Genetic Polymorphisms and Arrhythmia Susceptibility

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Over the past 10 years, remarkable advances have been made in identifying the genes responsible for primary electrical heart diseases, such as congenital long QT syndrome and Brugada syndrome. Basic and clinical studies on these inherited arrhythmias have provided significant insight into the molecular basis of cardiac electrophysiology and the mechanisms of arrhythmias. However, many studies of genotype–phenotype relationships in these diseases have revealed considerable phenotypic variability in individuals from the same kindred carrying the identical disease-associated DNA variant, as is commonly observed in other polygenic disorders. Furthermore, despite rapid progress in understanding the molecular basis of primary electrical heart diseases, there is little insight into the genetics of acquired arrhythmias. Recently, it has been recognized that common genetic polymorphisms in cardiac ion channel and other genes may modify cardiac excitability, which in turn predisposes affected individuals to arrhythmias in the presence of triggering factors, such as electrolyte abnormalities or drugs. This paper reviews the current understanding of the contribution of genetic polymorphisms to the pathophysiology of cardiac arrhythmias. (Circ J 2007; Suppl A: A-54–A-60)

Key Words: Arrhythmia susceptibility; Common arrhythmias; Genetic modifier; Polymorphism; QT prolongation; Single nucleotide polymorphisms

Primary electrical heart disease refers to a disease entity of rare, often familial, cardiac arrhythmias in the absence of structural cardiac abnormalities. It includes several hereditary arrhythmias including long-QT syndrome (LQTS), short-QT syndrome, Brugada syndrome (BS), and catecholaminergic polymorphic ventricular tachycardia. Most of these disorders are associated with mutations in the cardiac ion-channel genes, so they are referred to as cardiac ion channelopathies. Study of these rare diseases has been highly informative for basic and clinical electrophysiology, and a novel concept has recently emerged that common genetic variations might modify arrhythmia susceptibility in the general population. The finding that several drugs and electrolyte abnormalities are associated with development of cardiac arrhythmias has suggested a common genetic background in some individuals that predisposes them to arrhythmias in the presence of these triggering factors. With the availability of the human genome sequence, studies now focus on the identification of variations in the human genome and their contribution to arrhythmia predisposition.

Genetic Polymorphisms and Variable Penetrance

Mutations are generally defined as disease-associated alterations in DNA, occurring in less than 1% of the population. By contrast, polymorphisms are variations in the DNA sequence that have an allele frequency of at least 1% in a population, and are expected in approximately 1 in 1,000 base pairs of the human genome, differing in a polymorphic manner between 2 chromosomal homologues. As listed in Table 1, there are many different types of polymorphisms, including single nucleotide polymorphisms (SNPs), insertion/deletion variants, and microsatellite polymorphisms. Polymorphisms located within the coding region of a gene, such as non-synonymous SNPs, can directly influence the structure of its protein product, whereas others located within the regulatory region of a gene can influence the regulation of the expression levels of its protein product. These genetic variations may also alter phenotypic expression only under pathological conditions, such as during ischemia, or with the use of certain medications. However, a polymorphism does not necessarily mean that the variant is responsible for a clinical phenotype. Moreover, significant differences in polymorphic gene sites can be found in different ethnic backgrounds and may simply represent a genetic feature of a selected population rather than a susceptible allele.

In recent years, genetic approaches to understanding diversity in cardiac electrical function and susceptibility to cardiac arrhythmias have focused in particular on ion channels and gap junction proteins as key components in cardiac electrophysiology. Although mutations that cause the phenotype have been found in a single family or an individual in most cases, variations in genes linked to congenital arrhythmia syndromes may be relevant to more common acquired cardiac arrhythmias. Large population studies, in which cases of disease are compared with matched healthy controls from the same population, give a higher chance of detecting small genetic effects.

Systematic characterization of the clinical manifestations of genotyped families has revealed substantial intra- and interfamilial differences in phenotypic and disease expression, ranging from life-threatening arrhythmias to asymptomatic ECG changes. This phenomenon is referred to as “variable penetrance” and is typically observed in congenital LQTS. Variability elsewhere in the genome has been
**Table 1** Types of Gene Polymorphisms

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

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

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Fig 1. A mutation of SCN5A, L1825P, associated with cisapride-induced acquired long QT syndrome (LQTS). (A) Whole-cell Na current recorded from cells expressing wild-type (WT) or L1825P. Persistent non-inactivating Na current, characteristic of LQT3 mutations, is shown with an arrow. (B) Voltage-dependence of inactivation (Left) and activation (Right) is shifted in the hyperpolarizing and depolarizing directions, respectively. These biophysical abnormalities tend to reduce Na current. Therefore, L1825P exhibited the mixed channel dysfunction found in both LQT3 and Brugada syndrome.
assumed to contribute to this phenomenon. One possibility is that a modulator locus may reside in the same disease gene. Another possibility is that variability at 2 genetic loci might modulate an arrhythmogenic phenotype. One subset of such “double hit” cases is an especially severe or unusual phenotype in an individual with 2 abnormal alleles of the same gene, either by consanguinity (same abnormal allele) or by compound heterozygosity (different alleles).

**Acquired LQTS**

Acquired LQTS is often iatrogenic and associated with drugs, including familiar antibiotics, antihistamines, anti-psychotics, and antiarrhythmics. LQTS can also be manifested by electrolyte imbalances or bradycardia, especially in combination with the aforementioned drugs. Patients with acquired drug-induced torsades de pointes (TdP) share a number of clinical features with the congenital form of LQTS: female preponderance, apparent increased risk with hypokalemia, QT prolongation and TdP, and evidence of adrenergic activation prior to TdP. These findings, as well as the relatively unpredictable nature of drug-associated QT prolongation, suggest that there may be a population at risk because of genetic factors, but whose phenotype remains subclinical until drug challenge. It has been proposed that repolarization in the heart is accomplished by multiple redundant mechanisms, and that each 1 of the risk factors impairs 1 or more of these mechanisms to a variable extent. However, because of redundancy in the system, there is considerable “repolarization reserve”, and it is only when this reserve is exhausted by the presence of multiple risk factors that arrhythmias develop.

Following the concept of “repolarization reserve”, it is likely that the occurrence of TdP would be independent of specific drugs and more linked to a drug’s propensity to alter cardiac repolarization in individuals with a genetic predisposition to abnormal repolarization. Therefore, mutations can be identified not only in the $\text{IK}_r$ channel genes, but also in $\text{IK}_s$ or sodium channel genes in patients with drug-induced TdP. In fact, we found a novel $\text{SCN5A}$ mutation, L1825P, in a patient with cisapride-induced acquired LQTS (Fig 1). The heterologously expressed mutant L1825P showed mixed biophysical abnormalities of the persistent Na current that is a hallmark of LQT3 mutations and loss-of-function properties characteristic of BS. Liu et al recently explained the mechanism of QT prolongation and TdP caused by an $\text{IK}_s$ blocker in a patient with the $\text{SCN5A}$ mutation, L1825P. They showed that L1825P has a trafficking defect, and cisapride partially rescued the trafficking defect and the surface expression of the mutant channels, which led to an increase in the late Na current and QT prolongation.

The idea that common ion channel DNA variants may contribute to arrhythmia susceptibility has been driven by the identification of non-synonymous SNPs (Fig 2) and in vitro characterization showing subtle alterations differing from the wild type. In fact, genetic screening in 92 patients with drug-induced TdP demonstrated 6 genetic variants in 3 major LQTS genes ($\text{KCNQ1}$, $\text{KCNH2}$, and $\text{SCN5A}$) including mutations with mild biophysical phenotypes, which possibly confer an increased risk of TdP in response to drug challenge. However, none of these variants showed statistically significant differences in the allele frequency between normal and TdP populations. Moreover, it should...
be noted that functional studies of some ion channel SNPs are not comparable or may even produce contradictory results, probably because in vitro experiments are not standardized among different laboratories.

**Genetic Variations in Cardiac Sodium Channel Genes**

A recently identified SNP in the SCN5A gene, S1103Y, is associated with arrhythmia risk in African Americans. The Y1103-allele, carried by 13% of African Americans and overrepresented among arrhythmia patients of African decent (56.5%), was also linked to prolongation of the QT interval in an African American family. This SNP is found in approximately 19% of West Africans and Caribbeans, but not in Caucasians or Asians. More recently, S1103Y has been predominantly found in victims of sudden infant death syndrome, and the heterologously expressed S1103Y channel exhibited higher sensitivity to a lower intracellular pH than the wild type. These data suggest that the variant appears to confer susceptibility to acidosis-induced arrhythmia, indicating a gene–environment interaction.

H558R is one of the most prevalent SNPs of SCN5A. The electrophysiological characteristics of H558R do not differ from the wild type, but in vitro studies have reported that H558R modulates the trafficking of the SCN5A mutations T512I (responsible for isolated cardiac conduction defects) and M1766L (responsible for LQT3) when H558R and the mutations are on the same allele. More recent data show that H558R mitigates the trafficking abnormalities of a BS mutation, R282H, that is present on the non-mutant allele. These studies demonstrate the effect of genetic background on the phenotypic expression of a disease-causing mutation. However, population studies of congenital or acquired arrhythmias have shown no association between H558R and arrhythmias. Thus, the pathophysiological relevance of this SNP needs further elucidation.

Bezzina et al recently found a haplotype variant in the promoter region of SCN5A consisting of 6 polymorphisms in near-complete linkage disequilibrium (LD); an association of multiple loci on a chromosome caused by limited recombination between them. The allelic frequency of the variant haplotype (HapB) was 22% in the Asian population, but was absent in whites and blacks. Furthermore, HapB reduces transcription levels of SCN5A, and the promoter haplotype correlated well with PR and QRS durations. This study suggests that genetically-determined variable Na channel transcription is associated with variable conduction velocity, an important contributor to arrhythmia susceptibility.

**Genetic Variations in Cardiac Potassium Channel Genes**

Among 16 KCNQ1 mutations responsible for LQT1, 15 mutations were localized in the transmembrane domains and associated with a high percentage of symptomatic carriers and sudden deaths, whereas a missense mutation R555C at the C-terminal domain was associated with significantly less QT prolongation, and lower percentages of symptomatic carriers. R555C appeared to represent a forme fruste phenotype, a factor favoring acquired LQT syndrome. Sesti et al identified a SNP of KCNQ1 in a patient with sulfamethoxazole (SMZ)-associated LQTS (allelic frequency of 1.6% of the control population). Functional studies revealed that T8A channels were normal at baseline, but inhibited by SMZ at therapeutic levels that did not affect the wild-type channels. That study demonstrates that clinically silent DNA variations can increase the risk of life-threatening arrhythmias after drug exposure. Kubota et al reported that G643S a SNP of KCNQ1 (allelic frequency of 9% in the general Japanese population) decreased Iks current density in vitro. This SNP was in the LQT families studied and was mostly associated with a rather mild phenotype. Prolongation of the QT interval was often precipitated by hypokalemia and bradarrhythmias, implying that this polymorphism might be acting as a modifier gene in these LQT families.

With the KCNH2 (HERG) gene, several conflicting studies pertaining to K897T and the duration of QT interval in the healthy population have been reported. Pietila et al reported an association between the 897T-allele (allelic frequency of 16% in Finns) and prolongation of QTc interval in Finnish females. In a LQT2 family with the KCNH2 mutation A1116V, K897T is a genetic modifier that exaggerates the Iks reduction caused by A1116V, thus, only the individuals carrying both A1116V and K897T manifest QT prolongation. Bezzina et al found a significant association between the 897T-allele and shorter QTc intervals in healthy Caucasian groups, and the electrophysiological characterization of the K897T channel revealed gain-of-function properties leading to a shorter QT interval. More recently, a large LD-based SNP association study of LQTS genes showed that K897T is significantly associated with a shorter QTc interval.

**Genetic Variants of Other Genes**

Gap junctions are clusters of connexin (Cx) channels that span the closely opposed plasma membranes forming cell-to-cell pathways and facilitate action potential conduction. Cx40 is a gap junction protein predominantly expressed in the atrium and the specialized conduction system. There are 2 closely linked SNPs in the promoter region of Cx40, which lead to a substantial reduction in promoter activity. In 2 kindreds with atrial standstill, individuals with both the SCN5A mutation and the Cx40 promoter SNPs displayed the clinical phenotype, whereas individuals with a single defect are phenotypically indistinguishable from normal family members. These findings support the proposed interaction of the polymorphism with the mutation. By extension, multiple common variants in ion channel genes, or other genes that modulate cardiac electrophysiology, can be logically proposed as candidate modulators of clinical arrhythmia phenotypes.

More generally, this is an example of biology informing the identification of new candidate loci in which DNA variants might modulate a clinical phenotype. As complex signaling pathways determining integrated biologic responses (such as normal cardiac rhythm) are unraveled, each element is defined as such a candidate. Importantly, such candidate loci may involve modifier genes or signaling pathways heretofore not associated with arrhythmia phenotypes. Predicting the behavior of complex systems that have been perturbed by disease, drug administration, or functionally significant DNA variants is a major challenge to contemporary biology.
Altered myocardial repolarization is one of the important substrates of malignant ventricular arrhythmias, and rare gene variants affect repolarization in congenital LQTS. To investigate the influence of common gene variants on the QT interval, Pfeufer et al performed an LD-based SNP association study of 4 candidate genes \(^26\) (Fig 3). They initially genotyped 174 SNPs of 4 LQTS genes (\(KCNQ1\), \(KCNH2\), \(KCNE1\), and \(KCNE2\)) in 689 individuals, and successively screened 14 SNPs with suggestive linkage in a confirmatory sample of 3,277 individuals (total 3,966 individuals). They showed association to 1 SNP of \(KCNQ1\) (rs757092: +1.7 ms/allele) and 2 SNPs of \(KCNH2\) (K897T, rs1805123, –1.9 ms/allele; and rs3815459, +1.7 ms) (Fig 3).

These SNPs have additive effects on the QTc interval, showing a 10.5 ms difference in the QTc_RAS (QT interval corrected for rate, age and sex) between extreme-score groups (Table 2). This study is the first evidence that cardiac repolarization is heritable as a quantitative phenotypic trait.

To identify common genetic variants that modulate cardiac repolarization, Arking et al recently performed a genome-wide association study using 200 subjects at the extremes of a population-based QTc_RAS distribution of 3,966 subjects from the KORA cohort in Germany, with follow-on screening of selected markers in the remainder of the cohort. \(^29\) They identified \(NOS1AP\) (\(CAPON\)), a regulator of neuronal NOS, as a modulator of cardiac repolarization. Although the physiological roles of \(CAPON\) in the heart have not been extensively studied, a recent report showed that overexpression of \(CAPON\) in cardiomyocytes accelerated repolarization via a reduction of the L-type Ca current. \(^30\) As additional polymorphisms are elucidated, the true multigenic scope of arrhythmia susceptibility will emerge, but the clinical implication of individual polymorphisms will grow increasingly complex.

### Table 2 Genetic Polymorphisms

<table>
<thead>
<tr>
<th>SNP</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>QTc_RAS±SD</th>
<th>From total sample (n=3,966), n</th>
</tr>
</thead>
<tbody>
<tr>
<td>(KCNQ1) rs757092</td>
<td>GG</td>
<td>GA</td>
<td>AA</td>
<td>412.7±13.4</td>
<td>79</td>
</tr>
<tr>
<td>(KCNH2) rs1805123 (K897T)</td>
<td>CC</td>
<td>CA</td>
<td>AA</td>
<td>415.5±16.9</td>
<td>462</td>
</tr>
<tr>
<td>(KCNH2) rs3815459</td>
<td>GG</td>
<td>GA</td>
<td>AA</td>
<td>416.6±16.9</td>
<td>1,021</td>
</tr>
<tr>
<td>QT-prolongation score</td>
<td>3</td>
<td>418.3±17.8</td>
<td>1,132</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>419.3±16.9</td>
<td>641</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>423.2±19.4</td>
<td>135</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Effect of the 5 genotypic changes significant in the multivariate regression analysis from the 3 confirmed single nucleotide polymorphisms \(KCNQ1\)-rs757092, \(KCNH2\)-K897T, and \(KCNH2\)-rs3815459 was determined by a QT-prolongation score (\(p<0.00005\)). For each score-class the average QTc_RAS, standard deviation and the number of individuals are given. Individuals harboring the maximum possible number of 5 QT-prolonging alleles had on average a 10.5-ms longer QTc_RAS than individuals that had no QT-prolonging allele (0.95% of variance; \(p<0.00005\)).
Atrial Fibrillation (AF)

AF is the most common cardiac arrhythmia, and it increases in prevalence with advancing age to approximately 6% in individuals older than 65 years. Since the initial identification of the locus of familial AF on chromosome 10q22-24,7 further loci, including 4 relevant K channel genes, have been mapped. The KCNQ1 mutation, S140G, was identified in Chinese familial AF, and the heterologously expressed S140G channel revealed gain-of-function properties on the IkS current, in contrast to the loss-of-function effects of the KCNQ1 mutations previously described in LQT1 patients. In addition to monogenic diseases, Lai et al reported a non-synonymous SNP (G38S) of KCNE1 as a risk factor for AF susceptibility. The frequency of the 38G allele was significantly higher in the AF group than in the control group (76.4 vs 63.0%), although the functional significance of this SNP remains to be elucidated. More recently, Zeng et al have shown that none of the KCNQ1 and KCNE1 non-synonymous SNPs was associated with AF in the Chinese population, but the KCNE4 SNP E145D was associated with AF.

Limitations of Association Studies and Functional Assessment of SNPs

There are a number of potential limitations before the results of association studies can be integrated into clinical practice. First, the biological effects of a single polymorphism may be undetected, especially in the setting of multifactorial diseases, where the small additive effects of many factors may contribute to the disease phenotype. Second, the genetic heterogeneity of the population studied is also a major issue. Efforts should be made to match the subjects carefully by ethnic/geographic origin and other confounding variables (age, sex, smoking, cardiovascular disease, etc); thus, large-scale studies are required in order to detect even moderate associations.

Identification of non-synonymous SNPs and their subtle biophysical alterations characterized in vitro have prompted the idea that a common ion channel sequence variance may also contribute to a common arrhythmia and arrhythmia susceptibility. However, the results of ion channel alterations may be variable or even contradictory, because in vitro experiments are not standardized among different laboratories. For instance, Baroudi et al proposed an interesting mechanism underlying the apparent discrepancy between the severe clinical phenotype of an individual with BS carrying a T1620M mutation and its relatively minor biophysical abnormalities when expressed in cultured cells. Based on the fact that the proband carried an additional rare SNP R1232W, they constructed a double mutant (T1620M/R1232W) and characterized Na-channel function. In contrast to the relatively minor functional abnormalities of T1620M, they found that the double mutant channel molecule failed to reach the plasma membrane (defect of membrane trafficking), leading to total loss of the cardiac Na current. This observation supports the hypothesis that polymorphisms of ion channel genes can affect membrane trafficking or gating, thereby modulating the channel properties of the coexisting mutation. In order to confirm this observation, we constructed the same double mutation and performed patch clamp and confocal imaging to characterize its biophysical properties and subcellular distribution. To our surprise, the T1620M/R1232W channel elicited robust Na current, despite showing altered gating properties, and the subcellular distribution was nearly normal (data not shown). Unfortunately, such contradictions in vitro experiments are common among different laboratories, and this represents a major hurdle that needs to be overcome. Standardization of in vitro functional assessment of non-synonymous SNPs is needed to compare lab-specific results.

Conclusions

An important focus of future efforts will be to determine the mechanisms that control the expression of ion channel genes and the modulating factors that determine normal cardiac electrophysiology. Furthermore, it will be important to determine how genetic variations may cause arrhythmias. The identification of common variants that cause a subtle increase in the risk of life-threatening arrhythmias will facilitate prevention of sudden cardiac death through the rapid identification of populations at risk. Moreover, large databases with well-characterized drug responses may help define new drug targets and develop expedited technology to screen individuals with potential arrhythmogenic substrates, thereby leading to new treatment strategies.

Acknowledgments

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References