Clinical Genetics

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2.1 Introduction

Clinical geneticists are medical doctors that combine general knowledge of medicine with specific expertise in genetics, genetic diagnosis, and genetic aspects of disease. They are specifically trained in communicating the implications of genetic information and genetic disease to patients and their families. In addition, they are trained in syndrome diagnosis and clinical dysmorphology.

In some countries, clinical genetics has evolved as a recognized medical specialty, whereas in other countries genetic services are provided by traditional specialists with specific additional training in genetics. Usually, only university hospitals and sometimes large regional hospitals have departments of clinical genetics. This centralized approach may limit access to genetic services, but facilitates the availability of staff and resources that are necessary for conducting family investigations, that are usually beyond the scope of the individual medical specialist. By working on a consultant basis in different hospitals and by employing trained genetic nurses for specific tasks, clinical geneticists try to optimize access to genetic services.

With the very 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.

Over the last one and a half decade, 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 for not only rare monogenic disorders such as different types of cardiomyopathy, congenital long QT syndromes, and catecholaminergic polymorphic ventricular tachycardia, but also for more common complex disorders such as cardiovascular 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 causing the disease. 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 for 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

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

Cardiology is a third discipline of medicine where large-scale so-called presymptomatic testing of healthy at risk individuals has become available for some of the primary electric heart diseases and cardiomyopathies. An important driving force behind this development is the "sudden cardiac death phenotype," motivating both families and their treating cardiologists to undertake steps to identify individuals at increased risk of life-threatening arrhythmias at an early stage. 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 may be complicated, for example, by *genetic heterogeneity* (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.

2.1.1 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.

2.1.1.1 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.

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.

2.1.2 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

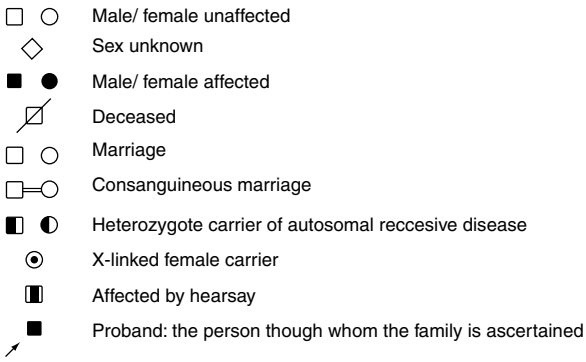

Fig. 2.1 Symbols used to denote individuals in a pedigree

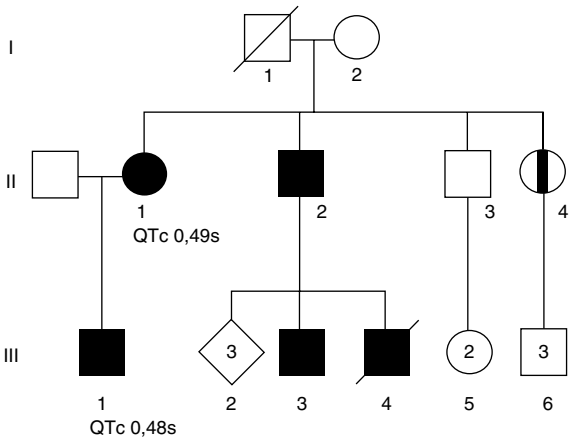

Fig. 2.2 Example of a small pedigree like one could draw up, while taking a family history, during consultation of a family suspected of long QT syndrome type 1. Footnotes with this pedigree could be: III-1 index patient (27-10-2000), syncope while playing soccer, spontaneous recovery, QTc 0.48 s, repolarization pattern compatible with lQT type I. I-1: no medical information, died in unilateral car accident at age 32. II-1: (02-3-1975), no symptoms, QTc 0,49 sec. II-2 (10-5-1977) known with seizures as a child. II-4: (13-6-1979)said to have fainted during exercise more than one occasion. III-4 sudden death, while swimming at age 12yrs. No other persons known with seizures ,syncope or sudden death known in the family

structure and asses the possible inheritance patterns.[4,5] 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 doing 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.

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. 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" (see 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.

2.1.3 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.[4] 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 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 exception 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.

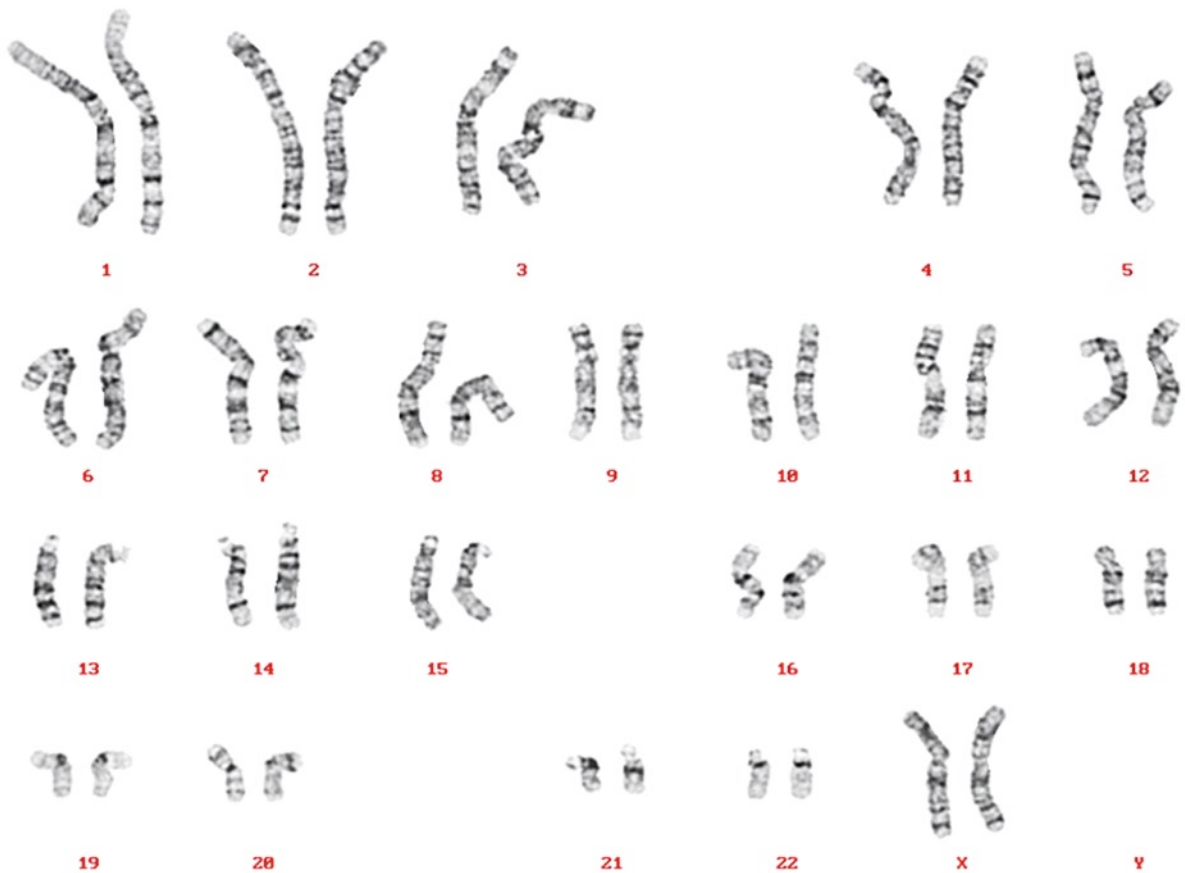

Fig. 2.3 Normal female karyogram (46, XX): the way the chromosomes are shown, when DNA is visualized through a light microscope

2.1.4 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 more or less random process is called homologous *recombination*.

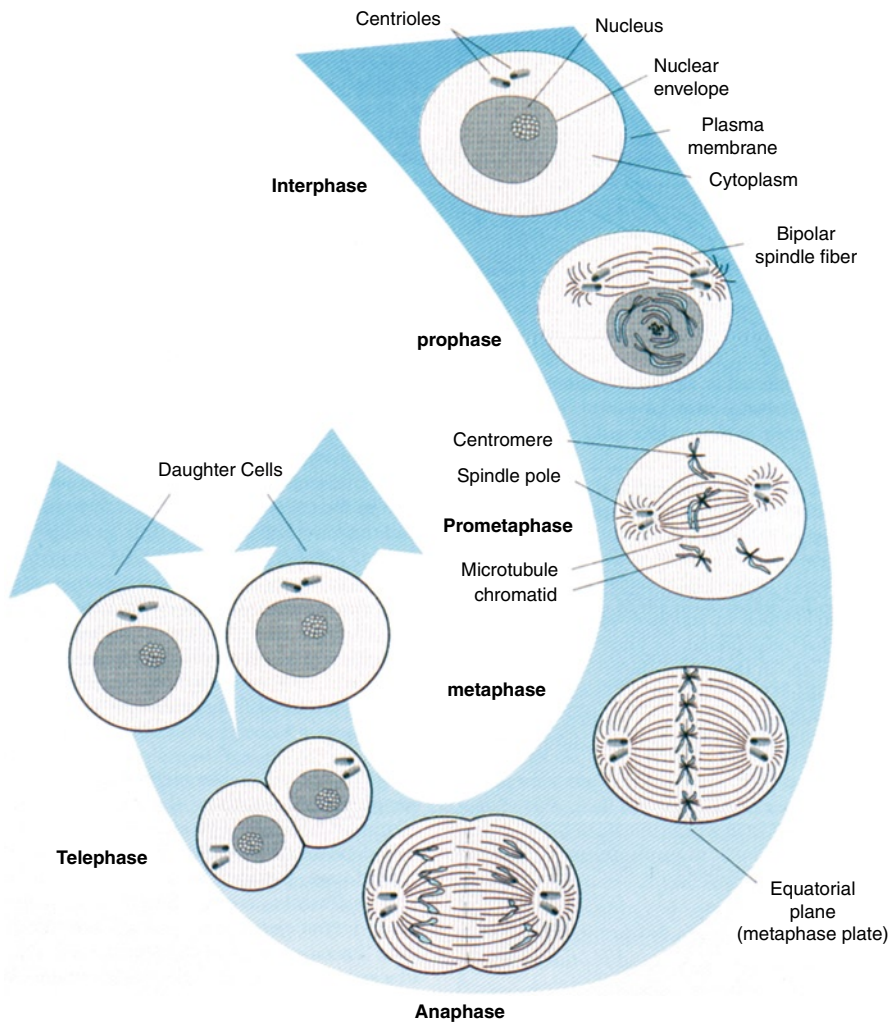

Fig. 2.4 Different stages of mitosis, leading to two daughter cells with exactly the same nuclear DnA content. (Reprinted with permission Jorde, Carey, Bamshad, White, Medical Genetics third edition, Mosby Elsevier 2006)

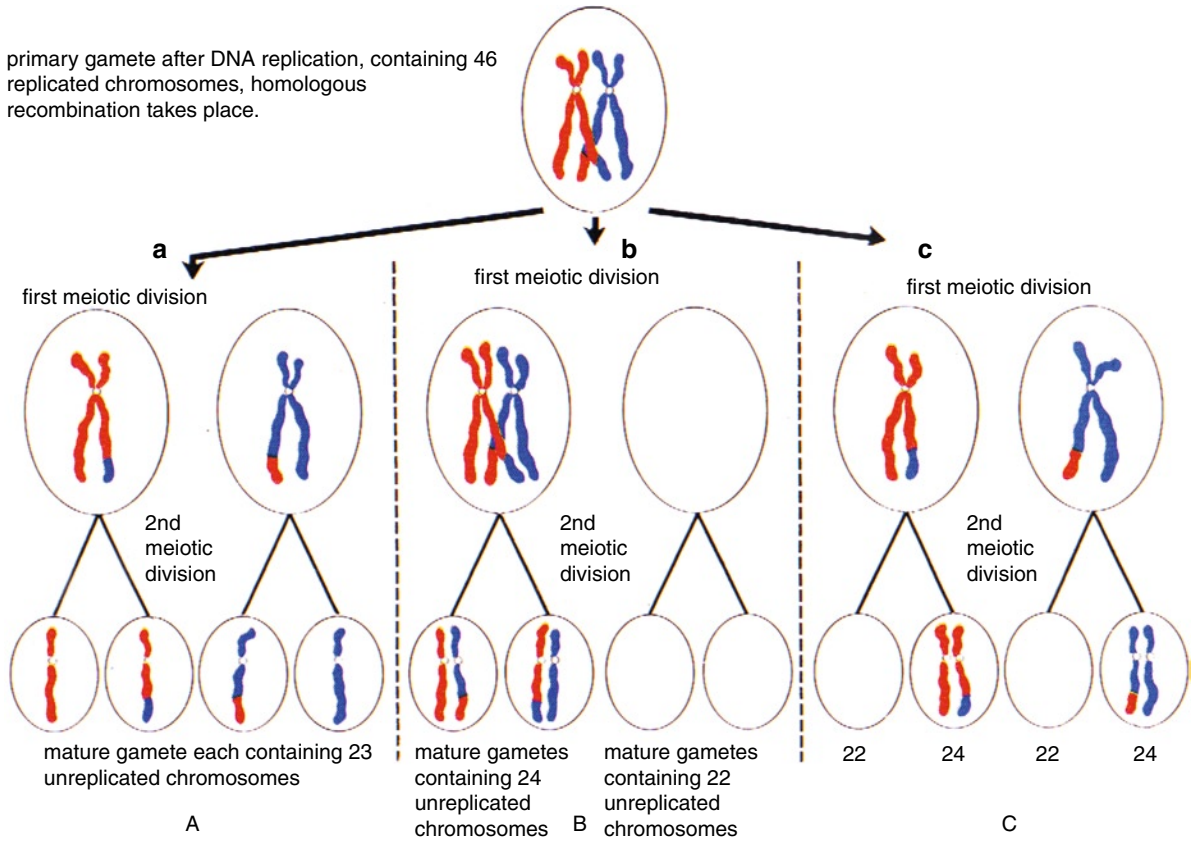

Fig. 2.5 Meiosis: (**a**) demonstrates the normal stages of meiosis (after division one the cell contains 23 replicated chromosomes – 22 autosomes and 1 sex-chromosome). (**b**) Demonstrates nondisjunction in meiosis 1 (the most frequent cause of for instance Down syndrome). (**c**) Demonstrates nondisjunction 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)

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.

2.1.4.1 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 in 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 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 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.

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

2.1.4.2 Inheritance Patterns

A genetic component plays a role in many diseases. Usually the genetic contribution to disease is appreciated when either a clear pattern of inheritance or significant familial clustering of a disease is noted.[5]

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 non-genetic 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. FH (familial hypercholesterolemia) as a result of mutations in the LDL receptor or MODY (maturity onset diabetes in the young), are examples of monogenic subtypes of diseases that most often have a complex etiology.

2.1.5 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.

2.1.5.1 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 to be 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)

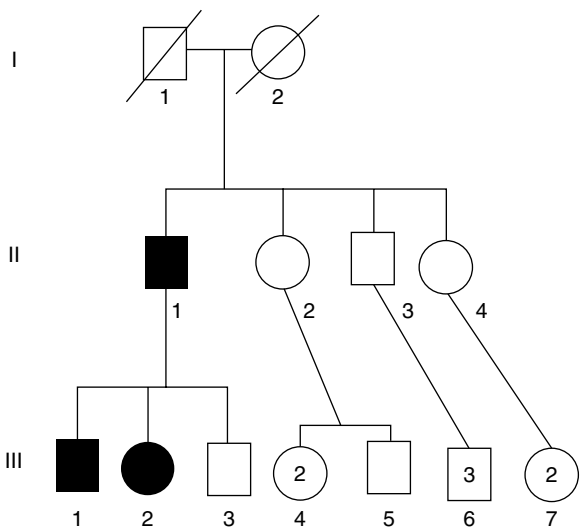

Fig. 2.6 Example of a small autosomal dominant pedigree, the observed male to male transmission (II-1 > III-1) excludes X-linked dominant inheritance. If we assume this disorder has full penetrance a the novo mutation must have occurred in II-1

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*).

2.1.5.2 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. 2.7). 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:

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

2.1.5.3 X-Linked Recessive Inheritance

X-linked disorders are caused by mutations on the X-chromosome. The X-chromosome does not contain

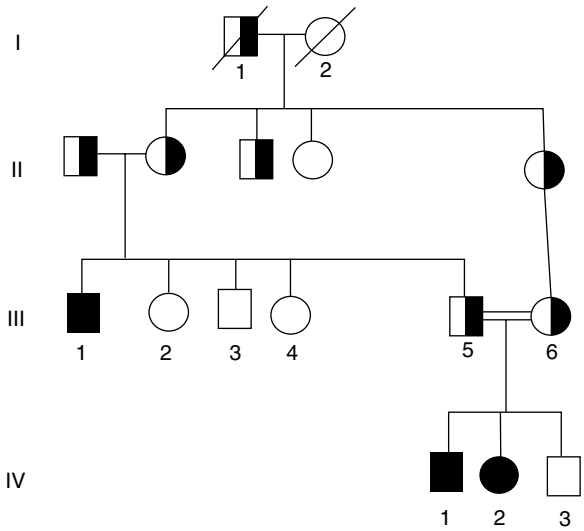

Fig. 2.7 Example of an autosomal recessive pedigree, illustrating the role of consanguinity in AR disease.III-5 and III-6 are first cousins. IV-1 and IV-2 inherited both their mutated alleles from a single heterozygous grandparent (in this case I-1). In this pedigree heterozygous carriers are indicated, usually heterozygous carriers for AR disorders can only be unambiguously identified by DNA analysis

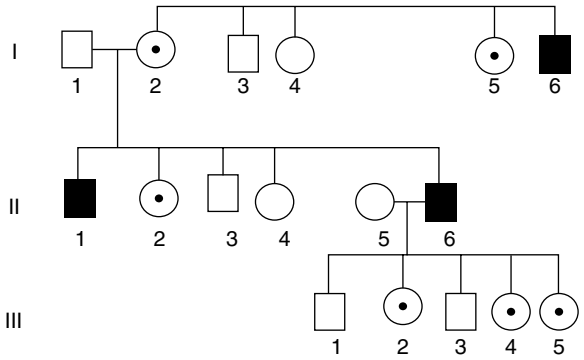

Fig. 2.8 Example of an X-linked recessive disorder, the disease is transmitted via apparently healthy heterozygous females, only hemizygous males manifest the disease. All daughters of an affected male will be carriers. Carrier females are indicated with dots within circles, usually DNA analysis will be required to unambiguosly identify carrier females

"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. 2.8):

- No male to male transmission occurs.
- Heterozygous females are healthy.
- All daughters of affected males will be 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.

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 a female like it does in males.

2.1.5.4 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. 2.9):

- 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.

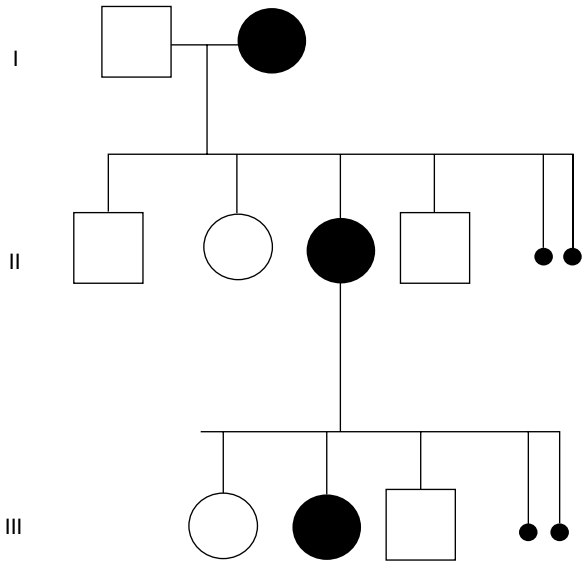

Fig. 2.9 Example pedigree of an X-linked dominant disorder with early lethality in males. Affected males that are conceived will miscarry (leading to skewed sex ratios in offspring). The black dots in the pedigree represent miscarriages. Based on such a limited pedigree, definite distinction from autosomal 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, whilst 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.

2.1.6 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 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.

2.1.6.1 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 will 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. 2.10). 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.
- 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.

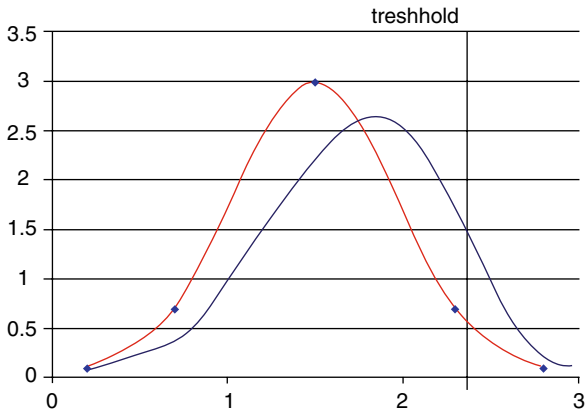

Fig. 2.10 Example of a liability distribution of a discontinuous multifactorial trait (a congenital heart defect) in a given population. The red curve is for the general population. The area under the red curve to the right of the threshold represents the proportion of individuals with CHD in the general 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

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 are 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.

2.1.6.2 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 RNA's and ribosomal RNA's).

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 non-penetrance will result if the threshold for disease expression is not reached.

2.1.7 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 (that is, 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}–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.

2.1.8 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 a 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.

2.1.9 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 dystrophin mutation will cause Duchenne muscular dystrophy in all hemizygous males. However, especially in autosomal dominant disease, penetrance is often reduced; for example, 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 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 non-penetrants. 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 non-penetrance 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 a 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.

2.2 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.[6,7] 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 selection 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.

The reverse situation needs also to be considered. Clinical information on history and clinical data such as, for example, T-wave morphology in patients suspected of having a form of long QT syndrome is very helpful in selecting the genes that should be analyzed first.[8] 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.

2.3 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.

2.3.1 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.

2.3.2 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.[9] 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.

2.4 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.[10] 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.

2.5 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.

2.5.1 Consanguinity

Consanguineous marriages are very common in some cultures and unusual in others.[11] 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.[12] 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 QT syndrome and hypertrophic cardiomyopathy.[13, 14]

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.

2.5.2 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 (future) family members. 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.

2.5.3 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.[15] Genetic counseling is offered by trained medical or paramedical professionals. Its goals are:

- 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 counselees choose a suitable course of action in view of their individual risk of disease, goals, personal and cultural values, religious beliefs, and facilitate this course of action
- Support counselees 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 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.

In the counseling process different stages can be discerned:

- *Diagnostic phase.* For meaningful genetic counseling, the genetic diagnosis has to be as precise as possible. As we are considering conditions that often run in families, medical information from other affected family members is necessary to arrive at the correct family diagnosis. In addition to a history, family history, physical examination, and cardiac investigations it may therefore be necessary to collect medical records or pathology reports of relatives of the counselee. If genetic testing has previously been performed in the family, it is vital that this information is retrieved.
- *Informative counseling.* During this stage, information on the condition, natural course, complications, prognosis, genetics, management, and treatment options are communicated with the counselee. Although standardized written information can be very helpful, it cannot fully replace counseling. Information should be tailored to the specific needs and knowledge of the counselee, and the counselor should verify whether vital information is understood.
- *Supportive counseling.* During this phase, the emphasis is on the decision-making process and coping. Counselees and their partners have to make choices with regard to, for example, reproductive issues or presymptomatic genetic testing. Choices have to be reinforced and counselees and their relatives have to be supported in coping with the genetic disease that runs in their family, and eventually with the outcome of genetic testing. The results of genetic testing in one individual often alter disease risks for relatives. It may be necessary to inform relatives about the (newly identified) genetic disease in their family, and the fact that they themselves are, or are not, at risk to be genetically predisposed to this disease. Counselees should be aided in communicating such difficult news to their relatives.
- *Follow-up.* People should be encouraged to return if important questions remain or if new issues arise. Ideal and easy solutions for problems related to inherited disease are rarely, if ever, available. Therefore, coping with genetic information may take quite some time, and what seems to be the best course of action may change over time. Moreover, new information on other affected persons in the family may alter earlier assumptions with respect to the genetic contribution to the disease. Such information may therefore change the conclusions of the genetic analysis, especially if risk estimates were based on empirical data.

It is customary that the conclusions of the genetic analysis are offered to the counselees in writing, to facilitate later decisions being made based on adequate medical and genetic information.

Dealing with unfavorable genetic information may be very distressing for the ones involved. In clinical genetics departments specially trained social workers

and/or psychologists are available to offer supportive care whenever needed.

Some paradigms are inherently associated with the genetic counseling process:

- 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.[16] 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.

2.6 Predictive Testing and the Dynamics of Family Studies

Predictive or presymptomatic testing is performed on symptom free or perceived symptom free individuals in order to find out whether or not they have inherited the predisposition for a genetic disease. 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.

2.6.1 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, 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 on genotype arises when non-penetrance 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.

2.6.2 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 socio-economic repercussions.[17] 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.

2.6.3 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.[18] Late onset disorders, or disorders that are not amenable to preventive treatment, are not to be tested in healthy children.[18] 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.[19] 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.

2.6.3.1 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 socio-economic 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.

2.6.4 Contacting Family Members

It is not customary for clinical geneticists to directly contact family members of their patients with genetic disease. Usually a patient is supplied with short written information for family members on the disease, management, genetic aspects, and the risks involved. It will be pointed out to the patient which family members should be contacted and should receive this written information. Subsequently, the family members themselves are left to decide whether or not they want to act upon it. This strategy, using these family letters guarantees "maximum nondirectiveness" toward family members and also, in a way, deals with issues of patient confidentiality, as the patient agrees to distribute general information on the disease within the family. An inventory of responses to these family letters demonstrated that this is an effective means to inform relatives.[20] Still, especially when potentially dangerous arrhythmia syndromes are involved, one could challenge the passive nature of this strategy. Is it correct to leave the responsibility to actually distribute the written information solely with the patient? Should clinical geneticists not at least check whether the information has been received by family members and has been understood? This issue has not yet been settled, but alternatives are certainly conceivable. For instance, in the Netherlands in familial

hypercholesterolemia (FH) family members are actively visited by genetic field workers.

2.6.5 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.[21, 22]

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.

2.6.6 Population Genetic Screening Issues

In screening for genetic disease whole populations, or specific subpopulations, are targeted.

Screening programs for inherited disease should meet with the same standards as any screening program, the only difference being that the outcome of screening may also have bearing on family members.

Above all, screening must produce a useful outcome, usually a form of early preventive treatment. If surveillance is the only screening outcome it must be well established that this surveillance leads to important health benefits. In genetic screening, apart from identifying individuals who are likely to develop the disease, a goal of the program could be the identification of carriers of frequent autosomal recessive disease, like cystic fibrosis or thalassemia. Here, the useful outcome would be the possibility of preconception counseling, and the possible prevention of the unexpected birth of

an affected child. This situation will not play a significant role in cardiogenetics as the only frequent autosomal recessive disorder associated with major cardiac problems, hemochromatosis, is a late onset disorder where preconception counseling would probably be without benefit.

The ethical framework for population screening requires that participation is voluntary, that easy understandable information is supplied to subjects about the goals of the program, that programs respect the privacy and autonomy of subjects, that screening results are confidential and that no pressure is exerted to follow a specific course of action based on screening results.

Organization and timing of screening are directly related to the goals of the program and to the question how subjects can be best recruited in order to maximize uptake of the program. For instance, mutations in long QT genes may be responsible for up to 10% of cases of sudden infant death syndrome.[23] This has led some professionals to propose ECG screening programs for neonates.[24] In developed countries, where programs for neonatal screening have already been implemented, it would seem logical to use the existing infrastructure for organizing such a screening program.

2.6.7 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. Actually, 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 readingframe) and larger rearrangements of genes will also lead to haploinsufficiency. Therefore, in most cases when such classes of mutations are detected in a candidate gene, one may assume that the mutation is causative for the disease phenotype.

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. Such mutations of unknown pathogenicity are called *unclassified variants* (UVs). Unfortunately, in clinical practice UVs occur rather frequently and cannot always be satisfactorily resolved.

Absence of the same UV in a large number of healthy controls is compatible with pathogenicity, but merely confirms that the UV is indeed rare. One would need to demonstrate that the UV is significantly more common in diseased than in healthy persons in order to prove the pathogenic potential. This is hardly ever possible in clinical practice. Proving that a UV always 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. Absence of the UV in an affected family member is, of course, strong evidence against the UV being the causal mutation. Importantly, if family history is negative, testing the healthy parents for presence of the UV can give vital information. If the UV turns out to be a de novo mutation, 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.

If the amino acid change is likely to change the three-dimensional structure of the protein, or if it is

located in a known binding site or important functional domain of the protein, and if it involves an evolutionary conserved residue, these are all indications that the UV is likely to be pathogenic, but none of these arguments does provide conclusive evidence. An amino acid residue is considered evolutionary conserved when all other species have the same amino acid at this position in the protein; in that case often other proteins of the same protein family also have the same amino acid at this position.

In a research setting it would often be possible to introduce the UV into a model system and observe its effect, but of course this is not possible in clinical practice.

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. Over-interpreting misses 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).

2.6.8 The Cardiogenetics Outclinic

Since the care for individuals with genetic cardiac disease and their relatives requires both cardiologic and genetic expertise, in the Netherlands multidisciplinary outpatient clinics for cardiogenetics have been set up. 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 co-operate 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 outclinics 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.

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