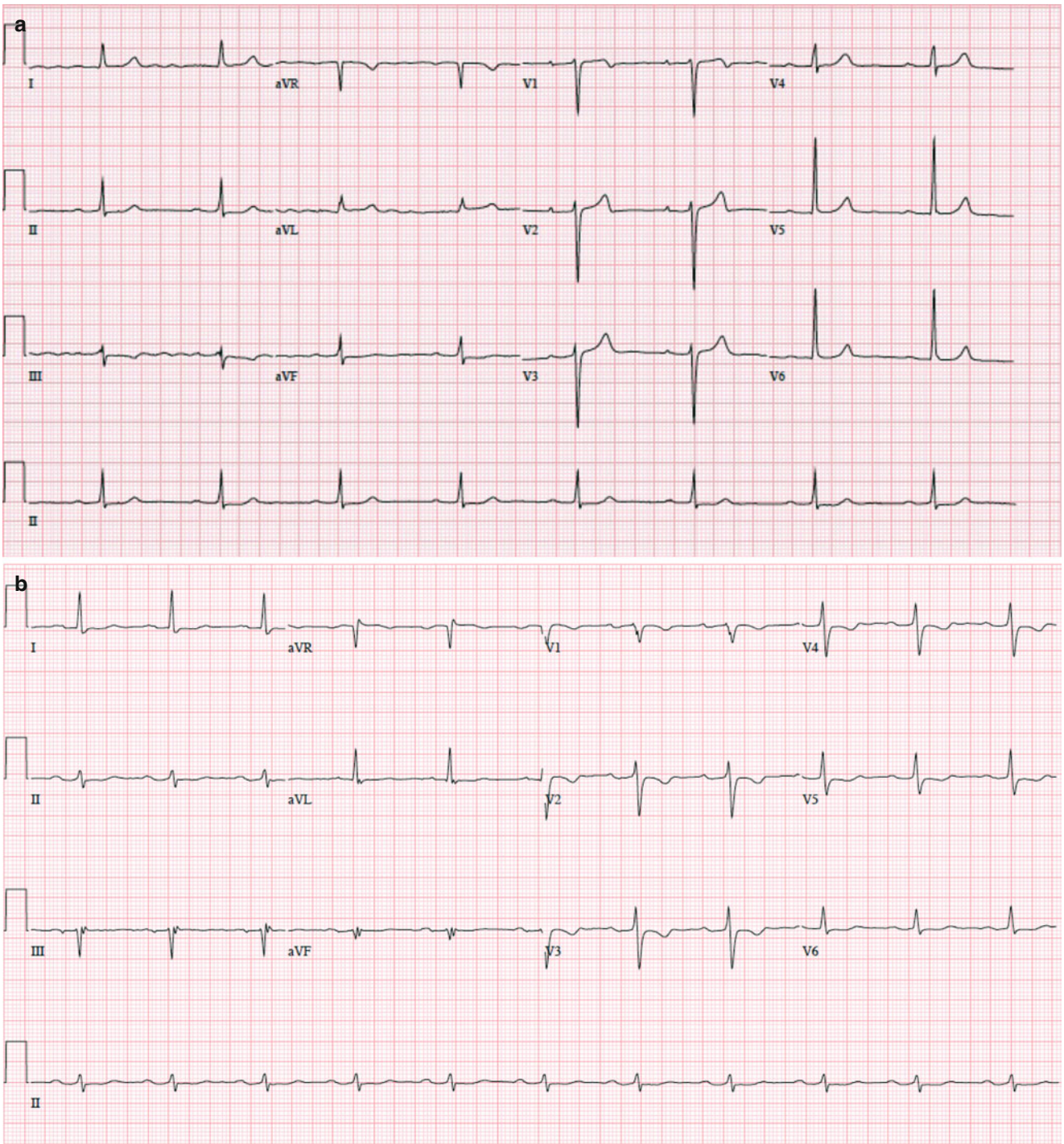

Fig. 16.1 (**a**) ECG obtained from a 50-year-old female with mild dilated cardiomyopathy (left ventricular ejection fraction, 52 %) and a truncating mutation in *LMNA* (Q410X) mutation. Note the low-voltage P-wave, prolonged PR interval, and narrow QRS, typical for *LMNA* disease. (**b**) ECG obtained from a 63-year-old female with structurally normal heart and a truncating mutation in *SCN5A* (R222X). Note the prolonged PR and QRS intervals and left-axis deviation, typical for *SCN5A* loss of function mutations

Clinical Therapy and Follow-up

The management of patients with CCD is aimed at alleviating symptoms and preventing SCD. No drug therapy is yet available. Pacemaker implantation is indicated in patients with symptomatic bradycardia as well as those at a high risk of complete AVB and SCD. Clinical guidelines on cardiac pacing are periodically updated and published [17]. With the exception of a few locus- and disease-specific management differences (e.g., *LMNA* mutations, muscular dystrophies;

discussed below), patients with suspected or established hereditary CCD are treated similarly as patients with other etiologies of CCD.

In the following situations, pacemaker implantation is recommended (Class I indication):

- Sinus node dysfunction with symptoms clearly attributed to bradycardia
- Third-degree or Mobitz II second-degree AVB, irrespective of symptoms
- Syncope with bundle-branch block and demonstration of conduction impairment below the AV node during an invasive electrophysiological study
- Alternating bundle-branch block (e.g., RBBB and LBBB), irrespective of symptoms

In the following situations, pacemaker implantation should be considered (Class IIa indication):

- Mobitz I second-degree AVB, with symptoms or invasive study showing a block below the AV node
- Syncope and demonstration of asymptomatic pauses for >6 s

Because of the progressive nature of the disease, a recent expert consensus statement on inherited arrhythmia syndromes also suggests that pacemaker implantation be considered (Class IIa) in the presence of bifascicular block with or without first-degree AVB [58].

Patients with manifest CCD as well as carriers of a pathogenic loss of function mutation in *SCN5A* should also avoid the use of medication with conduction-slowing properties, such as those listed in http://www.brugadadrugs.org [57].

Since the disease is progressive, patients with CCD should be periodically assessed with ECG and review of symptoms. Longer ECG recordings such as Holter monitoring and loop recording should be considered in symptomatic patients without ECG criteria for pacemaker implantation, to detect intermittent deterioration of the conduction abnormality. Patients reporting exercise intolerance should undergo exercise testing to detect chronotropic incompetence or exercise-induced AVB. Echocardiography should also be repeated in select patients, periodically in *LMNA, DES,* and *SCN5A* mutation carriers and whenever there is clinical suspicion of heart failure. The frequency of follow-up should be individualized, taking into account the severity of conduction anomalies, the rate of disease progression, the presence of symptoms, and the patient's age. Patients with muscular dystrophy and CCD should be monitored more closely. Patients should be advised to consult rapidly if they have a syncopal event, to assess the need for an urgent pacemaker implantation.

Molecular Diagnostics

Genetic testing should be considered in patients with primary CCD developing at a young age (<50 years old) with or without cardiomyopathy or congenital heart disease, especially in the presence of a positive family history [2]. The optimal diagnostic strategy remains unclear. As for other conditions, one should balance the desires for getting a higher yield yet avoiding the detection of variants of unknown significance. In patients with isolated CCD, sequencing of *TRPM4* and *SCN5A* is desirable. For those with associated DCM, sequencing of *LMNA, DES,* and *SCN5A* should be undertaken, while patients with CCD associated with congenital heart defects such as ASDs can be screened for mutations in *NKX2-5* and *TBX5*. Mutations in other genes have been identified in few families or isolated cases with CCD, with some supportive functional data. These genes often appear on next-generation sequencing (NGS) "arrhythmia" or "cardiac" panels. Data from the Netherlands suggest the yield of genetic testing in CCD (mostly limited to *SCN5A* sequencing) to be approximately 30 %, with a single recurrent *SCN5A* mutation (c.2582_2583delTT) accounting for most of the cases [39]. The yield of targeted genetic testing in large diverse populations and the added value of NGS remain to be explored.

Molecular Genetics

SCN5A

Mutations in *SCN5A* causing CCD were first identified by Schott [62]. The authors reported a large French family with autosomal dominant CCD presenting with first-degree AVB, LBBB, RBBB, or complete AVB requiring pacemaker implantation, in the absence of structural heart disease. Follow-up of the family showed a progressive disease. Using a targeted-linkage approach, the authors demonstrated a strong linkage to the 3p21 locus, which harbors *SCN5A*. Sequencing revealed a splice-site variant predicting skipping of exon 22. The authors also performed sequencing of *SCN5A* in an independent Dutch nuclear family with asymptomatic first-degree AVB, RBBB, and/or nonspecific intraventricular conduction delay and identified a frameshift variant predicting a premature stop codon and cosegregating with the phenotype. In sum, this study was the first to link CCD with *SCN5A*, which was earlier identified as a disease gene in long QT syndrome type 3 (LQT3) [83] and BS [20]. Similar to BS, *SCN5A* mutations causing CCD result in loss of function of Nav1.5, the major cardiac sodium channel responsible of cardiomyocyte depolarization. It is thus not uncommon for loss-of-function mutations to result in a mixed phenotype of BS and CCD, either in the same patient or in family members carrying the same variant. By contrast, LQT3 is caused by

gain of function *SCN5A* mutations, which result in impaired inactivation of Nav1.5 with increased late sodium current. Interestingly, mutations resulting in both a decrease in peak sodium current (loss of function) and increase in late current (gain of function) have been identified in families with CCD, BS, and LQT3 [59]. The 1795insD mutation is the best characterized example of such an *overlap syndrome* [56]. *SCN5A* mutations have also been observed in families with sinus node dysfunction, atrial fibrillation, as well as DCM, emphasizing the high heterogeneity of phenotypes associated with *SCN5A* [79]. Highlighting the important role of *SCN5A* and its product Nav1.5 in normal cardiac electrical function, common variants in the *SCN5A-SCN10A* locus have been associated with PR, QRS, and QT intervals as well as BS and ST-T voltages in multiple genome-wide association studies (GWAS) [4, 11, 40, 55, 66, 81].

Clinically, CCD associated with *SCN5A* mutations initially presents with a prolonged PR interval, wide QRS as well as left-axis deviation (Fig. 16.1b), which could progress to high-degree AVB. In addition to the general CCD management described in section "Clinical Therapy and Follow-up", patients with a pathogenic mutation in *SCN5A* should be counseled to avoid drugs with sodium-channel-blocking effects and should suppress fever, a potential trigger of arrhythmic events. Because of the possible overlap with other syndromes causing ventricular tachyarrhythmia, one should be attentive to investigate syncopal events appropriately, as these may sometimes be caused by malignant ventricular arrhythmia. While a standard pacemaker is usually the treatment of choice (when indicated, see above), some patients may benefit from an implantable cardioverter defibrillator (ICD), especially in overlap syndromes.

TRPM4

In 2009, mutations in *TRPM4* were identified as a cause of CCD [42]. In fact, the story behind this discovery dates back to the 1960s, when Combrink et al. [21] and later Steenkamp et al. [69] described large South African families with autosomal dominant CCD manifesting as RBBB, fascicular blocks, and SCD. Later, a large Lebanese family with a similar phenotype was reported [71]. In 1995, linkage analysis in both the South African and Lebanese families mapped the CCD phenotype to chromosome 19q13.3, which includes *TRPM4* [18, 23]. Sequencing of *TRPM4* identified two different missense mutations in these families, cosegregating with the CCD phenotype [42, 43]. Mutations were also identified in an additional large French family as well as smaller families and sporadic cases with CCD [22, 43, 68]. Based on recent data from relatively small cohorts without systematic cosegregation analysis, the estimated yield of TRPM4 testing in progressive CCD is about 15 % [22, 68]. Of note, the classic phenotype of *TRPM4*-related CCD is that of RBBB with or without fascicular block, which progresses to complete AVB, but rarely isolated LBBB.

TRPM4 encodes a Ca^{2+}-activated nonselective cation channel predominantly expressed in Purkinje fibers. The mechanism of *TRPM4*-related CCD is attenuated deSUMOylation of the protein, which results in decreased endocytosis, thus increasing channel density at the cell surface [42]. This increased cation channel density is thought to result in membrane depolarization, thus reducing the availability of Nav1.5, necessary for fast conduction in the specialized cardiac conduction system [1].

LMNA

Mutations in *LMNA* are associated with a wide spectrum of diseases, known as laminopathies. These include Hutchinson-Gilford progeria, autosomal recessive Charcot-Marie-Tooth, Emery-Dreifuss muscular dystrophy (EDMD), as well as DCM preceded by or accompanied with marked CCD. LMNA-related cardiomyopathy is a progressive disease which initially presents with CCD, typically sinus bradycardia, low-voltage P-waves, first-degree AVB, and initially a normal QRS (Fig. 16.1a). The disease is often accompanied by arrhythmia starting at an early stage (premature atrial complexes, atrial tachycardia or fibrillation, premature ventricular complexes or VT). With disease progression, the patient could present with complete AVB, malignant ventricular arrhythmia or SCD, and eventually DCM with heart failure or embolic stroke. Family data suggest that CCD typically precedes DCM by a median time interval of 7 years [19]. Patients with CCD and an LMNA mutation have a high risk of developing malignant ventricular tachyarrhythmia, even if left ventricular systolic function is preserved [3, 38]. In patients with an indication for pacemaker therapy and an *LMNA* mutation, ICD implantation should thus be considered [58], especially in the presence of additional risk factors such as male sex, nonsustained VT, left ventricular ejection fraction <45 %, and the presence of a non-missense mutation [76].

When no mutation is detected in *LMNA* by sequencing in a patient with a typical presentation (CCD, DCM, arrhythmia, and family history), one should consider testing for structural variants, such as a large deletion, using appropriate techniques (e.g., multiplex ligation-dependent probe amplification). Such an approach has been proven useful in some cases [36, 48, 78].

CCD Associated with Congenital Heart Defects: *NKX2-5* and *TBX5*

In 1998, Schott identified one missense and two nonsense variants in ***NKX2-5*** in four families affected with an

autosomal dominant form of congenital heart defects, mostly (27 of 33 cases) secundum ASDs but also a few with other defects with or without ASD [63]. All affected individuals had CCD manifesting as various degrees of AVB. Invasive electrophysiological studies performed in three patients revealed that the site of conduction delay was the AV node, and patients who were later followed up show progressive CCD. *NKX2-5* encodes a transcription factor involved in cardiac morphogenesis, specifically in septation during development and is also important for normal function of the AV node in postnatal life. Other groups also reported mutations in *NKX2-5* in smaller families with a similar phenotype, reproducing the original findings [67].

Holt-Oram syndrome (HOS) is an autosomal dominant disease affecting the heart and hand (heart and hand syndrome) and is caused by mutations in the transcription factor ***TBX5*** in >70 % of cases [9, 49]. Virtually all affected individuals have skeletal anomalies involving the radius, carpal, or hand bones, sometimes only seen on radiography. Most patients also have a congenital heart defect, typically a secundum ASD or VSD, but more severe lesions have been reported. Patients with the syndrome are also at risk for severe progressive CCD requiring pacemaker implantation, regardless of the presence of a structural defect. The exact prevalence of CCD in HOS has not been reported. Likewise, the prevalence of pathogenic *TBX5* mutations in suspected hereditary CCD is unknown. Both missense and truncating *TBX5* variants have been associated with HOS. The mutation type and the location of missense variants have been suggested as predicting the phenotype [10]. In the presence of a typical HOS and absence of mutation using sequencing, one should also consider testing for large deletions, which have been previously reported [15]. *TBX5* is critical for normal cardiac development in prenatal life, while its control of *SCN5A* expression makes it important in regulating cardiac conduction in postnatal life [5]. In addition to its involvement in HOS, GWAS identified common variations in the *TBX5* locus associated with both PR and QRS durations, again highlighting its role in normal cardiac conduction [24, 40, 66].

CCD Associated with Muscular Dystrophies: An Overview

Muscular dystrophies are a group of clinically and genetically heterogeneous inherited skeletal muscle diseases that often also affect the heart [34]. The prevalence, type, and severity of cardiac involvement depend on the specific muscular dystrophy. In the X-linked recessive *Duchenne and Becker dystrophies* caused by mutations in dystrophin (*DMD*), DCM is the predominant cardiac phenotype and CCD is infrequent. By contrast, the autosomal dominant *myotonic dystrophies* caused by repeat expansions in *DMPK* (type 1) or *CNBP* (type 2), CCD is very common and progressive while DCM is uncommon. In myotonic dystrophy type I (Steinert's disease), the majority of patients develop CCD. When CCD is severe (defined as nonsinus rhythm, PR >240 ms, QRS >120 ms, or second- or third-degree AVB), it is associated with an increased risk of SCD [35]. Interestingly, the number of CTG repeats in *DMPK* and the severity of the muscular phenotype are predictors of severe CCD. Because SCD is responsible for 30 % of mortality and that CCD is thought to play a major role in the mechanism of SCD, the threshold for pacemaker implantation should be low in patients affected with this disease. While the presence of second- or third-degree AVB is a clear indication for pacemaker implantation, the optimal approach for risk stratification and prophylactic device implantation in other patients remains unclear. Some experts suggest the use of an invasive electrophysiological study. A large nonrandomized study showed that an invasive electrophysiological study-guided device implant strategy was associated with increased survival, when compared to a conservative noninvasive strategy, after adjusting for baseline differences or matching using propensity scores [82]. Because of a risk of ventricular arrhythmia-mediated SCD, ICD implantation should also be considered instead of a standard pacemaker [12]. *EDMD* is a rare disease inherited as either X-linked recessive (caused by mutations in *EMD* or *FHL1*) or autosomal dominant or recessive (mutations in *LMNA*). EDMD is associated with CCD presenting with sinus bradycardia, atrial standstill, and AVB. Patients are also at risk of DCM and atrial arrhythmias with cardio-embolic stroke [14]. Both autosomal dominant and X-linked recessive EDMD forms are at risk, but *LMNA* mutation carriers are believed to be at a higher risk to have a cardiac involvement [13]. *LMNA* mutations can also cause autosomal dominant *limb girdle muscular dystrophy* (LGMD) type IB with a high prevalence of cardiac involvement (CCD and DCM). Other autosomal dominant and autosomal recessive subtypes of LGMD can be associated with DCM at various degrees but rarely with CCD. Other types of muscular dystrophies are rarely seen in the context of CCD or DCM.

Myofibrillar myopathy is another genetically heterogeneous neuromuscular disease associated with CCD with or without DCM. Its most common form observed in cardiogenetics is the autosomal dominant desmin-related myopathy. The latter is caused by mutation in *DES* and is characterized by isolated cardiac involvement (25 %), isolated neurological involvement (25 %), or both (50 %) [77]. Cardiac disease consists of cardiomyopathy (mainly DCM), CCD, supraventricular arrhythmias, as well as ventricular arrhythmias including a few cases of SCD despite a pacemaker. Considering the potential risk of SCD from ventricular arrhythmias, some clinicians suggest the use of an ICD in *DES* mutation carriers with a pacemaker indication [77]. Mitochondrial disease caused by mitochondrial DNA deletion can also present with both a neuromuscular defect and CCD with or without cardiomyopathy.

The *Kearns-Sayre syndrome* is the classic example, where rapidly progressive AVB is observed.

Management of patients with neuromuscular disease and CCD can be challenging given the limited available literature and the associated muscular morbidity. Given the increased risk of SCD in many of these diseases, clinical practice guidelines suggest a more aggressive approach than with other CCD patients. For instance, permanent pacemaker implantation may be considered for myotonic dystrophy and limb-girdle muscular dystrophy, with any degree of AV block (including first-degree AV block) or bifascicular block, with or without symptoms [26]. Such an aggressive approach based on little clinical data does not make a consensus among experts [17].

Other CCD Genes

Loss-of-function mutations in ***HCN4***, which encodes the major pacemaker channel protein in humans, have been identified in patients and families with sinus node dysfunction, sometimes in association with paroxysmal atrial fibrillation [50, 64]. The severity of the phenotype is highly variable and sometimes benign with isolated asymptomatic sinus bradycardia in a whole family [54]. Recently, loss-of-function mutations were identified in four families with sinus bradycardia in combination with left ventricular noncompaction cardiomyopathy [51] and mild aortic dilatation [80]. Of interest, a gain-of-function mutation in *HCN4* was identified in a familial form of inappropriate sinus tachycardia [8].

The cardiac voltage-gated sodium channel (Nav1.5) is part of a protein complex composed of the α-subunit (encoded by *SCN5A*), as well as β-subunits (e.g., *SCN1B*) and ancillary proteins. Following the association of *SCN5A* with CCD and BS, a candidate gene-sequencing study identified mutations in ***SCN1B*** in three small pedigrees affected by CCD with or without BS [84]. The investigators performed functional studies showing that coexpression of *SCN5A* with the mutant *SCN1B* resulted in a decreased sodium current as compared to coexpression of both wild-type proteins. Although these functional data are supportive, the lack of robust human genetic data (three small pedigrees and lack of convincing validation studies) makes one question the role of *SCN1B* in CCD.

The fast propagation of the electrical impulse in the His-Purkinje system depends on the availability of Nav1.5 and also high-conductance gap junctional channels. In a 6-year-old boy with CCD (LBBB and second-degree AVB) who later died suddenly, Makita et al. [47] identified a missense variant in ***GJA5***, which encodes the high-conductance gap junctional channel subunit connexin40. The variant was also present in his mother with documented CCD (LBBB) who later had a SCD, as well as in his 4-year-old sister with a QRS duration at the upper limit of normal. Expression of the mutant proteins showed a reduction in junctional conductance compared to wild-type connexin40. Somatic *GJA5* mutations in left atrial DNA have also been previously identified in patients with atrial fibrillation [31], although this finding was not reproduced in a larger cohort [60] (see Chap. 15 for discussion).

Recently, using linkage analysis and whole-exome sequencing, two groups identified missense variants in ***TNNI3K*** in families with supraventricular tachyarrhythmia and CCD, sometimes associated with DCM [73, 85]. *TNNI3K* encodes for the Troponin I-interacting kinase, a cardiac-specific kinase that was previously implicated in atrioventricular conduction in mice [44]. The pathophysiological mechanism implicating *TNNI3K* in arrhythmogenesis is an area of active investigation.

In 2001, Gollob identified missense mutations in ***PRKAG2*** in families with CCD and ventricular preexcitation with or without cardiac hypertrophy [30, 32]. Multiple families with mutations in *PRKAG2* and an identical phenotype have been identified since then. Sequencing of this gene should be performed in the presence of CCD in association with ventricular preexcitation and/or cardiac hypertrophy. *PRKAG2* encodes the gamma2 regulatory subunit of AMP-activated protein kinase, which is part of the AMP-activated protein kinase complex involved in cardiomyocyte metabolism and energetics.

Family Screening

Given the limited long-term data available on familial CCD, it is difficult to recommend a detailed family screening and follow-up algorithm. Instead, the clinician should adapt the follow-up plan to each patient and family. Below are some points for guidance depending on whether a pathogenic variant is identified and whether the disease is familial or sporadic.

When a pathogenic mutation is identified in the proband, cascade screening using mutation analysis in family members is recommended [2]. Mutation carriers should have a complete baseline cardiological evaluation, consisting of a review of symptoms, physical evaluation, ECG, and echocardiography. Exercise testing, Holter monitoring, or loop recoding are suggested if the patient reports any intermittent symptom. Given the progressive nature of the disease, mutation carriers need to be periodically evaluated (e.g., every 1–3 years) depending on age and the extent of ECG abnormalities, if present. Follow-up evaluation should include a review of symptoms and ECG. For patients with *LMNA* mutations, repeating the echocardiogram every 1–2 years is suggested. Patients should be advised to seek urgent medical attention if they present a syncopal event. Family members

that do not carry the mutation can be reassured, unless the pathogenicity of the variant is questionable. A baseline ECG is encouraged, while a more extensive evaluation should be performed if symptoms develop.

In genetically elusive unexplained CCD in a young patient without a clear familial disease, a baseline ECG should be performed in first-degree relatives. If the proband also has structural heart disease, an echocardiogram should also be performed in first-degree relatives. If the baseline evaluation is normal, the patient can be discharged from cardiological care and instructed to consult if symptoms develop (e.g., presyncope, syncope, exercise intolerance). If the baseline evaluation is abnormal, the patient should be treated accordingly and periodically followed up.

In genetically elusive CCD with a clear familial disease, advanced genetic testing (e.g., whole-exome sequencing, large-sequencing gene panel, and targeted deletion assays) with appropriate cosegregation analysis should be considered, recognizing that the yield is likely to be low. Clinical screening of first-degree relatives should be performed as above. However, if the baseline evaluation is normal, it is probably prudent to follow-up the patients periodically for a long term, unless the disease onset in affected family members is at a young age.

Summary and Take-Home Messages

- Cardiac conduction disease (CCD) is a clinically heterogeneous disorder involving genetic and nongenetic etiologies.
- A genetic etiology and genetic testing should be considered in the presence of a family history of CCD, cardiomyopathy, or congenital heart disease as well as in young patients (<50 years old) with unexplained severe sporadic CCD.
- Isolated CCD can be caused by mutations in *SCN5A* or *TRPM4*.
- CCD in association with dilated cardiomyopathy (DCM) can be caused by *LMNA* and *DES* mutations. Patients with such mutations and severe CCD are also at risk of ventricular arrhythmia. Implantable cardioverter defibrillator (ICD) therapy should be considered in such cases.
- CCD in association with congenital heart disease can be caused by mutations in *NKX2-5* and *TBX5*. The latter is invariably associated with upper limb skeletal anomalies (Holt-Oram syndrome).
- CCD in association with ventricular preexcitation or unexplained cardiac hypertrophy is suggestive for mutations in *PRKAG2*.
- CCD can accompany some muscular dystrophies (e.g., myotonic dystrophy, Emery-Dreifuss muscular dystrophy, limb-girdle muscular dystrophy type IB). In these cases, aggressive therapy with a pacemaker or ICD can be considered in early stages.
- Device therapy is the only available treatment for hereditary CCD. The decision to implant a device usually follows the same principles as with other causes of CCD, with the exceptions mentioned above.
- Family screening with genetic testing and/or phenotypic testing is recommended in established or suspected hereditary CCD.

References

1. Abriel H, Syam N, Sottas V, Amarouch MY, Rougier JS. TRPM4 channels in the cardiovascular system: physiology, pathophysiology, and pharmacology. Biochem Pharmacol. 2012;84(7):873–81.
2. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Europace. 2011;13(8):1077–109.
3. Anselme F, Moubarak G, Savoure A, Godin B, Borz B, Drouin-Garraud V, et al. Implantable cardioverter-defibrillators in lamin A/C mutation carriers with cardiac conduction disorders. Heart Rhythm. 2013;10(10):1492–8.
4. Arking DE, Pulit SL, Crotti L, van der Harst P, Munroe PB, Koopmann TT, et al. Genetic association study of QT interval highlights role for calcium signaling pathways in myocardial repolarization. Nat Genet. 2014;46(8):826–36.
5. Arnolds DE, Liu F, Fahrenbach JP, Kim GH, Schillinger KJ, Smemo S, et al. TBX5 drives Scn5a expression to regulate cardiac conduction system function. J Clin Invest. 2012;122(7):2509–18.
6. Aro AL, Anttonen O, Kerola T, Junttila MJ, Tikkanen JT, Rissanen HA, et al. Prognostic significance of prolonged PR interval in the general population. Eur Heart J. 2014;35(2):123–9.
7. Badheka AO, Singh V, Patel NJ, Deshmukh A, Shah N, Chothani A, et al. QRS duration on electrocardiography and cardiovascular mortality (from the National Health and Nutrition Examination Survey-III). Am J Cardiol. 2013;112(5):671–7.
8. Baruscotti M, Bucchi A, Milanesi R, Paina M, Barbuti A, Gnecchi-Ruscone T, et al. A gain-of-function mutation in the cardiac pacemaker HCN4 channel increasing cAMP sensitivity is associated with familial Inappropriate Sinus Tachycardia. Eur Heart J. 2015.
9. Basson CT, Bachinsky DR, Lin RC, Levi T, Elkins JA, Soults J, et al. Mutations in human TBX5 [corrected] cause limb and cardiac malformation in Holt-Oram syndrome. Nat Genet. 1997;15(1):30–5.
10. Basson CT, Huang T, Lin RC, Bachinsky DR, Weremowicz S, Vaglio A, et al. Different TBX5 interactions in heart and limb defined by Holt-Oram syndrome mutations. Proc Natl Acad Sci U S A. 1999;96(6):2919–24.
11. Bezzina CR, Barc J, Mizusawa Y, Remme CA, Gourraud JB, Simonet F, et al. Common variants at SCN5A-SCN10A and HEY2 are associated with Brugada syndrome, a rare disease with high risk of sudden cardiac death. Nat Genet. 2013;45(9):1044–9.
12. Bhakta D, Shen C, Kron J, Epstein AE, Pascuzzi RM, Groh WJ. Pacemaker and implantable cardioverter-defibrillator use in a US myotonic dystrophy type 1 population. J Cardiovasc Electrophysiol. 2011;22(12):1369–75.
13. Bonne G, Leturcq F, Ben YR. Emery-Dreifuss Muscular Dystrophy. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, et al., editors. GeneReviews(R). Seattle (WA); 1993.

14. Boriani G, Gallina M, Merlini L, Bonne G, Toniolo D, Amati S, et al. Clinical relevance of atrial fibrillation/flutter, stroke, pacemaker implant, and heart failure in Emery-Dreifuss muscular dystrophy: a long-term longitudinal study. Stroke. 2003;34(4):901–8.
15. Borozdin W. Bravo Ferrer Acosta AM, Bamshad MJ, Botzenhart EM, Froster UG, Lemke J, et al. Expanding the spectrum of TBX5 mutations in Holt-Oram syndrome: detection of two intragenic deletions by quantitative real time PCR, and report of eight novel point mutations. Hum Mutat. 2006;27(9):975–6.
16. Bradshaw PJ, Stobie P, Knuiman MW, Briffa TG, Hobbs MS. Trends in the incidence and prevalence of cardiac pacemaker insertions in an ageing population. Open Heart. 2014;1(1):e000177.
17. Brignole M, Auricchio A, Baron-Esquivias G, Bordachar P, Boriani G, Breithardt OA, et al. 2013 ESC guidelines on cardiac pacing and cardiac resynchronization therapy: the Task Force on cardiac pacing and resynchronization therapy of the European Society of Cardiology (ESC). Developed in collaboration with the European Heart Rhythm Association (EHRA). Eur Heart J. 2013;34(29):2281–329.
18. Brink PA, Ferreira A, Moolman JC, Weymar HW, van der Merwe PL, Corfield VA. Gene for progressive familial heart block type I maps to chromosome 19q13. Circulation. 1995;91(6):1633–40.
19. Brodt C, Siegfried JD, Hofmeyer M, Martel J, Rampersaud E, Li D, et al. Temporal relationship of conduction system disease and ventricular dysfunction in LMNA cardiomyopathy. J Card Fail. 2013;19(4):233–9.
20. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature. 1998;392(6673):293–6.
21. Combrink JM, Davis WH, Snyman HW. Familial bundle branch block. Am Heart J. 1962;64:397–400.
22. Daumy X, Amarouch MY, Lindenbaum P, Bonnaud S, Charpentier E, Bianchi B, et al. Targeted resequencing identifies TRPM4 as a major gene predisposing to progressive familial heart block type I. Int J Cardiol. 2016;207:349–58.
23. de Meeus A, Stephan E, Debrus S, Jean MK, Loiselet J, Weissenbach J, et al. An isolated cardiac conduction disease maps to chromosome 19q. Circ Res. 1995;77(4):735–40.
24. den Hoed M, Eijgelsheim M, Esko T, Brundel BJ, Peal DS, Evans DM, et al. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. Nat Genet. 2013;45(6):621–31.
25. Dhingra RC, Denes P, Wu D, Chuquimia R, Rosen KM. The significance of second degree atrioventricular block and bundle branch block. Observations regarding site and type of block. Circulation. 1974;49(4):638–46.
26. Epstein AE, DiMarco JP, Ellenbogen KA, Estes 3rd NA, Freedman RA, Gettes LS, et al. 2012 ACCF/AHA/HRS focused update incorporated into the ACCF/AHA/HRS 2008 guidelines for device-based therapy of cardiac rhythm abnormalities: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. J Am Coll Cardiol. 2013;61(3):e6–75.
27. Eriksson P, Hansson PO, Eriksson H, Dellborg M. Bundle-branch block in a general male population: the study of men born 1913. Circulation. 1998;98(22):2494–500.
28. Eriksson P, Wilhelmsen L, Rosengren A. Bundle-branch block in middle-aged men: risk of complications and death over 28 years. The Primary Prevention Study in Goteborg, Sweden. Eur Heart J. 2005;26(21):2300–6.
29. Fahy GJ, Pinski SL, Miller DP, McCabe N, Pye C, Walsh MJ, et al. Natural history of isolated bundle branch block. Am J Cardiol. 1996;77(14):1185–90.
30. Gollob MH, Green MS, Tang AS, Gollob T, Karibe A, Ali Hassan AS, et al. Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. N Engl J Med. 2001a;344(24):1823–31.
31. Gollob MH, Jones DL, Krahn AD, Danis L, Gong XQ, Shao Q, et al. Somatic mutations in the connexin 40 gene (GJA5) in atrial fibrillation. N Engl J Med. 2006;354(25):2677–88.
32. Gollob MH, Seger JJ, Gollob TN, Tapscott T, Gonzales O, Bachinski L, et al. Novel PRKAG2 mutation responsible for the genetic syndrome of ventricular preexcitation and conduction system disease with childhood onset and absence of cardiac hypertrophy. Circulation. 2001b;104(25):3030–3.
33. Gourraud JB, Kyndt F, Fouchard S, Rendu E, Jaafar P, Gully C, et al. Identification of a strong genetic background for progressive cardiac conduction defect by epidemiological approach. Heart. 2012;98(17):1305–10.
34. Groh WJ. Arrhythmias in the muscular dystrophies. Heart Rhythm. 2012;9(11):1890–5.
35. Groh WJ, Groh MR, Saha C, Kincaid JC, Simmons Z, Ciafaloni E, et al. Electrocardiographic abnormalities and sudden death in myotonic dystrophy type 1. N Engl J Med. 2008;358(25):2688–97.
36. Gupta P, Bilinska ZT, Sylvius N, Boudreau E, Veinot JP, Labib S, et al. Genetic and ultrastructural studies in dilated cardiomyopathy patients: a large deletion in the lamin A/C gene is associated with cardiomyocyte nuclear envelope disruption. Basic Res Cardiol. 2010;105(3):365–77.
37. Harbison J, Newton JL, Seifer C, Kenny RA. Stokes Adams attacks and cardiovascular syncope. Lancet. 2002;359(9301):158–60.
38. Hasselberg NE, Edvardsen T, Petri H, Berge KE, Leren TP, Bundgaard H, et al. Risk prediction of ventricular arrhythmias and myocardial function in Lamin A/C mutation positive subjects. Europace. 2014;16(4):563–71.
39. Hofman N, Tan HL, Alders M, Kolder I, de Haij S, Mannens MM, et al. Yield of molecular and clinical testing for arrhythmia syndromes: report of 15 years' experience. Circulation. 2013;128(14):1513–21.
40. Holm H, Gudbjartsson DF, Arnar DO, Thorleifsson G, Thorgeirsson G, Stefansdottir H, et al. Several common variants modulate heart rate, PR interval and QRS duration. Nat Genet. 2010;42(2):117–22.
41. Kandolin R, Lehtonen J, Kupari M. Cardiac sarcoidosis and giant cell myocarditis as causes of atrioventricular block in young and middle-aged adults. Circ Arrhythm Electrophysiol. 2011;4(3):303–9.
42. Kruse M, Schulze-Bahr E, Corfield V, Beckmann A, Stallmeyer B, Kurtbay G, et al. Impaired endocytosis of the ion channel TRPM4 is associated with human progressive familial heart block type I. J Clin Invest. 2009;119(9):2737–44.
43. Liu H, El Zein L, Kruse M, Guinamard R, Beckmann A, Bozio A, et al. Gain-of-function mutations in TRPM4 cause autosomal dominant isolated cardiac conduction disease. Circ Cardiovasc Genet. 2010;3(4):374–85.
44. Lodder EM, Scicluna BP, Milano A, Sun AY, Tang H, Remme CA, et al. Dissection of a quantitative trait locus for PR interval duration identifies Tnni3k as a novel modulator of cardiac conduction. PLoS Genet. 2012;8(12):e1003113.
45. Lynch HT, Mohiuddin S, Sketch MH, Krush AJ, Carter S, Runco V. Hereditary progressive atrioventricular conduction defect. A new syndrome? JAMA. 1973;225(12):1465–70.
46. Lynch RJ, Engle MA. Familial congenital complete heart block. Its occurrence in 2 children with another genetically determined anomaly. Am J Dis Child. 1961;102:210–7.
47. Makita N, Seki A, Sumitomo N, Chkourko H, Fukuhara S, Watanabe H, et al. A connexin40 mutation associated with a malignant variant of progressive familial heart block type I. Circ Arrhythm Electrophysiol. 2012;5(1):163–72.
48. Marsman RF, Bardai A, Postma AV, Res JC, Koopmann TT, Beekman L, et al. A complex double deletion in LMNA underlies progressive cardiac conduction disease, atrial arrhythmias, and sudden death. Circ Cardiovasc Genet. 2011;4(3):280–7.
49. McDermott DA, Fong JC, Basson CT. Holt-Oram syndrome. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, et al. editors. GeneReviews(R). Seattle (WA); 1993.

50. Milanesi R, Baruscotti M, Gnecchi-Ruscone T, DiFrancesco D. Familial sinus bradycardia associated with a mutation in the cardiac pacemaker channel. N Engl J Med. 2006;354(2):151–7.
51. Milano A, Vermeer AM, Lodder EM, Barc J, Verkerk AO, Postma AV, et al. HCN4 mutations in multiple families with bradycardia and left ventricular noncompaction cardiomyopathy. J Am Coll Cardiol. 2014;64(8):745–56.
52. Morquio L. Sur une maladie infantile caractérisée par des modifications permanentes du pouls, des attaques syncopales et épileptiformes. Arch Med Enfants. 1901;4:467–75.
53. Nery PB, Beanlands RS, Nair GM, Green M, Yang J, McArdle BA, et al. Atrioventricular block as the initial manifestation of cardiac sarcoidosis in middle-aged adults. J Cardiovasc Electrophysiol. 2014;25(8):875–81.
54. Nof E, Luria D, Brass D, Marek D, Lahat H, Reznik-Wolf H, et al. Point mutation in the HCN4 cardiac ion channel pore affecting synthesis, trafficking, and functional expression is associated with familial asymptomatic sinus bradycardia. Circulation. 2007;116(5):463–70.
55. Pfeufer A, van Noord C, Marciante KD, Arking DE, Larson MG, Smith AV, et al. Genome-wide association study of PR interval. Nat Genet. 2010;42(2):153–9.
56. Postema PG, Van den Berg M, Van Tintelen JP, Van den Heuvel F, Grundeken M, Hofman N, et al. Founder mutations in the Netherlands: SCN5a 1795insD, the first described arrhythmia overlap syndrome and one of the largest and best characterised families worldwide. Neth Hear J. 2009a;17(11):422–8.
57. Postema PG, Wolpert C, Amin AS, Probst V, Borggrefe M, Roden DM, et al. Drugs and Brugada syndrome patients: review of the literature, recommendations, and an up-to-date website (http://www.brugadadrugs.org/). Heart Rhythm. 2009b;6(9):1335–41.
58. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. Executive summary: HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. Europace. 2013;15(10):1389–406.
59. Remme CA, Verkerk AO, Nuyens D, van Ginneken AC, van Brunschot S, Belterman CN, et al. Overlap syndrome of cardiac sodium channel disease in mice carrying the equivalent mutation of human SCN5A-1795insD. Circulation. 2006;114(24):2584–94.
60. Roberts JD, Longoria J, Poon A, Gollob MH, Dewland TA, Kwok PY, et al. Targeted deep sequencing reveals no definitive evidence for somatic mosaicism in atrial fibrillation. Circ Cardiovasc Genet. 2015;8(1):50–7.
61. Rotman M, Triebwasser JH. A clinical and follow-up study of right and left bundle branch block. Circulation. 1975;51(3):477–84.
62. Schott JJ, Alshinawi C, Kyndt F, Probst V, Hoorntje TM, Hulsbeek M, et al. Cardiac conduction defects associate with mutations in SCN5A. Nat Genet. 1999;23(1):20–1.
63. Schott JJ, Benson DW, Basson CT, Pease W, Silberbach GM, Moak JP, et al. Congenital heart disease caused by mutations in the transcription factor NKX2-5. Science. 1998;281(5373):108–11.
64. Schulze-Bahr E, Neu A, Friederich P, Kaupp UB, Breithardt G, Pongs O, et al. Pacemaker channel dysfunction in a patient with sinus node disease. J Clin Invest. 2003;111(10):1537–45.
65. Silverman BG, Gross TP, Kaczmarek RG, Hamilton P, Hamburger S. The epidemiology of pacemaker implantation in the United States. Public Health Rep. 1995;110(1):42–6.
66. Sotoodehnia N, Isaacs A, de Bakker PI, Dorr M, Newton-Cheh C, Nolte IM, et al. Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction. Nat Genet. 2010;42(12):1068–76.
67. Stallmeyer B, Fenge H, Nowak-Gottl U, Schulze-Bahr E. Mutational spectrum in the cardiac transcription factor gene NKX2.5 (CSX) associated with congenital heart disease. Clin Genet. 2010;78(6):533–40.
68. Stallmeyer B, Zumhagen S, Denjoy I, Duthoit G, Hebert JL, Ferrer X, et al. Mutational spectrum in the Ca(2+)–activated cation channel gene TRPM4 in patients with cardiac conductance disturbances. Hum Mutat. 2012;33(1):109–17.
69. Steenkamp WF. Familial trifascicular block. Am Heart J. 1972;84(6):758–60.
70. Steere AC, Batsford WP, Weinberg M, Alexander J, Berger HJ, Wolfson S, et al. Lyme carditis: cardiac abnormalities of Lyme disease. Ann Intern Med. 1980;93(1):8–16.
71. Stephan E. Hereditary bundle branch system defect: survey of a family with four affected generations. Am Heart J. 1978;95(1):89–95.
72. Strasberg B, Amat YLF, Dhingra RC, Palileo E, Swiryn S, Bauernfeind R, et al. Natural history of chronic second-degree atrioventricular nodal block. Circulation. 1981;63(5):1043–9.
73. Theis JL, Zimmermann MT, Larsen BT, Rybakova IN, Long PA, Evans JM, et al. TNNI3K mutation in familial syndrome of conduction system disease, atrial tachyarrhythmia and dilated cardiomyopathy. Hum Mol Genet. 2014;23(21):5793–804.
74. Tresch DD, Fleg JL. Unexplained sinus bradycardia: clinical significance and long-term prognosis in apparently healthy persons older than 40 years. Am J Cardiol. 1986;58(10):1009–13.
75. van den Heuvel GCJ. Die ziekte van Stokes-Adams en een geval van aangeboren hartblok 1908.
76. van Rijsingen IA, Arbustini E, Elliott PM, Mogensen J, Hermans-van Ast JF, van der Kooi AJ, et al. Risk factors for malignant ventricular arrhythmias in lamin a/c mutation carriers a European cohort study. J Am Coll Cardiol. 2012;59(5):493–500.
77. van Spaendonck-Zwarts KY, van Hessem L, Jongbloed JD, de Walle HE, Capetanaki Y, van der Kooi AJ, et al. Desmin-related myopathy. Clin Genet. 2011;80(4):354–66.
78. van Tintelen JP, Tio RA, Kerstjens-Frederikse WS, van Berlo JH, Boven LG, et al. Severe myocardial fibrosis caused by a deletion of the 5′ end of the lamin A/C gene. J Am Coll Cardiol. 2007;49(25):2430–9.
79. Veerman CC, Wilde AA, Lodder EM. The cardiac sodium channel gene SCN5A and its gene product NaV1.5: role in physiology and pathophysiology. Gene. 2015;573(2):177–87.
80. Vermeer AM, Lodder EM, Thomas D, Duijkers FA, Marcelis C, van Gorselen EO, et al. Dilation of the aorta ascendens forms part of the clinical spectrum of HCN4 mutations. J Am Coll Cardiol. 2016;67(19):2313–5.
81. Verweij N, Mateo Leach I, Isaacs A, Arking DE, Bis JC, Pers TH, et al. Twenty-eight genetic loci associated with ST-T wave amplitudes of the electrocardiogram. Hum Mol Genet. 2016. (http://hmg.oxfordjournals.org/content/early/2016/03/23/hmg.ddw058.full)
82. Wahbi K, Meune C, Porcher R, Becane HM, Lazarus A, Laforet P, et al. Electrophysiological study with prophylactic pacing and survival in adults with myotonic dystrophy and conduction system disease. JAMA. 2012;307(12):1292–301.
83. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell. 1995;80(5):805–11.
84. Watanabe H, Koopmann TT, Le Scouarnec S, Yang T, Ingram CR, Schott JJ, et al. Sodium channel beta1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. J Clin Invest. 2008;118(6):2260–8.
85. Xi Y, Honeywell C, Zhang D, Schwartzentruber J, Beaulieu CL, Tetreault M, et al. Whole exome sequencing identifies the TNNI3K gene as a cause of familial conduction system disease and congenital junctional ectopic tachycardia. Int J Cardiol. 2015;185:114–6.
86. Xiong Y, Wang L, Liu W, Hankey GJ, Xu B, Wang S. The prognostic significance of right bundle branch block: a meta-analysis of prospective cohort studies. Clin Cardiol. 2015;38(10):604–13.
87. Zhang ZM, Rautaharju PM, Soliman EZ, Manson JE, Cain ME, Martin LW, et al. Mortality risk associated with bundle branch blocks and related repolarization abnormalities (from the Women's Health Initiative [WHI]). Am J Cardiol. 2012;110(10):1489–95.

Part IV

Hereditary Aortic Diseases

Heritable Thoracic Aortic Disorders

17

Barbara J.M. Mulder, Ingrid M.B.H. van de Laar, and Julie De Backer

Abstract

Heritable thoracic aortic disease (H-TAD) comprises a heterogeneous group of disorders with as a common denominator aortic aneurysm or dissection on one or several levels from the aortic annulus till the diaphragm. Depending on the presence or absence of manifestations in other organ systems, H-TAD can be further subdivided into syndromic and nonsyndromic H-TAD (NS H-TAD). For both clinical entities, multiple underlying gene defects have been identified, although we must recognize that in a substantial number of patients and families no causal mutation has been identified, defining them as "Heritable" but strictly speaking not (yet) as "Genetic."

Introduction

Heritable thoracic aortic disease (H-TAD) comprises a heterogeneous group of disorders with as a common denominator aortic aneurysm or dissection on one or several levels from the aortic annulus till the diaphragm. Depending on the presence or absence of manifestations in other organ systems, H-TAD can be further subdivided into syndromic and nonsyndromic H-TAD (NS H-TAD). For both clinical entities, multiple underlying gene defects have been identified, although we must recognize that in a substantial number of patients and families no causal mutation has been identified, defining them as "Heritable" but strictly speaking not (yet) as "Genetic."

Currently, known causal genes that have been identified so far in H-TAD can be grouped into those affecting structures (i.e., genes encoding extracellular matrix (ECM) components (*FBN1*, *COL3A1*, *MFAP5, ELN, and FBLN4*)) and those that affect the ability to modify structure in response to changes in mechanical load imposed on the aortic wall. The latter group can be divided into genes encoding various proteins involved in tumor growth factor (TGF)β signaling (*TGFBR1*, *TGFBR2*, *TGFB2*, *TGFB3*, and *SMAD3*) and genes encoding proteins involved in vascular smooth muscle cell (SMC) contractility (*ACTA2*, *MYH11*, MYLK, *PRKG1*, and *FLNA*). Table 17.1 provides a schematic overview of the currently known genes and their associated clinical entities.

Aneurysms and dissections in these disease entities will most commonly occur in the ascending thoracic part of the aorta, but the more distal locations in the aorta as well as involvement of branching vessels in some cases should also be acknowledged. Formerly, H-TADs were often classified as "Connective tissue disorders" since the initially identified molecular defects were located in genes encoding for structural components such as fibrillin 1 and collagen. More recent insights have clearly demonstrated, however, that other mechanisms, not always related to the "connective tissue," are equally important. Therefore, the term "H-TAD" seems more appropriate now.

In this chapter, we cover the clinical and molecular aspects of four relevant syndromic H-TAD entities: Marfan syndrome (MFS), vascular Ehlers–Danlos syndrome (EDS), Loeys–Dietz syndrome (LDS), and multisystemic SMC dysfunction syndrome as well as the current knowledge on NS H-TAD.

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Table 17.1 Schematic overview of heritable thoracic aortic disease (H-TAD) entities, according to the underlying gene defects and according to degree of manifestations outside the aorta (Syndromic Gradient, from left to right). Two major groups of gene mutations associated with H-TAD can be distinguished, namely those affecting structure (i.e., the ECM) and those that affect the ability to modify structure in response to changes in mechanical load imposed on the aortic wall (i.e., cell-signaling pathways and the contractile apparatus)

Gene name	Clinical entities associated with the gene – from highly syndromic on the left to nonsyndromic on the right		
H-TAD related to genes encoding components of the extracellular matrix			
FBN1	Neonatal MFS [1, 2]	Classic MFS/MFS features [2]	Isolated/Nonsyndromic H-TAD [3]
COL3A1	vEDS [4]		Isolated/Nonsyndromic H-TAD [5]
MFAP5		MFS features [6]	Isolated/Nonsyndromic H-TAD [6]
H-TAD related to genes encoding components of the TGF-β pathway			
TGFBR1	LDS; vEDS [7, 8]	Classic MFS/MFS features [9, 10]	Isolated/Nonsyndromic H-TAD [9, 10]
TGFBR2	LDS; vEDS [7, 8]	Classic MFS/MFS features [10–12]	Isolated/Nonsyndromic H-TAD [13, 14]
SMAD3	LDS [15, 16]	AOS, classic MFS/MFS features [17, 18]	Isolated/Nonsyndromic H-TAD [5, 19]
TGF-β2	LDS [15, 20]	Classic MFS/MFS features [21]	Isolated/Nonsyndromic H-TAD [22]
TGF-β3	LDS, syndrome presenting at birth with distal arthrogryposis, hypotonia, bifid uvula, a failure of normal postnatal muscle development [23]	MFS features [24, 25]	
H-TAD related to genes encoding proteins involved in the contractile apparatus of vascular smooth muscle cells			
ACTA2	multisystemic SMC dysfunction syndrome [26–28]	H-TAD with mild associated skin/ocular/vascular lesions [29]	Isolated/Nonsyndromic H-TAD [30]
MYLK			Isolated/Nonsyndromic H-TAD [31]
PRKG1			Isolated/Nonsyndromic H-TAD [32]
MYH11		H-TAD with Patent ductus arteriosus [33, 34]	Isolated/Nonsyndromic H-TAD [30]

AOS Aneurysm-Osteoarthritis syndrome, *H-TAD* Heritable-thoracic aortic disease, *MFS* Marfan syndrome, *LDS* Loeys–Dietz syndrome, *SMC* smooth muscle cell, *vEDS* vascular Ehlers–Danlos syndrome

Clinical Presentation

Marfan Syndrome

MFS (ORPHA 558, OMIM #154700) is a common yet under-recognized autosomal dominant systemic disorder of connective tissue, caused by heterozygous mutations in the *FBN1* gene at 15q21.1, which encodes the ECM protein fibrillin-1. The disorder shows characteristic but highly variable manifestations in mainly the cardiovascular, ocular, and musculoskeletal systems. It was first reported in 1896, when Antoine-Bernard Marfan described a young girl with unusual musculoskeletal features [35]. It was not until the mid-1950s that cardiovascular involvement in MFS was well recognized and described in Victor McKusick's monograph [36]. The age of onset of clinical manifestations is highly variable, ranging from severe cardiovascular involvement at birth in the neonatal form to patients developing manifestations only in mid-life. The estimated prevalence of MFS is 1 in 3000–5000 individuals, with no ethnic or gender predilection. About 25 % of patients represent new mutations. Prognosis is mainly determined by progressive dilation of the aorta, which may lead to aortic dissection and death at a young age. The mean survival of untreated patients is about 40 years. Fortunately, improved management and ongoing research have led to a significant increase in life expectancy of at least 30 years [37, 38]. A key factor in improving prognosis is early identification of patients with MFS. Precipitating factors reported to accelerate progressive dilatation or dissection include increased blood pressure, intense physical exercise, and pregnancy [39–42].

Loeys–Dietz Syndrome

In 2005, LDS (ORPHA 60030, OMIM # 609192, 610380, 610168, 608967, 615582) was recognized as a new disease entity. Currently, five types of LDS are described, labeled type 1 through 5, which are distinguished by their genetic cause. Regardless of the type, signs and symptoms of LDS can become apparent anytime ranging from early childhood to late adulthood, and the severity is variable. The prevalence of all types of LDS is currently unknown.

LDS is mainly characterized by aneurysms and/or dissections of the aortic root, although 50 % of LDS patients have aneurysms/dissections in other arteries, including cerebral, thoracic, and abdominal arteries. In most patients, arterial tortuosity of head and neck arteries is present. Fatal aortic events can occur at a young age and fatal aortic dissection

and rupture in the aortic root can occur with diameters smaller than 45 mm. In addition to the cardiovascular abnormalities, skeletal (pectus, scoliosis, joint laxity, osteoarthritis, arachnodactyly, and talipes equinovarus), craniofacial (hypertelorism, bifid uvula/cleft palate, and craniosynostosis), and cutaneous (translucent skin, easy bruising, and dystrophic scars) abnormalities can occur in varying severity among the different types of LDS.

The initial reports in LDS types 1 and 2 described a poor prognosis for LDS patients with a mean age at death of 26 years due to aortic dissection and cerebral hemorrhages as major causes of death. This figure may be somewhat biased by the clinical severity of individuals initially ascertained as having LDS. Also, there is a large inter- and intrafamilial variation [13, 43].

Vascular EDS

EDS is an inherited heterogeneous group of connective tissue disorders, comprising several different clinical subtypes. The prevalence is estimated at one in 10,000 to one in 25,000 for all types. The vascular subtype of EDS (vEDS, ORPHA286, *OMIM* #130050), formerly known as EDS type 4, is an autosomal dominant connective tissue disorder caused by mutations in the *COL3A1* gene. vEDS is clinically characterized by vascular, intestinal, and uterine fragility. The prevalence of vEDS has been estimated at 1/50,000 to 1/150,000 [44]. Actually, the true rate is unknown and estimates based on ascertained cases might significantly underestimate the true prevalence in the population. Studies of natural history in vEDS indicated that life span is significantly decreased, almost always related to arterial rupture. The largest (retrospective) series reporting on clinical events and survival mentions a median survival of 48 years. The age at death ranged from 6 to 73 years [4]. The mean age at first complication was 23.5 ± 11.1 years. Overall complications are rare in childhood but 25 % of patients have a first complication by the age of 20 years and more than 80 % have suffered from at least one complication by the age of 40 years. There are no gender differences. Bowel rupture affects about a quarter of affected individuals and in some it is the first indication of a connective tissue abnormality. Mortality related to intestinal rupture – which is more often amenable to surgical treatment – is significantly lower than mortality related to arterial complications (estimated at 3 %) [4]. A more recent smaller-scale study on 31 patients treated for vascular events showed slightly better survival rates with 68 % of patients surviving at the age of 50 years [45].

Pregnancy complications that included both intrapartum and peripartum vascular rupture could lead to death although recent data could not confirm pregnancy as a trigger for adverse outcome [46].

Multisystemic Smooth Muscle Dysfunction Syndrome

Multisystemic smooth muscle dysfunction (MSMD) syndrome (ORPHA404463, OMIM #613834) caused by specific de novo mutations in the *ACTA2* gene (R179H) is a rare disorder characterized by widespread SMC dysfunction manifesting in the iris, bladder, gastrointestinal (GI) tract and the vasculature [26, 47]. Most patients present in early infancy with congenital cardiovascular lesions including patent ductus arteriosus (PDA), aortic arch hypoplasia, aortic coarctation, or aortopulmonary window requiring surgery. Cerebrovascular lesions include typical vascular morphologic abnormalities and bilateral periventricular white matter hyperintensities. Hemiparesis in a 10-year-old and global neurodevelopmental delay has been reported [27]. Patients go on to develop fusiform ascending aortic aneurysms extending to the arch during childhood, also necessitating surgical repair. Type A aortic dissection has been reported in a 14-year-old boy [48]. The prevalence of the disease is unknown, but presumably very low with about 20 patients reported in literature worldwide. The outcome in these patients is poor – most known cases do not survive beyond the third decade of life and die from obstructive vascular lesions.

Nonsyndromic H-TAD

The majority of TAD patients will not present additional clinical features, related to an underlying syndromic entity. TAD can result from diverse aetiologies, including infectious agents and hemodynamic forces. The vast majority of descending thoracic aneurysms are associated with atherosclerosis and the risk factors for aneurysm formation are the same as those for atherosclerosis (e.g., hypertension, hypercholesterolemia, and smoking) [49]. Atherosclerosis is an infrequent cause of ascending thoracic aortic aneurysms; however [49], and underlying heritable forms should always be considered. It has been recognized for many years that up to 20 % of patients presenting with an aortic aneurysm/dissection have an affected first-degree relative in which case the term NS H-TAD applies (NS H-TAD, ORPHA91387, OMIM#132900; 607086; 607087; 609192; 610168; 611788; 613780; 614816; 615436; 615582; 616166). The younger the proband, the greater the chance of having affected relatives. We now know that simply asking about aortic disease is insufficient. One needs to query about intracerebral arterial disease, precocious coronary artery disease, congenital heart disease (specifically, coarctation and patent ductus), and bicuspid aortic valve (BAV) [50]

Aortic dilation progresses more rapidly in patients with familial aortopathy with a greater risk of aortic complications [51, 52].

Up to now, over a dozen genetic defects have been identified in NS H-TAD, many of whom are also encountered in the syndromic forms. Recent studies assessing the mutation uptake rate in these patients show figures varying between 15 and 20 % [5, 53].

The risk of aortic complications in NS H-TAD patients will be influenced by the underlying genetic defect, although data are lacking to confirm this. Evident additional patient-related factors for developing aortic complications include smoking, hypertension, and strenuous physical exercise as well as anatomical factors such as the presence of a BAV.

Clinical Diagnosis and Differential Diagnosis

Clinical Diagnosis

Marfan Syndrome

Early identification and establishment of the diagnosis in patients with MFS is of considerable importance because prophylactic surgery can prevent aortic dissection and rupture. Elucidation of the molecular mechanisms behind MFS will allow improvement in diagnostic testing, but so far the diagnosis of MFS has to be made on clinical grounds, following the revised Ghent criteria (Table 17.2) [1].

The diagnosis of MFS requires the coexistence of aortic root aneurysm or aortic dissection together with either a pathogenic *FBN1* mutation, ectopia lentis or a positive family history. The remaining cardinal manifestations of MFS are incorporated in a systemic score, where a systemic score of >7 also contributes to the diagnosis (Table 17.3) [1].

MFS shows a high penetrance, but marked inter- and intrafamilial variability. The disorder should be regarded as a spectrum of diverse and highly variable manifestations in the different organs, and not all patients display the classic habitus. Most of the manifestations have an age-dependent penetrance. Moreover, many of the physical findings are also encountered in the general population or in other syndromic H-TAD entities, such as LDS and EDS. The variability in clinical expression, with manifestations that emerge from childhood onwards, the presence of *FBN1* mutations in the different fibrillinopathies, and the high rate of de novo mutations can pose difficulties in establishing the diagnosis in some patients, particularly in younger individuals with few symptoms [2]. Some patients need follow-up before a definitive judgment can be made. A multidisciplinary approach, including clinical genetics, cardiology, ophthalmology, and radiology is essential in establishing the diagnosis, treatment, and follow-up. When the diagnosis has been established in an individual, first-degree relatives should be screened for the disorder as well.

Table 17.2 The revised Ghent criteria for diagnosis of Marfan syndrome

Family history	Aortic dilation ($Z \geq 2$) or dissection	Ectopia lentis	Systemic score (≥7 of 20)	Pathogenic-*FBN1* mutation
	X	X		
	X		X	
	X			X
		X		X
X	X			
X		X		
X			X	

Each line represents a possible combination leading to Marfan syndrome. Xao*: FBN1* mutation associated with aortic pathology

Cardiovascular System

The major sources of morbidity and mortality in MFS are due to manifestations in the cardiovascular system, of which aortic aneurysm and dissection are the most life-threatening. Dilatation of the sinus of Valsalva is found in up to 80 % of adults with MFS (Fig. 17.1), resulting in a typical pear shape of the aortic root. Aortic root aneurysm/dissection is a major criterion for the diagnosis of MFS (Table 17.2). The onset and progression of aortic dilatation is highly variable, in rare cases beginning in utero, while other individuals never develop dilatation to critical diameters. Normal aortic dimensions are dependent on body surface area (BSA)and age, and therefore the dimensions measured – especially in pediatric patients – have to be compared with age-dependent nomograms [54, 55]. However, in adults, aortic roots of ≥40-mm diameter can be considered dilated [56].

Without preventive surgery, dissections in MFS mostly constitute type A aortic dissections involving the aortic root,

Table 17.3 Scoring of the systemic features

Feature	Score
Wrist and thumb sign	3 (wrist or thumb: 1)
Pectus carinatum deformity	2 (pectus excavatum or chest asymmetry: 1)
Hindfoot deformity	2 (plain pes planus: 1)
Pneumothorax	2
Dural ectasia	2
Protrusio acetabuli	2
Reduced upper segment/lower segment ratio AND increased arm/height WITHOUT severe scoliosis	1
Scoliosis or thoracolumbar kyphosis	1
Reduced elbow extension	1
Facial features (3/5): dolichocephaly, enophthalmos, downslanting palpebral fissures, malar hypoplasia, retrognathia	1
Skin striae	1
Myopia >3 diopters	1
Mitral valve prolapse (all types)	1

in many cases propagating along the descending aorta. The risk of type A dissection clearly increases with increasing aortic root diameter, but dissection may occasionally occur in patients with no or only mild aortic dilatation. Other risk factors for dissection include the rate of aortic growth and a family history of aortic dissection.

As Marfan patients currently survive longer after surgical replacement of the aortic root, an increasing amount of patients develop aneurysms and/or dissections elsewhere in the arterial tree (Fig. 17.2) [57–59]. Apart from increasing diameters, the aorta also elongates, which forces the anatomically fixed aorta to curve and become tortuous. By means of magnetic resonance imaging (MRI), the tortuosity index can be measured. Apart from aortic diameters and tortuosity index, MRI may be useful to determine aortic elasticity in the distal aorta. A decreased local distensibility and increased flow wave velocity as an expression of decreased aortic elasticity can be found in many but not all unoperated patients with MFS. Patients with MFS with prior prophylactic aortic surgery are at a substantial risk for type B aortic dissection, even when the descending aorta is only slightly dilated.

In about 17 % of patients, the aorta distal from the root is the first site of complications [58]. Predictors for aortic growth and adverse events in the distal aorta include a larger aortic diameter, lower aortic distensibility, higher aortic tortuosity index, and previous aortic root replacement [60–62].

Eight to 15 % of Marfan patients require initial surgery in the descending aorta [57, 63]. Patients with initial type B aortic dissection are at a significantly higher risk for re-intervention (86 % for previous type B dissection vs. 42 % for previous type A dissection). The majority of re-interventions are required in patients with previous dissection (48 vs. 11 % re-intervention in the patients presenting with aortic aneurysm) [63]. A large contemporary series of 96 thoracoabdominal aortic aneurysm repairs in patients with MFS show an excellent survival rate of 97 % [64]. Recommendations are slightly different from the ones applied for the aortic root (see below): repair at the level of the thoracoabdominal in MFS is recommended when the aneurysm diameter exceeds 5.5 cm [65]. However, the high risk of surgical intervention at this level should be weighed against the risk of type B dissection, which may occur at substantially smaller aortic diameters.

The carotid or coronary arteries can also be involved, leading to cerebrovascular injury or myocardial infarction. Dilatation of the main pulmonary artery is a frequent finding in patients with MFS [66, 67]. However, patients may require surgery only in scarce circumstances, most likely because the formation of marked pulmonary aneurysm or dissection may lead to left ventricular (LV) dysfunction and increased pulmonary artery pressures.

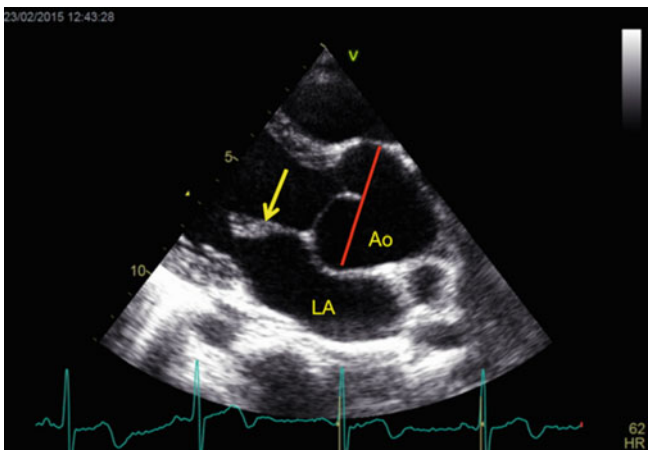

Fig. 17.1 Transthoracic echocardiography image of an aortic sinus of Valsalva aneurysm in a 15-year-old boy with Marfan syndrome. Note the pear-shaped aortic root and the thickened mitral valve leaflets (*arrow*). *Ao* aorta, *LA* left atrium

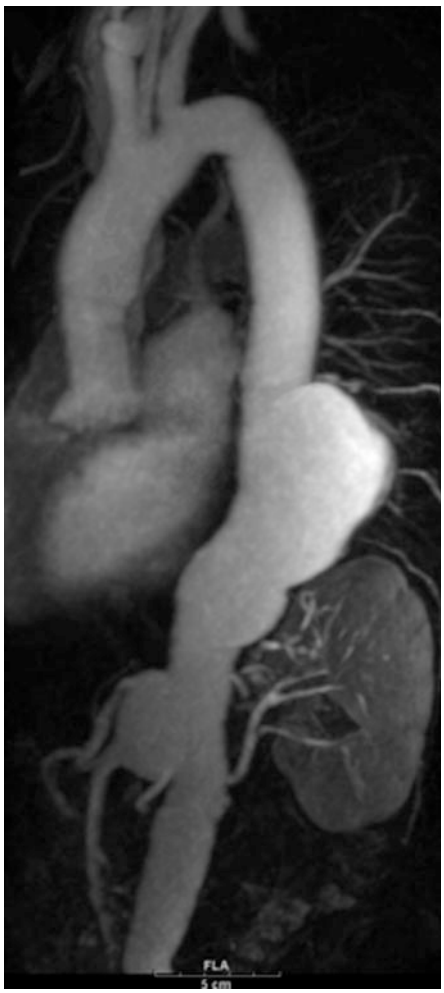

Fig. 17.2 MRI image of an aneurysm at the level of the descending thoracic aorta in a 52-year-old lady with Marfan syndrome who underwent previous replacement of the proximal descending and abdominal aorta

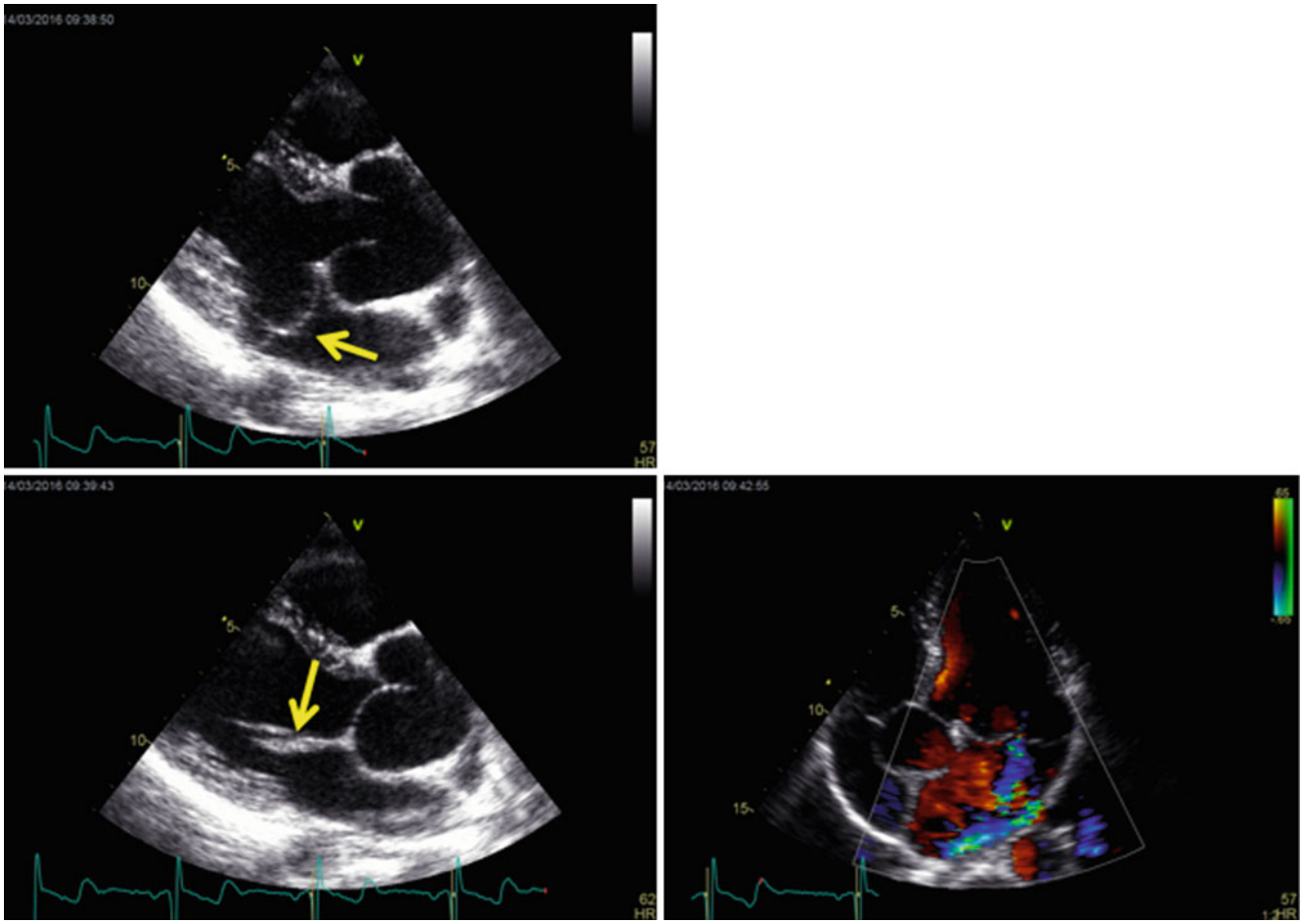

Fig. 17.3 Transthoracic echocardiographic images of mitral valve prolapse in a 15-year-old Marfan boy. Panel A shows the bulging of the mitral valve in the LA during systole. Panel B shows the thickened valve. Panel C shows the associated regurgitation on color flow

Within the heart, the atrioventricular valves are most often involved, with thickening and prolapse of mitral and/or tricuspid valves and subsequent regurgitation [68] (Fig. 17.3). Mitral valve prolapse accounts for one point in the systemic score. Aortic valve regurgitation usually arises in the context of stretching of the aortic annulus due to a dilated aortic root. LV failure may be the consequence.

Although not included in the diagnostic criteria for MFS, it has been speculated that a fibrillin defect in the myocardium may predispose MFS patients to LV dilation and reduced LV function. In several studies, evidence has been found for mild, but significant impairment of LV systolic and diastolic function in Marfan patients, not related to valvular heart disease [69–71].

Patients with a dilated aorta are usually asymptomatic. The presence of significant aortic, tricuspid, or mitral regurgitation may lead to symptoms of ventricular volume overload. Patients with MFS tend to feel fatigued, which may, at least partly, be explained by orthostatic hypotension. The combination of increased height and a structural abnormality of the blood vessels may cause impaired orthostatic tolerance. In Marfan patients, fatigue and low orthostatic tolerance have been correlated [72]. Patients can be educated in physical counterpressure maneuvers, such as leg crossing and muscle tensing, to counteract orthostatic drops in blood pressure.

Ocular System

Ocular lens dislocation (ectopia lentis), often bilateral and symmetric and mostly upward, is considered a major criterion and occurs in about 60 % of patients with MFS [73]. When dislocation of a lens is detected in the absence of a traumatic event (the most common cause), MFS should always be considered. Subluxation usually develops in childhood, but may first appear later in life. A slit-lamp examination is an essential part of the diagnostic examination. Myopia, often rapidly progressing during childhood, is the most common ocular finding in patients with MFS. It is associated with an increased length of the globe and an increased risk of retinal detachment [74]. The cornea can be flat and the iris or ciliary muscle may be hypoplastic [75]. A predisposition of cataracts and glaucoma exists. The lens dislocation,

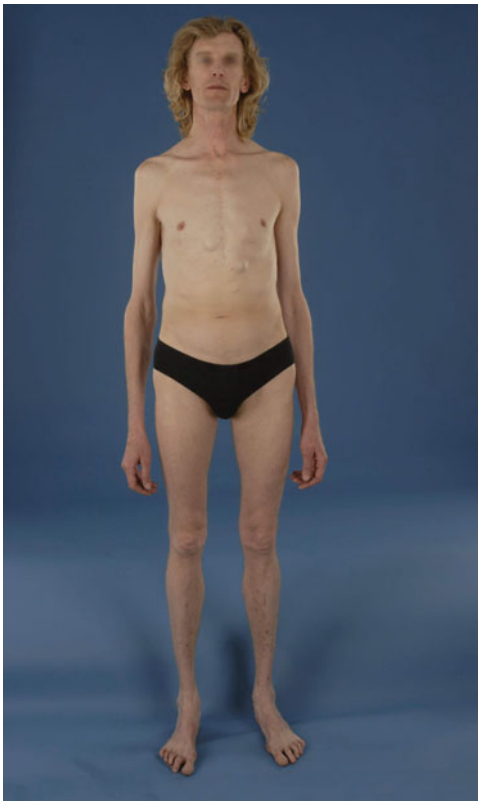

Fig. 17.4 Patient with a typical habitus of Marfan syndrome with overgrowth of long bones and hypoplasia of skeletal muscle and adipose tissue

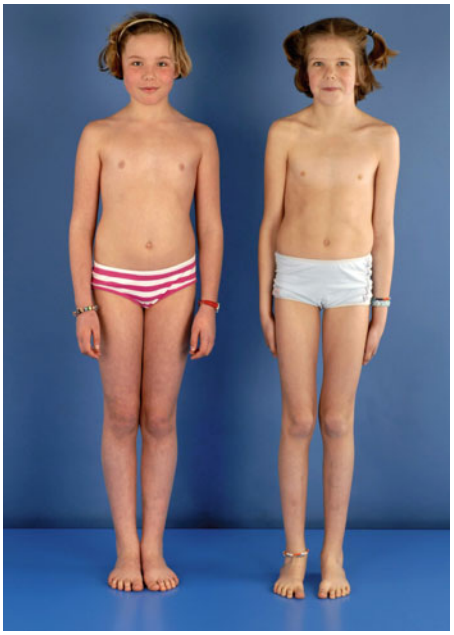

Fig. 17.5 The 8-year-old girl on the right has Marfan syndrome. She is 2 years younger than her sister on the left, though the girls have the same height

retinal detachment, cataract, and glaucoma may cause significant visual impairment.

Skeletal System

The most striking skeletal manifestation is the overgrowth of the long bones, leading to the characteristic appearance of patients with MFS. The extremities are disproportionately long for the size of the trunk (dolichostenomelia), which leads to an increased arm span-to-height and upper–lower segment ratio (Fig. 17.4. The fingers and toes are long and thin (arachnodactyly) and in combination with hypermobility of the joints this leads to the characteristic wrist (Walker-Murdoch) – and thumb (Steinberg) sign. Individuals with MFS are taller than predicted based on their nonaffected relatives; however, they are not necessarily tall compared to the general population (Fig. 17.5). Overgrowth of the rib cartilage can lead to pectus excavatum or pectus carinatum. Scoliosis, affecting around 60 % of patients, may lead to deformity, pain, and even respiratory problems. Additional skeletal manifestations include pes planus and an abnormally deep acetabulum (protrusion acetabuli) with accelerated erosion, which can be confirmed on radiographs. Typical facial features of MFS are a long and narrow face with underdeveloped cheekbones (malar hypoplasia), downward slanting palpebral fissures, enophthalmus, and retro- or micrognathia. A highly arched and narrow palate and tooth crowding are often present as well. Skeletal abnormalities in MFS emerge and may progress during childhood and adolescence, typically during periods of rapid growth. The most specific skeletal manifestations are incorporated in the systemic score (Table 17.3).

Pulmonary System

In the lungs, widening of distal airspaces and lung bullae or blebs may be present, particularly in the upper lobes, which can predispose to spontaneous pneumothorax [76]. In addition, pectus deformities and scoliosis may lead to significantly reduced lung capacity. The prevalence of obstructive sleep apnea syndrome is increased in MFS (almost one in three patients present at least mild sleep apnea) [77] and was related to aortic events in one study [78].

Dural Sac

Stretching and ballooning of the dural sac (dural ectasia) in the lumbosacral region is seen in about two thirds of patients with MFS. It can be assessed by lumbosacral imaging with MRI or computed tomography (CT) (Fig. 17.6) [79]. The presence of dural ectasia accounts for two points in the systemic score. Dural ectasia can also be present in other connective tissue disorders [80, 81], and in healthy individuals. Possible symptoms include back pain, and weakness, pain, and numbness in the proximal legs [82], although it is often asymptomatic. Bone erosion and nerve entrapment may occur.

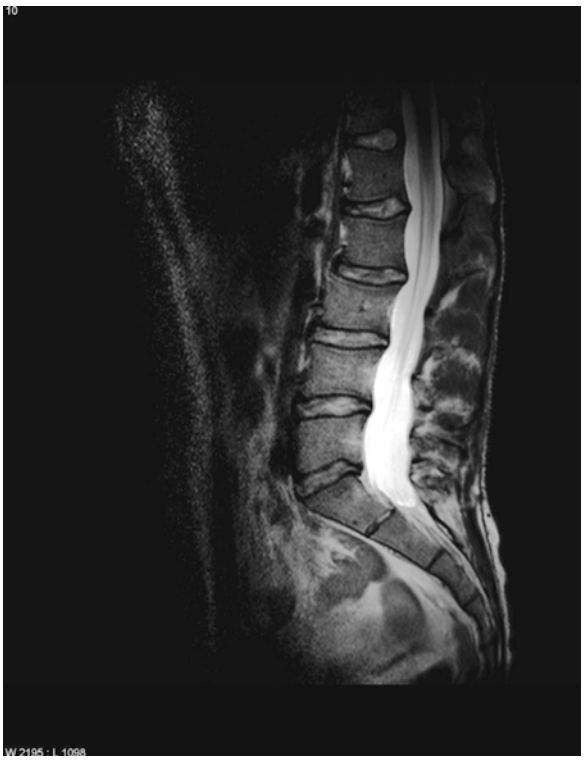

Fig. 17.6 Magnetic resonance imaging showing lumbar and sacral dural ectasia in a patient with Marfan syndrome

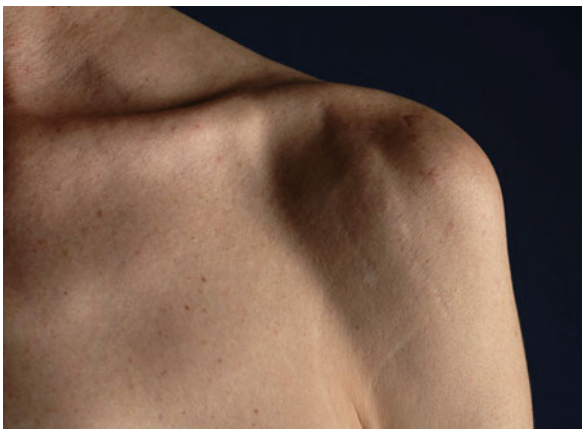

Fig. 17.7 Typical striae atrophicae on the anterior side of the shoulder of an adult patient with Marfan syndrome

Skin and Integuments

In contrast to many other connective tissue disorders, most patients with MFS have a normal skin texture and elasticity, although in some people the skin is unusually thin or elastic. A common feature in MFS is the presence of stretch marks (striae distensae) that are not associated with rapid weight gain, and at sites that are not typically stretched, such as the lumbar area and the anterior and posterior sides of the shoulders (Fig. 17.7). Inguinal and umbilical hernias, congenital or acquired, are also common. Only striae distensae are included in the systemic score.

Other

Hypoplasia of skeletal muscle and adipose tissue, often present in MFS, contributes to the slender and asthenic appearance of some patients (Fig. 17.4).

Loeys–Dietz Syndrome

There are no specific clinical criteria for the diagnosis of LDS. Patients with mutations in *TGFBR1*, *TGFBR2*, *SMAD3*, *TGFB2,* or *TGFB3* gene in combination with documented aneurysm or dissection or a family history of documented LDS is sufficient to establish the diagnosis of LDS [15]. The clinical LDS continuum is subdivided in multiple disease classes named LDS types 1–5. Ten years ago, Loeys et al. described mutations in TGFBR1 and TGFBR2 in syndromic H-TAD patients which are now considered to have LDS types 1 and 2, respectively [8, 15]. *SMAD3* mutations are identified in patients with aneurysms-osteoarthritis syndrome (AOS) [17]. Since the phenotype of individuals with *SMAD3* mutations shows significant overlap with the findings in patients with LDS, AOS is also indicated as LDS type 3. LDS 4 and 5 have now been allocated to mutations in the ligands of the TGFB receptors, *TGFB2* and *TGFB3,* respectively [20, 21, 83]. Recently, mutations in the *SMAD2* gene were identified in syndromic aneurysm patients but no LDS type has been assigned to this gene yet [84] (Table 17.4).

Cardiovascular System

All subtypes of LDS are characterized by aggressive and early aortic and arterial aneurysm and dissection, even in early childhood. A type A aortic dissection has been reported in individuals as young as 3 months of age [85]. Aneurysms are mainly present in the aortic root, at the level of the sinuses of Valsalva, and less commonly involves the descending or abdominal aorta. Arterial aneurysm can also be present in other arteries throughout the body and most commonly occur in the major arteries that arise from the aorta in the thorax and abdomen and those that supply the head and neck.

Dissections occur at aortic dimensions that are not considered hazardous in other connective tissue disorders such as MFS, that is, ≤45 mm, or without prior dilatation. In LDS type 5, no examples of early arterial dissection or dissection at small aortic dimension are reported so far.

Arterial tortuosity is most commonly observed in the vertebral and carotid arteries (Fig. 17.8), but can also be seen in the aorta or other arteries throughout the body. Tortuosity of the vertebral arteries is also present in MFS but its prevalence is much higher in LDS patients [86]. The severity of the arterial tortuosity is a poor prognostic factor and correlates with the degree of aortic dilation, and

Table 17.4 LDS subtypes and associated clinical entities

LDS type (OMIM#)	Orpha code	Gene (OMIM#)	Chromosome	Other disorders reported
LDS type 1 (609192)	60030	*TGFBR1* (190181)	9q22.33	Furlong syndrome, nonsyndromic H-TAD
LDS type 2 (610168)	60030	*TGFBR2* (190182)	3p24.1	nonsyndromic H-TAD , MFS2
LDS type 3 (613795)	284984	*SMAD3* (603109)	15q22.33	Aneurysm-osteoarthritis syndrome (AOS), nonsyndromic H-TAD
LDS type 4 (614816)	91387	*TGFB2* (190220)	1q41	MFS, nonsyndromic H-TAD
LDS type 5 (615582)	91387	*TGFB3* (190230)	14q24.3	Rienhoff syndrome, nonsyndromic H-TAD

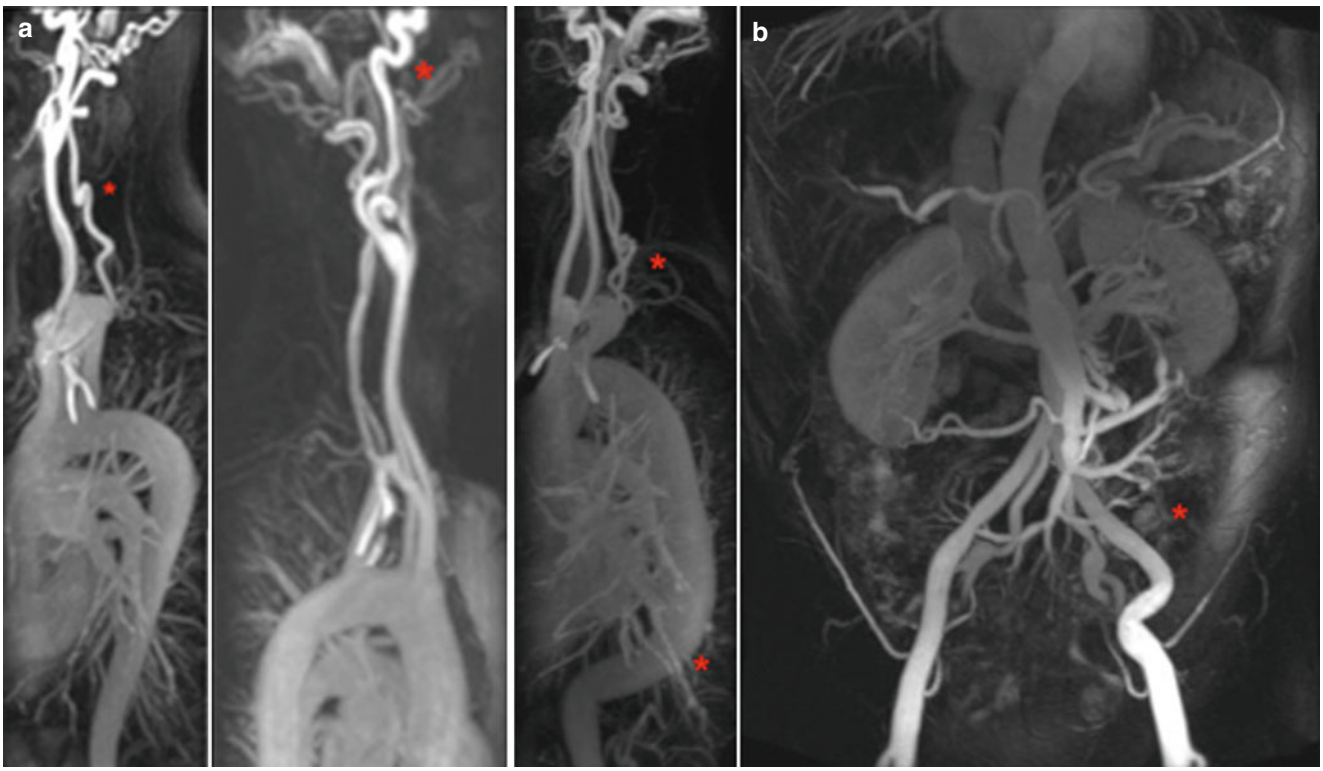

Fig. 17.8 MRI images of the head and neck vessels in two sisters with a TGFBR2 mutation (*panel A*) and of the upper thoracic aorta and head and neck vessels as well as the lower abdominal aorta and iliac arteries in a patient with a TGFBR1 mutation (*panel B*). Note the marked tortuosity of the marked vessels

younger age at dissection, cardiac surgery, and death [87]. Thus far, no arterial tortuosity is observed in LDS type 5 patients [23, 25, 83, 88].

Mitral valve prolapse and/or insufficiency appears less common in LDS than in MFS but can be seen in all types of LDS and ranges from mild-to-severe mitral valve disease. Congenital heart diseases are more prevalent in LDS than in the general population and include atrial septal defect (ASD), PDA, and BAV. In LDS type 3, atrial fibrillation (24 %) and LV hypertrophy (18 %) have been reported. Impaired LV systolic function has been reported in LDS type 1 [89].

For the most recently identified LDS genes, *TGFB2* and *TGFB3*, the aortic/arterial phenotypes seem less severe than LDS types 1–3 and a higher degree of non-penetrance is reported. However, these observations are based on limited data and detailed genotypic and phenotypic data have yet to emerge.

TGFBR1, *TGFBR2*, *SMAD3,* and *TGFB2* mutations have not only been associated with syndromic H-TAD presentations, rare mutations have also been described in NS H-TAD patients [5, 13, 19, 53].

Skeletal Anomalies

Skeletal manifestations that overlap with MFS include pectus deformity, scoliosis, flat feet, and arachnodactyly. Craniosynostosis occurs in LDS but rarely in MFS. Joint hypermobility is also common, including congenital hip dislocation, and recurrent or multiple joint subluxations. Paradoxically, contractures of extremities such as clubfoot, camptodactyly, and contractures of other joints are also described in LDS. In LDS types 1 and 2, cervical spine abnormalities, such as subluxations or instability, have been reported in 51 % of patients in the series reported by

MacCarrick [15]. Spondylolisthesis and scoliosis can be mild or severe and progressive.

Osteoarthritis is particularly present in LDS type 3. In the initial report of LDS3, it was noted that almost all *SMAD3* mutation carriers developed early-onset joint abnormalities including osteoarthritis and osteochondritis dissecans, meniscal lesions, and intervertebral disc degeneration [90]. Osteoarthritis mainly affects the spine, hands and/or wrists, and knees, but it is also reported in all other joints. These abnormalities may be present at a young age and may be the patient's presenting symptom. They seem to be discriminating clinical features in LDS type 3.

However, in recent years several studies have reported individuals with pathogenic *SMAD3* mutations without osteoarticular manifestations indicating that this finding is not mandatory [19, 91, 92]. When we include all published *SMAD3* mutation carriers where clinical information on osteoarticular phenotype is reported, the prevalence of osteoarthritis is still 63 %, indicating that osteoarthritis is an important diagnostic clue [19, 91–97].

Joint anomalies such as osteoarthritis, osteochondritis dissecans, and meniscal lesions are rarely described in LDS due to *TGFBR1*, *TGFBR2*, *TGFB2*, and *TGFB3* mutations, and MFS, but no systematic joint studies in these patients are reported. Further studies to establish the frequency of osteoarthritis and osteochondritis dissecans in these related syndromes are warranted [98, 99].

Craniofacial Abnormalities

The facial features in LDS patients include hypertelorism, and cleft palate. Uvula anomalies (ranging from bifid to broad) can be seen as the mildest form of cleft palate. It may be an easy diagnostic clue, as they only occur in LDS, but not in other syndromic or nonsyndromic forms of H-TAD.

There is a marked inter- and intrafamilial variability in facial features. In many cases, no craniofacial features are described. This might indicate that craniofacial anomalies are less frequent or milder than initially reported.

Skin and Integuments

Some features that are common in connective tissue disorders are also frequent in LDS such as inguinal, umbilical, and hiatal hernia, and thin translucent skin with a tendency to poor wound healing and atrophic scars. Some patients with *TGFBR1/2* mutations show significant clinical overlap with vascular EDS and have initially been designated as LDS type II [7].

Other

Apart from joint, skeletal, craniofacial, and cutaneous abnormalities pulmonary manifestations including spontaneous pneumothorax, restrictive lung disease, and obstructive sleep apnea are more frequent in LDS. Also immunological features such as allergic manifestations, especially asthma, food allergy, eczema, and allergic rhinitis, occur at a higher prevalence in LDS [100]. Autoimmune features, such as Sjogren's disease, rheumatoid arthritis, and Hashimoto's disease, have been described. GI disease including eosinophilic esophagitis, and inflammatory bowel disease such as ulcerative colitis and Crohn's disease are frequently seen in LDS [15, 95]. Infants and children with LDS often present with failure to thrive and constipation which might persist throughout life [15, 100]. Dural ectasia occurs with similar frequency and severity as in MFS [101]. In LDS type 3, neurological features such as muscle cramps, paresthesia, hypoesthesia, or gait disturbance have been described [95].

Other features, such as hydrocephalus, hypotonia, and headaches, are also part of the syndrome. Arnold Chiari type I malformation, developmental delay, defective tooth enamel, and osteoporosis have been rarely described in LDS types 1 and 2.

Vascular EDS

The clinical diagnosis of the vascular type of EDS is made on the basis of four clinical criteria: easy bruising, thin skin with visible veins, characteristic facial features, and rupture of arteries, uterus, or intestines [102]. The diagnosis is confirmed by the identification of a mutation in the *COL3A1* gene.

Cardiovascular System

In many vEDS patients, the diagnosis is made only after a catastrophic vascular complication or at postmortem examination. vEDS patients are at risk for aneurysms and rupture or dissection, especially of medium-sized arteries [4, 45]. Multiple locations (synchronous) or recurring ruptures or dissections in different anatomical regions in medium-sized arteries in individuals under the age 40 should raise this diagnostic consideration. The proximal and distal branches of the aortic arch, the descending thoracic aorta, and abdominal aorta are often affected, as well as vertebral and carotid arteries. Dissection and rupture often occur without preceding aneurysm formation, rendering management very challenging. In a recent literature review, Berqvist et al. reported arterial rupture without underlying aneurysm in 33 % of patients with a serious hemorrhagic complication [103]. Aneurysm and arterial-venous fistula in the cavernous portion of the carotid (often referred to as a carotid-cavernous sinus fistula, CCSF) is a rare condition with a higher-than expected prevalence in people with vEDS.

Mitral valve prolapse has been reported in several cases of vEDS [104, 105] but subsequent larger studies could not confirm this finding [106] indicating that mitral valve prolapse is an aspecific finding in vEDS.

Skin and Facial Characteristics

Distinctive facial features – although present in less than 30 % of the patients [107] – consist of an "old-looking" face, with prominent cheekbones, sunken or bulging eyes, a thin and pinched nose, as well as thin lips (Fig. 17.9). The skin on the extremities, especially the hands, appears aged (acrogeria) (Fig. 17.10). Unlike other types of EDS, affected individuals often have inelastic, thin, translucent skin [108]. Easy bruising may be prominent, especially in children.

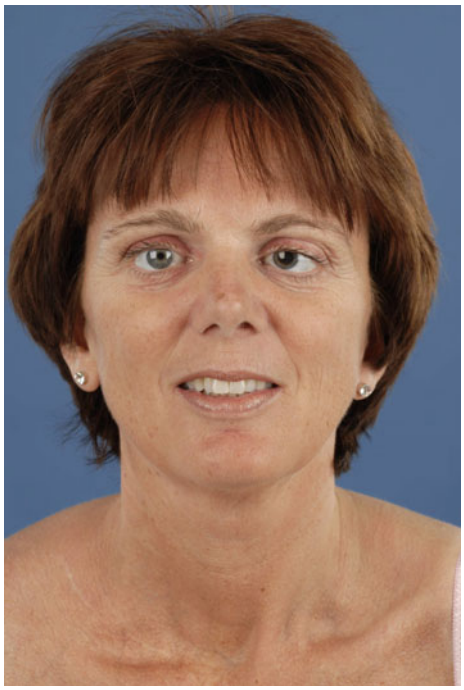

Fig. 17.9 Forty-three-year old with vascular Ehlers–Danlos syndrome. Typical facial features including protruding eyes and a thin and pinched nose. The left pupil is wide and unresponsive to light, resulting from a vascular complication and surgery

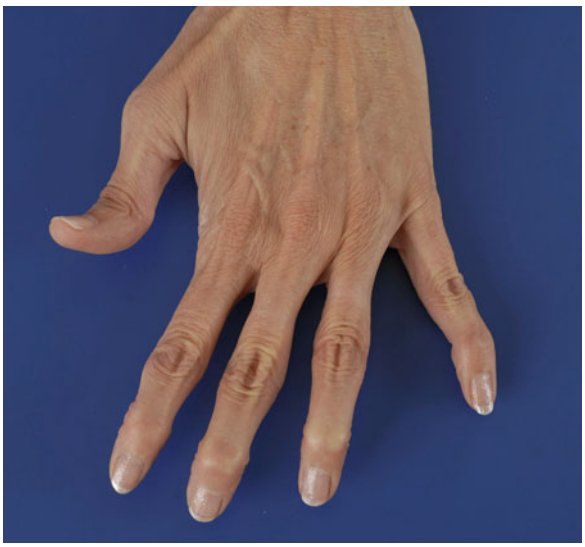

Fig. 17.10 Hand of the 43-year-old woman with vascular Ehlers–Danlos syndrome showing acrogeria

Skeletal System

Hypermobility of small joints can be present (Fig. 17.11), while hypermobility of large joints, characteristic of the more common forms of EDS, is unusual in the vascular type. Club feet and congenital hip dislocations are more prevalent in vEDS patients [4].

GI System

Rupture of the GI tract is another serious complication, occurring in about 25 % of affected individuals and being lethal in 3 % of cases [4]. It mostly occurs in the sigmoid colon, but the small intestine and stomach can also be affected.

Pregnancy

Pregnancy was initially considered as a potential trigger for uterine and vascular rupture and historical data reported pregnancy-related complications in 12 % [4]. Recent observations in a larger study, however, reported slightly lower percentages of pregnancy-related deaths in 5.3 % of pregnancies and found no difference in survival between parous and nulliparous women, suggesting that age is the main risk factor and not pregnancy itself [46]. The most common pregnancy-related complications in this study were third-/fourth-degree lacerations (20 %), arterial dissection/rupture (9.2 %), uterine rupture (2.6 %), and surgical complications (2.6 %). Preterm delivery (occurring in up to 19 % of cases) occurs more frequently in the setting of an affected fetus, owing to increased fragility of the membranes [109].

MSMD Syndrome

The specific missense de novo mutation, R179H in *ACTA2,* causes a syndrome characterized by dysfunction of SMCs throughout the body, with widespread manifestations [26].

Cardiovascular System

Patients reported so far invariably presented with a hemodynamically significant PDA requiring ligation in the neonatal period. Ascending aortic aneurysms develop during childhood and a majority of patients need surgery at a young age (<15 year). One 14-year-old boy reported by Ades developed a type A dissection at the age of 14 years [48]. Other common cardiovascular features include dilatation of the pulmonary arteries, dilatation of the aortic arch, suprarenal abdominal aorta, and head and neck vessels. Aortic coarctation and aortopulmonary window have also been reported.

Cerebrovascular System

Cerebrovascular abnormalities have been encountered on imaging studies in all patients, including fusiform

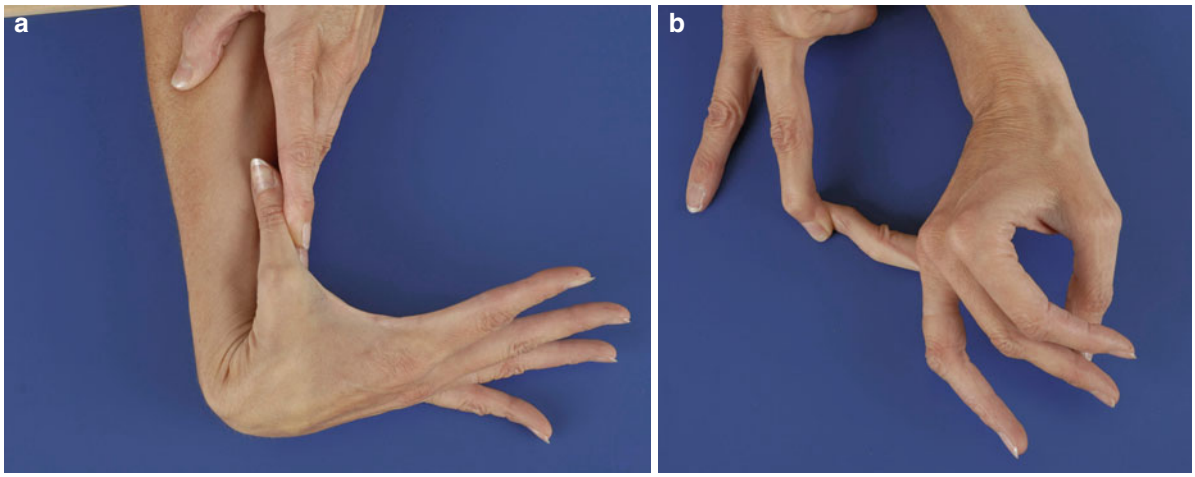

Fig. 17.11 Hypermobility of the wrist and fingers in vascular Ehlers–Danlos syndrome

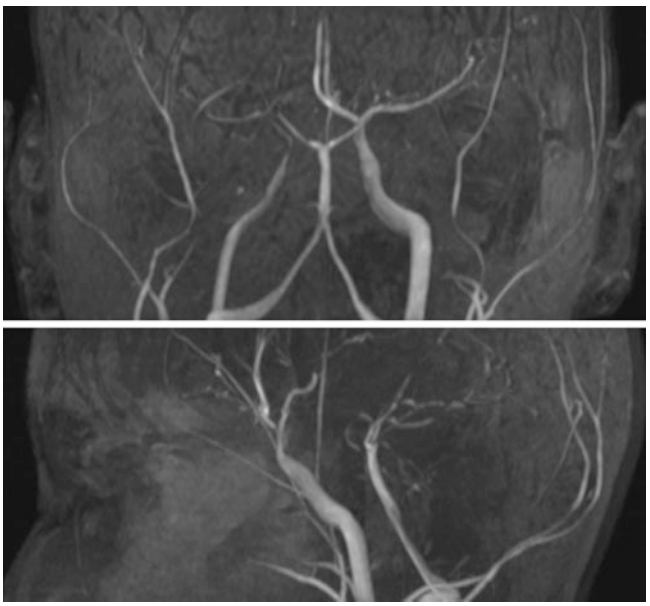

Fig. 17.12 MR angiography of the brain in a 17-year-old girl with multisystemic smooth muscle cell dysplasia syndrome. Note the dilatated left internal carotid artery to the terminal portion, occlusive disease of distal intracranial circulation, an abnormally straight course of intracranial arteries, and absence of "Moyamoya," collaterals.

dilatation of the internal carotids and stenoses at the more terminal portions of these same vessels, reminiscent but not entirely similar to what is seen in Moyamoya disease [27, 110] (Fig. 17.12). All patients for whom imaging was reported had bilateral periventricular white matter hyperintensities suggesting concurrent angiographically occult small-vessel disease [27]. Clinically, one young child has been reported with hemiparesis and global developmental delay has been reported in 2/13 patients in one series [27].

Other Organ System Manifestations

Congenital mydriasis or fixed dilated pupils are a feature shared by all patients with the R179H mutation [26, 111]. Other reported manifestations indicating SMC dysfunction include hypotonic bladder and malrotation, and hyperperistalsis of the GI tract.

Pulmonary manifestations include asthma, cystic lung disease in infancy, and primary pulmonary hypertension necessitating bilateral lung transplantation at the age of 18 months in one case [26, 28, 47].

Nonsyndromic H-TAD

About 20 % of individuals with a thoracic aortic aneurysm or a type A aortic dissection without a history of connective tissue disorder have an affected first-degree relative and therefore may have a genetic predisposition [52, 112]. NS H-TAD is diagnosed in the presence of familial dilatation and/or dissection of the thoracic aorta, in the absence of MFS, LDS, vEDS, or other syndromic features, and in the absence of other underlying factors for aortic disease such as atherosclerosis. NS H-TAD is inherited in an autosomal dominant manner with decreased penetrance and variable expression.

The genetic background of NS-HTAD is heterogeneous and, as can be appreciated from Table 17.1, mutations in nearly all genes reported in the setting of syndromic H-TAD entities may give rise to NS H-TAD. Mutations in the *ACTA2* gene (actin, α-2, smooth muscle, aorta; OMIM *102620) are most frequently encountered in the setting of NS H-TAD and responsible for 12–21 % of cases [29, 30, 113]. Mutations in genes encoding other proteins involved in SMC contraction also cause an inherited predisposition to thoracic aortic disease, including *MYH11, MYLK, and PRKG1* [31, 33, 34, 114]. Mutations in genes encoding components of the ECM can give rise to NS H-TAD as has

been illustrated by the identification of *FBN1* mutations in 3 % of a large cohort of NS H-TAD patients [115]. Additional evidence for the involvement of the ECM in H-TAD was demonstrated. Using a whole-exome-based strategy, Barbier and coworkers identified mutations in the *MFAP5* gene encoding for the MAGP2 protein. MAGP2 is an important protein, interacting with elastin fibers and the microfibrillar network in the ECM. The histology of the aorta was indistinguishable from MFS with cystic media degeneration and increased TGF-β signaling was evidenced with immunohistofluorescence staining indicating a very similar disease mechanism as in MFS [6].

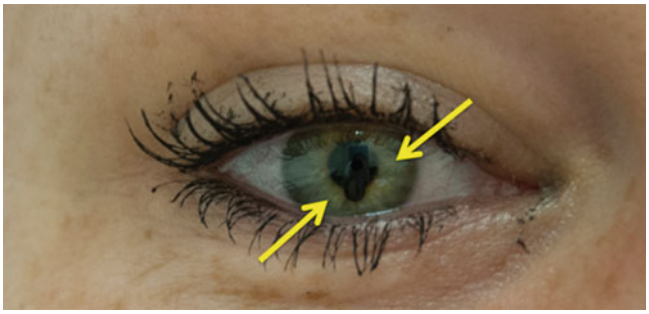

Fig. 17.13 Illustration of iris flocculi in a patient harboring a mutation on the ACTA2 gene

Cardiovascular System

Affected individuals have progressive aortic dilatation of the sinuses of Valsalva and/or ascending aorta and aortic dissection. In the majority of individuals with NS H-TAD, enlargement of the aorta precedes dissection [116]. In NS H-TAD, the onset and rate of progression of aortic dilatation is highly variable, with some individuals developing dilatation in childhood while others reach high age without aneurysms. A higher growth rate was observed in one study comparing familial to sporadic cases of TAD [51]. Individuals with familial H-TAD have a younger mean age at presentation than individuals with non-familial thoracic aortic aneurysms, but older than individuals with MFS [52]. Aortic dissection in childhood is rare.

A recent study describing aortic features in a large series of patients with *ACTA2* mutations indicated that aortic events occurred in 48 % of individuals, with the vast majority presenting with thoracic aortic dissections (88 %) associated with 25 % mortality. Type A dissections were more common than type B dissections (54 vs. 21 %), but the median age of onset of type B dissections was significantly younger than type A dissections (27 vs. 36 years). In this extensive series, the lifetime risk for an aortic event was 76 %, suggesting that additional environmental or genetic factors play a role in the expression of aortic disease in individuals with *ACTA2* mutations. Mutations disrupting p.R179 and p.R258 were associated with significantly increased risk for aortic events, whereas p.R185Q and p.R118Q mutations showed significantly lower risk of aortic events compared with other mutations [113]. Mitral valve prolapse in *ACTA2* mutation patients is reported in only 3 % of cases which is in contrast to MFS and LDS and approaches the prevalence in the general population [13, 113, 117].

Patients harboring specific *ACTA2* mutations also show an increased risk for early onset stroke or coronary artery disease [113]. Other features associated with *ACTA2* mutations include livedo reticularis and iris floccule (Fig. 17.13).

Patients with *MFAP5* mutations present with aortic root dilatation, mostly occurring at middle age and associated with various and very mild syndromic features in some individuals (pectus deformities, mitral valve prolapse). Interestingly, several patients also presented lone atrial fibrillation.

Sixty-three percent of the 31 patients reported with mutations in the *PRKG1* gene presented with aortic dissection, commonly at a young age (mean 31 years) [32].

Patients with *MAT2A* mutations have a predisposition for thoracic aortic aneurysms/dissections. BAVs are seen more frequently [118].

The majority of patients with a mutation in the *MYH11* gene also present a PDA [30, 33, 34, 114]. Aortic stiffness in mutation carriers is increased even in those without significant aortic dilatation [114].

NS H-TAD may also occur in association with BAV. BAV is the most common congenital heart defect and occurs in approximately 1–2 % of the general population. BAV is part of a spectrum of left-sided obstructive lesions, which also includes coarctation of the aorta and hypoplastic left heart syndrome, among others. A relatively high recurrence risk for relatives of patients with left-sided heart defects exists, and oligogenic or autosomal dominant inheritance with reduced penetrance has been suggested as the underlying genetic mechanism [119, 120]. Patients with BAV are at a risk of developing ascending aorta dilatation. Within families, some affected individuals only have the aortic dilatation, without associated structural cardiac abnormalities. Therefore, it is believed that in some families, BAV, aortic coarctation, associated cardiac lesions, and aortic aneurysms are part of a phenotypic spectrum and variable manifestations of the same gene defect. The underlying genetic basis is believed to be heterogeneous. Mutations in *NOTCH1* have been found in some patients who also had significant valve calcification and stenosis [121–123].

Other loci associated with H-TAD with or without BAV have been identified, and further locus heterogeneity is assumed. However, the genetic basis for the disorder remains unknown in the majority of families.

Differential Diagnosis

All entities described above show significant clinical and genetic overlap and should therefore always be considered in their respective differential diagnosis.

Genetic testing is important in the differential diagnosis and for some entities very characteristic clinical features can give the clue to the diagnosis as is, for example, the case for lens luxation, which is a feature unique to MFS. Diagnostic distinctions have prognostic value and may affect the clinical management and lifestyle of patients and are therefore of great importance. Table 17.5 provides an overview of the clinical and genetic findings of the most relevant differential diagnoses to be taken into account in

Table 17.5 Overview of clinical entities to be taken into account in the differential diagnosis of H-TAD (both syndromic and nonsyndromic). Distinctive cardiovascular and other clinical features are indicated in bold

Disorder	Gene(s)	Main cardiovascular features	Additional clinical features
Syndromic H-TAD			
Marfan	*FBN1* [124], *TGFBR1&2* [5, 11], *SMAD3* [5], *TGFB2* [21]	**Sinus of Valsalva Aneurysm**, Aortic Dissection, Mitral Valve Prolapse, Main Pulmonary Artery Dilatation, Left Ventricular Dysfunction	**Lens luxation**, skeletal features (arachnodactylia, pectus deformity, scoliosis, flat feet, increased armspan, dolichocephalia), dural ectasia, striae
Loeys–Dietz	*TGFBR1&2* [7, 8], *SMAD3, TGFB2* [20], *TGFB3*	**Sinus of Valsalva Aneurysm**, Aortic Dissection, Arterial Aneurysms and Dissections, **Arterial Tortuosity**, patent ductus arteriosus, atrial septal defect, bicuspid aortic valve	**Bifid uvula/cleft palate, hypertelorism**, pectus abnormalities, scoliosis, club feet
Vascular Ehlers–Danlos	*COL3A1*	**Arterial Rupture and dissection without preceding dilatation/ aneurysm**	**Gastrointestinal rupture, thin and translucent skin**, dystrophic scars, facial characteristics (Madonna face, thin lips, deep set eyes), club feet, uterine rupture
Multisystemic smooth muscle dysfunction syndrome	ACTA2 [26]	**Ascending aortic aneurysm**, aortic dissection, **patent ductus arteriosus**, aortic coarctation, aortopulmonary window, pulmonary arterial hypertension	**Congenital mydriasis**, malrotation of the gut, Moyamoya disease, periventricular white matter hyperintensities
Shprintzen–Goldberg syndrome	*SKI* [125, 126]	**Mild aortic root dilatation,** mitral valve prolapse	**Craniosynostosis**, distinctive craniofacial features, skeletal changes, neurologic abnormalities, mild-to-moderate intellectual disability
Arterial Tortuosity Syndrome	*SLC2A10* [127]	**Arterial Tortuosity**, Arterial Stenoses and Aneurysms, mild aortic root dilatation	Hyperlaxity of skin and joints, beaked nose, elongated face, micrognathia
Cutis Laxa Syndromes (autosomal dominant and recessive)	*ELN* [128], *FBLN4* [129], *FBLN5*	Mild aortic dilatation and tortuosity	Skin hyperlaxity, emphysema, downslanting palpebral fissures, inguinal hernia
Nonsyndromic TAAD			
	ACTA2 (10–21 %)	Thoracic Aortic Aneurysm/Dissection, cerebrovascular disease, coronary artery disease	Lack of Marfanoid skeletal features, **livedo reticularis, iris flocculi, coronary artery/cerebrovascular disease)**
	TGFBR1/2 (3–5 %)	Thoracic Aortic Aneurysm/Dissection	Lack of syndromal features
	FBN1 (3 %)	Sinus Valsalva Aneurysms	**Lack of syndromal features**
	MYLK	Thoracic Aortic Aneurysm/ Dissections often at low aortic diameters	
	SMAD3 (2 %)	Intracranial and other arterial aneurysms	
	$TGF_{\beta}2$	**Mitral valve prolapse**	
	NOTCH1	**Highly calcified Bicuspid Aortic Valve**	
	MYH11	**Patent ductus arteriosus**	
	PRKG1	**Aortic dissection at young age**	
	MAT2A	**Bicuspid aortic valve**	
	MFAP5	**Lone atrial fibrillation**	

H-TAD. The entities not already mentioned above are briefly listed below.

In an individual with Marfan-like skeletal manifestations, several disorders have to be considered. There is extensive overlap in clinical features between LDS and MFS, such as aortic root aneurysm and dissection, scoliosis, pectus deformity, and arachnodactyly. The main distinguishing features between LDS and MFS are the presence of the typical triad of hypertelorism, cleft palate/bifid uvula, and arterial tortuosity. Moreover, patients with LDS do not have ectopia lentis and the majority does not have the typical overgrowth of long bones as seen in MFS.

Mitral valve prolapse syndrome (MVPS) and MASS phenotype (MASS) are Marfan-like syndromes that exhibit aortic dilatation and mitral valve prolapse. Differentiating between MFS on the one hand and MVPS or MASS on the other hand can be particularly challenging in children and adolescents because of the age-dependant penetrance of many features in MFS. Unlike in MFS, the presence of ectopia lentis and aortic aneurysm precludes the diagnosis of MVPS and MASS. A recent study demonstrated that aortic dilatation in these clinical entities is mild and does not progress over time, as opposed to what is observed in MFS [130].

Arterial tortuosity syndrome, an autosomal recessive disorder, is characterized by generalized tortuosity of arteries, but can also present with other connective tissue findings such as cutis laxa, joint hypermobility, or skin hyperextensibility. Arterial stenoses may occur in the systemic as well as in the pulmonic vascular bed and mild aortic root dilatation has occasionally been reported. So far, no vascular ruptures have been reported in patients with this syndrome [131, 132].

Shprintzen-Goldberg syndrome is a rare craniosynostosis syndrome characterized by Marfanoid skeletal manifestations, exophthalmos, hypertelorism, downslanting palpebral fissures and other dysmorphic features, and developmental delay. The majority of patients do not show vascular involvement. Although a mutation in *FBN1* has been reported in two cases in the past presenting Marfanoid features with craniosynostosis [133], the actual underlying gene defect has recently been identified as heterozygous mutations in exon 1 of the *SKI* gene [125, 126]. The dysmorphic features in patients harboring *SKI* mutations are strikingly more severe than in those harboring *FBN1* mutations.

Congenital contractural arachnodactyly is a condition primarily affecting the skeleton with contractures of digits, elbows, and knees evident at birth, elongated long bones and kyphoscoliosis. In addition, the pinna of the ear is typically crumpled. Mitral valve prolapse and aortic root dilatation have been reported, with unknown frequency and generally in a milder degree than in MFS. Mutations in the *FBN2* gene account for about half of cases [134, 135].

Homocystinuria is a disorder caused by a deficiency of the cystathionine β-synthase (CBS) enzyme. Clinical features are variable, and include developmental delay, ectopia lentis, severe myopia, skeletal abnormalities (excessive height and long bone overgrowth), and thromboembolism. Homocystinuria is inherited in an autosomal recessive manner, the causative gene being the *CBS* gene, with mutations identified in over 95 % of patients [136, 137].

Clinical Therapy

Many aspects of treatment and medical management in H-TAD is based on the large body of knowledge obtained in MFS. This will therefore be discussed in more detail – only specific aspects related to treatment and management for the other entities will be mentioned below.

Marfan Syndrome

Medical Treatment

In patients with MFS, and especially in patients with aortic dissection, rigorous antihypertensive medical treatment is important, aiming at a systolic blood pressure less than 120 mm Hg. The most commonly prescribed drugs are β-adrenergic blockers, which reduce the aortic dilation rate in patients with MFS, due to its effects in reducing the blood pressure and the force of the LV ejection [138, 139]. Losartan, an angiotensin II receptor 1 blocker, might be an alternative or complementary therapy to β-blockers, since losartan reduces arterial pressure and potentially interferes with the pathophysiology of MFS by TGF-β antagonism. After evidence for effectiveness of losartan in a mouse model of MFS [140], a small pilot study in children and adults demonstrated a beneficial effect of losartan combined with β-blockers (n = 15) on aortic dilation rate compared with β-blockers alone (n = 13) after 35 months of echocardiographic follow-up [141]. Subsequently, eight randomized clinical trials were initiated to test losartan effectiveness; so far, four studies have been published [142]. The COMPARE trial confirmed these results in a larger cohort (n = 145) as measured by MRI, and additionally demonstrated the beneficial effect of losartan on the distal part of the aorta after aortic root surgery [143] . The Marfan Sartan trial evaluated the benefit of adding losartan to a high dose of β-blockers. Remarkably, in this cohort of 292 children and adults, aortic dilation rate was similar for the losartan- and placebo-treated group after 3.5 years of echocardiographic follow-up [144]. The Pediatric Heart Network Study demonstrated that both losartan and atenolol were equally effective in reducing aortic dilatation rate in a large, blinded trial including 608 children during 3 years by echocardiography [145]. The last published trial so far demonstrated in 140 Marfan patients aged 5–60 years that losartan was not inferior in respect to atenolol, and tended to be more favorable in the losartan monotherapy group when corrected for BSA or

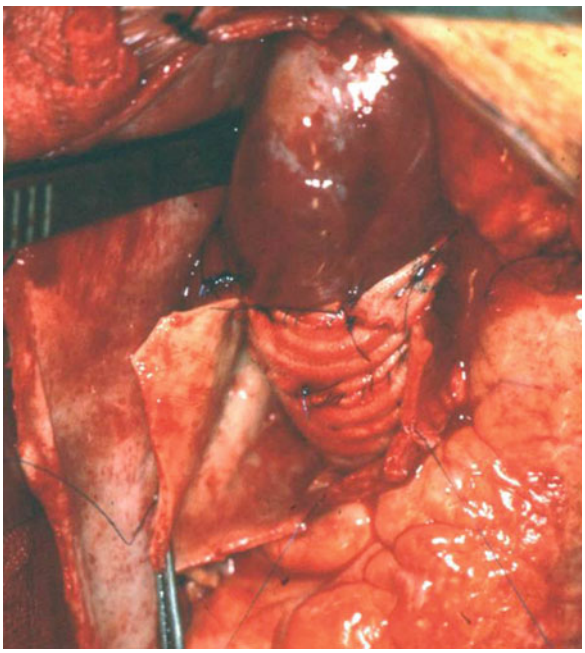

Fig. 17.14 Bentall procedure: the graft and mechanical valve have been incorporated and the coronary arteries have been re-implanted

Z-score measured by MRI over 3 years of follow-up [146]. The discrepancies in outcome between the studies may be explained by the different study designs [142, 147]. Until the results of the ongoing three trials and meta-analysis are known, we can conclude that losartan does not seem to be more effective in reducing the aortic dilation rate than a high dosage of β-blockers, but that losartan can safely be administered as an alternative or as an additive to β-blocker therapy, especially in patients with intolerance or side effects of β-blockers [142].

Surgical Treatment

The threshold diameter for aortic surgery is 50 mm for any level of the aorta, or 45 mm for the aortic root in combination with either a family history of dissection, progressive dilation of more than 2 mm/year, severe aortic or mitral valve regurgitation, or if pregnancy is desired. Lower thresholds for intervention may be considered according to BSA in patients of small stature or according to patient's preference [148]. On average, women have a smaller aorta (by 5 mm), which is only partly explained by a smaller BSA [149]. An indexed aortic diameter (adjusted for BSA) could be useful for operative decision making [150], surgery then would be indicated at an aortic diameter of 4.5 cm in patients with a BSA of 1.65 m^2, 5.0 cm at a BSA of 1.8 m^2, and 5.5 cm at a BSA of 2 m^2.

Over the past 30 years, the composite replacement of the aortic valve and ascending aorta ("Bentall procedure") (Fig. 17.14) has been a low risk and very durable operation for aortic root aneurysm in Marfan patients. In a series of 675 Marfan patients undergoing aortic root surgery, the operative mortality rate was 1.5 % for elective operations and 11.7 % for emergency operations [151]. However, in patients with initially normal aortic valves, valve-sparing operations with root replacement by a Dacron prosthesis and re-implantation of the coronary arteries into the prosthesis (the David procedure) have now become the preferred choice of surgery. Either type of aortic root replacement appears to be safe, reproducible, and associated with excellent 5–10-year results. Freedom from reoperation of the aortic valve after the David procedure was 94.8 %, with a slow progressive deterioration of aortic valve function after long-term follow-up [152]. A homograft or bioprosthetic valve may also be considered to avoid anticoagulant therapy.

Endovascular stenting is a minimally invasive surgical procedure with some advantages as compared to open repair in patients with abdominal aortic aneurysms [153]. Less information concerning the outcomes of endovascular stenting in Marfan patients is available. In ten Marfan patients treated with an endovascular stent procedure, the technical success rates were excellent. However, in Marfan patients with aortic dissection the use of endovascular stenting should be considered in life-threatening emergencies only, as a bridge to definite therapy, since these aortas dilate progressively, resulting in high endoleak rates, a 12 % mortality rate, and a 14–18 % need of a new surgical procedure [154–156].

Personalized external aortic root support (PEARS) is a novel surgical approach, stabilizing the aortic root and decreasing the risk of aortic dissection in patients with MFS. Although prospective follow-up data are currently lacking, in the first 30 selected patients operated with PEARS, the perioperative burden was less without any aortic or valvular event after 1.4–8.8 years of follow-up [157].

Follow-up

Optimal long-term outcome demands lifelong follow-up with imaging of the aortic root by means of echocardiography and the entire aorta by means of MRI at regular intervals. This is particularly true if a dissection has occurred and its stability is being monitored. Patients with mitral valve prolapse and moderate or severe mitral regurgitation should also be followed up with yearly echocardiography. Antihypertensive medical treatment, aiming at a systolic blood pressure less than 120 mm Hg, is important in all patients with MFS. After aortic dissection, systolic blood pressure should not exceed 110 mm Hg. Lifelong and regular follow-up of these patients requires involvement of trained specialists with ample expertise in a tertiary referral center.

Regular imaging of the aortic root and all other parts of the aorta is crucial in the follow-up of patients with MFS (Table 17.6).

Echocardiography in the parasternal long-axis view is mostly used for measurement of the aortic root (Figure). By

Table 17.6 Different imaging modalities

	Transthoracic echocardiography	Transesophageal echocardiography	Magnetic resonance imaging or computed tomography
Aortic root dilation	x		x
Presence and severity of aortic regurgitation	x	x	
Presence and severity of mitral regurgitation	x	x	
Reparability of mitral and aortic valves		x	
Dilation of the pulmonary trunk	x		x
Evidence of endocarditis	x	x	x
Presence of ascending aortic dissection	x	x	x
Intraoperative evaluation of aortic and mitral valve surgery		x	
Dimensions of major branches and arteries			x
Presence of lumbosacral dural ectasia			x
Aortic elasticity and tortuosity			x

means of Doppler echocardiography, the presence and hemodynamic consequences of aortic regurgitation, mitral valve prolapse, mitral regurgitation, and occasionally tricuspid valve prolapse can be assessed. MRI is particularly useful for imaging of the entire aorta, for patients with deformation of the chest wall and asymmetrical aortic roots (Fig. 17.15) [158]. Imaging of the entire aorta should be performed in every patient. When parts of the aorta are dilated, regular follow-up should be performed at least once every year. Even when the aorta shows no abnormalities, imaging should be repeated within 5 years. CT may be used when MRI cannot be performed because of contraindications or unavailability. With MRI, aortic elasticity can be measured and is often reduced. Aortic elasticity of the thoracic-descending aorta appeared to be an independent predictor for progressive-descending aortic dilation [159]. Holter monitoring should be performed in symptomatic patients, because ventricular arrhythmias, conduction disturbances, and sudden cardiac death may occasionally occur.

Lifestyle Advice

Patients should be advised to avoid both physical and emotional situations that increase blood pressure and heart rate dramatically. Furthermore, patients should be advised to avoid exertion at maximal capacity, competitive sports, contact sports, and isometric sports.

Endocarditis Prophylaxis

Endocarditis prophylaxis is recommended only in patients with a prosthetic valve and in patients with previous endocarditis, in patients with complete repair using prosthetic material (surgical or percutaneous) for up to 6 months after the procedure (until endothelialization), and ongoing only when a residual defect persists at the site of prosthetic material [160].

Pregnancy

For women with MFS, pregnancy presents a twofold problem: a 50 % chance that the child will be affected and an increased risk of aortic dissection during or (especially) shortly after pregnancy. Women with an aortic diameter above 45 mm are strongly discouraged to become pregnant before surgical repair. An aortic diameter below 40 mm rarely presents a problem, although a completely safe diameter does not exist. With an aorta between 40 and 45 mm, recent aortic growth and a family history of aortic events are important for advising pregnancy with or without preconception aortic repair [41]. A study on 55 pregnancies in 35 women with MFS showed an increased aortic growth rate of 0.3 mm/month, which decreased after delivery, but remained higher than the pre-pregnancy growth rate [42]. Two other smaller studies have reported no difference between the baseline and the pregnancy aortic dilation rate [161, 162]. Pregnancy, however, did influence long-term growth rate in Marfan women with an aortic root diameter above 40 mm (0.36vs. 0.14 mm/year in the childless Marfan women) [41].

In addition to cardiovascular complications, pregnancy in women with MFS is associated with a high rate of premature deliveries, premature rupture of membranes, and increased mortality in the offspring [161]. Especially the use of β-blockers is associated with intrauterine growth retardation [163].

Management of Other Manifestations

Periodic ophthalmic review is appropriate in childhood and early adolescence because ectopia lentis most often becomes evident in preschool years and may be slowly progressive. Adult patients should have ophthalmic screening at low frequency, because of the risk of glaucoma and cataracts. Most often, the myopia and lens dislocation can be managed with

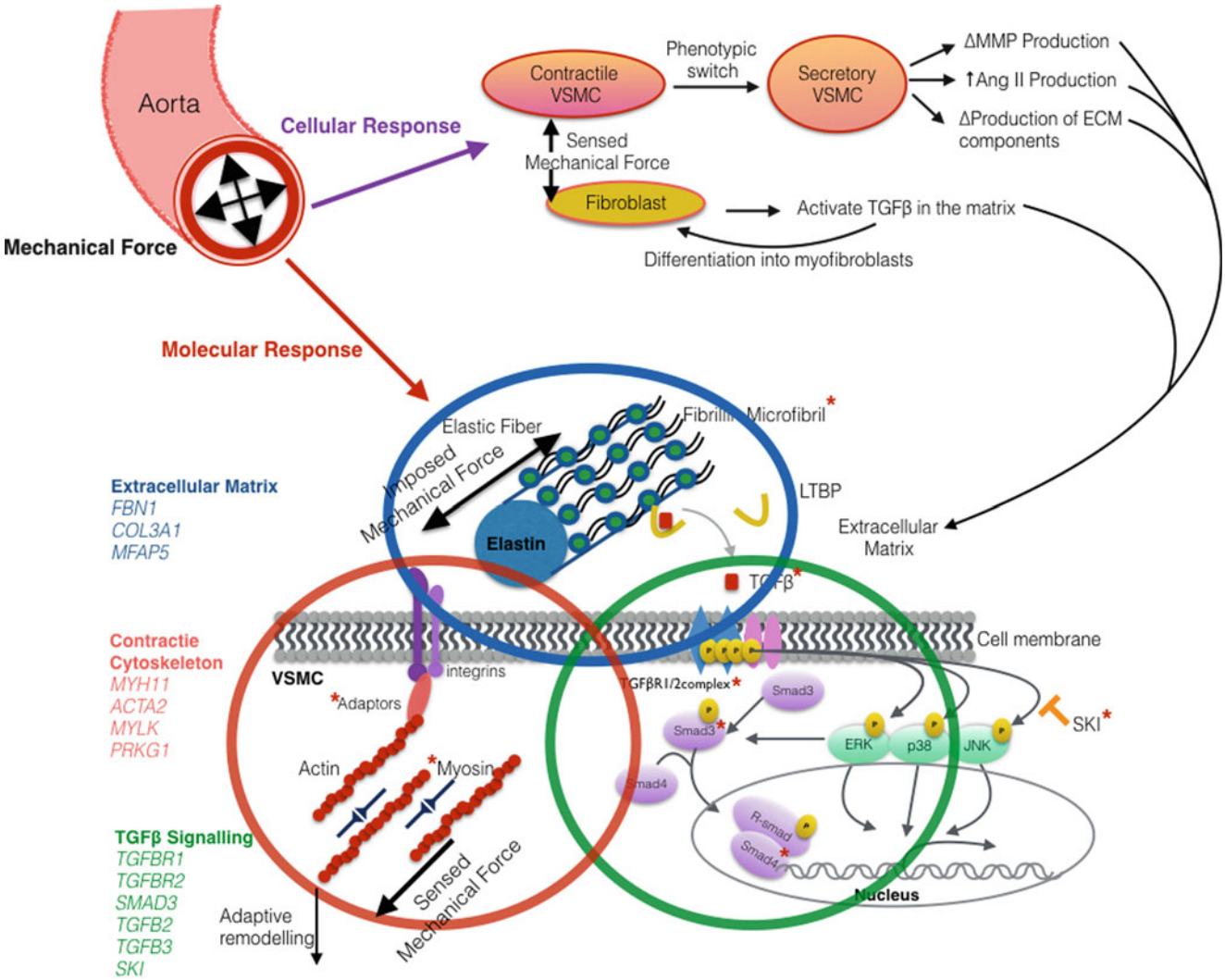

Fig. 17.15 Concept of mechanobiology underlying homeostasis in the thoracic aorta. Alterations, either due to higher imposed forces (hypertension) or due to (genetic) alterations in the various components required for proper sensing and/or transduction of the signal may lead to aneurysms/dissections. Mutations in the various components may affect the mechanical properties of the microfibrils or the signaling pathways, leading to an altered mechanotransduction signal and initiation of cellular response mechanisms including increased TGF-β signaling

eyeglasses. Sometimes, surgical intervention is necessary, including the implantation of artificial lenses. Growth should be monitored and the spine has to be evaluated for scoliosis. Surgical interventions are sometimes needed for stabilization of the spine or correction of severe pectus abnormalities, for medical as well as cosmetic reasons. The involvement of a skilled orthopedist is needed in these severe cases. Growth-reducing sex hormone therapy, starting before puberty, to limit adult height may be considered when an extreme height is anticipated.

Loeys–Dietz Syndrome

Careful follow-up of patients with LDS is mandatory. In 2014, extensive medical guidelines were provided both generalized and specific to LDS types 1–4, even though most experience is based on LDS types 1 and 2 [15]. We summarize the most important recommendations on the medical surveillance and treatments of LDS patients.

Cardiovascular Management

Many of the measures recommended for patients with MFS also apply to patients with LDS. Avoidance of isometric exercise, contact sports, competitive sports, and exercising to the point of exhaustion are indicated. Blood-pressure-lowering medication is advised in order to reduce hemodynamic stress. Beta-blockers are the standard-of-care treatment for individuals with syndromic aneurysmal disease but angiotensin receptor blockers can be considered due to their effects on the TGF-beta-signaling cascade. Also, angiotensin-converting enzyme (ACE) inhibitors have been

used in some institutions. All patients with LDS require at least yearly echocardiography to monitor the aortic root, ascending aorta, and heart valves. Congenital heart defects, arrhythmias, and heart failure should be managed according to protocols. Because the vascular pathology can be seen throughout the entire arterial tree, baseline surveillance includes imaging of the arterial tree from head through pelvis by magnetic resonance or CT angiography. Initially, these diagnostic investigations should be performed annually to determine the rate of progression. Thereafter, the frequency of head-to-pelvis imaging should be guided by progression rate, location, and size of aneurysm.

For LDS types 1–3, aortic root surgery in adolescents and adults is recommended at lower diameters than in other aortic aneurysm syndromes, that is, when maximal ascending aortic dimensions approach 4.0 cm, or when expanding more than 0.5 cm/year [15]. Surgical intervention at smaller dimensions may be indicated, based upon family history or personal risk assessments. In children, surgery should be considered when the maximal ascending aortic dimension exceeds the 99th percentile for age and BSA (in patients with severe craniofacial features) or 4.0 cm (in the presence of mild craniofacial features), and should preferably be performed when the size of the aortic annulus allows the insertion of a sufficiently sized graft to accommodate growth.

Valve-sparing aortic root replacement is the intervention of choice to avoid the need for anticoagulation. Aneurysms distant from the aortic root are often amenable to surgery as well. To prevent aneurysm rupture or dissection, elective vascular intervention in LDS patients should be considered when the arterial diameter exceeds two to three times the expected arterial diameter or in rapidly expanding arteries [15]. The reported risk of aortic surgery is approximately 1.7 % in LDS, and might be higher in the subset of patients with features overlapping with the vascular type EDS.

No guidelines have been suggested for LDS types 4 and 5; early studies suggest that the risk of aortic dissection may not occur at 4.0 cm but surgical intervention should be considered at 4.5 cm, depending on family history and/or adult aortic dimension. More phenotypic data are needed on these types of LDS in order to provide guidelines.

Management of Other Manifestations

In order to assess cervical spine instability, flexion-extension X-rays of the cervical spine should be performed at diagnosis. It is recommended to repeat imaging every 3–5 years during growth. Management of scoliosis and pectus deformities should be performed as for Marfan patient. McCarrick et al. also provided extensive guidelines for the management of allergies, gastroenterologic and nutritional, craniofacial, cutaneous, ophthalmologic, and pulmonary manifestations and the psychosocial implications of the diagnosis on a patient and its family.

Vascular EDS

Management of patients with vascular EDS is difficult due to the unpredictability of the events. The same rules with regard to blood-pressure control and lifestyle recommendations as mentioned for MFS apply here. Given the known tissue and especially vascular fragility, it is sensible to limit exposure to vigorous contact sports. Because of significant risks of arterial pathology and fragility, any sudden onset of unusual pain needs prompt and meticulous investigation, by both clinical examination and appropriate noninvasive imaging.

Antiplatelets and anticoagulants should be used only after careful consideration of the risks and benefits. This also applies to the use of non-steroidal anti-inflammatory drugs (NSAIDs). A Medical Alert Bracelet or the carrying of a note with essential medical information briefly notifies attending physicians of potential EDS vascular type complications. Some general guidelines for anesthesia and surgery have been suggested [164]. These include cross matching of adequate amounts of blood for transfusion, avoid intramuscular premedication, establish adequate peripheral venous access, and the avoidance of arterial lines and central venous lines whenever possible.

In general, the management of a vascular dissection or rupture should be conservative, whenever possible. Special surgical preventive measures need to be taken into account and surgery is more likely to be successful if the surgeon is well informed about the condition [45]. More recently developed techniques for endovascular repair have been used successfully in the right hands in small series [165]. The outcome of surgical management in such highly specialized centers is better than the average natural evolution but remains associated with high morbidity as demonstrated by complication in 46 % in a series of 31 patients from the Mayo clinic and in 33 % in nine patients from the Johns Hopkins hospital [45, 165]. Mortality of open surgery and endovascular procedures in a recent retrospective analysis was 30 and 24 %, respectively, and the overall mortality was 39 % [103]. As with all retrospective analyses, and especially in the case of rare disorders, selection bias cannot be excluded.

The pros and cons of serial vascular imaging are elusive, but are probably at least potentially beneficial. One should balance the risk of causing anxiety [103] against the potential benefits of detecting previously unknown aneurysms or progressive dilatations that are potentially treatable and potentially lifesaving [45]. So far, the reduction of mortality or morbidity by serial imaging capable of predicting potential early signs of arterial wall weakness has not been systematically explored in vEDS.

The only drug with a proven beneficial effect in vEDS is the selective B1 receptor blocker with B2 mimetic properties, celiprolol. A multicenter randomized open-label-controlled trial with celiprolol in 53 patients was ended

prematurely due to treatment benefit with a 36 % reduction in vascular events in the treated group as compared to the untreated group [166]. It needs to be acknowledged though that the occurrence rate of vascular events remained high at 20 % in the treated patient group.

MSMD Syndrome

No specific guidelines for the treatment and management of aortic aneurysms in patients with this recently identified disease entity are available yet – since aortic dissection has been reported in early stages, close surveillance and early surgical treatment seem appropriate. Treatment of associated lesions including patent ductus and coarctation repair needs to follow the respective guidelines.

Nonsyndromic H-TAD

When a patient is diagnosed with a thoracic aortic aneurysm, clinical evaluation including family history, physical examination, and ocular assessment is recommended to exclude underlying syndromic entities.

In contrast to patients with syndromic H-TAD entities who may come to medical attention through associated clinical manifestations, patients with nonsyndromic forms are often diagnosed on presentation with aortic events, which are overwhelmingly acute aortic dissections. In patients with *ACTA2* mutations, (complex) type B dissections appeared to be more common than type A aortic dissection, leading the authors to conclude that anyone presenting with an acute aortic dissection without syndromic features and with a family history of the disease, along with young people with aortic dissections, especially type B aortic dissections, should raise a suspicion for an underlying *ACTA2* mutation. Because these individuals present with complicated dissections often requiring surgical intervention, transfer to a tertiary-care center should be considered [113]. Since aortic disease in *ACTA2* mutation carriers often extends to the distal thoracic aorta, more extensive imaging with CT/MRI in these patients is recommended. In patients with *ACTA2* mutations, more extensive involvement of the aortic root, ascending aorta, and aortic arch should be taken into account when planning thoracic aortic repair, and consideration should be given to repairing all these regions even if a specific location is not yet enlarged [113].

Medications that reduce hemodynamic stress, such as β-adrenergic blockers**,** are recommended for individuals with H-TAD. Careful follow-up is warranted. Aortic surgery is recommended at ascending aortic diameters similar to MFS [92], and should be individualized for each patient, taking into account family history, rate of aortic growth, underlying gene defect, etc.

The threshold for surgical intervention may be guided by the aortic size at which other family members have had aortic complications, if known. If not known, then a size threshold of 4.5–5.0 cm for the ascending aorta and 5.5–6.0 cm for the descending thoracic aorta is reasonable because of the high complication rate of thoracoabdominal aortic surgery at this level [167]. The current guidelines of the American College of Cardiology [65] recommend prophylactic surgery based on different scenarios according to the underlying gene as different clinical courses are expected in patients with different gene mutations. The more recent European Society of Cardiology (ESC) guidelines on the management of aortic disease have not adopted these rules [148]. Recent studies indicate that the course of aortic disease related to mutations in the *TGFBR* genes appears to be less aggressive in at least a subset of patients [13, 43]. Studies are ongoing to assess whether clinical or biochemical parameters could help us to better estimate the risk in an individual patient.

Since TAA in the context of BAV appears to have more benign course with a subpopulation showing no growth over a 3-year period and a lower risk for dissection [168–170], thresholds for surgery of the ascending aorta in this setting are increased to 5.5 cm. In patients with additional risk factors including positive family history or increased growth rate (>3 mm/year), a threshold of 5.0 cm is recommended [148].

Molecular Diagnostics

Molecular confirmation of the correct diagnosis is increasingly important for gene-tailored patient management in H-TAD patients. Traditional genetic testing involved Sanger sequencing of a number of genes in a step-wise manner to try to identify the causal mutation. Currently, many genetic laboratories have introduced next-generation sequencing (NGS) into diagnostics, which changed the whole field of genetics. With NGS, or massive parallel sequencing, millions of small DNA fragments can be sequenced at the same time, creating a massive pool of data. Bioinformatic analyses are used to piece together these fragments by mapping the individual reads to the human reference genome providing accurate data on DNA variation. NGS can be used to sequence all 22,000 coding genes (whole exome) or targeted to small numbers of individual genes (panels).

Several studies have shown that the NGS approach including all syndromic and nonsyndromic genes for H-TAD is by far less time-consuming than consecutive Sanger sequencing of each TAD gene and as a consequence the labor costs also diminished proportionally.

Marfan Syndrome

The mutation detection rate for the *FBN1* gene in patients fulfilling the diagnostic criteria for MFS is about 90 % [171]. In the remaining 10 % of patients, no causal mutation or deletion in *FBN1* can be identified. In those cases, the causative mutation in *FBN1* might not be detectable with conventional techniques, or these patients may harbor a mutation in another (unknown) gene.

In a few *FBN1*-negative patients fulfilling the criteria of MFS, as well as in patients with "incomplete" MFS, mutations in genes involved in the transforming growth factor-β (TGF-β) pathway have been identified. A discriminative feature with MFS caused by *FBN1* mutations is ectopia lentis, which seems to be an exclusive feature for patients with *FBN1* mutations.

From a practical standpoint, single gene mutation screening of the *FBN1* gene may be considered in patients fulfilling the diagnostic criteria for MFS including ectopia lentis. In all others, more extensive NGS panel sequencing or targeted-exome sequencing including the *FBN1* gene may be more appropriate.

Loeys–Dietz Syndrome

In rare cases, careful workup by an experienced clinical geneticist or a cardiologist can help to make the correct clinical diagnosis and predict the molecular cause. However, LDS genes frequently lead to a range of phenotypes and in a majority of cases the features are less obvious or are evolving in children or young adults. Moreover, several LDS genes have been implicated in the pathogenesis of NS H-TAD.

In order to overcome these hurdles, targeted NGS panel of genes involved in H-TAD or whole-exome sequencing (WES) with a filter for the known H-TAD genes are advised in LDS patients.

Vascular EDS

As is the case with the other syndromes, isolated screening of the *COL3A1* gene may be considered in those cases with a very typical phenotype, for example, in those cases presenting with GI rupture or extensive ruptures/dissections in different arterial beds. In all other cases, extended genetic screening using NGS panels of targeted-exome sequencing seems more appropriate.

MSMD Syndrome

In patients with this distinct phenotype, specific mutational analysis of the *ACTA2* R179 mutation is indicated as a first step and if negative to be complemented with more extensive screening.

Nonsyndromic H-TAD

In several countries, target NGS of a panel of genes involved in H-TAD or WES with a filter of the known H-TAD genes is available. Deleterious mutations in cohorts of both syndromic and nonsyndromic H-TAD adults are identified in 4–27 % of patients using a targeted NGS panels with different numbers of TAAD genes [5, 53, 172, 173].

Lists of "core genes" and "additional genes" to be tested in the setting of H-TAD are provided in Arslan-Kirchner, M. et al. 2015. Clinical utility gene card for: Hereditary thoracic aortic aneurysm and dissection (TAAD) including NGS-based approaches. *European Journal of Human Genetics* [174].

The downside of more extended genetic screening is that many variants of unknown significance (around 20 %) are identified and it is challenging to assign causality with certainty to the found variants [53, 173]. Collaborative international networks including the ClinGen initiative are currently being installed with the aim to improve strategies for variant curation [175].

Molecular Genetics and Specific Consequences of the Genotype

Marfan Syndrome

Molecular Genetics

FBN1 is a large gene with 65 exons coding for fibrillin-1 [176, 177], a 320-kD glycoprotein consisting of 2871 amino acids. Fibrillins are large (~350,000 MW) structural macromolecules that contribute to the integrity and function of all connective tissues. They are considered to be "structural macromolecules" because, like the collagens, the fibrillins form fibers that are visible in transmission electron micrographs.

Fibrillin-1 is highly conserved among different species. The polypeptide comprises 47 repeated cysteine-rich motifs resembling epidermal growth factor (EGF-like) and an 8-cysteine motif (TB/8-cys). Forty-three of the 47 motifs contain a consensus sequence for calcium binding and are termed calcium-binding EGF-like motifs (cbEGF-like) (Fig. 17.1) [178, 179].

Fibrillin molecules polymerize to form the microfibrils, a constituent of the ECM. Microfibrils can associate with elastin to form elastic fibers. The microfibrils and elastic fibers have a widespread distribution in connective tissue

throughout the body, including the skin, vascular wall, tendons, fascia, alveolar wall, and ciliary zonules that suspend the ocular lens, where they provide force bearing structural support needed by these individual organ systems. Fibrillin microfibrils are organized to best suit the functional integrity of the tissue: for example, in tendons elastic fibers run parallel to the long axis whereas in muscular arteries, elastic fibers encircle the lumen (Sakai et al., Gene, in press).

In addition, it has become increasingly clear that fibrillin-rich microfibrils have functions that are not directly related to structural integrity but rather have to do with homeostasis of the elastic matrix, matrix-cell attachments, and regulation of cytokines [178, 179].

To date, about 2000 different mutations have been reported in *FBN1*. Most of the reported mutations are missense mutations affecting the conserved cysteine residues or residues of the consensus sequence of the cbEGF-like motifs. Nonsense mutations, mutations of splice sites associated with exon skipping, and more rarely small deletions are also found.[8] The majority of mutations are private mutations that are unique to a family or an individual patient. Approximately 25 % of cases of MFS are caused by de novo mutations. Most of the infants with a severe form of MFS are isolated (de novo) cases, which reflects the low likelihood of these patients to survive to reproductive age.

Many mutations in *FBN1* are believed to adversely affect the normal, wild-type gene product, that is, they are thought to have a dominant-negative effect. However, MFS and related disorders can also be caused by mutations that prevent or reduce the expression of the mutant allele. Haploinsufficiency may therefore also contribute to the pathogenesis [180].

Pathophysiology

The current knowledge of the role of fibrillin-1 in the pathogenesis of aortic aneurysm formation is at least trifold: (1) structural role in elastic fiber composition; (2) regulator of TGF-β signaling; and (3) role in mechanotransduction.

Early theories of disease pathogenesis in MFS assumed that the manifestations of the disease were caused by the loss of structural integrity of affected tissues, due to mutant fibrillin-1 in the microfibrils. It was thought that this resulted in weak tissue that could not withstand enduring stress over time. Some features of MFS could indeed be explained by these models, such as aortic aneurysms, ectopia lentis, and dural ectasia. However, other features including bone overgrowth, craniofacial features, and myxomatous changes of the mitral valve did not seem compatible with these theories. It has become increasingly clear that other mechanisms indeed contribute to the pathogenesis of MFS.

Animal studies have shown that fibrillin-rich microfibrils have an essential role in homeostasis of the elastic matrix during postnatal life [181]. Elastic fibers have intimate connections with adjacent vascular endothelial cells and SMCs, mediated by fibrillin-1. As a result of defective fibrillin-1, these connections may be absent or inadequate. In mice, this resulted in abortive matrix remodeling, characterized by overproduction of multiple structural components and matrix-degrading enzymes, including metalloproteinases 2 and 9. Subsequent events are infiltration of inflammatory cells, intimal hyperplasia, elastic fiber calcification, and structural collapse of the vessel wall leading to aneurysm formation [181]. These manifestations have also been observed in pathologic specimens from patients with MFS [182].

In addition to their structural function, fibrillin-rich microfibrils also play a significant role in the regulation of cytokines. TGF-βs are multifunctional cytokines that can induce many cellular events including proliferation, differentiation, cell cycle arrest, programmed cell death, and matrix deposition [183].The activation of TGF-β is limited by fibrillin-1. It was therefore hypothesized that abnormal fibrillin-1 or reduced levels of fibrillin-1 result in excessive amounts of active TGF-β [184]. Subsequently, an increased output of TGF-β-responsive genes, such as collagen and connective tissue growth factor, and altered cellular events lead to the phenotypic manifestations of MFS. TGF-β is a multifunctional peptide that controls proliferation, differentiation, and other functions in many cell types. Fibrillin-1 is homologous to the family of latent TGFβ-binding proteins (LTBPs), which serve to hold TGF-β in an inactive complex in various tissues, including the ECM [185]. Indeed, fibrillin-1 was shown to bind TGFβ and LTBPs [186, 187]. Hence, it was hypothesized that mutations in fibrillin-1 could lead to perturbed sequestration of the inactive TGF-β complex [184]. Indeed, increased TGF-β signaling has been demonstrated in several tissues in MFS patients and murine models for MFS.

Surprisingly, more recent studies demonstrated that an *Fbn1* mouse in which the LTBP binding site was deleted (*Fbn1H1Δ*) did not present features of MFS [188]. This observation refuted the importance of TGF-β sequestration by fibrillin-1 and an alternative hypothesis was proposed whereby mutant microfibrils influence TGF-β activation in a different way. Increased TGF-β signaling is now considered as the result of a final common pathway in the disease process. The role of the TGF-β-signaling pathway may also vary during the dynamic transition from aortic aneurysm predisposition to end-stage disease, such as dissection [189].

Moreover, fibrillins do not only contribute to cell signaling in the vessel wall through regulation of growth factor bioavailability, but these microfibrils are also important in *mechanotransduction* from the endothelium and ECM to the vascular SMCs. The process of mechanotransduction is critical to maintain homeostasis within the aortic wall by regulating aortic remodeling in response to hemodynamic stress. Mutations in fibrillin-1 may perturb this mechanism [190, 191]. Hence, a recent hypothesis states that the

mechanical state of the matrix is sensed by cells in the vessel wall, which consequently send a signal through integrins and the cytoskeleton, resulting in inappropriate remodeling and aneurysm formation and this via a common pathway of inappropriate TGF-β signaling. A schematic overview of the pathogenesis is provided in Fig. 17.15.

Genotype–Phenotype Correlation

No definitive genotype–phenotype correlations seem to be present in MFS [192, 193]. Therefore, the identification of a particular mutation in a patient has little prognostic value and cannot determine individual management. However, some generalizations in genotype–phenotype correlations can be made – an overview is provided in Table 17.7. Mutations causing in-frame loss or gain of central-coding sequence due to deletions, insertions, or splicing errors are associated with more severe disease. By contrast, mutations that create a premature termination codon leading to rapid degradation of the transcript can be associated with mild manifestations that may not fulfill the criteria for MFS [194–196]. The mutations that have been found in patients with a neonatal presentation of severe and rapidly progressive MFS, the so-called "neonatal MFS," are located in the central portion of the *FBN1* gene between exons 24 and 32, although many other patients with mutations in this region have a classic or mild phenotype [197].

A very recent study of *FBN1* mutation patients with MFS indicated that MFS patients with a haploinsufficient mutation are at an increased risk for cardiovascular death and aortic dissection compared to patients with a dominant-negative effect mutation [198].

Table 17.7 Main genotype–phenotype correlations in Marfan syndrome caused by *FBN1* mutations

Type of *FBN1* mutation	Phenotype
Cysteine substitutions in EGF-like domains [199]	High incidence of ectopia lentis; severe early onset in exons 26–32
Premature termination codons (PTCs) [200]	Low incidence of ectopia lentis; high incidence of large joint hypermobility; high incidence of skin striae; higher incidence of aortic dissection?
First 15 exons; arginine to cysteine mutations [201]	Predominant ectopia lentis
All mutation types [202]	Cysteine mutations correlate strongly with ectopia lentis; PTC mutations are associated with severe skeletal and skin phenotypes; mutations in exons 24–32 are associated with severe disease
Pediatric cohort; 33 % of mutations occurred in exons 24–32; incidence of PTCs was smaller than in adult cohort [2]	Lethal neonatal Marfan syndrome is a genuine clinical entity; clinical manifestations increase with age
All mutation types [203]	"Incomplete" or mild Marfan syndrome was associated with mutations in exons 59–65; mutations at the ends (in exons 1–15 and 59–65) may be milder than mutations in between
All mutation types [204]	Truncating and splicing mutations were associated with aortic events
Dominant Negative (DN) vs Haploinsufficient (HI) *FBN1* mutations [198]	Marfan syndrome patients with an HI mutation are at increased risk for cardiovascular death and aortic dissection compared to patients with a DN mutation

Loeys–Dietz Syndrome

Molecular Genetics

LDS types 1 and 2 are caused by mutations in the TGFβ receptor type I (*TGFBR1*) gene and TGFβ receptor type 2 (*TGFBR2*) gene. Several hundreds of inactivating mutations have been identified in *TGFBR1* or *TGFBR2*. These are mostly missense substitutions of evolutionary conserved residues that encode the intracellular serine threonine kinase domain of the receptors. No differences exist in clinical manifestation of patients with mutations in either gene. Mutations in *TGFBR1* and *TGFBR2* have also been reported in patients with familial TAAD, without the other features of the LDS [14] as well as in patients fulfilling the criteria for MFS [11]. There are no apparent differences in the type of mutations in these disorders as opposed to those found in LDS.

There is considerable intrafamilial variability in phenotype in LDS, and multiple cases of apparent non-penetrance have been reported [7, 9]. Most cases of severe LDS are due to de novo mutations.

LDS type 3 is caused by mutations in the SMAD family member 3 (*SMAD3*). Nowadays, 37 different *SMAD3* gene mutations have been published in the literature but many more unpublished *SMAD3* mutations have been identified. The *SMAD3* gene contains three main functional domains, namely the MH1, MH2 domain, and the linker region and mutations occur throughout the entire 9 exon-containing gene. The mutation spectrum encompasses both truncating and missense mutations, with the latter clustering within the MH2 protein domain [17]. The most likely effect of these mutations is a loss of function, with TGF-β signals not being propagated via SMAD3. Until now, no clear genotype–phenotype correlation has been established.

LDS types 4 and 5 are caused by mutations in the TGF-β-binding ligands *TGFB2* and *TGFB3*. Various *TGFB2/3* mutation types, that is, missense, frameshift, nonsense, and splice site mutations have been reported, most likely leading to loss of function of the respective protein. In LDS type 4, at least 17 distinct *TGFB2* mutations including three large deletions have been published In LDS type 5 thus far 11 distinct

TGFB3 mutations in 48 patients have been identified. Position p.Arg300 of the *TGFB3* gene seems to be a mutational hotspot since three different mutations (Arg300Gly, Arg300Gln, and Arg300Trp) are identified at this position in six independent individuals.

For all types of LDS, it is unknown why some mutations cause a severe LDS phenotype while others account for the mild end of the disease spectrum.

In a minority of cases, LDS results from a new gene mutation (de novo) and occurs in people with no history of the disorder in their family. More frequently, an affected person inherits the mutation from one affected parent.

Pathophysiology

All LDS genes are essential in TGF-beta signaling. At the aortic tissue level, a recurrent pattern of enhanced TGF-β signaling is observed despite a loss of function at the molecular level. Histology and immunohistochemistry of aorta fragments of LDS cases show upregulation of both upstream ligands and downstream targets of the TGF-β pathway. This observation is similar to patients with other syndromic and nonsyndromic aneurysms such as MFS, arterial tortuosity syndrome, aneurysms associated with BAV, and degenerative aneurismal aortic disease. This clearly indicates the existence of common (TGF-β-related) pathogenic mechanisms leading to arterial wall disease.

The precise mechanisms underlying the attenuation of TGF-β signaling remain elusive and a matter of debate. Several mechanisms that could explain this TGF-β paradox have been proposed but need experimental validation. These theories include altered receptor trafficking, impaired autoregulation of TGF-β signaling, alternative signaling cascades, or nonautonomous cellular events.

Vascular EDS

COL3A1 located on chromosome 2q24.3–q31 encodes type III collagen. There are N- and C-terminal propeptides coded, respectively, by five exons for the N propeptide and four exons for the C propeptide and in between an uninterrupted perfect triple helix coding for Gly XY triplets, in which X or Y are frequently lysine (4 %) or proline (10 %). Mutations of the triple helix are generally caused by missense point mutations converting glycine to a larger amino acid. Such errors distort the dimensions of the triple helix, interrupting helical winding and leading to incorporation of mutant alpha chains into mature triple helices. This leads to diminished collagen secretion and assembly, resulting in weakened tissues containing the mutant molecules. Similar effects arise from exon skips in which shortened triple helices are similarly disruptive. In the case of stop codon mutations or large deletions, dosage effects are exerted, by mechanisms of haploinsufficiency. An extensive list of *COL3A1* mutations and polymorphisms can be found at https://eds.gene.le.ac.uk/home.php?select_db=COL3A1

Pathophysiology

Despite the fact that vEDS and its underlying genetic defect have been known since early 1990s, there has been limited progress on understanding the disease mechanism beyond that of connective tissue weakness due to structural defects or reduced amounts of type III procollagen. Based on the recent established role of the altered TGFβ signaling in MFS and related thoracic aortic aneurysm disorders, the role of this mechanism was studied by Morissette and colleagues. They observed that mutations in *COL3A1* do not seem to alter the TGFβ-signaling pathway in dermal fibroblasts from vEDS patients [205]; data regarding TGFβ signaling in arterial tissue are unfortunately not yet available.

Genotype/Phenotype Correlation

Two recent studies [206, 207] indicated that individuals with missense mutations substituting glycine and splice site or in-frame insertions–deletions have a more severe and earlier onset of the disease than *COL3A1* null mutations, non-glycine mutations, or mutations in the N- or C-terminal part of *COL3A1*. The latter groups also had less digestive complications. Within the glycine-substituting group, substitutions for serine and arginine seem to have a better outcome than those for valine and aspartic acid [207] (Pepin et al.).

COL3A1 mutations are occasionally encountered in patients presenting with NS H-TAD [5, 208].

MSMD Syndrome

In patients presenting the characteristic phenotype of neonatal PDA, fixed congenital mydriasis, Moyamoya such as cerebrovascular disease and TAA, targeted mutation analysis of the *ACTA2* R179A mutation may be considered. The R179 mutation is located close to a key protein–protein interaction site on the macromolecular surface of α-actin, leading to the assumption that the mutation may disrupt critical interaction and disrupt downstream-signaling events necessary for SMC function. Analysis of the nucleotide sequence around the mutation failed to identify a mutable motif to explain the increased frequency of this mutation. Therefore, the recurrent identification of this mutation may be due to a recruitment bias resulting from the unique phenotype in these patients [26].

Nonsyndromic H-TAD

Molecular Genetics

As already mentioned and as indicated in Table 17.1, the genetic background of NS H-TAD is heterogeneous. In the majority of patients and families, the underlying genetic

basis is not found. Mutations in *FBN1, COL3A1, TGFBR1, TGFBR2, SMAD3, and TGFB2* have been reported in small proportions of patients with NS H-TAD [5, 9, 19, 115].

Pathophysiology

The identification and characterization of these genes suggests that altered ECM function and increased TGF-β signaling plays a role in pathogenesis. Another mechanism underlying NS H-TAD is through interaction with the vascular smooth muscle contractile apparatus. Several hypotheses exist to explain the link between H-TAD and impaired vascular SMC contractility. Mutations in genes involved in the SMC apparatus (*MYLK*, *MYH11*, *ACTA2*, and *PRKAG1*) may lead to upregulation of stress and repair pathways. In the vascular wall of patients harboring a *MYH11* mutation, ACE and insulin-like growth factor-1 (IGF-1) are upregulated [34]. Upregulation of ACE and IGF-1 activates the angiotensin II, phosphoinositide-3 kinase (PI3K), and canonical and noncanonical TGFβ pathways (SMAD2/3 and ERK, respectively), subsequently leading to increased SMC contractility and proliferation and upregulation of TGFβ [34, 209]. TGFβ can in itself induce a contractile phenotype of vascular SMCs, since it regulates transcription of contractile genes [210–212]. TGFβ upregulation has been demonstrated in the aortic wall of patients with missense mutations in *ACTA2* and *MYH11* [30]. However, Pannu and colleagues did not find increased CTGF staining or increased expression of CTGF and TGFβ1 in aortic tissue and SMCs of patients harboring *MYH11* mutations [14]. For *MYLK, FLNA,* and *PRKG1*, no data have been reported so far on a possible association with TGFβ signaling.

The cytoskeleton also plays a role in maintaining the ECM integrity. The cytoskeleton is involved in the assembly of fibronectin fibrils via integrin receptors [213]. A stable fibronectin fibrillar matrix is in turn indispensable for C-terminal association of fibrillin-1 monomers into bead-like structures and their linear assembly into microfibrils [214]. The inability of the contractile apparatus to exert its function can consequently affect the integrity of the ECM and this may thus indirectly trigger cellular response mechanisms, including increased TGFβ signaling.

Mutations leading to perturbed contractility of vascular SMCs may also impair mechanical homeostasis and lead to maladaptive remodeling, since mechanosensing requires intact load-bearing structures [191].

Genotype/Phenotype Correlations

In the era of personalized medicine, it is very tempting to say that the genotype may guide us in predicting the phenotype. Great caution is, however, warranted, especially since no large series of any of these diseases are currently available. An example to illustrate this is found in the identification of mutations in the *TGFBR1/2* genes. Initial reports of patients harboring these mutations indicated that the aortic phenotype was markedly more aggressive than the one common phenotype observed in MFS [7]. Subsequent observations could, however, not confirm these findings [13, 43], indicating that the phenotype in patients with *TGFBR* mutations may vary widely from severe syndromic presentations as in LDS to milder nonsyndromic phenotypes. Currently, efforts are undertaken to collect data on a larger scale in order to obtain data from representative cohorts of patients.

Within each H-TAD gene, genotype-specific phenotypes have been reported as, for example, in the *ACTA2* gene with the severe phenotype associated with the R179H mutation [26]. Similarly, more severe phenotypes have been reported for other substitutions at that position and for the R258 mutations [113].

Family Screening and Follow-Up in Relatives

A molecular diagnosis provides the opportunity of carrier testing in asymptomatic family members. However, genetic testing can have potential negative ethical, legal, and social implications. During pretest genetic counseling, the potential risks, benefits, and limitations of genetic testing need to be discussed, facilitating autonomous decision making. Predictive genetic testing of minors is generally accepted for childhood-onset conditions if preventative or therapeutic measurements are available to reduce morbidity or mortality. Accordingly, predictive testing seems justified in asymptomatic children at risk for MFS, LDS, and other H-TAD entities. Although clinical expression of these conditions is highly variable and age-dependent, severe cardiovascular manifestations have been observed in early childhood and children may benefit from early prophylactic treatment. Early knowledge of the diagnosis may also be useful for the timely treatment of skeletal and ocular manifestations in these disorders.

Testing in children for adult-onset disorders including vascular EDS is more controversial and needs to be decided on an individual basis. Patients (and their parents) should be informed of the fact that 12–24 % of individuals have a major complication by age 20 years [206] and that preventive treatment with celiprolol may be considered before the age of 18 years. Other potential benefits of testing for vEDS in minors include (1) elimination of concern for those children who do not have the familial *COL3A1* pathogenic variant; (2) awareness of and preparedness for potential complications; and (3) restriction of high-impact sports and high-risk activities for those with the pathogenic variant [215]. Given the opportunity to consider testing for children at 50 % risk of having inherited the pathogenic variant, parents usually do not wait until a complication arises or until the child reaches adulthood for a test to be performed [215].

In NS H-TAD, the medical benefit for genetic testing in children is less obvious and genetic testing is usually

postponed until adulthood to protect the child's future autonomy. We strongly recommend that testing of children always involves psychosocial support. In the many families without an identified causal mutation, it is not possible to determine which individuals are at risk of developing aneurysms and dissection. Therefore, all first-degree relatives (children, siblings, and parents) of H-TAD patients are advised to undergo regular aortic imaging, as recommended by the ESC and the American College of Cardiology (ACC) guidelines [65, 148]. There are no published recommendations at which age to start screening in children – in practice, we advise to initiate screening at 12–14 years; in families with early-onset dissections, this threshold may be lowered (10 years lower than the age at which dissection occurred in the family member). In clinical practice, clinicians have to weigh the probability of H-TAD diagnosis in the index patient. Family history, age, presence of hypertension, and aortic diameter may play a role in the decision-making process of the cardiologist. When a patient is highly suspected for H-TAD first-degree relatives should undergo regular aortic imaging, once every 5 years. In case of an increased aortic diameter, rapid growth, clinical suspicion of a more aggressive phenotype (e.g., LDS), or family history, more frequent monitoring can be considered. Echocardiography of the ascending aorta is indicated in all, but additional imaging by means of MRI /CT may be considered in high-risk patients.

Prenatal diagnosis (PND) and preimplantation diagnosis (PGD) can be offered to those H-TAD patients with a known mutation in most countries. A French study in MFS patients indicated that a majority of patients (74 %) was in favor of prenatal testing. The opinion of caregivers varied but most of them agreed that these issues should be addressed in a multidisciplinary team [216].

Summary/Take-Home Message

Heritable thoracic aortic disorders (H-TAD) comprise a heterogeneous group of disease entities with significant clinical and genetic overlap. Both syndromic and nonsyndromic forms are recognized. Identification of the underlying genetic defect is important for confirmation of the diagnosis and may help in risk stratification and guidance of management. A substantial proportion of (mainly nonsyndromic) families with H-TAD remains in whom the underlying gene defect has not been identified yet and in these cases clinical imaging of patients and relatives is the mainstay of follow-up.

NGS techniques have significantly improved the diagnostic yield in patients with H-TAD and NGS panel sequencing and/or targeted-exome sequencing is the method of choice for mutation screening.

Life expectancy in H-TAD patients is determined by the risk for aortic dissection and depends at least partly on the underlying molecular diagnosis. Timely diagnosis and treatment are crucial and should be performed in a multidisciplinary setting. Prophylactic aortic surgery is beyond doubt the most lifesaving treatment modality and more research is needed to better define the optimal thresholds for – especially descending – aortic surgery in the individual patient.

Take-Home Messages

- At least 20 % of patients presenting with thoracic aortic disease have an affected relative, and a detailed family history with clinical evaluation of first-degree relatives is an essential component of the evaluation.
- Both syndromic and nonsyndromic entities of H-TAD are recognized with significant clinical and genetic overlap. Careful clinical assessment of all patients is required to exclude syndromic entities. Clinical molecular testing is appropriate after pre-test genetic counseling.
- Over 15 genes have been identified so far that can cause Mendelian forms of thoracic aortic disease when mutated. These encode for components of the extracellular matrix, the TGFβ-signaling pathway or the smooth muscle cell contractile apparatus.
- Mutations lead to altered structural and functional properties in the aortic wall through interaction with mechanobiology.

References

1. Loeys BL, Dietz HC, Braverman AC, Callewaert BL, De Backer J, Devereux RB, et al. The revised Ghent nosology for the Marfan syndrome. J Med Genet. 2010;47(7):476–85.
2. Faivre L, Masurel-Paulet A, Collod-Beroud G, Callewaert BL, Child AH, Stheneur C, et al. Clinical and molecular study of 320 children with Marfan syndrome and related type I fibrillinopathies in a series of 1009 probands with pathogenic FBN1 mutations. Pediatrics. 2009a;123(1):391–8 Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=19117906&retmode=ref&cmd=prlinks.
3. Milewicz DM, Michael K, Fisher N, Coselli JS, Markello T, Biddinger A. Fibrillin-1 (FBN1) mutations in patients with thoracic aortic aneurysms. Circulation. 1996;94(11):2708–11.
4. Pepin M, Schwarze U, Superti-Furga A, Byers PH. Clinical and genetic features of Ehlers-Danlos syndrome type IV, the vascular type. N Engl J Med. 2000;342(10):673–80.
5. Campens L, Callewaert B, Muiño Mosquera L, Renard M, Symoens S, de Paepe A, et al. Gene panel sequencing in heritable thoracic aortic disorders and related entities – results of comprehensive testing in a cohort of 264 patients. Orphanet Journal of Rare Diseases. 2015a;10(1):9.
6. Barbier M, Gross M-S, Aubart M, Hanna N, Kessler K, Guo D-C, et al. MFAP5 loss-of-function mutations underscore the involvement of matrix alteration in the pathogenesis of familial thoracic aortic aneurysms and dissections. Am J Hum Genet. 2014;95(6):736–43.

7. Loeys BL, Schwarze U, Holm T, Callewaert BL, Thomas GH, Pannu H, et al. Aneurysm syndromes caused by mutations in the TGF-beta receptor. N Engl J Med. 2006;355(8):788–98.
8. Loeys BL, Chen J, Neptune ER, Judge DP, Podowski M, Holm T, et al. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. Nat Genet. 2005;37(3):275–81.
9. Stheneur C, Collod-Beroud G, Faivre L, Gouya L, Sultan G, Le Parc J-M, et al. Identification of 23 TGFBR2 and 6 TGFBR1 gene mutations and genotype-phenotype investigations in 457 patients with Marfan syndrome type I and II, Loeys-Dietz syndrome and related disorders. Hum Mutat. 2008;29(11):E284–95.
10. Singh KK, Rommel K, Mishra A, Karck M, Haverich A, Schmidtke J, et al. TGFBR1 and TGFBR2 mutations in patients with features of Marfan syndrome and Loeys-Dietz syndrome. Hum Mutat. 2006;27(8):770–7.
11. Mizuguchi T, Collod-Beroud G, Akiyama T, Abifadel M, Harada N, Morisaki T, et al. Heterozygous TGFBR2 mutations in Marfan syndrome. Nat Genet. 2004;36(8):855–60.
12. Disabella E, Grasso M, Marziliano N, Ansaldi S, Lucchelli C, Porcu E, et al. Two novel and one known mutation of the TGFBR2 gene in Marfan syndrome not associated with FBN1 gene defects. Eur J Hum Genet. 2006;14(1):34–8.
13. Attias D, Stheneur C, Roy C, Collod-Beroud G, Detaint D, Faivre L, et al. Comparison of clinical presentations and outcomes between patients with TGFBR2 and FBN1 mutations in Marfan syndrome and related disorders. Circulation. 2009;120(25):2541–9.
14. Pannu H. Mutations in transforming growth factor- receptor type II cause familial thoracic aortic aneurysms and dissections. Circulation. 2005;112(4):513–20.
15. MacCarrick G, Black JH, Bowdin S, El-Hamamsy I, Frischmeyer-Guerrerio PA, Guerrerio AL, et al. Loeys-Dietz syndrome: a primer for diagnosis and management. Genet Med. 2014;27.
16. van der Linde D, van de Laar IMBH, Bertoli-Avella AM, Oldenburg RA, Bekkers JA, Mattace-Raso FUS, et al. Aggressive cardiovascular phenotype of aneurysms-osteoarthritis syndrome caused by pathogenic SMAD3 variants. JAC. Elsevier Inc. 2012;60(5):397–403 Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=22633655&retmode=ref&cmd=prlinks.
17. van de Laar IMBH, Oldenburg RA, Pals G, Roos-Hesselink JW, de Graaf BM, Verhagen JMA, et al. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. Nat Genet. 2011a;43(2):121–6.
18. van de Laar IMBH, Oldenburg RA, Pals G, Roos-Hesselink JW, de Graaf BM, Verhagen JMA, et al. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. Nat Genet. 2011b;43(2):121–6.
19. Regalado ES, Guo D-C, Villamizar C, Avidan N, Gilchrist D, McGillivray B, et al. Exome sequencing identifies SMAD3 mutations as a cause of familial thoracic aortic aneurysm and dissection with intracranial and other arterial aneurysms. Circulation Research. 2011;109(6):680–6.
20. Lindsay ME, Schepers D, Bolar NA, Doyle JJ, Gallo E, Fert-Bober J, et al. Loss-of-function mutations in TGFB2 cause a syndromic presentation of thoracic aortic aneurysm. Nat Genet. Nature Publishing Group. 2012 :1–7. Available from: http://dx.doi.org/10.1038/ng.2349
21. Guo D-C, Hanna N, Regalado ES, Detaint D, Gong L, Varret M, et al. TGFB2 mutations cause familial thoracic aortic aneurysms and dissections associated with mild systemic features of Marfan syndrome. Nat Genet. Nature Publishing Group. 2012:1–8.
22. Renard M, Callewaert B, Malfait F, Campens L, Sharif S, Del Campo M, et al. Thoracic aortic-aneurysm and dissection in association with significant mitral valve disease caused by mutations in TGFB2. Int J Cardiol. Elsevier Ireland Ltd. 2012;165(3):584–587. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23102774&retmode=ref&cmd=prlinks
23. Rienhoff HY, Yeo C-Y, Morissette R, Khrebtukova I, Melnick J, Luo S, et al. A mutation in TGFB3 associated with a syndrome of low muscle mass, growth retardation, distal arthrogryposis and clinical features overlapping with Marfan and Loeys-Dietz syndrome. Am J Med Genet A. 2013;3.
24. Morisaki, H. et al., Pathogenic Mutations Found in 3 Japanese Families with MFS/LDS-like disorder. Abstract presentation at the 9th International Research Symposium on Marfan Syndrome and Related Disorders, 2014. Paris.
25. Matyas, G., Naef, P. & Oexle, K., De Novo TGFB3 Mutation in a Patient With Overgrowth and Loeys-Dietz Syndrome Features. Abstract presentation at the 9th International Research Symposium on Marfan Syndrome and Related Disorders, 2014. Paris
26. Milewicz DM, Ostergaard JR, Ala-Kokko LM, Khan N, Grange DK, Mendoza-Londono R, et al. De novo ACTA2 mutation causes a novel syndrome of multisystemic smooth muscle dysfunction. Am J Med Genet A. 2010;152A(10):2437–43.
27. Munot P, Saunders DE, Milewicz DM, Regalado ES, Ostergaard JR, Braun KP, et al. A novel distinctive cerebrovascular phenotype is associated with heterozygous Arg179 ACTA2 mutations. Brain. 2012;135(Pt 8):2506–14.
28. Meuwissen MEC, Lequin MH, Bindels-de Heus K, Bruggenwirth HT, Knapen MFCM, Dalinghaus M, et al. ACTA2 mutation with childhood cardiovascular, autonomic and brain anomalies and severe outcome. Am J Med Genet A. 2013;161A(6):1376–80.
29. Guo D-C, Pannu H, Tran-Fadulu V, Papke CL, Yu RK, Avidan N, et al. Mutations in smooth muscle α-actin (ACTA2) lead to thoracic aortic aneurysms and dissections. Nat Genet. 2007;39(12):1488–93.
30. Renard M, Callewaert B, Baetens M, Campens L, Macdermot K, Fryns J-P, et al. Novel MYH11 and ACTA2 mutations reveal a role for enhanced TGFbeta signaling in FTAAD. Int J Cardiol. 2011;165(2):314–21 Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=21937134&retmode=ref&cmd=prlinks.
31. Wang L, Guo D-C, Cao J, Gong L, Kamm KE, Regalado E, et al. Mutations in myosin light chain kinase cause familial aortic dissections. Am J Hum Genet. 2010;87(5):701–7.
32. Guo D-C, Regalado E, Casteel DE, Santos-Cortez RL, Gong L, Kim JJ, et al. Recurrent gain-of-function mutation in PRKG1 causes thoracic aortic aneurysms and acute aortic dissections. Am J Hum Genet. 2013;93(2):398–404.
33. Zhu L, Vranckx R, van Kien PK, Lalande A, Boisset N, Mathieu F, et al. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. Nat Genet. 2006;38(3):343–9.
34. Pannu H, Tran-Fadulu V, Papke CL, Scherer S, Liu Y, Presley C, et al. MYH11 mutations result in a distinct vascular pathology driven by insulin-like growth factor 1 and angiotensin II. Human Molecular Genetics. 2007;16(20):2453–62.
35. Marfan AB. Un cas de déformation congénitale des quatres membres, plus prononcée aux extrémités, caractérisée par l"allongement des avec un certain degré d"amincissement. Bull mem Soc Med Hop Paris. 1896;13:220–6.
36. McKusick VA. Heritable disorders of connective tissue. Mosby CA, editor. St Louis; 1956.
37. Pyeritz RE. Marfan syndrome: 30 years of research equals 30 years of additional life expectancy. Heart. 2008;95(3):173–5.
38. Silverman DI, Burton KJ, Gray J, Bosner MS, Kouchoukos NT, Roman MJ, et al. Life expectancy in the Marfan syndrome. AJC. 1995;75(2):157–60.

39. Braverman AC, Harris KM, Kovacs RJ, Maron BJ. Eligibility and disqualification recommendations for competitive athletes with cardiovascular abnormalities: Task Force 7: aortic diseases, including Marfan Syndrome: a scientific statement from the American Heart Association and American College of Cardiology. J Am Coll Cardiol. 2015.
40. Jondeau G, Detaint D, Tubach F, Arnoult F, Milleron O, Raoux F, et al. Aortic event rate in the Marfan population: a cohort study. Circulation. 2012;125(2):226–32.
41. Meijboom LJ. Pregnancy and aortic root growth in the Marfan syndrome: a prospective study. European Heart Journal. 2005; 26(9):914–20.
42. Donnelly RT, Pinto NM, Kocolas I, Yetman AT. The immediate and long-term impactof pregnancy on aortic growth rate and mortality in women with Marfan Syndrome. JAC. Elsevier Inc. 2012;60(3):224–9.
43. Teixido-Tura G, Franken R, Galuppo V, Gutiérrez García-Moreno L, Borregan M, Mulder BJM, et al. Heterogeneity of aortic disease severity in patients with Loeys-Dietz syndrome. Heart. 2016.
44. Byers PH. Ehlers-Danlos syndrome: recent advances and current understanding of the clinical and genetic heterogeneity. J Invest Dermatol. 1994;103(5 Suppl):47S–52S.
45. Oderich GS, Panneton JM, Bower TC, Lindor NM, Cherry KJ, Noel AA, et al. The spectrum, management and clinical outcome of Ehlers-Danlos syndrome type IV: a 30-year experience. YMVA. 2005;42(1):98–106.
46. Murray ML, Pepin M, Peterson S, Byers PH. Pregnancy-related deaths and complications in women with vascular Ehlers-Danlos syndrome. Genet Med. 2014;16(12):874–80.
47. Lemire BD, Buncic JR, Kennedy SJ, Dyack SJ, Teebi AS. Congenital mydriasis, patent ductus arteriosus, and congenital cystic lung disease: new syndromic spectrum? Am J Med Genet A. 2004;131(3):318–9.
48. Adès LC, Davies R, Haan EA, Holman KJ, Watson KC, Sreetharan D, et al. Aortic dissection, patent ductus arteriosus, iris hypoplasia and brachytelephalangy in a male adolescent. Clin Dysmorphol. 1999;8(4):269–76.
49. Isselbacher EM. Thoracic and abdominal aortic aneurysms. Circulation. 2005;111(6):816–28.
50. Pyeritz RE. Heritable thoracic aortic disorders. Curr Opin Cardiol. 2014;29(1):97–102.
51. Albornoz G, Coady M, Roberts M, Davies R, Tranquilli M, Rizzo J, et al. Familial thoracic aortic aneurysms and dissections—Incidence, modes of inheritance, and phenotypic patterns. Ann Thorac Surg. 2006;82(4):1400–5.
52. Coady MA, Rizzo JA, Goldstein LJ, Elefteriades JA. Natural history, pathogenesis, and etiology of thoracic aortic aneurysms and dissections. Cardiol Clin. 1999;17(4):615 –35–vii.
53. Wooderchak-Donahue W, VanSant-Webb C, Tvrdik T, Plant P, Lewis T, Stocks J, et al. Clinical utility of a next generation sequencing panel assay for Marfan and Marfan-like syndromes featuring aortopathy. Am J Med Genet A. 2015;5.
54. Rozendaal L, Groenink M, Naeff MS, Hennekam RC, Hart AA, van der Wall EE, et al. Marfan syndrome in children and adolescents: an adjusted nomogram for screening aortic root dilatation. Heart. 1998;79(1):69–72.
55. Groenink M, Rozendaal L, Naeff MS, Hennekam RC, Hart AA, van der Wall EE, et al. Marfan syndrome in children and adolescents: predictive and prognostic value of aortic root growth for screening for aortic complications. Heart. 1998;80(2):163–9.
56. Radonic T, de Witte P, Groenink M, de Bruin-Bon RACM, Timmermans J, Scholte AJH, et al. Critical appraisal of the revised Ghent criteria for diagnosis of Marfan syndrome. Clin Genet. 2011;80(4):346–53.
57. Finkbohner R, Johnston D, Crawford ES, Coselli J, Milewicz DM. Marfan syndrome. Long-term survival and complications after aortic aneurysm repair. Circulation. 1995;91(3):728–33.
58. Engelfriet PM. Beyond the root: dilatation of the distal aorta in Marfan's syndrome. Heart. 2006;92(9):1238–43.
59. Mulder BJM. The distal aorta in the Marfan syndrome. Neth Heart J. 2008;16(11):382–6.
60. Hartog den AW, Franken R, Zwinderman AH, Timmermans J, Scholte AJ, van den Berg MP, et al. The risk for type B aortic dissection in Marfan syndrome. JAC. 2015;65(3):246–54.
61. Nollen GJ, Groenink M, Tijssen JGP, Van Der Wall EE, Mulder BJM. Aortic stiffness and diameter predict progressive aortic dilatation in patients with Marfan syndrome. European Heart Journal. 2004;25(13):1146–52.
62. Franken R, Morabit el A, de Waard V, Timmermans J, Scholte AJ, van den Berg MP, et al. Increased aortic tortuosity indicates a more severe aortic phenotype in adults with Marfan syndrome. Int J Cardiol. 2015;194:7–12.
63. Schoenhoff FS, Jungi S, Czerny M, Roost E, Reineke D, Mátyás G, et al. Acute aortic dissection determines the fate of initially untreated aortic segments in Marfan syndrome. Circulation. 2013;127(15):1569–75.
64. Lemaire SA, la Cruz de KI, Coselli JS. The Thoracoabdominal Aorta in Marfan Syndrome. London: Springer London; 2014. pp. 423–434.
65. Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey DE, et al. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. Circulation. 2010;121:e266–369.
66. Sheikhzadeh S, De Backer J, Gorgan N, Rybczynski M, Hillebrand M, Schüler H, et al. The main pulmonary artery in adults: a controlled multicenter study with assessment of echocardiographic reference values, and the frequency of dilatation and aneurysm in Marfan syndrome. Orphanet J Rare Dis. 2014a;9(1):203.
67. Nollen GJ, van Schijndel KE, Timmermans J, Groenink M, Barentsz JO, van der Wall EE, et al. Pulmonary artery root dilatation in Marfan syndrome: quantitative assessment of an unknown criterion. Heart. 2002;87(5):470–1.
68. De Backer J, Loeys B, Devos D, Dietz H, de Sutter J, de Paepe A. A critical analysis of minor cardiovascular criteria in the diagnostic evaluation of patients with Marfan syndrome. Genet Med. 2006a;8(7):401–8 Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=16845272&retmode=ref&cmd=prlinks.
69. de Backer JF, Devos D, Segers P, Matthys D, François K, Gillebert TC, et al. Primary impairment of left ventricular function in Marfan syndrome☆. Int J Cardiol. 2006b;112(3):353–8 Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=16316698&retmode=ref&cmd=prlinks.
70. Meijboom LJ, Timmermans J, van Tintelen JP, Nollen GJ, De Backer J, van den Berg MP, et al. Evaluation of left ventricular dimensions and function in Marfan's syndrome without significant valvular regurgitation. Am J Cardiol. 2005a;95(6):795–7.
71. Campens L, Renard M, Trachet B, Segers P, Muiño Mosquera L, de Sutter J, et al. Intrinsic cardiomyopathy in Marfan syndrome: results from in-vivo and ex-vivo studies of the Fbn1(C1039G/+) model and longitudinal findings in humans. Pediatr Res. 2015b;78(3):256–63.
72. van Dijk N, Boer MC, Mulder BJM, van Montfrans GA, Wieling W. Is fatigue in Marfan syndrome related to orthostatic intolerance? Clin Auton Res. 2008;18(4):187–93.
73. Maumenee IH. The eye in the Marfan syndrome. Trans Am Ophthalmol Soc. 1981;79:684–733.

74. Dotrelova D, Karel I, Clupkova E. Retinal detachment in Marfan's syndrome. Characteristics and surgical results. Retina. 1997;17(5): 390–6.
75. Maumenee IH. The eye in the Marfan syndrome. Birth Defects Orig Artic Ser. 1982;18(6):515–24.
76. Wood JR, Bellamy D, Child AH, Citron KM. Pulmonary disease in patients with Marfan syndrome. Thorax. 1984;39(10):780–4.
77. Rybczynski M, Koschyk D, Karmeier A, Gessler N, Sheikhzadeh S, Bernhardt AMJ, et al. Frequency of sleep apnea in adults with the Marfan syndrome. AJC. Elsevier Inc; 2010;105(12):1836–1841. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=20538140&retmode=ref&cmd=prlinks
78. Kohler M, Pitcher A, Blair E, Risby P, Senn O, Forfar C, et al. The impact of obstructive sleep apnea on aortic disease in Marfan's syndrome. Respiration. 2013;86(1):39–44.
79. Oosterhof T, Groenink M, Hulsmans FJ, Mulder BJ, van der Wall EE, Smit R, et al. Quantitative assessment of dural ectasia as a marker for Marfan syndrome. Radiology. 2001;220(2):514–8.
80. Villeirs GM, Van Tongerloo AJ, Verstraete KL, Kunnen MF, de Paepe AM. Widening of the spinal canal and dural ectasia in Marfan's syndrome: assessment by CT. Neuroradiology. 1999; 41(11):850–4.
81. Sheikhzadeh S, Brockstaedt L, Habermann CR, Sondermann C, Bannas P, Mir TS, et al. Dural ectasia in Loeys-Dietz syndrome: comprehensive study of 30 patients with a TGFBR1 or TGFBR2 mutation. Clin Genet. 2014b;86(6):545–51.
82. Foran JRH, Pyeritz RE, Dietz HC, Sponseller PD. Characterization of the symptoms associated with dural ectasia in the Marfan patient. Am J Med Genet A. 2005;134A(1):58–65.
83. Bertoli-Avella AM, Gillis E, Morisaki H, Verhagen JMA, de Graaf BM, van de Beek G, et al. Mutations in a TGF-β Ligand, TGFB3, cause syndromic aortic aneurysms and dissections. J Am Coll Cardiol. 2015;65(13):1324–36.
84. Micha D, Guo D-C, Hilhorst-Hofstee Y, van Kooten F, Atmaja D, Overwater E, et al. SMAD2 mutations are associated with arterial aneurysms and dissections. Hum Mutat 2015.
85. Gupta-Malhotra, M. et al. Aortic dilatation in children with systemic hypertension. Journal of the American Society of Hypertension : JASH. 2014; 8(4): 239–45.
86. Kono AK, Higashi M, Morisaki H, Morisaki T, Tsutsumi Y, Akutsu K, et al. High prevalence of vertebral artery tortuosity of Loeys-Dietz syndrome in comparison with Marfan syndrome. Jpn J Radiol. 2010;28(4):273–7.
87. Morris SA, Orbach DB, GEVA T, Singh MN, Gauvreau K, Lacro RV. Increased vertebral artery tortuosity index is associated with adverse outcomes in children and young adults with connective tissue disorders. Circulation. 2011;124(4):388–96.
88. Kuechler A, Altmüller J, Nürnberg P, Kotthoff S, Kubisch C, Borck G. Exome sequencing identifies a novel heterozygous TGFB3 mutation in a disorder overlapping with Marfan and Loeys-Dietz syndrome. Mol Cell Probes. 2015;29(5):330–4.
89. Eckman PM, Hsich E, Rodriguez ER, Gonzalez-Stawinski GV, Moran R, Taylor DO. Impaired systolic function in Loeys-Dietz syndrome: a novel cardiomyopathy? Circ Heart Fail. 2009;2(6):707–8.
90. van de Laar IMBH, van der Linde D, Oei EHG, Bos PK, Bessems JH, Bierma-Zeinstra SM, et al. Phenotypic spectrum of the SMAD3-related aneurysms-osteoarthritis syndrome. J Med Genet. 2011c;49(1):47–57 Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=22167769&retmode=ref&cmd=prlinks.
91. Wischmeijer A, Van Laer L, Tortora G, Bolar NA, Van Camp G, Fransen E, et al. Thoracic aortic aneurysm in infancy in aneurysms-osteoarthritis syndrome due to a novel SMAD3 mutation: further delineation of the phenotype. Am J Med Genet A 2013.
92. Hilhorst-Hofstee Y, Scholte AJHA, Rijlaarsdam MEB, van Haeringen A, Kroft LJ, Reijnierse M, et al. An unanticipated copy number variant of chromosome 15 disrupting SMAD3 reveals a three-generation family at serious risk for aortic dissection. Clin Genet. 2013;83(4):337–44.
93. Fitzgerald-Butt SM, Klima J, Kelleher K, Chisolm D, McBride KL. Genetic knowledge and attitudes of parents of children with congenital heart defects. Am J Med Genet A 2014
94. Martens T, Van Herzeele I, De Ryck F, Renard M, de Paepe A, François K, et al. Multiple aneurysms in a patient with aneurysms-osteoarthritis syndrome. Ann Thorac Surg. Elsevier Inc; 2013;95(1):332–335. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23272854&retmode=ref&cmd=prlinks
95. Aubart M, Gobert D, Aubart-Cohen F, Detaint D, Hanna N, d'Indya H, et al. Early-onset osteoarthritis, Charcot-Marie-Tooth like neuropathy, autoimmune features, multiple arterial aneurysms and dissections: an unrecognized and life threatening condition. PLoS ONE. 2014;9(5):e96387.
96. Panesi P, Foffa I, Sabina S, Ait Alì L, Andreassi MG. Novel TGFBR2 and known missense SMAD3 mutations: two case reports of thoracic aortic aneurysms. Ann Thorac Surg. 2015;99(1): 303–5.
97. Berthet E, Hanna N, Giraud C, Soubrier M. A case of rheumatoid arthritis associated with SMAD3 gene mutation: a new clinical entity? J Rheumatol. 2015;42(3):556.
98. Grahame R, Pyeritz RE. The Marfan syndrome: joint and skin manifestations are prevalent and correlated. Br J Rheumatol. 1995;34(2):126–31.
99. Law C, Bunyan D, Castle B, Day L, Simpson I, Westwood G, et al. Clinical features in a family with an R460H mutation in transforming growth factor beta receptor 2 gene. J Med Genet. 2006;43(12):908–16.
100. Frischmeyer-Guerrerio PA, Guerrerio AL, Oswald G, Chichester K, Myers L, Halushka MK, et al. TGFβ receptor mutations impose a strong predisposition for human allergic disease. Sci Transl Med. 2013;5(195):195ra94.
101. Sheikhzadeh S, Sondermann C, Rybczynski M, Habermann CR, Brockstaedt L, Keyser B, et al. Comprehensive analysis of dural ectasia in 150 patients with a causative FBN1mutation. Clin Genet. 2013:n/a–n/a.
102. Beighton P, de Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet. 1998;77:31–7.
103. Bergqvist D, Björck M, Wanhainen A. Treatment of vascular Ehlers-Danlos syndrome: a systematic review. Ann Surg. 2013; 258(2):257–61.
104. Watanabe S, Ishimitsu T, Inoue K, Tomizawa T, Noguchi Y, Sugishita Y, et al. Type IV Ehlers-Danlos syndrome associated with mitral valve prolapse: a case report. J Cardiol Suppl. 1988;18:97–105 discussion106.
105. Jaffe AS, Geltman EM, Rodey GE, Uitto J. Mitral valve prolapse: a consistent manifestation of type IV Ehlers-Danlos syndrome. The pathogenetic role of the abnormal production of type III collagen. Circulation. 1981;64(1):121–5.
106. Dolan AL, Mishra MB, Chambers JB, Grahame R. Clinical and echocardiographic survey of the Ehlers-Danlos syndrome. Br J Rheumatol. 1997;36(4):459–62.
107. Zilocchi M, Macedo TA, Oderich GS, Vrtiska TJ, Biondetti PR, Stanson AW. Vascular Ehlers-Danlos syndrome: imaging findings. AJR Am J Roentgenol. 2007;189(3):712–9.
108. Heidbreder AE, Ringelstein EB, Dittrich R, Nabavi D, Metze D, Kuhlenbäumer G. Assessment of skin extensibility and joint hypermobility in patients with spontaneous cervical artery dissection and Ehlers-Danlos syndrome. J Clin Neurosci. 2008;15(6): 650–3.

109. Lind J, Wallenburg HCS. Pregnancy and the Ehlers-Danlos syndrome: a retrospective study in a Dutch population. Acta Obstet Gynecol Scand. 2002;81(4):293–300.
110. Khan N, Schinzel A, Shuknecht B, Baumann F, Ostergaard JR, Yonekawa Y. Moyamoya angiopathy with dolichoectatic internal carotid arteries, patent ductus arteriosus and pupillary dysfunction: a new genetic syndrome? Eur Neurol. 2004;51(2):72–7.
111. Roulez FMJ, Faes F, Delbeke P, Van Bogaert P, Rodesch G, De Zaeytijd J, et al. Congenital fixed dilated pupils due to ACTA2- multisystemic smooth muscle dysfunction syndrome. J Neuroophthalmol. 2014;34(2):137–43.
112. Biddinger A, Rocklin M, Coselli J, Milewicz DM. Familial thoracic aortic dilatations and dissections: a case control study. YMVA. 1997;25(3):506–11.
113. Regalado ES, Guo D, Prakash S, Bensend TA, Flynn K, Estrera A, et al. Aortic Disease Presentation and Outcome Associated with ACTA2 Mutations. Circ Cardiovas Genet. 2015a;8(3):457–64.
114. van Kien PK. Mapping of Familial Thoracic Aortic Aneurysm/Dissection With Patent Ductus Arteriosus to 16p12.2-p13.13. Circulation. 2005;112(2):200–6 Available from: http://circ.ahajournals.org/cgi/doi/10.1161/CIRCULATIONAHA.104.506345.
115. Regalado ES, Guo D-C, Santos-Cortez RLP, Hostetler E, Bensend TA, Pannu H, et al. Pathogenic FBN1 variants in familial thoracic aortic aneurysms and dissections. Clin Genet. 2015b:n/a–n/a. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=26621581&retmode=ref&cmd=prlinks
116. Milewicz DM, Chen H, Park ES, Petty EM, Zaghi H, Shashidhar G, et al. Reduced penetrance and variable expressivity of familial thoracic aortic aneurysms/dissections. AJC. 1998;82(4):474–9.
117. Freed LA, Levy D, Levine RA, Larson MG, Evans JC, Fuller DL, et al. Prevalence and clinical outcome of mitral-valve prolapse. N Engl J Med. 1999;341(1):1–7.
118. Guo D-C, Gong L, Regalado ES, Santos-Cortez RL, Zhao R, Cai B, et al. MAT2A mutations predispose individuals to thoracic aortic aneurysms. Am J Hum Genet. 2015;96(1):170–7.
119. Cripe L, Andelfinger G, Martin LJ, Shooner K, Benson DW. Bicuspid aortic valve is heritable. JAC. 2004;44(1):138–43.
120. McBride KL, Pignatelli R, Lewin M, Ho T, Fernbach S, Menesses A, et al. Inheritance analysis of congenital left ventricular outflow tract obstruction malformations: segregation, multiplex relative risk, and heritability. Am J Med Genet A. 2005;134A(2):180–6.
121. Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, King IN, et al. Mutations in NOTCH1 cause aortic valve disease. Nature. 2005;437(7056):270–4.
122. McKellar SH, Tester DJ, Yagubyan M, Majumdar R, Ackerman MJ, Sundt TM. Novel NOTCH1 mutations in patients with bicuspid aortic valve disease and thoracic aortic aneurysms. J Thorac Cardiovasc Surg. 2007;134(2):290–6.
123. Kerstjens-Frederikse WS, van de Laar IMBH, Vos YJ, Verhagen JMA, Berger RMF, Lichtenbelt KD, et al. Cardiovascular malformations caused by NOTCH1 mutations do not keep left: data on 428 probands with left-sided CHD and their families. Genet Med 2016
124. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature. 1991;352(6333):337–9.
125. Carmignac V, Thevenon J, Adès L, Callewaert B, Julia S, Thauvin-Robinet C, et al. In-frame mutations in exon 1 of SKI cause dominant Shprintzen-Goldberg syndrome. Am J Hum Genet. 2012;91(5):950–7 Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23103230&retmode=ref&cmd=prlinks.
126. Doyle AJ, Doyle JJ, Bessling SL, Maragh S, Lindsay ME, Schepers D, et al. Mutations in the TGF-β repressor SKI cause Shprintzen-Goldberg syndrome with aortic aneurysm. Nat Genet. 2012;44(11):1249–54.
127. Coucke PJ. Homozygosity mapping of a gene for arterial tortuosity syndrome to chromosome 20q13. J Med Genet. 2003;40(10):747–51.
128. Callewaert B, Renard M, Hucthagowder V, Albrecht B, Hausser I, Blair E, et al. New insights into the pathogenesis of autosomal-dominant cutis laxa with report of five ELN mutations. Hum Mutat. 2011;32(4):445–55.
129. Renard M, Holm T, Veith R, Callewaert BL. s LCAE, Baspinar O, et al. Altered TGFβ signaling and cardiovascular manifestations in patients with autosomal recessive cutis laxa type I caused by fibulin-4 deficiency. Eur J Hum Genet. 2010;18(8):895–901.
130. Rippe M, De Backer J, Kutsche K, Mosquera LM, Schüler H, Rybczynski M, et al. Mitral valve prolapse syndrome and MASS phenotype: stability of aortic dilatation but progression of mitral valve prolapse. IJCHA. The Authors. 2016;10:39–46.
131. Coucke PJ, Willaert A, Wessels MW, Callewaert B, Zoppi N, De Backer J, et al. Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome. Nat Genet. 2006;38(4):452–7.
132. Callewaert BL, Willaert A, Kerstjens-Frederikse WS, de Backer J, Devriendt K, Albrecht B, et al. Arterial tortuosity syndrome: clinical and molecular findings in 12 newly identified families. Hum Mutat. 2008;29(1):150–8.
133. Kosaki K, Takahashi D, Udaka T, Kosaki R, Matsumoto M, Ibe S, et al. Molecular pathology of Shprintzen-Goldberg syndrome. Am J Med Genet A. 2006;140(1):104–8 authorreply109–10.
134. Gupta PA, Wallis DD, Chin TO, Northrup H, Tran-Fadulu VT, Towbin JA, et al. FBN2 mutation associated with manifestations of Marfan syndrome and congenital contractural arachnodactyly. J Med Genet. 2004;41(5):e56.
135. Nishimura A, Sakai H, Ikegawa S, Kitoh H, Haga N, Ishikiriyama S, et al. FBN2, FBN1, TGFBR1, and TGFBR2 analyses in congenital contractural arachnodactyly. Am J Med Genet A. 2007;143A(7):694–8.
136. Kruger WD, Wang L, Jhee KH, Singh RH, Elsas LJ. Cystathionine beta-synthase deficiency in Georgia (USA): correlation of clinical and biochemical phenotype with genotype. Hum Mutat. 2003;22(6):434–41.
137. De Lucca M, Casique L. Characterization of cystathionine beta-synthase gene mutations in homocystinuric Venezuelan patients: identification of one novel mutation in exon 6. Mol Genet Metab. 2004;81(3):209–15.
138. Shores J, Berger KR, Murphy EA, Pyeritz RE. Progression of aortic dilatation and the benefit of long-term beta-adrenergic blockade in Marfan's syndrome. N Engl J Med. 1994;330(19):1335–41.
139. Engelfriet P, Mulder B. Is there benefit of beta-blocking agents in the treatment of patients with the Marfan syndrome? Int J Cardiol. 2007;114(3):300–2.
140. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science. 2006;312(5770):117–21.
141. Chiu H-H, Wu M-H, Wang J-K, Lu C-W, Chiu S-N, Chen C-A, et al. Losartan added to β-blockade therapy for aortic root dilation in Marfan syndrome: a randomized, open-label pilot study. Mayo Clinic Proceedings. 2013;88(3):271–6.
142. Franken R, Mulder BJM. Aortic disease: losartan versus atenolol in the Marfan aorta-how to treat? Nat Rev Cardiol. 2015.
143. Groenink M, Hartog den AW, Franken R, Radonic T, de Waard V, Timmermans J, et al. Losartan reduces aortic dilatation rate in adults with Marfan syndrome: a randomized controlled trial. Eur Heart J 2013.
144. Milleron O, Arnoult F, Ropers J, Aegerter P, Detaint D, Delorme G, et al. Marfan Sartan: a randomized, double-blind, placebo-controlled trial. Eur Heart J. 2015;36(32):2160–6.

145. Lacro RV, Dietz HC, Sleeper LA, Yetman AT, Bradley TJ, Colan SD, et al. Atenolol versus losartan in children and young adults with Marfan's syndrome. N Engl J Med. 2014;371(22):2061–71.
146. Forteza A, Evangelista A, Sánchez V, Teixido-Tura G, Sanz P, Gutiérrez L, et al. Efficacy of losartan vs. atenolol for the prevention of aortic dilation in Marfan syndrome: a randomized clinical trial. Eur Heart J. 2015.
147. De Backer J. Marfan and Sartans: time to wake up! European He art Journal. 2015.
148. Erbel, R. et al. 2014. ESC Guidelines on the diagnosis and treatment of aortic diseases: Document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The Task Force for the Diagnosis and Treatment of Aortic Diseases of the European Society of Cardiology (ESC). European Heart Journal. 2014;35(41): 2873–2926.
149. Meijboom LJ, Timmermans J, Zwinderman AH, Engelfriet PM, Mulder BJM. Aortic root growth in men and women with the Marfan's syndrome. Am J Cardiol. 2005b;96(10):1441–4.
150. Davies RR, Gallo A, Coady MA, Tellides G, Botta DM, Burke B, et al. Novel measurement of relative aortic size predicts rupture of thoracic aortic aneurysms. Ann Thorac Surg. 2006;81(1):169–77.
151. Gott VL, Greene PS, Alejo DE, Cameron DE, Naftel DC, Miller DC, et al. Replacement of the aortic root in patients with Marfan's syndrome. N Engl J Med. 1999;340(17):1307–13.
152. David TE, Feindel CM, David CM, Manlhiot C. A quarter of a century of experience with aortic valve-sparing operations. J Thorac Cardiovasc Surg. 2014;148(3):872 –9–discussion879–80.
153. Lederle FA, Freischlag JA, Kyriakides TC, Matsumura JS, Padberg FT, Kohler TR, et al. Long-term comparison of endovascular and open repair of abdominal aortic aneurysm. N Engl J Med. 2012;367(21):1988–97.
154. Preventza O, Mohammed S, Cheong BY, Gonzalez L, Ouzounian M, Livesay JJ, et al. Endovascular therapy in patients with genetically triggered thoracic aortic disease: applications and short- and mid-term outcomes. Eur J Cardiothorac Surg. 2014;46(2):248 –53–discussion253.
155. Pacini D, Parolari A, Berretta P, Di Bartolomeo R, Alamanni F, Bavaria J. Endovascular treatment for type B dissection in Marfan syndrome: is it worthwhile? Ann Thorac Surg. 2013;95(2): 737–49.
156. Nordon IM, Hinchliffe RJ, Holt PJ, Morgan R, Jahangiri M, Loftus IM, et al. Endovascular management of chronic aortic dissection in patients with Marfan syndrome. J Vasc Surg. 2009; 50(5):987–91.
157. Treasure, T., Takkenberg, J.J.M. & Pepper, J. Surgical management of aortic root disease in Marfan syndrome and other congenital disorders associated with aortic root aneurysms. Heart (British Cardiac Society). 2014;100(20):1571–76.
158. Meijboom LJ, Groenink M, van der Wall EE, Romkes H, Stoker J, Mulder BJ. Aortic root asymmetry in Marfan patients; evaluation by magnetic resonance imaging and comparison with standard echocardiography. Int J Card Imaging. 2000;16(3):161–8.
159. Nollen GJ. Aortic pressure-area relation in Marfan patients with and without blocking agents: a new non-invasive approach. Heart. 2004;90(3):314–318. Available from: http://heart.bmj.com/cgi/doi/10.1136/hrt.2003.010702
160. Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP, Guyton RA, et al. 2014 AHA/ACC guideline for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Thorac Cardiovasc Surg. 2014;148(1):e1–e132.
161. Meijboom LJ, Drenthen W, Pieper PG, Groenink M, van der Post JAM, Timmermans J, et al. Obstetric complications in Marfan syndrome. Int J Cardiol. 2006;110(1):53–9.
162. Rossiter JP, Repke JT, Morales AJ, Murphy EA, Pyeritz RE. A prospective longitudinal evaluation of pregnancy in the Marfan syndrome. Am J Obstet Gynecol. 1995;173(5):1599–606.
163. Ersbøll AS, Hedegaard M, Sondergaard L, Ersbøll M, Johansen M. Treatment with oral beta-blockers during pregnancy complicated by maternal heart disease increases the risk of fetal growth restriction. BJOG: Int J Obstet Gy. 2014;121(5):618–26.
164. Dolan P, Sisko F, Riley E. Anesthetic considerations for Ehlers-Danlos syndrome. Anesthesiology. 1980;52(3):266–9.
165. Brooke BS, Arnaoutakis G, McDonnell NB, Black JH. Contemporary management of vascular complications associated with Ehlers-Danlos syndrome. YMVA. Elsevier Inc. 2010;51(1):131–9.
166. Ong K-T, Perdu J, De Backer J, Bozec E, Collignon P, Emmerich J, et al. Effect of celiprolol on prevention of cardiovascular events in vascular Ehlers-Danlos syndrome: a prospective randomised, open, blinded-endpoints trial. Lancet. 2010;376(9751): 1476–84.
167. Boodhwani M, Andelfinger G, Leipsic J, Lindsay T, McMurtry MS, Therrien J, et al. Canadian Cardiovascular Society position statement on the management of thoracic aortic disease. Can J Cardiol. 2014;30:577–89.
168. Michelena HI, Desjardins VA, Avierinos J-F, Russo A, Nkomo VT, Sundt TM, et al. Natural history of asymptomatic patients with normally functioning or minimally dysfunctional bicuspid aortic valve in the community. Circulation. 2008;117(21): 2776–84.
169. Eleid MF, Forde I, Edwards WD, Maleszewski JJ, Suri RM, Schaff HV, et al. Type A aortic dissection in patients with bicuspid aortic valves: clinical and pathological comparison with tricuspid aortic valves. Heart. 2013;99(22):1668–74.
170. Detaint D, Michelena HI, Nkomo VT, Vahanian A, Jondeau G, Sarano ME. Aortic dilatation patterns and rates in adults with bicuspid aortic valves: a comparative study with Marfan syndrome and degenerative aortopathy. Heart. 2014;100(2):126–34.
171. Loeys B, de Backer J, van Acker P, Wettinck K, Pals G, Nuytinck L, et al. Comprehensive molecular screening of the FBN1 gene favors locus homogeneity of classical Marfan syndrome. Hum Mutat. 2004;24(2):140–6.
172. Proost, D. et al., Performant Mutation Identification Using Targeted Next-Generation Sequencing of 14 Thoracic Aortic Aneurysm Genes. Human Mutation. 2015;36(8): 808–14.
173. Ziganshin BA, Bailey AE, Coons C, Dykas D, Charilaou P, Tanriverdi LH, et al. Routine Genetic Testing for Thoracic Aortic Aneurysm and Dissection in a Clinical Setting. Ann Thorac Surg. 2015;100(5):1604–11.
174. Arslan-Kirchner M, Arbustini E, Boileau C, Charron P, Child AH, Collod-Beroud G, et al. Clinical utility gene card for: hereditary thoracic aortic aneurysm and dissection including next-generation sequencing-based approaches. Eur J Hum Genet. 2015;28.
175. Rehm HL, Berg JS, Brooks LD, Bustamante CD, Evans JP, Landrum MJ, et al. ClinGen--the Clinical Genome Resource. N Engl J Med. 2015;372(23):2235–42.
176. Corson GM, Chalberg SC, Dietz HC, Charbonneau NL, Sakai LY. Fibrillin binds calcium and is coded by cDNAs that reveal a multidomain structure and alternatively spliced exons at the 5′ end. Genomics. 1993;17(2):476–84.
177. Biery NJ, Eldadah ZA, Moore CS, Stetten G, Spencer F, Dietz HC. Revised genomic organization of FBN1 and significance for regulated gene expression. Genomics. 1999;56(1):70–7.
178. Sengle G. Sakai LY. The fibrillin microfibril scaffold: A niche for growth factors and mechanosensation? Matrix biology. journal of the International Society for Matrix Biology. 2015;47:3–12.
179. Hubmacher D, Tiedemann K, Reinhardt DP. Fibrillins: from biogenesis of microfibrils to signaling functions. Curr Top Dev Biol. 2006;75:93–123.
180. Judge DP, Biery NJ, Keene DR, Geubtner J, Myers L, Huso DL, et al. Evidence for a critical contribution of haploinsufficiency in the complex pathogenesis of Marfan syndrome. J Clin Invest. 2004;114(2):172–81.

181. Pereira L, Lee SY, Gayraud B, Andrikopoulos K, Shapiro SD, Bunton T, et al. Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. Proc Natl Acad Sci USA. 1999;96(7):3819–23.
182. Bunton TE, Biery NJ, Myers L, Gayraud B, Ramirez F, Dietz HC. Phenotypic alteration of vascular smooth muscle cells precedes elastolysis in a mouse model of Marfan syndrome. Circ Res. 2001;88(1):37–43.
183. Massagué J. The TGF-beta family of growth and differentiation factors. Cell. 1987;49(4):437–8.
184. Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, et al. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. Nat Genet. 2003; 33(3):407–11.
185. Isogai Z, Ono RN, Ushiro S, Keene DR, Chen Y, Mazzieri R, et al. Latent transforming growth factor beta-binding protein 1 interacts with fibrillin and is a microfibril-associated protein. J Biol Chem. 2003;278(4):2750–7.
186. Dallas SL, Miyazono K, Skerry TM, Mundy GR, Bonewald LF. Dual role for the latent transforming growth factor-beta binding protein in storage of latent TGF-beta in the extracellular matrix and as a structural matrix protein. J Cell Biol. 1995;131(2):539–49.
187. Saharinen J, Hyytiäinen M, Taipale J, Keski-Oja J. Latent transforming growth factor-beta binding proteins (LTBPs)--structural extracellular matrix proteins for targeting TGF-beta action. Cytokine Growth Factor Rev. 1999;10(2):99–117.
188. Charbonneau NL, Carlson EJ, Tufa S, Sengle G, Manalo EC, Carlberg VM, et al. In vivo studies of mutant fibrillin-1 microfibrils. J Biol Chem. 2010;285(32):24943–55.
189. Dietz H. A healthy tension in translational research. J Clin Invest. 2014;124(4):1425–9.
190. Jeremy RW, Robertson E, Lu Y, Hambly BD. Perturbations of mechanotransduction and aneurysm formation in heritable aortopathies. Int J Cardiol. 2013;169(1):7–16.
191. Humphrey JD, Milewicz DM, Tellides G, Schwartz MA. Cell biology. Dysfunctional mechanosensing in aneurysms. Science. 2014;344(6183):477–9.
192. Loeys B, Nuytinck L, Delvaux I, De Bie S, de Paepe A. Genotype and phenotype analysis of 171 patients referred for molecular study of the fibrillin-1 gene FBN1 because of suspected Marfan syndrome. Arch Intern Med. 2001;161(20):2447–54.
193. de Backer J, Nollen GJ, Devos D, Pals G, Coucke P, Verstraete K, et al. Variability of aortic stiffness is not associated with the fibrillin 1 genotype in patients with Marfan's syndrome. Heart. 2006c;92(7):977–8.
194. Nijbroek G, Sood S, McIntosh I, Francomano CA, Bull E, Pereira L, et al. Fifteen novel FBN1 mutations causing Marfan syndrome detected by heteroduplex analysis of genomic amplicons. Am J Hum Genet. 1995;57(1):8–21.
195. Tynan K, Comeau K, Pearson M, Wilgenbus P, Levitt D, Gasner C, et al. Mutation screening of complete fibrillin-1 coding sequence: report of five new mutations, including two in 8-cysteine domains. Hum Mol Genet. 1993;2(11):1813–21.
196. Dietz HC, McIntosh I, Sakai LY, Corson GM, Chalberg SC, Pyeritz RE, et al. Four novel FBN1 mutations: significance for mutant transcript level and EGF-like domain calcium binding in the pathogenesis of Marfan syndrome. Genomics. 1993;17(2): 468–75.
197. Faivre L, Collod-Beroud G, Callewaert B, Child A, Binquet C, Gautier E, et al. Clinical and mutation-type analysis from an international series of 198 probands with a pathogenic FBN1 exons 24-32 mutation. Eur J Hum Genet. 2009b;17(4):491–501.
198. Franken, R. et al., 2016. Genotype impacts survival in Marfan syndrome. European Heart Journal. Jan 18.
199. Schrijver I, Liu W, Brenn T, Furthmayr H, Francke U. Cysteine substitutions in epidermal growth factor-like domains of fibrillin-1: distinct effects on biochemical and clinical phenotypes. Am J Hum Genet. 1999;65(4):1007–20.
200. Schrijver I, Liu W, Odom R, Brenn T, Oefner P, Furthmayr H, et al. Premature termination mutations in FBN1: distinct effects on differential allelic expression and on protein and clinical phenotypes. Am J Hum Genet. 2002;71(2):223–37.
201. Comeglio P, Evans AL, Brice G, Cooling RJ, Child AH. Identification of FBN1 gene mutations in patients with ectopia lentis and marfanoid habitus. Br J Ophthalmol. 2002;86(12):1359–62.
202. Faivre L, Collod-Beroud G, Loeys BL, Child A, Binquet C, Gautier E, et al. Effect of mutation type and location on clinical outcome in 1,013 probands with Marfan syndrome or related phenotypes and FBN1 mutations: an international study. Am J Hum Genet. 2007;81(3):454–66.
203. Comeglio P, Johnson P, Arno G, Brice G, Evans A, Aragon-Martin J, et al. The importance of mutation detection in Marfan syndrome and Marfan-related disorders: report of 193 FBN1 mutations. Hum Mutat. 2007;28(9):928.
204. Baudhuin LM, Kotzer KE, Lagerstedt SA. Increased frequency of FBN1 truncating and splicing variants in Marfan syndrome patients with aortic events. Genet Med. 2015;17(3):177–87.
205. Morissette R, Schoenhoff F, Xu Z, Shilane DA, Griswold BF, Chen W, et al. Transforming Growth Factor-β (TGF-β) and Inflammation in Vascular (Type IV) Ehlers Danlos Syndrome. Cardiovascular Genetics: Circulation; 2014.
206. Frank M, Albuisson J, Ranque B, Golmard L, Mazzella J-M, Bal-Theoleyre L, et al. The type of variants at the COL3A1 gene associates with the phenotype and severity of vascular Ehlers-Danlos syndrome. Eur J Hum Genet. 2015;11.
207. Pepin MG, Schwarze U, Rice KM, Liu M, Leistritz D, Byers PH. Survival is affected by mutation type and molecular mechanism in vascular Ehlers-Danlos syndrome (EDS type IV). Genet Med. 2014;16(12):881–8.
208. Tromp G, Kuivaniemi H, Stolle C, Pope FM, Prockop DJ. Single base mutation in the type III procollagen gene that converts the codon for glycine 883 to aspartate in a mild variant of Ehlers-Danlos syndrome IV. J Biol Chem. 1989;264(32):19313–7.
209. Rodriguez-Vita J, Angiotensin II. Activates the Smad Pathway in Vascular Smooth Muscle Cells by a Transforming Growth Factor- -Independent Mechanism. Circulation. 2005;111(19): 2509–17.
210. Guo X, Chen S-Y. Transforming growth factor-β and smooth muscle differentiation. World J Biol Chem. 2012;3(3):41–52.
211. Wynne BM, Chiao C-W, Webb RC. Vascular Smooth Muscle Cell Signaling Mechanisms for Contraction to Angiotensin II and Endothelin-1. J Am Soc Hypertens. 2009;3(2):84–95.
212. Inamoto S, Kwartler CS, Lafont AL, Liang YY, Fadulu VT, Duraisamy S, et al. TGFBR2 mutations alter smooth muscle cell phenotype and predispose to thoracic aortic aneurysms and dissections. Cardiovasc Res. 2010;88(3):520–9.
213. Singh P, Carraher C, Schwarzbauer JE. Assembly of fibronectin extracellular matrix. Annu Rev Cell Dev Biol. 2010;26: 397–419.
214. Sabatier L, Chen D, Fagotto-Kaufmann C, Hubmacher D, McKee MD, Annis DS, et al. Fibrillin assembly requires fibronectin. Mol Biol Cell. 2009;20(3):846–58.
215. Pepin MG, Murray ML, Byers PH. Vascular Ehlers-Danlos Syndrome. In: RA P, MP A, HH A, editors. Gene Reviews. 1999 ed. Seattle: University of Washington, Seattle; pp. http://www.ncbi.nlm.nih.gov–books–NBK1494–.
216. Coron F, Rousseau T, Jondeau G, Gautier E, Binquet C, Gouya L, et al. What do French patients and geneticists think about prenatal and preimplantation diagnoses in Marfan syndrome? Prenat Diagn. 2012;32(13):1318–23.

Bicuspid Aortic Valve

18

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Abstract

An aortic valve typically consists of three valve leaflets, named after their orientation relative to the left and right coronary artery. In 0.5–2 % of the general population, the aortic valve comprises only two leaflets, which is termed a bicuspid aortic valve (BAV). BAV is believed to result from abnormal embryological fusion of two adjacent cusps, due to defective epithelial-to-mesenchymal transition in the outflow tract or abnormal activity of cardiac neural crest cells. Although intrinsically largely asymptomatic, it associates with severe cardiovascular complications such as aortic coarctation and thoracic aortic aneurysms and dissections. In the past, these manifestations accounted for a higher mortality and morbidity than all other congenital heart defects combined. As to significant advances in perioperative management, however, survival rates between BAV and tricuspid aortic valve individuals have now almost equaled. Further improvement of the existing interventions as well as discovery of novel therapeutic targets and accurate predictive biomarkers for BAV-related complications is still warranted though. Therefore, the condition's pathomechanisms are currently being extensively investigated. Although these investigations have been insightful to some extent, knowledge gaps have increasingly been exposed, highlighting the importance of future experiments digging into the etiology of BAV. In this chapter, a comprehensive overview on the clinical and yet unraveled molecular characteristics of BAV will be provided, as well as a reflection on the factors underlying its current etiological inscrutability.

Introduction

Cardiac valves act as one-way doors, ensuring blood to flow in a single direction through the heart. The heart's left ventricle is separated from the aorta by the aortic valve, a tricuspid valve which normally consists of three crescent-shaped leaflets, named after their orientation relative to the left and right coronary artery: the left coronary cusp (LCC), the right coronary cusp (RCC), and the non-coronary cusp (NCC). About 500 years ago, Leonardo Da Vinci first described individuals with an aortic valve with only two, unequally sized leaflets, which is termed a bicuspid aortic valve (BAV) [1]. BAV is believed to result from abnormal embryological fusion of two adjacent cusps. Pertaining to the orientation of the fused cusps, multiple classification systems have been put forward to accurately discriminate between different morphological BAV patterns.

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The Sievers classification is, by far, the most commonly used one [2]. It takes into account the number of raphes, that is, thin ridges of tissue representing the exact location of cusp fusion during valvulogenesis, the spatial position of the fused cusps, and the functional status of the valve. Most patients have a BAV with one raphe (88 %), stemming from fusion of either the RCC and LCC (R-L, 71 %) or the RCC and NCC (R-N, 14 %) (Fig. 18.1) [2]. Animal studies on their etiology have suggested that the R-N subtype results from defective epithelial-to-mesenchymal transition (EMT) in the outflow tract (OFT) during aortic cushion formation, whereas the R-L subtype is caused by defective OFT septation due to abnormal activity of neural crest cells [3].

With a prevalence of 0.5–2 % in the general population, BAV is considered the most common congenital cardiac malformation. Considerably more men are affected than women (3:1). Although being intrinsically asymptomatic, BAV associates with severe cardiovascular complications. In earlier years, these manifestations accounted for a higher mortality and morbidity than all other congenital heart defects combined [4]. Nowadays, significant advances in perioperative management have succeeded in roughly equaling the survival rates between BAV and tricuspid aortic valve (TAV) individuals [5, 6]. Further improvement of the existing interventions as well as discovery of novel therapeutic targets and accurate predictive biomarkers for BAV-related complications are still necessary though. Numerous investigative efforts into the condition's pathomechanisms have been instigated, which although insightful have increasingly exposed knowledge gaps and, hence, areas where further research is warranted. In this chapter, a comprehensive overview on the clinical and the so far unraveled molecular characteristics of BAV will be provided.

Clinical Presentation

The clinical presentation of BAV is exceedingly heterogeneous. While most BAV patients remain asymptomatic, approximately one third of patients develop cardiovascular complications due to the BAV itself or associated anomalies [4]. Aortic coarctation as well as thoracic aortic aneurysms (TAA) and dissections, usually above the sinotubular junction, are considered the most frequent associated lesions, but also hypoplastic left heart syndrome, ventricular septal defect (VSD), atrial septal defect (ASD), and patent ductus arteriosus are more commonly observed in BAV patients or their relatives [5, 7, 8]. Obviously, auxiliary valve dysfunction results from valve tightening (aortic stenosis, AS), leakiness (aortic regurgitation, AR), or infective endocarditis. BAV and its associated cardiovascular anomalies can present at any age, from prenatal stages to adulthood. About one half of the patients that undergo aortic valve surgery before age 70 have BAV. Over the past couple of years, some studies suggested associations between the morphological patterns of BAV and the occurrence of the valvular complications. More precisely, it has been shown that the R-L fusion subtype associates with AS, whereas the R-N and N-L subtypes increase susceptibility to AR [9, 10]. Though interesting, these findings emanated from relatively small patient cohorts, and conflicting results were found in other studies, as such warranting further replication.

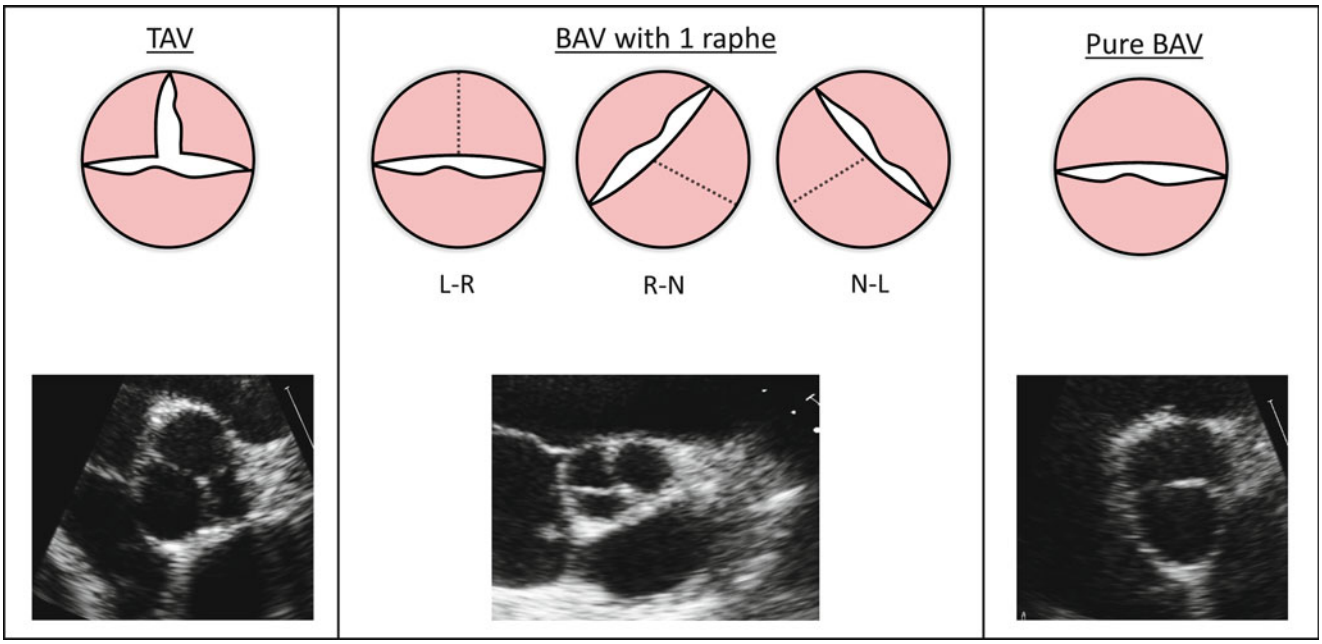

Fig. 18.1 Illustrations and transthoracic echocardiographies (TTE) of tricuspid and bicuspid aortic valves (BAV)

Of all established BAV-related complications, TAA – and particularly the resulting dissections when they are left untreated – pose the most serious threat. In clinical series and autopsy reports of patients with aortic dissection, BAV is reported in 4–9 % of the patients [11]. Among the young aortic dissection patients (<40 years), 9–28 % of the cases present with BAV. Overall, it is believed that BAV patients have an eight- to ninefold increased risk of deadly dissections compared to TAV individuals, occurring at considerably younger ages [7, 12]. As TAA by themselves generally remain unnoticed, careful cardiovascular monitoring of BAV patients is mandatory to prevent sudden cardiac death (SCD) because of aortic dissections or ruptures.

Clinical Diagnosis

Diagnosing BAV

Presently, BAV is being diagnosed at all ages, in very discrete clinical settings and often even accidently. Early detection, however, is critical to enable timely surveillance and recognition of the condition's concomitant cardiovascular complications. Based on auscultation and/or an individual's minor complaints (e.g., fatigue, dyspnea, palpitation) alone, BAV cannot reliably be diagnosed [4]. Nonetheless, perception of a mid-systolic ejection click at the apex frequently (in 60–70 % of the BAV cases) prompts clinicians to thoroughly inspect valve morphology through alternative, more rigorous techniques [13]. Echocardiography serves already more than 40 years as the first-line test because of its high accuracy at relatively low cost [14]. Whereas systolic long-axis views showing an eccentric leaflet closure plane and leaflet doming hint toward BAV, short-axis views are essential to firmly determine the number of valve leaflets, the fusion subtype, and presence of a raphe (Fig. 18.1) [15]. In recent years, particularly transesophageal echocardiography (TEE) has proven accurate, reaching sensitivities and specificities up to respectively 92 % and 96 % [16, 17]. Although TEE undeniably outperforms standard transthoracic echocardiography (TTE), it is invasive and requires sedation [18]. Hence, when improved diagnostic precision is warranted, the use of three-dimensional TTE is commonly preferred over TEE.

In patients with moderate-to-severe stenosis and/or calcification of the aortic valve, echocardiography-based differentiation between TAV and BAV is sometimes difficult (~70 % accuracy) [13]. In such instances, cardiovascular magnetic resonance (CMR) imaging has been reported to be more sensitive, yet less specific, than echocardiography (>90 % accuracy) [13, 19, 20].

Diagnosing BAV-Related Cardiovascular Manifestations

Apart from evaluating their valve morphology, it is warranted to check presumed BAV patients for the presence of TAA as well as AS, AR, ASD, VSD, and coarctation. Although careful echocardiographic studies usually allow to assess pathophysiological aortic root or aorta ascendens dimensions (>40 mm) (Fig. 18.2) [13, 21], computed tomography (CT) or CMR provide a (more) reliable impression of the full spectrum of BAV's associated complications. This has urged the European Society of Cardiology (ESC) and the American College of Cardiology/American Heart Association (ACC/AHA) to recommend them as complementary diagnostic tools [22, 23]. Owing to the recent improvements in CT with respect to radiation exposure, it is often the imaging method of choice. In children, however, CMR might still be preferred over CT.

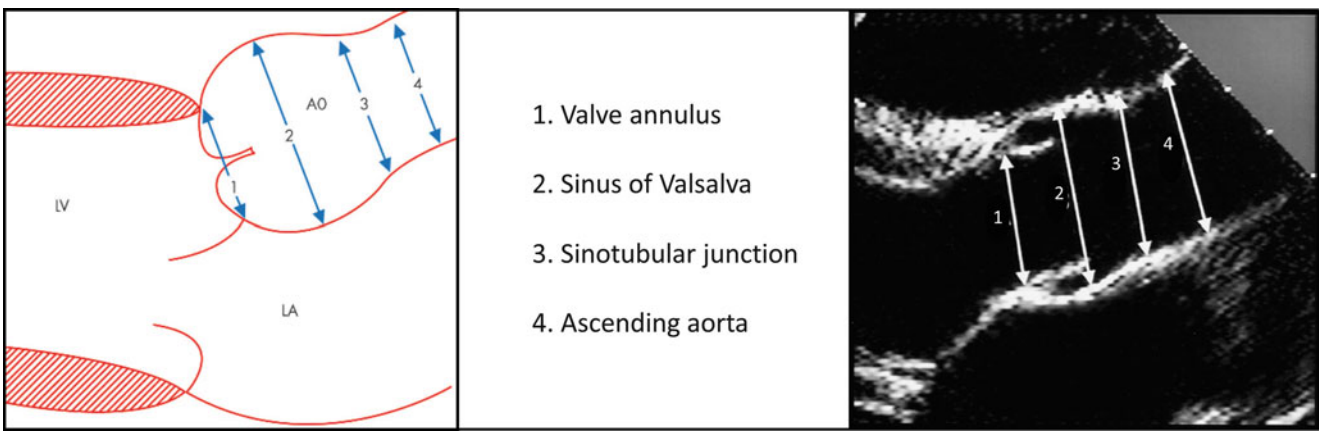

Fig. 18.2 Where to measure aortic dimensions in BAV patients by means of transthoracic echocardiography (Legend: Abbreviations: *LV* left ventricle, *LA* left atrium, *Ao* aorta)

Patient Management

Despite the fact that the risk for aortic dissection in BAV patients might be lower than in cases with Marfan syndrome (MFS), aortic dilatations and aneurysms entail a significant risk for acute aortic dissection and/or rupture, which – despite surgical progress – still associate with poor outcomes. Hitherto, the current medical therapies cannot prevent the progression of aortic dilatation or aneurysm formation in TAA patients in general, nor in BAV cases. As a result, much emphasis has been placed on cardiovascular monitoring and preventive surgery. Recent population-based studies revealed that in BAV cohorts the mortality rates have become similar to those in the general population [5, 6]. Given the high prevalence of BAV, universal application of imaging-based surveillance as well as preventive aortic surgery represent a costly endeavor for our society.

Cardiovascular Surveillance

To meticulously follow disease progression, serial echocardiography, when necessary complemented with CMR or CT, should be performed. The aortic growth rate is highly variable between BAV patients. It generally ranges from 0.2 to 0.9 mm/year [24–27], which is about fivefold higher than what is observed in TAV individuals [28]. In accordance with the law of Laplace, patients with larger aortas show faster expansion rates [29]. The current ESC and ACC/AHA guidelines advocate yearly cardiac imaging in BAV patients with an aortic root or ascending aorta with a diameter of ≥45 mm and a negative family history for aortic dissections, or considerably increasing interval changes in aortic dimensions [22, 23]. In those with smaller diameters, cardiac imaging every 2 years should be sufficient.

Medical Therapy

Due to histological similarities between aortic specimens of BAV and MFS patients [30], the current medical therapies for BAV resulted from extrapolating those for MFS. Noteworthy, the efficacy of these therapeutic strategies has not yet been demonstrated in large BAV cohorts and is even under debate in the MFS field [31]. Consequently, many efforts are in progress to acquire more evidence regarding the disease modifying capacities of the currently available drugs and to develop alternative MFS and/or BAV therapies.

The mainstay of the current therapeutic approach in MFS-related aortopathy is the administration of β-adrenergic receptor antagonists (β-blockers). Their role in BAV-related aortopathy remains controversial. Prophylactic β-blockers have been suggested to impact on aneurysm progression by reducing the mean arterial pressure and the systolic heart rate [32]. In spite of the established use of β-blockers in TAA management, clinical trials in MFS patients also revealed variable outcomes [33–35]. Additionally, in a recent 4D flow imaging study, in vivo 3D aortic blood flows were not significantly altered in treated BAV patients compared to their untreated counterparts [36]. To allow more powerful estimates of the protective effects of β-blockers on BAV-related aortopathy, the latter findings should be replicated in larger cohorts that are stratified for dosage in addition to treatment duration.

Administration of angiotensin II receptor blockers (ARBs) embodies the second therapeutic approach in patients with aortic dilatation. Angiotensin II binds and exerts its function through two G-protein-coupled receptors: AT1 and AT2. Via activation of AT1, the transforming growth factor (TGF-β) pathway becomes stimulated, initiating fibrosis [37]. In 2006, upregulated TGF-β signaling was pinpointed the key culprit in the pathogenesis of MFS-related aortopathy, urging development of TGF-β neutralizing therapies [38]. The AT1 blocker losartan had already proven capable of attenuating TGF-β signaling in certain animal models and was routinely used to treat hypertension, which rendered it the number-1 drug to test [39, 40]. While small studies in humans seemed promising [41–43], recent findings did not confirm the efficacy of losartan as to MFS-related TAA management [44–47]. A meta-analysis combining all individual trials (±2300 patients) is being conducted at the time of writing [48]. In BAV patients, there is no trial-based evidence for beneficence of ARBs, nor for preferred use of ARBs over β-blockers or vice versa. At present, a randomized multicenter trial is being conducted, addressing the efficacy of both β-blockers and ARB treatment in BAV patients (https://clinicaltrials.gov/ct2/show/NCT01202721).

The third potential therapeutic strategy involves administration of angiotensin-converting-enzyme inhibitors (ACEi), which block the processing of biologically inactive angiotensin I to its active form, that is, angiotensin II, which after binding to AT1 receptors leads to vasoconstriction. Consequently, ACEi decrease blood pressure by preventing the contraction of blood vessels. In MFS, they are mostly prescribed to patients who are intolerant of β-blockers [49]. Beneficence is under debate though, because ACEi also block the beneficial downstream AT2 pathway. As to BAV-related TAA, a recent study has revealed lack of significant ACEi efficacy [50]. Larger studies are necessary to shed a better light on the added value of ACEi therapy in BAV/TAA patients though.

Surgical Intervention

In the young, implantation of prosthetic valves yields suboptimal clinical outcomes because of the patients' enduring

growth. In addition, the anticoagulant therapy, warranted in patients with a mechanical valve, may prohibit sports participation or other activities. Hence, in BAV children and young adults with isolated AS, balloon valvuloplasty (i.e., widening of the stenotic aortic valve by inserting a balloon catheter) is designated the optimal treatment option [51]. In adults, aortic valve replacement is the preferred management strategy due to superior durability. To treat AR in the absence of AS, valve-sparing repair methods avoiding the anticoagulation-related risks that come with mechanical valve implantation have emerged. Their success, however, heavily depends on leaflet tissue quality, that is the degree of fibrosis and calcification [52]. Other options are bioprosthesis, human tissue valves, and the Ross-procedure (especially in children). As to surgical intervention for TAA, the precise timing is mostly being determined by pragmatically established, but systematically revised aortic diameter thresholds. In asymptomatic individuals, the current guidelines recommend elective aortic repair when proximal aortic diameters exceed 55 mm [53]. BAV patients in whom additional risk factors have been identified, including a positive family history for SCD due to dissections or ruptures, systemic hypertension, and aortic enlargement at an extremely rapid pace (≥5 mm/year), should undergo surgical intervention if the aortic dimensions ≥50 mm. A cutoff of 45 mm is advisable if aortic valve repair is anyway being performed because of severe AS or AR.

Taken together, surgical procedures for BAV and/or TAA should be tailored to the presence and degree of valvular dysfunction and aneurysmal disease as well as the desired anticoagulation status. In the early 1970s, the so-called Bentall and De Bono surgical procedures, which replace the aortic root, ascending aorta and/or aortic valve with, respectively, a Dacron prosthesis and/or mechanical valve, represented the golden standard [54, 55]. More recently, valve-sparing techniques such as David's or Yacoub's surgery are becoming increasingly popular, eliminating the need for life-long anticoagulation therapy [56]. Their mid-term results have proven excellent, with at least 90 % of the patients being free from reoperation on the aortic valve 10–15 years after surgery, but long-term results have yet to be evaluated [57].

Cardiovascular Management in Pregnancy

Pregnant women with BAV, and particularly those with severe AS, are at increased risk for cardiac and neonatal complications. Hence, female patients should be thoroughly monitored and counseled regarding potential threats and treatment options prior to, during, and after pregnancy. Recent guidelines recommend BAV females to undergo advanced imaging of the valve and thoracic aorta (CT or CMR) before pregnancy [58]. Upon observation of aortic diameters above 50 mm or symptomatic AS and AR, prepregnancy surgery should be considered. Nevertheless, in rare instances highly progressive symptoms may still develop during pregnancy, requiring balloon valvuloplasty or valve/aorta surgery. Only in the case of positive, well-substantiated benefit/risk ratios their execution is justified.

Of note, BAV in pregnancy might not cause major problems in the vast majority of patients. The latter cases are generally not reported in the literature as they do not require specific interventions, possibly biasing the estimated complication frequencies (10–30 % of BAV/AS) [59].

Sports Recommendations

In about 5–8 % of the athletes below 40 years, SCD during sports is caused by AS and/or ruptured TAA, which are commonly associated with BAV [60, 61]. As such, once athletes have been diagnosed with BAV, specific guidelines on exercise should be followed to prevent SCD. According to the 2015 ACC/AHA guidelines, BAV athletes without valve problems and with aortic dimensions ≤40 mm can participate in all competitive sports [62]. For those with a mildly to moderately dilated aorta (40–45 mm), participation in low-intensity competitive athletics with a low likelihood of bodily contact may be considered. Intense weight training has to be avoided. Finally, athletes with a markedly dilated aorta (>45 mm) should not participate in any competitive sports. They can participate safely in recreational golfing or billiards, for example, though.

Molecular Genetics

BAV may occur both sporadically or familial, but its high heritability (89 %) indicates that disease determination is largely genetic [63]. In rare extended families, an autosomal dominant inheritance pattern with reduced penetrance and variable expressivity has been observed [64, 65]. Also high genetic heterogeneity has been established, further complicating the etiology of BAV [66].

Historically, aberrant postvalvular hemodynamics were thought to be the sole trigger for TAA development in BAV patients. Over the last decade, however, increasing evidence for the existence of common genetic defects causing BAV and TAA has emerged [67]. Arguments in favor of this hypothesis include identification of several pedigrees in which BAV and TAA either cooccur or manifest as a single disease entity [68], progression of aneurysm formation after valve replacement [1] and shared embryonic origin of the aortic valve as well as the ascending aorta [69]. Most likely, the etiology of BAV/TAA is complex in nature, with genetic predisposing factors working in combination with abnormal flow patterns.

Pertaining to genetics, BAV likely is a consequence of mutations in diverse genes encoding transcription factors, extracellular matrix components, or proteins acting in signaling pathways that regulate cell proliferation, differentiation, adhesion, or apoptosis [70]. The high clinical and genetic heterogeneity of BAV and its accompanying complications, however, have significantly burdened gene identification. As an example, family-based linkage studies have successfully pinpointed BAV loci (chr18q, 5q, & 13q) almost 10 years ago [70]. Nonetheless, the major underlying BAV culprits have still not been identified. Even the recent advent of next-generation sequencing did not (yet) prominently move the BAV genetics field forward. In the following sections, we report on the genetic breakthroughs so far.

BAV Genes in Humans

NOTCH1

To date, the only firmly established BAV gene is *NOTCH1* (OMIM *190198). In 2005, dominantly inherited loss-of-function mutations were first described in two unrelated BAV families suffering from early onset aortic valve calcification [71]. Meanwhile, additional *NOTCH1* mutations (i.e., missense, splice site, nonsense, and frameshift) have been identified in up to 13 % of the familial and 4 % of the sporadic BAV, AS, or BAV/AS cases [72]. Reduced penetrance (~16 %) has been documented. Although TAA has repeatedly been reported in a fraction of the mutation carriers, *NOTCH1* mutations are not considered as a major cause of noncalcified, nonstenotic BAV with highly penetrant TAA [73]. Occasionally, *NOTCH1* mutations do cause non-BAV/AS left-sided cardiovascular pathologies though, for example, (BAV/)coarctation and hypoplastic left heart syndrome [72, 74, 75].

NOTCH1 encodes a 300-kDa single-pass transmembrane receptor consisting of an extracellular domain with 36 epidermal growth factor-like and three NOTCH/Lin repeats, in addition to an intracellular transactivating domain containing six ankyrin repeats. Upon binding of its ligands (i.e., DLL1, DLL3, DLL4, JAG1, and JAG2), NOTCH1 gets cleaved by ADAM metalloproteases and converts into a transcriptional coactivator [76]. Influencing embryonic cell fate decisions, proper NOTCH signaling is crucial for multiple developmental processes, including cardiovascular development [77, 78]. Experimental evidence increasingly converges on abnormal EMT as the key culprit for aortic valve malformations in *NOTCH1* mutation carriers. Constitutional Notch1 null mice die early due to severe cardiac defects attributed to abnormal EMT [79]. Furthermore, in aortic endothelial cells of *NOTCH1* mutation carriers, ligand-to-NOTCH binding cannot activate EMT [80]. Most recently, also excessive mesenchyme proliferation has been observed in cardiac-specific *Notch1* mutant mice who present with valve dysmorphology (e.g., BAV) [81]. It has been suggested that whereas Dll4-Notch1 signaling mediates EMT, Jag1-Notch1 signaling restricts post-EMT valve mesenchyme proliferation. Interestingly, besides the single mechanisms as such, also crosstalk between both signaling cascades might be affected in *NOTCH1*-related congenital heart disease. Further investigation with respect to these assumptions, however, is warranted.

Scientists have also succeeded in shedding light on the molecular mechanisms underlying aortic valve calcification in *NOTCH1* mutation carriers. Under normal physiological circumstances, NOTCH1 suppresses valve calcification by inducing the expression of *HEY1/2*. In turn, they act through BMP2 to suppress activation of Runx2, a transcriptional activator of osteoblast development [82–86]. Obviously, loss of NOTCH1 disables its protective capacity relating to vascular/valvular calcification, which fits the pathological hallmarks of *NOTCH1* patients.

GATA5 and Related Transcription Factors

The search for rare *GATA5* sequence variants in humans with BAV was instigated by the discovery of hypoplastic hearts and partially penetrant R-N BAV (~25 %) in endocardial cell-specific Gata5 knockout mice [87]. To date, several rare heterozygous mutations in *GATA5* (OMIM *611496), which encodes a cardiac transcription factor, have been described in BAV patients [88–91]. No prominent clustering in specific protein domains was observed, yet nearly all mutations were shown to drastically reduce the protein's transcriptional potency. Comparable *GATA5* mutations have also been identified in other, strikingly dissimilar cardiovascular disorders: isolated VSD [92], dilated cardiomyopathy [93], lone atrial fibrillation [94], and tetralogy of fallot [95]. The pronounced discrepancy in the *GATA5*-related phenotypes remains to be explained, but one likely hypothesis states that other genetic determinants and/or environmental risk factors influence an individual's ultimate phenotypic outcome.

The GATA5 zinc finger transcription factor is exclusively expressed in the endocardial cells and cushions of both the atrioventricular canal and the OFT [96]. In embryonic mice, loss of Gata5 reduces Jag1 expression while increasing Rbpj-κ expression, respectively a ligand and repressor of Notch, cumulatively resulting in ~20–30 % downregulation of the Notch signaling pathway [87]. In contrast to what has been observed in Notch1 knockout mice, EMT was not altered. This might be explained by (partial) compensation for loss of Gata5 by closely related cardiac Gata transcription factors [87]. Besides Notch-related genes, also *Nos3* and *Tbx20* were suggested to be downstream targets of Gata5. Interestingly, mutations in *TBX20* have been linked with valve and septal defects in humans [97] and Nos3−/− mice display partially penetrant R-N BAV (see section, BAV in animal models) [98].

GATA6 is closely related to *GATA5*, but has a somewhat different expression pattern. Besides in the fetal heart, it is

also highly expressed in the embryonic pancreas. In concordance with this profile, *de novo GATA6* (OMIM *601656) mutations have been identified in patients with pancreatic agenesis and, in the majority of the cases (92 %), congenital heart defects, including ASD, VSD, tetralogy of fallot, patent ductus arteriosus or double outlet right ventricle [99]. Although one of the parents of a patient with *GATA6*-related ASD was found to have BAV [100], and heterozygous Gata6 knockout mice show partially penetrant BAV [101], substantial evidence for a role of heterozygous *GATA6* mutations in humans with BAV lacks.

One more crucial GATA transcription factor for heart development exists: GATA4. Similarly to genetic defects in *GATA6*, *GATA4* mutations (OMIM *600576) cause atrial and VSD, yet no BAV [102].

SMAD6

In 2012, heterozygous missense mutations in the *SMAD6* gene (OMIM *602931) were identified in two sporadic BAV patients with mild to moderate AS [103]. In one of these patients, also coarctation was observed. *SMAD6* is highly expressed in the cardiac valves and OFT of the human embryonic heart. Its encoded protein consists of two large domains, MH1 and MH2. Via the latter protein domain, SMAD6 interacts with bone morphogenetic protein (BMP) type I receptors to inhibit BMP signaling [104]. The described mutations both locate to this MH2 domain and lead to inefficient inhibition of BMP signaling when compared to wild-type protein, suggesting that *SMAD6* mutations cause BAV through a loss of function mechanism [103]. A causal link between *SMAD6* mutations and BAV/AS development is further supported by the fact that Smad6 deficient mice mirror the human phenotype with aortic ossification and multiple congenital cardiovascular abnormalities including thickening of the cardiac valves and OFT septation defects [105]. Interestingly, BMP signaling has been documented to crosstalk with NOTCH as well as canonical and non-canonical TGF-β signaling, highlighting the emergence of convergent pathomechanisms for BAV [106].

Occasional Findings in *NKX2.5* and *MATR3*

NKX2.5 has been considered a plausible candidate gene for BAV because of multiple reasons: it encodes an important transcription regulator involved in cardiac morphogenesis, BAV has been reported in 11 % of the Nkx2.5+/– mice [107], and finally, the human *NKX2.5* gene maps to chr5q34, a linked but yet unexplained locus for BAV. Whereas more than 50 mutations spread over the various functional domains of NKX2.5 (OMIM *600584) have been identified in patients with ASD, VSD, hypoplastic left heart syndrome or tetralogy of fallot [108], only two have been reported in BAV patients. A heterozygous missense mutation (p.Arg25Cys) that had previously been identified in a non-BAV individual with cardiac disease was found in a Down syndrome patient with aneurysm of the membranous septum, aortic coarctation, and BAV [109]. The other mutation (p.Lys192*) segregated in a three-generational autosomal dominant BAV family [110]. Functional analyses of the latter genetic defect revealed almost complete depletion of transcriptional activity compared to wild-type NKX2.5 as well as loss of the synergistic transcriptional activation of NKX2.5 and GATA5, supporting variant pathogenicity. Yet, owing to the very few *NKX2.5* mutations that have been reported in BAV patients to date, *NKX2.5* is currently not unambiguously recognized as a human BAV gene.

A direct relationship between genetic variability in *MATR3* and BAV development was put forward with the identification of a *de novo* translocation disrupting the 5′ UTR of *AHDC1* and the 3′ UTR of *MATR3* (OMIM *164015) in an individual with BAV, coarctation of the aorta and patent ductus arteriosus in addition to pervasive developmental delay [111]. Whereas *AHDC1* loss of function most probably accounts for the observed developmental impairment [112], subtle perturbations in the level and/or function of the nuclear matrix protein MATR3 have been proposed to explain the cardiovascular manifestations [111]. Arguments in favor of this assumption include strong expression of *Matr3* in the developing mouse heart and the presence of BAV in 15 % of heterozygous Matr3 3′ truncated transgenic mice. Missense *MATR3* mutations in humans, however, cause a slowly progressive form of amyotrophic lateral sclerosis, a devastating neuromuscular disorder [113]. Taken together, more supportive evidence is needed, for example, through the identification of additional BAV patients with *MATR3* mutations, to truly establish an association between BAV and genetic variability in *MATR3*.

BAV in Syndromic Disease

Several reports on incidental BAV recognition in clinically and pathomechanistically diverse disease entities exist, for example, in chr22q11.2 deletion syndrome [114], familial left ventricular noncompaction (*MYH7*) [115], non-syndromic TAA (*ACTA2*) [116], Joubert syndrome (unknown genetic defect) [117], and joint dislocation associated with congenital heart disease (*B3GAT3*) [118]. Whether BAV truly belongs to the phenotypic spectrum of these disorders is yet surrounded by uncertainty. Hence, more interesting to follow-up might be the established, markedly increased prevalence of BAV in Loeys Dietz syndrome (LDS) and Turner syndrome. Whereas the majority of the underlying genetic disease causes of the latter syndromes have been identified, the precise mechanisms with respect to increased BAV susceptibility in these patients remain largely elusive.

Although some studies have suggested that BAV presents in up to 5 % of the MFS cases [119], this finding has not been observed in large observational MFS studies. With MFS being caused by mutations in the fibrillin-1 encoding *FBN1*

gene (OMIM *134797), resulting in dysregulation of the TGF-β signaling pathway and impaired extracellular matrix integrity [31, 120], some studies aimed at unraveling the link between *FBN1* and BAV, thus far with inconclusive results. On one hand, decreased fibrillin-1 levels have been observed in the aorta and pulmonary artery of patients with BAV [121] and rare BAV/TAA patients (in whom a clinical MFS diagnosis was excluded) with *FBN1* missense mutations have been reported [122]. The latter *FBN1* missense mutations seem rather mild variants with few other MFS outward features but in association with a BAV sufficient to cause aortic aneurysm. On the other hand, a significant association between single nucleotide polymorphisms in or spanning the *FBN1* gene and BAV could not be established [123, 124], whereas genetic *FBN1* variability did associate with increased TAA risk [124]. Fewer studies have investigated the link between BAV and LDS, a connective tissue disorder that clinically resembles MFS but presents with additional features such as hypertelorism, craniosynostosis, bifid uvula, cleft palate, and arterial tortuosity. The incidence of BAV in LDS patients exceeds that seen in the general population by five times [125]. To date, mutations in six genes (i.e., *TGFBR1, TGFBR2, SMAD2, SMAD3, TGFB2* and *TGFB3*) causing LDS have been reported, all resulting in a pathological increase in TGF-β signaling [31]. Mutation analysis of *TGFBR1* and *TGFBR2* in BAV cohorts only yielded a single hit (p.Val387Met) of unknown significance [74, 126, 127]. *SMAD2, SMAD3, TGFB2*, and *TGFB3* have not yet systematically been screened in BAV cohorts as far as we know, but LDS patients with mutations in those genes occasionally present BAV.

BAV (±30 %), as well as TAA and aortic coarctation, is remarkably frequent in Turner syndrome, which is caused by either partial or complete absence of one X-chromosome [128]. Apart from the aforementioned cardiovascular defects, affected females generally present with a webbed neck, low-set ears, short stature, diabetes, and low thyroid hormone levels. It is expected that the complex phenotype of Turner syndrome results from loss of function of multiple X-linked genes, including at least one yet to be identified gene that explains the cardiovascular manifestations [129]. A remarkably higher prevalence of BAV in subjects missing only the short arm of the X-chromosome (Xp) has been observed, suggesting Xp location of such gene(s) [130]. Of note, existence of an X-linked BAV gene might, at least partially, explain the prominent male predominance of BAV.

BAV in Animal Models

Besides the already above-described ones, several more mouse genes have been linked to partially penetrant BAV in mouse knockout models. Additionally, concurrent targeting of multiple genes belonging to the Robo-Slit signaling pathway was shown to cause BAV as well.

The Nos3 null mouse was one of the first discovered BAV animal models [98]. *Nos3* is highly expressed in the endothelial cells of the aortic valve. It encodes endothelial nitric oxide synthase (eNos), which is a downstream target of Gata5 and Notch1 [87, 131]. Approximately 27 % of the eNos knockout mice develop BAV [132]. Backcrossing Notch1+/– into a Nos3 null background yielded a significantly higher penetrance of BAV (~73 %), further underlining genetic interaction between the eNos and Notch1 signaling pathways [132]. Interestingly, adult compound –/– Nos3; +/–Notch1 mice have also been found to develop dilation of the aortic sinus, independent of BAV-related hemodynamic disturbances [133]. Consistent with the mouse findings, decreased protein expression of eNOS was observed in aortic endothelial cells of patients with BAV [134]. Moreover, in BAV patients a significant inverse correlation between eNOS expression and aortic diameters was observed [134]. Nevertheless, no BAV causing *NOS3* mutations have been identified so far in humans.

BAV also frequently (~78 %) presents in mice lacking *Alk2* in the endocardial cushion mesenchyme [135], though not in neural crest-specific knockouts [136], highlighting a cell-autonomous origin of *Alk2*-related BAV development. *Alk2* encodes the activin receptor type I (AcvrI), which has a key role in the BMP signaling pathway. To be exact, AcvrI activation has been shown to stimulate EMT in the aortic valve cushions [137]. In humans, a gain-of-function *ALK2* mutation causes a very rare connective tissue disorder characterized by progressive ectopic ossification of skeletal muscles, fascia, tendons, and ligaments [138]. BAV has not been reported in these patients.

Mice depleted for *Hoxa1*, coding for another essential cardiovascular transcription factor, show a variety of heart defects, including partially penetrant BAV (25 %) [139]. During early embryogenesis, *Hoxa1* is expressed in precursors of the cardiac neural crest cells, where it acts upstream of genes driving neural crest specification and maturation [139]. In humans, recessive *HOXA1* mutations cause a complex phenotype characterized by horizontal gaze abnormalities, deafness, facial weakness, hypoventilation, malformations of the internal carotid arteries and OFT, mental retardation, and autism [140]. BAV did not present in *HOXA1* cases, nor have *HOXA1* mutations been observed in BAV patients.

Mouse embryos lacking endocardial Brg1 show thickened cardiac valves that frequently (~35 %) are bicuspid [141]. Brg1 almost entirely makes up the core ATPase subunit of the Brg1-associated factor chromatin remodeling complex, which facilitates activation and repression of genes through ATP-dependent alteration of the chromatin structure. In the cardiovascular system, Brg1 has particularly been shown to be pivotal in regulating proliferation, differentia-

tion, and apoptosis of neural crest cells as well as EMT [141, 142]. Gain and loss of function mutations in the human ortholog of *Brg1*, *SMARCA4*, respectively, cause syndromic mental retardation or rhabdoid tumor predisposition syndrome [143, 144]. BAV does not belong to the phenotypic spectrum of either one of both.

Finally, BAV has been observed in mice depleted for *Robo* and/or *Slit* genes [145]. Whereas BAV presents in all compound Robo1; Robo2 mutants, the aortic valves in single Robo1 or Robo2 mutant mice appear normal, suggesting functional Robo1/2 redundancy in the heart. Of note, BAV was also observed in Slit2 knockout mice, yet with significantly reduced penetrance. The exact mechanisms that interconnect Robo-Slit dysfunction and BAV formation remain to be elucidated. Crosstalk between Robo-Slit and Notch signaling might be part of the explanation, as Robo1 deficiency was shown to impact on *Notch1/2* expression levels [145]. Furthermore, genes relating to the Notch or Robi-Slit pathways show strikingly similar expression patterns in the developing heart. To date, no human disorders have firmly been linked to mutations in *ROBO1*, *ROBO2,* or *SLIT2*.

Molecular Diagnostics

Molecular diagnostic testing encompasses systematic screening of patients and their relatives for the presence of pathogenic variants in known causal genes, enabling early disease management stratification by discriminating between at-risk individuals and those likely to be unaffected.

As discussed in the sections above, the genetic etiology of BAV is considered exceedingly heterogeneous and is, based on the large number of genetically unexplained familial cases, far from solved. Moreover, genetic defects in the most established BAV culprits (i.e., *NOTCH1*, *GATA5*, *SMAD6*) are rare, hardly ever fully penetrant, and associate with exceedingly diverse phenotypes, making interpretation of their pathogenic nature and genetic counseling challenging. Establishment of variant recurrence among multiple families, mutation clustering in certain protein domains, and genotype–phenotype correlations would facilitate the latter process. Routine testing of the known BAV genes in a molecular diagnostic setting, which is still in its infancy, therefore serves a dual purpose, that is, risk stratification as well as expansion and validation of the mutational and phenotypic spectrum of the known BAV genes.

In genetically heterogeneous disorders such as BAV, molecular diagnostic testing by means of gene panel sequencing has proven most proficient [146]. However, as presently only a small number of BAV genes has to be screened and their mutation frequencies are low, the approach is of too little yield to be cost efficient. Consequently, BAV genes are presently being included in large next-generation sequencing panels encompassing genes causal for a variety of congenital heart disorders and/or aortopathies. With the declining cost of exome sequencing, it will likely also replace gene panel sequencing in a diagnostic setting in the near future. Molecular tests to identify patients at risk for specific BAV-related complications, such as AS, AR, or TAA, will require considerably more time and research because their underlying genetic determinants are even more enigmatic. Until the genetic etiology of BAV and its associated complications has been considerably further elucidated, careful cardiovascular surveillance will likely remain an important clinical strategy in the majority of BAV families (see section, "Family Screening").

Family Screening

The ESC (http://www.escardio.org/) and ACC/AHA guidelines acknowledge the involvement of genetics in the disease etiology and recommend imaging-based evaluation for BAV and TAA of first-degree relatives of BAV cases [32]. Whether monitoring should start as soon as possible or from 18 years onwards is still under debate. Once BAV has been diagnosed, standard surveillance and management guidelines should be followed (see section, "Patient Management"). As to TAA monitoring following TAV establishment, no formal recommendations have been drafted. As aortopathy can take years to develop, regular (i.e., every 3–5 years) interval follow-up is advisable though.

At present, imaging-based family screening is in practice still predominantly tailored to relatives of either BAV patients with severe cardiovascular complications or a positive anamnesis for BAV or TAA.

Conclusions

Due to the high prevalence of BAV, and the life-threatening cardiovascular complications that associate with it, BAV represents a major public health problem. Over the past couple of years extensive efforts aiming at unraveling the disorder's etiology have revealed valuable insights, typically pinpointing EMT malfunction or distortion of the normal cardiac neural crest cell activity as the key disease culprits. Yet, the genetic and pathomechanistic picture is still far from complete, hampering development of novel therapies. These are definitely needed, as major uncertainty surrounds the usefulness of the current medical therapies for BAV. Investigations into the basic genetic defects as well as dysfunctional cellular mechanisms and signaling pathways underlying BAV will definitely continue in the upcoming years. Their adequate design necessitates acknowledgment of the factors underlying the current inscrutability of BAV genetics. Some hypotheses can already be put forward. As extended families segregating BAV in a Mendelian manner are rare, gene identification approaches in groups of unrelated BAV patients, commonly taking advantage of the

advent of next-generation sequencing technologies, have increasingly gained momentum. In the latter studies, genetic heterogeneity might jeopardize success. Recent findings suggest that distinct BAV subtypes result from different pathomechanisms [3]. Moreover, occurrence of particular cardiovascular complications might correlate with the underlying genetic BAV cause. Considering BAV as a single disease entity can thus unnecessarily compromise homogeneity of patients under study. To increase the probability of finding multiple cases with pathogenic mutations in the same gene in future gene identification studies, it could be advantageous to stratify patient populations for well-defined endophenotypes as to valve morphology and coexisting morbidity. Additionally, one might consider to select cases with a positive family history who have been confronted with prominent valve dysfunction or aortopathy early in life. These individuals are situated at the severest end of the phenotypic BAV spectrum, rendering a major contribution of genetic factors to the disease etiology very likely. A second hypothesis states that oligogenic inheritance accounts for a substantial fraction of the genetic etiology of BAV, implying that one single patient should carry rare mutations in multiple genes, whether or not belonging to one biological pathway. Supportive evidence encompasses low penetrance of BAV in the so far described single gene knockout mice, whereas compound knockouts are more consistently affected [132, 145]. Furthermore, oligogenic inheritance in other left ventricular OFT malformations has already been demonstrated [147]. Consequently, future BAV analysis strategies should also be tailored toward non-Mendelian inheritance patterns.

Take Home Message

- Bicuspid aortic valve:
  - Is the most common congenital heart disorder
  - Is most often clinically silent, but associates with a variety of severe cardiovascular complications
  - Is currently managed by means of:
    - Interval cardiovascular monitoring to diagnose concomitant cardiovascular features
    - Medical therapy (although efficacy remains to be proven)
    - Surgical intervention
  - Presents with high heritability, which is indicative of a role for genetic factors
  - Is genetically far from explained, but seems complex and exceedingly heterogeneous
  - Likely results from defective endothelial-to-mesenchymal transition and/or altered cardiac neural crest cell activity

References

1. Braverman AC, Guven H, Beardslee MA, Makan M, Kates AM, Moon MR. The bicuspid aortic valve. Curr Probl Cardiol. 2005; 30(9):470–522.
2. Sievers HH, Schmidtke C. A classification system for the bicuspid aortic valve from 304 surgical specimens. J Thorac Cardiovasc Surg. 2007;133(5):1226–33.
3. Fernandez B, Duran AC, Fernandez-Gallego T, Fernandez MC, Such M, Arque JM, et al. Bicuspid aortic valves with different spatial orientations of the leaflets are distinct etiological entities. J Am Coll Cardiol. 2009;54(24):2312–8.
4. Ward C. Clinical significance of the bicuspid aortic valve. Heart. 2000;83(1):81–5.
5. Tzemos N, Therrien J, Yip J, Thanassoulis G, Tremblay S, Jamorski MT, et al. Outcomes in adults with bicuspid aortic valves. JAMA. 2008;300(11):1317–25.
6. Michelena HI, Desjardins VA, Avierinos JF, Russo A, Nkomo VT, Sundt TM, et al. Natural history of asymptomatic patients with normally functioning or minimally dysfunctional bicuspid aortic valve in the community. Circulation. 2008;117(21):2776–84.
7. Michelena HI, Khanna AD, Mahoney D, Margaryan E, Topilsky Y, Suri RM, et al. Incidence of aortic complications in patients with bicuspid aortic valves. JAMA. 2011;306(10):1104–12.
8. Fedak PW, Verma S, David TE, Leask RL, Weisel RD, Butany J. Clinical and pathophysiological implications of a bicuspid aortic valve. Circulation. 2002;106(8):900–4.
9. Kang JW, Song HG, Yang DH, Baek S, Kim DH, Song JM, et al. Association between bicuspid aortic valve phenotype and patterns of valvular dysfunction and bicuspid aortopathy: comprehensive evaluation using MDCT and echocardiography. JACC Cardiovasc Imaging. 2013;6(2):150–61.
10. Kim JS, Ko SM, Chee HK, Shin JK, Song MG, Shin HJ. Relationship between bicuspid aortic valve phenotype, valvular function, and ascending aortic dimensions. J Heart Valve Dis. 2014;23(4):406–13.
11. Braverman AC. Aortic involvement in patients with a bicuspid aortic valve. Heart. 2011;97(6):506–13.
12. Edwards WD, Leaf DS, Edwards JE. Dissecting aortic aneurysm associated with congenital bicuspid aortic valve. Circulation. 1978;57(5):1022–5.
13. Michelena HI, Prakash SK, Della Corte A, Bissell MM, Anavekar N, Mathieu P, et al. Bicuspid aortic valve: identifying knowledge gaps and rising to the challenge from the International Bicuspid Aortic Valve Consortium (BAVCon). Circulation. 2014;129(25): 2691–704.
14. Nanda NC, Gramiak R, Manning J, Mahoney EB, Lipchik EO, DeWeese JA. Echocardiographic recognition of the congenital bicuspid aortic valve. Circulation. 1974;49(5):870–5.
15. Freeman RV, Otto CM. Bicuspid aortic valve and aortopathy: see the first, then look at the second. JACC Cardiovasc Imaging. 2013;6(2):162–4.
16. Yousry M, Rickenlund A, Petrini J, Jenner J, Liska J, Eriksson P, et al. Aortic valve type and calcification as assessed by transthoracic and transoesophageal echocardiography. Clin Physiol Funct Imaging. 2015;35(4):306–13.
17. Takeda H, Muro T, Saito T, Hyodo E, Ehara S, Hanatani A, et al. Diagnostic accuracy of transthoracic and transesophageal echocardiography for the diagnosis of bicuspid aortic valve: comparison with operative findings. Osaka City Med J. 2013;59(2):69–78.
18. Cote G, Denault A. Transesophageal echocardiography-related complications. Can J Anaesth. 2008;55(9):622–47.
19. Joziasse IC, Vink A, Cramer MJ, van Oosterhout MF, van Herwerden LA, Heijmen R, et al. Bicuspid stenotic aortic valves: clinical characteristics and morphological assessment using MRI and echocardiography. Neth Heart J. 2011;19(3):119–25.

20. van der Wall EE. Bicuspid aortic valve; optimal diagnosis and latest interventional treatment. Neth Heart J. 2015;23(3):149–50.
21. Mordi I, Tzemos N. Bicuspid aortic valve disease: a comprehensive review. Cardiol Res Pract. 2012;2012:196037.
22. Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin 3rd JP, Guyton RA, et al. 2014 AHA/ACC Guideline for the Management of Patients With Valvular Heart Disease: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129(23):2440–92.
23. Joint Task Force on the Management of Valvular Heart Disease of the European Society of C, European Association for Cardio-Thoracic S, Vahanian A, Alfieri O, Andreotti F, Antunes MJ, et al. Guidelines on the management of valvular heart disease (version 2012). Eur Heart J. 2012;33(19):2451–96.
24. Ferencik M, Pape LA. Changes in size of ascending aorta and aortic valve function with time in patients with congenitally bicuspid aortic valves. Am J Cardiol. 2003;92(1):43–6.
25. Dore A, Brochu MC, Baril JF, Guertin MC, Mercier LA. Progressive dilation of the diameter of the aortic root in adults with a bicuspid aortic valve. Cardiol Young. 2003;13(6):526–31.
26. Detaint D, Michelena HI, Nkomo VT, Vahanian A, Jondeau G, Sarano ME. Aortic dilatation patterns and rates in adults with bicuspid aortic valves: a comparative study with Marfan syndrome and degenerative aortopathy. Heart. 2014;100(2):126–34.
27. Yap SC, Kouwenhoven GC, Takkenberg JJ, Galema TW, Meijboom FJ, van Domburg R, et al. Congenital aortic stenosis in adults: rate of progression and predictors of clinical outcome. Int J Cardiol. 2007;122(3):224–31.
28. Etz CD, Zoli S, Brenner R, Roder F, Bischoff M, Bodian CA, et al. When to operate on the bicuspid valve patient with a modestly dilated ascending aorta. Ann Thorac Surg. 2010;90(6):1884–90; discussion 91–2.
29. Shimada I, Rooney SJ, Pagano D, Farneti PA, Davies P, Guest PJ, et al. Prediction of thoracic aortic aneurysm expansion: validation of formulae describing growth. Ann Thorac Surg. 1999;67(6):1968–70; discussion 79–80.
30. Niwa K, Perloff JK, Bhuta SM, Laks H, Drinkwater DC, Child JS, et al. Structural abnormalities of great arterial walls in congenital heart disease: light and electron microscopic analyses. Circulation. 2001;103(3):393–400.
31. Verstraeten A, Alaerts M, van Laer L, Loeys B. Marfan syndrome and related disorders: 25 years of gene discovery. Hum Mutat. 2016;37(6):524–31.
32. Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey Jr DE, et al. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. Circulation. 2010;121(13):e266–369.
33. Gersony DR, McClaughlin MA, Jin Z, Gersony WM. The effect of beta-blocker therapy on clinical outcome in patients with Marfan's syndrome: a meta-analysis. Int J Cardiol. 2007;114(3):303–8.
34. Plein A, Calmont A, Fantin A, Denti L, Anderson NA, Scambler PJ, et al. Neural crest-derived SEMA3C activates endothelial NRP1 for cardiac outflow tract septation. J Clin Invest. 2015;125(7):2661–76.
35. Kioussi C, Briata P, Baek SH, Rose DW, Hamblet NS, Herman T, et al. Identification of a Wnt/Dvl/beta-Catenin → Pitx2 pathway mediating cell-type-specific proliferation during development. Cell. 2002;111(5):673–85.
36. Allen BD, Markl M, Barker AJ, van Ooij P, Carr JC, Malaisrie SC, et al. Influence of beta-blocker therapy on aortic blood flow in patients with bicuspid aortic valve. Int J Cardiovasc Imaging. 2016;32(4):621–8.
37. Habashi JP, Doyle JJ, Holm TM, Aziz H, Schoenhoff F, Bedja D, et al. Angiotensin II type 2 receptor signaling attenuates aortic aneurysm in mice through ERK antagonism. Science. 2011;332(6027):361–5.
38. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science. 2006;312(5770):117–21.
39. Lim DS, Lutucuta S, Bachireddy P, Youker K, Evans A, Entman M, et al. Angiotensin II blockade reverses myocardial fibrosis in a transgenic mouse model of human hypertrophic cardiomyopathy. Circulation. 2001;103(6):789–91.
40. Martin B, Brenneman R, Becker KG, Gucek M, Cole RN, Maudsley S. iTRAQ analysis of complex proteome alterations in 3xTgAD Alzheimer's mice: understanding the interface between physiology and disease. PLoS One. 2008;3(7):e2750.
41. Brooke BS, Habashi JP, Judge DP, Patel N, Loeys B, Dietz HC, 3rd. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. N Engl J Med. 2008;358(26):2787–95.
42. Chiu HH, Wu MH, Wang JK, Lu CW, Chiu SN, Chen CA, et al. Losartan added to beta-blockade therapy for aortic root dilation in Marfan syndrome: a randomized, open-label pilot study. Mayo Clin Proc. 2013;88(3):271–6.
43. Pees C, Laccone F, Hagl M, Debrauwer V, Moser E, Michel-Behnke I. Usefulness of losartan on the size of the ascending aorta in an unselected cohort of children, adolescents, and young adults with Marfan syndrome. Am J Cardiol. 2013;112(9):1477–83.
44. Bhatt AB, Buck JS, Zuflacht JP, Milian J, Kadivar S, Gauvreau K, et al. Distinct effects of losartan and atenolol on vascular stiffness in Marfan syndrome. Vasc Med. 2015;20(4):317–25.
45. Forteza A, Evangelista A, Sanchez V, Teixido-Tura G, Sanz P, Gutierrez L, et al. Efficacy of losartan vs. atenolol for the prevention of aortic dilation in Marfan syndrome: a randomized clinical trial. Eur Heart J. 2015;37(12):978–85.
46. Milleron O, Arnoult F, Ropers J, Aegerter P, Detaint D, Delorme G, et al. Marfan Sartan: a randomized, double-blind, placebo-controlled trial. Eur Heart J. 2015;36(32):2160–6.
47. Lacro RV, Dietz HC, Sleeper LA, Yetman AT, Bradley TJ, Colan SD, et al. Atenolol versus losartan in children and young adults with Marfan's syndrome. N Engl J Med. 2014;371(22):2061–71.
48. Pitcher A, Emberson J, Lacro RV, Sleeper LA, Stylianou M, Mahony L, et al. Design and rationale of a prospective, collaborative meta-analysis of all randomized controlled trials of angiotensin receptor antagonists in Marfan syndrome, based on individual patient data: A report from the Marfan Treatment Trialists' Collaboration. Am Heart J. 2015;169(5):605–12.
49. Feiner L, Webber AL, Brown CB, Lu MM, Jia L, Feinstein P, et al. Targeted disruption of semaphorin 3C leads to persistent truncus arteriosus and aortic arch interruption. Development. 2001;128(16):3061–70.
50. Ohnemus D, Oster ME, Gatlin S, Jokhadar M, Mahle WT. The effect of angiotensin-converting enzyme inhibitors on the rate of ascending aorta dilation in patients with bicuspid aortic valve. Congenit Heart Dis. 2015;10(1):E1–5.
51. Siu SC, Silversides CK. Bicuspid aortic valve disease. J Am Coll Cardiol. 2010;55(25):2789–800.
52. Della Corte A, Body SC, Booher AM, Schaefers HJ, Milewski RK, Michelena HI, et al. Surgical treatment of bicuspid aortic valve disease: knowledge gaps and research perspectives. J Thorac Cardiovasc Surg. 2014;147(6):1749–57, 57 e1.

53. Accf/Aha/Aats/Acr/Asa/Sca/Scai/Sir/Sts/Svm Guidelines For The D, Management Of Patients With Thoracic Aortic Disease Representative M, Hiratzka LF, Creager MA, Isselbacher EM, Svensson LG, et al. Surgery for Aortic Dilatation in Patients With Bicuspid Aortic Valves: A Statement of Clarification From the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2016; 133(7):680–6.
54. Bentall H, De Bono A. A technique for complete replacement of the ascending aorta. Thorax. 1968;23(4):338–9.
55. Gott VL, Greene PS, Alejo DE, Cameron DE, Naftel DC, Miller DC, et al. Replacement of the aortic root in patients with Marfan's syndrome. N Engl J Med. 1999;340(17):1307–13.
56. Benedetto U, Melina G, Takkenberg JJ, Roscitano A, Angeloni E, Sinatra R. Surgical management of aortic root disease in Marfan syndrome: a systematic review and meta-analysis. Heart. 2011; 97(12):955–8.
57. David TE. Aortic valve sparing operations: outcomes at 20 years. Ann Cardiothorac Surg. 2013;2(1):24–9.
58. European Society of G, Association for European Paediatric C, German Society for Gender M, Regitz-Zagrosek V, Blomstrom Lundqvist C, Borghi C, et al. ESC Guidelines on the management of cardiovascular diseases during pregnancy: the Task Force on the Management of Cardiovascular Diseases during Pregnancy of the European Society of Cardiology (ESC). Eur Heart J. 2011; 32(24):3147–97.
59. Lésniak-Sobelga A, Kostklewicz M, Wisniowska-Smialek S, Rubis P, Podolec P. Outcome of pregnancy in patients with bicuspid aortic valve – a study of 89 patients. J Rare Cardiovasc Dis. 2014;2(1):9–14.
60. Maron BJ, Pelliccia A. The heart of trained athletes: cardiac remodeling and the risks of sports, including sudden death. Circulation. 2006;114(15):1633–44.
61. Maron BJ, Zipes DP, Kovacs RJ, American Heart Association E, Arrhythmias Committee of Council on Clinical Cardiology CoCDiYCoC, Stroke Nursing CoFG, et al. Eligibility and disqualification recommendations for competitive athletes with cardiovascular Aanormalities: preamble, principles, and general considerations: a scientific statement from the American Heart Association and American College of Cardiology. Circulation. 2015;132(22):e256–61.
62. Braverman AC, Harris KM, Kovacs RJ, Maron BJ. Eligibility and disqualification recommendations for competitive athletes with cardiovascular abnormalities: Task Force 7: aortic diseases, including Marfan Syndrome: a scientific statement from the American Heart Association and American College of Cardiology. J Am Coll Cardiol. 2015;66(21):2398–405.
63. Cripe L, Andelfinger G, Martin LJ, Shooner K, Benson DW. Bicuspid aortic valve is heritable. J Am Coll Cardiol. 2004;44(1): 138–43.
64. Huntington K, Hunter AG, Chan KL. A prospective study to assess the frequency of familial clustering of congenital bicuspid aortic valve. J Am Coll Cardiol. 1997;30(7):1809–12.
65. Clementi M, Notari L, Borghi A, Tenconi R. Familial congenital bicuspid aortic valve: a disorder of uncertain inheritance. Am J Med Genet. 1996;62(4):336–8.
66. Ellison JW, Yagubyan M, Majumdar R, Sarkar G, Bolander ME, Atkinson EJ, et al. Evidence of genetic locus heterogeneity for familial bicuspid aortic valve. J Surg Res. 2007;142(1):28–31.
67. Hinton RB. Bicuspid aortic valve and thoracic aortic aneurysm: three patient populations, two disease phenotypes, and one shared genotype. Cardiology research and practice. 2012;2012:926975.
68. Loscalzo ML, Goh DL, Loeys B, Kent KC, Spevak PJ, Dietz HC. Familial thoracic aortic dilation and bicommissural aortic valve: a prospective analysis of natural history and inheritance. Am J Med Genet A. 2007;143A(17):1960–7.
69. Jain R, Engleka KA, Rentschler SL, Manderfield LJ, Li L, Yuan L, et al. Cardiac neural crest orchestrates remodeling and functional maturation of mouse semilunar valves. J Clin Invest. 2011;121(1): 422–30.
70. Martin LJ, Ramachandran V, Cripe LH, Hinton RB, Andelfinger G, Tabangin M, et al. Evidence in favor of linkage to human chromosomal regions 18q, 5q and 13q for bicuspid aortic valve and associated cardiovascular malformations. Hum Genet. 2007; 121(2):275–84.
71. Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, King IN, et al. Mutations in NOTCH1 cause aortic valve disease. Nature. 2005;437(7056):270–4.
72. Kerstjens-Frederikse WS, van de Laar IM, Vos YJ, Verhagen JM, Berger RM, Lichtenbelt KD, et al. Cardiovascular malformations caused by NOTCH1 mutations do not keep left: data on 428 probands with left-sided CHD and their families. Genet Med. 2016. doi:10.1038/gim.2015.193.
73. Kent KC, Crenshaw ML, Goh DL, Dietz HC. Genotype-phenotype correlation in patients with bicuspid aortic valve and aneurysm. J Thorac Cardiovasc Surg. 2013;146(1):158–65 e1.
74. Foffa I, Ait Ali L, Panesi P, Mariani M, Festa P, Botto N, et al. Sequencing of NOTCH1, GATA5, TGFBR1 and TGFBR2 genes in familial cases of bicuspid aortic valve. BMC Med Genet. 2013;14:44.
75. McBride KL, Riley MF, Zender GA, Fitzgerald-Butt SM, Towbin JA, Belmont JW, et al. NOTCH1 mutations in individuals with left ventricular outflow tract malformations reduce ligand-induced signaling. Hum Mol Genet. 2008;17(18):2886–93.
76. Andersson ER, Sandberg R, Lendahl U. Notch signaling: simplicity in design, versatility in function. Development. 2011;138(17): 3593–612.
77. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science. 1999;284(5415):770–6.
78. High FA, Epstein JA. The multifaceted role of Notch in cardiac development and disease. Nat Rev Genet. 2008;9(1):49–61.
79. Timmerman LA, Grego-Bessa J, Raya A, Bertran E, Perez-Pomares JM, Diez J, et al. Notch promotes epithelial-mesenchymal transition during cardiac development and oncogenic transformation. Genes Dev. 2004;18(1):99–115.
80. Kostina AS, Uspensky Vcapital Ie C, Irtyuga OB, Ignatieva EV, Freylikhman O, Gavriliuk ND, et al. Notch-dependent EMT is attenuated in patients with aortic aneurysm and bicuspid aortic valve. Biochim Biophys Acta. 2016;1862(4):733–40.
81. MacGrogan D, D'Amato G, Travisano S, Martinez-Poveda B, de Luxan G, Del Monte-Nieto G, et al. Sequential ligand-dependent Notch signaling activation regulates valve primordium formation and morphogenesis. Circ Res. 2016;118(10): 1480–97.
82. Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell. 1997; 89(5):747–54.
83. Kaden JJ, Bickelhaupt S, Grobholz R, Vahl CF, Hagl S, Brueckmann M, et al. Expression of bone sialoprotein and bone morphogenetic protein-2 in calcific aortic stenosis. J Heart Valve Dis. 2004;13(4):560–6.
84. Mohler 3rd ER, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS. Bone formation and inflammation in cardiac valves. Circulation. 2001;103(11):1522–8.
85. Acharya A, Hans CP, Koenig SN, Nichols HA, Galindo CL, Garner HR, et al. Inhibitory role of Notch1 in calcific aortic valve disease. PLoS One. 2011;6(11):e27743.
86. Nigam V, Srivastava D. Notch1 represses osteogenic pathways in aortic valve cells. J Mol Cell Cardiol. 2009;47(6):828–34.
87. Laforest B, Andelfinger G, Nemer M. Loss of Gata5 in mice leads to bicuspid aortic valve. J Clin Invest. 2011;121(7):2876–87.

88. Padang R, Bagnall RD, Richmond DR, Bannon PG, Semsarian C. Rare non-synonymous variations in the transcriptional activation domains of GATA5 in bicuspid aortic valve disease. J Mol Cell Cardiol. 2012;53(2):277–81.
89. Bonachea EM, Chang SW, Zender G, LaHaye S, Fitzgerald-Butt S, McBride KL, et al. Rare GATA5 sequence variants identified in individuals with bicuspid aortic valve. Pediatr Res. 2014;76(2):211–6.
90. Shi LM, Tao JW, Qiu XB, Wang J, Yuan F, Xu L, et al. GATA5 loss-of-function mutations associated with congenital bicuspid aortic valve. Int J Mol Med. 2014;33(5):1219–26.
91. Martin M, Alonso-Montes C, Florez JP, Pichel IA, Rozado J, Andia JB, et al. Bicuspid aortic valve syndrome: a heterogeneous and still unknown condition. Int J Cardiol. 2014;177(3):1105.
92. Wei D, Bao H, Zhou N, Zheng GF, Liu XY, Yang YQ. GATA5 loss-of-function mutation responsible for the congenital ventriculoseptal defect. Pediatr Cardiol. 2013;34(3):504–11.
93. Zhang XL, Dai N, Tang K, Chen YQ, Chen W, Wang J, et al. GATA5 loss-of-function mutation in familial dilated cardiomyopathy. Int J Mol Med. 2015;35(3):763–70.
94. Wang XH, Huang CX, Wang Q, Li RG, Xu YJ, Liu X, et al. A novel GATA5 loss-of-function mutation underlies lone atrial fibrillation. Int J Mol Med. 2013;31(1):43–50.
95. Wei D, Bao H, Liu XY, Zhou N, Wang Q, Li RG, et al. GATA5 loss-of-function mutations underlie tetralogy of fallot. Int J Med Sci. 2013;10(1):34–42.
96. Nemer G, Nemer M. Cooperative interaction between GATA5 and NF-ATc regulates endothelial-endocardial differentiation of cardiogenic cells. Development. 2002;129(17):4045–55.
97. Kirk EP, Sunde M, Costa MW, Rankin SA, Wolstein O, Castro ML, et al. Mutations in cardiac T-box factor gene TBX20 are associated with diverse cardiac pathologies, including defects of septation and valvulogenesis and cardiomyopathy. Am J Hum Genet. 2007;81(2):280–91.
98. Lee TC, Zhao YD, Courtman DW, Stewart DJ. Abnormal aortic valve development in mice lacking endothelial nitric oxide synthase. Circulation. 2000;101(20):2345–8.
99. Chao CS, McKnight KD, Cox KL, Chang AL, Kim SK, Feldman BJ. Novel GATA6 mutations in patients with pancreatic agenesis and congenital heart malformations. PLoS One. 2015;10(2) e0118449.
100. Lin X, Huo Z, Liu X, Zhang Y, Li L, Zhao H, et al. A novel GATA6 mutation in patients with tetralogy of Fallot or atrial septal defect. J Hum Genet. 2010;55(10):662–7.
101. Laforest B, Nemer M. Genetic insights into bicuspid aortic valve formation. Cardiol Res Pract 2012;2012:180297.
102. Garg V, Kathiriya IS, Barnes R, Schluterman MK, King IN, Butler CA, et al. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. Nature. 2003;424(6947):443–7.
103. Tan HL, Glen E, Topf A, Hall D, O'Sullivan JJ, Sneddon L, et al. Nonsynonymous variants in the SMAD6 gene predispose to congenital cardiovascular malformation. Hum Mutat. 2012;33(4):720–7.
104. Hanyu A, Ishidou Y, Ebisawa T, Shimanuki T, Imamura T, Miyazono K. The N domain of Smad7 is essential for specific inhibition of transforming growth factor-beta signaling. J Cell Biol. 2001;155(6):1017–27.
105. Galvin KM, Donovan MJ, Lynch CA, Meyer RI, Paul RJ, Lorenz JN, et al. A role for smad6 in development and homeostasis of the cardiovascular system. Nat Genet. 2000;24(2):171–4.
106. Chen G, Deng C, Li YP. TGF-beta and BMP signaling in osteoblast differentiation and bone formation. Int J Biol Sci. 2012;8(2):272–88.
107. Biben C, Weber R, Kesteven S, Stanley E, McDonald L, Elliott DA, et al. Cardiac septal and valvular dysmorphogenesis in mice heterozygous for mutations in the homeobox gene Nkx2-5. Circ Res. 2000;87(10):888–95.
108. Chung IM, Rajakumar G. Genetics of congenital heart defects: The NKX2-5 gene, a key player. Genes (Basel). 2016;7(2).
109. Beffagna G, Cecchetto A, Dal Bianco L, Lorenzon A, Angelini A, Padalino M, et al. R25C mutation in the NKX2.5 gene in Italian patients affected with non-syndromic and syndromic congenital heart disease. J Cardiovasc Med (Hagerstown). 2013;14(8):582–6.
110. Qu XK, Qiu XB, Yuan F, Wang J, Zhao CM, Liu XY, et al. A novel NKX2.5 loss-of-function mutation associated with congenital bicuspid aortic valve. Am J Cardiol. 2014;114(12):1891–5.
111. Quintero-Rivera F, Xi QJ, Keppler-Noreuil KM, Lee JH, Higgins AW, Anchan RM, et al. MATR3 disruption in human and mouse associated with bicuspid aortic valve, aortic coarctation and patent ductus arteriosus. Hum Mol Genet. 2015;24(8):2375–89.
112. Xia F, Bainbridge MN, Tan TY, Wangler MF, Scheuerle AE, Zackai EH, et al. De novo truncating mutations in AHDC1 in individuals with syndromic expressive language delay, hypotonia, and sleep apnea. Am J Hum Genet. 2014;94(5):784–9.
113. Johnson JO, Pioro EP, Boehringer A, Chia R, Feit H, Renton AE, et al. Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis. Nat Neurosci. 2014;17(5):664–6.
114. Ben-Shachar S, Ou Z, Shaw CA, Belmont JW, Patel MS, Hummel M, et al. 22q11.2 distal deletion: a recurrent genomic disorder distinct from DiGeorge syndrome and velocardiofacial syndrome. Am J Hum Genet. 2008;82(1):214–21.
115. Basu R, Hazra S, Shanks M, Paterson DI, Oudit GY. Novel mutation in exon 14 of the sarcomere gene MYH7 in familial left ventricular noncompaction with bicuspid aortic valve. Circ Heart Fail. 2014;7(6):1059–62.
116. Guo DC, Papke CL, Tran-Fadulu V, Regalado ES, Avidan N, Johnson RJ, et al. Mutations in smooth muscle alpha-actin (ACTA2) cause coronary artery disease, stroke, and Moyamoya disease, along with thoracic aortic disease. Am J Hum Genet. 2009;84(5):617–27.
117. Karp N, Grosse-Wortmann L, Bowdin S. Severe aortic stenosis, bicuspid aortic valve and atrial septal defect in a child with Joubert Syndrome and Related Disorders (JSRD) - a case report and review of congenital heart defects reported in the human ciliopathies. Eur J Med Genet. 2012;55(11):605–10.
118. Baasanjav S, Al-Gazali L, Hashiguchi T, Mizumoto S, Fischer B, Horn D, et al. Faulty initiation of proteoglycan synthesis causes cardiac and joint defects. Am J Hum Genet. 2011;89(1):15–27.
119. Nistri S, Porciani MC, Attanasio M, Abbate R, Gensini GF, Pepe G. Association of Marfan syndrome and bicuspid aortic valve: frequency and outcome. Int J Cardiol. 2012;155(2):324–5.
120. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature. 1991;352(6333):337–9.
121. Fedak PW, de Sa MP, Verma S, Nili N, Kazemian P, Butany J, et al. Vascular matrix remodeling in patients with bicuspid aortic valve malformations: implications for aortic dilatation. J Thorac Cardiovasc Surg. 2003;126(3):797–806.
122. Pepe G, Nistri S, Giusti B, Sticchi E, Attanasio M, Porciani C, et al. Identification of fibrillin 1 gene mutations in patients with bicuspid aortic valve (BAV) without Marfan syndrome. BMC Med Genet. 2014;15:23.
123. Lesauskaite V, Sepetiene R, Jariene G, Patamsyte V, Zukovas G, Grabauskyte I, et al. FBN1 polymorphisms in patients with the dilatative pathology of the ascending thoracic aorta. Eur J Cardiothorac Surg. 2015;47(4):e124–30.
124. Lemaire SA, McDonald ML, Guo DC, Russell L, Miller 3rd CC, Johnson RJ, et al. Genome-wide association study identifies a susceptibility locus for thoracic aortic aneurysms and aortic dissec-

tions spanning FBN1 at 15q21.1. Nat Genet. 2011;43(10): 996–1000.
125. Loeys BL, Dietz HC. Loeys-Dietz Syndrome. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, et al., editors. GeneReviews(R). Seattle (WA)2008.
126. Arrington CB, Sower CT, Chuckwuk N, Stevens J, Leppert MF, Yetman AT, et al. Absence of TGFBR1 and TGFBR2 mutations in patients with bicuspid aortic valve and aortic dilation. Am J Cardiol. 2008;102(5):629–31.
127. Girdauskas E, Schulz S, Borger MA, Mierzwa M, Kuntze T. Transforming growth factor-beta receptor type II mutation in a patient with bicuspid aortic valve disease and intraoperative aortic dissection. Ann Thorac Surg. 2011;91(5):e70–1.
128. Olivieri LJ, Baba RY, Arai AE, Bandettini WP, Rosing DR, Bakalov V, et al. Spectrum of aortic valve abnormalities associated with aortic dilation across age groups in Turner syndrome. Circ Cardiovasc Imaging. 2013;6(6):1018–23.
129. Mortensen KH, Andersen NH, Gravholt CH. Cardiovascular phenotype in Turner syndrome--integrating cardiology, genetics, and endocrinology. Endocr Rev. 2012;33(5):677–714.
130. Bondy C, Bakalov VK, Cheng C, Olivieri L, Rosing DR, Arai AE. Bicuspid aortic valve and aortic coarctation are linked to deletion of the X chromosome short arm in Turner syndrome. J Med Genet. 2013;50(10):662–5.
131. Chang AC, Fu Y, Garside VC, Niessen K, Chang L, Fuller M, et al. Notch initiates the endothelial-to-mesenchymal transition in the atrioventricular canal through autocrine activation of soluble guanylyl cyclase. Dev Cell. 2011;21(2):288–300.
132. Bosse K, Hans CP, Zhao N, Koenig SN, Huang N, Guggilam A, et al. Endothelial nitric oxide signaling regulates Notch1 in aortic valve disease. J Mol Cell Cardiol. 2013;60:27–35.
133. Koenig SN, Bosse KM, Nadorlik HA, Lilly B, Garg V. Evidence of aortopathy in mice with haploinsufficiency of in -null background. J Cardiovasc Dev Dis. 2015;2(1):17–30.
134. Aicher D, Urbich C, Zeiher A, Dimmeler S, Schafers HJ. Endothelial nitric oxide synthase in bicuspid aortic valve disease. Ann Thorac Surg. 2007;83(4):1290–4.
135. Thomas PS, Sridurongrit S, Ruiz-Lozano P, Kaartinen V. Deficient signaling via Alk2 (Acvr1) leads to bicuspid aortic valve development. PLoS One. 2012;7(4):e35539.
136. Kaartinen V, Dudas M, Nagy A, Sridurongrit S, Lu MM, Epstein JA. Cardiac outflow tract defects in mice lacking ALK2 in neural crest cells. Development. 2004;131(14):3481–90.
137. Wang J, Sridurongrit S, Dudas M, Thomas P, Nagy A, Schneider MD, et al. Atrioventricular cushion transformation is mediated by ALK2 in the developing mouse heart. Dev Biol. 2005;286(1): 299–310.
138. Shore EM, Xu M, Feldman GJ, Fenstermacher DA, Cho TJ, Choi IH, et al. A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. Nat Genet. 2006;38(5):525–7.
139. Makki N, Capecchi MR. Cardiovascular defects in a mouse model of HOXA1 syndrome. Hum Mol Genet. 2012;21(1):26–31.
140. Tischfield MA, Bosley TM, Salih MA, Alorainy IA, Sener EC, Nester MJ, et al. Homozygous HOXA1 mutations disrupt human brainstem, inner ear, cardiovascular and cognitive development. Nat Genet. 2005;37(10):1035–7.
141. Akerberg BN, Sarangam ML, Stankunas K. Endocardial Brg1 disruption illustrates the developmental origins of semilunar valve disease. Dev Biol. 2015;407(1):158–72.
142. Li W, Xiong Y, Shang C, Twu KY, Hang CT, Yang J, et al. Brg1 governs distinct pathways to direct multiple aspects of mammalian neural crest cell development. Proc Natl Acad Sci U S A. 2013;110(5):1738–43.
143. Schneppenheim R, Fruhwald MC, Gesk S, Hasselblatt M, Jeibmann A, Kordes U, et al. Germline nonsense mutation and somatic inactivation of SMARCA4/BRG1 in a family with rhabdoid tumor predisposition syndrome. Am J Hum Genet. 2010; 86(2):279–84.
144. Tsurusaki Y, Okamoto N, Ohashi H, Kosho T, Imai Y, Hibi-Ko Y, et al. Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome. Nat Genet. 2012;44(4): 376–8.
145. Mommersteeg MT, Yeh ML, Parnavelas JG, Andrews WD. Disrupted Slit-Robo signalling results in membranous ventricular septum defects and bicuspid aortic valves. Cardiovasc Res. 2015; 106(1):55–66.
146. Xue Y, Ankala A, Wilcox WR, Hegde MR. Solving the molecular diagnostic testing conundrum for Mendelian disorders in the era of next-generation sequencing: single-gene, gene panel, or exome/genome sequencing. Genet Med. 2015;17(6):444–51.
147. McBride KL, Pignatelli R, Lewin M, Ho T, Fernbach S, Menesses A, et al. Inheritance analysis of congenital left ventricular outflow tract obstruction malformations: Segregation, multiplex relative risk, and heritability. Am J Med Genet A. 2005;134A(2):180–6.

Part V

Sudden Cardiac Death in the Young

Sudden Cardiac Death in the Young; Epidemiology and Cardiogenetic Evaluation of Victims and Their Relatives

19

Anneke Hendrix, Michiel L. Bots, and Arend Mosterd

Abstract

Sudden death (SD) is defined as "a nontraumatic, unexpected fatal event occurring within 1 hour after the onset of symptoms in an apparently healthy subject." "If death is not witnessed, the definition applies when the victim was in good health 24 hours before the event" (Eur Heart J 36(41):2793–2786, 2015). The sudden deaths can be subdivided into noncardiac deaths, sudden cardiac deaths (SCD) and the sudden unexplained deaths (SUD). The latter two categories comprise the cardiac deaths due to inherited diseases and are discussed further in this chapter.

Introduction

Sudden death (SD) is defined as "a nontraumatic, unexpected fatal event occurring within 1 hour after the onset of symptoms in an apparently healthy subject." "If death is not witnessed, the definition applies when the victim was in good health 24 hours before the event" [1]. The sudden deaths can be subdivided into noncardiac deaths, sudden cardiac deaths (SCD) and the sudden unexplained deaths (SUD). The latter two categories comprise the cardiac deaths due to inherited diseases and are discussed further in this chapter.

The sudden death of a young person has an enormous impact on those who are left behind. During the last 10–15 years, it has become clear that in 50–70 % of SCD and SUD victims aged 40 years or younger, potential inherited cardiac disease can be identified as the cause of sudden death [2–4]. *Cardiomyopathies* (e.g., hypertrophic cardiomyopathy [HCM]) or *primary arrhythmia syndromes* (e.g., congenital long-QT syndrome [LQTS]) can cause fatal arrhythmias that may lead to sudden death. Premature *coronary artery disease*, as observed in *familial hypercholesterolemia* (FH), is another cause of sudden death in the young. Relatives of young SCD victims have an increased risk of carrying the inherited predisposition to develop cardiac disease [5–12]. Furthermore, a family history of sudden death is associated with an increased risk of sudden death among adult family members [13–15]. Increasingly, genetic testing is available for inherited cardiac diseases and new mutations, accounting for specific phenotypes, are being discovered [16–18].

Diagnostic evaluation of first-degree relatives followed by early treatment may reduce the risk of SCD in patients with inherited cardiac diseases [19–22]. However, as sudden cardiac arrest in the young is often the first "symptom" of inherited cardiac disease and early identification is difficult in apparently healthy individuals [23–25], presymptomatic cardiogenetic evaluation has been recommended for first-degree relatives of SCD victims with possible inherited diseases to prevent SCD.

In this chapter, we will present an overview of the epidemiology of SCD in the young and discuss the potential benefits of presymptomatic cardiogenetic evaluation of first-degree relatives. In addition, we will discuss *preparticipation screening* of young *athletes*.

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Definitions

The terminology that is used to describe SCD is often confusing due to the variety of definitions that are used in the literature. SCD was recently defined as "sudden death due to any cardiac disease or vascular anomaly, or when no extra cardiac cause could be identified at postmortem investigation". The term *sudden unexplained death syndrome* (SUDS) is used when the cause of death remains unknown and no postmortem investigation has been performed [1]. In the absence of structural abnormalities on postmortem investigation and with a negative toxicologic screening, the term sudden arrhythmic death syndrome (SADS) is used [1, 5, 8].

The term "*sudden death* in the young" covers different age categories, but in general comprises victims between 1 and 40 years of age. The general *definition* of *sudden infant death syndrome* (SIDS) is "sudden unexpected death of an infant <1 year of age, with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including performance of a complete postmortem investigation and review of the circumstances of death and the clinical history" [26]. The distinction between these two age categories (<1 year and >1 year) is based on the differences in incidence and causes. For example, the occurrence of SIDS is strongly associated with sleeping position (after an international public health campaign to place infants on their back while sleeping, the incidence of SIDS decreased with 50–90 %) [27]. Although there is an overlap between the causes of death in SIDS (e.g., the congenital LQTS) and sudden death in the young, we will focus on SCD victims older than 1 year in this chapter.

Incidence

In the general population (over all ages), SCD accounts for approximately 1 death per 1000 person-years. In the young (<40 years), the *incidence* of SCD is estimated to be 100-fold lower and lies between 0.8 and 3.7 per 100,000 person-years (Fig. 19.1) [11, 28–30]. The population-based incidence of *SADS* is estimated to be 0.16–0.43/100,000 person-years [31, 32].

Incidence estimates vary considerably between studies. The collection of data on this topic is complicated, because most cases of sudden death occur out of hospital and often information needs to be collected retrospectively [34]. In addition, *traumatic deaths* like car accidents or drownings can initially be caused by a cardiac arrhythmia but are often not taken into account in incidence estimates. As no nationwide registrations of victims of SCD or SUD of 1–40 years exist, present studies are often restricted to regional observations, where socioeconomical status, racial differences, and the presence of founder mutations predisposing to a specific cardiogenetic disorder might influence the occurrence of

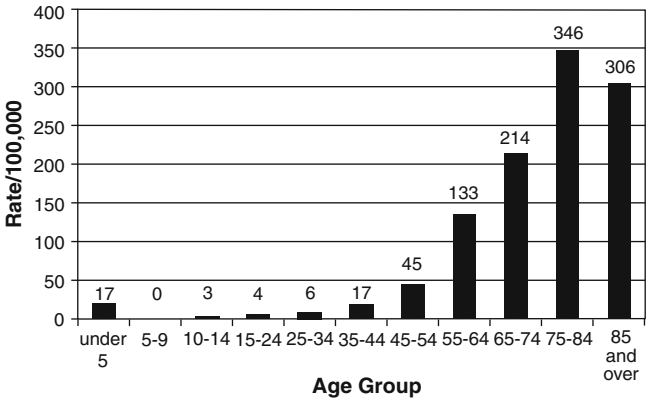

Fig. 19.1 Annual incidence of sudden cardiac death among residents of Multnomah County by age-groups, Oregon (population 660,486) (Adapted from Chugh et al. [33])

SCD [35, 36]. From studies based on *death certificate diagnosis*, absolute numbers of sudden deaths in the young population may be adequately derived, but the proportion of cardiovascular deaths may be unreliable due to misclassification [37, 38].

Causes

SCD in persons older than 40 years is mainly due to coronary artery disease that can result in myocardial ischemia and fatal arrhythmias. It has been estimated that 80 % of the cardiac deaths in victims over 40 years of age are caused by coronary artery disease, 10–15 % by cardiomyopathies, and 5 % by other (less common) causes [39]. However, in the young (1–40 years), inherited cardiac causes are more frequently observed. A review of the literature was performed that included articles from 1980 to 2007 on *causes* of death in the young. All studies were included in which postmortem investigation was performed in >70 % of the sudden death victims [28]. Seventeen publications were identified, including 3528 cases of SD in the age group of 1–40 years that were collected between 1967 and 2004. The most common causes of SD in persons aged 1–40 years were atherosclerotic coronary artery disease (accounting for 23 % of the cases), followed by the autopsy negative deaths (including the primary arrhythmia syndromes) (16 %) and cardiomyopathies (13 %). In athletes, cardiomyopathies were the most common causes of SD (accounting for 48 % of the cases), followed by (nonatherosclerotic) coronary pathology (e.g., coronary artery aneurysm and vasculitis) (16 %) and atherosclerotic coronary artery disease (CAD) (7 %). A considerable proportion of the sudden deaths remained unexplained, accounting for 16 % of the SCD in the general population and 4 % in the athlete population. This latter group comprises primary arrhythmia syndromes [e.g., LQTS, catecholaminergic polymorphic ventricular tachycardia (CPVT) and the Brugada syndrome (BS)]

Table 19.1 Prevalence (%) with 95% confidence interval (95% CI) of reported causes of sudden death, by study population [28]

	General population		athletes	
Causes of death	N	% (95 %CI)	N	% (95 %CI)
Sudden cardiac death				
Atherosclerotic disease	726	23 (22–25)	27	7 (5–10)
Conduction disorders	44	1 (1–2)	5	1 (0–3)
Myocarditis	195	6 (5–7)	16	4 (2–6)
Cardiomyopathy[a]	397	13 (11–14)	181	48 (43–53)
Coronary pathology (nonischemic)[b]	73	2 (2–3)	61	16 (12–20)
Congenital cardiac diseases[c]	37	1 (1–2)	2	1 (0–1)
Valve abnormalities[d]	120	4 (3–5)	22	6 (4–8)
Other cardiovascular diseases	230	7 (6–8)	8	2 (1–4)
Sudden death with unknown cause	519	16 (15–18)	16	4 (2–6)
Sudden noncardiac death				
Respiratory[e]	244	8 (7–9)	8	2 (1–4)
Neurologic[f]	289	9 (8–10)	2	1 (0–1)
Other noncardiac deaths	249	8 (7–9)	22	6 (4–8)
Abdominal aneurysms	27	1 (1–1)	8	2 (1–4)
Total sudden deaths	3150	100	378	100

[a]Arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, left ventricular hypertrophy, diffuse fibrosis, endocardial fibroelastosis, myocardial fibrosis, idiopathic myocardial scarring, right ventricular dysplasia, fibroelastosis cordis
[b]Coronary abnormalities, coronary bridging, vasculitis, coronary artery aneurysm
[c]Marfan syndrome, tetralogy of Fallot
[d]Mitral valve prolapse, mitral valve insufficiency, aortic valve insufficiency
[e]Asthma, pulmonary embolism
[f]Epilepsy, subarachnoidal hemorrhage, intracranial hemorrhage, meningitis

[40, 41]. The proportion of deaths due to coronary artery disease increases with age. In addition, *myocarditis* and *primary arrhythmia syndromes* were relatively more common in the younger sudden death cases (1–25 years) (Table 19.1) [23, 28, 42].

Demographics

Information on regional, racial, and gender differences in SCD in the young is scarce. However, it seems that the incidence and causes of SCD (over all ages) differ among regions and populations [43, 44]. This might be due to several factors including the regional distribution of age and gender and the prevalence of inherited cardiac diseases and coronary artery disease. Several studies reported clustering of inherited diseases in populations (and regions); in southeast Asia for example, especially in Cambodia, Philippines, Thailand, and Japan, the incidence of nocturnal sudden death among young men was estimated to be as high as 26–38 per 100,000 person-years. Cardiogenetic evaluation suggested that a *primary arrhythmia syndrome* similar to the BS is underlying these sudden deaths [45–48]. Furthermore, SCD occurs more frequently in African Americans than in white Americans [42]. HCM is the most common cause of SCD in the athletes in the United States, while in the Veneto region in Italy, *arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C)* is accounting for the majority of deaths among athletes [49–51].

Overall, *SCD* in the young (1–40 years) is more common in men (2.27 per 100,000 person-years) than in women (0.95 per 100,000 person-years) [28]. This difference can partly be explained by the high proportion of deaths due to coronary artery disease which is increasing over age (especially >30 years) [28, 42] and perhaps because women are relatively protected for the development of atherosclerosis in the premenopausal period [52–54].

Postmortem Diagnosis

A dedicated and focused *postmortem investigation* as compared to a "routine" cardiac postmortem investigation is essential in detecting potential inherited cardiac diseases in sudden death victims. Postmortem investigation includes investigation of the circumstances of death, verification of the victim's medical history and family history, postmortem investigation, and DNA storage. [55, 56]

Circumstances of Death, Verification of the Victim's Medical History and Family History

An effort should be made to obtain relevant information from health care professionals (e.g., resuscitation team) and other witnesses of the event regarding the circumstances of

death (e.g., occurring during sleep, emotional stress, or exercise), the type and duration of *preceding symptoms* (e.g., chest pain, dizziness, nausea, fever, or headache), and the location of the fatal event. Relatives and general practitioners can be a useful source of information on medical and family history. Circumstances of death can provide important clues to the underlying causes of death, since triggers of sudden cardiac arrest might be specific for the underlying disease. Arrhythmias may be triggered by exercise in patients with *HCM, ARVD/C, LQTS type 1*, or *CPVT*, while in patients with *BS* or *LQTS* type 3, fatal arrhythmias more often occur during sleep. Furthermore, information on the victim's medical history should be collected (e.g., recent infections, surgical operations, or comorbidities (e.g., hypertension, neuromuscular diseases, asthma, or epilepsy). Medication use as well as cigarette exposure, alcohol, and substance abuse should be reported.

Besides, the victim's *family history* with respect to sudden death and inherited cardiac or neuromuscular diseases can reveal important additional information. When available, *antemortem investigations* (e.g., electrocardiogram, echocardiography, results of exercise testing, or CT scan) should be investigated [55, 56].

Postmortem Investigation

Recently, international *guidelines* for postmortem investigation in SCD/SUD in the young have been published [55, 57]. When postmortem investigation is limited to macroscopic examination of the heart without histological sampling of cardiac tissue or *toxicological examination*, focal cardiac abnormalities or extracardiac causes such as an intoxication may remain undetected. In 79 % of the cases of SCD without macroscopic abnormalities, *histopathological examination* of the heart revealed local pathology like focal *myocarditis* or the presence of conduction system abnormalities [58]. Revision of the heart by a cardiac expert pathologist is recommended when no cause of death can be established. A toxicological examination for drugs (e.g., opiates, amphetamine), alcohol, and medication may be considered necessary in cases with no structural abnormalities at autopsy [59, 60].

DNA Storage

Storage of the victim's *DNA* enables genetic testing when relatives consult a cardiologist or clinical–geneticist for cardiogenetic evaluation. Postmortem investigation allows tissue to be collected and stored in a tissue bank. Usually, only *paraffin-embedded tissue* is stored when a SCD victim is autopsied, which is not an optimal source for extensive genetic testing. Guidelines have supported the notion that it is desirable to store EDTA-blood and/or frozen muscle, liver, or spleen tissue that can be obtained during postmortem investigation [1, 58, 61]. When no postmortem investigation is performed, a skin *biopsy* might be taken (with permission from the victim's relatives). Prior to the biopsy, the skin needs to be disinfected with alcohol. The obtained tissue can be temporarily stored in a sterile vial with physiological isotonic saline before sending it to a DNA laboratory [62].

Cardiogenetic Evaluation of First-Degree Relatives of Young Sudden Cardiac Death Victims

Cardiogenetic Clinic

When an inherited cardiac disease is suspected or when the cause of death remains unknown, relatives should be referred for *cardiogenetic evaluation* that comprises cardiological assessment and/or genetic testing [1, 57]. Cardiogenetic evaluation entails several aspects that need careful consideration upfront. These include difficulties in establishing a final diagnosis, the interpretation of genetic test results, and the ethical considerations concerning genetic testing [63]. Relatives should be informed about the advantages and disadvantages of cardiogenetic evaluation (see Chap. 2). Therefore, dedicated *cardiogenetic outpatient clinics* have been established that provide integrated cardiogenetic care by combining expertise from the fields of ethics, genetics, and cardiology.

Genetic Testing in SCD and SADS Victims

Recent cardiogenetic developments have resulted in the identification of many different mutated genes related to specific cardiac pathology, and consequently lead to a better understanding of the pathophysiology of clinical syndromes such as HCM and LQTS [64]. However, *genetic testing* does not always reveal a genetic mutation when an inherited cardiac disease is suspected [65–67].

Not all causative mutations have been discovered yet and many clinical syndromes show genetic heterogeneity. In SADS victims, genetic testing revealed a mutation in LQTS-associated genes in 20 % of the victims and a mutation in *CPVT*-associated genes in 14 % of the victims [40, 41]. Genetic testing in SCD and SADS victims is generally guided by the antemortem and postmortem findings and by the cardiological evaluation of the relatives.

Evaluation of First-Degree Relatives

Because most inherited heart diseases show an *autosomal dominant* pattern of inheritance, first-degree relatives of *SCD*

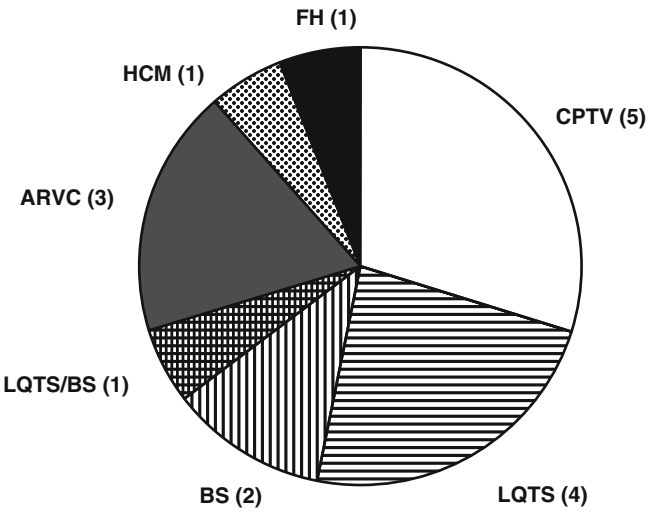

Fig. 19.2 Diagnosis after evaluation of families with ≥1 SUD victim *FH* familiar hypercholesterolemia, *HCM* hypertrophic cardiomyopathy, *ARVC* arrythmogenic right ventricular cardiomyopathy, *LQTS* long-QT syndrome, *BS* Brugada syndrome, *CPVT* catecholaminergic polymorphic ventricular tachycardia (Adapted from Tan et al. [6])

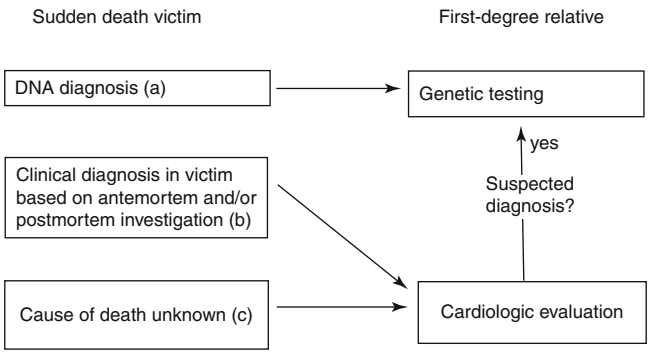

Fig. 19.3 Flow chart, cardiogenetic evaluation of first-degree relatives

victims with genetic disease have a 50 % risk of being a carrier of the same disease [64]. As mentioned earlier, former studies showed that, with thorough clinical assessment of first-degree *relatives* of SUD victims, a cause of death can be established in 22–53 % of the families [5–8]. A Dutch investigation of 43 families of *SUD* victims, of whom 22 were autopsied, found an inherited cardiac disease in 17 of the 43 families that explained the sudden death of the victims (Fig. 19.2) [6]. Furthermore, a study executed in the United Kingdom revealed an inherited disease in 53 % of the 57 families of SADS victims aged 4–64 years [8].

The yield of genetic testing in relatives is high when the causative mutation is known [68]. However, it should be realized that a relative carrying the mutation of an inherited cardiac disease will not necessarily develop signs or symptoms of the clinical syndrome that is associated with the mutation. *Penetrance* of genetic mutations may vary among individuals of the same family and sometimes secondary factors (or genes) can influence the *phenotype expression* [64, 66].

Three scenarios for the cardiogenetic evaluation of first-degree *relatives* of young *SCD/SUD* victims can be distinguished (Fig. 19.3):

(a) The causative mutation in the SCD victim is known.
(b) An inherited cardiac disease is suspected in the victim, but not established by genetic testing.
(c) The cause of the victim's death is unknown (with or without extensive postmortem investigation).

Scenario (a) allows for a targeted approach by genetic testing of the victim's relatives. Cascade screening, starting with genetic testing of the (genetically) first-degree relatives (which include the parents, children, brothers, and sisters) of an affected individual will genetically identify the causative mutation in one or more of the relatives. Subsequently, the screening can be extended to the connecting branch of the pedigree [66]. Consequently, the absence of the mutation rules out the presence of the disease, and no further investigation of the pedigree is needed. In case a causative mutation is present in a relative, cardiologic evaluation and/or diagnostic follow-up is usually indicated.

In scenario (b), the *cardiogenetic evaluation* of relatives is more complicated. The postmortem findings in the victim may raise the suspicion of an inherited cardiac disease. Based on this, targeted cardiologic evaluation of the relatives can be performed. Based on the results of the cardiologic evaluation of relatives, targeted genetic testing can be performed in the victim (if the victim's DNA is available) or in the clinically affected relative (with cardiac abnormalities).

If a mutation is found, cascade screening in the pedigree is recommended (see scenario a). In case no mutation can be detected, cardiologic evaluation (guided by the findings in the affected relative or postmortem findings in the sudden death victim) of all first-degree relatives may be considered. As the penetrance of a causative mutation may differ among individuals, the absence of abnormalities on cardiologic evaluation does not automatically rule out the presence of an inherited disease. In some diseases, symptoms develop only at older age (e.g., *HCM, DCM*, and *ARVD/C*), which may mandate follow-up in these individuals.

In scenario (c), the cause of sudden death is unknown and no clues are available for a specific diagnosis, which makes *cardiogenetic evaluation* less feasible. A cardiac examination of the relatives may reveal a relevant diagnosis. Examination should include the following aspects; (1) medical history, (2) physical examination, (3) standard resting 12 lead electrocardiogram and 12 lead electrocardiogram with specific right precordial positioning of the leads (leads – V1, V2, 1V1, and 1V2), (4) echocardiography, (5) Holter recording, (6) exercise

testing, and (7) measurement of serum lipid levels [69]. If the initial examination raises the possibility of a specific genetic disorder, additional investigations may be indicated, which may include provocation testing (e.g., ajmaline challenging), cardiac MRI, and genetic testing [5–8].

Cost-Effectiveness of Cardiogenetic Evaluation of First-Degree Relatives

A formal evaluation of the above-mentioned scenarios to identify inherited cardiac diseases in relatives of young sudden death victims has not been performed. The *cost-effectiveness* of cardiogenetic evaluation of relatives of SCD/SUD victims depends on the balance between the probability of identifying the causative mutation and its associated therapeutic (risk reduction through treatment) and prognostic consequences (risk when no treatment is given). This can differ from situation to situation. Since the costs of *genetic testing* are associated with the number and size of the analyzed genes, a targeted genetic evaluation of relatives (e.g., scenarios a and b) is likely to result in a higher yield of genetic testing and lower costs [70]. When the cause of death is unknown (scenario c), genetic evaluation is less (cost)-effective [71]. Limited analyses of only those genes that are responsible for the major part of the clinical syndromes seem to increase efficiency. To date, no studies on the yield and cost-effectiveness of *cardiogenetic evaluation* of relatives of *SCD/SUD* victims have been published. A cost-effectiveness analysis through modeling is necessary to provide additional information on the value of cardiogenetic evaluation of relatives for different diseases and scenarios [28]. Based on these future studies, recommendations can be drafted regarding the cardiogenetic evaluation of relatives of SCD/SUD victims.

Preparticipation Screening of Athletes

Being physically active is generally regarded as the best way to prevent (cardiovascular) disease, but vigorous activity can also acutely and transiently increase the risk of acute cardiac events in susceptible persons [72]. *Physical activity* can trigger fatal arrhythmias in persons with cardiomyopathies (e.g., *HCM* or *ARVD/C*), CPVT, and LQTS or provoke coronary plaque rupture in those with coronary artery disease [72, 73]. The sudden death of an apparently healthy *athlete* inevitably leads to discussions if death could have been prevented by preparticipation screening.

Approximately 5–14 % of all SCDs in the young occur during physical activity [74–76]. However, it is still largely unclear whether young athletes have an increased risk of SCD compared to nonathletes. An Italian study reported that the risk of SD was 2.5 (CI: 1.8–3.4) times higher in young athletes than in nonathletes [77]. In this study, events not directly associated with physical activity were also taken into account. The physician's Health Study reported a 16.9 times higher risk of sudden death (CI: 10.5–27.0) during physical activity and the 30 min after physical activity than during episodes of low activity, but an association between the frequency of physical activity and the long-term risk of sudden death could not be established [76]. A moderate to intensive level of physical activity was associated with a significant decrease in SCD in a study of 7735 middle-aged men [72].

In 2015, the European Society of Cardiology issued recommendations for routine preparticipation cardiovascular screening of young competitive athletes [1]. It is recommended that asymptomatic adults who are moderately active, should be evaluated according to a risk assessment scheme. The evaluation consists of a questionnaire, physical examination, an electrocardiogram and a risk SCORE [78]. An electrocardiogram (although recommended for specific subgroups) is not a standard part of the *preparticipation screening* recommended by the American Heart Association that essentially relies on medical history and physical examination [79]. The European recommendations are largely based on the Italian experience, suggesting that the introduction of a screening program in young athletes led to a decline in the incidence of SCD. In Italy, 55 cases of SCD aged 12–35 years among screened athletes were registered between 1997 and 2004. The incidence of SCD dropped from 4.19 (CI: 1.78–7.59) to 0.87 (CI: 0.46–1.28) per 100,000 athletes per year [29].

The European preparticipation screening recommendations have led to a stream of pro-contra discussions [80, 81]. The main criticisms of the recommendations are the lack of randomized studies to support the recommendations, the absence of validated questionnaires and electrocardiographic criteria, and the potential of false-positive findings among screened athletes, especially given the rare occurrence of SCD in young athletes [80]. The interpretation of the electrocardiogram of athletes is hampered by physiological adaptation to systematic training, known as the athlete's heart [82]. Up to 40 % of the electrocardiograms taken in athletes demonstrate variations that can be deemed abnormal, such as sinus bradycardia, atrial fibrillation, and ST-segment changes in the right precordial leads [78]. Furthermore, it has been estimated that only a small proportion of the athletes who suddenly died would have been previously identified as being at increased risk of SCD by the preparticipation program [80].

The Netherlands Institute for Public Health and Environment calculated that a randomized study of the effects of *preparticipation screening* (assuming a 50 % reduction in the rate of SCD from 4 to 2 per 100,000 athletes per year, with 80 % power) would mandate two groups with 1,200,000 person-years of follow-up. Taking this into

account, it is unlikely that definitive evidence supporting the use of preparticipation screening will ever be presented.

Raising awareness of the potential consequences of symptoms during exercise (e.g., collapse, chest discomfort), improving the availability of automated external defibrillators, and careful cardiogenetic evaluation of young SCD victims and their relatives may constitute sound alternatives to mandatory preparticipation screening. Given the higher absolute number of acute cardiac arrests in older athletes, in whom coronary artery disease is far more common, this group should not be neglected [83].

Take Home Message

- The *incidence* of *sudden cardiac death* in persons 40 years or younger is approximately 0.8–1.6 per 100,000 person-years.
- Sudden cardiac death or *sudden unexplained death* in the young is frequently caused by inherited cardiac diseases.
- A thorough *postmortem investigation* of young SCD/SUD victims is desirable to establish the cause of death.
- Standardization of *postmortem investigation* is important to compare studies on the causes of sudden cardiac death and sudden unexplained death and to enable pooling of information from various sources.
- *Relatives* of sudden cardiac death victims with diagnosed inherited diseases have a high risk of being a carrier of an inherited cardiac disease, because most inherited cardiac diseases show an autosomal dominant pattern of inheritance.
- If postmortem investigation does not reveal any structural abnormalities in young victims or when no postmortem examination is performed in SUD victims, *cardiogenetic evaluation* of relatives should be considered.
- The *cost-effectiveness* of screening of relatives of sudden cardiac and sudden unexplained death victims needs to be analyzed in future studies.
- Given the rare occurrence of SCD in young athletes, the preventive effects of routine *preparticipation screening* is likely to be limited.
- Raising awareness of the potential consequences of symptoms during exercise (e.g., collaps, chest discomfort), improving the availability of automated external defibrillators, and careful cardiogenetic evaluation of young SCD/SUD victims and their relatives may constitute sound alternatives to mandatory preparticipation screening.

References

1. Priori SG, Blomstrom-Lundqvist C. Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: the task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European Society of Cardiology (ESC) endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). Eur Heart J. 2015;36(41):2793–86.
2. Wilde AA, Bezzina CR. Genetics of cardiac arrhythmias. Heart. 2005;91(10):1352–8.
3. Wilde AA, van Langen IM, Mannens MM, Waalewijn RA, Maes A. Sudden death at young age and the importance of molecular-pathologic investigation. Ned Tijdschr Geneeskd. 2005;149(29):1601–4.
4. Vaartjes I, Hendrix A. Sudden death in persons younger than 40 years of age: incidence and causes. Eur J Cardiovasc Prev Rehabil. 2009;16(5):592–6.
5. Behr E, Wood DA, Wright M, et al. Cardiological assessment of first-degree relatives in sudden arrhythmic death syndrome. Lancet. 2003;362(9394):1457–9.
6. Tan HL, Hofman N, van Langen IM, van der Wal AC, Wilde AA. Sudden unexplained death: heritability and diagnostic yield of cardiological and genetic examination in surviving relatives. Circulation. 2005;112(2):207–13.
7. Hofman N, Tan HL, Clur SA, Alders M, van Langen IM, Wilde AA. Contribution of inherited heart disease to sudden cardiac death in childhood. Pediatrics. 2007;120(4):e967–73.
8. Behr ER, Dalageorgou C, Christiansen M, et al. Sudden arrhythmic death syndrome: familial evaluation identifies inheritable heart disease in the majority of families. Eur Heart J. 2008;29(13):1670–80.
9. Schwartz K, Carrier L, Guicheney P, Komajda M. Molecular basis of familial cardiomyopathies. Circulation. 1995;91(2):532–40.
10. Garson Jr A, Dick M, Fournier A, et al. The long QT syndrome in children. An international study of 287 patients. Circulation. 1993;87(6):1866–72.
11. Vd Werf C, Hendrix A, et al. Improving usual care after sudden death in the young with focus on inherited cardiac diseases (the CAREFUL study): a community-based intervention study. Europace 2015 Apr 1
12. Cann F, Corbett M et al. Phenotype driven molecular autopsy for sudden cardiac death. Clin Genet. 2016 Mar 22.
13. Friedlander Y, Siscovick DS, Weinmann S, et al. Family history as a risk factor for primary cardiac arrest. Circulation. 1998;97(2):155–60.
14. Jouven X, Desnos M, Guerot C, Ducimetiere P. Predicting sudden death in the population: the Paris prospective study I. Circulation. 1999;99(15):1978–83.
15. Elliott PM, Poloniecki J, Dickie S, et al. Sudden death in hypertrophic cardiomyopathy: identification of high risk patients. J Am Coll Cardiol. 2000;36(7):2212–8.
16. Alders M, Koopmann TT, Christiaans I, et al. Haplotype-sharing analysis implicates chromosome 7q36 harboring DPP6 in familial idiopathic ventricular fibrillation. Am J Hum Genet. 2009;84(4):468–76.
17. Lehnart SE, Ackerman MJ, Benson DW, et al. Inherited arrhythmias: a national heart, lung, and blood institute and office of rare diseases workshop consensus report about the diagnosis, phenotyping, molecular mechanisms, and therapeutic approaches for primary cardiomyopathies of gene mutations affecting ion channel function. Circulation. 2007;116(20):2325–45.
18. Priori SG, Wilde AA, et al. Executive summary: HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. Europace. 2013;15(10):1389–406.

19. Maron BJ, Spirito P, Shen WK, et al. Implantable cardioverter-defibrillators and prevention of sudden cardiac death in hypertrophic cardiomyopathy. JAMA. 2007;298(4):405–12.
20. Watanabe H, Chopra N, Laver D, et al. Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. Nat Med. 2009;15(4):380–3.
21. Hobbs JB, Peterson DR, Moss AJ, et al. Risk of aborted cardiac arrest or sudden cardiac death during adolescence in the long-QT syndrome. JAMA. 2006;296(10):1249–54.
22. Moss AJ, Zareba W, Hall WJ, et al. Effectiveness and limitations of beta-blocker therapy in congenital long-QT syndrome. Circulation. 2000;101(6):616–23.
23. Drory Y, Turetz Y, Hiss Y, et al. Sudden unexpected death in persons less than 40 years of age. Am J Cardiol. 1991;68(13):1388–92.
24. Amital H, Glikson M, Burstein M, et al. Clinical characteristics of unexpected death among young enlisted military personnel: results of a three-decade retrospective surveillance. Chest. 2004;126(2):528–33.
25. Wisten A, Forsberg H, Krantz P, Messner T. Sudden cardiac death in 15-35-year olds in Sweden during 1992–99. J Intern Med. 2002;252(6):529–36.
26. Krous HF, Beckwith JB, Byard RW, et al. Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. Pediatrics. 2004;114(1):234–8.
27. Moon RY, Horne RS, Hauck FR. Sudden infant death syndrome. Lancet. 2007;370(9598):1578–87.
28. Vaartjes I, Hendrix A, Hertogh EM, et al. Sudden death in persons younger than 40 years: causes and incidence. Eur J Cardiovasc Prev and Prehab. 2009;16(5):592–6.
29. Corrado D, Basso C, Pavei A, Michieli P, Schiavon M, Thiene G. Trends in sudden cardiovascular death in young competitive athletes after implementation of a preparticipation screening program. JAMA. 2006;296(13):1593–601.
30. Winkel BG, Holst AG. Nationwide study of sudden cardiac death in persons aged 1–35 years. Eur Heart J. 2011;32(8):983–90.
31. Morentin B, Suarez-Mier MP, Aguilera B. Sudden unexplained death among persons 1-35 years old. Forensic Sci Int. 2003;135(3):213–7.
32. Behr ER, Casey A, Sheppard M, et al. Sudden arrhythmic death syndrome: a national survey of sudden unexplained cardiac death. Heart. 2007;93(5):601–5.
33. Chugh SS, Jui J, Gunson K, et al. Current burden of sudden cardiac death: multiple source surveillance versus retrospective death certificate-based review in a large U.S. community. J Am Coll Cardiol. 2004;44(6):1268–75.
34. de Vreede-Swagemakers JJ, Gorgels AP, Dubois-Arbouw WI, et al. Out-of-hospital cardiac arrest in the 1990's: a population-based study in the Maastricht area on incidence, characteristics and survival. J Am Coll Cardiol. 1997;30(6):1500–5.
35. Corrado D, Basso C, Thiene G. Sudden cardiac death in young people with apparently normal heart. Cardiovasc Res. 2001;50(2):399–408.
36. Maron BJ. Sudden death in young athletes. N Engl J Med. 2003;349(11):1064–75.
37. Iribarren C, Crow RS, Hannan PJ, Jacobs Jr DR, Luepker RV. Validation of death certificate diagnosis of out-of-hospital sudden cardiac death. Am J Cardiol. 1998;82(1):50–3.
38. Fox CS, Evans JC, Larson MG, et al. A comparison of death certificate out-of-hospital coronary heart disease death with physician-adjudicated sudden cardiac death. Am J Cardiol. 2005;95(7):856–9.
39. Huikuri HV, Castellanos A, Myerburg RJ. Sudden death due to cardiac arrhythmias. N Engl J Med. 2001;345(20):1473–82.
40. Tester DJ, Ackerman MJ. Post-mortem long QT syndrome genetic testing for sudden unexplained death in the young. J Am Coll Cardiol. 2007;49(2):240–6.
41. Tester DJ, Spoon DB, Valdivia HH, Makielski JC, Ackerman MJ. Targeted mutational analysis of the RyR2-encoded cardiac ryanodine receptor in sudden unexplained death: a molecular post-mortem investigation of 49 medical examiner/coroner's cases. Mayo Clin Proc. 2004;79(11):1380–4.
42. Zheng ZJ, Croft JB, Giles WH, Mensah GA. Out-of-hospital cardiac deaths in adolescents and young adults in the United States, 1989 to 1998. Am J Prev Med. 2005;29(5 Suppl 1):36–41.
43. Soo L, Huff N, Gray D, Hampton JR. Geographical distribution of cardiac arrest in Nottinghamshire. Resuscitation. 2001;48(2):137–47.
44. Nichol G, Thomas E, Callaway CW, et al. Regional variation in out-of-hospital cardiac arrest incidence and outcome. JAMA. 2008;300(12):1423–31.
45. Roberts R, Brugada R. Genetics and arrhythmias. Annu Rev Med. 2003;54:257–67.
46. Vatta M, Dumaine R, Varghese G, et al. Genetic and biophysical basis of sudden unexplained nocturnal death syndrome (SUNDS), a disease allelic to Brugada syndrome. Hum Mol Genet. 2002;11(3):337–45.
47. Tungsanga K, Sriboonlue P. Sudden unexplained death syndrome in north-east Thailand. Int J Epidemiol. 1993;22(1):81–7.
48. Tatsanavivat P, Chiravatkul A, Klungboonkrong V, et al. Sudden and unexplained deaths in sleep
49. Maron BJ, Doerer JJ, Haas TS, Tierney DM, Mueller FO. Sudden deaths in young competitive athletes: analysis of 1866 deaths in the United States, 1980–2006. Circulation. 2009;119(8):1085–92.
50. Corrado D, Basso C, Schiavon M, Thiene G. Screening for hypertrophic cardiomyopathy in young athletes. N Engl J Med. 1998;339(6):364–9.
51. Maron BJ, Shirani J, Poliac LC, Mathenge R, Roberts WC, Mueller FO. Sudden death in young competitive athletes. Clinical, demographic, and pathological profiles. JAMA. 1996;27(3):199–204.
52. Gordon T, Kannel WB, Hjortland MC, McNamara PM. Menopause and coronary heart disease. The Framingham Study. Ann Intern Med. 1978;89(2):157–61.
53. Atsma F, Bartelink ML, Grobbee DE, van der Schouw YT. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. Menopause. 2006;13(2):265–79.
54. Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE, Hennekens CH. Menopause and the risk of coronary heart disease in women. N Engl J Med. 1987;316(18):1105–10.
55. Basso C, Burke M, Fornes P, et al. Guidelines for post-mortem investigation investigation of sudden cardiac death. Virchows Arch. 2008;452(1):11–8.
56. de la Grandmaison GL. Is there progress in the post-mortem investigation diagnosis of sudden unexpected death in adults? Forensic Sci Int. 2006;156(2–3):138–44.
57. Ackerman MJ, Priori SG. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Heart Rhythm. 2011;8(8):1308–39.
58. Carturan E, Tester DJ, Brost BC, Basso C, Thiene G, Ackerman MJ. Post-mortem genetic testing for conventional post-mortem investigation-negative sudden unexplained death: an evaluation of different DNA extraction protocols and the feasibility of mutational analysis from archival paraffin-embedded heart tissue. Am J Clin Pathol. 2008;129(3):391–7.
59. Safranek DJ, Eisenberg MS, Larsen MP. The epidemiology of cardiac arrest in young adults. Ann Emerg Med. 1992;21(9):1102–6.
60. van Ingen G, van Loenen AC, Voortman M, Zweipfenning PG, Meijer CJ. Recommendations for toxicologic studies in sudden, unexpected death. Ned Tijdschr Geneeskd. 1996;140(4):179–81.

61. Ackerman MJ, Tester DJ, Driscoll DJ. Molecular post-mortem investigation of sudden unexplained death in the young. Am J Forensic Med Pathol. 2001;22(2):105–11.
62. Christiaans I, Langen IM, Wilde AA. Plotselinge dood op jonge leeftijd, de noodzaak van obductie en erfelijkheidsonderzoek. Medisch Contact. 2009;61:1253–6.
63. Hendriks KS, Hendriks MM, Birnie E, et al. Familial disease with a risk of sudden death: a longitudinal study of the psychological consequences of predictive testing for long QT syndrome. Heart Rhythm. 2008;5(5):719–24.
64. Priori SG, Barhanin J, Hauer RN, et al. Genetic and molecular basis of cardiac arrhythmias: impact on clinical management parts I and II. Circulation. 1999;99(4):518–28.
65. Priori SG, Napolitano C. Role of genetic analyses in cardiology: part I: mendelian diseases: cardiac channelopathies. Circulation. 2006;113(8):1130–5.
66. Clinical indications for genetic testing in familial sudden cardiac death syndromes: an HRUK position statement. Heart. 2008;94(4): 502–7.
67. Lakdawala NK, Funke BH, Baxter S, et al. Genetic testing for dilated cardiomyopathy in clinical practice. J Card Fail. 2012;18(4): 296–303.
68. Basso C, Calabrese F, Corrado D, Thiene G. Post-mortem diagnosis in sudden cardiac death victims: macroscopic, microscopic and molecular findings. Cardiovasc Res. 2001;50(2): 290–300.
69. Sangwatanaroj S, Prechawat S, Sunsaneewitayakul B, Sitthisook S, Tosukhowong P, Tungsanga K. New electrocardiographic leads and the procainamide test for the detection of the Brugada sign in sudden unexplained death syndrome survivors and their relatives. Eur Heart J. 2001;22(24):2290–6.
70. Wilde AA, Pinto YM. Cost-effectiveness of genotyping in inherited arrhythmia syndromes, are we getting value for the money? Circ Arrhythmia electrophysiol. 2009;2:1–3.
71. Bai R, Napolitano C, Bloise R, Monteforte N, Priori SG. Yield of genetic screening in inherited cardiac channelopathies. Circ Arrhythmia Electrophysiol. 2009;2:6–15.
72. Thompson PD, Franklin BA, Balady GJ, et al. Exercise and acute cardiovascular events placing the risks into perspective: a scientific statement from the American Heart Association Council on nutrition, physical activity, and metabolism and the Council on Clinical Cardiology. Circulation. 2007;115(17):2358–68.
73. Mittleman MA, Maclure M, Tofler GH, Sherwood JB, Goldberg RJ, Muller JE. Triggering of acute myocardial infarction by heavy physical exertion. Protection against triggering by regular exertion. Determinants of Myocardial Infarction Onset Study Investigators. N Engl J Med. 1993;329(23):1677–83.
74. Burke AP, Farb A, Virmani R, Goodin J, Smialek JE. Sports-related and non-sports-related sudden cardiac death in young adults. Am Heart J. 1991;121(2 Pt 1):568–75.
75. Quigley F, Greene M, O'Connor D, Kelly F. A survey of the causes of sudden cardiac death in the under 35-year-age group. Ir Med J. 2005;98(8):232–5.
76. Albert CM, Mittleman MA, Chae C, Lee IM, Hennekens CH, Manson JE. Triggering of sudden death from cardiac causes by vigorous exertion. N Engl J Med. 2000;343(19):1355–61.
77. Corrado D, Basso C, Rizzoli G, Schiavon M, Thiene G. Does sports activity enhance the risk of sudden death in adolescents and young adults? J Am Coll Cardiol. 2003;42(11):1959–63.
78. Corrado D, Pelliccia A, Bjornstad HH, et al. Cardiovascular preparticipation screening of young competitive athletes for prevention of sudden death: proposal for a common European protocol. Consensus Statement of the Study Group of Sport Cardiology of the Working Group of Cardiac Rehabilitation and Exercise Physiology and the Working Group of Myocardial and Pericardial Diseases of the European Society of Cardiology. Eur Heart J. 2005;26(5):516–24.
79. AHA guideline, Maron et al., circulation 2015.
80. Viskin S. Antagonist: routine screening of all athletes prior to participation in competitive sports should be mandatory to prevent sudden cardiac death. Heart Rhythm. 2007;4(4):525–8.
81. Corrado D, Thiene G. Protagonist: routine screening of all athletes prior to participation in competitive sports should be mandatory to prevent sudden cardiac death. Heart Rhythm. 2007;4(4):520–4.
82. Maron BJ, Pelliccia A. The heart of trained athletes: cardiac remodeling and the risks of sports, including sudden death. Circulation. 2006;114(15):1633–44.
83. Mohlenkamp S, Lehmann N, Breuckmann F, et al. Running: the risk of coronary events: prevalence and prognostic relevance of coronary atherosclerosis in marathon runners. Eur Heart J. 2008;29(15):1903–10.

Part VI

Miscellaneous

The Genetics of Mitral Valve Prolapse

20

Toon Oomen and J. Peter van Tintelen

Abstract

Mitral valve prolapse (MVP) is a common valvular heart disease with a diverse clinical presentation. It mostly occurs as a solitary phenomenon and in a small subset of cases it is part of a systemic disorder, most often a connective tissue disease. A myxomatous mitral valve is a common form of MVP. Complications in MVP are rare, yet these can be severe. Endocarditis or sudden cardiac death has been recognized as such.

Bileaflet involvement, female sex and substrate related fibrosis and mechanical effects may trigger ventricular premature contractions that may predispose to sustained ventricular arrhythmias in a minority of patients.

MVP can be found in up to half of first degree adult relatives, suggesting a genetic contribution. Until today, three chromosomal loci have been identified in autosomal dominant MVP with *DSCH1* as the sole gene identified in autosomal dominant disease. Filamin A *(FLNA)* has been identified in X-linked myxomatous MVP, suggesting a underlying mechanism in the regulation of the valvular cytoskeleton. In familial disease or male patients with severe MVP or sudden death, genetic screening should be considered.

To more precisely define patients at risk for sudden cardiac death more genetic as well as clinical research is warranted. A known relation with connective tissue diseases underscore the value of specialized clinics for this entity in cardiogenetic centres.

Introduction

Mitral valve prolapse (MVP) is one of the most common forms of valvular heart disease. It has long been recognized as an auscultatory phenomenon. It was not until 1966 that Barlow discovered the reason for the often-heard midsystolic click [1, 2]. Shortly afterwards, the introduction of echocardiography led to large – numbers of patients diagnosed with MVP. Due to incorrect echocardiographic definitions and selection bias of studied populations, prevalences of up to 35 % were reported in the 1970s and early 1980s [3]. A redefinition of echocardiographic criteria due to improved knowledge of mitral valve architecture provided a more accurate insight into the extent of the problem. Currently, the prevalence of MVP is known to range from about 0.5–2.5 % in the general population [3–7]. It is equally distributed between men and women, yet patients with MVP tend to have a leaner stature [6].

Mitral valve prolapse (MVP) is defined as the billowing of one or both mitral valve leaflets across the plane of the mitral valve annulus into the left atrium during systole. By definition, the leaflets should reach more than 2 mm above the annular plane on the parasternal long axis view with echocardiography (Figs. 20.1 and 20.2).

The clinical presentation can be very diverse, ranging from an incidental finding within asymptomatic patients to dramatic cases with severe mitral regurgitation, heart

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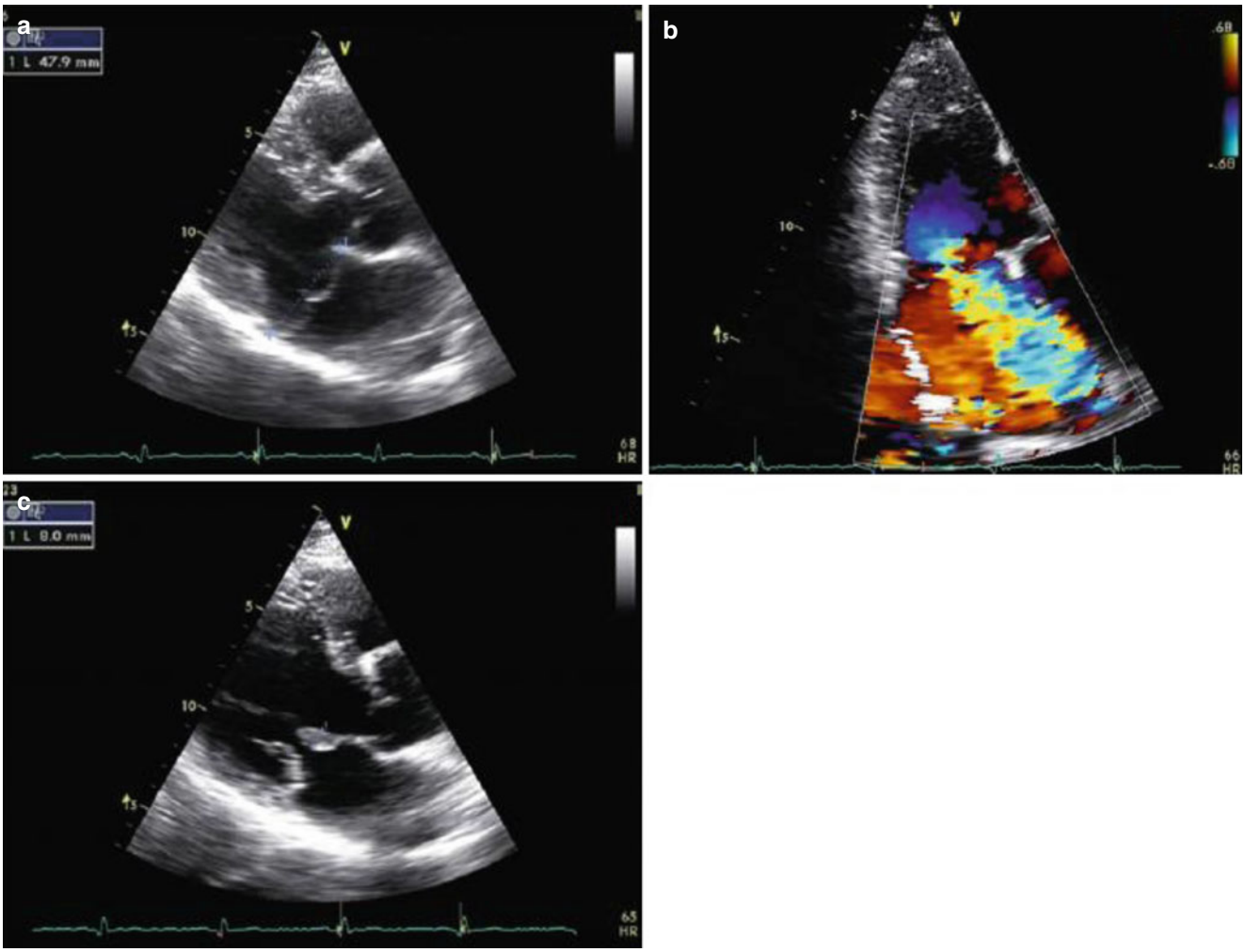


Fig. 20.1 (**a**) Parasternal long axis echocardiography showing classical mitral valve prolapse. Both posterior and anterior myxomatous mitral valve leaflets are billowing up to 6.5 mm in the left atrium during systole. (**b**) Apical three-chamber echocardiography with color Doppler flow measurement showing moderate to severe mitral regurgitation in the same patient. The left atrium is enlarged. (**c**) Parasternal long axis demonstrating myxomatous tips of both anterior and posterior mitral valves measuring 8 mm

failure, bacterial endocarditis, or even sudden cardiac death.

MVP may present as part of a systemic disorder or as a solitary phenomenon. It may occur more frequently in connective tissue disorders such as Marfan syndrome, Ehlers Danlos syndrome, and osteogenesis imperfecta. However, in most cases, it presents as a solitary entity; only a small minority, up to 1–2 % of all patients with MVP, do have a connective tissue disorder. This chapter, however, focuses on the solitary forms that appear to be one of the most common Mendelian cardiovascular abnormalities in humans and it discusses the epidemiologic aspects of MVP, its pathophysiology, and the current status of genetic knowledge of this intriguing valvular disorder.

Clinical Aspects, Complications and Pathophysiology of Mitral Valve Disease

The solitary forms of mitral valve disease are referred to as the classical prolapse, with the valve leaflet thickness exceeding 5 mm on echocardiography, and the nonclassical form, with leaflet thickness of less than 5 mm (both in the presence of a systolic upward displacement of 2 mm). Leaflet thickening or myxomatous degeneration is characterized by expansion of the spongiosa layer due to accumulation of proteoglycans. Also, structural alterations of collagen in all components of the valvular system and chordae can be found. It is thought that the mechanism underlying the expansion of the spongiosa layer is the result of a dysregulation of the balance between matrix protein synthesis and degradation [6].

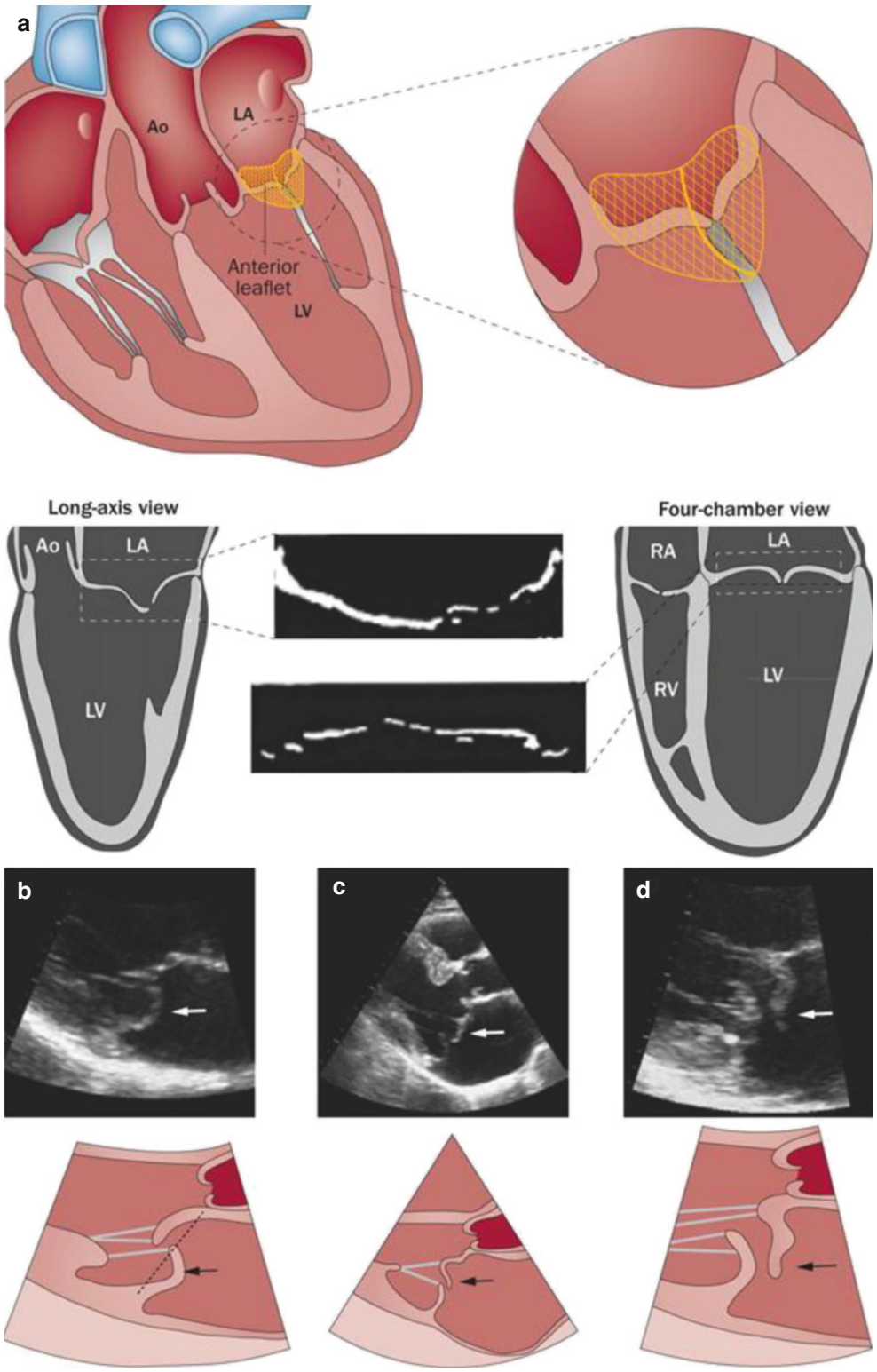


Fig. 20.2 Echocardiographic diagnosis of mitral valve prolapse. (**a**) Diagnosis of mitral valve prolapse must take into account the normal saddle shape of the valve and annulus, which produces opposite leaflet–annular relationships in perpendicular views. Mitral valve prolapse is most specifically diagnosed by leaflet displacement above the annular high points, imaged in long-axis views; and by leaflet misalignment at their point of coaptation. (**b**) Parasternal long-axis echocardiographic view of posterior leaflet prolapse (*arrows*) beyond the annular hinge points (*dashed line*). (**c**) Anterior leaflet prolapse and partial flail (partial eversion of the leaflet tip into the dilated LA; *arrows*) relative to the posterior leaflet, which is restricted, tethered by the dilated LV. These opposite leaflet displacements increase the regurgitant gap between the leaflets. (**d**) Patient with extensive leaflet thickening and anterior leaflet flail (arrows). Abbreviations: *Ao* aorta, *LA* left atrium, *LV* left ventricle, *RA* right atrium, *RV* right ventricle (From Levine et al. [8])

From the pathologic anatomical point of view, accumulation of proteoglycans (myxomatous mitral valve) is the most common cause of MVP, leading to leaflet thickening and redundancy, chordeal elongation and interchordal hookings and annular dilatation [7].

The clinical presentation of MVP is extremely heterogeneous, and to date, hardly any specific set of predictors for disease progression has been identified (see section, "Ventricular Arrhythmias and Sudden Cardiac Death in MVP").

The diagnosis is made by physical examination. Typically, a midsystolic click is heard, often followed by a late systolic murmur [3]. The diagnosis is confirmed by two-dimensional echocardiography. MVP generally has a good prognosis although in 25 % of patients, MVP may progress to significant mitral regurgitation [9].

Complications such as severe mitral insufficiency, heart failure, thromboembolic complications, and sudden cardiac death are rare, especially in patients with nonclassical prolapse. Freed and coworkers, from the Framingham study group, found that complications affected only 3 % of all patients with MVP [6]. However, patients with classical prolapse carry a 14-fold risk for complications [10]. It is not surprising that patients above 50 years who have a decreased left ventricular function, moderate to severe mitral regurgitation, and atrial fibrillation exhibit more complications.

The risk of infective endocarditis is raised three to eight times; in a population study (Olmsted USA), where nearly 900 MVP patients were identified and followed up, the 15-year cohort risk of infectious endocarditis after MVP diagnosis was 1.1 %±0.4 % [11]. However, current guidelines for infectious endocarditis no longer advocate the use of prophylactic antibiotics in patients with MVP. Only patients who are known to have had endocarditis should receive infective endocarditis prophylaxis when appropriate [12].

Mitral regurgitation may among other things lead to atrial arrhythmias including atrial fibrillation and therefore to thromboembolic complications. Antithrombotic medication, on the other hand, should be given only if classical risk factors unrelated to MVP are present. Nowadays, mitral valve reconstruction or replacement is advocated when moderate to severe mitral regurgitation is present [13].

Ventricular Arrhythmias and Sudden Cardiac Death in MVP

Sudden cardiac death (SCD) occurs twice as often in patients with myxomatous valve disease /MVP as compared to the general population, with sudden death rates of 0.2–0.4 % per year [10, 14]. SCD is found more often in patients with impaired left ventricular function, moderate to severe mitral regurgitation, and redundant chordae [15]. Interestingly, in a series of 200 victims of sudden cardiac death younger than 35 years, MVP was the only cardiac abnormality that could be found in as many as 10 % of cases [16]. In a SCD series of persons <40 years of age, recently studied by Basso et al., 7 % of cases (13 % females) had MVP as a sole anomaly [17]. This might be age dependent; in a recent sudden unexplained death syndrome general population study (mean age 70 ± 15 years) MVP was observed in 2.3 % of individuals prior to the sudden cardiac arrest event [18]. This percentage is similar to, for example, the Framingham Heart offspring study (2.4 %) [6].

The involvement of two leaflets might also contribute to life-threatening arrhythmias and sudden cardiac death: in a series of 24 otherwise unexplained OHCA cases, 42 % had bileaflet MVP. In addition to these abnormalities, patients with life-threatening arrhythmias were (mainly) females, more often demonstrated T-wave abnormalities (biphasic, or inverted T waves) and complex ventricular ectopy (multiform premature ventricular complexes, ventricular bigeminy, ventricular tachycardia or ventricular fibrillation) [19, 20]. Also at a population level, bileaflet MVP was associated with a higher level of ventricular tachycardias as compared to single leaflet MVP and controls [19]. These individuals with bileaflet MVP, however, did not appear to portend a poor prognosis when compared to single leaflet MVP or controls. Interestingly, bileaflet MVP was associated with a lower rate of all-cause mortality [19]. These data suggest that bileaflet MVP may in a subset of cases be associated with structural changes that predispose to ventricular tachycardia, but also reassure that, at least at the population level, incidentally noted MVP does not signal an elevated risk of fatal arrhythmias or mortality.

Although patients with MVP more often exhibit atrial and ventricular arrhythmias during Holter monitoring, the exact mechanism for pro-arrhythmia in MVP is not yet completely understood [21].

Mechanical stress on the papillary muscles resulting in fibrosis may contribute. On MRI, late gadolinium enhancement suggesting fibrosis can be found in the papillary muscles adjacent to the chordae tendineae in MVP patients with complex ventricular arrhythmias [22]. These findings and additional pathological evidence of myocardial fibrosis in one or both papillary muscles and adjacent LV free wall and the inferobasal wall were recently described [17, 23].

Endocardial friction lesions from mechanical contact from the relapsing leaflets may also play a role; not only in inducing fibrosis, but they also might trigger premature contractions resulting in ventricular arrhythmias [24]. So, a combination of substrate related fibrosis and mechanical effects may trigger premature contractions that subsequently may predispose to ventricular arrhythmias.

These different observations suggest that there is a specific subgroup of patients with MVP that may be at particular risk of SCD, that is, female, with not just an echocardio-

graphic diagnosis of MVP, but also bileaflet or posterior myxoid degeneration, ECG repolarization abnormalities and polymorphic/RBBB morphology complex ventricular arrhythmias [17, 20].

Treatment of Ventricular Arrhythmias in MVP

According to the ESC guidelines, the effectiveness of mitral valve repair or replacement to reduce the risk of SCD in patients with MVP, severe mitral regurgitation, and serious ventricular arrhythmia is not well established (Class IIb; level of evidence C) www.escardio.org/guidelines. A subset of patients with malignant ventricular arrhythmias may probably benefit from ablation therapy [20, 25].

Genetic Aspects of Mitral Valve Disease

Mitral valve prolapse has for many years been known to be familial in a subset of cases with an autosomal dominant mode of inheritance with reduced penetrance, influenced by age and sex [26–29]. Most cases of MVP come along with a family history of valvular disease. MVP was found in 46 % of first-degree relatives over 20 years, whereas only 16 % of patients below that age were affected, suggesting progressive disease with age-dependent penetrance [30]. A recent population study showed that parental MVP is associated with an odds of about 5 for MVP in offspring, also suggesting a genetic contribution to MVP.

Therefore, cardiac screening of first degree family members may be considered in patients with classical MVP. When conducting family studies, echocardiography should be performed in all cases. Holter monitoring can be performed in the presence of complaints or a family history of sudden cardiac death.

Until today, three loci for genetic myxomatous autosomal dominant MVP have been identified, on chromosomes 16p11.2–p12.1, 11p15.4, and 13q31.3–q32.1 (MMVP1 [31], MMVP2 [32], and MMPV3 [33],). Only the gene underlying MMVP2 has been identified so far. This gene, *DCHS1*, was identified in a large family and its role was confirmed in two smaller families. Extensive functional analysis suggested a role for this protein in the development of cardiac valves [34].

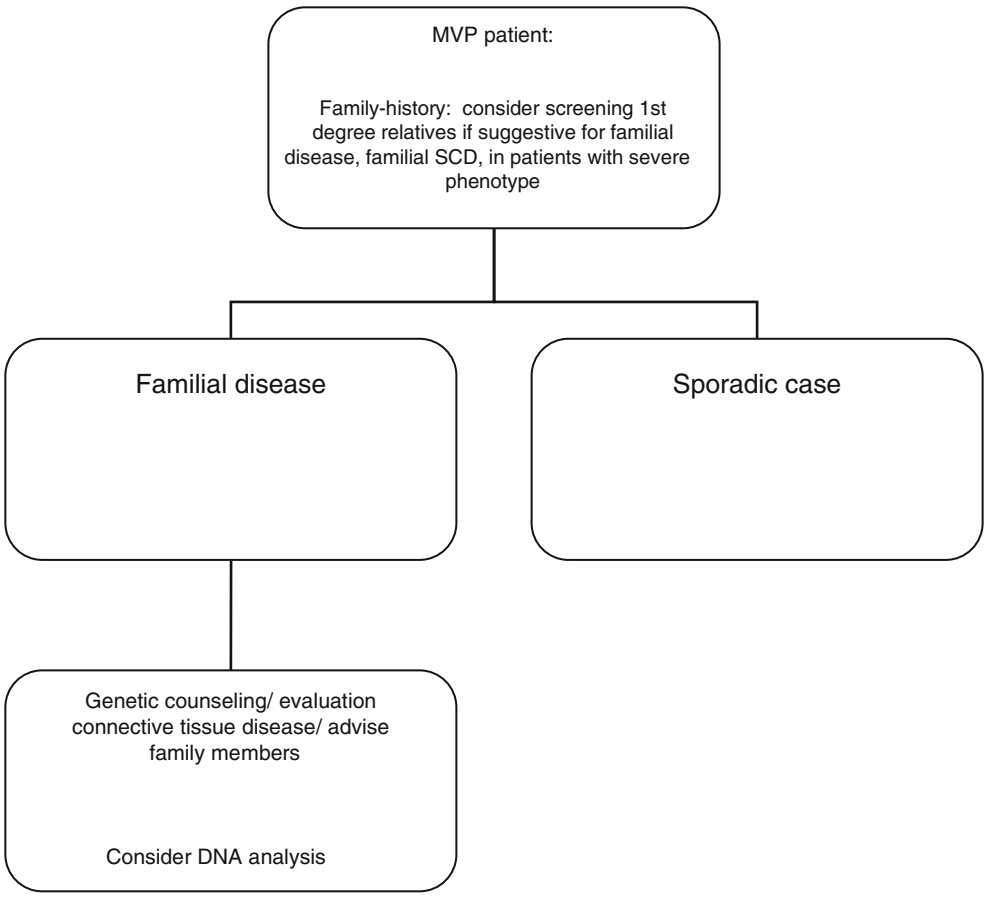
The gene-encoding Filamin A (*FLNA*) has been identified in X-linked myxomatous mitral valves in different unrelated families [35, 36]. Males carrying *FLNA* mutations exhibit a severe phenotype, often manifesting at young age (neonatally to 40 years), while females (heterozygotes) show milder manifestations of the disorder [36]. Also, other structural cardiac abnormalities like atrial and ventricular septal defects and aortic root dilatation can be found in mutation carriers. Filamin A is an actin-binding protein that plays a pivotal role in cell motility and membrane stability. Filamin A may contribute to the development of myxomatous changes of the cardiac valves by the regulation of transforming growth factor-β (TGF-β) signaling through its interaction with Smads activated by TGF-β receptors [37, 38]. Defective signaling cascades that involve members of the TGF-β superfamily have been described in impaired remodeling of cardiac valves during development.

The exact role of monogenic MVP still has to be elucidated: the larger pedigrees studied suggest that rare, high penetrant genes may indeed underlie disease phenotype, while familial clustering in the population may be due to either highly penetrant rare alleles with a strong effect size or (multiple common) variants with smaller effect sizes.

Molecular Diagnostics

The role of molecular genetics in MVP is limited because only two genes have been identified so far. The selection for targeted screening of one of those genes can be based upon family history or the results of evaluation of family members. These genes may also be included in larger panels that are generally designed to evaluate generalized connective tissue diseases like marfan and ehlers-danlos syndromes that may also be associated with MVP. The specific clinical features of these disorders can, however, also be recognized by careful clinical evaluation. Clinical genetics centers often offer special clinics for diagnosing these patients.

Family Screening and Follow-Up in Relatives



Summary/Take Home Message

Mitral valve prolapse is the most common valvular disorder with a strong genetic contribution. The course of disease is benign in most cases, but serious complications such as heart failure, severe mitral regurgitation, bacterial endocarditis, and ventricular arrhythmias/sudden cardiac death occur especially in patients with myxomatous degenerated valves. Bileaflet involvement, female sex, and substrate-related fibrosis and mechanical effects may trigger premature contractions that predispose to ventricular arrhythmias in a minority of patients. Until now, three chromosomal loci have been identified in autosomal dominant MVP with *DSCH1* as the sole gene identified in dominant disease. Filamin A (*FLNA*) has been identified in X-linked myxomatous MVP, suggesting an underlying mechanism in the regulation of the valvular cytoskeleton. More genetic and clinical research is warranted to more precisely define patients at risk for this potentially lethal condition. First-degree relatives of patients with classical MVP and a history suggestive for familial disease and/or SCD should undergo cardiac screening. In familial disease or male patients with severe MVP or SCD, genetic screening should be considered.

Take Home Message

- MVP is a common, generally benign valvular disorder, with a familial character in a subset of cases
- Sudden cardiac death rates are 0.2–0.4 %/year
- Malignant arrhythmias infrequently occur: preliminary studies suggest that the substrate for arrhythmias seems related to fibrosis and mechanical effects that trigger premature contractions.
- Female patients with MVP, in particular those with bileaflet disease or posterior myxoid degeneration, repolarization abnormalities on ECG, and/or polymorphic/RBBB morphology complex ventricular arrhythmias may be at risk of SCD.
- Cardiac screening should be considered in first-degree family members; particularly if a family history is positive for mitral valve disease and/or sudden cardiac death.
- Genetic screening/clinical genetic evaluation should be considered in males with severe (myxomatous) disease/X-linked pedigrees, clear familial cases.

References

1. Barlow JB, Bosman CK. Aneurysmal protrusion of the posterior leaflet of the mitral valve. An auscultatory-electrocardiographic syndrome. Am Heart J. 1966;71:166–78.
2. Cheng TO(1). John B. Barlow: the man and his syndrome. Int J Cardiol. 2014;177(2):311–6.
3. Weisse AB. Mitral valve prolapse: now you see it; now you don't: recalling the discovery, rise and decline of a diagnosis. Am J Cardiol. 2007;99:129–33.
4. Turker Y, Turker Y, Baltaci D, et al. The prevalence and clinical characteristics of mitral valve prolapse in a large population-based epidemiologic study: the MELEN study. Eur Rev Med Pharmacol Sci. 2015;19:2208–12.
5. Hepner AD, Ahmadi-Kashani M, Movahed MR. The prevalence of mitral valve prolapse in patients undergoing echocardiography for clinical reason. Int J Cardiol. 2007;123:55–7.
6. Freed LA, Levy D, Levine RA, et al. Prevalence and clinical outcome of mitral-valve prolapse. N Engl J Med. 1999;341:1–7.
7. Davies MJ, Moore BP, Braimbridge MV. The floppy mitral valve. Study of incidence, pathology, and complications in surgical, necropsy, and forensic material. Br Heart J. 1978;40:468–81.
8. Levine RA, Hagége AA, Judge DP, et al. Mitral valve disease–morphology and mechanisms. Nat Rev Cardiol. 2015;12:689–710.
9. Delling FN, Rong J, Larson MG, et al. Evolution of mitral valve prolapse: insights from the Framingham heart study. Circulation. 2016;133:1688–95.
10. Nishimura RA, McGoon MD, Shub C, et al. Echocardiographically documented mitral-valve prolapse. Long-term follow-up of 237 patients. N Engl J Med. 1985;313:1305–9.
11. Katan O, Michelena HI, Avierinos JF, et al. Incidence and predictors of infective endocarditis in mitral valve prolapse: a population-based study. Mayo Clin Proc. 2016;91:336–42.
12. Wilson W, Taubert KA, Gewitz M, et al. Prevention of infective endocarditis: guidelines from the American Heart Association: a guideline from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. Circulation. 2007;116:1736–54.
13. RO B, BA C, Chatterjee K, et al. Focused update incorporated into the ACC/AHA 2006 guidelines for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 1998 Guidelines for the Management of Patients With Valvular Heart Disease): endorsed by the Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *Circulation*. 2008;118:e523–661.
14. Düren DR, Becker AE, Dunning AJ. Long-term follow-up of idiopathic mitral valve prolapse in 300 patients: a prospective study. J Am Coll Cardiol. 1988;11:42–7.
15. Kligfield P, Levy D, Devereux RB, et al. Arrhythmias and sudden death in mitral valve prolapse. Am Heart J. 1987;113:1298–307.
16. Basso C, Corrado D, Thiene G. Cardiovascular causes of sudden death in young individuals including athletes. Cardiol Rev. 1999;7:127–35.
17. Basso C, Perazzolo Marra M, Rizzo S, et al. Arrhythmic mitral valve prolapse and sudden cardiac death. Circulation. 2015;132:556–66.
18. Narayanan K, Uy-Evanado A, Teodorescu C, et al. Mitral valve prolapse and sudden cardiac arrest in the community. Heart Rhythm. 2016;13:498–503.
19. Nordhues BD, Siontis KC, Scott CG, et al. Bileaflet mitral valve prolapse and risk of ventricular dysrhythmias and death. J Cardiovasc Electrophysiol. 2016;27:463–8.
20. Sriram CS, Syed FF, Ferguson ME, et al. Malignant bileaflet mitral valve prolapse syndrome in patients with otherwise idiopathic out-of-hospital cardiac arrest. J Am Coll Cardiol. 2013;62:222–30.
21. Hayek E, Gring CN, Griffin BP. Mitral valve prolapse. Lancet. 2005;365:507–18.
22. Han Y, Peters DC, Salton CJ, et al. Cardiovascular magnetic resonance characterization of mitral valve prolapse. JACC Cardiovasc Imaging. 2008;1:294–303.
23. Sheppard MN, Steriotis AK, Sharma S. Letter by Sheppard et al regarding article, "Arrhythmic mitral valve prolapse and sudden cardiac death". Circulation. 2016;133:e458.
24. Sniezek-Maciejewska M, Dubiel JP, et al. Ventricular arrhythmias and the autonomic tone in patients with mitral valve prolapse. Clin Cardiol. 1992;15:720–4.
25. Syed FF, Ackerman MJ, McLeod CJ, et al. Sites of successful ventricular fibrillation ablation in bileaflet mitral valve prolapse Syndrome. Circ Arrhythm Electrophysiol. 2016 May;9(5). pii:e004005.
26. Devereux RB, Brown WT, Kramer-Fox R, et al. Inheritance of mitral valve prolapse: effect of age and sex on gene expression. Ann Intern Med. 1982;97:826–32.
27. Strahan NV, Murphy EA, Fortuin NJ, et al. Inheritance of the mitral valve prolapse syndrome. Discussion of a three-dimensional penetrance model. Am J Med. 1983;74:967–72.
28. Weiss AN, Mimbs JW, Ludbrook PA, et al. Echocardiographic detection of mitral valve prolapse. Exclusion of false positive diagnosis and determination of inheritance. Circulation. 1975;52:1091–6.
29. Delling FN, Rong J, Larson MG, et al. Familial clustering of mitral valve prolapse in the community. Circulation. 2015;131:263–8.
30. Grau JB, Pirelli L, Yu PJ, et al. The genetics of mitral valve prolapse. Clin Genet. 2007;72:288–95.
31. Disse S, Abergel E, Berrebi A, et al. Mapping of a first locus for autosomal dominant myxomatous mitral-valve prolapse to chromosome 16p11.2–p12.1. Am J Hum Genet. 1999;65:1242–51.
32. Freed LA, Acierno Jr JS, Dai D, et al. A locus for autosomal dominant mitral valve prolapse on chromosome 11p15.4. Am J Hum Genet. 2003;72:1551–9.
33. Nesta F, Leyne M, Yosefy C, et al. New locus for autosomal dominant mitral valve prolapse on chromosome 13: clinical insights from genetic studies. Circulation. 2005;112:2022–30.
34. Durst R, Sauls K, Peal DS, et al. Mutations in DCHS1 cause mitral valve prolapse. Nature. 2015;525:109–13.
35. Kyndt F, Gueffet JP, Probst V, et al. Mutations in the gene encoding filamin A as a cause for familial cardiac valvular dystrophy. Circulation. 2007;115:40–9.
36. Aalberts JJ, van Tintelen JP, Oomen T, et al. Screening of TGFBR1, TGFBR2, and FLNA in familial mitral valve prolapse. Am J Med Genet A. 2014;164A:113–9.
37. Sasaki A, Masuda Y, Ohta Y, et al. Filamin associates with Smads and regulates transforming growth factor-beta signaling. J Biol Chem. 2001;276:17871–7.
38. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature. 2003;425:577–84.

Genetic Disorders of Lipoprotein Metabolism: Diagnosis and Management

21

A.J. Cupido, R.M. Stoekenbroek, and J.J.P. Kastelein

Abstract

Disorders of lipoprotein metabolism are major contributors to cardiovascular disease (CVD). Dyslipidemia refers to elevated LDL-C levels, triglycerides, or remnant cholesterol, and decreased HDL-C. Most cases of CVD are multifactorial and/or polygenic in origin. However, (mono)genic causes can be suspected in individuals with early-onset CVD or with specific clinical hallmarks. The first step in the diagnostic workup of dyslipidemias is to exclude secondary dyslipidemias by obtaining a medical history and through biochemical testing. Specialized biochemical tests or genetic tests can often help in establishing a definite diagnosis.

Treatment consists of lifestyle modifications, usually in combination with pharmacological agents such as statins. However, advances in gene technologies have enabled a rapid increase in the repertoire of available treatment options.

This chapter provides an extensive overview of lipoprotein metabolism, followed by an overview of mono- and polygenic disorders of lipoprotein metabolism, including underlying causes, clinical- and diagnostic characteristics, and available treatment options.

Abbreviations

ABC	Adenosine triphosphate (ATP) binding cassette
ACAT	Acyl-coenzyme A: cholesterol O-acyltransferase
Apo-A1	Apolipoprotein A1
BASs	Bile acid sequestrants
CAD	Coronary artery disease
CE	Cholesterylester
CETP	Cholesterylester transfer protein
cIMT	Carotid intima media thickness
CHD	Coronary heart disease
CVD	Cardiovascular disease
EMA	European Medicines Agency
FCH	Familial combined hyperlipidemia
FD	Familial dysbetalipoproteinemia
FDA	Food and Drug Administration
FDB	Familial defective apolipoprotein B
FH	Familial hypercholesterolemia
FHTG	Familial hypertriglyceridemia
HDL-C	High-density lipoprotein cholesterol
HL	Hepatic lipase
HMG-CoA	3-Hydroxyl-3-methylglutaryl coenzyme A
IDL	Intermediate-density lipoprotein
LCAT	Lecithin: cholesteryl acyltransferase
LDL-C	Low-density lipoprotein cholesterol
LDL-R	Low-density lipoprotein receptor
LDLRAP	LDL-receptor-adapting protein
LIPC	Gene-encoding hepatic lipase
LIPG	Gene-encoding endothelial lipase
LPL	Lipoprotein lipase
NPC1L1	Niemann-Pick C1 like 1
PCSK9	Proprotein convertase subtilisin/kexin type 9
PLTP	Phospholipid transfer protein

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RCT	Reverse cholesterol transport
SNP	Single nucleotide polymorphism
SR-B1	Scavenger receptor B1
TC	Total cholesterol
VLDL	Very low-density lipoprotein

Introduction

Atherosclerosis, leading to ischemic manifestations in different vascular beds, is the leading cause of morbidity and mortality worldwide. It is a multifactorial disease, driven by a combination of genetic, environmental, and behavioral factors. The process of atherosclerosis accelerates in the presence of classical risk factors such as *dyslipidemia*, hypertension, diabetes mellitus, obesity, and smoking. Dyslipidemia is one of the major contributors to atherosclerosis and includes both elevated low-density lipoprotein cholesterol (*LDL-C*) and remnant cholesterol levels, as well as decreased high-density lipoprotein cholesterol (*HDL-C*) levels [1]. The crucial role of increased plasma LDL-C levels in the pathogenesis of atherosclerosis has been well established. This also applies to the pharmacological reduction of plasma LDL-C levels accomplished by 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or *statins*. A large prospective meta-analysis including over 90,000 individuals demonstrated that an LDL-C reduction of 1 mmol/L is associated with a 21 % reduction in major cardiovascular events [2]. In addition, decreased plasma HDL-C levels are an independent predictor of cardiovascular disease (CVD), as has been unequivocally established by numerous epidemiological studies. Almost 40 % of patients with premature coronary artery disease (CAD) have low HDL-C levels, either alone or in conjunction with hypertriglyceridemia or combined hyperlipidemia [3]. Furthermore, it has been estimated that each 0.03 mmol/L (1 mg/dL) increase in HDL-C is associated with a 2 % reduction CAD risk in men and a 3 % reduction in women [4]. However, whether raising HDL-C by pharmacological means will result in cardiovascular benefit is questionable. A recent meta-regression analysis of 108 randomized controlled trials, including more than 300,000 patients using several lipid-modifying interventions, did not show a relationship between treatment-induced increases in HDL-C and a decrease in coronary heart disease events or deaths when corrected for concurrent LDL-C reductions [5]. Nevertheless, this study does not prove that increasing HDL-C in selected patients with low HDL-C levels has no value [6]. In addition, these studies evaluated only HDL-C concentrations and did not address HDL functionality.

Finally, *hypertriglyceridemia* also influences CVD risk. Several epidemiological and genetic studies have indicated elevated plasma triglyceride levels as an independent risk factor for CVD [7–13]. This also applies to remnant cholesterol [11]. For individuals with high triglycerides in the general population, the risks for myocardial infarction, ischemic heart disease, ischemic stroke, and all-cause mortality are significantly increased [11].

Although dyslipidemia has a largely polygenic background, a number of *monogenetic disorders* have been identified. This chapter provides an overview of genetic causes underlying disturbances in lipid and lipoprotein metabolism, in which the focus will be primarily on these monogenetic disorders. The chapter starts with a global overview of lipid and lipoprotein metabolism, followed by the genetic background of disturbances in LDL-C and HDL-C levels, respectively. Finally, genetic causes of disorders in triglyceride metabolism are also discussed. For each of these categories, genetics, clinical phenotype, diagnosis, and management will be addressed.

Structure of Lipids and Lipoproteins

Cholesterol and *triglycerides* exert essential functions in body cell membranes and in hormone and energy homeostasis. Due to their hydrophobic properties, cholesterol and triglycerides are transported in large macromolecular complexes, the so-called lipoproteins. *Lipoproteins* contain a core of hydrophobic lipids surrounded by hydrophilic molecules such as phospholipids, unesterified cholesterol, and *apolipoproteins*. The latter are proteins that provide structural integrity to the lipoprotein and serve as ligands for binding to specific receptors. Based on their relative density, lipoproteins can be categorized into five major classes: chylomicrons, very low-density lipoproteins (*VLDL*), intermediate-density lipoproteins (*IDL*), *LDL*, and *HDL*. The first two categories are large, buoyant triglyceride-rich particles, whereas the latter three are dense, cholesterol-rich particles. When fasting, plasma cholesterol levels are usually a reflection of the amount of LDL in the plasma, whereas plasma triglyceride levels reflect the amount of VLDL.

Lipid and Lipoprotein Metabolism

The liver and the intestine are the most important sources of lipoproteins. Their transport and metabolism is generally divided into three systems: absorption of exogenous and endogenous lipids and lipoproteins, endogenous synthesis of lipids and lipoproteins, and *reverse cholesterol transport (RCT)*. These processes are depicted in Fig. 21.1.

Absorption of Exogenous and Endogenous Lipids

The average Western diet consists of a daily intake of approximately 100 g of fat and 500 mg of cholesterol. Phospholipids

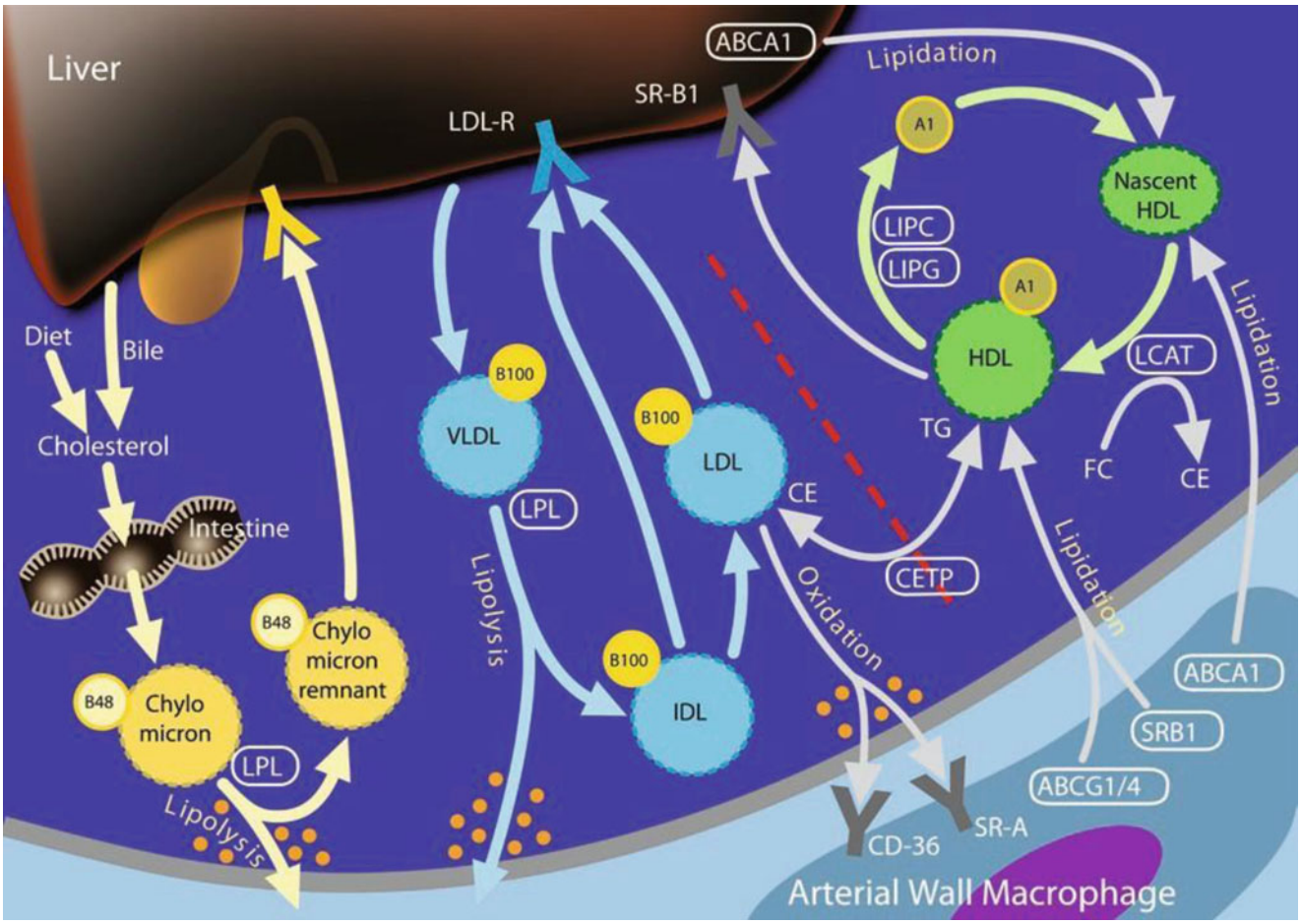

Fig 21.1 Overview of lipoprotein metabolism. Dietary lipids and cholesterol from the hepatic bile are absorbed in the intestine, packaged into chylomicrons, and secreted into the lymph, which drains into the systemic circulation. In the bloodstream, the triglyceride-rich (*TG*) chylomicrons are hydrolyzed through the action of lipoprotein lipase (*LPL*) and the removed TGs and free fatty acids are taken up by extrahepatic tissues such as the liver and muscles. The chylomicrons remnants are taken up by the liver for further processing. In the fasting state, the liver assembles TG-rich very low-density lipoprotein (*VLDL*). Also VLDL are hydrolyzed by LPL and thereby transformed to smaller VLDL remnants, IDL. Half of the IDL are directly taken up by the liver through binding of the LDL-R, whereas the other half is converted to cholesterol-rich LDL. Most of the plasma LDL-C is cleared from the circulation by binding to the LDL-R of the liver. Of the remaining LDL, some subfractions are especially prone to oxidative modification and then taken up by scavenger receptors (CD-36) of arterial wall macrophages resulting in foam cells and atherosclerotic plaques. High-density lipoprotein (*HDL*) is responsible for the RCT from extrahepatic tissues to the liver. Nascent HDL is formed from lipid-poor Apo-A1, which is secreted by the liver and intestine and which is lipidated through interaction with ABCA1. Nascent HDL is also generated from surface components shed during lipolysis of TG-rich lipoproteins by LPL (not depicted). After lipidation, LCAT esterifies free cholesterol (*FC*) to cholesterylesters (*CE*) which migrates into the core of the HDL making them larger spherical particles. These larger HDL particles acquire additional lipids from extrahepatic tissues, including arterial wall macrophages, by receptor-mediated pathways such as ABCG1, ABCG4, and SR-B1, as well as from lipolysis of TG-rich lipoproteins and passive diffusion (not depicted). The HDL particles can be metabolized in several ways. First, they can deliver CE to the liver by binding to SR-B1 on the hepatocyte surface. In the liver, the cholesterol can be processed and eliminated. Alternatively, CE in HDL can be exchanged for TG in apo-B-containing lipoproteins, by the action of CETP. The TG-enriched HDL is hydrolyzed by LIPC and LIPG to smaller HDL and lipid-poor Apo-A1 particles. These can be either recycled to acquire cholesterol or excreted from the body through the kidneys

and bile acids, present in hepatic bile, emulsify lipids from food to form micelles within the intestinal lumen. Hepatic bile also delivers significant amounts of unesterified cholesterol to these micelles.

Pancreatic lipases secreted into the intestinal lumen digest dietary lipids to chemical entities that can be absorbed by enterocytes. Fatty acids and monoacylglycerides are almost entirely absorbed through both passive diffusion and carrier-mediated processes [14]. By contrast, *cholesterol absorption* is an active process, mediated by several transporter proteins which are located at the intestinal brush-border membrane. Cholesterol and sterols derived from plants are taken up by the enterocyte through the recently identified Niemann-Pick C Like 1 (NPC1L1) transporter [15], whereas the ATP-binding cassette transporters (ABC) G5 and G8 actively secrete plant sterols, and to a lesser extent cholesterol, back into the intestinal lumen [16]. Of note, NPC1L1 and ABCG5 and G8 are also located in the liver, where they are involved

in hepatic cholesterol trafficking to the bile [16, 17]. Intestinal cholesterol absorption exhibits on average about 50 % efficiency, with large interindividual variation, ranging from 20 % to 80 % [18]. Free cholesterol that has entered the enterocyte is either reesterified intracellularly by Acyl-coenzyme A: cholesterol acyltransferase (ACAT) 2 and then packaged into chylomicrons, or trafficked toward the basolaterally located ATP-binding cassette transport protein A1 (ABCA1) protein for HDL formation.

Chylomicrons consist for approximately 80–95 % of triglycerides and apolipoprotein B48 (apo-B48) as their structural surface protein. They are secreted into the lymph, which drains directly into the systemic circulation. In the bloodstream, chylomicrons are hydrolyzed, that is, triglycerides and free fatty acids (FFAs) are removed from the core of the chylomicrons, by *lipoprotein lipase (LPL)*, thereby generating remnant particles. LPL is anchored to the endothelial surface by proteoglycans and/or by the recently identified anchoring protein GPIHBP1 (glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1) [19]. LPL requires apolipoprotein CII as a cofactor for adequate hydrolysis. The removed triglycerides and FFAs are taken up by the liver and muscle, whereas the chylomicrons remnants are taken up by the liver for further processing, as described below. Chylomicrons have a short half-life in the circulation, averaging approximately 10–20 min, provided that clearance is undisturbed. Hence, chylomicrons are not present in the bloodstream in the fasting state. However, when postprandial levels of chylomicrons and their remnants remain high, due to intestinal overproduction or delayed clearance, this can promote delivery of chylomicrons and their remnants to the arterial endothelium, with subsequent generation of *foam cells* and fatty streaks and eventually *atherosclerotic plaque formation*.

Endogenous Synthesis of Lipids and Lipoproteins

In the fasting state, the liver assembles VLDL-C by combining triglycerides, phospholipids, apolipoprotein B100 (apo-B100), and cholesterylesters (CEs). The latter originate either from de novo synthesis and subsequent esterification by ACAT2 or from remnant particles that have been taken up from the circulation. Like chylomicrons, VLDLs are triglyceride-rich particles secreted into the bloodstream, where they are hydrolyzed by LPL and thereby transformed to smaller and denser VLDL remnants, IDL, and finally LDL particles. In general, half of the VLDL remnants are directly taken up by the liver through binding to the *LDL receptor* (LDL-R), whereas the other half is converted to LDL.

LDL is the most abundant cholesterol-carrying particle in humans and accounts for more than 75 % of plasma cholesterol. Mediated by *apo-B100*, most of plasma LDL is cleared from the circulation by the LDL-R, which is located at the surface of hepatocytes and internalized entirely (lipoprotein + receptor) upon binding of LDL. The remaining LDL particles are delivered to peripheral tissues such as the adrenals and gonads for the synthesis of steroid and sex hormones. In hepatic endosomes, LDL is degraded to amino acids and free cholesterol, whereas the LDL-R is scavenged back to the cell surface for the uptake of additional LDL particles. Approximately 70–80 % of the LDL catabolism takes place via the LDL-R. The remaining part is cleared via non-specific routes.

The recently identified proprotein convertase subtilisin/kexin type 9 (*PCSK9*) protein plays a pivotal role in LDL metabolism by promoting degradation of LDL-R instead of recycling it back to the cell surface, thereby reducing the number of available LDL receptors at the surface of hepatocytes [20]. It primarily does so by acting on the LDL receptor as a secreted factor and the expression is – similar to LDL-R – modulated by intracellular cholesterol levels. Because of this effect of cholesterol at the transcriptional level, statins increase *PCSK9* expression, thereby partially counteracting their effect in terms of upregulating LDL receptor expression. Therefore, inhibiting PCSK9 could lower LDL-C levels by increasing the available pool of LDL receptors and work synergistically with statins. PCSK9 inhibitors have emerged as the prime candidate to further reduce CVD risk, as will be discussed in section, "Management."

Finally, LDL is not a homogeneous lipoprotein fraction, as it consists of several subfractions with varying mass and density. *Small-dense LDL* is particularly associated with atherosclerotic disease. This subfraction is mostly prevalent in subjects with elevated triglyceride levels. Small-dense LDL particles are prone to oxidative modification, resulting in uptake by scavenger receptors of arterial macrophages, which express a strong affinity for these so-called ox-LDL particles. Since a negative feedback system for these scavenger receptors is lacking, unlimited amounts of ox-LDL can be taken up by these macrophages, which transform into foam cells and atherosclerotic plaques.

HDL Metabolism and RCT

HDL is a highly heterogeneous class of lipoprotein particles that differ in protein component and lipid composition, size, shape, density, and charge. In addition to the observational support for the atheroprotective role of HDL, numerous *in vitro* and *in vivo* animal studies have demonstrated various mechanisms through which HDL exerts its beneficial effects on the arterial wall. The most widely acknowledged mechanism is its role in *RCT*. This involves the ability of HDL to

stimulate efflux of cholesterol from peripheral tissues, transport in the plasma, and uptake by the liver, followed by biliary excretion and elimination via the feces. Specifically, the efflux of cholesterol from macrophage foam cells in the artery wall is thought to be central to the antiatherogenic properties of HDL. In addition, putative atheroprotective properties of HDL include its ability to improve endothelial function, inhibit LDL oxidation, and induce several anti-apoptotic, anti-inflammatory, and antithrombotic effects. However, whether stimulation of these processes results in clinical benefit in humans is as of yet not resolved [21].

The process of RCT starts by lipidation of *apolipoprotein A1* (apo-A1) through interaction with the ABCA1. Apo-AI is the most important structural protein of HDL and comprises approximately 70 % of the proteins in HDL-C. It is synthesized by the liver and intestine and released into the circulation either in a free non-lipidated form or incorporated in small discoid particles, rich in phospholipids and poor in cholesterol, the so-called nascent or pre-β-HDL. Nascent HDL is also generated from redundant surface components shed during lipolysis of triglyceride-rich lipoproteins such as chylomicrons and VLDL by LPL. The *ABCA1 transporter* resides at the cellular membrane and facilitates the transfer of free cholesterol and phospholipids from intracellular lipid pools to apo-A1. New insights suggest that, in contrast to previous opinion, hepatic ABCA1 appears to be critical for the initial lipidation of lipid-poor apo-A1, protecting it from rapid degradation and allowing it to go on to form mature HDL. Conversely, macrophage ABCA1 appears to contribute little to bulk lipidation of HDL and therefore to plasma HDL-C levels, but does seem to be important for protection against atherosclerosis [22]. After lipidation, lecithin: cholesteryl acyltransferase (*LCAT*) subsequently esterifies the externalized free cholesterol to cholesterylesters on the surface of HDL on activation by its cofactor apo-A1. The esterified cholesterol then migrates into the core of the HDL and as larger amounts of cholesterylesters become incorporated, HDL becomes a larger spherical particle. These larger so-called HDL-3 and HDL-2 particles acquire additional free cholesterol and phospholipids from extrahepatic tissues, including macrophage foam cells, by means of passive diffusion or receptor-mediated pathways, such as ABCG1, ABCG4, and scavenger receptor B1 (SR-B1), as well as from lipolysis of triglyceride-rich lipoproteins [23]. The HDL-3 and HDL-2 particles can be metabolized in several ways. First, they can directly deliver cholesterylesters to the liver by binding to *SR-B1* on the hepatocyte surface. In the liver, the cholesterol can be processed and eliminated as bile or converted to cholesterol-containing steroids. Once the HDL particle is delipidated, it dissociates from SR-B1 and can then reinitiate another cycle of RCT. Alternatively, cholesterylesters in HDL can be exchanged for triglycerides in apo-B-containing lipoproteins such as LDL, by the action of *cholesterylester transfer protein (CETP)*, after which these cholesterylesters are available for hepatic clearance via the LDL receptor. However, if a population of apo-B-containing lipoproteins enriched with cholesterylesters by CETP interacts with macrophages in arterial walls and promotes net cholesterol uptake, this process is potentially atherogenic. Whether the sum effect of CETP activity in humans is pro- or antiatherogenic is not clear. Most experimental evidence in animals favors a pro-atherogenic role for CETP. The triglyceride-enriched HDL is a substrate for hydrolysis by *hepatic lipase* (LIPC) while phospholipids are mainly hydrolyzed by *endothelial lipase* (LIPG). In this way, HDL is remodeled to lipid poor Apo-A1 and smaller HDL particles which can either be recycled to acquire cholesterol from extrahepatic tissues or dissociated apo-A1 is excreted from the body through the kidneys. *Phospholipid transfer protein (PLTP)* also plays a major role in HDL metabolism in various ways. PLTP facilitates the transfer of phospholipids from triglyceride-rich lipoproteins during lipolysis and evidence has accumulated over the years that PLTP can also remodel HDL particles [24].

Genetic Causes of Elevated LDL-C Levels

Mutations in genes involved in LDL metabolism can result in increased plasma LDL-C concentrations. Three genes have been characterized: the *LDL-R* gene, the *Apo-B* gene, and most recently the *PCSK9* gene [25]. These genes are involved in *autosomal dominant hypercholesterolemia*. The single known *autosomal recessive* form of *hypercholesterolemia* (ARH) is caused by failing internalization of the LDL-R/LDL particle complex in the hepatocytes and is caused by a mutation in the ARH gene [26, 27].

Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) is the most common autosomal dominant inherited disorder of metabolism. Approximately 1:500 people are affected, resulting in almost ten million patients worldwide. In some populations, this prevalence is higher due to a founder effect [28]. Homozygosity is rare, with an average of one per 160,000–300,000, with higher frequencies in populations where a founder effect or high rates of consanguinity are present [29]. FH subjects are characterized by plasma LDL-C levels above the 95th percentile for age and gender, due to impaired internalization of LDL particles caused by functional alterations in the LDL receptor [30]. Moreover, the decrease in the hepatic cholesterol pool stimulates cholesterol synthesis, resulting in increased production of VLDL, which further increases LDL-C levels.

Genetics

The molecular defect underlying FH most often consists of a mutation in the *LDL-R gene*, located on chromosome 19p13 [30]. At present, over 1000 different mutations in the LDL-R or promoter region leading to an FH phenotype have been described [31], 91 % of which are point mutations [32].

In addition, mutations in the *PCSK9 gene* on chromosome 1 are a rare cause of the FH phenotype, accounting for <1 % of cases [33]. To date, eight hypercholesterolemic missense mutations in *PCSK9* have been reported [34]. These "gain-of-function" mutations cause hypercholesterolemia due to PCSK9-induced enhanced degradation of LDL receptors, thereby decreasing the available pool of hepatic LDL receptors. Finally, mutations in the LDL-R-binding domain of apo-B100 can result in a phenotype which resembles FH, approximately in 5 % of FH cases [33]. This is outlined in the next paragraph on familial defective apolipoprotein B (FDB).

Clinical Characteristics

A hallmark of FH is plasma LDL-C levels above the 95th percentile for age and gender. This induces accelerated deposition of cholesterol in arterial walls and other tissues, resulting in the clinical hallmarks of FH: premature atherosclerosis, *tendon xanthomas, xanthelasma*, and corneal arcus [30]. However, these clinical characteristics (Figs. 21.2 and 21.3) are not exclusively associated with or may not be present in every patient with FH. Moreover, the age of appearance of these symptoms varies depending on the severity of the phenotype. If untreated, approximately 50 % of male and 30 % of female heterozygous FH patients will develop symptomatic CVD before the age of 50 years [35]. However, the onset and progression of atherosclerotic disease varies considerably between FH individuals and within families. It was shown that event-free survival depends more on actual LDL-C levels caused by the mutation, rather than the type of mutation itself [36].

Patients with homozygous FH have plasma cholesterol levels >13 mmol/L. If untreated, patients suffer from CVD before 20 years of age and generally do not survive past 30 years of age [29].

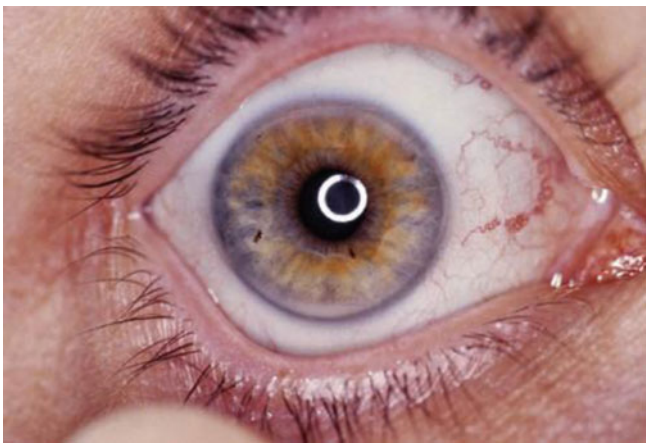

Fig. 21.3 Arcus lipoides

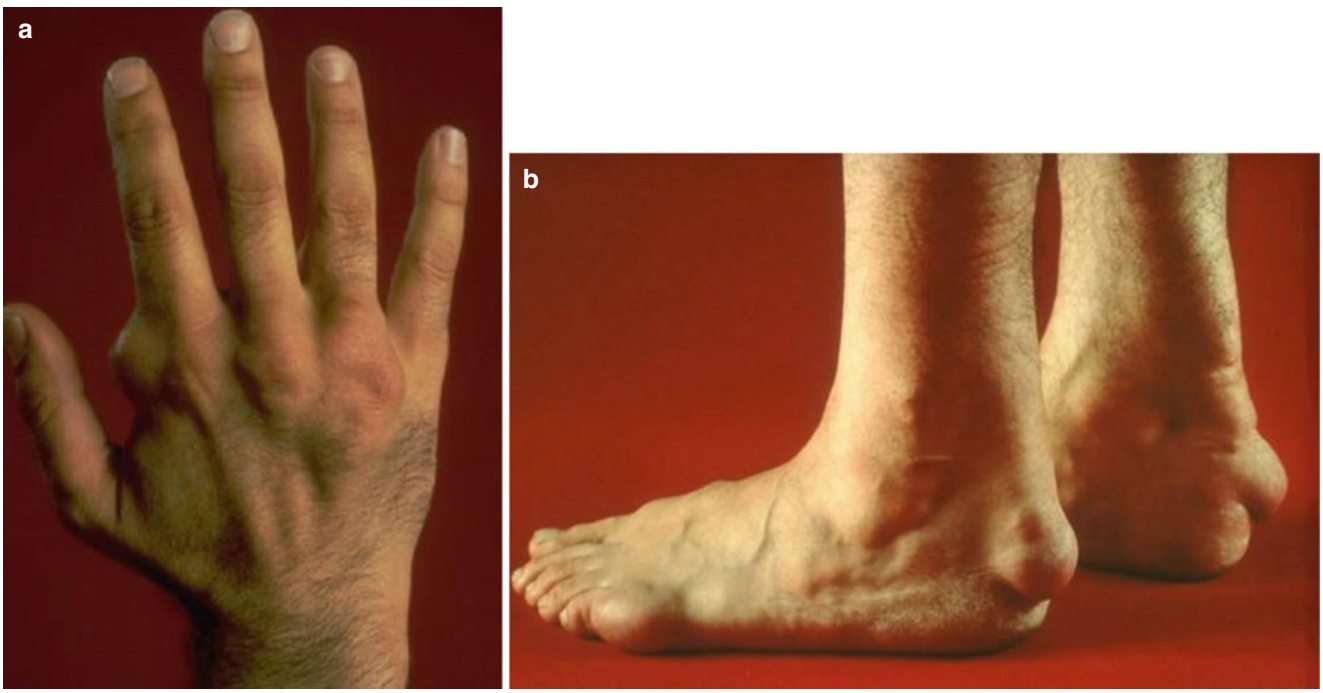

Fig. 21.2 Skin xanthomas (**a**) Xanthomas of the hand and (**b**) Achilles tendon xanthomas

Although cardiovascular events are rare in children heterozygous for FH, affected children were already shown to have an impaired endothelial function [35], as well as an increased *carotid intima-media thickness (cIMT)* [37], when compared to their unaffected siblings, which is indicative for an early onset of subclinical atherosclerosis. Based on these findings, current guidelines advise early pharmacological cholesterol-lowering in children with FH, as discussed below.

Diagnosis

FH is usually diagnosed on the basis of clinical features. Several clinical tools have been developed, with different diagnostic criteria, some of which combined with DNA analysis (reviewed in Reference ([38])). In the Netherlands, the algorithm of the Dutch Lipid Network is used, as shown in Fig. 21.4. The primary clinical diagnostic criteria are elevated LDL-C levels above the 95th percentile for age and gender, the presence of tendon xanthomata in the patient or a

Family history*	
I First-degree relative with CVD < 60 years of age	1
II First-degree relative with plasma LDL-cholesterol levels > 5 mmol/l	
III First-degree relative with an corneal arcus < 45 years and/or tendon xanthomas IV Children < 18 years of age with plasma LDL-cholesterol levels > 3.5 mmol/l	2
Personal Medical History	
I First-degree relative with CHD < 60 years of age	2
II Cerebro-vascular event or peripheral arterial disease < 60 years of age	1
Physical Examination	
I Presence of tendon xanthomas	6
II Presence of corneal arcus < 45 years of age	4
Laboratory parameters	
I LDL-cholesterol > 8.5 mmol/l	8
II LDL-cholesterol 6.5 -8.4 mmol/l	5
III LDL-cholesterol 5.0 -6.4 mmol/l	3
IV LDL-cholesterol 4.0 -4.9 mmol/l	1
FH DIAGNOSIS	
Almost certain score = 8 Likely score= 6-7 Possible score= 3-5	
Additional DNA testing is advised if the score > 6	

* In this category, only the highest applicable number should be scored; the highest score for family history is 2.

Fig. 21.4 Diagnostic algorithm for familial hypercholesterolemia

first-degree relative, and a pattern of autosomal dominant inheritance of premature coronary heart disease or hypercholesterolemia. The diagnosis can be confirmed through genetic testing of the *LDLR, APO-B, and PCSK9* genes. However, careful selection of patients is important given the costs of genetic analysis. Although the current yield is relatively low (between 54 % and 70 %), tools to aid physicians in the decision for referral are currently being developed [39].

Early diagnosis – preferably during childhood – of FH is recommendable to enable prompt treatment. Nationally organized genetic cascade-screening programs have been implemented in the Netherlands, Spain, and Wales, in addition to initiatives on a smaller scale in various other countries [40].

Management

Treatment with *high-dose statins* is currently the most effective strategy to reduce CVD risk in FH patients [41]. Lowering LDL-c levels remains the primary target for therapy. Recent studies have confirmed that the inverse relationship between LDL-c and CVD risk persists even at very low LDL-c levels, without apparent adverse effects. In response, updated clinical guidelines no longer advocate the use of LDL-c treatment targets but instead argue for treatment with high-intensity statins from the start of therapy [42]. In addition, patients are treated in combination with lifestyle modifications aimed to reduce the risk of other atherogenic factors. Finally, several new pharmacological agents have been developed to optimize cholesterol-lowering treatment in those who do not reach acceptable LDL-C levels or who are unable to tolerate high doses of statins. However, current guidelines do not recommend the routine use of nonstatin drugs in addition to high-intensity statin therapy.

PCSK9 inhibition represents the latest advancement in cholesterol-lowering drugs [43]. Although several approaches to PCSK9 inhibition are currently being developed and evaluated in clinical studies, monoclonal antibodies are most advanced in clinical development. By virtue of their ability to prevent lysosomal degradation of the LDL receptor, PCSK9 inhibitors increase the available pool of hepatic LDL receptors. Recent meta-analyses [44, 45] of clinical trials comprising >10,000 individuals have unequivocally demonstrated the efficacy of PCSK9 inhibitors in improving lipid profiles: mean LDL reductions were observed of 50 %, whereas HDL increased by 6 % and Lp(a) decreased by 26 %. Preliminary evidence suggests that these improved lipid profiles translate into improved CVD outcomes, and large-scale clinical outcome trials are currently ongoing to substantiate these findings. Importantly, PCSK9 inhibitors have shown to be generally safe and well tolerated. Based on these promising results, both the Food and Drug Administration (FDA) and European Medicines Agency (EMA) have approved alirocumab and evolocumab for several categories of patients including hetero- and homozygous FH patients who fail to meet LDL goals as well as statin-intolerant patients.

Ezetimibe is a cholesterol absorption-inhibiting compound, which acts by blocking the intestinal NPC1L1 protein. As of yet, ezetimibe is the only nonstatin drug which has been shown to improve clinical outcomes when added to statins in clinical trials (Improve it). These results were somewhat surprising, given that earlier studies, using surrogate end points by ultrasound (ENHANCE), did not find a benefit in terms of primary end points [46].

Bile acid sequestrants (BASs) bind bile acids in the intestine and subsequently increase hepatic conversion of cholesterol into bile acids. The resulting decrease in hepatic cholesterol content results in increased hepatic LDL-R expression. *Colesevelam* is a novel BAS with a more favorable side-effect profile, as it is thought to bind with higher affinity compared to other BAS [47]. It is currently being evaluated in patients with FH with higher than acceptable LDL-C levels, despite a maximally tolerated and stable dose of statin and ezetimibe. Although Colesevelam effectively lowered LDL-C levels, to date no data on clinical end points are available [46]. Moreover, it showed adverse effects similar to other BASs, including increased serum triglyceride levels and reduced intestinal uptake of several drugs, which means that these drugs should be taken more than 4 h before Colesevelam [46].

Selective inhibition of apo-B100 mRNA synthesis by *antisense oligonucleotides (ASOs)* is an entirely new approach to lower cholesterol levels. ASOs bind to a complementary mRNA sequence by Watson-Crick hybridization, resulting in selective degradation of the targeted mRNA sequence and thereby in a reduction in apo-B100 synthesis. The drug is administered subcutaneously once a week or less and induces an approximately 50 % LDL-C reduction in FH [48]. The most common adverse events are mild injection-site reactions, as well as modest increases in liver enzymes, as seen with all other lipid-lowering drugs. Mipomersen is an ASO that in 2013 was approved by the FDA for use in patients with homozygous FH (HoFH), although it is still not approved in Europe. It lowered LDL-C with 25 % in patients already receiving lipid-lowering drugs, while also reducing Apo-B and Lp(a) and having no effect on HDL-C levels. Benefits in terms of clinical outcomes remain to be confirmed in clinical trials. Side effects, including flu-like symptoms and injection-site reactions, as well as adverse hepatic effects, could reduce compliance [46].

Gene therapy is currently under investigation as treatment option in HoFH. While the first trial in the early 1990s showed disappointing results, new advances paved the road to new trials. In gene therapy, patients are being treated with a recombinant adeno-asssociated virus (AAV) vector loaded with a functional transgene, for example, an LDL-R expressing transgene. The newest vector AAV8 is of interest because of its strong liver tropism and relatively low seroprevalence in Western populations, which is important because of immune responses. Clinical testing in mice showed promising results, with a total cholesterol of 227 versus 1032 mg/dL at day 56 post-vector administration. Metabolic effects were

maintained for up to 20 weeks. Preliminary clinical trials with HoFH patients are currently ongoing. Of note, an AAV1 vector expressing LPL was the first gene therapy agent approved in the Western world to treat LPL-deficient patients (reviewed below) [49].

Lomitapide is an oral small-molecule inhibitor of the microsomal triglyceride transfer protein (MTP), which facilitates the assembly of apo-B-containing lipoproteins. This leads to reductions in lipoprotein secretion and lowers LDL-C levels. After a single-arm, open-label, 78-week phase 3 trial including 29 patients, which showed promising results [50], Lomitapide was approved for treatment of HoFH. A larger registry (LOWER) was started in 2014 to evaluate the clinical long-term safety and effectiveness, including at least 300 patients for follow-up for at least 10 years [51].

Finally, with respect to treatment of *children with FH*, several statin trials have been performed over the past decade [52]. On the basis of these studies, showing that statin treatment lowers LDL-C safely and effectively in children with FH [52, 53], and a study demonstrating reduced cIMT progression in FH adolescents on statin therapy [54], current guidelines in the USA and Europe recommend initial statin treatment in children with heterozygous FH from the age of 10 years [33]. Another international workforce published a consensus-based guideline applicable for most patients, which advocates for lifestyle modifications and consideration of the use of statin monotherapy starting from the age of 8 years and the eventual addition of ezetimibe or a BAS from the age of 10 years. For patients with homozygous FH, treatment with statins should start as early as possible [55]. In the USA, pravastatin is approved from the age of 8 years, while in Europe rosuvastatin is approved from age 6 years. Ezetimibe is approved from age 10 years in both the USA and Europe. Pediatric trials of PCSK9 inhibitors are underway or planned. In the event of homozygous FH and rapidly progressive atherosclerosis, lomitapide and mipomersen should be considered, although both drugs yet have to be tested in children, especially when apheresis is not an option [56].

In addition to pharmacological treatment of hypercholesterolemia, management should also comprise screening of first-degree relatives (referral to review [33]).

Familial Defective Apolipoprotein B

Familial Defective Apolipoprotein B (FDB) is an autosomal dominant disorder, which resembles the clinical phenotype of FH. The mechanism underlying the hypercholesterolemia is defective binding of apo-B100 of the LDL particle to the LDL receptor. The estimated prevalence is 1:500 in Central Europe and 1:700 in Northern America. Due to founder effects, prevalence up to 1:200 was observed in certain regions of Europe [57]. However, the exact prevalence remains unknown, since it frequently overlaps with that of FH, Therefore, the prevalence of the phenotype of FH due to mutations in *LDLR, PCSK9, or Apo-B100* is around 1:250.

Genetics

FDB is caused by mutations in the *apo-B100* gene located on chromosome 2p23–24. So far, 11 functional mutations at the *apo-B* locus have been identified. The R3500Q mutation is the most frequent one, with a prevalence of 1:600–700 in Caucasians [32].

Clinical Characteristics, Diagnosis, and Management

FDB is clinically indistinguishable from FH [58], although with slightly lower LDL-C levels [59]. FDB is diagnosed by genotyping or according to clinical diagnostic criteria for FH. Equal to FH, patients with FDB are treated with lipid-lowering medication combined with lifestyle modification.

Autosomal Recessive Hypercholesterolemia

ARH is the single known recessive disorder causing hypercholesterolemia. Only about 50 individuals with ARH have been identified worldwide, although the disease is not uncommon on the island of Sardinia with a frequency of 1:40.000 for homozygotes and compound heterozygotes and even 1:143 for heterozygotes [60]. In ARH, hepatic endocytosis of the LDL-R/LDL particle complex, mediated by the *LDL-R-adapting protein (LDLRAP)* is disrupted [27, 61].

Genetics

To date, 17 mutations in the *ARH* gene, located on chromosome 1p35–36.1, have been identified, the great majority being truncating mutations [26].

Clinical Characteristics

ARH is characterized by a phenotype, which resembles homozygous FH, consisting of severe hypercholesterolemia, large xanthomas, and premature CVD, although the phenotype in ARH is slightly milder, since patients tend to have higher HDL-C levels and are more responsive to lipid-lowering therapy and express a longer event-free survival when compared to homozygous FH patients [62]. The presence of residual LDL-R activity, as demonstrated in skin fibroblasts, might explain the favorable plasma cholesterol concentrations and the response to cholesterol-lowering medication in patients with ARH [63]. In general, no clinical symptoms before the age of 20 years are present in ARH. Heterozygous carriers of the *ARH* mutation have slightly elevated lipid levels within the normal range.

Diagnosis

ARH is diagnosed by genetic testing. Affected individuals meet clinical criteria for homozygous FH, as described in

section, "Clinical Characteristics"; however, based on the clinical evaluation of first-degree relatives, a lipid disorder of recessive origin, rather than homozygous FH, should be considered.

Management

Patients with ARH are sensitive to treatment with statins and a cholesterol-lowering diet [64]. A case study of a Sardinian patient treated with rosuvastatin 60 mg combined with ezetimibe showed an 81 % reduction of LDL-C. Although it is just one case, it is in line with another Lebanese patient being treated with rosuvastatin 80 mg and ezetimibe, showing a reduction of even 90 % [60].

Familial Combined Hyperlipidemia

Familial combined hyperlipidemia (FCH) is a relatively common lipoprotein disorder, with a prevalence of 1:200. The disease is based on increased *VLDL synthesis*, due to an overproduction of apo-B100, sometimes combined with delayed hepatic clearance of VLDL [65].

Genetics

Initially, FCH was considered an autosomal dominant monogenetic disorder; however, the hereditary background might be *polygenetic*, as a handful of families display a convincingly autosomal dominant mode of inheritance, whereas in others, a multifactorial basis is considered to be more likely. FCH might be best conceptualized as a phenotype with a common clinical presentation but with variable predisposing causes. Rare large-effect mutations are found in a fraction of patients, while multiple independently segregating small-effect mutations accumulate in a patient's genome, thereby raising LDL-C and triglycerides further [66].

Clinical Characteristics

FCH is phenotypically heterogeneous and, in most individuals, not manifest until adulthood. It is characterized by elevated LDL-C and/or triglyceride levels, a tendency to decreased HDL-C levels, and is often accompanied by central obesity, insulin resistance, and hypertension. In addition, there are no clinical stigmata such as in FH. Different phenotypes can be expressed within members of one affected family. In most cases, apo-B100 levels are elevated above 1.2 g/L and plasma triglycerides are mildly to moderately increased; however, cholesterol and triglyceride levels can vary over time within affected individuals. FCH patients have an increased risk of premature CVD [67].

Diagnosis

FCH is diagnosed based on a combination of plasma lipid abnormalities and a positive family history of dyslipidemia: either solitary elevated LDL-C, triglycerides or both, with or without premature CVD in the index patient or family members. A nomogram has been developed to aid physicians in estimating the probability of FCH based on clinical criteria [68].

Management

Untreated FCH patients are prone to premature CVD; therefore, aggressive lipid-lowering treatment equal to that for FH is required. Most FCH patients are treated with high-dose statins, with a target LDL-C of 2.5 or 1.8 mmol/L in subjects with CVD. In case triglyceride levels are elevated as well, patients can be treated with a *fibrate* on top of the statin, but not Gemfibrozil, since that combination increases the risk of rhabdomyolysis [69]. In addition, FCH patients should be treated with lifestyle modification, also in order to target the accompanying symptoms of obesity, insulin resistance, and hypertension. Finally, since FCH patients have functioning LDL receptors, the response to dietary interventions and pharmacological cholesterol lowering is generally better than observed in FH.

Sitosterolemia

Sitosterolemia is a rare autosomal recessive disorder characterized by premature atherosclerosis. Although hypercholesterolemia is not obligatory, elevations in LDL-C levels may be observed. The underlying defect in sitosterolemia is hyperabsorption of *plant sterols* and *sterols* and decreased biliary secretion of both cholesterol and plant sterols [70]. Plant sterols are structurally similar to cholesterol and are derived solely from the diet. Normally, plasma plant sterol levels in humans are extremely low due to active efflux, as achieved by the *ABCG5/G8 transporters*. In sitosterolemia, this mechanism is disrupted. The exact prevalence is unknown; approximately 50 cases have been described worldwide.

Genetics

The *ABCG5* and *ABCG8* transporter genes are arranged in a head-to-head configuration on chromosome 2p21 [71]. Mutations in either the *ABCG5* or the *ABCG8* gene can cause sitosterolemia [70, 72]. All of the missense mutations in either *ABCG5* or *ABCG8* studied to date either prevent the formation of the obligate heterodimer or block the efficient trafficking of the heterodimer to the plasma membrane [16].

Clinical Characteristics

Sitosterolemia is characterized by xanthomas, arthralgias, anemia, and premature atherosclerosis [73]. Plasma cholesterol levels are not necessarily elevated; however, affected individuals are highly sensitive to dietary cholesterol and

become markedly hypercholesterolemic when fed a high-cholesterol diet [74].

Diagnosis

The disease should be suspected in patients who develop xanthomas in early childhood, despite normal or only moderately elevated plasma cholesterol concentrations. Sitosterolemia can be diagnosed by genetic analysis or by plasma plant sterol levels exceeding 0.024 mM (1 mg/dL).

Management

Affected individuals should be restricted from a cholesterol- and plant sterol-rich diet, as well as from plant sterol-enriched food products. In addition, subjects benefit from treatment with ezetimibe, a cholesterol absorption inhibitor, which also inhibits intestinal absorption of plant sterols [75], alone or combined with a BAS [76]. Statins are not effective in sitosterolemia.

Genetic Causes of HDL-C Disorders

Disorders of HDL-C identified in humans may result from interaction between genetic and environmental factors. Plasma HDL-C levels are under strong genetic influence, with heritability estimates ranging between 40 % and 60 % [77]. Several monogenetic defects in various proteins involved in HDL metabolism have been identified in humans to date. The genes encoding apolipoprotein-AI, ABCA1, and LCAT are essential for the de novo synthesis of HDL. A complete lack of any of these factors confers severe HDL deficiency, which is referred to as familial hypoalphalipoproteinemia. By contrast, CETP deficiency mostly induces accumulation of HDL in the circulation, the so-called hyperalphalipoproteinemia. However, the vast majority of cases with HDL-C deficiency, defined as an age- and sex-adjusted plasma HDL-C concentration below the tenth percentile, are polygenic and/or multifactorial in origin. Decreased HDL levels are often found in patients with genetically disturbed metabolic pathways such as hypertriglyceridemia, diabetes mellitus type 2, and obesity and metabolic syndrome [78]. In addition, multiple other factors have been identified to negatively influence HDL-C levels, such as smoking, physical inactivity, anabolic steroids, and certain medication or diseases such as rheumatoid arthritis and systemic lupus erythematosus [79].

The first step in the diagnostic workup of HDL deficiency consists of exclusion of these underlying conditions. Patients with a virtual absence of HDL must undergo careful physical examination to unravel the clinical characteristics of certain HDL deficiency syndromes as described below. In addition, family studies should be initiated to show segregation of low HDL in the family. Definitive diagnosis requires specialized biochemical tests and the demonstration of a functionally relevant mutation in an HDL gene [78].

To date, no routinely used drug is able to increase HDL-C in patients with specific familial HDL deficiency syndromes so that the prevention of CVD in these patients must be focused on the avoidance and treatment of additional risk factors. In general, several lifestyle and pharmacologic interventions have shown to modestly increase HDL, although the impact of these interventions on the functional quality of HDL is unclear. Lifestyle modifications such as weight reduction, exercise, and smoking cessation can increase HDL levels by approximately 10–15 %. In addition, pharmacologic therapies with niacin, fibrates, and statins, alone or in combination, raise HDL. Niacin therapy is the most effective pharmacological agent currently available and results in significant HDL increases of 15–35 %. Several mechanisms have been suggested, although it is not exactly clear how niacin raises HDL. The most common reason for treatment failure is inability to tolerate cutaneous flushing. This can be reduced by prescribing the long-acting form, or by administering premedication with aspirin, or may diminish spontaneously after several days of therapy, as patients develop tolerance. Niacin used as monotherapy has shown benefit with regard to CHD risk reduction [80]. However, two recent clinical trials (AIM-HIGH, ([81]) HPS2 THRIVE [82]) in which niacin was added to statins in patients with established CVD and well-controlled LDL failed to confirm the clinical benefit in terms of CVD prevention, despite markedly increasing HDL levels. Fibric acid derivatives increase the synthesis of apo-A1, enhancing the formation of new HDL particles and raising HDL by 5–20 %, with the largest increases seen in patients with hypertriglyceridemia. Triglycerides are reduced by 20–50 %, but LDL is changed minimally, if at all, and are sometimes increased. To date, trial results are mixed, with two trials reporting a significant reduction in their primary outcome, while three others did not. Overall, patients with high triglyceride and low HDL cholesterol seem to benefit from fibrates, but it is debatable whether this is due to an increase in HDL concentration [83].

Current treatment guidelines do not recommend specific HDL treatment goals, because it remains to be determined whether pharmacologically increasing HDL will translate into clinically meaningful CVD risk reduction [84]. However, promising new agents, which target both quantity and quality of HDL particles, are currently under development including CETP inhibitors, apo-A1 and HDL mimetics, intravenous apo-A1 (Milano) infusion, and agonists of PPAR-alpha, LRH-1 and LXR [85]. CETP inhibitors, like torcetrapib, dalcetrapib (JTT- 705), evacetrapib and anacetrapib, are powerful HDL raisers. However, all CETP inhibitor clinical trials have failed [86]. A phase III trial on anacetrapib is underway and should be completed in 2017 [83].

Table 21.1 Clinical hallmarks of familial HDL deficiency syndromes [78]

Apo-A1 deficiency		Tangier disease	Fish-eye disease	Familial LCAT deficiency
Affected gene	*APO-A1*	*ABCA1*	*LCAT*	*LCAT*
Enlarged tonsils	No	Occasionally	No	No
Hepato/splenomegaly	No	Occasionally	No	No
Neuropathy	No	Occasionally	No	No
Corneal opacities	+++	+	+++	+++
Xanthomas	Occasionally	No	No	Occasionally
Xanthelasma	Occasionally	No	No	No
Nephropathy	No	No	No	Yes
Hemolytic anemia	No	No	No	Yes

In this section, we focus on the established monogenetic disorders of HDL metabolism including Apo-A1, ABCA1, and LCAT. Also genetic disorders of CETP will be discussed.

Apolipoprotein AI Deficiency

Apo-A1 is the major protein component of HDL-C in plasma and plays a central role in cholesterol efflux from tissues to the liver for excretion. Apo-A1 deficiency is a rare autosomal recessive inherited disorder characterized by decreased HDL-C levels.

Genetics

The *apo-A1* gene is located on the long arm of chromosome 11, adjacent to the genes encoding the apolipoproteins C-III and IV. Of the approximately 70 reported distinctive mutations of this gene, mostly found in heterozygous state, some are functionally relevant, that is, are associated with reduced levels of apo-A1 and HDL-C [87].

Clinical Characteristics

Heterozygous carriers of a functionally relevant mutation usually present with half normal apo-A1 and HDL-C levels. Some mutations can even lead to more pronounced decreases. In most cases, heterozygous carriers of apo-A1 variants do not present with specific clinical symptoms. Important exceptions are some structural apo-A1 variants with amino acid substitutions in the N-terminus, which have been detected in patients with familial amyloidosis [88]. Surprisingly, susceptibility for premature coronary heart disease has been shown to differ markedly between apo-A1 variants. Low HDL-C levels due to heterozygosity for a specific apo-A1 mutation (p.L178P) were associated with vascular dysfunction, accelerated carotid arterial wall thickness, and an increased incidence of premature vascular events compared with their family controls [89]. By contrast, despite very low HDL levels, carriers of the *apo-A1* (p.R173C) Milano mutant did not differ from controls in terms of vascular function [90] and arterial wall thickness [91]. These differences are likely due to the profoundly different effects of the mutations at the protein level.

Patients with complete apo-A1 deficiency, due to homozygosity or compound heterozygosity, present with a virtually absent HDL-C. In adult patients, variable clinical manifestations have been described, such as abnormalities of the skin (xanthelasma and xanthomas) and/or eyes (corneal opacities) [78] (see Table 21.1). Remarkably, only 11 of the 25 reported cases with complete apo-A1 deficiency suffered from premature cardiovascular events. However, the remaining 14 cases were almost all below the age of 50 and may have been too young for clinical manifestations of atherosclerosis to occur. In addition, this small number of cases and differences in the type of apo-A1 gene defect makes conclusions on the susceptibility to premature coronary heart disease in these specific patients difficult [92]. Mendelian randomization studies suggest no relationship between HDL-C levels and CAD [93].

Diagnosis

The diagnosis of apo-A1 deficiency requires sequencing of the apo-A1 gene and the demonstration of a functionally relevant mutation.

Management

Since no routinely used drug is able to increase HDL-C levels in patients with familial low HDL cholesterol, the prevention of CVD in these patients must be focused on the avoidance and treatment of additional risk factors and the use of statins to obtain very low LDL-C levels [78].

ABCA1 Deficiency and Tangier Disease

ABCA1 mediates the efflux of cholesterol and phospholipids from peripheral tissues to lipid-poor apo-A1 in plasma and thereby plays a central role in forming HDL. Functionally relevant mutations in the *ABCA1* gene lead to cholesterol efflux defects, which subsequently cause low HDL-C and apo-A1

levels. Complete ABCA1 deficiency is the underlying cause of *Tangier disease*. This rare autosomal recessive disorder has been diagnosed in about 70 patients worldwide.

Genetics

The *ABCA1* gene resides on chromosome 9q31. To date, more than 90 mutations and several common and rare variants have been described in the *ABCA1* gene, with a wide range of biochemical and clinical phenotypes [94]. Several recent genome-wide association studies have identified common variants in *ABCA1* as a significant source of variation in plasma HDL cholesterol levels across multiple ethnic groups [95, 96] establishing *ABCA1* as a major gene influencing HDL levels in humans [97].

Clinical Characteristics

Heterozygote carriers of functionally relevant *ABCA1* mutations can present with a broad range of plasma HDL-C levels ranging from 30 to 83 % of age- and sex-matched controls [97]. However, the majority of these mutations are associated with an approximately 50 % reduction in serum HDL-C and apo-A1 levels and increased triglycerides. LDL levels are typically within the normal range.

Tangier disease, which is caused by complete ABCA1 deficiency due to homozygosity or compound heterozygosity, is characterized by profoundly decreased HDL-C plasma and apo-A1 levels. Frequently, serum levels of total and LDL cholesterol are also low, whereas serum levels of triglycerides are mildly elevated. The clinical presentation of Tangier disease varies considerably and if present clinical symptoms can be isolated or combined (see Table 21.1). It is likely that this phenotypic heterogeneity might at least in part be accounted for by the nature of the mutation and its effect on the protein [98]. Presenting features of Tangier disease include enlarged orange tonsils, hepatomegaly, and splenomegaly. Also, lymph nodes can have bright yellow streaks and morphologic characteristics as those present in the tonsils. A symptom with significant implications for quality of life is a peripheral neuropathy, which, however, has a highly variable expression. These clinical symptoms result from the accumulation of cholesterylesters in reticuloendothelial cells, that is, macrophages, Kupffer cells or histiocytes, leading to the accumulation of these cells in various organs [78]. Despite the known role of ABCA1 in determining plasma HDL levels, the impact of ABCA1 on atherosclerosis remains controversial and incompletely understood [99]. Prior to the identification of *ABCA1* mutations as the genetic basis of Tangier disease in 1999, patients were identified based on their clinical phenotype, that is, extremely low HDL cholesterol in homozygotes, with the offspring and parents of homozygotes being obligate heterozygotes. Considering the wide variation in phenotype, misclassification of patients was likely and this complicated accurate CAD risk estimation. Since the assignment of disease has been based on genotype, allowing a more unambiguous diagnosis, several studies have addressed the risk for CAD in these patients. Large family studies, studying several mutations, showed a more than threefold excess of CAD and increased carotid arterial wall thickness in affected family members when compared to unaffected members [100, 101]. In both studies, levels of cholesterol efflux correlated well with HDL-C levels and there was a strong correlation between levels of cholesterol efflux and CAD and/or carotid arterial wall thickness. However, these family studies potentially suffer from selection bias, as only families with the most severe phenotypes may have presented at clinics. Also, CAD risk estimates were based on few individuals and were not adjusted for age and other cardiovascular risk factors. Bypassing these problems, seven different *ABCA1* mutations were studied in two different population cohorts and a large case-control study, including a total of 109 heterozygotes, 6666 ischemic heart disease cases, and a total of 41,961 participants [102]. Four mutations were found to be associated with an average of 30 % reduction in HDL-C and decreased cholesterol efflux. Carriers of these four mutations, however, did not display an increased risk of CVD. However, this conclusion should also be interpreted with caution as the variants studied were mild mutations, giving relatively small reductions in HDL cholesterol levels and cholesterol efflux [103]. The findings are also conflicting with several reports showing that common genetic variations of the ABCA1 gene influence the risk of CAD in the general population [102, 104, 105]. Interestingly, these associations with atherosclerosis are independent of effects on HDL levels. These findings, of an altered risk for CAD but without corresponding differences in lipid levels, suggest that although ABCA1 may be an important atherosclerosis susceptibility locus, the mechanism by which it exerts this effect is not necessarily by altering steady-state HDL-C levels. In conclusion, any specific *ABCA1* variant must be considered in relation to its impact on protein function, as different variants will have different effects on HDL and susceptibility to atherosclerosis [106].

Diagnosis

The findings of virtually absent HDL-C and low levels of apo-A1 are not sufficient to diagnose Tangier disease, which ultimately requires *ABCA1* gene sequence analysis. Cholesterol efflux defects can be demonstrated with the cholesterol efflux assay on cultivated skin fibroblasts. However, even in the absence of coding sequence mutations in *ABCA1*, cellular cholesterol efflux defects are a common feature in subjects with low HDL [107]. Foam cell formation, responsible for the clinical symptoms in Tangier disease, can be detected in the rectal mucosa by endoscopic examination as pale mucosa with studded 1–2-mm discrete orange brown spots [78].

Management

To date, no specific treatment for Tangier disease exists. It is advised to identify and tightly regulate modifiable cardiovascular risk factors and possibly institute statin therapy as a means to drive LDL-C levels down even further.

Familial LCAT Deficiency and Fish-Eye Disease

Lecithin: cholesterol acyltransferase (LCAT) plays a key role in the maturation of small HDL by means of esterification of free cholesterol, primarily at the surface of the HDL particle (the so-called alpha-LCAT activity) but also on lipids transported by apo-B-containing lipoproteins (the so-called beta-LCAT activity). After esterification, the CE molecules migrate to the inner core of the lipoprotein, promoting further cholesterol efflux from peripheral tissues and leading to larger, cholesterylester-enriched HDL particles. Mutations in the *LCAT* gene causing LCAT deficiency represent another rare autosomal recessive disorder that underlies HDL deficiency. Low HDL-C values result from defective HDL maturation followed by rapid clearance of nascent HDL particles from the circulation. Depending on the mutation, patients with complete LCAT deficiency present with one of the two clinical phenotypes, *familial LCAT deficiency* (FLD) or *fish-eye disease* (FED).

Genetics

The gene encoding *LCAT* is located on chromosome 16, locus 16q22.1. Mutations in *LCAT* account for approximately 4 % of low HDL [107]. Thus far, over 80 mutations in the *LCAT* gene have been described in reports that predominantly investigated single cases or small nuclear families [92].

Clinical Characteristics

Heterozygous carriers of *LCAT* mutations lack clinical symptoms, although frequently they present with half normal HDL-C levels and mild hypertriglyceridemia [92]. Homozygous or compound heterozygous patients with mutations in the *LCAT* gene present with one of two clinical phenotypes, familial LCAT deficiency or fish-eye disease. In FLD, both alpha-LCAT, which is specific for HDL, and beta-LCAT, which is specific for VLDL and LDL, are deficient, that is, the deficient esterification is generalized. By contrast, patients with FED have a selective alpha-LCAT deficiency. Because LCAT is still partly active, these patients have, in general, a less severe phenotype. Both FLD and FED are characterized by corneal opacifications, which become apparent after the third decade (see Table 21.1). In addition, FLD is characterized by hemolytic anemia, and the deposition of foam cells in bone marrow, spleen, and particularly in kidneys. Progressive renal disease, with proteinuria and hematuria, which progresses to terminal renal insufficiency, has been described in a high percentage of these patients [65] (see Table 21.1). Biochemically, FLD and FED are both characterized by variable loss of LCAT activity and *HDL deficiency* (5–10 % of normal HDL-C levels). Serum levels of apo-A1 are usually decreased but not as low as in patients with apo-A1 deficiency or Tangier disease. Additionally, hypertriglyceridemia is observed [92].

The association between *LCAT* gene mutations and atherosclerosis is still controversial, both because of the limited number of carriers and because of variable results in the studies performed. A 25-year follow-up of nine heterozygote family members [108], as well as a large family study, including 68 carriers of *LCAT* defects of which 59 heterozygotes and 74 family controls [92] which measured carotid arterial wall thickness indicated that heterozygous carriers of *LCAT* defects may have an increased risk of atherosclerotic vascular disease. Another study including 45 carriers of *LCAT* mutations reported an increase in aortic pulse wave velocity with both ultrasound and MRI, indicating increased arterial stiffness and carotid wall thickening [109]. However, a study including 540 carriers from the IMPROVE study who underwent ultrasound showed no increase in intima wall thickening [110].

Diagnosis

The identification of LCAT deficiency needs either genetic testing or measurement of LCAT activity. Depending on the kind of mutation, immunoassays of LCAT detect either no, or slightly reduced concentrations of LCAT protein in plasma. Routine lipid and lipoprotein analyses do not help to distinguish patients with FLD and FED. However, patients with FLD show an increased proportion of unesterified cholesterol in plasma (80–100 % instead of normal <30 %). By contrast, the plasma of patients with FED has a normal or slightly elevated (up to 70 %) unesterified cholesterol/cholesterol ester ratio [78].

Management

Only symptomatic treatments exist for LCAT deficiency. Because deposition of highly abnormal apo-B- containing lipoproteins in the kidneys of FLD patients has been implicated as the pathogenetic factor in the formation of renal disease, therapies that reduce the concentration of apo-B-containing lipoproteins (such as a fat-restricted diet and statins) are at least theoretically useful [78]. Recombinant LCAT therapy has been suggested as possible acute treatment for acute coronary syndrome [111]. The effects of long-term rLCAT therapy have not been investigated yet.

Genetic Disorders of CETP

As a regulator of cholesterol flux through the RCT system, CETP, may be viewed as potentially having both pro-atherogenic and antiatherogenic properties (see Fig. 21.1). By facilitating the exchange of cholesterylesters for triglycerides between HDL and Apo-B-containing lipoproteins (LDL and VLDL), CETP may decrease direct RCT via the HDL/hepatic SR-B1 route. In addition, pro-atherogenic effects of CETP activity may result from a reduction in overall HDL levels, potentially reducing cellular cholesterol efflux from the arterial wall, and from an increase in atherogenic LDL levels. However, the potentially pro-atherogenic activities of CETP may, to a large extent, be neutralized by an increase in indirect RCT via the LDL/hepatic LDL receptor route [112].

Genetics

CETP is encoded by a gene located on the long arm of chromosome 16. Several mutations of the *CETP* gene have been associated with altered CETP activity and HDL-c levels. Recent genome-wide association studies have reported that *CETP* genotypes are associated with HDL-C levels more strongly than any other locus across the genome [95, 96].

Clinical Characteristics

Significant differences between ethnic groups with regard to allele frequencies of *CETP* polymorphisms exist [113]. Particularly in Japan, *CETP* gene defects are common and there are appreciable numbers of individuals who are homozygous for mutations in the *CETP* gene. Not surprisingly, functional mutations of the *CETP* gene can produce significant changes in lipid and lipoprotein metabolism. Not all *CETP* gene mutations have an as-dramatic effect on CETP protein levels as the ones described above. Various single nucleotide polymorphisms of the *CETP* gene are associated with only small changes in plasma CETP levels and subsequently HDL-C levels, in either direction [113]. Consequently, the role of *CETP* mutations on cardiovascular risk profiles is complex. Although sparse, there is evidence emerging from clinical trials that elevated CETP levels, regardless of the cause, are associated with an increased risk of CVD [114–116]. However, studies on individuals with CETP protein deficiency, arising from different genetic mutations, have reported ambiguous findings on the relationship between CETP protein deficiency and CAD risk. In some studies, CETP-deficient patients were thought to have an increased CAD risk [117] but, conversely, this concept was not supported by others [118, 119]. In addition, a recent meta-analysis was published that involved a total of more than 113,000 individuals and six *CETP* polymorphisms [120]. Three common *CETP* gene variants (TaqIB, I405V, and −629C > A) were consistently associated with a decreased CETP concentrations, modestly increased HDL-C and apo-A1 levels, and weakly decreased triglycerides and coronary risk. Data were insufficient for informative per-allele estimates in relation to three uncommon *CETP* variants (p. D442G, p.−631C > A, and p.R451Q). However, they were associated with mean differences in HDL-C of 13.4 %, −0.7 %, and −8.8 %, respectively, compared with controls. Thus, from the results of studies on individuals with CETP protein deficiency arising from genetic mutations, the relationship between CETP and the risk of CVD is not entirely conclusive. Overall cardiovascular risk is presumably dependent not only on the effect of CETP deficiency on overall levels of HDL-C but also on the effect on functionality of the HDL particles. Moreover, additional factors affecting the metabolic setting of the *CETP* gene mutation probably also play an important role. It was shown that high HDL-C resulting from simultaneous presence of *CETP*- and *LIPC* gene variants did not protect against CAD. By contrast, an increased risk for CAD was found in these patients [121]. In addition, high triglyceride levels have been suggested to enhance the effect of CETP concentration on CHD risk [116]. Also, potential joint effects of *CETP* genotypes with environmental determinants of HDL-C levels (e.g., exercise and alcohol) on the risk of coronary disease have been reported [122].

Genetic Causes of Elevated Triglycerides

Severely elevated *triglyceride* concentrations are a risk factor for developing *pancreatitis* and in the absence of other causes such as diabetes mellitus, alcohol abuse, chronic renal failure, or hypothyroidism, generally point to genetic disorders of triglyceride-rich lipoprotein-modulating enzymes or apolipoproteins. Mutations in several genes have been described, of which the *LPL*, *apo-CII*, and *Apo-E* genes are the most important ones. Very recently, the *GPIHBP1 gene* has been introduced as a contributor to *primary hypertriglyceridemia* [19, 123]. Two other new contributors described in a few families are the *APO-A5 gene* and the *LMF1 gene* [124]. On the other hand, loss-of-function mutations in apoC3 are associated with low levels of triglycerides and a reduced risk of CVD [13]. Next to the monogenic causes, polygenetic causes are also familial due to clustering of genetic mutations within families. Susceptibility then results from the accumulation of these mutations, because individual variants are insufficient to actually raise triglyceride levels significantly [124].

Regardless of its origin, the management of hypertriglyceridemia consists of therapeutic lifestyle changes aiming at dietary and weight control, as well as pharmacological treatment with fibrates, niacin, or high doses of fish oil, alone or in various combinations. In case triglyceride levels exceed

10 mmol/L (800 mg/dL), combinations of different drugs are usually required, in order to reduce the risk of pancreatitis [125]. The benefit of treating mild-to-moderate triglyceride elevations is less clear [126]. If hypertriglyceridemia is a comorbidity, statins can lower triglyceride levels by 20–40 % [127]. *Fibrates* lower triglyceride levels by approximately 40–60 % and modestly raise HDL-C levels by approximately 15–25 % [127]. Patients who do not respond to *fibrates* can be treated with *niacin*, which lowers triglyceride levels by 30–50 %, raises HDL-C levels by 20–30 %, and lowers LDL-C levels by 5–25 % [127, 128]. They are reviewed above. Fish oil with 2–4 g of *omega-3 fatty acids* daily can reduce triglyceride levels by 15–50 %, depending on the dosage and different formulations [129]. Moreover, few adverse effects have been reported, mostly gastrointestinal. Over-the-counter preparations usually contain far less than these required amounts [130]. The new drug *lomitapide* (reviewed above) has also been shown to reduce triglyceride levels up to 40 % [124]. Loss-of-function mutations of Apolipoprotein CIII (APOCIII) are associated with low levels of triglycerides and decreased incidence of CVD [13]. As a result, a new second-generation ASO inhibiting APOCIII is currently under investigation in phase 2 studies [131]. Of note, in patients with *diabetes mellitus*, optimizing glycemic control might help to lower triglyceride levels without additional medication for hypertriglyceridemia.

LPL Deficiency and Apo-CII Deficiency

Plasma LPL and its cofactor apo-CII are involved in the hydrolysis of triglyceride-rich particles such as chylomicrons and VLDL. Genetic *LPL deficiency* is a rare autosomal recessive disorder causing severe hypertriglyceridemia. Estimations of prevalence vary between 1:500,000 in the general population and 1:5000 in French Quebec. The incidence of apo-CII deficiency is even lower than that of LPL deficiency.

Genetics

The *LPL* gene is located on chromosome 8p22 [132]. More than 114 mutations have been described [124]. The *apo-CII* gene is located on chromosome 19, in which at least 13 mutations have been described [133].

Clinical Characteristics

Affected individuals have insufficient capacity to hydrolyze triglycerides, resulting in extremely high plasma triglyceride concentrations, often accompanied by recurrent episodes of *pancreatitis*. LPL deficiency typically manifests itself in early childhood with severe and repetitive colicky pain in the abdomen, acute pancreatitis, and failure to thrive. Eruptive xanthomas (Fig. 21.4), lipemia retinalis, and hepatosplenomegaly can also be present. Plasma is lipemic, reflecting increased plasma levels of both chylomicrons and VLDL. Loss-of-function mutations in *LPL* are associated with an increased risk of CVD, while gain-of-function mutations are protective [134–136]. To date, the only *apo-CII* mutation described to cause early atherosclerosis is the *apo-CII* St Michel mutation [137].

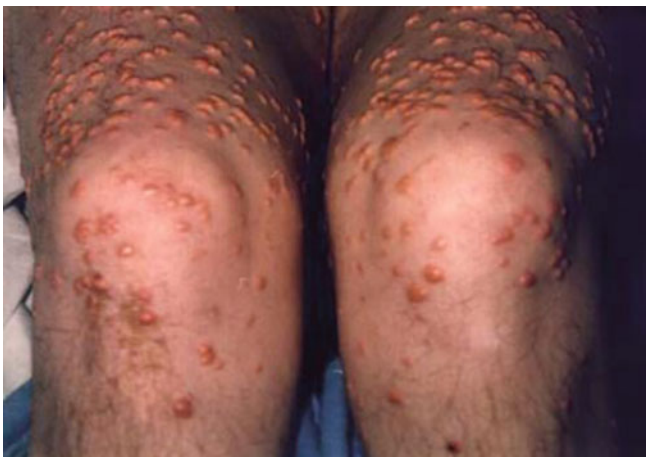

Fig. 21.5 Eruptive xanthomas

Diagnosis

Genetic *LPL* and *apo-CII deficiency* are diagnosed by genotyping, combined with the phenotype as described above. Apo-CII deficiency can also be diagnosed by a post-heparin LPL activity assay, in which the patient's post-heparin plasma is mixed with that of a nonaffected individual. In this experiment, triglyceride levels will decrease rapidly in an apo-CII-deficient patient, in contrast to subjects with LPL deficiency.

Treatment

Treatment consists of *dietary fat restriction*. Hypertriglyceridemia is treated as described above; however, in genetic *LPL* and *apo-CII* deficiency, most of these strategies do not result in a substantial reduction in triglyceride levels. Nevertheless, promising new compounds for the treatment of this patient group are under investigation, such as *ibrolipim*, a pharmacological stimulator of tissue LPL formation, *LPL gene therapy* [138], and *antisense apo-CIII therapy* [139]. *Alipogene tiparvovec* is an adeno-associated virus serotype 1-based gene therapy being the first gene therapy to be approved in the Western world. In clinical studies with 27 patients, it lowered plasma triglyceride levels for up to 26 weeks and after even 6 years of follow-up there were still clinically relevant reductions in the incidence of pancreatitis and acute abdominal pain events [140]. It is now approved for a small subset of patients with familial LPL deficiency suffering from recurrent severe pancreatitis under

a fat-restricted diet. However, clinical experience is limited and further research should be conducted to assess the long-term safety.

Familial Dysbetalipoproteinemia (Apo E2/E2 Deficiency)

Familial dysbetalipoproteinemia (FD) is characterized by the defective clearance of VLDL- and *chylomicron- remnant particles* caused by homozygosity for apoE2, the type of apoE unable to bind to its receptor. There are three common apoE isoforms: apoE3, apoE2, and apoE4 [141]. Although approximately 0.5 % of the population worldwide is homozygous for apoE2, only a small minority develops FD with an estimated prevalence of 1–2:10,000. This is due to the necessity of concomitant environmental, hormonal, and possibly genetic factors, inducing VLDL or chylomicron overproduction, such as a high caloric diet or alcohol abuse, diabetes mellitus, obesity, hypothyroidism, renal disease, or estrogen deficiency.

Genetics

Most people have an *apoE3/E3* genotype, with a ~55 % prevalence; however, *apoE4* and *apoE2* also exist, with an estimated frequency of 0.5 % for *apoE2/E2*, 15 % for *apoE2/E3*, 25 % for *apoE3/E4*, 1–2 % for *apoE4/E4*, and 3–4 % for *apoE2/E4*. *ApoE2* differs from *ApoE3* by a single substitution of cysteine for arginine at residue 158.

Less common, dominant-negative mutations may also cause the disorder (*ApoE3*-Leiden or *s*-Lys146 > Gln) [142].

Clinical Characteristics

Clinically, apoE2/E2 patients present with *tubero-eruptive xanthomas* (see Fig. 21.5), palmar streaks, elevated TC, and triglyceride concentrations, and are at a high risk for premature CVD and peripheral vascular disease [143]. Tubero-eruptive xanthomas begin as clusters of small papules on elbows, knees, or buttocks and can grow to the size of small grapes. Palmar xanthomas are orange yellow discolorations of palm and wrist creases. Both are pathognomonic for FD, but their absence does not exclude the disorder. Plasma TC concentrations usually exceed 8.0 mmol/L (300 mg/dL) and may approach 26.0 mmol/L (1000 mg/dL).

Triglyceride concentrations are within the same range. Dyslipidemia in FD rarely manifests before adulthood. The average age of clinically overt vascular disease is approximately 40 years in men and 59 in women.

Diagnosis

FD can be diagnosed either by lipoprotein ultracentrifugation and electrophoresis with a *VLDL/triglyceride ratio* >0.3 or by *apoE* genotyping. However, the absence of apoE2/E2 does not rule out the disease, as other genetic causes might also give rise to this trait.

Management

Treatment of FD is aimed at reducing the overproduction of VLDL and/or chylomicrons, by means of dietary restrictions, including alcohol intake and weight reduction, combined with pharmacological treatment with statins, alone or combined with other compounds, as described above. Recently, a European cross-sectional study including 305 patients from seven academic hospitals in four European countries found that the majority of FD patients have non-HDL-C levels above the threshold of 3.3 mmol/L. However, less than half of these patients were adequately treated, increasing their cardiovascular risk [144].

Familial Combined Hyperlipidemia

FCH is discussed in section, "Familial Combined Hyperlipidemia."

Familial Hypertriglyceridemia

Familial hypertriglyceridemia (FHTG) is a common disorder causing hypertriglyceridemia with the prevalence of 1:500. The genetic basis seems to be based on an accumulation of common and rare genetic mutations that increase susceptibility [145], and the onset of disease depends on the presence of certain lifestyle factors. FHTG is discussed due to its high prevalence. The metabolic defect is a combination of hepatic VLDL overproduction and decreased catabolism of both VLDL and chylomicrons.

Clinical Characteristics

Typically, patients have moderately elevated plasma triglycerides, 3–10 mmol/L, often accompanied by low HDL-C levels. FHTG is associated with obesity, insulin resistance, hypertension, and hyperuricemia. The onset of hypertriglyceridemia is usually in adult age, when lifestyle factors, which increase triglyceride levels, such as obesity, become more prominent. When the hypertriglyceridemia becomes more severe, the clinical picture can resemble that of LPL deficiency. The association with CVD is weak, at most.

Diagnosis

FHTG is diagnosed by exclusion of other causes of hypertriglyceridemia. A first-degree family member with the same disorder is useful for the diagnosis. FCH and FD should definitely be excluded, since these disorders are associated with a more pronounced CVD risk and therefore require a more stringent therapy.

Management

The first line of treatment is lifestyle modification, possibly combined with pharmacological treatment in case of more severe hypertriglyceridemia.

Finally, mutations in the recently identified *GPIHBP1 protein* might be a cause of severe hypertriglyceridemia, resembling LPL or apo-CII deficiency [19]. This protein is thought to anchor LPL on the luminal surface of capillaries, where lipolysis of triglyceride-rich particles takes place. At present, two mutations have been described in humans: the p.G56R [146, 147] and p.Q115P mutation [123], of which only the latter was proven to be causal. In addition, the *LMF1* and the *APO-A5* gene have also recently been identified as interesting candidates for severe *hypertriglyceridemia* [124, 148].

Summary

Disorders of lipoprotein metabolism are major contributors to CVD, a leading cause of mortality and morbidity worldwide. Dyslipidemia includes both elevated LDL-C levels, elevated triglycerides, and elevated remnant cholesterol, as well as decreased HDL-C levels.

LDL mediates cholesterol transport from the liver to peripheral tissues, including macrophages in the arterial wall, which, after uptake and accumulation of cholesterol, can transform into foam cells and atherosclerotic plaques. Conversely, HDL is thought to exert beneficial effects on the arterial wall through its role in the RCT, which involves the transport of cholesterol from peripheral tissues to the liver followed by biliary excretion and elimination via the feces.

The crucial role of increased plasma LDL-C levels in the pathogenesis of atherosclerosis has been firmly established, as well as the beneficial effects of LDL-C reduction accomplished by HMG-CoA reductase inhibitors or statins. In addition, decreased plasma HDL-C levels are an established independent predictor of CVD. However, pharmacological raising of plasma HDL levels has failed to reduce cardiovascular events thus far. It is therefore uncertain whether HDL plays a causative role in CVD protection or if it is merely an epiphenomenon or nonfunctional biomarker. Finally, the relationship between hypertriglyceridemia and CVD risk also remains to be elucidated.

Most cases of CVD are multifactorial and/or polygenic in origin. However, when CVD occurs at young age, a number of monogenetic disorders of lipoprotein are frequently seen. These monogenetic disorders of lipoproteins are the primary focus of this chapter.

Regarding LDL metabolism, mutations in four genes are currently identified to result in increased plasma LDL-C concentrations, namely the LDL-R gene, Apo-B gene, ARH gene, and most recently the *PCSK9 gene*. Clinical hallmarks of these disorders, of which familial hypercholesterolemia is the most frequent and well known, are elevated plasma LDL-C levels and, consequently, premature atherosclerosis.

Table 21.2 Underlying causes of dyslipidemias

Elevated LDL-C levels	Decreased HDL-C levels	Elevated TG levels
Hypothyroidism	Obesity	Obesity
Kidney diseases	Diabetes mellitus	Diabetes mellitus
Certain medications, e.g., Corticosteroids Thiazide diuretics	Metabolic syndrome Certain medication Underlying diseases, e.g., Rheumatoid arthritis Systemic lupus erythematosus	Metabolic syndrome Alcohol abuse Chronic renal failure Hypothyroidism

To date, several rare monogenetic defects in various proteins involved in HDL metabolism have been identified in humans. The genes encoding apolipoprotein-AI, ABCA1, and LCAT, respectively, are essential for the de novo synthesis of HDL. A complete lack of any of these factors confers severe HDL deficiency, which is referred to as familial hypoalphalipoproteinemia. By contrast, CETP deficiency mostly induces accumulation of HDL in the circulation. Although FHA patients display extremely low plasma HDL-C levels, the association of these genetic disorders with atherosclerosis is disputed. Since HDL is a heterogeneous class of lipoprotein particles, these different classes may have different associations with disease. Furthermore, the functionality of the HDL particles, rather than their abundance, may be an important determinant of their antiatherogenic effects.

Finally, severely elevated triglyceride concentrations can be induced by mutations in several genes, of which the *LPL*, *apo-CII*, and *Apo-E* genes are the most important ones. Despite the unclear role of hypertriglyceridemia in atherogenesis, severely elevated triglyceride levels confer a health risk due to the increased risk of pancreatitis.

In general, the vast majority of dyslipidemia are polygenic and/or multifactorial in origin.

The first step in the diagnostic workup of dyslipidemias consists of the exclusion of underlying conditions through careful medical history taking, physical examination, and biochemical testing (see Table 21.2). Suggestive for a genetic cause are the presence of specific clinical hallmarks (see text and Table 21.1) and/or the presence of familial dyslipidemias/premature atherosclerosis. In these cases, specialized biochemical tests and/or the demonstration of a functionally relevant mutation in the involved genes should be performed to obtain a definitive diagnosis. In addition, family studies should be initiated to evaluate the inheritance pattern of the phenotype (see Table 21.3).

Table 21.3 Steps in the diagnostic workup of dyslipidemias [65]

1. Exclude underlying conditions
2. Suspected genetic cause? Profoundly decreased HDL-C levels? (<fifth percentile adjusted for age and sex) Presence of specific clinical hallmarks? (see text and Table 21.1) Presence of familiar dyslipidemias/premature atherosclerosis?
3. Perform specialized biochemical tests and/or specific HDL gene sequencing
4. Initiate family studies

Treatment consists of lifestyle modifications such as weight reduction, exercise, and smoking cessation to improve other atherogenic risk factors, possibly in combination with pharmacological agents. High-dose statins are currently the most effective pharmacological strategy to reduce CVD risk. Also in case of low HDL and hypertriglyceridemia, statin monotherapy, possibly in combination with other agents, reduces the risk of CVD. However, it should be noted that many new therapies are becoming available that could change current treatment guidelines in the years to come.

References

1. Yusuf S, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet [Internet]. Elsevier BV; 2004;364(9438):937–52. Available from: doi:10.1016/s0140-6736(04)17018-9.
2. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet (London, England) [Internet]; 2005 [cited 2016 Apr 28];366(9493):1267–78. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16214597
3. Genest JJ, Martin-Munley SS, McNamara JR, Ordovas JM, Jenner J, Myers RH, et al. Familial lipoprotein disorders in patients with premature coronary artery disease. Circulation [Internet]. Ovid Technologies (Wolters Kluwer Health); 1992;85(6):2025–33. Available from: doi:10.1161/01.cir.85.6.2025.
4. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation [Internet]. Ovid Technologies (Wolters Kluwer Health); 1989;79(1):8–15. Available from: doi:10.1161/01.cir.79.1.8.
5. Briel M, Ferreira-Gonzalez I, You JJ, Karanicolas PJ, Akl EA, Wu P, et al. Association between change in high density lipoprotein cholesterol and cardiovascular disease morbidity and mortality: systematic review and meta-regression analysis. BMJ [Internet]. BMJ 2009;338(feb16 1):b92–b92. Available from: doi:10.1136/bmj.b92.
6. Ghali WA, Rodondi N. HDL cholesterol and cardiovascular risk. BMJ [Internet]. BMJ; 2009;338(feb16 1):a3065–a3065. Available from: doi:10.1136/bmj.a3065.
7. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk [Internet]. Ovid Technologies (Wolters Kluwer Health); 1996;3(2):213–9. Available from: doi:10.1097/00043798-199604000-00014.
8. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. JAMA [Internet]. American Medical Association (AMA); 2007;298(3):309. Available from: doi:10.1001/jama.298.3.309.
9. Nordestgaard BG, Benn M, Schnohr P, Tybjærg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA [Internet]. American Medical Association (AMA); 2007;298(3):299. Available from: doi:10.1001/jama.298.3.299.
10. Stauffer ME, Weisenfluh L, Morrison A. Association between triglycerides and cardiovascular events in primary populations: a meta-regression analysis and synthesis of evidence. Vasc Health Risk Manag. 2013;9(1):671–80.
11. Nordestgaard BG. Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease. Circ Res [Internet]. 2016;118(4):547–563. Available from: http://circres.ahajournals.org/lookup/doi/10.1161/CIRCRESAHA.115.306249
12. Article O. Coding variation in *ANGPTL4*, *LPL*, and *SVEP1* and the risk of coronary disease. N Engl J Med [Internet]. 2016;NEJMoa1507652. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1507652
13. Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjærg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. N Engl J Med [Internet]. 2014;371(1):32–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24941082
14. Neeli I, Siddiqi SA, Siddiqi S, Mahan J, Lagakos WS, Binas B, et al. Liver fatty acid-binding protein initiates budding of prechylomicron transport vesicles from intestinal endoplasmic reticulum. J Biol Chem [Internet]. American Society for Biochemistry & Molecular Biology (ASBMB); 2007;282(25):17974–84. Available from: doi:10.1074/jbc.m610765200.
15. Davies JP, Levy B, Ioannou YA. Evidence for a Niemann–Pick C (NPC) gene family: identification and characterization of NPC1L1. Genomics [Internet]. Elsevier BV; 2000;65(2):137–45. Available from: doi:10.1006/geno.2000.6151.
16. Graf GA, Cohen JC, Hobbs HH. Missense mutations in ABCG5 and ABCG8 disrupt heterodimerization and trafficking. J Biol Chem [Internet]. American Society for Biochemistry & Molecular Biology (ASBMB); 2004;279(23):24881–8. Available from: doi:10.1074/jbc.m402634200.
17. Temel RE, Tang W, Ma Y, Rudel LL, Willingham MC, Ioannou YA, et al. Hepatic Niemann-Pick C1–like 1 regulates biliary cholesterol concentration and is a target of ezetimibe. J Clin Invest [Internet]. American Society for Clinical Investigation; 2007;117(7):1968–78. Available from: doi:10.1172/jci30060.
18. Grundy SM. Absorption and metabolism of dietary cholesterol. Annu Rev Nutr [Internet]. Annual Reviews; 1983;3(1):71–96. Available from: doi:10.1146/annurev.nu.03.070183.000443.
19. Beigneux AP, Davies BSJ, Gin P, Weinstein MM, Farber E, Qiao X, et al. Glycosylphosphatidylinositol-Anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. Cell Metab [Internet]. Elsevier BV; 2007;5(4):279–91. Available from: doi:10.1016/j.cmet.2007.02.002.
20. Lambert G, Sjouke B, Choque B, Kastelein JJP, Hovingh GK. The PCSK9 decade. J Lipid Res. 2012;53:2515–24.
21. Tuteja S, Rader DJ. High-density lipoproteins in the prevention of cardiovascular disease: changing the paradigm. Clin Pharmacol Ther [Internet]. 2014 [cited 2016 May 1];96(1):48–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24713591
22. Lewis GF, Rader DJ. New insights into the regulation of HDL metabolism and reverse cholesterol transport. Circ Res [Internet].

Ovid Technologies (Wolters Kluwer Health); 2005;96(12):1221–32. Available from: doi:10.1161/01.res.0000170946.56981.5c.
23. Tall AR. Cholesterol efflux pathways and other potential mechanisms involved in the athero-protective effect of high density lipoproteins. J Intern Med [Internet]. Wiley-Blackwell; 2008;263(3):256–73. Available from: doi:10.1111/j.1365-2796.2007.01898.x.
24. van Tol A. Phospholipid transfer protein. Curr Opin Lipidol [Internet]. Ovid Technologies (Wolters Kluwer Health); 2002;13(2):135–9. Available from: doi:10.1097/00041433-200204000-00004.
25. Abifadel M, Varret M, Rabès J-P, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet [Internet]. Nature Publishing Group; 2003;34(2):154–6. Available from: doi:10.1038/ng1161.
26. Quagliarini F, Vallvé J-C, Campagna F, Alvaro A, Fuentes-Jimenez FJ, Sirinian MI, et al. Autosomal recessive hypercholesterolemia in Spanish kindred due to a large deletion in the ARH gene. Mol Genet Metab [Internet]. Elsevier BV; 2007;92(3):243–8. Available from: doi:10.1016/j.ymgme.2007.06.012.
27. Garcia CK, Willund K, Arca M, Zuliani G, Fellin R, Maioli M, et al. Autosomal recessive hypercholesterolemia caused by mutations in a putative LDL receptor adaptor protein. Science (80-) [Internet]. American Association for the Advancement of Science (AAAS); 2001;292(5520):1394–8. Available from: doi:10.1126/science.1060458.
28. Leitersdorf E, Tobin EJ, Davignon J, Hobbs HH. Common low-density lipoprotein receptor mutations in the French Canadian population. J Clin Invest [Internet]. American Society for Clinical Investigation; 1990;85(4):1014–23. Available from: doi:10.1172/jci114531.
29. Raal FJ, Sjouke B, Hovingh GK, Isaac BF. Phenotype diversity among patients with homozygous familial hypercholesterolemia: a cohort study. Atherosclerosis 2016;248:238–44. DOI: 10.1016/j.atherosclerosis.2016.03.009
30. Goldstein JL, Hobbs HH, Brown MS. Familial hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, et al., editors. The metabolic basis of inherited disease. 8th ed. New York: McGraw-Hill; 2001. p. 2863–913.
31. Leigh SEA, Foster AH, Whittall RA, Hubbart CS, Humphries SE. Update and analysis of the University College London low density lipoprotein receptor familial hypercholesterolemia database. Ann Hum Genet [Internet]. Wiley-Blackwell; 2008;72(4):485–98. Available from: doi:10.1111/j.1469-1809.2008.00436.x.
32. Varret M, Abifadel M, Rabès J-P, Boileau C. Genetic heterogeneity of autosomal dominant hypercholesterolemia. Clin Genet [Internet]. Wiley-Blackwell; 2007;73(1):1–13. Available from: doi:10.1111/j.1399-0004.2007.00915.x.
33. Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolaemia in children and adolescents : gaining decades of life by optimizing detection and treatment. Eur Heart J. 2015;36:2425–37.
34. Allard D, Amsellem S, Abifadel M, Trillard M, Devillers M, Luc G, et al. Novel mutations of thePCSK9 gene cause variable phenotype of autosomal dominant hypercholesterolemia. Hum Mutat [Internet]. Wiley-Blackwell; 2005;26(5):497. Available from: doi:10.1002/humu.9383.
35. Slack J. Risks of ischæmic heart-disease in familial hyperlipoproteinæmic states. Lancet [Internet]. Elsevier BV; 1969;294(7635):1380–2. Available from: doi:10.1016/s0140-6736(69)90930-1.
36. Souverein OW, Defesche JC, Zwinderman AH, Kastelein JJP, Tanck MWT. Influence of LDL-receptor mutation type on age at first cardiovascular event in patients with familial hypercholesterolaemia. Eur Heart J [Internet]. Oxford University Press (OUP); 2007;28(3):299–304. Available from: doi:10.1093/eurheartj/ehl366.
37. Wiegman A, de Groot E, Hutten BA, Rodenburg J, Gort J, Bakker HD, et al. Arterial intima-media thickness in children heterozygous for familial hypercholesterolaemia. Lancet [Internet]. Elsevier BV; 2004;363(9406):369–70. Available from: doi:10.1016/s0140-6736(04)15467-6.
38. Huijgen R, Vissers MN, Defesche JC, Lansberg PJ, Kastelein JJP, Hutten BA. Familial hypercholesterolemia: current treatment and advances in management. Expert Rev Cardiovasc Ther [Internet]. Informa Healthcare; 2008;6(4):567–81. Available from: doi:10.1586/14779072.6.4.567.
39. Besseling J, Reitsma JB, Gaudet D, Brisson D, Kastelein JJP, Hovingh GK, et al. Selection of individuals for genetic testing for familial hypercholesterolaemia: development and external validation of a prediction model for the presence of a mutation causing familial hypercholesterolaemia. Eur Heart J [Internet]. 2016 [cited 2016 May 29]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/27044878
40. Hovingh GK, Davidson MH, Kastelein JJP, O' Connor AM. Clinical update diagnosis and treatment of familial hypercholesterolaemia. Eur Heart J. 2013;34:962–71.
41. Prospective Studies Collaboration, Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, et al. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet (London, England) [Internet]. 2007 [cited 2016 Apr 28];370(9602):1829–39. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18061058
42. Besseling J, Sjouke B, Kastelein JJP. Screening and treatment of familial hypercholesterolemia e Lessons from the past and opportunities for the future (based on the Anitschkow Lecture 2014). Atherosclerosis [Internet]. Elsevier Ltd; 2015;241(2):597–606. Available from: doi:10.1016/j.atherosclerosis.2015.06.011.
43. Stoekenbroek RM, Kastelein JJP, Huijgen R. PCSK9 inhibition : the way forward in the treatment of dyslipidemia. BMC Med [Internet]. BMC Medicine; 2015;1–6. Available from: doi:10.1186/s12916-015-0503-4.
44. Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, Robinson J, et al. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. N Engl J Med [Internet]. 2015 [cited 2016 May 1];372(16):1500–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25773607
45. Zhang X-L, Zhu Q-Q, Zhu L, Chen J-Z, Chen Q-H, Li G-N, et al. Safety and efficacy of anti-PCSK9 antibodies: a meta-analysis of 25 randomized, controlled trials. BMC Med [Internet]. BioMed Central; 2015 [cited 2016 May 1];13(1):123. Available from: http://www.biomedcentral.com/1741-7015/13/123
46. Reiner Ž. Management of patients with familial hypercholesterolaemia. Nat Publ Gr [Internet]. Nature Publishing Group; 2015;12(10):565–75. Available from: doi:10.1038/nrcardio.2015.92.
47. Florentin M, Liberopoulos EN, Mikhailidis DP, Elisaf MS. Colesevelam hydrochloride in clinical practice: a new approach in the treatment of hypercholesterolaemia. Curr Med Res Opin [Internet]. Informa Healthcare; 2008;24(4):995–1009. Available from: doi:10.1185/030079908x280446.
48. Kastelein JJP, Wedel MK, Baker BF, Su J, Bradley JD, Yu RZ, et al. Potent reduction of apolipoprotein B and low-density lipoprotein cholesterol by short-term administration of an antisense inhibitor of apolipoprotein B. Circulation [Internet]. Ovid Technologies (Wolters Kluwer Health); 2006;114(16):1729–35. Available from: doi:10.1161/circulationaha.105.606442.
49. Ajufo E, Cuchel M. Recent developments in gene therapy for homozygous familial hypercholesterolemia. Curr Atheroscler Rep [Internet]. Current Atherosclerosis Reports; 2016; Available from: doi:10.1007/s11883-016-0579-0.

50. Averna M, Cefalù AB, Stefanutti C, Di Giacomo S, Sirtori CR, Vigna G. Nutrition, metabolism & cardiovascular diseases individual analysis of patients with HoFH participating in a phase 3 trial with lomitapide : the Italian cohort. Nutr Metab Cardiovasc Dis [Internet]. Elsevier B.V; 2016;26(1):36–44. Available from: doi:10.1016/j.numecd.2015.11.001.
51. Blom DJ, Fayad ZA, Kastelein JJP, Larrey D, Makris L, Schwamlein C, et al. LOWER, a registry of lomitapide-treated patients with homozygous familial hypercholesterolemia : Rationale and design. J Clin Lipidol [Internet]. Elsevier Inc; 2016;10(2):273–82. Available from: doi:10.1016/j.jacl.2015.11.011.
52. Avis HJ, Vissers MN, Stein EA, Wijburg FA, Trip MD, Kastelein JJP, et al. A systematic review and meta-analysis of statin therapy in children with familial hypercholesterolemia. Arterioscler Thromb Vasc Biol [Internet]. Ovid Technologies (Wolters Kluwer Health); 2007;27(8):1803–10. Available from: doi:10.1161/atvbaha.107.145151.
53. Arambepola C, Farmer AJ, Perera R, Neil HAW. Statin treatment for children and adolescents with heterozygous familial hypercholesterolaemia: a systematic review and meta-analysis. Atherosclerosis [Internet]. Elsevier BV; 2007;195(2):339–47. Available from: doi:10.1016/j.atherosclerosis.2006.09.030.
54. Rodenburg J, Vissers MN, Wiegman A, van Trotsenburg ASP, van der Graaf A, de Groot E, et al. Statin treatment in children with familial hypercholesterolemia: the younger, the better. Circulation [Internet]. Ovid Technologies (Wolters Kluwer Health); 2007;116(6):664–8. Available from: doi:10.1161/circulationaha.106.671016.
55. Reiner Ž. Impact of early evidence of atherosclerotic changes on early treatment in children with familial hypercholesterolemia. Circ Res. 2014;114(2):233–235.
56. Watts GF, Gidding S, Wierzbicki AS, Toth PP, Alonso R, Brown WV, et al. Integrated guidance on the care of familial hypercholesterolemia from the International FH Foundation. J Clin Lipidol [Internet]. Mosby, Inc; 2014;8(2):148–72. Available from: doi:10.1016/j.jacl.2014.01.002.
57. Miserez AR, Muller PY. Familial defective apolipoprotein B-100: a mutation emerged in the Mesolithic ancestors of Celtic peoples? Atherosclerosis [Internet]. Elsevier BV; 2000;148(2):433–6. Available from: doi:10.1016/s0021-9150(99)00470-0.
58. Defesche JC, Pricker KL, Hayden MR, van der Ende BE, Kastelein JJ. Familial defective apolipoprotein B-100 is clinically indistinguishable from familial hypercholesterolemia. Arch Intern Med [Internet]. American Medical Association (AMA); 1993;153(20):2349. Available from: doi:10.1001/archinte.1993.00410200071008.
59. Miserez AR, Keller U. Differences in the phenotypic characteristics of subjects with familial defective apolipoprotein B-100 and familial hypercholesterolemia. Arterioscler Thromb Vasc Biol [Internet]. Ovid Technologies (Wolters Kluwer Health); 1995;15(10):1719–29. Available from: doi:10.1161/01.atv.15.10.1719.
60. Muntoni S, Pisciotta L, Muntoni S, Bertolini S. Pharmacological treatment of a Sardinian patient affected by Autosomal Recessive Hypercholesterolemia (ARH). J Clin Lipidol [Internet]. Mosby, Inc; 2015;9(1):103–6. Available from: doi:10.1016/j.jacl.2014.08.009.
61. Eden ER, Sun X-M, Patel DD, Soutar AK. Adaptor protein Disabled-2 modulates low density lipoprotein receptor synthesis in fibroblasts from patients with autosomal recessive hypercholesterolaemia. Hum Mol Genet [Internet]. Oxford University Press (OUP); 2007;16(22):2751–9. Available from: doi:10.1093/hmg/ddm232.
62. Cohen JC, Kimmel M, Polanski A, Hobbs HH. Molecular mechanisms of autosomal recessive hypercholesterolemia. Curr Opin Lipidol [Internet]. Ovid Technologies (Wolters Kluwer Health); 2003;14(2):121–7. Available from: doi:10.1097/00041433-200304000-00002.
63. Wilund KR, Campagna F, Arca M, Zuiliani G, Fellin R, Ho YK, et al. Molecular mechanisms of autosomal recessive hypercholesterolemia. Hum Mol Genet [Internet]. Oxford University Press (OUP); 2002;11(24):3019–30. Available from: doi:10.1093/hmg/11.24.3019.
64. Rodenburg J, Wiegman A, Vissers MN, Kastelein JJP, Stalenhoef AFH. A boy with autosomal recessive hypercholesterolaemia. Neth J Med [Internet]. 2004 [cited 2016 Apr 28];62(3):89–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15209474
65. Wierzbicki A, Graham C, Young I, Nicholls D. Familial combined hyperlipidaemia: under – defined and under – diagnosed? Curr Vasc Pharmacol [Internet]. Bentham Science Publishers Ltd.; 2008;6(1):13–22.Availablefrom:doi:10.2174/157016108783331268.
66. Brahm AJ, Hegele RA. Combined hyperlipidemia : familial but not (usually) monogenic. Curr Opin Lipidol. 2016;27:131–40.
67. Schaefer EJ, Genest JJ, Ordovas JM, Salem DN, Wilson PWF. Familial lipoprotein disorders and premature coronary artery disease. Atherosclerosis [Internet]. Elsevier BV; 1994;108:S41–54. Available from: doi:10.1016/0021-9150(94)90152-x.
68. Veerkamp MJ. Nomogram to diagnose familial combined hyperlipidemia on the basis of results of a 5-year follow-up study. Circulation [Internet]. Ovid Technologies (Wolters Kluwer Health); 2004;109(24):2980–5. Available from: doi:10.1161/01.cir.0000130646.93255.86.
69. Amend KL, Landon J, Thyagarajan V, Niemcryk S, McAfee A. Incidence of hospitalized rhabdomyolysis with statin and fibrate use in an insured US population. Ann Pharmacother [Internet]. 2011 [cited 2016 May 1];45(10):1230–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21917557
70. Berge KE, Tian H, Graf GA, Yu L, Grishin N V, Schultz J, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science [Internet]. 2000 [cited 2016 Apr 28];290(5497):1771–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11099417
71. Lu K, Lee M-H, Yu H, Zhou Y, Sandell SA, Salen G, et al. Molecular cloning, genomic organization, genetic variations, and characterization of murine sterolin genes Abcg5 and Abcg8. J Lipid Res [Internet]. 2002 [cited 2016 Apr 28];43(4):565–78. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11907139
72. Hubacek JA, Berge KE, Cohen JC, Hobbs HH. Mutations in ATP-cassette binding proteins G5 (ABCG5) and G8 (ABCG8) causing sitosterolemia. Hum Mutat [Internet]. Wiley-Blackwell; 2001;18(4):359–60. Available from: doi:10.1002/humu.1206.
73. Bhattacharyya AK, Connor WE. β-Sitosterolemia and Xanthomatosis. J Clin Invest [Internet]. American Society for Clinical Investigation; 1974;53(4):1033–43. Available from: doi:10.1172/jci107640.
74. Salen G, Shefer S, Nguyen L, Ness GC, Tint GS, Shore V. Sitosterolemia. J Lipid Res [Internet]. 1992 [cited 2016 Apr 28];33(7):945–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1431587
75. Salen G, von Bergmann K, Lütjohann D, Kwiterovich P, Kane J, Patel SB, et al. Ezetimibe effectively reduces plasma plant sterols in patients with sitosterolemia. Circulation [Internet]. 2004 [cited 2016 Apr 28];109(8):966–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14769702
76. Salen G, Starc T, Sisk CM, Patel SB. Intestinal cholesterol absorption inhibitor ezetimibe added to cholestyramine for sitosterolemia and xanthomatosis. Gastroenterology [Internet]. Elsevier BV; 2006;130(6):1853–7. Available from: doi:10.1053/j.gastro.2006.02.027.
77. Wang X, Paigen B. Genetics of variation in HDL cholesterol in humans and mice. Circ Res [Internet]. 2005 [cited 2016 Apr 28];96(1):27–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15637305
78. von Eckardstein A. Differential diagnosis of familial high density lipoprotein deficiency syndromes. Atherosclerosis [Internet].

Elsevier BV; 2006;186(2):231–9. Available from: doi:10.1016/j.atherosclerosis.2005.10.033.
79. Khovidhunkit W, Memon RA, Feingold KR, Grunfeld C. Infection and inflammation-induced proatherogenic changes of lipoproteins. J Infect Dis [Internet]. Oxford University Press (OUP); 2000;181(s3):S462–72. Available from: doi:10.1086/315611.
80. Canner PL, Berge KG, Wenger NK, Stamler J, Friedman L, Prineas RJ, et al. Fifteen year mortality in coronary drug project patients: long-term benefit with niacin. J Am Coll Cardiol [Internet]. Elsevier BV; 1986;8(6):1245–55. Available from: doi:10.1016/s0735-1097(86)80293-5.
81. AIM-HIGH Investigators, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med [Internet]. 2011 [cited 2016 May 1];365(24):2255–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22085343
82. HPS2-THRIVE Collaborative Group, Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, et al. Effects of extended-release niacin with laropiprant in high-risk patients. N Engl J Med [Internet]. 2014 [cited 2016 May 1];371(3):203–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25014686
83. Barter PJ, Rye K. Targeting high-density lipoproteins to reduce cardiovascular risk: what is the evidence ? Clin Ther [Internet]. Elsevier; 2015;37(12):2716–31. Available from: doi:10.1016/j.clinthera.2015.07.021.
84. Link JJ, Rohatgi A, de Lemos JA. HDL cholesterol: physiology, pathophysiology, and management. Curr Probl Cardiol [Internet]. Elsevier BV; 2007;32(5):268–314. Available from: doi:10.1016/j.cpcardiol.2007.01.004.
85. Hausenloy DJ, Yellon DM. Targeting residual cardiovascular risk: raising high-density lipoprotein cholesterol levels. Heart [Internet]. BMJ; 2008;94(6):706–14. Available from: doi:10.1136/hrt.2007.125401.
86. Kosmas C, DeJesus E, Rosario D, Vittorio T. CETP inhibition: past failures and future hopes. Clin Med Insights Cardiol. 2016;10:37–42.
87. Pisciotta L, Fasano T, Calabresi L, Bellocchio A, Fresa R, Borrini C, et al. A novel mutation of the apolipoprotein A-I gene in a family with familial combined hyperlipidemia. Atherosclerosis [Internet]. Elsevier BV; 2008;198(1):145–51. Available from: doi:10.1016/j.atherosclerosis.2007.09.017.
88. Joy T, Wang J, Hahn A, Hegele RA. Apoa1 related amyloidosis: a case report and literature review. Clin Biochem [Internet]. Elsevier BV; 2003;36(8):641–5. Available from: doi:10.1016/s0009-9120(03)00110-3.
89. Hovingh GK, Brownlie A, Bisoendial RJ, Dube MP, Levels JHM, Petersen W, et al. A novel apoA-I mutation (L178P) leads to endothelial dysfunction, increased arterial wall thickness, and premature coronary artery disease. J Am Coll Cardiol [Internet]. Elsevier BV; 2004;44(7):1429–35. Available from: doi:10.1016/j.jacc.2004.06.070.
90. Gomaraschi M, Baldassarre D, Amato M, Eligini S, Conca P, Sirtori CR, et al. Normal vascular function despite low levels of high-density lipoprotein cholesterol in carriers of the apolipoprotein A-IMilano mutant. Circulation [Internet]. Ovid Technologies (Wolters Kluwer Health); 2007;116(19):2165–72. Available from: doi:10.1161/circulationaha.107.705657.
91. Sirtori CR, Calabresi L, Franceschini G, Baldassarre D, Amato M, Johansson J, et al. Cardiovascular status of carriers of the apolipoprotein A-IMilano mutant : the Limone sul Garda study. Circulation [Internet]. Ovid Technologies (Wolters Kluwer Health); 2001;103(15):1949–54. Available from: doi:10.1161/01.cir.103.15.1949.
92. Hovingh GK, de Groot E, van der Steeg W, Boekholdt SM, Hutten BA, Kuivenhoven JA, et al. Inherited disorders of HDL metabolism and atherosclerosis. Curr Opin Lipidol [Internet]. Ovid Technologies (Wolters Kluwer Health); 2005;16(2):139–45. Available from: doi:10.1097/01.mol.0000162318.47172.ef.
93. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet (London, England) [Internet]. 2012 [cited 2016 May 1];380(9841):572–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22607825
94. Negi SI, Brautbar A, Virani SS, Anand A, Polisecki E, Asztalos BF, et al. Case study a novel mutation in the ABCA1 gene causing an atypical phenotype of Tangier disease. J Clin Lipidol [Internet]. Mosby, Inc; 2013;7(1):82–7. Available from: doi:10.1016/j.jacl.2012.09.004.
95. Kathiresan S, Melander O, Guiducci C, Surti A, Burtt NP, Rieder MJ, et al. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet [Internet]. Nature Publishing Group; 2008;40(2):189–97. Available from: doi:10.1038/ng.75.
96. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet [Internet]. Nature Publishing Group; 2008;40(2):161–9. Available from: doi:10.1038/ng.76.
97. Singaraja RR, Visscher H, James ER, Chroni A, Coutinho JM, Brunham LR, et al. Specific mutations in ABCA1 have discrete effects on ABCA1 function and lipid phenotypes both in vivo and in vitro. Circ Res [Internet]. 2006 [cited 2016 Apr 28];99(4):389–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16873719
98. Singaraja RR, Brunham LR, Visscher H, Kastelein JJP, Hayden MR. Efflux and atherosclerosis: the clinical and biochemical impact of variations in the ABCA1 gene. Arterioscler Thromb Vasc Biol [Internet]. 2003 [cited 2016 Apr 28];23(8):1322–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12763760
99. Rosenson RS, Jr Brewer HB, Ansell BJ, Barter P, Chapman MJ, Heinecke JW, et al. Dysfunctional HDL and atherosclerotic cardiovascular disease. Nat Publ Gr [Internet]. Nature Publishing Group; 2015;13(01):48–60. Available from: doi:10.1038/nrcardio.2015.124.
100. Clee SM, Kastelein JJP, van Dam M, Marcil M, Roomp K, Zwarts KY, et al. Age and residual cholesterol efflux affect HDL cholesterol levels and coronary artery disease in ABCA1 heterozygotes. J Clin Invest [Internet]. American Society for Clinical Investigation; 2000;106(10):1263–70. Available from: doi:10.1172/jci10727.
101. van Dam MJ, de Groot E, Clee SM, Hovingh GK, Roelants R, Brooks-Wilson A, et al. Association between increased arterial-wall thickness and impairment in ABCA1-driven cholesterol efflux: an observational study. Lancet [Internet]. Elsevier BV; 2002;359(9300):37–41. Available from: doi:10.1016/s0140-6736(02)07277-x.
102. Frikke-Schmidt R, Nordestgaard BG, Stene MCA, Sethi AA, Remaley AT, Schnohr P, et al. Association of loss-of-function mutations in the ABCA1 gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. JAMA [Internet]. 2008 [cited 2016 Apr 28];299(21):2524–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18523221
103. Brunham LR, Singaraja RR, Duong M, Timmins JM, Fievet C, Bissada N, et al. Tissue-specific roles of ABCA1 influence susceptibility to atherosclerosis. Arterioscler Thromb Vasc Biol [Internet]. Ovid Technologies (Wolters Kluwer Health); 2009;29(4):548–54. Available from: doi:10.1161/atvbaha.108.182303.
104. Clee SM, Zwinderman AH, Engert JC, Zwarts KY, Molhuizen HOF, Roomp K, et al. Common genetic variation in ABCA1 is associated with altered lipoprotein levels and a modified risk for coronary artery disease. Circulation [Internet]. Ovid Technologies (Wolters Kluwer Health); 2001;103(9):1198–205. Available from: doi:10.1161/01.cir.103.9.1198.
105. Zwarts KY, Clee SM, Zwinderman AH, Engert JC, Singaraja R, Loubser O, et al. ABCA1 regulatory variants influence coronary

artery disease independent of effects on plasma lipid levels. Clin Genet [Internet]. Wiley-Blackwell; 2002;61(2):115–25. Available from: doi:10.1034/j.1399-0004.2002.610206.x.
106. Brunham LR, Kastelein JJP, Hayden MR. ABCA1 gene mutations, HDL cholesterol levels, and risk of ischemic heart disease. JAMA [Internet]. 2008 [cited 2016 Apr 28];300(17):1997–8; author reply 1998. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18984885
107. Kiss RS, Kavaslar N, Okuhira K -I, Freeman MW, Walter S, Milne RW, et al. Genetic etiology of isolated low HDL syndrome: incidence and heterogeneity of efflux defects. Arterioscler Thromb Vasc Biol [Internet]. Ovid Technologies (Wolters Kluwer Health); 2007;27(5):1139–45. Available from: doi:10.1161/atvbaha.106.137646.
108. Ayyobi AF, McGladdery SH, Chan S, John Mancini GB, Hill JS, Frohlich JJ. Lecithin: cholesterol acyltransferase (LCAT) deficiency and risk of vascular disease: 25 year follow-up. Atherosclerosis [Internet]. Elsevier BV; 2004;177(2):361–6. Available from: doi:10.1016/j.atherosclerosis.2004.07.018.
109. Van Den Bogaard B, Holleboom AG, Duivenvoorden R, Hutten BA, Kastelein JJP, Hovingh GK, et al. Patients with low HDL-cholesterol caused by mutations in LCAT have increased arterial stiffness. Atherosclerosis [Internet]. Elsevier Ltd; 2012;225(2):481–5. Available from: doi:10.1016/j.atherosclerosis.2012.09.022.
110. Calabresi L, Baldassarre D, Simonelli S, Gomaraschi M, Amato M, Castelnuovo S, et al. Plasma lecithin : cholesterol acyltransferase and carotid intima-media thickness in European individuals at high cardiovascular risk. J Lipid Res. 2011;52:1569–74.
111. Saeedi R, Li M, Frohlich J. A review on lecithin : cholesterol acyltransferase de fi ciency. Clin Biochem. 2015;48:472–5.
112. Shah PK. Inhibition of CETP as a novel therapeutic strategy for reducing the risk of atherosclerotic disease. Eur Heart J [Internet]. Oxford University Press (OUP); 2006;28(1):5–12. Available from: doi:10.1093/eurheartj/ehl392.
113. Tsai MY, Johnson C, Kao WHL, Sharrett AR, Arends VL, Kronmal R, et al. Cholesteryl ester transfer protein genetic polymorphisms, HDL cholesterol, and subclinical cardiovascular disease in the multi-ethnic study of atherosclerosis. Atherosclerosis [Internet]. Elsevier BV; 2008;200(2):359–67. Available from: doi:10.1016/j.atherosclerosis.2007.12.038.
114. Klerkx AHEM, de Grooth GJ, Zwinderman AH, Jukema JW, Kuivenhoven JA, Kastelein JJP. Cholesteryl ester transfer protein concentration is associated with progression of atherosclerosis and response to pravastatin in men with coronary artery disease (REGRESS). Eur J Clin Invest [Internet]. Wiley-Blackwell; 2004;34(1):21–8. Available from: doi:10.1111/j.1365-2362.2004.01281.x.
115. Smilde TJ, van Wissen S, Awollersheim H, Trip MD, Kastelein JJP, Stalenhoef AFH. Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolemia (ASAP): a prospective, randomised, double-blind trial. Lancet [Internet]. Elsevier BV; 2001;357(9256):577–81. Available from: doi:10.1016/s0140-6736(00)04053-8.
116. Boekholdt SM, Kuivenhoven J-A, Wareham NJ, Peters RJG, Jukema JW, Luben R, et al. Plasma levels of cholesteryl ester transfer protein and the risk of future coronary artery disease in apparently healthy men and women: the prospective EPIC (European Prospective Investigation into Cancer and nutrition)-Norfolk population study. Circulation [Internet]. 2004 [cited 2016 Apr 28];110(11):1418–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15337694
117. Agerholm-Larsen B, Tybjarg-Hansen A, Schnohr P, Steffensen R, Nordestgaard BG. Common cholesteryl ester transfer protein mutations, decreased HDL cholesterol, and possible decreased risk of ischemic heart disease : the Copenhagen City heart study. Circulation [Internet]. Ovid Technologies (Wolters Kluwer Health); 2000;102(18):2197–203. Available from: doi:10.1161/01.cir.102.18.2197.
118. Curb JD, Abbott RD, Rodriguez BL, Masaki K, Chen R, Sharp DS, et al. A prospective study of HDL-C and cholesteryl ester transfer protein gene mutations and the risk of coronary heart disease in the elderly. J Lipid Res [Internet]. 2004 [cited 2016 Apr 28];45(5):948–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14967821
119. Moriyama Y, Okamura T, Inazu A, Doi M, Iso H, Mouri Y, et al. A low prevalence of coronary heart disease among subjects with increased high-density lipoprotein cholesterol levels, including those with plasma cholesteryl ester transfer protein deficiency. Prev Med (Baltim) [Internet]. Elsevier BV; 1998;27(5):659–67. Available from: doi:10.1006/pmed.1998.0340.
120. Thompson A, Di Angelantonio E, Sarwar N, Erqou S, Saleheen D, Dullaart RPF, et al. Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. JAMA [Internet]. American Medical Association (AMA); 2008;299(23):2777. Available from: doi:10.1001/jama.299.23.2777.
121. van Acker BAC, Botma G-J, Zwinderman AH, Kuivenhoven JA, Dallinga-Thie GM, Sijbrands EJG, et al. High HDL cholesterol does not protect against coronary artery disease when associated with combined cholesteryl ester transfer protein and hepatic lipase gene variants. Atherosclerosis [Internet]. Elsevier BV; 2008;200(1):161–7. Available from: doi:10.1016/j.atherosclerosis.2007.11.019.
122. Mukherjee M, Shetty KR. Variations in high-density lipoprotein cholesterol in relation to physical activity and Taq 1B polymorphism of the cholesteryl ester transfer protein gene. Clin Genet [Internet]. Wiley-Blackwell; 2004;65(5):412–8. Available from: doi:10.1111/j.0009-9163.2004.0237.x.
123. Beigneux AP, Franssen R, Bensadoun A, Gin P, Melford K, Peter J, et al. Chylomicronemia with a mutant GPIHBP1 (Q115P) that cannot bind lipoprotein lipase. Arterioscler Thromb Vasc Biol [Internet]. Ovid Technologies (Wolters Kluwer Health); 2009;29(6):956–62. Available from: doi:10.1161/atvbaha.109.186577.
124. Brahm AJ, Hegele RA. Chylomicronaemia – current diagnosis and future therapies. Nat Publ Gr [Internet]. Nature Publishing Group; 2015;11(6):352–62. Available from: doi:10.1038/nrendo.2015.26.
125. Brunzell JD. Hypertriglyceridemia. N Engl J Med [Internet]. New England Journal of Medicine (NEJM/MMS); 2007;357(10):1009–17. Available from: doi:10.1056/nejmcp070061.
126. Birjmohun RS, Hutten BA, Kastelein JJP, Stroes ESG. Efficacy and safety of high-density lipoprotein cholesterol-increasing compounds: a meta-analysis of randomized controlled trials. J Am Coll Cardiol [Internet]. 2005 [cited 2016 Apr 28];45(2):185–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15653014
127. Grundy SM, Cleeman JI, Merz CNB, Brewer HB, Clark LT, Hunninghake DB, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation [Internet]. 2004 [cited 2016 Apr 28];110(2):227–39. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15249516
128. McKenney J. New perspectives on the use of niacin in the treatment of lipid disorders. Arch Intern Med [Internet]. American Medical Association (AMA); 2004;164(7):697. Available from: doi:10.1001/archinte.164.7.697.
129. Pirillo A, Catapano AL. Update on the management of severe hypertriglyceridemia – focus on free fatty acid forms of omega-3. Drug Des Devel Ther. 2015;9:2129–37.
130. Hooper L, Ness A, Higgins JPT, Moore T, Ebrahim S. GISSI-Prevenzione trial. Lancet [Internet]. Elsevier BV; 1999;354(9189):1557. Available from: doi:10.1016/s0140-6736(05)76587-9.
131. Moens SJB, van Capelleveen JC, Stroes ESG. Inhibition of ApoCIII : the next PCSK9 ? Curr Opin Lipidol. 2014;25(6):418–22.
132. Merkel M, Eckel RH, Goldberg IJ. Lipoprotein lipase: genetics, lipid uptake, and regulation. J Lipid Res [Internet]. 2002 [cited

2016 Apr 28];43(12):1997–2006. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12454259
133. Lam C-W, Yuen Y-P, Cheng W-F, Chan Y-W, Tong S-F. Missense mutation Leu72Pro located on the carboxyl terminal amphipathic helix of apolipoprotein C-II causes familial chylomicronemia syndrome. Clin Chim Acta [Internet]. Elsevier BV; 2006;364(1-2):256–9. Available from: doi:10.1016/j.cca.2005.07.025.
134. Benlian P, De Gennes JL, Foubert L, Zhang H, Gagné SE, Hayden M. Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. N Engl J Med [Internet]. New England Journal of Medicine (NEJM/MMS); 1996;335(12):848–54. Available from: doi:10.1056/nejm199609193351203.
135. Saika Y, Sakai N, Takahashi M, Maruyama T, Kihara S, Ouchi N, et al. Novel LPL mutation (L303F) found in a patient associated with coronary artery disease and severe systemic atherosclerosis. Eur J Clin Invest [Internet]. Wiley-Blackwell; 2003;33(3):216–22. Available from: doi:10.1046/j.1365-2362.2003.01129.x.
136. Myocardial Infarction Genetics, CARDIoGRAM Exome Consortia Investigators. Coding variation in ANGPTL4, LPL, and SVEP1 and the risk of coronary disease. N Engl J Med [Internet]. 2016 [cited 2016 May 1];374(12):1134–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26934567
137. Connelly PW, Maguire GF, Little JA. Apolipoprotein CIISt. Michael. Familial apolipoprotein CII deficiency associated with premature vascular disease. J Clin Invest [Internet]. American Society for Clinical Investigation; 1987;80(6):1597–606. Available from: doi:10.1172/jci113246.
138. Stroes ES, Nierman MC, Meulenberg JJ, Franssen R, Twisk J, Henny CP, et al. Intramuscular administration of AAV1-Lipoprotein lipaseS447X lowers triglycerides in lipoprotein lipase-deficient patients. Arterioscler Thromb Vasc Biol [Internet]. Ovid Technologies (Wolters Kluwer Health); 2008;28(12):2303–4. Available from: doi:10.1161/atvbaha.108.175620.
139. Franssen R, Visser ME, Kuivenhoven JA, Kastelein JJP, Dallinga-Thie GM, Stroes ESG. Role of lipoprotein lipase in triglyceride metabolism: potential therapeutic target. Future Lipidol [Internet]. Future Medicine Ltd; 2008;3(4):385–97. Available from: doi:10.2217/17460875.3.4.385.
140. Scott LJ. Alipogene tiparvovec: a review of its use in adults with familial lipoprotein lipase deficiency. Drugs. 2015;75:175–82.
141. Yuan G, Al-Shali KZ, Hegele RA. Hypertriglyceridemia: its etiology, effects and treatment. Can Med Assoc J [Internet]. 8872147 Canada, Inc.; 2007;176(8):1113–20. Available from: doi:10.1503/cmaj.060963.
142. Smelt AHM, de Beer F. Apolipoprotein E and familial dysbetalipoproteinemia: clinical, biochemical, and genetic aspects. Semin Vasc Med [Internet]. Thieme Publishing Group; 2004;4(03):249–57. Available from: doi:10.1055/s-2004-861492.
143. Walden CC, Hegele RA. Apolipoprotein E in hyperlipidemia. Ann Intern Med [Internet]. 1994 [cited 2016 Apr 28];120(12):1026–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8185134
144. Koopal C, Retterstøl K, Sjouke B, Hovingh GK, Ros E, De Graaf J, et al. Vascular risk factors , vascular disease, lipids and lipid targets in patients with familial dysbetalipoproteinemia : a European cross- sectional study. Atherosclerosis [Internet]. Elsevier Ltd; 2015;240(1):90–7. Available from: doi:10.1016/j.atherosclerosis.2015.02.046.
145. Lewis GF, Xiao C, Hegele RA. Hypertriglyceridemia in the genomic era: a new paradigm. Endocr Rev [Internet]. 2015;36(1):131–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25554923
146. Wang J, Hegele RA. Homozygous missense mutation (G56R) in glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPI-HBP1) in two siblings with fasting chylomicronemia (MIM 144650). Lipids Heal Dis [Internet]. Springer Science + Business Media; 2007;6(1):23. Available from: doi:10.1186/1476-511x-6-23.
147. Gin P, Beigneux AP, Davies B, Young MF, Ryan RO, Bensadoun A, et al. Normal binding of lipoprotein lipase, chylomicrons, and apo-AV to GPIHBP1 containing a G56R amino acid substitution. Biochim Biophys Acta – Mol Cell Biol Lipids [Internet]. Elsevier BV; 2007;1771(12):1464–8. Available from: doi:10.1016/j.bbalip.2007.10.005.
148. Péterfy M, Ben-Zeev O, Mao HZ, Weissglas-Volkov D, Aouizerat BE, Pullinger CR, et al. Mutations in LMF1 cause combined lipase deficiency and severe hypertriglyceridemia. Nat Genet [Internet]. Nature Publishing Group; 2007;39(12):1483–7. Available from: doi:10.1038/ng.2007.24.

Genetics of (Premature) Coronary Artery Disease

22

Jeanette Erdmann and Heribert Schunkert

Abstract

Coronary artery disease (CAD) and its major complication, myocardial infarction (MI), remain the number one cause of death in industrialized society, causing approximately one in every six deaths in the United States in 2010. CAD is the clinical manifestation of a chronic pathomorphological process that occurs in the vascular wall. Carl Müller (1886–1983) was the first to identify a link between high plasma cholesterol, xanthoma, and premature coronary heart disease in 1939, providing early evidence of a genetic component of CAD and its association with cholesterol. Today, it is well established that CAD arises from the interaction of multiple genetic and environmental factors. Likewise, a multifactorial etiology applies to many of the underlying cardiovascular risk factors, including hypercholesterolemia, hypertension, diabetes mellitus, and smoking.

Introduction

Coronary artery disease (CAD) and its major complication, myocardial infarction (MI), remain the number one cause of death in industrialized society, causing approximately one in every six deaths in the United States in 2010 [1]. CAD is the clinical manifestation of a chronic pathomorphological process that occurs in the vascular wall [2]. Carl Müller (1886–1983) was the first to identify a link between high plasma cholesterol, xanthoma, and premature coronary heart disease in 1939, providing early evidence of a genetic component of CAD and its association with cholesterol [3]. Today, it is well established that CAD arises from the interaction of multiple genetic and environmental factors. Likewise, a multifactorial etiology applies to many of the underlying cardiovascular risk factors, including hypercholesterolemia, hypertension, diabetes mellitus, and smoking. Thus, endogenous (genetic) and exogenous (nutrition, physical activity, therapy, etc.) factors all contribute to the development of atherosclerotic lesions, directly in the arterial wall, indirectly via traditional risk factors, or interactively by augmentation or amelioration of other contributing processes [2]. On a cellular level, atherosclerosis is a complex process characterized by endothelial dysfunction, lipid and matrix accumulation, migration and local transformation of circulating cells, smooth muscle cell (SMC) proliferation, calcification, inflammation, and finally, thrombus formation [2]. In this scenario, the potential influence of genetically modulated mechanisms may occur at multiple points during the development of the disease.

For years, evaluation of the family history served as a guide for assessing a patient's genetic risk for coronary events. While a positive family history exists in 20–30 % of cases, modern molecular genetics revealed that genetic variants affecting the risk of CAD may be fairly common in our population. Indeed, the number of risk alleles identified since the year 2007 implies that basically all individuals share a variable number of genetic risk factors.

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For example, 75 % of Western Europeans carry at least one variant of the chromosome 9p21.3 risk allele, which increases the probability of CAD by 25 %, irrespective of family history [4].

Thus, genetic factors may play a variable role in nearly all cases of CAD, even when the family history is negative. Nevertheless, rare cases of families with multiple affected members have allowed for the identification of specific molecular gene defects that have become novel targets for risk prediction and enhanced our understanding of the pathophysiology of this disease.

Importance of Family History

Assessment of the family history is fundamental for understanding the genetic components of the complex disease processes leading to CAD. Familial predisposition is assumed when MI is diagnosed before 55 years in a male first-degree relative or before 65 years in a female first-degree relative. The Framingham Heart Study revealed that this type of positive family history for premature MI increases the risk to a slightly different extent depending on parental premature CAD (1.45-fold) or sibling CAD (1.99-fold) (Fig. 22.1).

Moreover, familial risk was found to increase with decreasing age of the onset of disease in the affected relatives [5–7]. To a lesser degree, genetic factors affecting the risk of MI can be traced in affected second-degree relatives [8]. In families with several affected family members, traditional cardiovascular risk factors are often observed with increased frequency [9].

Furthermore, lifestyle habits associated with a raised incidence of MI (e.g., smoking) are more frequently shared in affected family members. Interestingly, both the Northwick Park Heart Study and the Reykjavik Cohort Study revealed that the increase in risk in terms of a positive family history remains highly significant (odds ratio 1.5–1.8) even after adjustment for traditional risk factors [10, 11]. Thus, the increased risk related to a positive family history is partially independent of traditional risk factors, suggesting that unrelated mechanisms may be causative in this respect [12].

Furthermore, a high rate of reoccurrence of MI was found in identical twins with MI. In these cases, a positive family history was related to an eightfold increased probability of death due to MI before the age of 55 years when the twin was affected at an early age as well [13]. The highest risk related to family history, however, is observed in families with a rare autosomal-dominant pattern of inheritance for MI [14, 15].

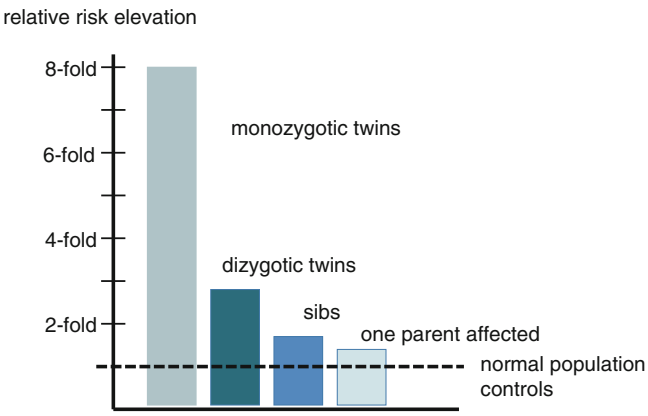

Fig. 22.1 The relative increase in the risk of myocardial infarction (MI)/coronary artery disease (CAD) is shown in relation to different familial backgrounds. The risk for monozygotic (MZ) and dizygotic (DZ) twins is based on the hypothesis that the partner twin died of MI at the age of 55 years

Familial Forms of Coronary Artery Disease

Some families present with an extremely high prevalence of CAD/MI. With the exception of two large families studied by Wang et al. [14] and Erdmann et al. [15] (see the next section), most of these families could not be systematically analyzed genetically due to the high lethality of the disease. In the German MI Family Study, we specifically looked for MI in large families with at least four surviving affected individuals. Overall, these families represent less than 0.1 % of cases of MI. Based on the analyses of 19 family pedigrees and statistical simulations, the presence of an autosomal-dominant inheritance pattern was probable in all cases. These family pedigrees will hopefully extend the knowledge of genes involved in MI in the near future. However, as was shown for familial hypercholesterolemia, in a proportion of the extended families, complex oligogenic inheritance can masquerade as monogenic disease [16, 17].

MEF2A

Wang et al. reported a mutation in the gene of the transcription factor MEF2A in a family with an autosomal-dominant form of MI. This marked the first time that a familial genetic defect was shown to give rise to MI in humans [14]. A 21-bp deletion in the gene appeared to result in abnormal epithelium of the coronary walls, thus favoring plaque deposition that may ultimately lead to MI. Interestingly, the same pathway is crucial for preventing apoptosis in endothelial cells and death due to vascular obstruction in mice. However, at present, genetic studies do not support the significance of this gene with respect to CAD/MI morbidity in humans, as several reports showed no association between single nucleotide polymorphisms (SNPs) in the MEF2A gene and CAD/MI in other families in large case–control studies or genome-wide association studies (GWAS) [18, 19].

GUCY1A3

Recently, the *GUCY1A3* gene, which encodes the α1-subunit of the soluble guanylyl cyclase (sGC) heterodimer consisting of the α1- and β1-subunits, was linked to MI through a family study performed by our group [15]. The sGC complex acts as the receptor for nitric oxide (NO) and catalyzes the formation of the second messenger cGMP [20]. A loss-of-function (LOF) mutation was detected in GUCY1A3 in an extended family with a history of MI upon exome sequencing of three affected family members. The mutation in GUCY1A3 impairs its function by reducing the protein content of the α1-subunit, abolishing the activity of the sGC enzyme and reducing the production of cGMP [15]. Moreover, mice deficient in the α1-subunit displayed accelerated thrombus formation in the microcirculation upon local trauma. A contributory role of CCT7, which stabilizes the α1ß1 sGC dimer [21], was also established based on the identification of a second mutation found within the same family. Additionally, a number of rare variants in GUCY1A3 with potential functional relevance were identified in MI patients [15]. Interestingly, in addition to these rare mutations, common variants in the GUCY1A3 gene are significantly associated with CAD on a genome-wide basis (OR = 1.08; $p = 4.57 \times 10^{-9}$), as reported by a GWAS meta-analysis performed by the CARDIoGRAM+C4D consortium (Fig. 22.2) [22]. Further genetic evidence pointing to critical involvement of the NO-sGC-cGMP pathway in mediating CAD and MI risk has been shown by identifying the NOS3 gene, encoding the endothelial NO synthase, as a further CAD risk gene by GWAS [20, 23, 24].

Heritability Estimates of Coronary Artery Disease

The classic measure of the genetic component of a phenotype (trait), termed "heritability", is defined as the percentage of the total variance of the trait that is explained by inheritance. By examining the increased similarity of trait values in related individuals compared with unrelated or less-related individuals, one can estimate the heritability. The simplest conceptual study design in man is the comparison of MZ and DZ twins. MZ twins share 100 % of their genes, whereas DZ twins share, on average, 50 % of their genes. If a trait has a genetic component, MZ twins are more likely to resemble each other than DZ twins.

Because of the assumptions that are required to estimate heritability, the calculated numbers must be considered rough proxies. In particular, the high prevalence of risk alleles in apparently healthy subjects within a population may result in underestimation of the true role of the genetic factors involved.

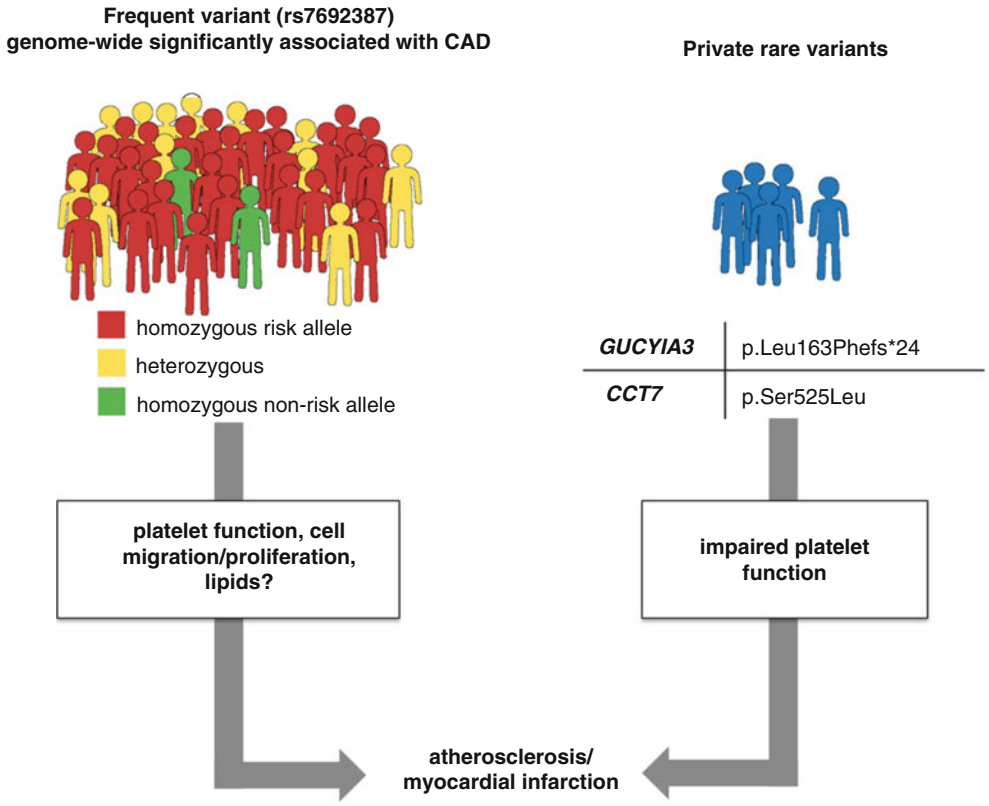

Fig. 22.2 Both frequent and rare variants in the *GUCY1A3* gene are involved in atherosclerosis and MI. Digenic rare variants in GUCY1A3 and its chaperonin protein CCTη with large effects highlight the crucial role of cGMP in atherosclerosis and MI due to altered inhibition of platelet aggregation. Common variants with moderate effects, such as rs7692387, are significantly associated with CAD and MI. Studies to elucidate their pathophysiological roles are ongoing (Figure taken from Wobst et al. [20])

Heritability of Coronary Anatomy and Pathology

We recently demonstrated that the heritability estimates of CAD depend in part on the pattern of coronary morphology. Particularly, left main disease and proximal coronary artery stenoses displayed high reoccurrence rates in affected siblings. The heritability estimate for ostial and proximal coronary stenoses was found to be $h^2 = 0.32$, indicating that approximately one third of the variability in this phenotype is explained by genetic factors ($p = 0.008$). Likewise, a highly significant heritability was found for the ecstatic form of coronary atherosclerosis and extraluminal calcification of the coronary arteries, as well as the abdominal aorta [25]. Thus, in addition to family history, knowledge of the coronary pathology in an affected family member may enhance risk prediction in first-degree relatives [26].

Genes Affecting Coronary Artery Disease

Over the past three decades, a great deal of research has focused on defining the genetic components of MI, CAD, and their risk factors. Initially, this research focused on candidate genes that hypothetically might affect known traits involved in the atherosclerotic process, including the renin–angiotensin system, lipoprotein metabolism, inflammation, or coagulation. However, the findings of many of these candidate gene studies were not replicated in consecutive studies. Consequently, at the beginning of the twenty-first century, novel strategies for gene identification were undertaken that allowed exploration of the entire genome. Without a priori hypothesis, genome-wide linkage analyses were performed that searched the entire genome for chromosomal regions shared by affected family members. While these studies resulted in the identification of several chromosomal loci harboring MI genes, these regions were too large for the elucidation of specific causative genes or molecular variants [27].

These efforts resulted in technological and methodological advances that initiated the advent of GWAS in 2005 [28], and a new era of exploration of CAD and MI only 2 years later [29–32]. Within the last decade, these GWAS have reproducibly identified hundreds of gene variants associated with a broad spectrum of disorders such as coronary heart disease, high blood pressure, hypercholesterolemia, and diabetes mellitus. Surprisingly, most of the genes identified thus far were not expected to play a role in the development of atherosclerosis. Thus, an important task for the immediate future is to understand the fundamental pathophysiological mechanisms affected by these genes. Another difficulty in this research is that, unlike Mendelian traits, genetic studies on complex cardiovascular disorders are complicated by variable cosegregation of the risk allele and the disease. In fact, many genetic variants associated with these disorders were found to be relatively common in the overall population and therefore, albeit to variable degrees, prevalent in both healthy and affected individuals. Accordingly, functional information on these genetic factors and the related gene expression as well as protein expression patterns is crucial. Subsequently, genetic research may enhance diagnostic testing and identification of new treatment targets.

Genome-Wide Association Studies for CAD and MI: Novel Insights

Currently, commercially available DNA arrays allow genotyping of up to 4,300,000 SNPs for statistical analysis [33]. To further increase the power of GWAS, an in-silico method called imputation can be used. This method, based on the overall haplotype structure of the human genome, allows for the inference of missing genotypes, harmonizes datasets for meta-analyses, and increases the overall number of markers available for association testing [34]. Based on the latest reference dataset from the 1000 Genomes Project [35], one can currently integrate more than 39 million variants for analysis [36].

Since 2007, ten single GWAS and four large-scale GWAS meta-analyses have identified 57 loci displaying genome-wide significance (Fig. 22.3). Most of these loci are characterized by common variants (minor allele frequency = 10–90 %) and small effect sizes (OR = 1.05–1.30) (Fig. 22.4). Overall, these loci explain ~15 % of the heritability of CAD and MI. For most of these loci, the underlying pathomechanism connecting the association signal to the disease is unknown. However, approximately one third of these loci display pleiotropic effects and associate with traditional risk factors, such as levels of low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and others [23].

Annotation of CAD Loci

In the last decade, GWAS have undoubtedly revolutionized the identification of genetic variants underlying an increased risk of complex diseases such as CAD. However, the functional interpretation of these loci has just begun [37]. The next step in unraveling the genetic causes of CAD is to analyze GWAS findings in detail, that is, to map the genetic loci to genes and pathways. Getting from GWAS loci to the disturbed genetic mechanisms is, however, not straightforward, and cannot be accomplished solely using GWAS data. Indeed, identifying the underlying genetic cause is even more difficult, as most SNPs associated with complex disease lie in the noncoding regions of the genome and may have an effect on more than one gene, including genes that are not necessarily close neighbors. Recent efforts to better understand the genet-

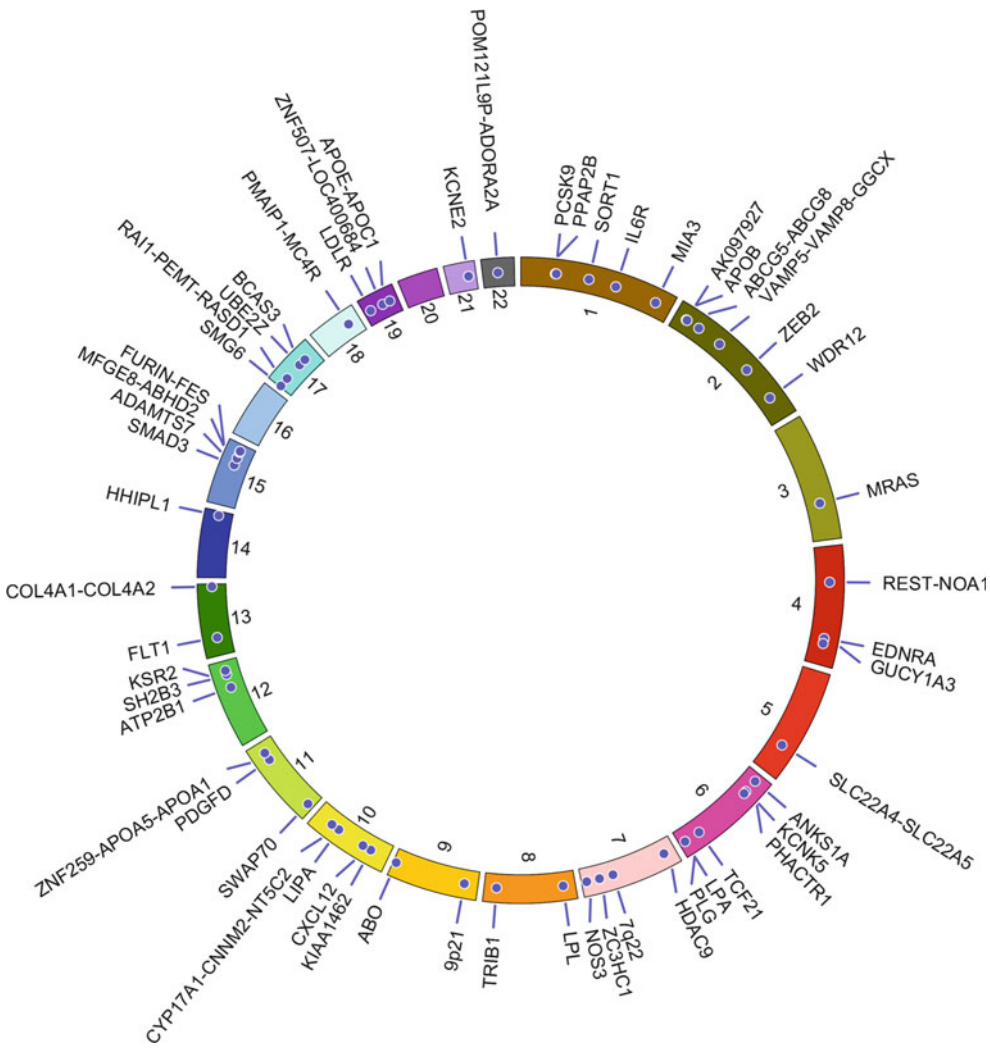

Fig. 22.3 Circos plot representing the genome-wide significant gene regions ($p < 5 \times 10^{-8}$) associated with CAD and MI (March 2016)

ics underlying GWAS signals resulted in a detailed annotation of CAD loci by combining different datasets. More and more publicly available "omics" datasets, for example, ENCODE data, have become available, facilitating in-depth loci characterization [38–40]. In a recent study performed by Brænne et al., all SNPs within known CAD loci (linkage disequilibrium, LD: r2 >0.8) were annotated with respect to their protein-coding sequence and influence on gene expression [41]. Influence on gene expression was estimated through quantitative trait loci, mi-RNA binding sites, or location within a promoter or other regulatory region. Based on the results of these in-silico studies (the workflow is depicted in Fig. 22.5), it is evident that most loci have an effect on regulatory gene function and do not act directly at the protein level. In addition, new genes have been functionally linked to known GWAS loci and a substantial number of genes, previously assigned to the loci (based on proximity), have not been validated [41]. Hence, systematic characterization of GWAS loci is necessary to better understand the disease pathways and develop new therapeutic treatment options [42].

Chromosome 9p21.3

Each C-allele of SNP rs1333049 (G/C, MAF 0.46 in HapMap CEU), representing the CAD/MI locus on 9p21.3, is associated with a 25 % increase in the risk for CAD/MI (95 % CI [1.16–1.35]). The high frequency of the risk allele (approximately 75 % of all individuals in a Caucasian population carry at least one risk allele) explains why the proportion of CAD/MI risk that can be attributed to carrying the rs1333049 C-allele is fairly high (22 %), even after adjustment for cardiovascular risk scores [29].

Pleiotropic Effects of Chromosome 9p21.3

Data show that this locus not only affects CAD/MI risk but also affects the risk of abdominal aortic aneurysm, intracranial aneurysm, peripheral arterial disease, and cardioembolic stroke in many populations [43]. In addition, Matarin et al. [44] and Gschwendtner et al. [45] reported that the 9p21.3

Region	Genes	Freq (%)	OR
6q25.3	LPA	0.013	1.64
6q25.3	LPA	0.055	1.37
9p21.3	CDKN2A/2B	0.489	1.21
22q11.23	POM121L9P	0.971	1.20
21q22.11	APOE	0.926	1.15
2q33	WDR12, NBEAL1	0.111	1.14
7q36.1	NOS3	0.065	1.14
19q13.31	SMARCA4, LDLR	0.900	1.14
6p24.1	PHACTR1	0.432	1.14
1p32.2	PPAP2B	0.915	1.13
11q23.3	APOA5–A4–C3–A1	0.132	1.13
1p13.3	SORT1	0.786	1.12
21q22.11	SLC5A3, KCNE2	0.130	1.12
2q22.3	ZEB2–AS1,ZEB2	0.088	1.10
6p24.1	PHACTR1	0.392	1.10
15q26.1	MFGE8, ABHD2	0.902	1.10
1q41	MIA3	0.654	1.08
17q23.2	BCAS3	0.147	1.08
9q34.2	ABO	0.192	1.08
10q24.32	CYP17A1	0.873	1.08
15q25.1	ADAMTS7	0.638	1.08
5q31.1	SLC22A4/A5	0.133	1.08
7q22.3	BCAP29	0.800	1.08
10q11.21	CXCL12	0.817	1.08
15q25.1	MORF4L1	0.564	1.08
1p32.3	PCSK9	0.848	1.08
3q22.3	MRAS	0.162	1.08
7p21.1	HDAC9	0.200	1.08
7q32.2	ZC3HC1	0.687	1.07
13q34	COL4A1/A2	0.761	1.07
4q31.22	EDNRA	0.795	1.07
8p21.3	LPL	0.845	1.07
10q23.31	LIPA	0.376	1.07
15q22.33	SMAD3	0.794	1.07
2p24.1	APOB	0.830	1.07
6p21.31	ANKS1A	0.750	1.07
6p21.2	KCNK5	0.807	1.07
6q23.2	TCF21	0.685	1.07
12q24.12	SH2B3, ATXN2	0.433	1.07
4q32.1	GUCY1A3	0.797	1.07
10q11.21	KIAA1462	0.418	1.06
11q22.3	PDGFD	0.331	1.06
6q26	PLG	0.720	1.06
2p21	ABCG5, ABCG8	0.294	1.06
4q12	NOA1, REST	0.215	1.06
2p11.2	VAMP5/8, GGCX	0.447	1.06
15q26.1	FURIN, FES	0.445	1.06
17p11.2	RASD1, PEMT	0.576	1.06
17q21.32	UBE2Z	0.530	1.06
18q21.32	PMAIP1,MC4R	0.257	1.06
14q32.2	HHIPL1	0.423	1.06
1q21.3	IL6R	0.447	1.06
11p15.4	SWAP70	0.550	1.06
17p13.3	SMG6	0.357	1.05
13q12.3	FLT1	0.341	1.05
8q24.13	TRIB1	0.538	1.05

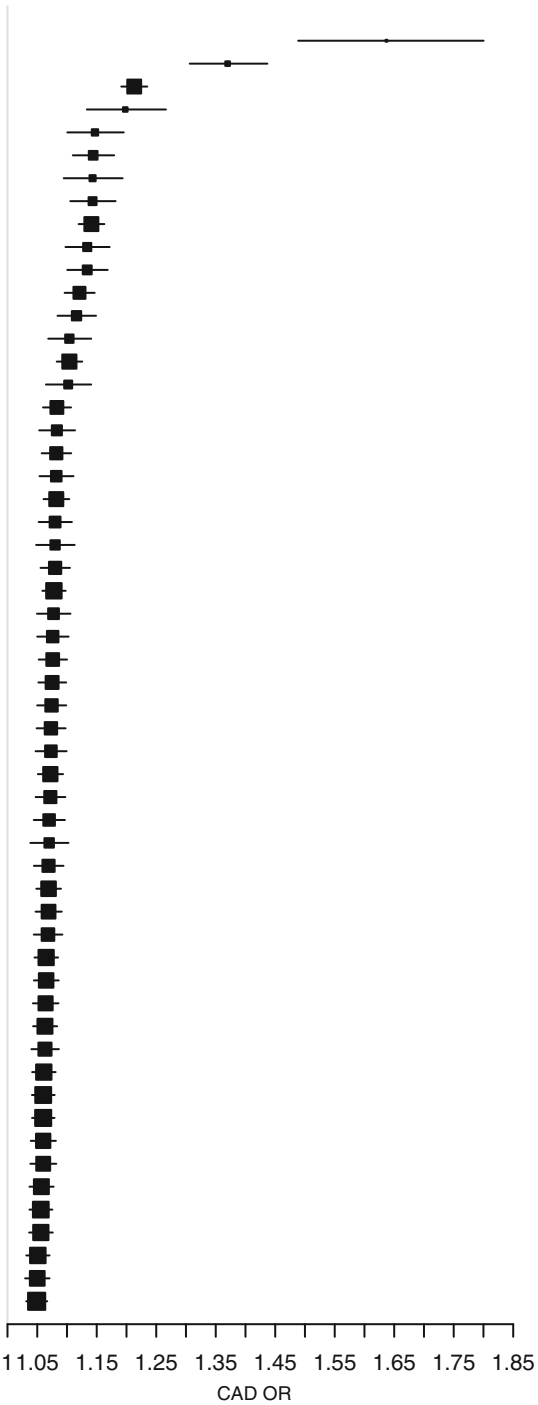

Fig. 22.4 All 56 common alleles genome-wide significantly associated with CAD. The figure shows the chromosomal location, genes in the vicinity, the frequency of the risk allele, and the odds ratio for CAD

region represents a major risk locus for atherosclerotic stroke [45]. The effect of this locus on stroke appears to be independent of its relationship to CAD and other stroke risk factors and further supports a broad role for the 9p21 region in arterial disease. Recently, additional associations were reported between common variants located in 9p21.3 as well as a broad range of phenotypes not directly connected to atherosclerosis. The spectrum of these diseases ranges from periodontitis to various human cancers, including glioma, basal cell carcinoma, and familial melanoma. Interestingly, the CDKN2A/2B tumor suppressor genes located in this region encode critical regulators of cell cycle and/or apoptosis. Of note, the CAD/MI risk haplotype does not appear to overlap completely with the cancer locus. While the associations of many of the phenotypes with 9p21.3 are only descriptive in nature, functional studies will help to unravel their relationship. For example, the same locus on chromosome 9p21.3 was also reported to be associated with type 2 diabetes mellitus (T2DM) in three out of five GWAS. However, more detailed studies revealed that neighboring LD blocks, but not the same SNPs, are responsible for T2DM (a risk factor for CAD) and CAD/MI (Fig. 22.6) [46].

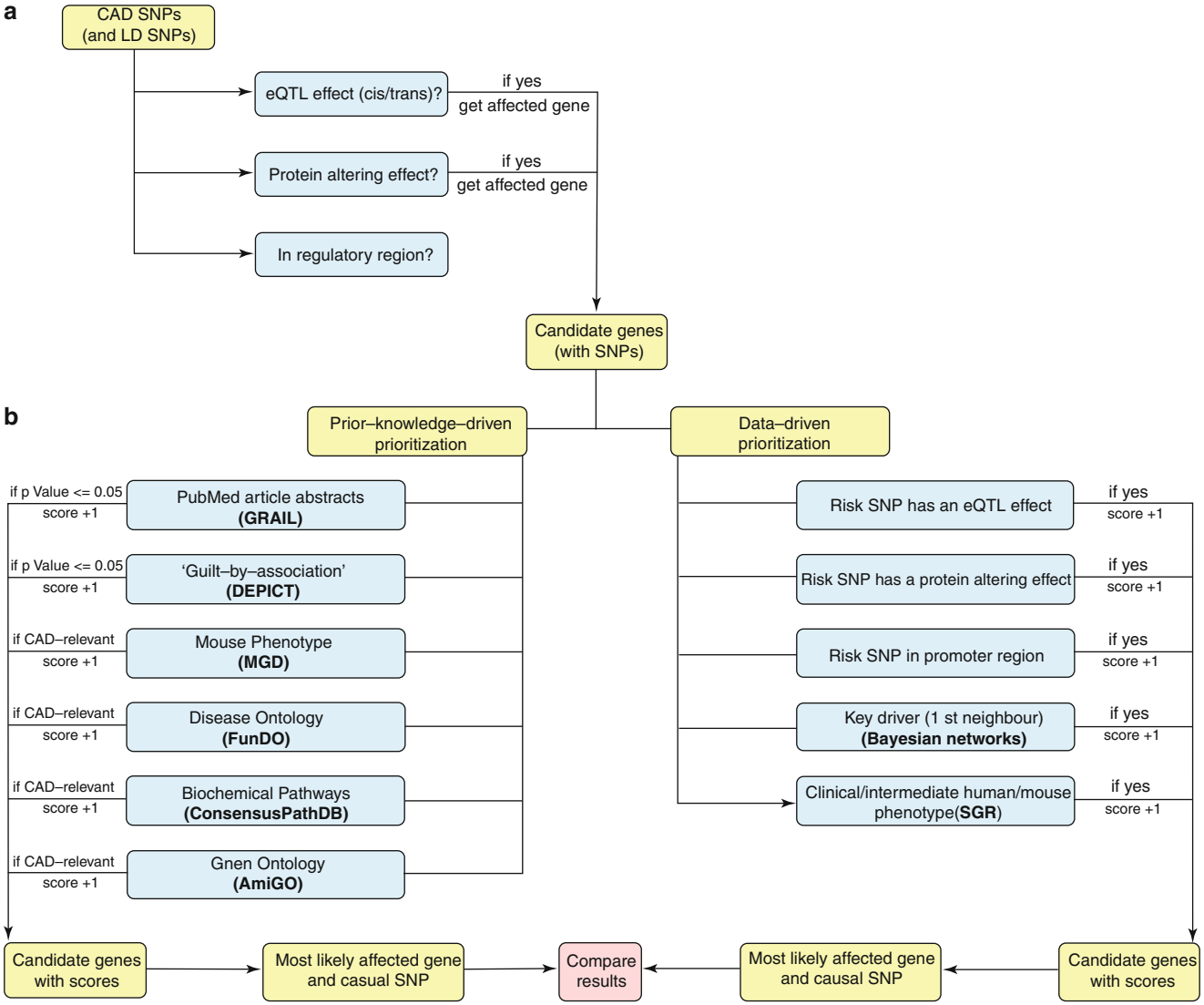

Fig. 22.5 Workflow of the bioinformatic pipeline to annotate SNPs and their potential functional impact. Detailed information can be found in Braenne et al. [41] (Figure taken from Braenne et al. [41])

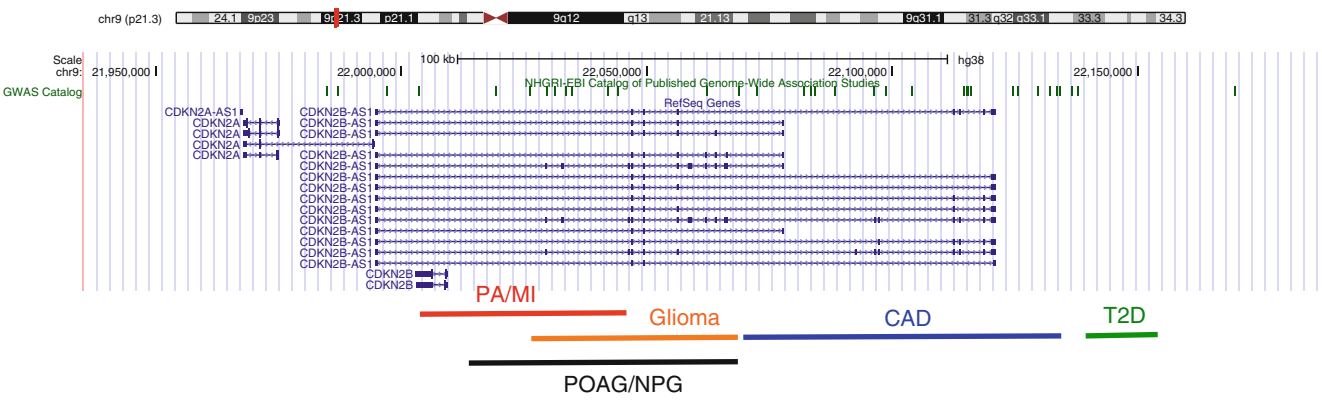

Fig. 22.6 The chromosome 9p21 locus haplotype structure, and pleiotropic effects (Modified from [46])

Pathophysiology Behind Chromosome 9p21.3

The first insights into the pathophysiological mechanisms of 9p21.3 in CAD/MI came from Broadbent et al. [47], who described lncRNA ANRIL, a long noncoding RNA that collocates with the CAD high-risk haplotype at chromosome 9p21.3. This transcript is expressed in tissues and cell types that are affected by atherosclerosis, and is therefore a prime candidate for the chromosome 9p21.3 CAD/MI locus [47]. Liu et al. [48] analyzed the expression of 9p21 transcripts in purified peripheral blood T-cells from healthy probands [48]. They found significantly reduced expression of all INK4/ARF transcripts (p15(INK4b), p16(INK4a), ARF, and ANRIL) in subjects with CAD, stroke, and aortic aneurysm, while the expression of methylthioadenosine phosphorylase (MTAP) was not influenced by the genotype. A more detailed analysis by Jarinova et al. [49]. using reporter gene expression analysis in primary aortic smooth muscle revealed that a conserved sequence within the 9p21.3 locus has enhancer activity [49]. Furthermore, whole blood RNA expression of short ANRIL variants was increased by 2.2-fold, whereas expression of the long ANRIL variant was decreased by 1.2-fold in healthy subjects homozygous for the risk allele. Moreover, relevant to atherosclerosis, genome-wide expression profiling demonstrated upregulation of gene sets modulating cellular proliferation in carriers of the risk allele. These results suggest that, in risk-allele carriers, the activity of an enhancer element is altered, thus promoting atherosclerosis by regulating expression of ANRIL, which in turn leads to altered expression of genes controlling cellular proliferation pathways. More insights into the pathogenetic mechanisms behind the chromosome 9p21.3 locus were reported by Visel et al.[50], who showed that deletion of the orthologous 70 kb noncoding CAD risk interval on mouse chromosome 4 affects the cardiac expression of neighboring genes, as well as the proliferation properties of vascular cells. Particularly, the cardiac expression levels of two genes near the noncoding interval, Cdkn2a and Cdkn2b, are severely reduced in chr4Δ70kb/Δ70kb mice, indicating that distant-acting gene regulators are located in the noncoding CAD risk interval. Primary cultures of chr4Δ70kb/Δ70kb aortic SMCs exhibited excessive proliferation and diminished senescence, a cellular phenotype consistent with accelerated CAD pathogenesis [50]. More recently, chromatin conformation capture analysis identified interactions between sequences at the 9p21.3 locus and sequences in the vicinity of the genes encoding CDKN2A and CDKN2B and MTAP in the short range, and between IFNW1 and interferon-α21 (IFNA21) in the long range, approximately one million base pairs upstream on chromosome 9 [51]. This finding is remarkable because it suggests that the influence of the enhancer sequences at 9p21.3 acts at considerably greater distances than previously thought. A recent review by Holdt and Teupser [52] describes in more detail the emerging concept suggesting that *ANRIL* might constitute a regulator of epigenetic modification and thus modulate cardiovascular risk [52]. However, at present (almost 10 years after the first report) the underlying pathomechanism explaining the strong association between SNPs at the 9p21 locus and CAD, as well as other diseases, is still not fully understood [53].

SORT1: LDL-C and Beyond

A locus in 1p13.3, represented by the SNP rs599839, was initially identified through a GWAS for CAD [29]. Interestingly, this locus has been linked with LDL-C in several other studies [54]. A minor allele in European populations (A/G, MAF 0.28 in HapMap CEU), SNP rs599839 is associated with a lower risk of CAD and lower levels of LDL-C. SNP rs599839 is responsible for approximately 1 % of the variation in circulating LDL-cholesterol levels, equivalent to more established genes for LDL regulation, particularly APOE. SNP rs599839 lies in an approximately 97-kb large haplotype block on 1p13.3. This chromosomal region harbors four genes: proline/serine-rich coiled coil protein 1 (PSRC1), cadherin EGF LAG seven-pass G-type receptor 2 (CELSR2), myosin-binding protein H-like (MYBPHL), and sortilin 1 (SORT1). The hepatic mRNA expression levels of PSRC1, CELSR2, and SORT1 were shown to correlate with LDL-C plasma levels in a mouse model of cardiovascular disease as well as in a human cohort. The CAD risk allele (A) was associated with lower levels of CELSR2 and SORT1 expression and with higher LDL-C levels. Both genes fall into the category of cell surface receptor-linked signal transduction [55]. SORT1 is a transmembrane receptor protein that binds to a variety of different ligands and is involved in the endocytosis and intracellular degradation of lipoprotein lipase (LPL) [56], a rate-limiting enzyme of triglyceride hydrolysis in lipoproteins. Recently, SORT1 was also linked to the endocytosis of APOA-V-containing chylomicrons [57]. Studies from our group confirmed association of the G allele of SNP rs599839 with higher sortilin mRNA levels in whole blood RNA [58]. Furthermore, we showed that overexpression of sortilin in transfected cells leads to increased uptake of LDL particles into these cells. One possible explanation for association of the chromosome 1p13 variant with LDL-C and CAD might therefore be increased sortilin expression leading to enhanced LDL uptake into tissues, which in turn results in lower LDL-C levels and subsequently lower risk of CAD [58]. Musunuru et al. reported that a common noncoding polymorphism at the 1p13 locus, rs12740374 (in high LD with rs599839), creates a C/EBP (CCAAT/enhancer-binding protein) transcription factor binding site and alters hepatic expression of the SORT1 gene. Moreover, small interfering RNA (siRNA) knockdown and viral

overexpression in mouse liver demonstrated that Sort1 alters plasma LDL-C and very low-density lipoprotein (VLDL) particle levels by modulating hepatic VLDL secretion. Thus, Musunuru et al. provided functional evidence of a novel regulatory pathway for lipoprotein metabolism and suggested that modulation of this pathway may alter risk for MI in humans [59]. However, sortilin also appears to be involved in the development of atherosclerosis by mechanisms not directly involving LDL cholesterol, but possibly resulting from the attenuated secretion of proinflammatory cytokines, such as IL6 and TNFα, which accompanies sortilin deficiency in immune cells. In conclusion, the data indicate that sortilin plays an important role in the development of cardiovascular disease and functions beyond regulating LDL-cholesterol levels [60].

ADAMTS-7: Protective Role in Atherosclerosis

At the ADAMTS-7 locus, the major allele (A) of the most significant SNP identified, rs3825807, was associated with an 8 % increase in the odds of developing CAD [61]. Interestingly, in subgroup analysis of MI and angiographic CAD phenotypes, this variant showed a greater association with atherosclerosis than MI (OR = 1.20 vs. 1.08) [61, 62]. Additional GWAS reported the association of ADAMTS-7 with human coronary calcification [63]. Variant rs3825807 is a nonsynonymous polymorphism, with an adenine (A) to guanine (G) change resulting in a serine (Ser) to proline (Pro) substitution in the pro-domain of ADAMTS-7. ADAMTS-7 is a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family member. Vascular smooth muscle cells (VSMCs) are key cells in migration and proliferation during the development of atherosclerosis, calcification, and restenosis. Previous studies sought to understand the underlying pathomechanisms involving ADAMTS-7 in VSMCs and their possible role in vascular disorders. Wang et al. reported previously the involvement of ADAMTS-7 in VSMC migration [64]. ADAMTS-7 was shown to promote neo-intima formation upon arterial injury through the degradation of cartilage oligomeric matrix protein (COMP). Furthermore, a recent study showed that rs3825807 has an effect on ADAMTS-7 maturation, thrombospondin-5 cleavage, and VSMC migration, as well as plays a potentially protective role against atherosclerosis and CAD [65]. Also, it was recently shown that ADAMTS-7 promotes VSMC and aortic calcification by altering the balance between the osteogenic protein BMP-2 and its natural inhibitor COMP [66]. Thus, based on these studies, there is increasing evidence linking ADAMTS-7 with VSMC function, which may explain, at least in part, its role in the development of atherosclerosis and vascular remodeling as well as calcification. However, the precise mechanisms linking ADAMTS-7 in vivo with vascular remodeling and atherosclerosis remain unclear. Very recently, a study conducted by our group employed ADAMTS-7 knockout (KO) mice to reveal the role of ADAMTS-7 in vascular remodeling. We demonstrated that Adamts-7-deficient mice are resistant to neo-intima formation after vascular injury. Thrombospondin-1 was identified as a potential substrate for ADAMTS-7 using liquid chromatography-tandem mass spectrometry secretome analysis. Furthermore, we demonstrated that the C-terminus of ADAMTS-7 directly associates with and degrades thrombospondin-1 in vivo and in vitro [67]. Interestingly, at the same time, another group published findings on the protective effects of ADAMTS-7 inhibition on atherosclerosis in mice lacking Adamts-7 in an atherogenic background (Apoe-KO and/or LDL-KO) [68]. Thus, all findings reported thus far pinpoint a key role of ADAMTS-7 in VSMC function. ADAMTS-7 deficiency is beneficial for atherosclerosis and vascular remodeling, and its inhibition might represent a promising therapeutic target.

ZC3HC1: A Single Nonsynonymous SNP Hit

This locus on chromosome 7 was reported in 2011 and contains only one genome-wide significant SNP, rs11556924 ($p = 2.22 \times 10^{-9}$, C to T), associated with CAD/MI [61]. rs11556924 lies within the coding region of the gene *ZC3HC1*, and there are no other SNPs in high LD (Fig. 22.7). The wild-type allele, C, is associated with a higher risk of CAD/ MI, and the minor allele, T, has a frequency of 15.58 %. The SNP is nonsynonymous, causing substitution of arginine with histidine in the encoded protein at position 363. Although there is an eQTL effect of the SNP rs11556924 on the neighboring gene *KLHDC10* [69], *ZC3HC1* is believed to be the better candidate gene for functional analyses, as the SNP leads to an amino acid exchange in the encoded protein [41].

ZC3HC1 encodes the protein NIPA (Nuclear interaction partner of ALK), which is involved in cell cycle control. It was first described in 2003 as a possible nuclear downstream target of the oncogenic tyrosine kinase nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) [70]. Although NIPA directly binds NPM-ALK, it is not phosphorylated by this kinase. In subsequent studies, NIPA was found to be part of an SCF-type E3-ubiquitin ligase that interacts with cyclin B1 [71]. Cyclin B1 is a cell cycle regulating protein. During interphase, cyclin B1 is localized in the cytoplasm. Prior to mitosis, it is upregulated and transported to the nucleus. SCF-NIPA contributes to the regulation of cyclin B1 by targeting nuclear cyclin B1 and leading to its degradation [71]. Upon entry into M-phase, cyclin B1 enters the nucleus, whereupon NIPA is inactivated through phosphorylation.

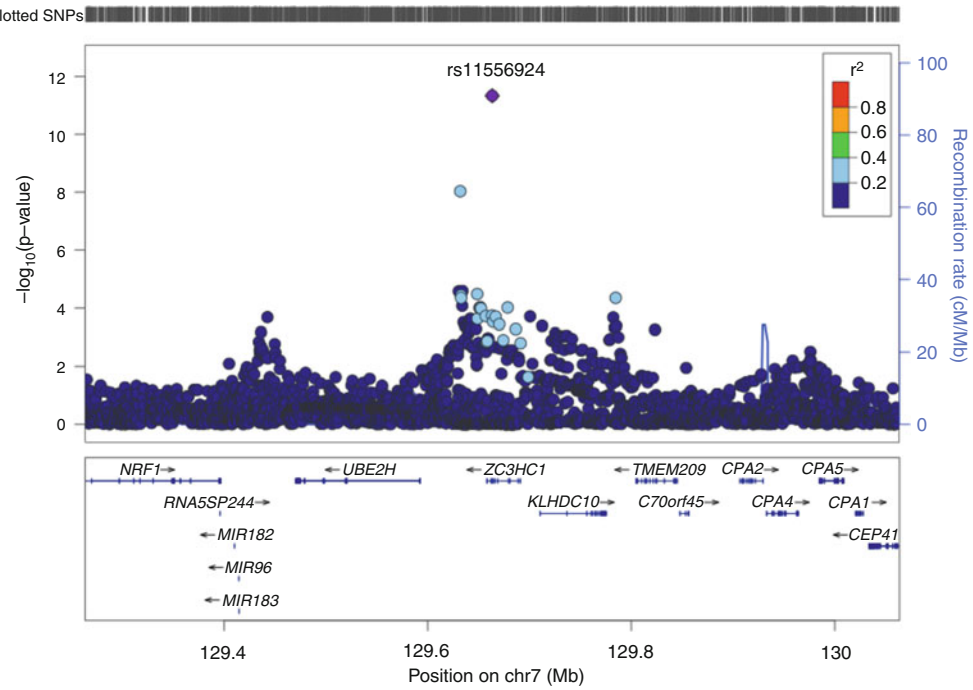

Fig. 22.7 Association plot for rs11556924 on human chromosome 7 based on 1000G imputed meta-analysis data (Nikpay et al. [23]). No further SNPs in high LD have been reported. The SNP rs11556924 is a missense variant leading to the replacement of arginine with histidine at position 363 (p.R363H)

After inactivation of NIPA, cyclin B1 can carry out its function in promoting early events in mitosis [72].

Recent publications linked rs11556924 to hypertension in a Finnish population [73] and to a greater carotid intima-media thickness (cIMT) [74]. Another study in a Japanese population reported the SNP to be associated with atrial fibrillation, a common consequence of CAD/MI [75]. López-Mejías et al. found the homozygous nonrisk genotype to be associated with higher cIMT, an indicator of atherosclerosis. Yamase et al. also found the nonrisk allele to represent a risk factor for atrial fibrillation, whereas Kunnas et al. reported the homozygous risk genotype to be correlated with higher blood pressure. Although these publications appear to report contradictory results, they consolidate the link between the locus and CVD.

Chromosome 6q26–27: Haplotype Approach Links Risk SNPs to Lp(a)

Using a genome-wide haplotype approach, we were able to identify the SLC22A3-LPAL2-LPA gene cluster as a strong susceptibility locus for CAD [76]. Two haplotypes consisting of four SNPs (rs2048327 in the SLC22A3 gene, rs3127599 in the LPAL2 gene, and rs7767084 and rs10755578 in the LPA gene) were consistently associated with CAD/MI risk (CTTG haplotype, OR = 1.2 [95 % CI, 1.13–1.28]; CCTC haplotype, OR = 1.82 [95 % CI, 1.57–2.12]). Interestingly, this locus was not identified in previous GWAS that focused on univariate analyses of SNPs. The proposed approach in the paper by Tregouet et al. may have wide utility for analyzing GWA data for other complex traits. The haplotype association analysis was performed using a sliding-windows approach. The locus partly overlaps the LPA gene, which encodes apolipoprotein(a), the main protein of lipoprotein(a) (Lp(a)), a well-known risk factor for CAD. Indeed, Tregouet et al. showed that the haplotypes associated with CAD were also associated with the highest Lp(a) levels, and, after adjustment for Lp(a) levels, were no longer associated with CAD, suggesting that their relation to risk is mediated by an effect on Lp(a) levels. Genetic variants, particularly a kringle repeat polymorphism, also affect the size of Lp(a) particles, and recent studies suggested that small Lp(a) particle size may be an independent risk marker. In light of these findings, Clarke et al. identified new risk variants (rs10455872 and rs3798220) with low allele frequency but strong effects on CAD (2.5-fold risk increase in individuals who carried at least two of these risk alleles) [77].

Genetic Architecture of (Premature) Coronary Artery Disease

Interestingly, the genetic components reflected by the multiple genetic variants identified in GWAS cannot explain familial clustering of the disease as indicated by a positive family history (Fig. 22.8). As mentioned, the variants identified in GWAS were characterized by a high allele frequency (i.e., each individual in a population is affected by multiple risk alleles to a greater or lesser degree) and a small effect size (i.e., only the cumulative effect of multiple variants may be of clinical relevance). A positive family history, on the other hand, appears to be mediated by rare deleterious muta-

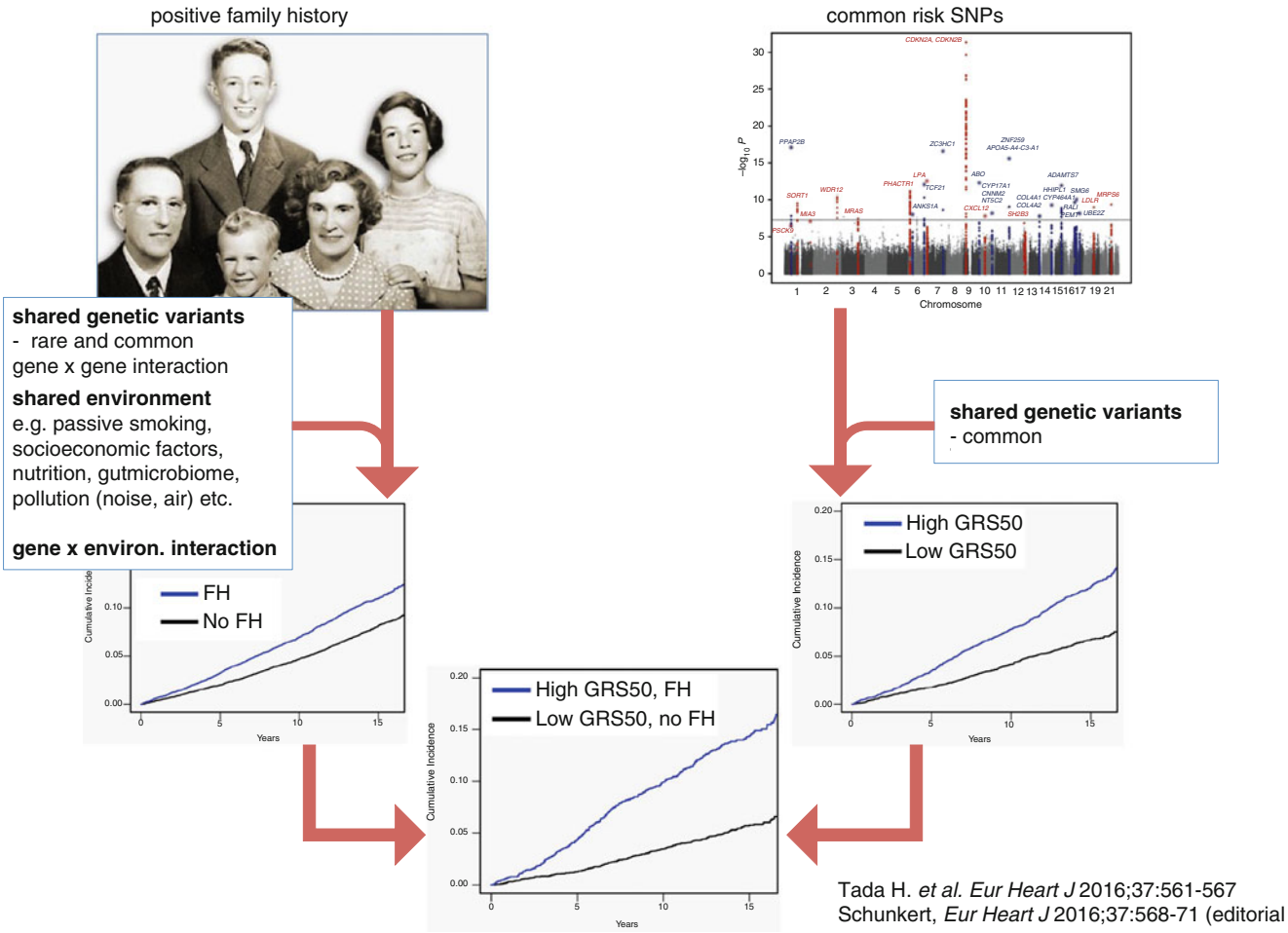

Fig. 22.8 Positive family history (FH) and common risk alleles (genetic risk score, GRS 50) confer additive information in predicting CAD risk (Figure taken from Schunkert et al [78])

tions with a more profound effect [79, 80] or by specific interactions between more common genetic variants (epistasis) [81, 82]. Not surprisingly, the heritability of CAD and MI is only partially explained by currently known risk alleles [83, 84].

Remarkably, there appears to be a marked overlap between the few genes that showed cosegregation in family-based studies and those identified in GWAS. Indeed, almost all genes causing monogenic forms of CAD or MI also produce a signal in GWAS (i.e., *GUCY1A3*, *LDLR*, *PCSK9*, *APOB*, and *LPA*), giving rise to an allelic series ranging from rare, damaging alleles with profound effects to common alleles with mild effects. While monogenic forms are explained by rare damaging mutations in the coding sequence (Fig. 22.9: upper corner on the left side), GWAS signals most often arise from frequent variants with smaller effects in the very same genes or their regulatory regions (Fig. 22.9: lower corner of the right side) [15, 16, 22, 86].

Genetic Variants and Relevance for Therapeutic Development

Although over the last decades, much research has focused on the identification of biological risk factors and developing medicines to modify them, actually only few medicines (e.g., aspirin, statins, and anti-hypertensive agents) have been proven to reduce the risk of CAD or MI. A novel promising approach for identifying potential therapeutical targets for drug development is through leveraging the human genome. This is based on the presence of naturally occurring genetic variations that can be found in nearly every gene, including genes that are potential drug-specific targets. If these deoxyribonucleic acid (DNA) sequence variation modulate the function or expression of a gene, then the phenotypic consequences of this variation in the human population could predict whether a drug will safely reduce disease risk. Of significant interest are so-called "human knockouts" [87], people with biallelic LOF muta-

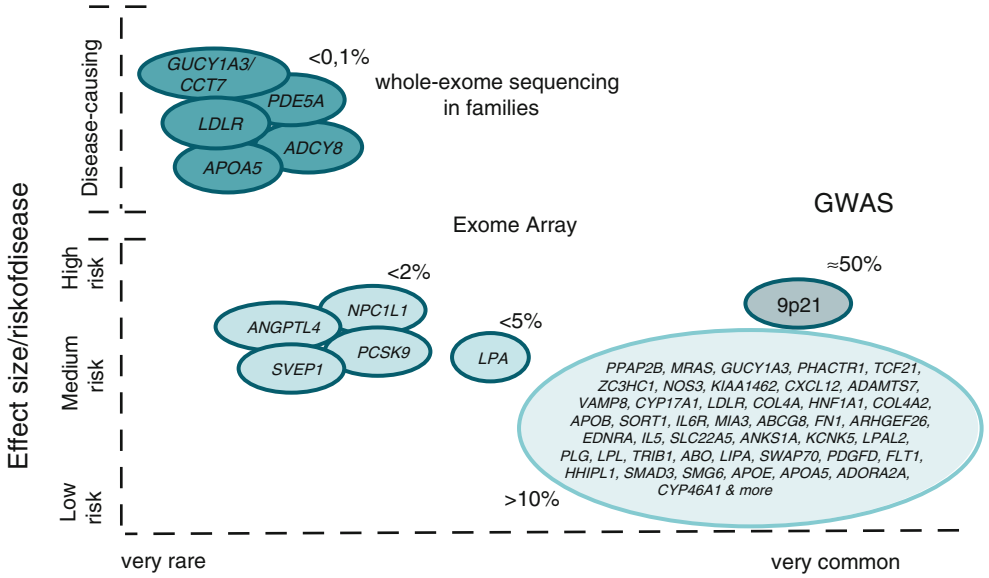

Fig. 22.9 Summary of the genetic architecture of CAD and MI. Rare, coding variants in GUCY1A3, ADCY8, PDE5A, LDLR, and APOA5 have been identified by whole-exome sequencing (WES) in affected families. These variants confer large risk effects, displaying an almost monogenic inheritance pattern [15]. Low-frequency variants in ANGPTL4, NPC1L1, PCSK9, and SVEP1 have been identified by WES or large-scale genotyping of the exome array [85]. Common variants in more than 50 genetic loci, conferring a low-risk effect, have been identified by GWAS (Kessler [84] #2205)

Table 22.1 Examples of genes affecting CAD and MI risk identified by large-scale array-based and deep-sequencing projects with relevance for therapeutic development

Gene	PCSK9	NPC1L1	LPA	LPL	APOC3	ANGPTL4	ASGR1
Frequency	1 in 50 blacks	1 in 150	1 in 13	1 in 10	1 in 150	1 in 500	1 in 120
Phenotype	LDL	LDL	Lp(a)	TG	TG	TG	non-HDL-C
Risk	80 % lower risk	53 % lower risk	14 % higher risk	17 % lower risk	40 % lower risk	57 % lower risk	34 % lower risk
Therapy	Evolocumab Bococizumab Alirocumab	Ezetimibe	Antisense in development	?	Antisense in development	Monoclonal antibodies in development	?
References	[88]	[89]	[77]	[85]	[90]	[85, 91]	[92]

LPL lipoprotein lipase, *TG* triglycerides

tions. This innovative approach – the druggable genome – has now been applied to several drug-gene pairs. Some of these drugs are already in clinical use (such as ezetimibe, targeting NPC1L1, and statins, targeting HMGCR), and some are in development (including drugs targeting PCSK9, APOC3, CETP, and just very recently ASGR1) (Table 22.1) [93].

General Lessons from Modern CAD Genetics

A summary of the current data on genes affecting the risk of MI/CAD is as follows:

- The precise mechanisms linking chromosomal loci and disease manifestation are still unclear for almost all of the loci. However, it is foreseeable that mechanistic insights will be gained in the near future.
- Traditional risk factors mediate risk only partially, since the majority of loci (2/3) display no association with intermediate phenotypes.
- The genetic risk conferred by newly discovered loci is independent of the risk conferred by a positive family history. Thus, the molecular-genetic information for risk prediction goes beyond that of all traditional risk factors.
- Each of the currently known common risk alleles increases the probability of CAD by a relatively small margin, that is, 5–30 % per allele. In other words, individuals who are homozygous for the risk allele on chromosome 9p21.3 carry a 50 % increased risk compared with the 25 % of the European population who do not carry this allele.
- The high frequency of risk alleles, on the other hand, explains why the implications of the recently identified genetic factors at the population level are substantial, even though an affected individual carries only a relatively moderate risk increase.

This wealth of new information on the heritable aspects of CAD opens multiple avenues for scientific exploration. From a clinical point of view, the immediate needs concern risk prediction and (preventive) therapy for atherosclerosis (see the next section).

Is Genetic Risk Prediction Feasible?

While CAD is a chronic process, its clinical manifestation may occur suddenly and fatally in the form of a MI. Thus, there is a strong clinical demand for predicting disease onset and (preventive) therapy for atherosclerosis. A simplified view of genetic risk calculation could be a count of risk alleles, similar to the quantitative assessment of cholesterol levels in a population. The underlying assumption, that risk conferred by some alleles can be balanced by "protective" alleles at other loci, does not take into account that biological mechanisms, as well as effect sizes, at various loci are likely to be different. Thus, the development of GRS needs careful prospective testing. An open question for estimating genetic risk is the definition of a "control sample" free of genetic predisposition. The number of known chromosomal loci implicated in CAD is growing constantly, and the frequency of most of these alleles is high. Even in a "healthy" Western European population sample, the average number of currently known risk alleles is >50. Thus, if a group of individuals does not carry a specific risk allele, it cannot be expected that their genetic risk is "zero" but rather at the "population average." This population average is, however, heavily inflated by a multitude of perhaps untested genetically predisposing factors, minus the effect of the tested allele. Vice versa, the effect of a traditional risk factor, such as smoking, in a person who luckily does not carry any genetic predisposition, that is, none of the CAD risk alleles, cannot be known. Is it outrageous to hypothesize that CAD could be eliminated altogether if the effects of susceptibility genes could be entirely neutralized? In this respect, it may be noteworthy that some mammalian species (mice) or vascular beds (internal mammary artery) do not develop atherosclerosis; in other words, even the presence of multiple established risk factors does not automatically result in the manifestation of CAD. Currently, only a relatively limited fraction (~15 %) of the overall genetic risk (heritability) of CAD is explained by the identified loci. This can be partly explained by the limited power of individual GWAS to detect such loci. Global consortia (such as CARDIoGRAM and, later, CARDIoGRAM+C4D) are in the process of analyzing genome-wide information from more than 80,000 cases of CAD and over 120,000 controls, and have identified additional loci harboring even more common variants. Moreover, an increasing effort has been made to elucidate the role of rare variants, and this has been aided by the novel information on such variants coming out of the 1000 Genomes Project (http:// www.1000genomes.org) and genotyping the exome array [85]. In parallel, statistical methods have been developed that make use of SNPs for risk prediction even when their statistical level of association with disease does not reach the conservative genome-wide significance threshold of $p < 5 \times 10^{-8}$. These algorithms take into account that analysis of all SNPs with association at significance levels of $p < 5 \times 10^{-6}$ or $p < 5 \times 10^{-5}$ will include multiple falsely associated SNPs. However, the predictive information derived from the large number of remaining truly associated SNPs may go far beyond the information derived from the relatively few "established" SNPs. Together, genetic susceptibility for MI, as well as for related risk factors, will soon become more transparent. In practical terms, the challenge is to utilize genomic information for the refinement of clinically utilized risk scores. These scores are largely dominated by the predictive information of age and gender and based on prediction of short-term risks. It is obvious that a man in his 70s has a higher risk than a young woman over the next 10 years, regardless of the genetic risk burden these two subjects may carry. The clinically relevant question is, what difference do genetic factors make in refining risk prediction in patients with similar characteristics (e.g., two middle-aged men) to better target future preventive measures? Epidemiological studies with prospective DNA and data collection are ongoing to address these clinically important issues. The first promising results have been published already: a study of the placebo arms of four statin therapy trials demonstrated that GRS adequately stratified CAD risk (HR for intermediate risk vs. low risk = 1.34; 95 % CI, 1.22–1.47; HR for high-risk vs. low risk = 1.72; 95 % CI, 1.55–1.92). The analysis of the so-called ASCOT trial reported that individuals at high genetic risk for CHD derive greater benefit from statin therapy than those at low genetic risk. Indeed, the number needed to treat to prevent one event was 100 in those with low genetic risk, but 33 in those with high genetic risk [94]. Such prospective studies will help to understand the degree to which molecular-genetic prediction of CAD can improve personalized risk assessment beyond that approximated from family history or risk scores such as the Framingham or Euro Score.

Cardiovascular System Genetics

System genetics offer the potential to provide new insights into our understanding of the pathogenesis of CAD. The rationale for this approach is based on the hypothesis that an interacting network precipitates complex diseases such as atherosclerosis. Players in this network modulate one another at multiple levels, including the genome, transcriptome (mRNA and miRNAs), methylome/epigenome, proteome, and metabolome. The challenge is to comprehend the connections and interactions between individual con-

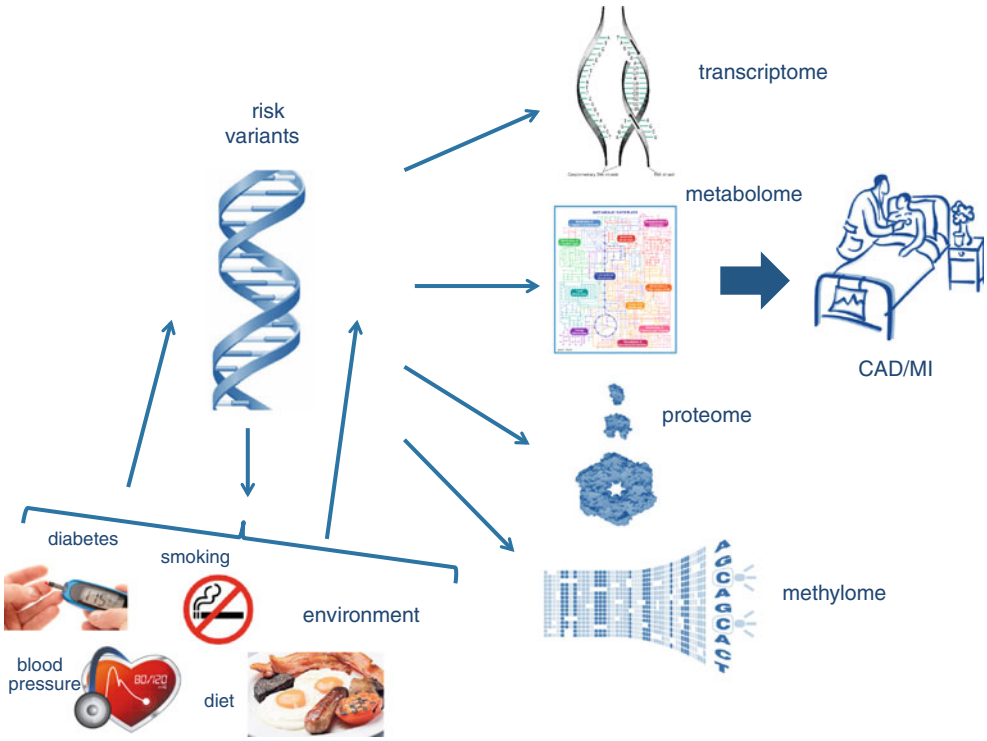

Fig. 22.10 Integrative view of genetic risk variants affecting gene expression or function in the context of traditional risk factors and hitherto unspecified environmental cofactors. Ultimately, biological networks may malfunction resulting in the precipitation of CAD

stituents of this network. Specifically, it is of great interest to understand the communication between genetic (SNPs, CNVs), traditional, and environmental risk factors (SNPs, CNVs) at the level of the cell, tissue, and organ to ultimately describe the entire organism with respect to its predisposition to develop disease (Fig. 22.10). The intention is to identify the biological networks that connect the different system elements, thereby defining the characteristics that describe the overall system. This information can then be used to derive mechanistic information on biological processes as well as identify potential target sites for therapeutic intervention [37, 95, 96].

Summary/Take Home Message

Molecular-genetic approaches applied to the study of CAD will continue to identify genes and pathways involved in predisposition to and pathophysiology of this often life-threatening condition.

Moreover, future gene expression profiling studies will refine our understanding of the nature of atherosclerotic lesions within the vascular wall and promise discovery and validation of targets for therapeutic intervention.

Opportunities to translate genetic, genomic, proteomic, and metabolomic information into cardiovascular clinical practice have never been greater, but their implementation requires validation in large independent cohorts, which can be achieved only through collaborative effort, such as CARDIoGRAM [61] or CARDIoGRAMplusC4D [23]. Their continued success will depend on ongoing cooperation within the cardiovascular research community.

Advice for the Clinical Practise

Based on recent studies, risk prediction using GRS is feasible; however, the use of a GRS in clinical practice for risk reclassification in intermediate risk patients is not yet routine.

Genetic analysis (either specific SNPs or specific rare molecular gene defects) in patients with a strong family history of CAD after exclusion of familial hypercholesterolemia or in unaffected relatives is not yet recommended in a clinical settings, because individual findings are still difficult to interpret. However, in a research setting, identification and analysis of patients with a strong family history are of utmost importance to further unravel the pathogenesis of the disease.

References

1. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, et al. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. Circulation. 2014;129(3): e28–e292.
2. Lusis AJ. Atherosclerosis. Nature. 2000;407(6801):233–41.

3. Müller C. Angina pectoris in hereditary xanthomatosis. Arch Intern Med. 1939;64(4):675–700.
4. Schunkert H, Gotz A, Braund P, McGinnis R, Tregouet DA, Mangino M, et al. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. Circulation. 2008;117(13):1675–84.
5. Murabito JM, Pencina MJ, Nam BH, D'Agostino Sr RB, Wang TJ, Lloyd-Jones D, et al. Sibling cardiovascular disease as a risk factor for cardiovascular disease in middle-aged adults. JAMA. 2005;294(24):3117–23.
6. Myers RH, Kiely DK, Cupples LA, Kannel WB. Parental history is an independent risk factor for coronary artery disease: the Framingham Study. Am Heart J. 1990;120(4):963–9.
7. Lloyd-Jones DM, Nam BH, D'Agostino Sr RB, Levy D, Murabito JM, Wang TJ, et al. Parental cardiovascular disease as a risk factor for cardiovascular disease in middle-aged adults: a prospective study of parents and offspring. JAMA. 2004;291(18):2204–11.
8. Horne BD, Camp NJ, Muhlestein JB, Cannon-Albright LA. Identification of excess clustering of coronary heart diseases among extended pedigrees in a genealogical population database. Am Heart J. 2006;152(2):305–11.
9. Hengstenberg C, Holmer SR, Mayer B, Engel S, Schneider A, Lowel H, et al. Siblings of myocardial infarction patients are overlooked in primary prevention of cardiovascular disease. Eur Heart J. 2001;22(11):926–33.
10. Andresdottir MB, Sigurdsson G, Sigvaldason H, Gudnason V, Reykjavik CS. Fifteen percent of myocardial infarctions and coronary revascularizations explained by family history unrelated to conventional risk factors. The Reykjavik Cohort Study. Eur Heart J. 2002;23(21):1655–63.
11. Hawe E, Talmud PJ, Miller GJ, Humphries SE. Second Northwick Park Heart S. Family history is a coronary heart disease risk factor in the Second Northwick Park Heart Study. Ann Hum Genet. 2003;67(Pt 2):97–106.
12. Boer JM, Feskens EJ, Verschuren WM, Seidell JC, Kromhout D. The joint impact of family history of myocardial infarction and other risk factors on 12-year coronary heart disease mortality. Epidemiology. 1999;10(6):767–70.
13. Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. N Engl J Med. 1994;330(15):1041–6.
14. Wang L, Fan C, Topol SE, Topol EJ, Wang Q. Mutation of MEF2A in an inherited disorder with features of coronary artery disease. Science. 2003;302(5650):1578–81.
15. Erdmann J, Stark K, Esslinger UB, Rumpf PM, Koesling D, de Wit C, et al. Dysfunctional nitric oxide signalling increases risk of myocardial infarction. Nature. 2013;504(7480):432–6.
16. Stitziel NO, Peloso GM, Abifadel M, Cefalu AB, Fouchier S, Motazacker MM, et al. Exome sequencing in suspected monogenic dyslipidemias. Circ Cardiovasc Genet. 2015;8(2):343–50.
17. Schunkert H, Bourier F. Deciphering unexplained familial dyslipidemias: do we have the tools? Circ Cardiovasc Genet. 2015;8(2):250–2.
18. Lieb W, Mayer B, Konig IR, Borwitzky I, Gotz A, Kain S, et al. Lack of association between the MEF2A gene and myocardial infarction. Circulation. 2008;117(2):185–91.
19. Guella I, Rimoldi V, Asselta R, Ardissino D, Francolini M, Martinelli N, et al. Association and functional analyses of MEF2A as a susceptibility gene for premature myocardial infarction and coronary artery disease. Circ Cardiovasc Genet. 2009;2(2):165–72.
20. Wobst J, Kessler T, Dang TA, Erdmann J, Schunkert H. Role of sGC-dependent NO signalling and myocardial infarction risk. J Mol Med. 2015;93(4):383–94.
21. Hanafy KA, Martin E, Murad F. CCTeta, a novel soluble guanylyl cyclase-interacting protein. J Biol Chem. 2004;279(45):46946–53.
22. Consortium CAD, Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet. 2013;45(1):25–33.
23. Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nat Genet. 2015;47(10):1121–30.
24. Wobst J, Rumpf PM, Dang TA, Segura-Puimedon M, Erdmann J, Schunkert H. Molecular variants of soluble guanylyl cyclase affecting cardiovascular risk. CircJ. 2015;79(3):463–9.
25. Fischer M, Broeckel U, Holmer S, Baessler A, Hengstenberg C, Mayer B, et al. Distinct heritable patterns of angiographic coronary artery disease in families with myocardial infarction. Circulation. 2005;111(7):855–62.
26. Fischer M, Mayer B, Baessler A, Riegger G, Erdmann J, Hengstenberg C, et al. Familial aggregation of left main coronary artery disease and future risk of coronary events in asymptomatic siblings of affected patients. Eur Heart J. 2007;28(20):2432–7.
27. Broeckel U, Hengstenberg C, Mayer B, Maresso K, Gaudet D, Seda O, et al. A locus on chromosome 10 influences C-reactive protein levels in two independent populations. Hum Genet. 2007;122(1):95–102.
28. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, et al. Complement factor H polymorphism in age-related macular degeneration. Science. 2005;308(5720):385–9.
29. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, et al. Genomewide association analysis of coronary artery disease. N Engl J Med. 2007;357(5):443–53.
30. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al. A common allele on chromosome 9 associated with coronary heart disease. Science. 2007;316(5830):1488–91.
31. Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science. 2007;316(5830):1491–3.
32. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447(7145):661–78.
33. Xing C, Huang J, Hsu YH, DeStefano AL, Heard-Costa NL, Wolf PA, et al Evaluation of power of the Illumina HumanOmni5M-4v1 BeadChip to detect risk variants for human complex diseases. Eur J Hum Genet. 2016;24(7):1029–34.
34. Marchini J, Howie B. Genotype imputation for genome-wide association studies. Nat Rev Genet. 2010;11(7):499–511.
35. The Genomes Project C. A global reference for human genetic variation. Nature. 2015;526(7571):68–74.
36. Porcu E, Sanna S, Fuchsberger C, Fritsche LG. Genotype imputation in genome-wide association studies. Curr Protoc Hum Genet. 2013;Chapter 1:Unit 1 25.
37. Bjorkegren JL, Kovacic JC, Dudley JT, Schadt EE. Genome-wide significant loci: how important are they? Systems genetics to understand heritability of coronary artery disease and other common complex disorders. J Am Coll Cardiol. 2015;65(8):830–45.
38. Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, et al. Systematic localization of common disease-associated variation in regulatory DNA. Science. 2012;337(6099):1190–5.
39. Nicolae DL, Gamazon E, Zhang W, Duan S, Dolan ME, Cox NJ. Trait-associated SNPs are more likely to be eQTLs: annotation to enhance discovery from GWAS. PLoS Genet. 2010;6(4):e1000888.
40. Schaub MA, Boyle AP, Kundaje A, Batzoglou S, Snyder M. Linking disease associations with regulatory information in the human genome. Genome Res. 2012;22(9):1748–59.
41. Braenne I, Civelek M, Vilne B, Di Narzo A, Johnson AD, Zhao Y, et al. Prediction of causal candidate genes in coronary artery disease loci. Arterioscler Thromb Vasc Biol. 2015;35(10):2207–17.

42. Miller CL, Pjanic M, Quertermous T. From Locus Association to Mechanism of Gene Causality: the devil is in the details. Arterioscler Thromb Vasc Biol. 2015;35(10):2079–80.
43. Helgadottir A, Thorleifsson G, Magnusson KP, Gretarsdottir S, Steinthorsdottir V, Manolescu A, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet. 2008;40(2): 217–24.
44. Matarin M, Brown WM, Singleton A, Hardy JA, Meschia JF; ISGS investigators. Whole genome analyses suggest ischemic stroke and heart disease share an association with polymorphisms on chromosome 9p21. Stroke. 2008;39(5):1586–9.
45. Gschwendtner A, Bevan S, Cole JW, Plourde A, Matarin M, Ross-Adams H, et al. Sequence variants on chromosome 9p21.3 confer risk for atherosclerotic stroke. Ann Neurol. 2009;65(5):531–9.
46. Chen HH, Almontashiri NA, Antoine D, Stewart AF. Functional genomics of the 9p21.3 locus for atherosclerosis: clarity or confusion? Curr Cardiol Rep. 2014;16(7):502.
47. Broadbent HM, Peden JF, Lorkowski S, Goel A, Ongen H, Green F, et al. Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. Hum Mol Genet. 2008;17(6):806–14.
48. Liu Y, Sanoff HK, Cho H, Burd CE, Torrice C, Mohlke KL, et al. INK4/ARF transcript expression is associated with chromosome 9p21 variants linked to atherosclerosis. PLoS One. 2009;4(4):e5027.
49. Jarinova O, Stewart AF, Roberts R, Wells G, Lau P, Naing T, et al. Functional analysis of the chromosome 9p21.3 coronary artery disease risk locus. Arterioscler Thromb Vasc Biol. 2009;29(10):1671–7.
50. Visel A, Zhu Y, May D, Afzal V, Gong E, Attanasio C, et al. Targeted deletion of the 9p21 non-coding coronary artery disease risk interval in mice. Nature. 2010;464(7287):409–12.
51. Harismendy O, Notani D, Song X, Rahim NG, Tanasa B, Heintzman N, et al. 9p21 DNA variants associated with coronary artery disease impair interferon-gamma signalling response. Nature. 2011; 470(7333):264–8.
52. Holdt LM, Teupser D. Recent studies of the human chromosome 9p21 locus, which is associated with atherosclerosis in human populations. Arterioscler Thromb Vasc Biol. 2012;32(2):196–206.
53. Hannou SA, Wouters K, Paumelle R, Staels B. Functional genomics of the CDKN2A/B locus in cardiovascular and metabolic disease: what have we learned from GWASs? Trends Endocrinol Metab. 2015;26(4):176–84.
54. Samani NJ, Braund PS, Erdmann J, Gotz A, Tomaszewski M, Linsel-Nitschke P, et al. The novel genetic variant predisposing to coronary artery disease in the region of the PSRC1 and CELSR2 genes on chromosome 1 associates with serum cholesterol. J Mol Med. 2008;86(11):1233–41.
55. Schadt EE, Molony C, Chudin E, Hao K, Yang X, Lum PY, et al. Mapping the genetic architecture of gene expression in human liver. PLoS Biol. 2008;6(5):e107.
56. Nielsen MS, Jacobsen C, Olivecrona G, Gliemann J, Petersen CM. Sortilin/neurotensin receptor-3 binds and mediates degradation of lipoprotein lipase. J Biol Chem. 1999;274(13):8832–6.
57. Nilsson SK, Christensen S, Raarup MK, Ryan RO, Nielsen MS, Olivecrona G. Endocytosis of apolipoprotein A-V by members of the low density lipoprotein receptor and the VPS10p domain receptor families. J Biol Chem. 2008;283(38):25920–7.
58. Linsel-Nitschke P, Heeren J, Aherrahrou Z, Bruse P, Gieger C, Illig T, et al. Genetic variation at chromosome 1p13.3 affects sortilin mRNA expression, cellular LDL-uptake and serum LDL levels which translates to the risk of coronary artery disease. Atherosclerosis. 2010;208(1):183–9.
59. Musunuru K, Strong A, Frank-Kamenetsky M, Lee NE, Ahfeldt T, Sachs KV, et al. From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus. Nature. 2010;466(7307):714–9.
60. Kjolby M, Nielsen MS, Petersen CM. Sortilin, encoded by the cardiovascular risk gene SORT1, and its suggested functions in cardiovascular disease. Curr Atheroscler Rep. 2015;17(4):1–9.
61. Schunkert H, Konig IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet. 2011; 43(4):333–8.
62. Reilly MP, Li M, He J, Ferguson JF, Stylianou IM, Mehta NN, et al. Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. Lancet. 2011;377(9763):383–92.
63. O'Donnell CJ, Kavousi M, Smith AV, Kardia SL, Feitosa MF, Hwang SJ, et al. Genome-wide association study for coronary artery calcification with follow-up in myocardial infarction. Circulation. 2011;124(25):2855–64.
64. Wang L, Zheng J, Bai X, Liu B, Liu CJ, Xu Q, et al. ADAMTS-7 mediates vascular smooth muscle cell migration and neointima formation in balloon-injured rat arteries. Circ Res. 2009;104(5): 688–98.
65. Pu X, Xiao Q, Kiechl S, Chan K, Ng FL, Gor S, et al. ADAMTS7 cleavage and vascular smooth muscle cell migration is affected by a coronary-artery-disease-associated variant. Am J Hum Genet. 2013;92(3):366–74.
66. Du Y, Gao C, Liu Z, Wang L, Liu B, He F, et al. Upregulation of a disintegrin and metalloproteinase with thrombospondin motifs-7 by miR-29 repression mediates vascular smooth muscle calcification. Arterioscler Thromb Vasc Biol. 2012;32(11):2580–8.
67. Kessler T, Zhang L, Liu Z, Yin X, Huang Y, Wang Y, et al. ADAMTS-7 inhibits re-endothelialization of injured arteries and promotes vascular remodeling through cleavage of thrombospondin-1. Circulation. 2015;131(13):1191–201.
68. Bauer RC, Tohyama J, Cui J, Cheng L, Yang J, Zhang X, et al. Knockout of Adamts7, a novel coronary artery disease locus in humans, reduces atherosclerosis in mice. Circulation. 2015;131(13): 1202–13.
69. Erbilgin A, Civelek M, Romanoski CE, Pan C, Hagopian R, Berliner JA, et al. Identification of CAD candidate genes in GWAS loci and their expression in vascular cells. J Lipid Res. 2013; 54(7):1894–905.
70. Ouyang T, Bai RY, Bassermann F, von Klitzing C, Klumpen S, Miething C, et al. Identification and characterization of a nuclear interacting partner of anaplastic lymphoma kinase (NIPA). J Biol Chem. 2003;278(32):30028–36.
71. Bassermann F, von Klitzing C, Munch S, Bai RY, Kawaguchi H, Morris SW, et al. NIPA defines an SCF-type mammalian E3 ligase that regulates mitotic entry. Cell. 2005;122(1):45–57.
72. Bassermann F, Peschel C, Duyster J. Mitotic entry: a matter of oscillating destruction. Cell Cycle. 2005;4(11):1515–7.
73. Kunnas T, Nikkari ST. Association of Zinc Finger, C3HC-Type Containing 1 (ZC3HC1) rs11556924 genetic variant with hypertension in a finnish population, the TAMRISK Study. Medicine (Baltimore). 2015;94(32):e1221.
74. Lopez-Mejias R, Genre F, Garcia-Bermudez M, Corrales A, Gonzalez-Juanatey C, Llorca J, et al. The ZC3HC1 rs11556924 polymorphism is associated with increased carotid intima-media thickness in patients with rheumatoid arthritis. Arthritis Res Ther. 2013;15(5):R152.
75. Yamase Y, Kato K, Horibe H, Ueyama C, Fujimaki T, Oguri M, et al. Association of genetic variants with atrial fibrillation. Biol Reprod. 2016;4(2):178–82.
76. Tregouet DA, Konig IR, Erdmann J, Munteanu A, Braund PS, Hall AS, et al. Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease. Nat Genet. 2009;41(3):283–5.

77. Clarke R, Peden JF, Hopewell JC, Kyriakou T, Goel A, Heath SC, et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. N Engl J Med. 2009;361(26):2518–28.
78. Schunkert H. Family or SNPs: what counts for hereditary risk of coronary artery disease? Eur Heart J. 2016;37(6):568–71.
79. Braenne I, Reiz B, Medack A, Kleinecke M, Fischer M, Tuna S, et al. Whole-exome sequencing in an extended family with myocardial infarction unmasks familial hypercholesterolemia. BMC Cardiovasc Disord. 2014;14:108.
80. Braenne I, Kleinecke M, Reiz B, Graf E, Strom T, Wieland T, et al Systematic analysis of variants related to familial hypercholesterolemia in families with premature myocardial infarction. Eur J Hum Genet. 2016;24(2):191–7.
81. Lin C, Chu CM, Lin J, Yang HY, Su SL. Gene-gene and gene-environment interactions in meta-analysis of genetic association studies. PLoS One. 2015;10(4):e0124967.
82. Mascheretti S, Bureau A, Trezzi V, Giorda R, Marino C. An assessment of gene-by-gene interactions as a tool to unfold missing heritability in dyslexia. Hum Genet. 2015;134(7):749–60.
83. Kessler T, Kaess B, Bourier F, Erdmann J, Schunkert H. Genetic analyses as basis for a personalized medicine in patients with coronary artery disease. Herz. 2014;39(2):186–93.
84. Kessler T, Vilne B, Schunkert H. The impact of genome-wide association studies on the pathophysiology and therapy of cardiovascular disease. EMBO Mol Med. 2016;8(7):688–701.
85. Myocardial Infarction G, Investigators CAEC. Coding variation in ANGPTL4, LPL, and SVEP1 and the risk of coronary disease. N Engl J Med. 2016;374(12):1134–44.
86. Do R, Stitziel NO, Won HH, Jorgensen AB, Duga S, Angelica Merlini P, et al. Exome sequencing identifies rare LDLR and APOA5 alleles conferring risk for myocardial infarction. Nature. 2015;518(7537):102–6.
87. Alkuraya FS. Human knockout research: new horizons and opportunities. Trends Genet. 2015;31(2):108–15.
88. Kathiresan S. Myocardial Infarction Genetics C. A PCSK9 missense variant associated with a reduced risk of early-onset myocardial infarction. N Engl J Med. 2008;358(21):2299–300.
89. Myocardial Infarction Genetics Consortium I, Stitziel NO, Won HH, Morrison AC, Peloso GM, Do R, et al. Inactivating mutations in NPC1L1 and protection from coronary heart disease. N Engl J Med. 2014;371(22):2072–82.
90. Tg, Hdl Working Group of the Exome Sequencing Project NHL, Blood I, Crosby J, GM P, PL A, et al. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. N Engl J Med. 2014;371(1):22–31.
91. Dewey FE, Gusarova V, O'Dushlaine C, Gottesman O, Trejos J, Hunt C, et al. Inactivating variants in ANGPTL4 and risk of coronary artery disease. N Engl J Med. 2016;374(12):1123–33.
92. Nioi P, Sigurdsson A, Thorleifsson G, Helgason H, Agustsdottir AB, Norddahl GL, et al. Variant ASGR1 associated with a reduced risk of coronary artery disease. N Engl J Med. 2016;374(22):2131–41.
93. Kathiresan S. Developing medicines that mimic the natural successes of the human genome: lessons from NPC1L1, HMGCR, PCSK9, APOC3, and CETP. J Am Coll Cardiol. 2015;65(15):1562–6.
94. Mega JL, Stitziel NO, Smith JG, Chasman DI, Caulfield MJ, Devlin JJ, et al. Genetic risk, coronary heart disease events, and the clinical benefit of statin therapy: an analysis of primary and secondary prevention trials. Lancet. 2015;385(9984):2264–71.
95. MacLellan WR, Wang Y, Lusis AJ. Systems-based approaches to cardiovascular disease. Nat Rev Cardiol. 2012;9(3):172–84.
96. Schadt EE, Bjorkegren JL. NEW: network-enabled wisdom in biology, medicine, and health care. Sci Transl Med. 2012;4(115):115rv1.

Hereditary Neuromuscular Diseases and Cardiac Involvement

23

A.J. van der Kooi, K. Wahbi, G. Bonne, and M. de Visser

Abstract

Neuromuscular disorders comprise a large group of diseases caused by dysfunction of motor neurons, peripheral nerves, and skeletal muscles. A fair proportion of neuromuscular disorders have a genetic cause. The incidence and prevalence of cardiomyopathies associated with inherited neuromuscular diseases, particularly with muscular dystrophies, have until recently been underestimated, even though cardiac involvement is either the direct or indirect cause of death in many of these diseases.

Introduction

This chapter focuses on the primary cardiac involvement in hereditary neuromuscular diseases, that is, the primary cardiac changes which are caused by the same genetic anomalies that damage skeletal muscle or nerves. Cardiac involvement can manifest itself as impulse generation or conduction defects, focal or diffuse myocardial thickening, dilation of the cardiac cavities, relaxation abnormality, hypertrophic, dilated, noncompaction or restrictive cardiomyopathy, Takotsubo phenomenon, secondary-valve insufficiency, intracardiac thrombus formation, or heart failure with systolic or diastolic dysfunction [1]. Secondary cardiac involvement in neuromuscular disorders ultimately manifesting with cor pulmonale is not addressed in detail. Chest-wall disorders (e.g., in spinal muscular atrophy type 2 or congenital myopathies/dystrophies) or respiratory muscle weakness (e.g., in *Pompe*'s disease) reduce the pulmonary vascular bed and cause pulmonary hypertension, chronic hypoxia, and hypercapnia. Respiratory muscle weakness is confirmed by pulmonary function tests that show a significant reduction of maximal respiratory pressures and vital capacity (VC) consistent with a restrictive ventilatory defect. In such cases, nocturnal ventilatory support is indicated.

This chapter reviews the probability and severity of cardiac disease in each type of hereditary neuromuscular disease and provides recommendations for management.

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Muscle Disorders

Muscular Dystrophies

Muscular dystrophies represent a clinically and genetically heterogeneous group of disorders, characterized by muscle wasting and weakness of variable distribution and severity and dystrophic changes in skeletal muscle. They can be caused by mutations in genes encoding sarcolemma-associated proteins, such as dystrophin and the dystrophin-associated glycoprotein complex, and genes encoding proteins of the nuclear envelope.

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Sarcolemma-Associated Proteins

Dystrophinopathic Cardiomyopathy

Dystrophinopathic cardiomyopathy is caused by a defect in the *dystrophin* gene on the X-chromosome encoding for the protein dystrophin. Dystrophin is expressed in heart, skeletal muscle, neural tissues, and smooth muscle, but progressive tissue damage is confined to heart and skeletal muscle. Dystrophin, together with other cytoskeletal proteins, provides mechanical support for the sarcolemma. A change in the amount, size, or function of dystrophin causes a structurally weak sarcolemma, which ruptures under mechanical stress, allowing uncontrolled focal ingress of extracellular fluid components, especially calcium, into the muscle fiber interior [2, 3]. Mutations of the dystrophin gene can result in different disorders manifesting with skeletal muscle involvement and/or cardiomyopathy: *Duchenne* muscular dystrophy (complete absence of dystrophin), *Becker* muscular dystrophy (qualitative and/or quantitative abnormalities of dystrophin), *X-linked dilated cardiomyopathy* (XL-LDC) (dystrophin abnormalities confined to the myocardium), and symptomatic carrier (DMD/BMD) manifesting with cardiomyopathy. The lack of dystrophin in cardiac muscle leads to progressive cardiomyocyte degeneration and fibrosis. The posterior basal segment of the left ventricle is consistently the first site in which hypokinesis is detected on echocardiography. The characteristic electrocardiographic (ECG) alterations consist of tall right precordial R-waves (R/S ratio greater than 1 in V1) and deep Q-waves (greater than 3 mm) in the left precordial and limb leads. A progressive, global hypokinesia with ventricular dilatation then evolves. The right side of the heart is rarely affected, and in particular the right atrium rarely demonstrates any echocardiographic abnormality. The most frequent cardiac abnormality in DMD is sinus tachycardia, occurring in childhood and persisting throughout life. Another rhythm abnormality frequently found is atrial premature beats. The incidence of cardiac conduction defects remained 6–13 % throughout life [4]. AV blocks are only rarely found. Ventricular arrhythmias are infrequent at the early stages, but their incidence increases with the progression of the disease.

Duchenne Muscular Dystrophy

Duchenne muscular dystrophy is caused by frame-shift mutations in the dystrophin gene, causing complete absence of the dystrophin protein. DMD has an incidence of one in 2500 to 3500 live male births [5]. Predominantly, boys are affected in this X-linked disorder. The onset of disease is usually between 2 and 5 years of age with progressive symmetrical proximal weakness, legs more than arms, a characteristic hypertrophic appearance of the calves, and marked elevation of serum creatine kinase (CK) activity. Wheelchair dependency occurred around the age of 10–12 years, and corticosteroid treatment delays this to a median of 14.5 years [6]. The introduction of ventilatory support led to a considerable extension of the life expectancy, from a mean age of death of 14.4 years in the 1960s to 25.3 years for those who were ventilated since 1990 [7]. From a clinical perspective, a progressive dilated cardiomyopathy eventually occurs in all boys with DMD. Symptoms of overt cardiac failure are rare, probably because boys typically have a severely restricted physical ability. Left untreated, cardiomyopathy makes a significant contribution to early mortality [7]. Sudden cardiac death events and significant Holter findings are rare in DMD patients with a left ventricular ejection fraction (LVEF) >35 % [8].

Management Patients should have a cardiac investigation (ECG and echocardiography) at diagnosis. Cardiac investigations should be performed every 2 years up to the age of 10 years, before any surgery, and annually after age 10 years, or more frequently, if an abnormal echocardiogram is identified [9]. Assessment and treatment of respiratory function should be performed in parallel with the cardiological investigations [7]. Cardiac magnetic resonance (CMR) imaging with gadolinium is an additional emerging tool that is rapidly becoming a preferred method for cardiac monitoring in boys with DMD. Increased gadolinium enhancement on CMR is one of the earliest findings of cardiac involvement, and is thought to reflect myocardial damage and fibrosis. Newer cardiac guidelines will probably include this new technique [10]. Initial treatment with angiotensin-converting enzyme (ACE) inhibitors and/or beta-blockers is indicated in case of impaired left ventricular function. In the only randomized trial on cardiac prevention in the disease, there is also evidence indicating that treatment even before any impairment of ventricular function is detectable on echocardiogram may delay the onset and progression of cardiomyopathy and improve long-term survival (92.9 % of the treated group were alive at 10 years vs. 65.5 % of the untreated group) [11, 12]. A recent trial compared ACE inhibitor and angiotensin receptor blocker (ARB), which turned out equally effective [13]. Eplerone treatment seems to strengthen the effect of both [14]. The age at which such therapy should begin remains an important question. A recent working group was of the opinion that ACE inhibitor/ARB use in DMD should begin by 10 years of age [10]. Data from retrospective studies on the effect of corticosteroids showed that 5 % of treated patients versus 58 % of untreated patients developed cardiomyopathy, indicating a beneficial effect of corticosteroid treatment on the development and progression of cardiomyopathy [15]. There are concerns about the possible impact of ACE inhibition on left ventricular development in very young children [9]. Anticoagulant therapy should be considered in patients with severe cardiac dysfunction to prevent systemic thromboembolic events [16]. DMD patients are

rarely eligible for cardiac transplantation due to other complications including scoliosis and respiratory insufficiency [9]. Recently, the use of ventricular-assist devices as a destination therapy (DT) as an alternative to cardiac transplantation in DMD patients has been described. [17]

Becker Muscular Dystrophy

Becker muscular dystrophy is caused by in-frame mutations in the dystrophin gene, which lead to reduced or otherwise altered dystrophin protein expression. The incidence of BMD is onethird of that of DMD, a much higher figure than was previously thought, implying that BMD has been underdiagnosed in the past [18]. The clinical picture is characterized by later age of onset and slower rate of progression as compared to DMD. However, the spectrum of BMD encompasses a variety of phenotypes, including an intermediate form between BMD and DMD ("outliers"), a "quadriceps-only" form, and a very mild form in which BMD may manifest itself with myalgias and muscle cramps, exercise intolerance and myoglobinuria, or asymptomatic elevation of the serum CK activity. In most cases, the first symptoms were noticed between the 6th and 18th year of life with a mean age of onset of 11.1 years [19]. The age of loss of ambulation varies from 10 to 78 years (mean age is in the fourth decade). Becker cardiomyopathy evolves in the same manner as DMD cardiomyopathy. In BMD, the severity of cardiac disease does not correlate with that of skeletal muscle weakness [20]. A severe dilated cardiomyopathy can occur in patients with BMD with relatively preserved muscle function.

Management Patients with Becker muscular dystrophy should have cardiac evaluation (ECG and echocardiography) at diagnosis. Subsequent 5-yearly and preferably 2-yearly screening is recommended [9]. When progressive abnormality is found, they should be seen more regularly and treated with ACE inhibitors and, if indicated, beta-blockers. Cardiac transplantation may be a viable treatment option in this group of patients [21, 22].

X-Linked Dilated Cardiomyopathy

There are descriptions of male patients who present with early-onset dilated cardiomyopathy and do not develop or have only mild skeletal muscle weakness [23–26]. Many but not all affected patients have an increased serum CK activity [26, 27]. The disease is being referred to as XL-LDC. X-LDC may be caused by the presence of a single-point mutation at the first exon–intron boundary or a nonsense mutation in exon 29, by a rearrangement downstream from the 5′ end of intron 11 or by a deletion in the mid-rod domain of the dystrophin gene. What all these mutations have in common is that they show a different pattern of expression in cardiac as compared to skeletal muscle [28].

Management Patients should have cardiac evaluation (ECG and echocardiography) at diagnosis. Subsequent 5-yearly and preferably 2-yearly screening is recommended. When progressive abnormality is found, they should be seen more regularly and treated with ACE inhibitors and, if indicated, beta-blockers. Cardiac transplantation may be a viable treatment in this group of patients.

Female Carriers of Duchenne and Becker Muscular Dystrophy

Carriers of DMD and BMD are at risk of developing dilated cardiomyopathy. The cumulative risk of cardiomyopathy is estimated to be between 7 % and 10 % [29–31]. DCM is more frequently found in carriers who have symptomatic weakness. Cardiac abnormalities in DMD/BMD carriers are progressive, as in patients with DMD/BMD [32].

Management Echocardiography and ECG are recommended in carriers of DMD and BMD at diagnosis and at least every 5 years thereafter, or more frequently in patients with abnormalities on investigation. There is no indication to test them presymptomatically before the age of 16 years [9]. Clinical geneticists should refer women for cardiac evaluation when carriership is diagnosed. Carriers manifesting severe skeletal muscle symptoms or cardiac symptoms require more frequent investigation. Once significant abnormalities are detected, patients may benefit from treatment with ACE inhibitors and beta-blockers, if indicated. Ultimately, cardiac transplantation may be appropriate [9, 33].

Dystrophin-Associated Glycoprotein Complex Cardiomyopathies

Sarcoglycanopathies (limb-girdle muscular dystrophy *(LGMD) types 2C, D, E, and F*) constitute about 25 % of the LGMD group and are inherited in an autosomal recessive manner. LGMDs constitute a heterogeneous group of disorders characterized by progressive weakness of the limb-girdle muscles, that is, the muscles of hip region and upper leg, and shoulder region and proximal arm. LGMD 2C-F are caused by defects in alpha-, beta-, gamma-, and delta-sarcoglycan, which are part of the sarcoglycan transmembrane component of the dystrophin-associated glycoprotein complex. All types of sarcoglycanopathies can be associated with cardiomyopathy. The clinical course is comparable to that of patients with Duchenne muscular dystrophy or severe Becker muscular dystrophy. The frequency of DCM is around 20 % and DCM is progressive over time [34]. There were no significant differences in median age or severity of disease between patients with or without DCM.

Management It is recommended to investigate sarcoglycanopathy patients with the same frequency as patients with DMD/BMD (see above) [9]. Present perception is that the

incidence of tachy- or brady-arrhythmias in sarcoglycanopathies is low but the issue has not been fully resolved. Arrhythmia surveillance with Holter ECG or other ambulatory ECG registrations is justified. Standard therapy should be effective in these patients with evidence of cardiomyopathy, but trial-based evidence of efficacy is lacking.

Other Plasma Membrane Proteins

Caveolinopathies Caveolins are the structural proteins that are necessary for the formation of caveolae membrane domains. Caveolae are vesicular organelles (50–100 nm in diameter) that are particularly abundant in cells of the cardiovascular system. In these cell types, caveolae function both in protein trafficking and in signal transduction [35]. The gene encoding for caveolin-3, the muscle-specific form of the caveolin family, is located on chromosome 3. Cardiac myocytes and skeletal muscle fibers express caveolin-3. In skeletal muscle, caveolin-3 is partly associated with the complex of dystrophin-associated proteins. Caveolin-3 mutations, predominantly autosomal dominant but occasionally autosomal recessive, may cause a variety of phenotypes, including LGMD1C, distal myopathy, rippling muscle disease, myoglobinuria, and asymptomatic hyperCKemia. The incidence is unknown. There seems to be no evidence to suggest that cardiac surveillance is indicated routinely in LGMD1C [9, 36]. However, several recent observations of familial hypertrophic cardiomyopathy [36], sudden death, possibly due to arrhythmia [37], and long QT syndrome [38] associated with caveolin-3 mutations suggest that cardiac involvement is a feature of caveolin-3 deficiency, and careful cardiac assessment of these patients seems reasonable [39].

Proteins with Enzymatic Activity

LGMD2I/MDC1C

LGMD2I, caused by mutations in the *fukutin-related protein* (FKRP) gene, is an autosomal recessive disorder. The *FKRP* gene is a homolog of the fukutin gene encoding for the FKRP. FKRP is a putative glycosyltransferase whose precise function is uncertain. It has been localized in the Golgi apparatus and is involved in the glycosylation processing of α-dystroglycan, an indispensable molecule for binding laminin alpha2. FKRP is ubiquitously expressed. Mutations in the *FKRP* gene located on chromosome 19q13 give rise to a spectrum of phenotypes including a form of *congenital muscular dystrophy (MDC1C), Walker-Warburg* phenotype, and a relatively mild form of LGMD2I. The most common mutation is the c.826C>A mutation. Patients with a homozygous C826A mutation generally exhibit milder and late-onset muscular dystrophy, whereas the compound heterozygous mutations are associated with more severe and early-onset type of muscular dystrophy phenotypically related to Duchenne muscular dystrophy [40, 41]. There are considerable regional differences. In the Netherlands, for example, LGMD2I was diagnosed in only 8 % of all LGMD families, whereas in the United Kingdom and Denmark, LGMD2I is considered the most frequent cause of LGMD [41–43].

Left ventricular hypokinesis, dilated cardiomyopathy, and heart failure have been reported in 30–80 % of LGMD2I patients, regardless of the gene mutation and the severity of the muscular disease, suggesting that all patients should be referred for cardiac evaluation [44, 45].

Management It is recommended to subject all patients with LGDM2I to evaluation for cardiac involvement (ECG and echocardiography) at diagnosis. After that, 2-year screening seems reasonable [9].

Fukuyama Congenital Muscular Dystrophy

Fukuyama congenital muscular dystrophy (FCMD) is an autosomal recessive disorder, caused by mutations in the fukutin gene on chromosome 9q31 [46]. Its protein product, fukutin, has sequence homologies with bacterial glycosyltransferase, but its precise function is unknown. FCMD also belongs to the group of disorders associated with glycosylation defects of α-dystroglycan. The disorder is particularly frequent in Japan where its incidence is 40 % of that of Duchenne muscular dystrophy while it is rare in Western countries [47]. FCMD is clinically characterized by a triad of mental retardation, brain deformities, and congenital muscular dystrophy.

In contrast with the severe dystrophic involvement of skeletal muscle, cardiac involvement is quite rare. Typically, patients are able to sit but never attain independent ambulation. By contrast, the mildest fukutin-related phenotype, *LGMD2M*, presents with minimal muscle weakness, dilated cardiomyopathy, and normal intelligence [48]. This suggests that late-onset LGMD patients with mutations in the fukutin protein should be evaluated with ECG and echocardiography at diagnosis. After that, 2-year screening seems reasonable.

Inner Nuclear Membrane Proteins

Emery-Dreifuss muscular dystrophy (EDMD) can present as an X-linked or autosomal dominant disorder. The disease is characterized by early contractures, and a humeroperoneal distribution of muscle weakness. Both emerin and lamin A/C, the causative genes in X-linked and autosomal dominant EDMD, respectively, are nuclear lamina genes. Defects in these genes cause conduction disorders and cardiomyopathy. In case of more prominent limb-girdle muscle weakness in the presence of a lamin A/C mutation, the disorder is called LGMD1B.

X-Linked EDMD

The gene locus for this entity is located at Xq28, and the *gene* (*EMD* previously named *STA*), which is 2100 bp in

length and consists of six exons encoding a 254 amino acid serine-rich protein, is called emerin [49]. Emerin mutations identified to date include a few missense mutations, and the majority are nonsense, splice site, or small deletions/insertions that ultimately result in premature translation termination and complete absence of emerin expression on both Western blotting and immunohistochemistry. The function of the *emerin* protein, which is ubiquitously expressed in all tissues [50] and in all vertebrates, remains to be fully elucidated.

Clinically, the disorder is characterized by early contractures of the Achilles tendons, elbows, and posterior cervical muscles, often before there is any significant weakness. Subsequently, limitation of neck flexion develops, but later forward flexion of the entire spine becomes limited [51, 52]. Muscle wasting and weakness with a distinctive humeroperoneal distribution early in the course of the disease is slowly progressive. Weakness later extends to the proximal limb-girdle musculature, but is rarely profound. Onset in the first few years of life is not exceptional [53, 54]. The variability of the clinical severity in individual members of the same family appears to be much greater as compared to other forms of muscular dystrophy (even compared to Becker muscular dystrophy). Only very rarely, ambulation is lost as a result of muscle weakness or contractures [53]. Very rare cases seem to be completely asymptomatic still in the fourth decade of life [53].

Cardiac features usually occur in patients' (early) teens or twenties, but a boy as young as age 5 years, in whom the heart was involved, has been reported [53]. Cardiac involvement is characterized by cardiac conduction defects, ranging from sinus bradycardia, prolongation of the PR interval on ECG to complete heart block. Atrial paralysis is almost pathognomonic of EDMD. The finding of a dilated right atrium on echocardiography and isolated atrial paralysis with absent "P"-waves on ECG should always prompt the exclusion of EDMD [55]. The severity of heart disease does not correlate with the degree of skeletal muscle involvement, and cardiac involvement can be very prominent [56]. EDMD affects the atria, and right heart involvement predominates. There is progressive replacement of the normal myocardium by fibrous and adipose tissue, which results in the loss of atrial contractility (atrial paralysis) and atrial dilatation. Evidence of left ventricular dysfunction (in addition to the invariable involvement of the conduction system) was reported by some groups but not by others [53].

As with DMD, there may be some female carriers of this X-linked disease who manifest cardiac disease, in particular, atrial paralysis, albeit usually at a later age than male subjects [56, 57]. No association with any sign of muscle weakness, wasting, or contractures appears to be present [53]. Published cases of manifesting carriers may have been diluted by cases of dominant disease.

Management Cardiological evaluation at diagnosis and annually thereafter using 12-lead ECG (preferably at 50 mm/s) requires expert assessment as ECG changes may be subtle and difficult to interpret [9]. Holter monitoring should be recommended annually for tachy- or brady-arrhythmias. Echocardiography can be done on a less regular basis. Permanent pacemaker implantation is justified, even in asymptomatic patients [9] when ECG begins to show abnormalities of sinus-node or AV node disease. However, nocturnal AV-Wenkebach may be a normal finding in young people. In the presence of sinoatrial or AV-nodal conduction abnormalities on surface ECG, invasive electrophysiology testing probably adds little to the decision to or timing of pacemaker implantation. However, such testing may have a role in determining the optimum mode of and sites for pacing [9]. Whether implantable defibrillators may be a more appropriate form of management than pacemakers when anti-bradycardia pacing is indicated for these patients is unclear.

It is recommended to establish the carrier status in females at risk and to offer them periodic ECG surveillance including 24-h ambulatory Holter monitoring to detect atrial or AV-nodal conduction disease [58]. There is a need for more systematic study of the natural history of cardiac involvement in X-linked EDMD carriers.

Autosomal Dominant EDMD/LGMD1B/L-CMD

Mutations in the *LMNA* gene on chromosome 1q11-q23 [59] encoding *lamins A and C* by alternative splicing cause primary laminopathies including various types of lipodystrophies, muscular dystrophies (EDMD2, LGMD1B, L-CMD) and *progeroid syndromes*, *mandibulo-acral dysplasia*, dilated cardiomyopathies, *neuropathy*, restrictive *dermopathy*, and *arthropathy* with tendinous calcifications. When looking at *LMNA*-related muscular dystrophies, most cases will have an EDMD phenotype, but in some instances, a limb-girdle phenotype, referred to as LGMD1B, is found as well as congenital forms of muscular dystrophy, that is, L-CMD.

Lamins are nuclear intermediate filaments that form the *nuclear lamina,* which lines the inner nuclear membrane. Lamin proteins have been shown to bind to chromatin and to several inner nuclear membrane proteins.

The pattern and severity of cardiac disease is thought to be more severe in the autosomal dominant form as compared to the X-linked EDMD. Among patients with AD-EDMD, 35 % will develop a progressive and potentially life-limiting dilated cardiomyopathy by middle age. Ventricular dysrhythmias are also significant in laminopathy patients and are an important cause of sudden death, despite pacing suggesting that implantation of an ICD is warranted when there is an indication for pacemaker implantation [60, 61].

Management ECG at diagnosis and yearly thereafter. Holter monitoring for tachy- or brady-arrhythmias and echocardiography annually. Prevention of sudden death, which is mostly caused by ventricular tachyarrhythmia, is complex and should be based on implantable defibrillators rather than pacemakers despite the greater risk for complications [62]. These patients should be managed in specialized centers and their data collated to contribute to further evidence in the future. In the meantime, there is a strong indication for defibrillator implantation to be considered when anti-bradycardia pacing is indicated, left ventricular function is severely impaired or in patients with sustained ventricular tachycardia. Four risk factors for malignant ventricular tachyarrhythmias have been identified and can help selecting patients for prophylactic implantations of cardiodefibrillators: (non)sustained ventricular tachycardia, male gender, nonmissense mutations, and mild ventricular dysfunction (left ventricular ejection fraction of <45 %) [63]. These recommendations need to be validated over time through the collection of high-quality prospective data.[11]

Nucleotide Repeat Disorders with Myotonia

Myotonic Dystrophy

Myotonic dystrophy type 1 (*DM1*, also known as *dystrophia myotonica* or *Steinert's* disease) is an autosomal dominant multisystem disorder, and the most common myopathy presenting in adults (incidence one in 8000 live births, prevalence is approximately five per 100,000 in most American and European populations).

DM1 is caused by an aberrantly expanded CTG repeat in the 3′-untranslated region of the DM protein kinase (DMPK) gene on chromosome 19q13.3. The mutated *DMPK* gene produces an altered version of messenger RNA, which interacts with certain proteins to form clumps within the cell. The abnormal clumps interfere with the production of many other proteins. The severity of the disease is related to the repeat length, which can expand from generation to generation (anticipation), and varies from very severe, often lethal congenital DM to late-onset mild muscle weakness, myotonia, and cataract. Patients with adolescent-onset DM1 characteristically manifest with myotonia (delayed muscle relaxation after contraction), progressive weakness and atrophy of the skeletal muscles, with predominant distal weakness, and facial involvement with involvement of systems other than skeletal muscle, such as the heart, endocrine glands, central nervous system, and smooth muscle.

Myocardial fibrosis and degeneration of the cardiac-conduction system occur in the majority of patients. Approximately, 90 % show ECG abnormalities, commonly, prolongation of the PR interval, and QRS duration. Arrhythmias can occur, including sinus-node dysfunction, progressive heart block, atrial tachycardia, flutter or fibrillation, and ventricular tachycardia or fibrillation [64]. Patients with adult DM1 are at high risk for arrhythmias and sudden death [65, 66]. A rhythm other than sinus, PR interval of 240 ms or more, QRS duration of 120 ms or more, or second-degree or third-degree atrioventricular block and a diagnosis of atrial tachyarrhythmia (sustained atrial tachycardia, flutter, or fibrillation) predict sudden death [65]. Cardiomyopathy and congestive heart failure occur far less frequently than conduction disturbances. The most prevalent echocardiographic changes are mitral valve prolapse and septal and myocardial fibrosis.

Management Cardiac evaluation includes annual ECG, and Holter monitoring if annual ECG shows increasing PR or QRS intervals or other evidence of increased risk of bradycardia. Echocardiogram should be performed at diagnosis in myotonic dystrophy. Invasive measurement of the HV interval may help decide the need for pacing in borderline cases. If atrial tachyarrhythmias (atrial flutter, fibrillation) become symptomatic, antiarrhythmic treatment may be justified [9]. However, antiarrhythmic drugs may aggravate any preexisting tendency to bradycardia or ventricular tachyarrhythmias. Prophylactic pacing remains the first-line treatment for the prevention of sudden death, which is mainly related to conduction defects in the disease. A strategy based on invasive electrophysiological study in patients with mild conduction defects on their ECG has been associated with an improvement of overall long-term survival related to major reduction of sudden death [67]. A subset of patients remains, however, exposed to sudden death despite permanent pacing, particularly those with nonsustained tachycardia or severe ventricular dysfunction for whom implantable cardiac defibrillators should be discussed.

Myotonic Dystrophy Type 2

Myotonic dystrophy type 2 (DM2), also called *proximal myotonic myopathy (PROMM)*, is present in a large number of families of northern European ancestry. In Germany, it has the same prevalence as DM1. DM2 is caused by an expanded CCTG tetra-nucleotide repeat in the first intron of the zinc finger protein 9 (ZNF9) gene on chromosome 3q21.

DM2 shares many features with DM1, but the patients have less symptomatic distal, facial, and bulbar weakness, and less pronounced clinical myotonia. Important other differences include the absence of a congenital form of DM2, an apparent lack of mental retardation in juvenile cases, and less evident excessive daytime sleepiness.

The heart involvement is comparable to that in DM1 in its characteristics, though less frequent and delayed in the course of the disease.

Ion Channel Disorder Associated with Periodic Paralysis and Heart Involvement

Anderson Syndrome

Anderson syndrome is a very rare disease, and characterized by the clinical triad of dyskalemic paralytic attack, ventricular ectopy, and potential dysmorphic features. It is inherited as an autosomal dominant trait. Mutations in the potassium-channel gene *KCNJ2*, which encodes for the Kir2.1 potassium channel generating the I_{K1} current, have been found. Cardiac disturbances may comprise the long QT syndrome (type 7), ventricular extrasystoles, or tachycardia [68]. Tachydysrhythmia may cause syncopal attacks and sudden death. The cardiac symptoms are provoked or worsened by hypokalemia and digitalis. The *paralytic attacks* may be hyperkalemic or hypokalemic, and therefore the response to oral potassium is unpredictable.

Myofibrillar Myopathies

The term *myofibrillar myopathies (MFMs)* was proposed as a noncommittal designation for a group of chronic neuromuscular diseases associated with common morphologic features, consisting of a distinct pathologic pattern of myofibrillar disorganization that begins at the Z-disk and is followed by accumulation of myofibrillar degradation products and ectopic expression of diverse proteins. These disorders are transmitted by autosomal dominant inheritance, and typically manifest as distal myopathies, but may also affect proximal muscles [69]. The median age of onset is 55 years (range 7–77). Serum CK activity is normal or slightly elevated. Cardiomyopathy, often of the arrhythmogenic type, is a frequent associated feature, particularly for patients with mutations in the *DES* gene who develop in a majority of cases complete atrioventricular blocks and ventricular tachyarrhythmias [70, 71]. Mutations in the *desmin, αB-crystallin, myotilin, ZASP, filamin C, FHL1, and BAG3* genes have been identified in about half of the patients. Mutations in myotilin cause *LGMD1A*. Cardiac involvement in desminopathies resembles cardiac involvement in laminopathies, the same guidelines for follow-up and management seem to be appropriate, with a strong indication for prophylactic implantation of a defibrillator.

Congenital Myopathies

Central Core Disease

The core myopathies central core disease (CCD) and multiminicore disease are heterogeneous congenital myopathies. They are most frequently caused by mutations in the *ryanodin receptor (RYR1).* Mutations encoding *selenoprotein (SEPN1)* can also be found [72]. CCD can present with hypotonia and weakness in the neonatal period and a nonprogressive course, but also with milder phenotypes [73]. It is inherited in an autosomal dominant or autosomal recessive fashion. Dysmorphic features may develop secondary to muscle weakness. An association with the potentially fatal malignant hyperthermia syndrome is well known. Serum CK activity is usually normal. Muscle biopsies reveal well-demarcated cores within most muscle fibers. Cardiac involvement in CCD is rare.

Nemaline Rod Myopathy

Defects in ten thin filament protein genes, including skeletal *α-actin (ACTA1), nebulin (NEB), α-tropomyosin (TPM3), β-tropomyosin (TPM2), troponin T (TNNT1), and cofilin-2 (CFL2)*, *KBTBD13*, *KLHL40* en *41*, and *LMOD3,* have so far been shown to result in nemaline myopathy [72]. Nemaline myopathy is characterized by the presence of rod-shaped structures in the muscle fibers.

The clinical spectrum of nemaline myopathies is wide, ranging from severe, often fatal conditions with prenatal-onset to early childhood-onset conditions of varying severity. Disproportionately severe axial and respiratory muscle involvement is common in all variants, and is often the long-term prognostic determinant. The condition is otherwise essentially stable, though in a few patients with mutations in *ACTA1*, severe progression of weakness in late childhood has been noticed.

Primary cardiac involvement is rare. However, several cases with nemaline myopathy and predominantly hypertrophic cardiomyopathy have been described [74, 75].

Myosin-Storage Myopathy

Mutations in the myosin heavy-chain gene *MYH7* cause myosin-storage myopathy. MYH7 is the most frequent cause of hypertrophic cardiomyopathy. Cardiomyopathy has been described in combination with this disorder [76, 77].

Centronuclear Myopathy with Cardiomyopathy

Centronuclear myopathies (CNMs) are characterized by muscle weakness and increased numbers of central nuclei within myofibers. It is a very rare disorder, which can present with divers phenotypes. Striated muscle preferentially expressed protein kinase (*SPEG*), the product of SPEG complex locus (*SPEG*), was identified as an MTM1-interacting protein. *SPEG* is present in cardiac muscle, where it plays a critical role. Recently, recessively inherited *SPEG* mutations were demonstrated in CNM with cardiomyopathy [78].

Metabolic Disorders Affecting Muscle

Lysosomal Glycogenosis

Pompe's Disease or Glycogen-Storage Disease Type II

Pompe's disease is a rare autosomal recessive disorder caused by mutations in the gene that encodes for *α-glucosidase*. Alpha-glucosidase deficiency causes glycogen to accumulate in various tissues and disrupt function of skeletal and cardiac muscle in particular. Presentation in infancy is associated with respiratory failure, cardiomyopathy, and severe muscle weakness. Juvenile- or adult-onset cases typically present with proximal muscle weakness, and often develop respiratory insufficiency or exertional dyspnea due to diaphragmatic involvement [68]. Cardiac involvement in glycogenosis type II comprises cardiomyopathy, arrhythmias, and cardiac decompensation. The cardiac involvement depends on the residual acid alpha-glucosidase activity and the age at symptom onset. In the late-onset forms, cardiac involvement is rare [79].

Management Until recently, treatment was focused on supportive measures, and infants diagnosed with classical Pompe's disease usually died within the first year of life. The introduction of enzyme replacement therapy (ERT) with recombinant α-glucosidase has dramatically improved the life expectancy of infantile-onset disease with anecdotal improvements in respiratory and motor function observed in juvenile- or adult-onset cases [80]. Cardiac assessment in infants with glycogenosis type II should involve an echocardiogram at diagnosis, followed by checkups at quarterly intervals during the first 2 years of treatment with ERT, and then at 6 monthly intervals. For adult patients, it is advocated to perform an electrocardiogram at least once in routine clinical follow-up. Additional echocardiography seems indicated only in those patients with abnormal ECG findings, a history of cardiac disease or evident cardiac symptoms [81].

Danon Disease

Danon disease is caused by a primary deficiency of a major lysosomal membrane glycoprotein, *LAMP2 (lysosome-associated membrane protein 2*). This is a rare X-linked dominant disorder, characterized by hypertrophic cardiomyopathy, skeletal myopathy, and variable degree of mental retardation, with autophagic vacuoles in skeletal and cardiac muscles. Males are more affected than females. In probands, cardiac symptoms, such as exertional dyspnea, start in teenage years. The association of hypertrophic cardiomyopathy and cardiac arrhythmia is common, and patients typically die of cardiac failure or cardiac arrest in their fourth decade. Problems with the electrical activity in the heart can occur, presenting as "Wolff-Parkinson-White" syndrome. The myopathy is usually mild. Serum CK activities are five to ten times elevated [82]. Milder variants of the disease have been described [83, 84].

Management Patients should have a cardiac investigation, including ECG and echocardiography at diagnosis. Cardiac investigations should be performed every 1–2 years or more frequently, if an abnormal echocardiogram is identified.

Mitochondrial Disorders

Primary Disorders of Mitochondrial Function

These are caused by mutations in both mitochondrial and nuclear genes encoding mitochondrial proteins. They are an increasingly recognized cause of multisystem diseases that have disorders of the central nervous system and skeletal muscle as their predominant manifestations. Because of its dependence on oxidative metabolism, the heart is also frequently involved in *mitochondrial* disease (see Chap. 24). Several mitochondrial syndromes that involve the heart include *Kearns-Sayre syndrome, MELAS* (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes), and *MERFF* (myoclonic epilepsy with ragged red fibers). Several types of cardiac abnormality including hypertrophic cardiomyopathy, dilated cardiomyopathy, *Wolff-Parkinson-White syndrome*, and cardiac arrhythmia have been described [85]. Conduction disturbances may be an important cause of mortality in patients with Kearns-Sayre syndrome. The following parameters have proven their ability to stratify the long-term risk for cardiac life-threatening complications in any adult patient with mitochondrial disease: left ventricular hypertrophy, ventricular premature contractions, diabetes, and intraventricular conduction blocks [86].

Management Patients should have cardiac evaluation (ECG, echocardiography, and Holter ECG) at diagnosis. After that, they should be screened for the development of cardiomyopathy and electrical complications with time intervals that depend on initial evaluation, at least yearly for patients with *Kearns-Sayre syndrome* or *MELAS* with hypertrophic cardiomyopathy. Timely placement of a pacemaker can be life-saving in the presence of conduction block. In patients with isolated cardiomyopathy, cardiac transplantation may be required [87].

Carnitine Deficiency

Carnitine plays an essential role in the transfer of long-chain fatty acids across the inner mitochondrial mem-

brane. This transfer requires enzymes and transporters that accumulate carnitine within the cell (*OCTN2 carnitine transporter*), conjugate it with long-chain fatty acids (*carnitine palmitoyl transferase 1, CPT1*), transfer the acylcarnitine across the inner plasma membrane (*carnitine-acylcarnitine translocase, CACT*), and conjugate the fatty acid back to coenzyme A for subsequent beta-oxidation (*carnitine palmitoyl transferase 2, CPT2*). Deficiency of the OCTN2 carnitine transporter causes primary carnitine deficiency, characterized by increased loss of carnitine in the urine and decreased carnitine accumulation in tissues. Patients can present with hypoketotic hypoglycemia and hepatic encephalopathy, or with muscle weakness and cardiomyopathy. This disease responds to carnitine supplementation.

CACT deficiency presents in most cases in the neonatal period with hypoglycemia, hyperammonemia, and cardiomyopathy with arrhythmia leading to cardiac arrest. Plasma carnitine levels are extremely low.

In CPT1 deficiency, the skeletal muscle and heart are usually unaffected. In adults with deficiency of CPT2, rhabdomyolysis triggered by prolonged exercise may occur. More severe variants of CPT2 deficiency present in the neonatal period similar to CACT deficiency. Treatment for the deficiency of CPT2 and CACT consists of a low-fat diet supplemented with medium-chain triglycerides that can be metabolized by mitochondria.

Friedreich's Ataxia

Friedreich's ataxia is an autosomal recessive disorder, in most cases caused by a homozygous-expanded GAA repeat (55–1700, normal 7–33) localized in the intron of the frataxin gene on chromosome 9q13. There is an inverse correlation between the length of the GAA repeat and onset of the disease, progression, and the occurrence of cardiomyopathy [88]. The *frataxin gene* encodes for the frataxin protein located at the inner mitochondrial membrane, the function of which remains to be elucidated.

The estimated prevalence is two to three per 100,000 inhabitants. Onset of the disease is usually between 5 and 25 years. Progressive gait ataxia and ataxia of the legs are the first manifestations of the disease. Subsequently, cerebellar dysarthria, ataxia of the arms, oculomotor disturbances, pyramidal features, and sensory abnormalities due to involvement of the posterior columns and the peripheral nerves occur. Hypertrophic cardiomyopathy is observed in 60–70 % and can even precede cerebellar ataxia. In later stages, dilated cardiomyopathy may develop. Most patients are wheelchair-bound after a disease duration of 8–15 years. There is a great range in age of death (30–70 years), dependent on the occurrence of cardiac involvement.

Management Cardiac evaluation should take place at diagnosis, with re-screening every 3–5 years when no abnormalities are found.

Barth Syndrome

Barth syndrome is an extremely rare X-linked cardioskeletal myopathy caused by a deficiency in tafazzin. *Tafazzin*, a phospholipid acyltransferase, is involved in acyl-specific remodelling of cardiolipin, which promotes structural uniformity and molecular symmetry among the cardiolipin molecular species. Inhibition of this pathway leads to changes in mitochondrial architecture and function [89]. Patients have variable clinical findings, often including heart failure, myopathy, cyclic neutropenia, growth retardation, and organic aciduria. Female carriers are not affected. Affected boys usually die of heart failure in infancy or early childhood, but there may be relative improvement in those who survive to later childhood [90].

Management Patients should have a cardiac investigation, including ECG and echocardiography at diagnosis and thereafter every 1–2 years, or more frequently, if an abnormal echocardiogram is identified.

Neuropathies

Familial Amyloid Polyneuropathy

Familial amyloid polyneuropathy designates a group of dominantly inherited neuropathies, with extracellular deposition of amyloid substance in various tissues. The three main precursor proteins encountered in these disorders are *transthyretin (TTR), apolipoprotein A1 or not gelsolin*. TTR-associated neuropathies are by far the most frequent type with a severe sensorimotor and autonomic neuropathy as the hallmark of the disease, most often associated with cardiac manifestations. First described in Portugal, the disorder was subsequently reported across the world, although Portugal, Japan, and Sweden are the three main areas of prevalence. In the past years, an increasing number of mutations has been identified in the TTR gene, along with a larger clinical spectrum than initially thought. Variable age of onset and penetrance are also largely reported with unclear phenotypic–genotypic correlations. Over the last 15 years, liver transplantation has enabled improved prognosis of this devastating condition. However, in some patients with substantial cardiac involvement prior to liver transplantation, the cardiac condition continues to worsen, as measured by left ventricular wall thickness and ejection fraction [91]. These findings have led to a very small number of combined liver and heart transplantations in cases

of hereditary amyloidosis with cardiac involvement. Recently, new therapeutic strategies have emerged, which intend to stabilize TTR or to silence the TTR gene.

Management Patients should undergo cardiac evaluations including ECG, echocardiography, and when possible cardiac MRI at diagnosis and at least on a yearly basis during follow-up. Besides etiological treatment of cardiomyopathy, which remains liver transplantation, the treatment of heart failure symptoms includes loop diuretics and spironolactone with a close surveillance of blood pressure and blood ionogram since those patients are prone to hypotension and renal failure in combination with ACE inhibitors. Beta-blockers are not recommended since they are often poorly tolerated as they blunt compensatory tachycardia drive and induce greater negative inotropic effects in amyloid-infiltrated hearts [92]. Prophylactic pacing is generally recommended in patients with conduction defect on their ECG regarding the high risk of progression to complete atrioventricular blocks and sudden death [93].

Charcot-Marie-Tooth Disease Type 2 Caused by Lamin A/C Mutations

Charcot-Marie-Tooth (CMT) disease comprises a group of clinically and genetically heterogeneous hereditary motor and sensory neuropathies, which are clinically characterized by distal muscle weakness and wasting, sensory disturbances, and foot and hand deformities. An axonal subtype, CMT2, is defined by (near-) normal nerve conduction velocities in combination with the loss of large myelinated fibers and axonal degeneration on nerve biopsy. CMT2 phenotypes are characterized by a large genetic heterogeneity. In one autosomal recessive subtype of CMT2, mutations in the lamin A/C gene have been found. Mutations in this gene also cause AD-EDMD, LGMD1B, and dilated cardiomyopathy with conduction defects, and therefore similar cardiac involvement may be anticipated, although, as yet, it has not been described.

Refsum's Disease

This is a rare autosomal recessive peroxisomal disorder. The classic triad encompasses ataxia, retinitis pigmentosa, and polyneuropathy. Refsum's disease is caused by an inborn error in the metabolism of a fatty acid, called phytanic acid. All patients have markedly increased serum concentrations of phytanic acid. Cardiomyopathy can occur in the course of the disease, mostly at an advanced stage of the disease. Chronic dietary treatment by restricting the exogenous sources of phytanic acid and its precursor phytol results in clinical improvement.

Summary

A fair proportion of the neuromuscular disorders have a genetic cause. Molecular genetics evaluation can reveal a pathogenic mutation in many cases. Heart involvement is either the direct or indirect cause of death in many of these diseases. It is also of importance to consider the presence of cardiac abnormalities in patients with inherited neuromuscular disease who are to be given a general anesthetic, because arrhythmias and conduction abnormalities may be precipitated perioperatively.

Cardiac involvement related to the primary skeletal muscle disorder can manifest itself as impulse generation or conduction defects, or cardiomyopathy.

Patients with neuromuscular disorders, known to be associated with cardiac pathology, should be referred to a cardiologist for extensive evaluation of ventricular function, impulse formation, and conduction diseases. Vice versa in patients presenting with dilated cardiomyopathies due to a gene defect related to a neuromuscular disorder or without a detected genetic cause should be investigated for neuromuscular disease, Table 23.1

Table 23.1 Frequency, type, and implications of cardiac involvement in different neuromuscular disorders

Disease (gene)	Cardiac involvement	% of patients with cardiac involvement	Age at onset	Morbidity/mortality	Evaluation	Management
Duchenne muscular dystrophy (dystrophin)	ECG abnormalities; DCM	Abnormal ECG >90 %; abnormal echocardiography >90 %	Detectable from the age of 6 years onwards	Cardiac death 30–40 %,	ECG and echocardiography at diagnosis, 2-yearly before 10 years, annually thereafter	ACE inhibitors, beta-blockers
Becker muscular dystrophy (dystrophin)	ECG abnormalities; HCM and DCM	ECG abnormal – 90 %, echocardiography abnormal – 65 %	Variable, may be disproportionate to skeletal involvement	Cardiac death in up to 50 %	ECG and echocardiography every 2–5 years	ACE inhibitors, beta-blockers, heart transplantation in end-stage DCM in patients with relatively preserved skeletal muscle function
DMD/BMD carriers (dystrophin)	ECG abnormalities; DCM	7–10 % dilated cardiomyopathy, ECG abnormalities 20–90 %	Variable, may be disproportionate to skeletal muscle involvement		ECHO and ECG at diagnosis, or after the age of 16 years and at least every 5 years thereafter	ACE inhibitors, beta-blockers, cardiac transplantation in end-stage DCM
X-DCM (dystrophin)	ECG abnormalities; DCM	100 % by definition	No evident muscle weakness	Cardiac transplantation sometimes necessary	ECG and echocardiography every 2–5 years	ACE inhibitors, beta-blockers. Cardiac transplantation in end-stage DCM
Sarcoglycanopathies (LGMD2C-F) (sarcoglycans)	ECG abnormalities; DCM	20–25 %	Variable	Cardiac transplantation sometimes necessary	ECG and echocardiography every 2–5 years	ACE inhibitors, beta-blockers. Cardiac transplantation in end-stage DCM
LGMD1C (Caveolin-3)	HCM, long QT	Case reports				
LGMD2I/MDC1C	ECG abnormalities	One third of adult-onset	Possibly related to		ECG and	ACE inhibitors
(FKRP)	DCM	Cases	severity of overall disease		echocardiography at diagnosis, every 2-year thereafter	beta-blockers
Fukutin congenital muscular dystrophy/ LGMD2M (fukutin)	DCM	Case reports			Late-onset cases: ECG and echocardiography at	ACE inhibitors, beta-blockers diagnosis, every 2-year thereafter
X-EDMD (emerin)	AV block, atrial paralysis, atrial flutter and fibrillation	>95 % by the age of 30 years	10–39	SCD common in nonpaced individuals	ECG and Holter at diagnosis and annually thereafter	Pacemaker or cardiac defibrillator

(continued)

Table 23.1 (continued)

Disease (gene)	Cardiac involvement	% of patients with cardiac involvement	Age at onset	Morbidity/mortality	Evaluation	Management
AD-EDMD/LGMD1B/myofibrillar myopathies (lamin A/C desmin, alpha-B-crystallin, myotilin)	AV block, atrial flutter and fibrillation; DCM	Rhythm and conduction disturbances >95 % by the age of 30 years, DCM 35 %		SCD despite pacing, heart failure	ECG and Holter at diagnosis and annually thereafter	ICD, ACE inhibitors, beta-blockers, cardiac transplantation in end-stage DCM
DM1/DM2 (DMPK)	AV conduction disturbances, atrial flutter and fibrillation, ventricular tachyarrhythmias	90 % ECG abnormalities		SCD 30 %	ECG and Holter at diagnosis and annually thereafter, echocardiogram	Pacemaker/
Anderson syndrome (KCNJ2)	Long QT syndrome, ventricular extrasystoles, or tachycardia			Syncopal attacks sudden death; provoked by hypokalemia and digitalis		
Congenital myopathy	HCM, fatal	Rare, only case				
RYR1, SEPN1, TTN	Cardiomyopathy	reports				
Pompe's disease (alpha-glucosidase)	Cardiomyopathy in neonatal/childhood cases	Rare in adult-onset Cases			In infants echocardiography with 3–6 months interval; ECG at least once in adult-onset cases	Enzyme replacement therapy
Danon disease (LAMP2)	HCM			Cardiac failure or cardiac arrest in 4th decade	ECG and echocardiography at diagnosis. every 1–2 years thereafter	Cardiac transplantation in end-stage HCM
Mitochondrial	HCM, DCM Wolff-Parkinson-White syndrome, and cardiac arrhythmia, conduction disturbances				ECG, Holter ECG and echocardiography at diagnosis and during follow up according to initial risk stratification	Pacemaker, cardiac transplantation in end-stage DCM
Carnitine deficiency	Cardiomyopathy, arrhythmia					Dietary treatment
Barth syndrome (tafazzin)	DCM, HCM			Heart failure in infancy or early childhood	ECG and echocardiography at diagnosis and thereafter every 1–2 years	
Familial amyloid neuropathy (TTR, apolipoprotein A1, gelsolin)	HCM, DCM					Liver (+heart) transplantation

Disease (gene)	Cardiac involvement	% of patients with cardiac involvement	Age at onset	Morbidity/mortality	Evaluation	Management
Refsum disease	HCM, DCM		Later stages of the disease			Dietary treatment
Friedreich's ataxia (frataxin)	HCM, DCM	60–70 %		Cardiac involvement determines age of death	ECG and echocardiography at diagnosis, re-screening every 3–5 years	Idebenone

DCM denotes dilated cardiomyopathy; HCM denotes hypertrophic cardiomyopathy; ACE denotes angiotensin-converting enzyme; SCD denotes sudden cardiac death; AV denotes atrioventricular; X denotes X-linked; AD denotes autosomal dominant; DM1 denotes myotonic dystrophy type 1; DM2 denotes myotonic dystrophy type 2

References

1. Finsterer J, Stollberger C. Cardiac involvement in primary myopathies. Cardiology. 2000;94(1):1–11.
2. Mokri B, Engel AG. Duchenne dystrophy: electron microscopic findings pointing to a basic or early abnormality in the plasma membrane of the muscle fiber. Neurology. 1975;25(12): 1111–20.
3. Head SI, Williams DA, Stephenson DG. Abnormalities in structure and function of limb skeletal muscle fibres of dystrophic mdx mice. Proc Biol Sci. 1992;248(1322):163–9.
4. Nigro G, Comi LI, Politano L, Bain RJI. The incidence and evolution of cardiomyopathy in Duchene muscular dystrophy. Int J Cardiol. 1990;26:271–7.
5. van Essen AJ, Busch HF, te Meerman GJ, ten Kate LP. Birth and population prevalence of Duchenne muscular dystrophy in The Netherlands. Hum Genet. 1992;88(3):258–66.
6. Ricotti V, Ridout DA, Scott E, Quinlivan R, Robb SA, Manzur AY, Muntoni F, NorthStar Clinical Network. Long-term benefits and adverse effects of intermittent versus daily glucocorticoids in boys with Duchenne muscular dystrophy. J Neurol Neurosurg Psychiatry. 2013;84(6):698–705.
7. Eagle M, Baudouin SV, Chandler C, Giddings DR, Bullock R, Bushby K. Survival in Duchenne muscular dystrophy: improvements in life expectancy since 1967 and the impact of home nocturnal ventilation. Neuromuscul Disord. 2002;12(10):926–9.
8. Villa CR, Czosek RJ, Ahmed H, Khoury PR, Anderson JB, Knilans TK, Jefferies JL, Wong B, Spar DS. Ambulatory monitoring and arrhythmic outcomes in pediatric and adolescent patients with duchenne muscular dystrophy. J Am Heart Assoc. 2015;5(1).
9. Bushby K, Muntoni F, Bourke JP. 107th ENMC international workshop: the management of cardiac involvement in muscular dystrophy and myotonic dystrophy. 7th–9th June 2002, Naarden, the Netherlands. Neuromuscul Disord. 2003;13(2):166–72.
10. McNally EM, Kaltman JR, Benson DW, et al; Working Group of the National Heart, Lung, and Blood Institute; Parent Project Muscular Dystrophy.Contemporary cardiac issues in Duchenne muscular dystrophy. Working Group of the National Heart, Lung, and Blood Institute in collaboration with Parent Project Muscular Dystrophy. Circulation 2015;131(18):1590-1598.
11. Duboc D, Meune C, Lerebours G, Devaux JY, Vaksmann G, Becane HM. Effect of perindopril on the onset and progression of left ventricular dysfunction in Duchenne muscular dystrophy. J Am Coll Cardiol. 2005;45(6):855–7.
12. Duboc D, Meune C, Pierre B, et al. Perindopril preventive treatment on mortality in Duchenne muscular dystrophy: 10 years' follow-up. Am Heart J. 2007;154(3):596–602.
13. Allen HD, Flanigan KM, Thrush PT, et al. A randomized, double-blind trial of lisinopril and losartan for the treatment of cardiomyopathy in duchenne muscular dystrophy. PLoS Curr. 2013;12:5.
14. Raman SV, Hor KN, Mazur W, et al. Eplerenone for early cardiomyopathy in Duchenne muscular dystrophy: a randomized, double-blind, placebo-controlled trial. Lancet Neurol. 2015;14(2):153–61.
15. Silversides CK, Webb GD, Harris VA, Biggar DW. Effects of deflazacort on left ventricular function in patients with Duchenne muscular dystrophy. Am J Cardiol. 2003;91(6):769–72.
16. American Academy of Pediatrics Section on Cardiology and Cardiac Surgery. Cardiovascular health supervision for individuals affected by Duchenne or Becker muscular dystrophy. Pediatrics. 2005;116(6):1569–73.
17. Iodice F, Testa G, Averardi M, Brancaccio G, Amodeo A, Cogo P. Implantation of a left ventricular assist device as a destination therapy in Duchenne muscular dystrophy patients with end stage cardiac failure: management and lessons learned. Neuromuscul Disord. 2015;25(1):19–23.
18. Bushby KM, Thambyayah M, Gardner-Medwin D. Prevalence and incidence of Becker muscular dystrophy. Lancet. 1991;337(8748): 1022–4.
19. Emery AE, Skinner R. Clinical studies in benign (Becker type) X-linked muscular dystrophy. *Clin Genet*. 1976;10(4):189–201.
20. Hoogerwaard EM, De Voogt WG, Wilde AAM, et al. Evolution of cardiac abnormalities in Becker muscular dystrophy over a 13-year period. J Neurol. 1997;244:657–63.
21. Casazza F, Brambilla G, Salvato A, Morandi L, Gronda E, Bonacina E. Dilated cardiomyopathy and successful cardiac transplantation in Becker's muscular distrophy. Follow-up after two years. G Ital Cardiol. 1988;18(9):753–7.
22. Connuck DM, Sleeper LA, Colan SD, et al. Characteristics and outcomes of cardiomyopathy in children with Duchenne or Becker muscular dystrophy: a comparative study from the Pediatric Cardiomyopathy Registry. Am Heart J. 2008;155(6):998–1005.
23. Muntoni F, Cau M, Ganau A, et al. Deletion of the dystrophin muscle-promotor region associated with X-linked dilated cardiomyopathy. N Engl J Med. 1993;329:921–5.
24. Palmucci L, Doriguzzi C, Mongini T, et al. Dilating cardiomyopathy as the expression of Xp21 Becker type muscular dystrophy. J Neurol Sci. 1992;111(2):218–21.
25. Towbin JA. Fielding Hejtmancik J, Brink P, et al. *X-linked dilated cardiomyopathy Circulation* 1993;87:1854-1865.
26. Milasin J, Muntoni F, Severini GM, et al. A point mutation in the 5′ splice site of the dystrophin gene first intron responsible for X-linked dilated cardiomyopathy. Hum Mol Genet. 1996;5(1):73–9.
27. Muntoni F, Di LA, Porcu M, et al. Dystrophin gene abnormalities in two patients with idiopathic dilated cardiomyopathy. Heart. 1997;78(6):608–12.
28. Ferlini A, Galié N, Merlini L, Sewry C, Branzi A, Muntoni F. A novel Alu-like element rearranged in the dystrophin gene causes a splicing mutation in a family with X-linked dilated cardiomyopathy. Am J Hum Genet. 1998;63(2):436–46.
29. Hoogerwaard EM, van der Wouw PA, Wilde AAM, et al. Cardiac involvement in carriers of Duchenne and Becker muscular dystrophy. Neurobiol Dis. 1999;9:347–51.
30. Politano L, Nigro V, Nigro G, et al. Development of cardiomyopathy in female carriers of Duchenne and Becker muscular dystrophy. JAMA. 1996;275:1335–8.
31. Grain L, Cortina-Borja M, Forfar C, Hilton-Jones D, Hopkin J, Burch M. Cardiac abnormalities and skeletal muscle weakness in carriers of Duchenne and Becker muscular dystrophies and controls. Neuromuscul Disord. 2001;11(2):186–91.
32. Schade van Westrum SM, Hoogerwaard EM, Dekker L, et al. Cardiac abnormalities in a follow-up study on carriers of Duchenne and Becker muscular dystrophy. Neurology. 2011;77(1):62–6.
33. Rees W, Schuler S, Hummel M, Hetzer R. Heart transplantation in patients with muscular dystrophy associated with end-stage cardiomyopathy. J Heart Lung Transplant. 1993;12(5):804–7.
34. Schade van Westrum SM, Dekker LR, de Voogt WG, Wilde AA, Ginjaar IB, de Visser M, van der Kooi AJ. Cardiac involvement in Dutch patients with sarcoglycanopathy: a cross-sectional cohort and follow-up study. Muscle Nerve. 2014;50(6):909–13.
35. Williams TM, Lisanti MP. The Caveolin genes: from cell biology to medicine. Ann Med. 2004;36(8):584–95.
36. Hayashi T, Arimura T, Ueda K, et al. Identification and functional analysis of a caveolin-3 mutation associated with familial hypertrophic cardiomyopathy. Biochem Biophys Res Commun. 2004; 313(1):178–84.
37. Cronk LB, Ye B, Kaku T, et al. Novel mechanism for sudden infant death syndrome: persistent late sodium current secondary to mutations in caveolin-3. Heart Rhythm. 2007;4(2):161–6.
38. Vatta M, Ackerman MJ, Ye B, et al. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. Circulation. 2006;114(20):2104–12.

39. Goodwin FC, Muntoni F. Cardiac involvement in muscular dystrophies: molecular mechanisms. Muscle Nerve. 2005;32(5):577–88.
40. Brockington M, Yuva Y, Prandini P, et al. Mutations in the fukutin-related protein gene (FKRP) identify limb girdle muscular dystrophy 2I as a milder allelic variant of congenital muscular dystrophy MDC1C. Hum Mol Genet. 2001;10(25):2851–9.
41. Poppe M, Cree L, Bourke J, et al. The phenotype of limb-girdle muscular dystrophy type 2I. Neurology. 2003;60(8):1246–51.
42. van der Kooi AJ, Frankhuizen WS, Barth PG, et al. Limb-girdle muscular dystrophy in the Netherlands: gene defect identified in half the families. Neurology. 2007;68(24):2125–8.
43. Sveen ML, Schwartz M, Vissing J. High prevalence and phenotype-genotype correlations of limb girdle muscular dystrophy type 2I in Denmark. Ann Neurol. 2006;59(5):808–15.
44. Wahbi K, Meune C, Hamouda e H, et al. Cardiac assessment of limb-girdle muscular dystrophy 2I patients: an echography, Holter ECG and magnetic resonance imaging study. Neuromuscul Disord. 2008;18(8):650–5.
45. Petri H, Sveen ML, Thune JJ, Vissing C, Dahlqvist JR, Witting N, Bundgaard H, Køber L, Vissing J. Progression of cardiac involvement in patients with limb-girdle type 2 and Becker muscular dystrophies: a 9-year follow-up study. Int J Cardiol. 2015;182:403–11.
46. Kobayashi K, Nakahori Y, Miyake M, et al. An ancient retrotransposal insertion causes Fukuyama-type congenital muscular dystrophy. Nature. 1998;394(6691):388–92.
47. Toda T, Kobayashi K, Kondo-Iida E, Sasaki J, Nakamura Y. The Fukuyama congenital muscular dystrophy story. Neuromuscul Disord. 2000;10(3):153–9.
48. Murakami T, Hayashi YK, Noguchi S, et al. Fukutin gene mutations cause dilated cardiomyopathy with minimal muscle weakness. Ann Neurol. 2006;60(5):597–602.
49. Bione S, Maestrini E, Rivella S, et al. Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. Nat Genet. 1994;8:323–7.
50. Manilal S, Nguyen TM, Sewry CA, Morris GE. The Emery-Dreifuss muscular dystrophy protein, emerin, is a nuclear membrane protein. Hum Mol Genet. 1996;5(6):801–8.
51. Yates JRW. Workshop report: European workshop on Emery-Dreifuss muscular dystrophy. Neuromuscul Disord. 1991;1:393–6.
52. Emery AE. Emery-Dreifuss muscular dystrophy – a 40 year retrospective. Neuromuscul Disord. 2000;10(4–5):228–32.
53. Wehnert M, Muntoni F. 60th ENMC International Workshop: non X-linked Emery-Dreifuss Muscular Dystrophy 5–7 June 1998, Naarden, The Netherlands. *Neuromuscul Disord*. 1999;9(2):115–21.
54. Talkop UA, Talvik I, Sonajalg M, et al. Early onset of cardiomyopathy in two brothers with X-linked Emery-Dreifuss muscular dystrophy. Neuromuscul Disord. 2002;12(9):878–81.
55. Buckley AE, Dean J, Mahy IR. Cardiac involvement in Emery Dreifuss muscular dystrophy: a case series. Heart. 1999;82(1):105–8.
56. Sakata K, Shimizu M, Ino H, et al. High incidence of sudden cardiac death with conduction disturbances and atrial cardiomyopathy caused by a nonsense mutation in the STA gene. *Circulation*. 2005;111(25):3352–8.
57. Fishbein MC, Siegel RJ, Thompson CE, Hopkins LC. Sudden death of a carrier of X-linked Emery-Dreifuss muscular dystrophy. Ann Intern Med. 1993;119(9):900–5.
58. Anderson LVB. Multiplex Western blot analysis of the muscular dystrophy proteins. In: Bushby KMD, Anderson LVB, editors. Muscular dystrophy: Methods and protocols. Totowa: Humana Press; 2001. p. 369–86.
59. Bonne G, Di Barletta MR, Varnous S, et al. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery- Dreifuss muscular dystrophy. Nat Genet. 1999;21(3):285–8.
60. Goodwin FC, Muntoni F. Cardiac involvement in muscular dystrophies: molecular mechanisms. Muscle Nerve. 2005;32(5):577–88.
61. van Berlo JH, de Voogt WG, van der Kooi AJ, et al. Meta-analysis of clinical characteristics of 299 carriers of LMNA gene mutations: do lamin A/C mutations portend a high risk of sudden death? J Mol Med. 2005;83(1):79–83.
62. Curtis JP, Luebbert JJ, Wang Y, et al. Association of physician certification and outcomes among patients receiving an implantable cardioverter-defibrillator. JAMA. 2009;301(16):1661–70.
63. Van Rijsingen IA, Arbustini E, Elliott PM, et al. Risk factors for malignant ventricular arrhythmias in lamin a/c mutation carriers: a European cohort study. J Am Coll Cardiol. 2012;59(5):493–500.
64. Sovari AA, Bodine CK, Farokhi F. Cardiovascular manifestations of myotonic dystrophy-1. Cardiol Rev. 2007;15(4):191–4.
65. Groh WJ, Groh MR, Saha C, et al. Electrocardiographic abnormalities and sudden death in myotonic dystrophy type 1. N Engl J Med. 2008;358(25):2688–97.
66. Hermans MC, Faber CG, Pinto YM. Sudden death in myotonic dystrophy. N Engl J Med. 2008;359(15):1626–8.
67. Wahbi K, Meune C, Porcher R, et al. Electrophysiological study with prophylactic pacing and survival in adults with myotonic dystrophy and conduction system disease. JAMA. 2012;307(12):1292–301.
68. Hagemans ML, Hop WJ, Van Doorn PA, Reuser AJ, Van der Ploeg AT. Course of disability and respiratory function in untreated late-onset Pompe disease. Neurology. 2006;66(4):581–3.
69. Selcen D. Myofibrillar myopathies. Curr Opin Neurol. 2008;21(5):585–9.
70. van Spaendonck-Zwarts K, van Hessem L, van der Kooi AJ, et al. Desmin-related myopathy: a review and meta-analysis. Clin Genet. 2011;80(4):354–66.
71. Wahbi K, Béhin A, Charron P, Dunand M, Richard P, Meune C, Vicart P, Laforêt P, Stojkovic T, Bécane HM, Kuntzer T, Duboc D. High cardiovascular morbidity and mortality in myofibrillar myopathies due to DES gene mutations: a 10-year longitudinal study. Neuromuscul Disord. 2012;22(3):211–8.
72. Sewry CA, Jimenez-Mallebrera C, Muntoni F. Congenital myopathies. Curr Opin Neurol. 2008;21(5):569–75.
73. Jungbluth H, Sewry CA, Muntoni F. Core myopathies. Semin Pediatr Neurol. 2011;18(4):239–49.
74. Feng JJ, Marston S. Genotype-phenotype correlations in ACTA1 mutations that cause congenital myopathies. Neuromuscul Disord. 2009;19(1):6–16.
75. D'Amico A, Graziano C, Pacileo G, et al. Fatal hypertrophic cardiomyopathy and nemaline myopathy associated with ACTA1 K336E mutation. Neuromuscul Disord. 2006;16(9–10):548–52.
76. Tajsharghi H, Oldfors A, Macleod DP, Swash M. Homozygous mutation in MYH7 in myosin storage myopathy and cardiomyopathy. Neurology. 2007;68(12):962.
77. Uro-Coste E, Arne-Bes MC, Pellissier JF, et al. Striking phenotypic variability in two familial cases of myosin storage myopathy with a MYH7 Leu1793pro mutation. Neuromuscul Disord. 2009;19(2):163–6.
78. Agrawal PB, Pierson CR, Joshi M, Liu X, Ravenscroft G, Moghadaszadeh B, Talabere T, Viola M, Swanson LC, Haliloğlu G, Talim B, Yau KS, Allcock RJ, Laing NG, Perrella MA, Beggs AH. SPEG interacts with myotubularin, and its deficiency causes centronuclear myopathy with dilated cardiomyopathy. Am J Hum Genet. 2014;95(2):218–26.
79. Sacconi S, Wahbi K, Theodore G, Garcia J, Salviati L, Bouhour F, Vial C, Duboc D, Laforêt P, Desnuelle C. Atrio-ventricular block requiring pacemaker in patients with late onset Pompe disease. Neuromuscul Disord. 2014;24(7):648–50.
80. Van der Beek NA, Hagemans ML, Van der Ploeg AT, Reuser AJ, Van Doorn PA. Pompe disease (glycogen storage disease type II): clinical features and enzyme replacement therapy. Acta Neurol Belg. 2006;106(2):82–6.
81. Van der Beek NA, Soliman OI, van Capelle CI, et al. Cardiac evaluation in children and adults with Pompe disease sharing the com-

mon c.-32–13T>G genotype rarely reveals abnormalities. J Neurol Sci. 2008;275(1–2):46–50.
82. Danon MJ, Oh SJ, DiMauro S, et al. Lysosomal glycogen storage disease with normal acid maltase. Neurology. 1981;31(1):51–7.
83. van der Kooi AJ, van Langen IM, Aronica E, et al. Extension of the clinical spectrum of Danon disease. Neurology. 2008;70(16):1358–9.
84. Cetin H, Wöhrer A, Rittelmeyer I, Gencik M, Zulehner G, Zimprich F, Ströbel T, Zimprich A. The c.65-2A>G splice site mutation is associated with a mild phenotype in Danon disease due to the transcription of normal LAMP2 mRNA. Clin Genet. 2016.
85. Ozawa T, Tanaka M, Sugiyama S, Hattori K, Ito T, Ohno K, Takahashi A, Sato W, Takada G, Mayumi B, et al. Multiple mitochondrial DNA deletions exist in cardiomyocytes of patients with hypertrophic or dilated cardiomyopathy. Biochem Biophys Res Commun. 1990;170(2):830–6.
86. Wahbi K, Bougouin W, Béhin A, et al. Long-term cardiac prognosis and risk stratification in 260 adults presenting with mitochondrial diseases. Eur Heart J. 2015;36(42):2886–93.
87. Tranchant C, Mousson B, Mohr M, et al. Cardiac transplantation in an incomplete Kearns-Sayre syndrome with mitochondrial DNA deletion. Neuromuscul Disord. 1993;3(5–6):561–6.
88. Bit-Avragim N, Perrot A, Schols L, et al. The GAA repeat expansion in intron 1 of the frataxin gene is related to the severity of cardiac manifestation in patients with Friedreich's ataxia. J Mol Med. 2001;78(11):626–32.
89. Barth PG, Valianpour F, Bowen VM, et al. X-linked cardioskeletal myopathy and neutropenia (Barth syndrome): an update. Am J Med Genet A. 2004;126A(4):349–54.
90. Barth PG, Wanders RJ, Vreken P. X-linked cardioskeletal myopathy and neutropenia (Barth syndrome)-MIM 302060. J Pediatr. 1999;135(3):273–6.
91. Stangou AJ, Hawkins PN, Heaton ND, et al. Progressive cardiac amyloidosis following liver transplantation for familial amyloid polyneuropathy: implications for amyloid fibrillogenesis. Transplantation. 1998;66(2):229–33.
92. Ruberg FL, Berk JL. Transthyretin (TTR) cardiac amyloidosis. Circulation. 2012;126(10):1286–300.
93. Algalarrondo V, Dinanian S, Juin C, Chemla D, Bennani SL, Sebag C, Planté V, Le Guludec D, Samuel D, Adams D, Slama MS. Prophylactic pacemaker implantation in familial amyloid polyneuropathy. Heart Rhythm. 2012;9(7):1069–75.

Future of Cardiogenetics

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Abstract

Cardiogenetics faces two major challenges: identifying index cases and making optimal use of sequencing technologies. Nonexpert physicians need to be actively engaged to identify index cases, not only in cardiology and clinical genetics but also in primary care, population screening, at postmortem, and elsewhere in health care. Training, including e-modules, is needed to update knowledge and skills. An active approach to test family members of diagnosed cases may save lives. With decreasing prices of sequencing technologies, the temptation to sequence everything that is possible might lead to many variants of unknown significance. Patients and their families would be better served by targeted analysis of variants of high predictive value and selecting the DNA test based on the phenotype. Data sharing and more detailed and precise phenotyping will help to better understand the current variants of unknown significance.

Introduction

Over the last two decades, cardiogenetics has developed as a health service at the interface of cardiology and genetics. The 23 chapters in this book discuss disorders, some of which may be rare, but the sum of which deserves serious attention in health care. Cardiac disorders often follow a well-known pattern (risk associated with age, obesity, smoking, sedentary lifestyle, diabetes) but if the usual pattern does not fit, a cardiogenetic diagnosis may apply, requiring a treatment that is different and often disease-specific. Since many of these conditions are autosomal dominant (with reduced penetrance), presymptomatic diagnosis in family members may provide a platform for early prevention strategies.

In this final chapter, we will discuss challenges for the future. The first challenge is how the increasing body of knowledge can be translated from science to implementation at a population level. To avoid that science is "lost in translation," an active approach to identify index cases is needed, in cardiology, clinical genetics, primary care, population screening, at postmortem, and elsewhere in health care. Furthermore, a challenge is how new technologies can be implemented in health care in a responsible manner, including genome sequencing technologies and imaging, with the ultimate goal to improve the care of patients and their families with inherited heart diseases.

The First Step

Diagnosis in the Mainstream of Medicine

Translation of cardiogenetic knowledge to health care requires that *index cases* are *recognized*. For cardiology and clinical genetics, diagnostic skills and technical support may

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be relatively well developed. The first contact in health care, however, can be in primary care, in a public health screening program, at the ultrasound unit, or at postmortem. Many stakeholders are involved in these different settings. Therefore, a transdisciplinary collaborative effort is required for a beneficial effect of cardiogenetics in daily medical practice. Current diagnostic workup of suspected ischemic heart disease in primary care often applies a limited set of parameters to assess risk. In daily primary care practice, important subsets of patients at high risk based on a family history of premature heart disease, but otherwise limited conventional risk profile, are withheld further diagnostics.

Current genomic literacy of nongeneticist health care providers is limited. Up-to-date CVD guidelines could be of great value since they are frequently checked in daily practice, could be used in educational activities, and are used to attune between primary and secondary care. While not all (rare) diseases can be recognized, the red flag or gut feeling "this is not a regular cardiovascular problem" should be developed for all health care workers. Electronic health records with integrated ICT tools will help daily practice to register family history, store, and analyze imaging results, and provide decision support. Consequently awareness and information on possibilities for referral to centers with adequate diagnostic facilities can then be provided. The future health care worker can go to *online resources* to quickly look up which diagnoses should be considered, to whom he can refer, and which tests are available. Postgraduate training will update health care workers on the current status of cardiogenetics. Given the fast developments, not only face-to-face training sessions are needed but also e-learning is needed to enable noncardiogeneticists to update their skills (Houwink et al. [8]).

Population Screening

Apart from recognizing patients who visit a clinic with *symptoms* of cardiogenetic disorders, it is conceivable that all *asymptomatic* persons at a certain age (newborn) or in a certain setting (working place, sports) would be *offered* screening for cardiogenetic risks. Ever increasing genome technological capability will force a discussion of including genetic tests in these screening programs (Henneman et al. [7]). In general, population screening programs are implemented only after careful consideration whether the pros outweigh the cons, a systematic evaluation of which may use the framework developed by Wilson and Jungner in 1968, or frameworks that elaborated these criteria [22]. Criteria include whether the condition screened for is an important health problem, the natural history is adequately understood, treatment is available, good tests are available, and the cost of case finding is economically in balance relative to medical expenditure.

Familial Hypercholesterolemia (FH) as Example

For the field of cardiogenetics, the assessment of cholesterol levels and subsequent DNA testing to recognize familial hypercholesterolemia (FH) may serve as a historical example. Most people with FH are undiagnosed or only diagnosed after their first coronary event. There is a need for earlier detection. The first step should be effective family history taking, the primary care physician would be the first health care worker in most cases where people with (early) disease symptoms could come. Up to 1 in 200–500 individuals carry a mutation in genes associated with FH (*LDLR, APOB, PCSK9*) (Kassner et al. [12]). Early identification followed by lipid lowering treatment, increasing levels of physical activity and not smoking can substantially reduce mortality and morbidity. A cascade-screening program in the Netherlands identified 28.000 persons affected (Carpay et al. [2]). It has been estimated that this represents 50–71 % of the patients in the country (Carpay et al. [2], Norderstgaard et al. [13]). Other countries have reported lower proportions recognized, even <1 % (Nordestgaard et al. [13]). Several types of FH-screening programs exist, but a recent debate focused on the question whether all 10–12 year old children should be screened. In children, opportunities for CHD prevention are greatest (Wiegman et al. [21]). In the USA in 2003, the CDC began funding integrating genomics into chronic disease programs where possible (St. Pierre [17]). A statewide universal screening of school children would optimize prevention for all FH children. However, in such an endeavor different professional groups come together, each with their own practices, cultures, and structures. Decisions must be made on the type of the test used (first cholesterol, DNA as second tier?). For the future, this primary preventive approach may be suitable for more conditions. Presymptomatic genetic testing of minors is, however, often not encouraged, but it may be acceptable if preventive actions can be initiated before adulthood [3]. In many cardiogenetic conditions indeed, prevention should start before adulthood.

Opportunistic Screening

Sequencing technologies would make it possible to investigate cardiogenes whenever sequencing is performed: opportunistic screening (Green et al. [4]). It has been argued that the ethics of opportunistic screening requires further debate, and informed decision making for the individuals undergoing testing should be facilitated; however, the technical possibilities exist already. Professionals need to develop recommendations for handling genomic findings not related to the initial clinical question, in line with the legal and cultural particularities of individual states (Hehir-Kwa et al. [6]).

Postmortem Cardiogenetic Diagnostics

The first cardiovascular event in a lifetime can lead to sudden death. Pathologists and coroners play an important role to recognize cardiogenetic conditions, particularly in sudden cardiac death in the young aged under 35 years, and thus to contribute to prevention in relatives. Countries differ in their postmortem health services. Protocols should include the possibility of reporting of the results of postmortem investigations to relatives, freezing tissue, or isolating DNA (Semsarian et al. [16], Bagnall et al. [1]). The autopsy rate in young sudden death cases could be increased, and more families should undergo cardiogenetic evaluation to detect inherited cardiac diseases (van der Werf et al. [19]). Presymptomatic diagnosis in healthy relatives will make targeted treatment possible, such as implantable cardioverter defibrillator placement. This can save future lives (Stattin et al. [18]).

New Methods

Pipelines for Multiple Variants in Multiple Genes

Whole genome sequencing techniques have entered diagnostic laboratories, making it possible to investigate simultaneously many potential causes of a specific disorder (Haas et al. [5]). The cost of sequencing has decreased rapidly (https://www.genome.gov/sequencingcosts/), thus making it possible to do the test first and depend less on a differential diagnosis of an expert cardiogeneticist. Many people who lost a family member due to sudden (cardiac?) death did not have access to genomic testing, but with pipelines for hundreds of genes implicated in cardiogenetics, they may qualify for a broad DNA test to confirm or exclude an increased risk for themselves. A major challenge will be the interpretation of variants. Only if the predictive value of a variant is high, a (preventive) treatment should be proposed, but what does this mean for the pipelines to be built? It has been suggested to use a targeted approach first in order to avoid unsolicited findings or findings that cannot be interpreted, and that known genetic variants with limited or no clinical utility should be filtered out (van El et al. [20]).

Interpreting Variants

A major challenge in the current era is determining the pathogenicity of identified variants. Caution is needed as variants of unknown significance (VUS) can be detected in a high percentage of probands (Nunn et al. [14]), with an increase in VUS as more genes are screened. A mutation in genes associated with cardiomyopathies and ion channelopathies is not always a disease causing mutation, and so measures to determine pathogenicity need to be improved as we look ahead at whole exome and genome approaches. Proband genetic testing results in a probabilistic outcome, with the need for accumulating evidence for disease causality (Ingles [10]). Population databases may help to determine whether a variant was frequently seen in healthy persons or only in affected families. Sharing of data is more important than ever not only for research but for clinical purposes as well. Initiatives such as The Global Alliance for Genomics and Health (genomicsandhealth.org) and The Clinical Genome Resource (clinicalgenome.org) call for the establishment of a common framework of harmonized approaches to enable effective and responsible sharing of genomic and clinical data, and by catalyzing data-sharing projects that drive and demonstrate the value of data sharing. Functional data can indicate whether a variant has phenotypical consequences in animal models, or even in in silico tools such as myocytes on-a-chip. Rapid functional assays for determining disease causality of variants are currently lacking and will be an important component of future cardiogenetic evaluation. Newer and faster animal models such as zebrafish, and utility of induced pluripotent stem cells derived from the patient, may be the types of methods which may help to elucidate the functional consequences of the identified genetic variants.

The Choice of the Genetic Test

With the explosion of next-generation sequencing technologies, the temptation will be to screen all genes. The choice of which genetic test is chosen for the individual patient will be more important than ever. Unnecessary screening of genes will likely result in more VUS, more incidental, and secondary findings. As such, the principle of determining the most appropriate genetic tested based on the patient's phenotype will be essential. Targeted genetic sequencing of the top ten hypertrophic cardiomyopathy genes may be the most appropriate test for someone with classical HCM and a strong family history. In contrast, an unexplained sudden death in a young person may require a genetic testing panel of 50–60 genes covering the main primary arrhythmia disease genes for LQTS, BrS, and CPVT. The clear tradeoff is that in all cardiogenetic diseases, as more disease genes are screened, the greater likelihood of VUS results (Ingles [11]).

Improving Detection of the Cardiac Phenotype: "Deep Phenotyping"

One of the major challenges relating to cardiogenetics in coming years is to match the depth of genetic sequencing with more detailed and precise phenotyping (Semsarian [15]). Most clinicians agree that measuring a QT interval on an ECG or measuring a left ventricular wall thickness on an echocardiogram are very basic and superficial measures for

long QT syndrome and HCM respectively. Newer clinical diagnostics will be required to phenotype cardiogenetic patients in more detail. This may include more precise imaging techniques, use of serum biomarkers of disease, and more ambulatory measures such as 12 lead Holter monitoring in Brugada syndrome patients. Such newer approaches will facilitate better definition of the cardiac phenotype, allow better targeting of genetic testing, and may have implications for the choice of therapeutic interventions and overall prognosis.

Imaging

Cardiac magnetic resonance imaging and cardiac computed tomographic angiography are increasingly available for non-invasive diagnosis of cardiovascular disease. Structural anomalies identified while using advanced imaging techniques may help to diagnose hypertrophic cardiomyopathy, FH causing premature coronary disease, and systemic connective tissue diseases. If young high-risk individuals are to profit most from imaging, a strategy is needed to timely recognize these high-risk patients in clinical practice through an increase in genomic literacy of all relevant health care providers (Houwink et al. [9]). An integrated and multidisciplinary strategy is needed for timely detection of cardiovascular disease using advanced imaging and genomics techniques.

Summary/Take Home Message

Cardiogenetics has developed over the last two decades to a very valuable health service. The main challenges ahead are to make this service well known to all who could potentially benefit. A wider circle of health care workers need education and training in cardiogenetics to improve the recognition of index cases. Testing their healthy family members will contribute to save future lives. Technological developments using sequencing technologies currently lead to many VUS. Data-sharing strategies, functional studies, and deep phenotyping are needed to understand their significance. Filtering known variants will be needed. Genetic variants with limited or no clinical utility should be filtered out.

- The first step to identify index cases requires mainstreaming of cardiogenetics including increased awareness in primary care and with pathologists.
- Sequencing techniques will make it possible to investigate multiple variants in multiple genes. The filtering of known variants with known predictive value will be a challenge.
- The choice of which genetic test will continue to rely heavily on the phenotype of the index case and the relevant family history.
- The complexities of cardiac genetic testing highlight the importance of a specialized multidisciplinary approach to care for patients and their families.

References

1. Bagnall RD, Weintraub RG, Ingles J, Duflou J, Yeates L, Lam L, et al. A Prospective Study of Sudden Cardiac Death among Children and Young Adults. N Engl J Med. 2016;374:2441–52.
2. Carpay MEM, van der Horst A, Hoebee B. Eindrapportage bevolkingsonderzoek naar familiaire hypercholesterolemie. Organisatie en opbrengsten. RIVM Briefrapport 2014–0152. 2014. Bilthoven, RIVM.
3. European Society of Human Genetics. Genetic testing in asymptomatic minors: recommendations of the European Society of Human Genetics. Eur J Hum Genet. 2009;17:720–1.
4. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med. 2013;15:565–74.
5. Haas J, Frese KS, Peil B, Kloos W, Keller A, Nietsch R, et al. Atlas of the clinical genetics of human dilated cardiomyopathy. Eur Heart J. 2015;36:1123–35a.
6. Hehir-Kwa JY, Claustres M, Hastings RJ, van Ravenswaaij-Arts C, Christenhusz G, Genuardi M, et al. Towards a European consensus for reporting incidental findings during clinical NGS testing. Eur J Hum Genet. 2015;23:1601–6.
7. Henneman L, McBride CM, Cornel MC, Duquette D, Qureshi N. Screening for familial hypercholesterolemia in children: What can we learn from adult screening programs? Healthcare. 2015;3:1018–30.
8. Houwink EJ, Muijtjens AM, van Teeffelen SR, Henneman L, Rethans JJ, Jacobi F, et al. Effect of comprehensive oncogenetics training interventions for general practitioners, evaluated at multiple performance levels. PLoS One. 2015;10(4):e0122648.
9. Houwink EJ, Sollie AW, Numans ME, Cornel MC. Proposed roadmap to stepwise integration of genetics in family medicine and clinical research. Clin Transl Med. 2013;2:5.
10. Ingles J, Semsarian C. Conveying a probabilistic genetic test result to families with an inherited heart disease. Heart Rhythm. 2014a;11:1073–8.
11. Ingles J, Semsarian C. The value of cardiac genetic testing. Trends Cardiovasc Med. 2014b;24:217–24.
12. Kassner U, Wuhle-Demuth M, Missala I, Humphries SE, Steinhagen-Thiessen E, Demuth I. Clinical utility gene card for: Hyperlipoproteinemia, TYPE II. Eur J Hum Genet. 2014. doi:10.1038/ejhg.2013.271.
13. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, OS D, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease consensus statement of the European Atherosclerosis Society. Eur Heart J. 2013;34:3478–90.
14. Nunn LM, Lopes LR, Syrris P, Murphy C, Plagnol V, Firman E, et al. Diagnostic yield of molecular autopsy in patients with sudden arrhythmic death syndrome using targeted exome sequencing. Europace. 2015; pii: euv285.
15. Semsarian C, Ingles J. Determining pathogenicity in cardiac genetic testing: filling in the blank spaces. Trends Cardiovasc Med. 2015a;25:653–4.

16. Semsarian C, Ingles J, Wilde AA. Sudden cardiac death in the young: the molecular autopsy and a practical approach to surviving relatives. Eur Heart J. 2015b;36:1290–6.
17. St. Pierre J, Bach J, Duquette D, Oehlke K, Nystrom R, Silvey K, et al. Strategies, actions, and outcomes of pilot state programs in public health genomics, 2003–2008. Prev Chronic Dis. 2014; 11:E97.
18. Stattin EL, Westin IM, Cederquist K, Jonasson J, Jonsson BA, Mörner S, et al. Genetic screening in sudden cardiac death in the young can save future lives. Int J Legal Med. 2016;130:59–66.
19. van der Werf C, Hendrix A, Birnie E, Bots ML, Vink A, Bardai A, et al. Improving usual care after sudden death in the young with focus on inherited cardiac diseases (the CAREFUL study): a community-based intervention study. Europace. 2015; pii: euv059.
20. Van El CG, Cornel MC, Borry P, Hastings RJ, Fellmann F, Hodgson SV, et al. Whole-genome sequencing in health care: recommendations of the European Society of Human Genetics. Eur J Hum Genet. 2013;21:580–4.
21. Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. Eur Heart J. 2015;36:2425–37.
22. Wilson JMG, Jungner G. Principles and practice of screening for disease, vol. 68. Geneva: World Health Organization; 1968.

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