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Single Nucleotide Polymorphisms in Health and Cardiac Disease

Eric Schulze-Bahr

Introduction

The prevalence of many complex human diseases such as asthma, cardiovascular disease, and diabetes has risen greatly over the past two decades in developed countries. In addition, the genetic causes of monogenic diseases have been identified, leading to a better understanding of their pathogenesis and to the development of preventive strategies, diagnostic tools, and treatment. Considerable effort has been made to detect genetic loci contributing to quantitative phenotypes and complex arrhythmogenic diseases. Genetic association and linkage studies 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. Predisposition to arrhythmia, e.g., acquired QT prolongation or torsade de pointes (TdP) during treatment with cardiac and noncardiac drugs, is still a major challenge for physicians. Recent advances in the knowledge of the genomic and physiological regulation of myocardial repolarization suggest that common alterations of cardiac (ion channel) genes are associated with slight electrophysiological changes and an increased susceptibility for ventricular arrhythmia. The extent to which common genetic factors play a role is under current investigation and remains to be determined. The availability of extensive catalogues of SNPs in cardiac and noncardiac genes across the human genome is applicable for further genetic and functional studies to address the issue of genetically determined arrhythmogenesis.

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

The annotated draft sequence of approximately three billion base pairs (bp) of the human genome has been completed much sooner than expected.[1,2] This was a major scientific and technological development for researchers with an interest in the molecular bases of rare and common disorders, since awareness of the genomic diversity and molecular differences is expected to help in understanding the role of genetic contributions between individuals and disease. 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.[3] However, due to a lower degree of heterozygosity and less genetic information when compared to 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 to unique personal features (e.g., eye color, height) and alterations of regular physiological function, varied response to environmental conditions, and predisposition for certain diseases. Of the approximately 10^6 million SNPs in the human genome, only a fraction has been 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 depend on the nucleotide subtype, their genomic location and effect on protein structure/function, their abundance (allele frequency), and their contribution to subchromosomal compartments of SNPs in linkage disequilibrium (haplotypes). The SNPs differ in their location within the genomic sequence (coding vs. noncoding areas), in the type of nucleotide exchange and the consequences for the amino acid sequence, and in their frequency (relative occurrence) in the human genome (Table 17–1). Polymorphisms that potentially have the greatest impact of phenotypic disease are rare within the genome.[4] Current estimates of the degree of diversity range from 1 : 500 to 1 : 1000, resulting in millions of variants in the human genome. 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. Because SNPs and haplotypes can vary in their prevalence among different populations, an 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[2,5–8] along with improved methods to screen immense numbers of SNP candidates. Nearly three million variants have been reported and are catalogued in public databases (http://www.ncbi.nlm.nih.gov/SNP/) (http://www.ncbi.nlm.nih.gov/projects/SNP/). Newer techniques allow high-throughput genotyping to study simultaneously large numbers of SNP loci

Table 17–1. Types and sequence location of DNA variation.[a]

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, nonsynonymous	Coding, nonconserved	Altered amino acid chain, mostly similar protein properties	Low	Low (to high)
Missense, nonsynonymous	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	Noncoding, 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)	Noncoding, Splice/Lariat sites	Altered amino acid chain, failed recognition of exonic structure	Low	Low to high, depending on site
Intronic nucleotide exchange (>40 bp)	Noncoding, between introns	Unchanged amino acid chain, rarely abnormal splicing or mRNA instability, site for gene rearrangements	Medium	Very low
Intergenic nucleotide exchange	Noncoding, between genes	Unchanged amino acid chain, may affect gene expression, site for gross rearrangements	High	Very low

Source: From Kääb and Schulze-Bahr.[4]

[a]UTR, untranslated region (5′ or 3′ region of a gene); bp, base pairs.

(currently 500K per chip) and are based on matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF; e.g., Sequenom MassARRAY), pyrosequencing, or hybridization. There are, however, some inherent limitations to SNP studies. The two major issues are *statistical power* and *replication* of genetic findings in another population. In association studies, the prevalence of genetic marker alleles in unrelated subjects with a certain phenotype and (unaffected) controls will be compared, with the aim of correlating differences in disease frequencies between groups (or in trait levels for continuously varying characters) and allele frequencies for an SNP. Thus, the frequencies of the two variant forms (alleles) of an SNP are of primary interest for the 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. Studies with small sample sizes may result in *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 the odds ratio and 95% confidence interval is recommended.[9] The term β (beta) 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 indicate that small differences are statistically significant and thus commit *type I errors* (i.e., declaring a statistically significant difference when it may not be present). The term α (alpha) refers to the chance of making a *type I error*; usually, a level of 0.05 or less is chosen.

Proposed guidelines have been developed that should facilitate the quality of association studies,[10,11] 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. 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,[12–15] 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.[4] Discovery of meaningful SNP markers, e.g., indicating an elevated risk of sudden cardiac death (SCD), is still far from being established. Common weaknesses of many association studies include a study design that fails 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 the interpretation of the 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 maintaining data quality, and, so far, only a few studies merit these criteria. A shift from case–control and cohort studies toward a family-based association designs has therefore 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 Single Nucleotide Polymorphisms in Arrhythmias: Toward Common Genetic Constellations for Arrhythmogenesis

Phenotypic variation in the development of arrhythmia is well known from families with inherited, arrhythmogenic disorders that have

demonstrated an important phenotypic spectrum of the same mutation in affected family members.[16,17] These observations are also seen in patients with more polygenic disorders, such as myocardial infarction, for which not every patient develops ventricular fibrillation during acute ischemia.[18] 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.[18] Thus, development of arrhythmia may have a common substrate modification in both, rare inherited 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 conditions—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 as key features that alter ion channel gene expression. Downregulation 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." Cellular abnormalities through disturbances in electrical cell–cell coupling and a local reduction of conduction velocity facilitate reentrant ventricular arrhythmias. These cellular abnormalities can be found in the structurally diseased heart. The extent of the genetically controlled variation is not clear to date, but it is of potential interest and has recently been investigated.

Population-based studies demonstrated an increased risk of SCD among patients with a parental history of cardiac arrest,[19,20] but a clearly defined genetic basis is not known.[21] In contrast to patients with cardiac dysfunction, in patients without intraventricular conduction defects or a normal cardiac function, QTc prolongation is a nonnegligible 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 of 6.7 years) and patients had a 3-fold increased risk for a prolonged QTc interval.[21–23] Recently, a quantitative influence of ion channel gene variation on myocellular repolarization was described in twins[14] and in the general population.[12,13,24] Genomic studies are currently making progress in narrowing these candidate gene regions and in identifying these variants (SNPs or haplotype constellations) in coding and noncoding sequences. Also, patients with drug-induced QT prolongation have been investigated for the presence of (clinically inapparent) gene mutations in long QT syndrome (LQTS) genes. In 10–15% of patients such mutations can be found.[25–27] Recently, common protein variants (SNPs) in cardiac ion channels have been identified (Table 17–2), which also may have a potential impact on susceptibility to arrhythmia and pharmacogenetic strategies for circumvention of arrhythmia as well as for therapy. Yang *et al.* screened the coding regions of the three major LQT genes (LQT1-3) in 92 patients with drug-induced LQTS and additional controls.[28] The allele frequencies of three common, nonsynonymous polymorphisms (*SCN5A*-H558R, *SCN5A*-R34C, *HERG*-K897T) did not significantly differ between the two groups. Similar findings were reported by Paulussen *et al.*[29] and indicate no particular concomitant effect of the presence of an LQTS gene polymorphism and the occurrence of TdP.

These observations and more general considerations led to the concept that beyond the "rare disease paradigm" [meaning that rare gene mutations cause (rare) Mendelian disorders] a "common variant–common disease hypothesis" may be present. Here, common SNPs in ion channel genes may determine arrhythmogenesis. It is anticipated that not a single SNP, but several SNPs in one or more genes together exhibit a detectable, functional effect. For severe atrial conduction impairment, a heterozygous mutation (D1275N) in the cardiac sodium channel gene *SCN5A* has been reported that was probably aggravated by the presence of two SNPs within regulatory regions of gap junction protein connexin 40.[30,31] For patients with Brugada syndrome as well as control individuals, an *SCN5A* promoter haplotype (so-called HapB) was associated with longer PR and QRS interval durations and response to sodium channel blockers.[32] These examples of genomic interplay are assumed to

TABLE 17–2. Ion channel gene variants identified in patients with drug-induced QT prolongation or torsade de pointes occurrence.

Gene	Current	Amino acid alteration	Drug/setting	Functional assay	Minor allele	Reference
KCNE2	I_{Kr}	T8E	Trimethoprim (TMX)/sulfamethoxazole (SMX); quinidine; amiodarone	E8-MiRP1 weakly reduced I_{Kr} current peak density; Chinese hamster ovary (CHO) cells; SMX and TMX had almost no effecton on wild-type channels, but SMX was reported to inhibit more than 50% of A8-MiRP1 at −40 mV; mutant channels were four times more sensitive to SMX than wild type	1.6%	29, 45, 46
	I_{Kr}	Q9E	Clarithromycin, low K^+		Rare in whites, but not in Afro-Americans	33, 45
	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	46
	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	46
	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	46
KCNH2	I_{Kr}	M124T	Probucol	Coexpression 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	47
	I_{Kr}	R328C	Not reported		Rare	48
	I_{Kr}	P347S	Cisapride/clarithromycin		Rare	29, 49
	I_{Kr}	R486H	Quinidine		Rare[a]	25
	I_{Kr}	A561P	Clobutinol	P561-hERG led to an intracellular trafficking defect; when coexpressed with wild-type hERG, voltage dependence was shifted toward more negative potentials (3–3.5 mV); clobutinol further blocked heteromeric channels	Rare[a]	50 51
	I_{Kr}	R784W	Amiodarone	W784-hERG mediated a reduced I_{Kr} current (by ~75%) and a positive shift of voltage dependence of activation	Rare	28
KCNQ1	I_{Ks}	R243H	Halofantrine; hydrochinine		Rare[a,b]	25
	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[a]	52
	I_{Ks}	R555C	Terfenadine		Rare	53, 54
	I_{Ks}	R583C	Dofetilide	C583-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	28
SCN5A	I_{Na}	G615E	Quinidine	E615-SCN5A was indistinguishable from wild-type mediated I_{Na} currents (tsa-201 cells)	Rare	28
	I_{Na}	L618F	Quinidine	F618-SCN5A was indistinguishable from wild-type mediated I_{Na} currents (tsa-201 cells)	Rare	28
	I_{Na}	S1103Y	Amiodarone	Y1103-SCN5A mediated an increased I_{Na} channel activation (HEK 293)	7–10% (Afro-Americans or West Africans/Caribbeans only)	28

Table 17–2. *Continued*

Gene	Current	Amino acid alteration	Drug/setting	Functional assay	Minor allele	Reference
	I_{Na}	F1250L	Sotalol	L1250-SCN5A was indistinguishable from wild-type mediated I_{Na} currents (tsa-201 cells)	Rare	28
	I_{Na}	P1825L	Cisapride	The C-terminal mutant L1825-SCN5A mediated I_{Na} current with slow decay and prominent TTX- insensitive, noninactivating 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)		55 56
				L1825-SCN5A channels showed impairment of intracellular trafficking (CHO cells) and failed to generate QT prolongation; exposure with cisapride rescued cell surface expression of L1825-SCN5A and exaggerated the LQT3 phenotype		

[a]Heterozygous mutation carriers may have a normal QT interval; for SCN5A, amino acid residues are numerated according to their position on the long splice variant.
[b]Reported from recessive forms of long QT syndrome.

alter the designation of a specific phenotype from mild to pronounced, even in carriers of the same mutation. Typically, more frequently occurring SNPs should be used, but large numbers of cases and controls would still be needed because of their low phenotypic impact. The following assumptions underlie these investigations:

1. An important fraction of susceptibility to a given disease may be explained by the relatively modest effects of a small number of relatively common variants.
2. Many of these common variants are either SNPs themselves or genomic markers in linkage disequilibrium with the functional variant, and arise at rates lower than the rate at which new SNPs appear.
3. Rates at which SNPs become common are low enough that many of the SNP carriers inherited this variant from a single ancestor.
4. Recombination rates at SNP loci are low; therefore carriers of a common disease-linked variant will have the same SNP (haplotype) pattern that appeared on the ancestral chromosomal locus near the causal variant.

Ethnicity-specific and population-specific differences in the frequency of an SNP allele in cardiac ion channel genes have to be considered, since they recently became evident.[33] The SNP *KCNH2*-K897T (rs1805123) has been investigated in different studies to address an effect on QT interval duration. So far, no convincing data were reported, because some,[12,35,35] but not all studies[28,36,37] suggested an effect on QTc interval duration. *SCN5A*-S1103Y (also *SCN5A*-S1102Y, referring to the shorter splice form of *SCN5A*) is another frequent LQT gene polymorphism that has been identified primarily in West Africans and Caribbeans (ca. 19%) and in Afro-Americans (ca. 13%), but is very rare in whites, Asians, and Hispanics.[38] Y1103 allele carriers (YY or SY) were reported to have a higher relative risk for ventricular arrhythmia, which was not linked to baseline repolarization parameters[38] and sudden cardiac death in infants (SIDS).[39,40,41]

The majority is related to sudden cardiac death in myocardial ischemia or during heart failure. The instability of membrane electrophysiological properties of myocardium and local conduction impairment led to lethal ventricular arrhythmias. Since ATP-sensitive potassium (KATP) channels are involved in membrane regulation during metabolic stress, studies focused on identifying variants in the *KCNJ11* gene associated with SCD after

myocardial infarction.[42] 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 nonsynonymous polymorphisms (R371H, P266T) in two highly conserved pore regions are known that show altered modulation by intracellular ATP and protons and differences in channel density[43] and, thus, are potential candidates for genetically determined electrophysiological differences under ischemic conditions.

Future Directions

Recent advances in our knowledge of the genomic structure of ion channel genes and their physiological role in myocardial repolarization have shown that genetic alterations of these key molecular components are associated with slight *in vitro* effects and changes in finetuning normal repolarization. The extent to which minor genetic factors are associated with susceptibility to arrhythmias remains to be determined, but initial evidence for this is present. Following the concept of "repolarization reserve,"[44] it is likely that TdP as well as arrhythmia during acute myocardial infarction or drug response (as well as side effects) are also dependent on individual genetic backgrounds. The genetic factors involved in arrhythmogenesis switch from gene identification and single pathway understanding to genomic medicine by integrating complex gene and environmental information. Future research will

1. Identify all relevant genes and their genomic structure for repolarization.
2. Determine the extent of the variability of the QT interval and of the response to action potential prolongation that is genetically controlled.
3. Investigate the role of functionally relevant SNPs and haplotype constellations in LQTS and other gene loci for their quantitative contribution to repolarization.
4. Integrate identified genetic factors with other known factors for TdP 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, will enable researchers to lower the costs of complex genotyping and to implement these data into a personalized, genomic-oriented medicine.

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References

1. Venter JC, Adams MD, Myers EW, *et al.* The sequence of the human genome. *Science* 2001; 291(5507):1304–1351.
2. Lander ES, Linton LM, Birren B, *et al.* Initial sequencing and analysis of the human genome. *Nature* 2001;409(6822):860–921.
3. Botstein D, White RL, Skolnick M, *et al.* Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 1980;32(3):314–331.
4. Kääb S, Schulze-Bahr E. Susceptibility genes and modifiers for cardiac arrhythmias. *Cardiovasc Res* 2005;67(3):397–413.
5. Altshuler D, Pollara VJ, Cowles CR, *et al.* An SNP map of the human genome generated by reduced representation shotgun sequencing. *Nature* 2000; 407(6803):513–516.
6. Lindblad-Toh K, Winchester E, Daly MJ, *et al.* Large-scale discovery and genotyping of single-nucleotide polymorphisms in the mouse. *Nat Genet* 2000;24(4):381–386.
7. Mullikin JC, Hunt SE, Cole CG, *et al.* An SNP map of human chromosome 22. *Nature* 2000;407(6803): 516–520.
8. Sachidanandam R, Weissman D, Schmidt SC, *et al.* A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 2001;409(6822):928–933.
9. Au WW, Oh HY, Grady J, *et al.* Usefulness of genetic susceptibility and biomarkers for evaluation of environmental health risk. *Environ Mol Mutagen* 2001;37(3):215–225.
10. Editorial. Freely associating. *Nat Genet* 1999;22: 1–2.

11. Cooper DN, Nussbaum RL, Krawczak M. Proposed guidelines for papers describing DNA polymorphism-disease associations. *Hum Genet* 2002;110(3):207–208.
12. Pfeufer A, Jalilzadeh S, Perz S, *et al*. Common variants in myocardial ion channel genes modify the QT interval in the general population: Results from the KORA study. *Circ Res* 2005;96(6):693–701.
13. Arking DE, Pfeufer A, Post W, *et al*. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. *Nat Genet* 2006; 38(6):644–651.
14. Busjahn A, Knoblauch H, Faulhaber HD, *et al*. QT interval is linked to 2 long-QT syndrome loci in normal subjects. *Circulation* 1999;99(24):3161–3164.
15. Newton-Cheh C, Larson MG, Corey DC, *et al*. QT interval is a heritable quantitative trait with evidence of linkage to chromosome 3 in a genome-wide linkage analysis: The Framingham Heart Study. *Heart Rhythm* 2005;2(3):277–284.
16. Kaufman ES, Priori SG, Napolitano C, *et al*. Electrocardiographic prediction of abnormal genotype in congenital long QT syndrome: Experience in 101 related family members. *J Cardiovasc Electrophysiol* 2001;12(4):455–461.
17. Zareba W, Moss AJ, Sheu G, *et al*. Location of mutation in the KCNQ1 and phenotypic presentation of long QT syndrome. *J Cardiovasc Electrophysiol* 2003;14(11):1149–1153.
18. Dekker LR, Bezzina CR, Henriques JP, *et al*. Familial sudden death is an important risk factor for primary ventricular fibrillation: A case-control study in acute myocardial infarction patients. *Circulation* 2006;114(11):1140–1145.
19. Spooner PM, Albert C, Benjamin EJ, *et al*. Sudden cardiac death, genes, and arrhythmogenesis: Consideration of new population and mechanistic approaches from a national heart, lung, and blood institute workshop, part I. *Circulation* 2001;103(19): 2361–2364.
20. Jouven X, Desnos M, Guerot C, *et al*. Predicting sudden death in the population: The Paris Prospective Study I. *Circulation* 1999;99(15):1978–1983.
21. de Bruyne MC, Hoes AW, Kors JA, *et al*. Prolonged QT interval predicts cardiac and all-cause mortality in the elderly. The Rotterdam Study. *Eur Heart J* 1999;20(4):278–284.
22. Algra A, Tijssen JG, Roelandt JR, *et al*. QTc prolongation measured by standard 12-lead electrocardiography is an independent risk factor for sudden death due to cardiac arrest. *Circulation* 1991;83(6):1888–1894.
23. Straus SM, Kors JA, De Bruin ML, *et al*. Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. *J Am Coll Cardiol* 2006; 47(2):362–367.
24. Newton-Cheh C, Larson MG, Corey DC, *et al*. QT interval is a heritable quantitative trait with evidence of linkage to chromosome 3 in a genome-wide linkage analysis: The Framingham Heart Study. *Heart Rhythm* 2005;2(3):277–284.
25. Schulze-Bahr E, Haverkamp W, Hördt M, *et al*. Do mutations in cardiac ion channel genes predispose to drug-induced (acquired) long-QT syndrome? *Circulation* 1997;96(8):I–210(Suppl.).
26. Roden DM, Viswanathan PC. Genetics of acquired long QT syndrome. *J Clin Invest* 2005;115(8):2025–2032.
27. Roden DM. Long QT syndrome: Reduced repolarization reserve and the genetic link. *J Intern Med* 2006;259(1):59–69.
28. Yang P, Kanki H, Drolet B, *et al*. Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. *Circulation* 2002; 105(16):1943–1948.
29. Paulussen ADC, Gilissen RAHJ, Armstrong M, *et al*. Genetic variations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in drug-induced long QT syndrome patients. *J Mol Med* 2004;82(3):182–188.
30. Groenewegen WA, Firouzi M, Bezzina CR, *et al*. A cardiac sodium channel mutation cosegregates with a rare connexin40 genotype in familial atrial standstill. *Circ Res* 2003;92(1):14–22.
31. Makita N, Sasaki K, Groenewegen WA, *et al*. Congenital atrial standstill associated with coinheritance of a novel SCN5A mutation and connexin 40 polymorphisms. *Heart Rhythm* 2005;2(10):1128–1134.
32. Bezzina CR, Shimizu W, Yang P, *et al*. Common sodium channel promoter haplotype in asian subjects underlies variability in cardiac conduction. *Circulation* 2006;113(3):338–344.
33. Ackerman MJ, Tester DJ, Jones GS, *et al*. Ethnic differences in cardiac potassium channel variants: Implications for genetic susceptibility to sudden cardiac death and genetic testing for congenital long QT syndrome. *Mayo Clinic Proc* 2003;78(12): 1479–1487.
34. Bezzina CR, Verkerk AO, Busjahn A, *et al*. A common polymorphism in KCNH2 (HERG) hastens cardiac repolarization. *Cardiovasc Res* 2003;59(1): 27–36.
35. Gouas L, Nicaud V, Berthet M, *et al*. Association of KCNQ1, KCNE1, KCNH2 and SCN5A polymorphisms with QTc interval length in a healthy

population. *Eur J Hum Genet* 2005;13(11):1213–1222.
36. Paavonen KJ, Chapman H, Laitinen PJ, *et al.* Functional characterization of the common amino acid 897 polymorphism of the cardiac potassium channel KCNH2 (HERG). *Cardiovasc Res* 2003; 59(3):603–611.
37. Pietila E, Fodstad H, Niskasaari E, *et al.* Association between HERG K897T polymorphism and QT interval in middle-aged Finnish women. *J Am Coll Cardiol* 2002;40(3):511–514.
38. Splawski I, Timothy KW, Tateyama M, *et al.* Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia. *Science* 2002;297(5585):1333–1336.
39. Burke A, Creighton W, Mont E, Li L, Hogan S, Kutys R, *et al.* Role of SCN5A Y1102 polymorphism in sudden cardiac death in blacks. *Circulation* 2005;112(6):798–802.
40. Plant LD, Bowers PN, Liu Q, Morgan T, Zhang T, State MW, *et al.* A common cardiac sodium channel variant associated with sudden infant death in African Americans, SCN5A S1103Y. *J Clin Invest* 2006;116(2):430–435.
41. Etzrodt D, Schulze-Bahr E. Letter regarding article by Burke *et al.*, "role of SCN5A Y1102 polymorphism in sudden cardiac death in blacks." *Circulation* 2006;113(15):e709.
42. Jeron A, Hengstenberg C, Holmer S, *et al.* KCNJ11 polymorphisms and sudden cardiac death in patients with acute myocardial infarction. *J Mol Cell Cardiol* 2004;36(2):287–293.
43. Cui N, Li L, Wang X, *et al.* Elimination of allosteric modulation of myocardial KATP channels by ATP and protons in two Kir6.2 polymorphisms found in sudden cardiac death. *Physiol Genomics* 2006;25(1): 105–115.
44. Roden DM. Taking the "idio" out of "idiosyncratic": Predicting torsades de pointes. *Pacing Clin Electrophysiol* 1998;21(5):1029–1034.
45. Abbott GW, Sesti F, Splawski I, *et al.* MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 1999;97(2): 175–187.
46. Sesti F, Abbott GW, Wei J, *et al.* A common polymorphism associated with antibiotic-induced cardiac arrhythmia. *Proc Natl Acad Sci USA* 2000; 97(19):10613–10618.
47. Hayashi K, Shimizu M, Ino H, *et al.* Probucol aggravates long QT syndrome associated with a novel missense mutation M124T in the N-terminus of HERG. *Clin Sci (Lond)* 2004;107(2): 175–182.
48. Splawski I, Shen J, Timothy KW, *et al.* Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. *Genomics* 1998;51(1): 86–97.
49. Piquette RK. Torsade de pointes induced by cisapride/clarithromycin interaction. *Ann Pharmacother* 1999;33(1):22–26.
50. Bellocq C, Wilders R, Schott JJ, *et al.* A common antitussive drug, clobutinol, precipitates the long QT syndrome 2. *Mol Pharmacol* 2004;66(5):1093–1102.
51. Chevalier P, Rodriguez C, Bontemps L, *et al.* Noninvasive testing of acquired long QT syndrome: Evidence for multiple arrhythmogenic substrates. *Cardiovasc Res* 2001;50(2):386–398.
52. Napolitano C, Schwartz PJ, Brown AM, *et al.* Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias. *J Cardiovasc Electrophysiol* 2000;11(6):691–696.
53. Berthet M, Denjoy I, Donger C, *et al.* C-terminal HERG mutations: The role of hypokalemia and a KCNQ1-associated mutation in cardiac event occurrence. *Circulation* 1999;99(11):1464–1470.
54. Donger C, Denjoy I, Berthet M, *et al.* KVLQT1 C-terminal missense mutation causes a forme fruste long-QT syndrome. *Circulation* 1997;96(9):2778–2781.
55. Makita N, Horie M, Nakamura T, *et al.* Drug-induced long-QT syndrome associated with a subclinical SCN5A mutation. *Circulation* 2002;106(10): 1269–1274.
56. Liu J, Laurita KR. The mechanism of pause-induced torsade de pointes in long QT syndrome. *J Cardiovasc Electrophysiol* 2005;16(9):981–987.