21
Single Nucleotide Polymorphisms in Health and Cardiac Disease

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Abstract

Recent advances in knowledge of the genome structure of ion channel genes and their physiologic role in myocardial repolarization have shown that genetic alterations of these key molecular components are associated with slight in-vitro effects and changes in fine tune of normal repolarization. It is expected that next-generation sequencing technologies (e.g., targeted resequencing of ion channel genes) will booster knowledge for individual arrhythmia predisposition and will enable researchers to lower the costs of complex genotyping and to implement these data into a personalized, genomic-oriented medicine. In this chapter, the role of natural genomic variation according to two main hypotheses, i.e. the 'common variant-common disease' hypothesis and the 'rare variants in common disease' hypothesis will be discussed upon current knowledge.

Keywords

Human genome • SNP • Complex heart diseases • Arrhythmias • QT interval • Repolarisation • Genetic predisposition

Prevalence of many common and genetically complex human diseases such as asthma, cardiovascular disease, and diabetes has risen greatly over the past two decades in developed countries and significantly contribute to the society's health burden. In addition, the genetic causes of monogenic diseases have been increasingly identified. In general, these conditions are declared as 'rare diseases' (RD) and are defined by the EU as rare when they affect less than one persons in 2,000. The fact that there are more than 8,000 such diseases means that the overall number of patients is considerable despite the low prevalence of individual clinical cases. It is estimated that more than 80 % of rare diseases are genetic in origin, yet in most patients the aetiology of their disease remains undetected. However, large scale investigations (e.g., exome sequencing) has been made to better understand their pathogenesis, and to develop preventive strategies, diagnostic tools, and treatment. Together with deciphering the human genome and its natural complexity of variance [1–3], considerable effort has been made to detect genetic loci contributing to quantitative phenotypes and complex arrhythmogenic diseases.

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Since the introduction of high-throughput DNA sequencing technologies, the costs of re-sequencing (i.e., non-repetitive portions) human genome felt down, and it has been reasoned that in a few years from now, costs will drop down to less than 1,000 US$. Therefore, the question is no longer whether, but when deep sequencing approaches will become routine in the diagnosis of genetic disorders and SNP detection. Since the first description of next-generation sequencing (NGS) systems 14–15 and the development of methods for genome partitioning [4, 5], numerous groups have successfully combined these methods to identify the molecular causes of monogenic disorders. In consequence, whole exome sequencing has been established as a potent and affordable strategy to identify disease-causing mutations in the 1 % of the human genome that codes for protein [6]. Thus, it is likely that whole genome approach will replace whole-exome sequence analysis in order to identify disease-causing mutations; by the way, also neutral variability in the human genome (e.g., see http://www.1000genomes.org/) will be investigated and further available to facilitate knowledge of disease modifying variants in non-coding sequences. This may further allow systematic elucidation of monogenic disorders as a clue to understand the pathogenesis of complex diseases [7].

Genetic association and linkage studies thereby comprise the two dominant strategies: association studies aim to find disease-predisposing alleles (from single nucleotide polymorphisms (SNPs) or microsatellite markers) at the population level, whereas linkage studies focus on familial segregation. A novel approach is a family-based association study design [8, 9]. Arrhythmia predisposition, e.g. acquired QT prolongation or torsade de pointes during treatment with cardiac and non-cardiac drugs, is still a major challenge for physicians. Recent advances in knowledge on the genomic and physiologic regulation of myocardial repolarization suggest that common alterations of cardiac (ion channel) genes might be associated with slight electrophysiologic changes and an increased susceptibility for ventricular arrhythmia. The extent, to which common genetic factors play a role, is under current investigations and remains to be determined. To date, no prospective data are available that link presence of certain SNP genotypes with a favorable or worse arrhythmia outcome.

Human Genome and Single Nucleotide Polymorphisms (SNPs): A Revival in Genomic Medicine

The annotated draft sequence of approximately three billion base pairs (bp) of the human genome has been completed earlier than expected [1, 3]. This was a major scientific and technologic development for researchers with an interest in the molecular bases of rare and common disorders, since awareness of the genomic diversity and molecular differences are expected to help in the understanding of role of a genetic contribution between individuals and disease [2]. The variations at the nucleotide level are implemented to determine the physiological differences and individual phenotypic variance, including major biological functions at the cellular and body level. **Single nucleotide polymorphisms (SNPs)** were the first type of genetic markers that were used to make chromosomal genetic maps [10]. However, due to their lower degree of heterozygosity and genetic informativeness when compared with polymorphic length (repeat) markers, SNPs became temporarily less attractive, until the completion of the human genome was done. In general, SNPs are single nucleotide base substitutions at a certain gene or genomic position and represent the major part of interindividual variability that accounts for only 0.1 % of genome sequences between individuals in health and disease. These small differences in the genetic code can be linked with unique personal features (e.g., eye color, tallness, …) and alterations of regular physiologic function, varied response to environmental conditions and predisposition for certain diseases. Of the approximate 10^6 million SNPs in the human genome, only a fraction is directly associated with functional significance and related to complex traits so far. Thus, the complexity of the entire human genome map is undermined by distinct effects of SNPs that

Table 21–1 Types and sequence location of DNA variation [11]

Polymorphism type	Sequence location	Predicted protein and potential functional effects	Occurrence in genome	Potential disease impact
Nonsense	Coding	Prematurely truncated, most likely loss of protein function	Very low	High
Missense, non-synonymous	Coding, non-conserved	Altered amino acid chain, mostly similar protein properties	Low	Low (to high)
Missense, non-synonymous	Coding, conserved	Altered amino acid chain, mostly different protein properties	Low	Medium to high
Rearrangements (insertion/deletion)	Coding	Altered amino acid chain, mostly different protein properties	Low	High
Sense, synonymous	Coding	Unchanged amino acid chain, rarely an effect on exon splicing	Medium	Low (to medium)
Promotor and regulatory sequences	Non-coding, promotor/UTR	Unchanged amino acid chain, but may affect gene expression	Low to medium	Low to high, depending on site
Intronic nucleotide exchange (<40 bp)	Non-coding, splice/lariat sites	Altered amino acid chain, failed recognition of exonic structure	Low	Low to high, depending on site
Intronic nucleotide exchange (>40 bp)	Non-coding, between introns	Unchanged amino acid chain, rarely abnormal splicing or mRNA instability, site for gene rearrangements	Medium	Very low
Intergenic nucleotide exchange	Non-coding, between genes	Unchanged amino acid chain, may effect gene expression, site for gross rearrangements	High	Very low

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Abbreviations: *UTR* untranslated region (5′ or 3′ region of a gene), *bp* base pairs

depend on the nucleotide subtype, their genomic location and effect on the protein structure/function, their abundance (allele frequency) and contribution to subchromosomal compartments of SNPs in linkage disequilibrium (haplotypes). SNPs differ from their location within the genomic sequence (coding vs. non-coding areas), from the type of nucleotide exchange and the consequence for the amino acid sequence, and from the frequency (relative occurrence) in the human genome (Table 21.1). Polymorphisms with the potentially highest phenotypic disease impact are rare within the genome [11]. A well-recognized example is variation in the Factor V gene (e.g., the Leiden variant) and its association with deep vein thrombosis. An understanding of the genetic diversity and of its contribution to variations in normal and abnormal physiology will have a potentially powerful effect on cardiovascular and genomic medicine.

Genetic association studies (or: case-control studies) are an analysis of statistically significant relationships between SNP alleles and phenotypic differences. The power of a genetic association study is a direct function of the number and quality of the SNPs used to screen a population for phenotypic variability. SNPs and haplotypes can vary in their prevalence among different populations. Thus, a SNP associated with a particular phenotype or quantitative trait in one population may not have the same frequency or effect in another population, e.g., when the population is of different ethnicity, age or gender. Large datasets of chromosomal SNPs have been published since 2000 [1, 13–17], along with improved methods to screen immense numbers of SNP candidates. More than three million variants have been reported and are catalogued in public databases (e.g., http://www.ncbi.nlm.nih.gov/projects/SNP/). Newer techniques allow high-throughput genotyping to study simultaneously large numbers of SNP loci (currently: >4.0 M markers per sample/chip; e.g., HumanOmni5-Quad, Illumina Inc.) and are based on matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF; e.g., Sequenom MassARRAY), pyrosequencing, or hybridisation.

A huge and as yet unsolved problem is the identification of clinically relevant mutations in

a plethora of functionally neutral single nucleotide polymorphisms. Common SNPs can be filtered out through comparison with genomes and exomes from healthy individuals (e.g., http://www.1000genomes.org/) [18] or dbSNP (see http://www.ncbi.nlm.nih.gov/projects/SNP/, despite an increasing contamination with clinically relevant mutations), but this approach is not possible for the many rare SNPs in the human genome. Indeed, comprehensive NGS-based reanalyses have recently found that 12 % of the previously reported mutations are not disease-causing itself [19]. In principle, large-scale whole-genome sequencing may reduce the number of novel variants from 3.4 million to a mere 150.000 per genome. Therefore, sequencing 100,000 individuals and comparing the results with their complete medical records (e.g., see http://www.personalgenomes.org/), would identify the vast majority of changes that do not give rise to disease. A comparable project is the NHLBI GO Exome Sequencing Project that focuses to discover novel genes and mechanisms contributing to heart, lung and blood disorders by pioneering the application of next-generation sequencing of the protein coding regions of the human genome across diverse, richly-phenotyped populations. These datasets and findings – obtained from potentially affected individuals – are also shared with the scientific community (Exome Variant Server; http://evs.gs.washington.edu/EVS/) and now showed that rare variants (e.g., less than 1 in 1,000 alleles) can be commonly identified in many cardiovascular genes. The presence of these databases, in contrary, enhances the need for certified and proven mutation databases for genes, such as such as the Human Gene Mutation Database (HGMD, see http://www.hgmd.org/), or potentially the Human Variome Project (http://www.humanvariomeproject.org/) or the Human Genome Organization (http://www.hugo-international.org/). Without theses, the clear clinical significance of many genetic variants and the role of the relevant genes in disease may remain uncertain for a long time. Similarly to SNPs and recently being more recognized [2], also many genomic imbalances were recurrently detected and were found in both, patients and healthy individuals (see central databases like Decipher, http://decipher.sanger.ac.uk/ or the Database of Genomic Structural Variations, http://www.ncbi.nlm.nih.gov/dbvar).

The clinical use of SNPs is still far away from being established, at least in arrhythmia prediction. This might be related to some inherent limitations with SNP studies [20, 21]. The two major issues are *statistical power* and *replication* of genetic findings in another, independent population set of same origin to avoid population stratification. In association studies, the prevalence of genetic marker alleles in unrelated subjects with a certain phenotype and (unaffected) controls will be compared and aim to correlate differences in disease frequencies between groups (or in trait levels for continuously varying characters) with differences in allele frequencies at an SNP. Thus, the frequencies of the two variant forms (alleles) of an SNP are of primary interest for identification of genes affecting disease. The traditional 'case-control' approach assumes that any noted difference in allele frequencies is related to the outcome measured and that there are no unobserved confounding effects. Unfortunately, allele frequencies are known to vary widely within and between populations, irrespective of disease status. For an appropriate study, an adequate sample size of the groups and a relatively high frequency of the minor SNP allele (to facilitate detection of allele frequency differences between the investigated populations) are needed. Usually, haplotype tagging SNPs (tagSNPs) were selected on chip-based arrays to systematically analyze nearly every genes approach. Typical criteria for tagSNP selection are a pairwise-only tagging with $r^2 > 0.8$ and a minor allele frequency (MAF) >0.1. Studies with small sample sizes may commit **type II errors**, i.e., not declaring a statistically significant result when there may be a difference. These underpowered studies can be misleading because genes may be undetected, and reporting of the odds ratio and 95 %-confidence interval are recommended [22]. The term β is defined as the chance of making a type II error. Values for β are typically 10–20 %, meaning a power $(1 - \beta)$ between 80 and 90 %. In contrast, a sample size that is much larger than required may declare small differences to be statistically significant and thus commit **type I errors** (i.e., declaring a statistically significant difference when it may

not be present). The term α refers to the chance of making a type I error; usually, a level of 0.05 or less is chosen. Due to the increasing, but also inconsistent number of GWAS publications, proposed guidelines have been developed which should facilitate the quality of association studies [23, 24], including strategies to ascertain *heritability and exact phenotyping of a trait*, to perform *population stratification of cases and controls* (ethnicity, age and gender distribution), *to select physiologically and genetically meaningful markers*, to address the *probability of association*, and to *replicate* initial results in independent studies [25, 26]. A p-value $<5\times10^{8}$ is considered as statistically significant for GWAS results. This quite stringent significance threshold, that is frequently used when studying samples of European ancestry, accounts for about 1,000,000 independent common variant tests in the human genome. To date, only a few of the several thousand published association studies strictly meet the criteria to ascertain a ('true') genetic association. For arrhythmogenic disorders, first studies exist [27–30], but the majority of data is still unreplicated by independent approaches. Differences in study outcome may be related to population stratification, study design, still inappropriate marker selection, and lack of statistical power [11]. Discovery of meaningful SNP markers [31], e.g., indicating an elevated risk of SCD, is still far from being established. Common weaknesses of many association studies include study design failing to adequately identify true positives while eliminating false positives, poorly defined phenotypes and sampling from heterogeneous patient populations, inappropriately matched controls, small sample sizes relative to the magnitude of the genetic effects, failure to account for multiple testing, population and sample stratification, failure to replicate marginal findings and overemphasizing interpretation of study results. In the past, the optimum study design for association studies has been discussed because, often, studies were prone to population stratification and biased or spurious results. Thus, replication of the findings from genetic association studies in other populations became a cornerstone for the data quality, and, so far, only a few studies merit these criteria. In this line, a shift from case-control and cohort studies towards family-based association designs has been noted. These study designs have fewer problems with population stratification, but have greater genotyping and sampling requirements, and data can be difficult or impossible to gather.

Analysis of SNPs in Cardiac Arrhythmogenesis: Towards a Dissection of Common ECG Traits

Phenotypic variation in arrhythmia development is well known from families with inherited, arrhythmogenic disorders that have demonstrated an important phenotypic spectrum of the same mutation in affected family members [32, 33]. Recent reports have highlighted the importance of a family history of sudden death as a risk for **ventricular fibrillation** (VF) in patients experiencing acute myocardial infarction (AMI), pointing to the possibility of a genetic predisposition. Familial aggregation demonstrated an increased risk of SCD among patients with a parental history of cardiac arrest [34, 35], but a clearly defined genetic basis is not known to date [36]. Sudden death was found to share the same profile of risk factors for coronary artery disease and, thus, was not specifically predictable in the general population. These observations are also recognized from in patients with more polygenic disorders, such as myocardial infarction, for which not every patient develops ventricular fibrillation during acute ischemia [37, 38]. In a case-control study in patients with a first ST-elevation myocardial infarction (STEMI) and similar infarct sizes and locations, it was recently shown that (cumulative) ST-segment elevation was significantly higher among cases and that familial sudden death occurred more frequently among cases than controls [37]. Two population-based studies of the late 1990s demonstrated an increased risk of SCD in first-degree relatives of SCD victims and provided some evidence that genetic components may be involved in SCD of unknown (probably atherosclerotic) origin [35, 39]. A family history of MI/SCD was associated

with SCD (RR = 1.57), after adjustment for other common risk factors and person–years at risk among (first degree) relatives [39]. After differentiating between family history of MI or of SCD, the positive family history of early-onset SCD finally was associated with a 2.7-fold increase in risk of SCD. In victims of VF in the setting of their first, acute MI it has been also reported that SCD of degree relative is a strong risk factor for ventricular fibrillation (OR = 2.72) [37].

Thus, arrhythmia development may have a common and modifiable substrate in both, rare inherited (monogenic) and common (polygenic) forms of various arrhythmias and a positive family history can be noted in both. In addition, multiple factors – such as age, gender, and environmental condition – play an important role in the modulation of the phenotype. Structural and electrical remodeling during acute ischemia, altered hemodynamic loads, or changes in neurohormonal signaling are recognized key features that alter ion channel gene expression. Down-regulation of major repolarizing potassium currents, I_{to}, I_{Kr}, I_{Ks}, and I_{K1}, has been described in several models of heart failure and resembles a condition of "acquired QT prolongation" and reduced, but reversible repolarisation reserve [40]. Cellular abnormalities through disturbances in the electrical cell-cell coupling and a local reduction of conduction velocity facilitate re-entrant ventricular arrhythmias. These cellular abnormalities can be found in the structurally diseased heart. The extent of genetically controlled variation is not clear to date, but it is of potential interest and under recent investigations. Of note, some studies already focused on associations of SNPs in ion channel genes and a relation with myocardial infarction. Since KATP channels are involved in membrane regulation during metabolic stress, studies focused to identify variants in the *KCNJ11* gene associated with SCD after myocardial infarction [41]. These channels are composed of four pore-forming Kir6.2 (KCNJ11) subunits and four sulfonylurea receptor subunits (SUR2A); sarcolemmal KATP channels regulate membrane potential and action potential duration, whereas the mitochondrial KATP channels are involved in ischemic preconditioning. So far, two non-synonymous polymorphisms (R371H, P266T) in two highly conserved pore regions are known that showed altered modulation by intracellular ATP and protons and differences in channel density [42] and, thus, are potential candidates for genetically determined electrophysiologic differences under ischemic conditions. Interestingly, mutations in the *KCNJ8* gene have been associated with idiopathic ventricular fibrillation [43–45]. Phase 2 re-entry is a key mechanism for ventricular fibrillation complicating acute myocardial infarction as well as arrhythmias associated with Brugada syndrome. In this line, a heterozygous SCN5A gene mutation (G400A, located in *cis* with the H558R polymorphism) was reported in a patient who developed an arrhythmic electrical storm during acute myocardial infarction and suggested a hidden genetic predisposition to the severity of arrhythmias that in the setting of acute myocardial ischemia [46]. Another study indicated significant changes (up to 63 % down-regulation) of sodium channel transcription in dependence of the SNP composition within the potential SCN5A promoter region when investigated by transient transfection of promoter-reporter constructs in CHO cells or in neonatal cardiomyocytes [47]. These results may further support a concept of interindividual variability in transcription of this cardiac ion channel gene and arrhythmogenesis.

Population-based studies for SCD are ongoing to highlight potential causes among patients with a positive parental history of cardiac arrest [34, 35], but a clearly defined genetic basis is not known to date [36]. In contrast to patients with cardiac dysfunction, in patients without intraventricular conduction defects or a normal cardiac function, QTc prolongation is a non-negligible risk factor for sudden cardiac death independent of age, history of myocardial infarction, heart rate, and drug use. This has been shown in the Rotterdam Study, a prospective population-based cohort study, in which 125 patients died of sudden cardiac death (mean follow-up 6.7 years) and a prolonged QTc interval had a threefold increased risk [36, 48, 49]. So far, first GWAS have been directed to evaluate the role of common genetic variation in modulation of SCD or VF risk [38, 50, 51]. Since the causes and confounding factors for SCD are

diverse, it is not unexpected to note that in two GWAS studies reported [38, 50], did not confirm and replicate each other, i.e., the SNP at chromosome 2q24.2 (rs4665058) at the *BAZ2B* gene locus was not seen in the Dutch case-control set. Similarly, the *CXADR* gene signal was not detected in the study involving European ancestors; this may be due to several factors, including not only insufficient statistical power and random chance, but also differences in study design (population stratification) and phenotype definition. Future studies, entailing expression quantitative trait locus (eQTL) analysis in cardiac tissue as well as the genome-wide identification by ChiP-Seq of regulatory regions occupied by transcriptional enhancers and transcription factors [52] will shed additional light on the pathophysiology.

Recently, a quantitative influence of ion channel gene variation on the myocellular repolarization has been described in twins [29] and in the general population [27, 28, 30]. Of note, the heritability reflecting the degree of variance in ECG indices between individuals is for the QTc interval in the range of 25–50 % and for the PR interval between 34 and 40 %, at least depending on the population set studied, see [53]. Genomic studies are currently on the way to narrow candidate these gene regions and to identify these variants (SNPs or haplotype constellations) in coding and non-coding sequences. An example has been shown very recently [54]; in the study by Amin and co-workers sequence variance of the 3′-UTR at the LQT-1 (*KCNQ1*) locus was investigated by microRNA binding sites that may influence *KCNQ1* expression of mutant or wild-type allele. As a novel concept, three single nucleotide polymorphisms (rs2519184, rs8234, and rs10798) were associated in an allele-specific manner with QTc and symptom occurrence and, intriguingly, with concordant, but altered gene expression upon luciferase reporter assays. These data raised the idea that clinical disease expression may be a function of the ratio between normal and mutant allele expression and other factors [54].

It has long been surmised that **drug-induced torsade de pointes** is an acquired condition that may occur in the context of a mutation encoding for a cardiac ion channel gene responsible for repolarization. This was enhanced due to the recognition of LQTS gene mutation carriers with normal or nearly normal ECGs (incomplete or non-penetrance) [55, 56]. First reports on patients with drug-induced QT interval prolongation and LQTS ion channel gene mutations and were reported nearly a decade ago [57–59] and are listed in Table 21.2. In at least 15–20 % of patients LQTS genes mutations can be found [60], even some reports are indicative for a higher ratio [73, 74]. Altogether, these factors further diminish 'repolarization reserve' [75] to a critical extent and allow the generation of afterdepolarizations and triggered activity preceding torsade de pointes. The observation that in the majority of patients with idiosyncratic drug reactions and TdP development a LQTS gene mutation cannot be found, is possibly related to undetected mutations in these, predisposing variants or other target genes responsible for cardiac repolarization [11].

Heritability and a **quantitative influence on the QT interval** has been described particularly in twins [29] as well as in the general population [27, 28, 30, 76]. In consequence, a 'common variant – common phenotype' hypothesis has been proposed that implies the influence of frequent (ion channel and other) genetic variance (e.g., SNPs) to modulate the QT interval in terms of a quantitative trait. The expectation is that multiple SNPs, whether alone or in combination, have protective or deleterious effects on the QT interval [27]. A series of genome-wide or candidate gene SNP studies have been conducted and tested for the propensity of single SNPs to modulate repolarization or the arrhythmia phenotype. In Fig. 21.1, those with a significant p-value ($<10^{-8}$) are shown. Some of them are known in close location of cardiac ion channel and LQT genes, others not. Meanwhile, also in cardiac ion channels common protein variants (non-synonymous SNPs) have been identified and were seen in a setting with QT drug prolonging drugs (Table 21.2). Yang et al. screened the coding regions of the three major LQT genes (LQT1-3) in 92 patients with drug-induced LQTS and controls [59]. The allele frequencies of three, common, non-synonymous polymorphisms (SCN5A-H558R, SCN5A-R34C, HERG-K897T), however, did not significantly differ between the

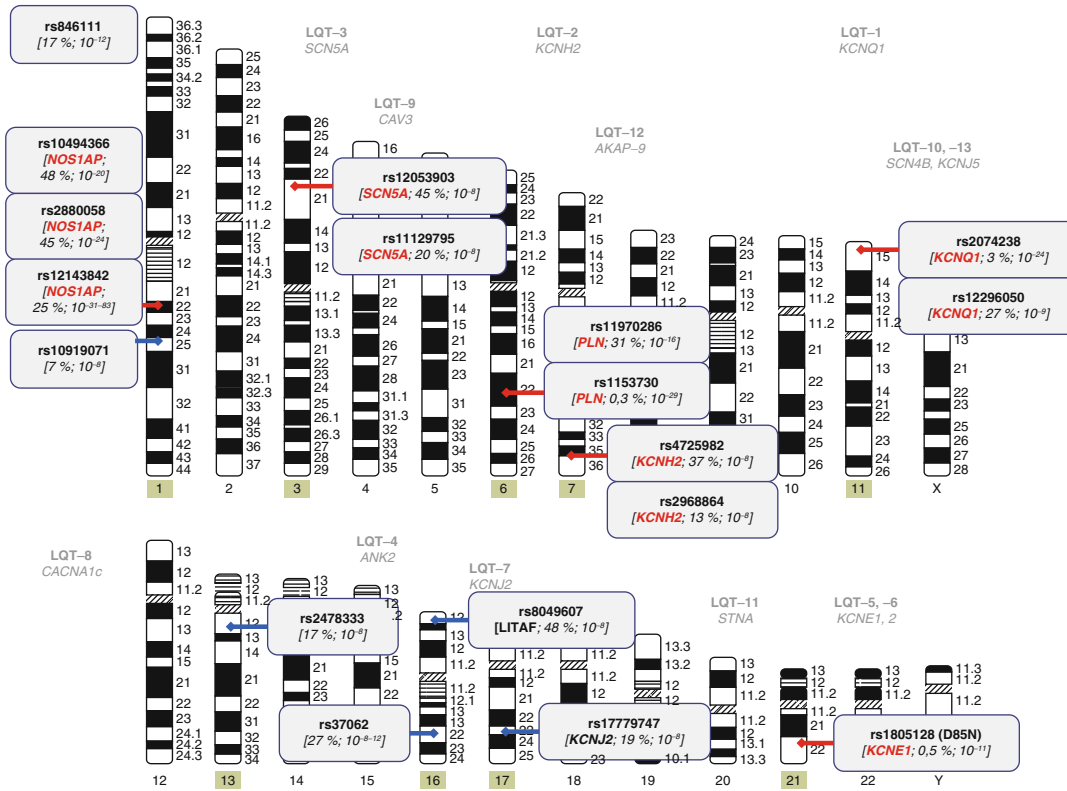

Figure 21–1. Human chromosomes (ideograms) and SNPs (rs identifier; gene symbol in *red*; minor allele frequency [%]; p-value, only $<10^{-8}$) with a reported effect on QT interval duration

two groups. Similar findings were reported by others [77]. In the African population, a particular SNP in the cardiac sodium channel gene *SCN5A* (LQT-3) gene was reported to predispose to prolong the QT interval, which appears to be ethnic-related (SCN5A_S1102Y) [78]. This SNP that was primarily found in West African and Caribbean (10.1 % frequency for Y1102 allele) increases the risk of cardiac arrhythmias in the presence of drugs such as amiodarone. SCN5A_Y1102 itself does not cause LQTS, but induces a small and potentially inherent and chronic risk of acquired arrhythmia in the setting of additional risk factors, such as medications, hypokalemia, or structural heart disease. Additional, longitudinal studies are still required to confirm the predictive utility of the Y1102 allele. Meanwhile, an association of SCN5A_Y1102 with sudden cardiac death and sudden infant death in blacks [79–81] has been described; in-vitro data indicate susceptibility for repolarization delay and arrhythmia during acidotic/ischemic environmental conditions. Recently, another propensity for ventricular arrhythmias in black patients with heart failure and reduced ejection fraction was proposed [82]. Moreover, gene-gene interaction intragenic variance may affect function of wild-type sodium (SCN5A) channels and modulate the cardiac arrhythmia phenotype. This particularly refers to naturally occurring splice variants of LQTS genes [83–85] upon quantitative mRNA analysis from the cardiac tissue and to the observation that these common variants had a different electrophysiologic behavior than full-length clones. Of interest, baseline differences in isoform-mediated sodium currents were profoundly modulated when a common SCN5A polymorphism (SCN5A_H558R) was present and exhibited functional differences. In addition to SCN5A_Y1102, a series of other, non-synonymous SNPs have been noticed in the sodium channel [86, 87]

Table 21–2 Ion channel gene variants identified in patients with drug-induced QT prolongation or TdP occurrence

Gene	Current	Amino acid alteration	Drug/setting	Functional assay	Minor allele	Reference
KCNE2	I_{Kr}	T8E	Trimethoprim (TMX)/ sulfa-methoxazole (SMX); quinidine; amiodarone	E8-MiRP1 weakly reduced I_{Kr} current peak density; (CHO cells); SMX and TMX had almost no effect on wild-type channels, but SMX was reported to inhibit more than 50 % of A8-MiRP1 at −40 mV. Mutant channels were 4× more sensitive to SMX than wild-type	1.6 %	[60–62]
	I_{Kr}	Q9E	Clarithromycin, low K^+		Rare in Caucasians, but not in Afro-Americans	[62, 63]
	I_{Kr}	M54T	Procainamide	T54-MiRP1 significantly reduced I_{Kr} current peak density (CHO cells). No influence on drug-related channel inhibition was seen	Rare	[61]
	I_{Kr}	M57T	Oxatomide	T57-MiRP1 significantly reduced I_{Kr} current peak density (CHO cells). No influence on drug-related channel inhibition was seen	Rare	[61]
	I_{Kr}	A116V	Quinidine	V116-MiRP1 significantly reduced I_{Kr} current peak density (CHO cells). No influence on drug-related channel inhibition was seen	Rare	[61]
KCNH2	I_{Kr}	M124T	Probucol	Co-expression of wild-type HERG and T124-HERG resulted in markedly smaller amplitudes of I_{Kr} (Xenopus oocytes). Probucol decreased the amplitude of the HERG tail current, decelerated the rate of channel activation, accelerated the rate of channel deactivation, and shifted the reversal potential to a more positive value	Rare	[64]
	I_{Kr}	R328C	Not reported		Rare	[65]
	I_{Kr}	P347S	Cisapride/clarithromycin		Rare	[60, 66]
	I_{Kr}	R486H	Quinidine		Rare[b]	[57]
	I_{Kr}	A561P	Clobutinol	P561-HERG led to an intracellular trafficking defect; when co-expressed with wild-type HERG, voltage-dependence was shifted towards more negative potentials (3–3.5-mV). Clobutinol further blocked heteromeric channels	Rare[b]	[67, 68]
	I_{Kr}	R784W	Amiodarone	W784-HERG mediated a reduced I_{Kr} current (by ~75 %) and a positive shift of voltage dependence of activation	Rare	[59]
KCNQ1	I_{Ks}	R243H	Halofantrine; hydrochinine		Rare[a, b]	[57]
	I_{Ks}	Y315C	Cisapride	In-vitro expression of mutant KvLQT1 protein showed a severe loss of current with a dominant negative effect on the WT-KvLQT1 channel	Rare[b]	[58]
	I_{Ks}	R555C	Terfenadine		Rare	[69, 70]
	I_{Ks}	R583C	Dofetilide	C583C-KvLQT1 mediated I_{Ks} was reduced by ~50 % compared with wild-type, and the voltage dependence of activation was shifted positively by 19.6 mV (CHO cells)	Rare	[59]
SCN5A	I_{Na}	G615E	Quinidine	E615-SCN5A indistinguishable from wild-type mediated I_{Na} currents (tsa-201 cells)	Rare	[59]
	I_{Na}	L618F	Quinidine	E615-SCN5A indistinguishable from wild-type mediated I_{Na} currents (tsa-201 cells)	Rare	[59]
	I_{Na}	S1103Y	Amiodarone	Y1103-SCN5A mediated an increased I_{Na} channel activation (HEK 293)	7–10 % (Afro-Americans or West Africans/ Caribbeans only)	[59]
	I_{Na}	F1250L	Sotalol	E615-SCN5A indistinguishable from wild-type mediated I_{Na} currents (tsa-201 cells)	Rare	[59]
	I_{Na}	P1825L	Cisapride	The C-terminal mutant P1825-SCN5A mediated I_{Na} current with slow decay and prominent TTX-insensitive, non-inactivating component (gain-of-function), a reduced peak density (loss of function), shifted voltage dependence of activation (more positive potentials) and of inactivation (more negative potentials) (tsA-201 cells)		[71]
				P1825-SCN5A channels showed impairment of intracellular trafficking (CHO cells) and failed to generate QT prolongation. Exposure with cisapride rescued cell surface expression of P1825-SCN5A and exaggerating the LQT3 phenotype		[72]

[a]Reported from recessive forms of LQTS

[b]Heterozygous mutation carriers may have a normal QT interval; for SCN5A, amino acid residues are numerated according to position on the long splice variant

and other LQTS genes [61, 63], but a role as a modulating component of repolarization is undetermined. Very recently, a set of 176 drug-induced LQTS patients as genotyped for a total of 1,424 single nucleotide polymorphisms in 18 candidate genes (among them 1,386 SNPs tagging common haplotype blocks) were compared to controls [88]. After all, the *KCNE1* gene polymorphism D85N (rs1805128) was present in 8.6 % of cases, 2.9 % of drug-exposed controls, and 1.8 % of population controls (odds ratio of 9.0) and suggestive for a susceptibility allele that may be associated with the rare adverse drug reaction torsade de pointes.

In the first study of the general (healthy) population, the influence of 174 (out of around 270 possible) common LQTS gene variants (LQT-1, -2, -5, -6) on the QT interval were investigated in a total of 3,966 unrelated individuals from the general population [27]. Using a two-step design and a population-based linear regression formula to calculate gender- and age-specific QT values (named QTc_RAS), four SNPs (one in intron 1 of LQT-1 gene, one 5′ of LQT-5, KCNH2_K897T, and another one in the same KCNH2 haploblock, respectively) were detected with a slightly lower QTc_RAS value (for each of the four SNPs < 2.0 ms). Genetic association data were not reported for the more commonly used QT formulas. In another publication [28], confirmed linkage was found between a 5′ SNP (rs10494366) in NOS1AP gene with QT interval with an average genetic effect for QTc_RAS of ~4 ms. NOS1AP (CAPON) is a regulator of neuronal nitric oxide synthase, as a new target that potentially is involved in modulating cardiac repolarization and the minor allele of the NOS1AP genetic variant was reported to explain around 1.5 % of QT interval variation [28]. In a subsequent study, replication of this association was addressed in the Old Order Amish, a genetically isolated population, where a heritability of the QT interval was 0.50–0.09 [89]. Two of the four NOS1AP SNPs were significantly associated with variation in adjusted QT interval and explained a fraction of 0.9 % of QT interval variability, with an average genetic effect on adjusted QT of 6.1 ms [89]. Subsequently, NOS1AP SNPs were proposed to influence the clinical course in congenital LQTS and sudden cardiac death in blacks [90, 91], probably through the modulation of L-type channel activity [92]. Meanwhile, increasing numbers of publications using GWAS approaches in large population sets [93–95] are indicating the presence of small-sized SNP effects (1–10 ms) on the QT interval (see Fig. 21.1 and [53]). The majority of these SNPs are located in intronic regions and further studies are needed to evaluate the mechanisms for repolarisation modulation.

Future Directions

Recent advances in knowledge of the genome structure of ion channel genes and their physiologic role in myocardial repolarization have shown that genetic alterations of these key molecular components are associated with slight in-vitro effects and changes in fine tune of normal repolarization. The extent to which minor genetic factors altogether are associated with susceptibility to arrhythmias remains to be determined, but first evidence is present. Following the concept of 'repolarization reserve' [40], it is likely that torsade de pointes occurrence, arrhythmia occurrence during acute myocardial infarction or drug response (as well as side effects) are also dependent on an individual genetic background. Genetics of arrhythmogenesis switches from gene identification and single pathway understanding to genomic medicine by integrating complex gene and environmental information. Future research will

- identify all relevant genes and their genomic structure for repolarization,
- determine the extent of the variability of the QT interval and of the response to action potential-prolongation that is genetically controlled,
- investigate the role of functionally relevant SNPs and haplotype constellations in LQTS and other gene loci for their quantitative contribution to repolarization,
- integrate identified genetic factors with other known factors for cardiac risk, according to their relative importance, in a network algorithm for arrhythmogenesis.

These data should be available within the next few years and advances, along with additional technological improvements in DNA analysis and data management. It is expected that next-generation sequencing technologies (e.g., targeted re-sequencing of ion channel genes) will booster knowledge for individual arrhythmia predisposition and will enable researchers to lower the costs of complex genotyping and to implement these data into a personalized, genomic-oriented medicine. Apart from the 'common variant-common disease' hypothesis, the increasing evidence for a role of 'rare variants in common disease' will be elucidated [96–99].

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