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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)[1] short-QT syndrome[2] Brugada syndrome (BS)[3] and cathecholaminergic polymorphic ventricular tachycardia[4,5] 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[6,7] 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[8] Variability elsewhere in the genome has been

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Table 1 Types of Gene Polymorphisms[6,7]

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 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 midium*	*Low to high, depending on site*
Intoronic 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*
Intoronic 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*

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

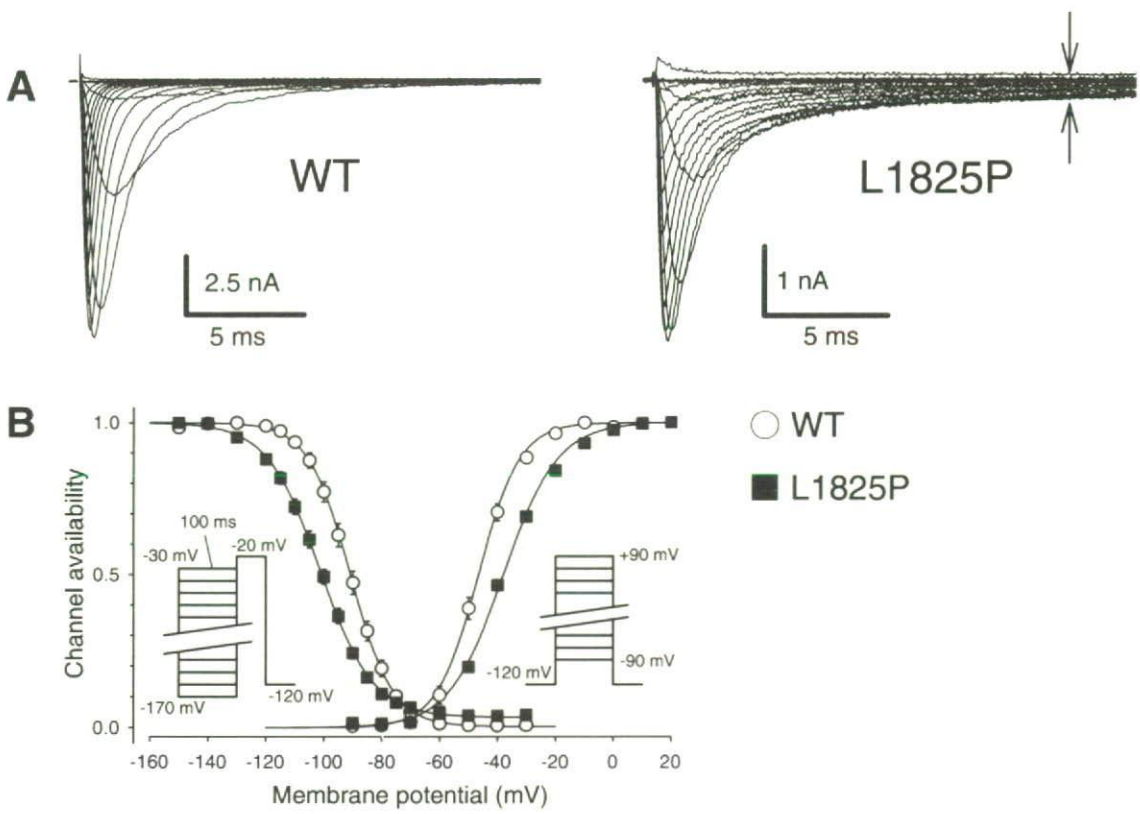

Fig 1. A mutation of *SCN5A*, L1825P, associated with cisapride-induced acquired long QT syndrome (LQTS). (A) Whole-cell Na current recorded from cells expressing wild-type (WT) or L1825P. Persistent non-inactivating Na current, characteristic of LQT3 mutations, is shown with an arrow. (B) Voltage-dependence 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.

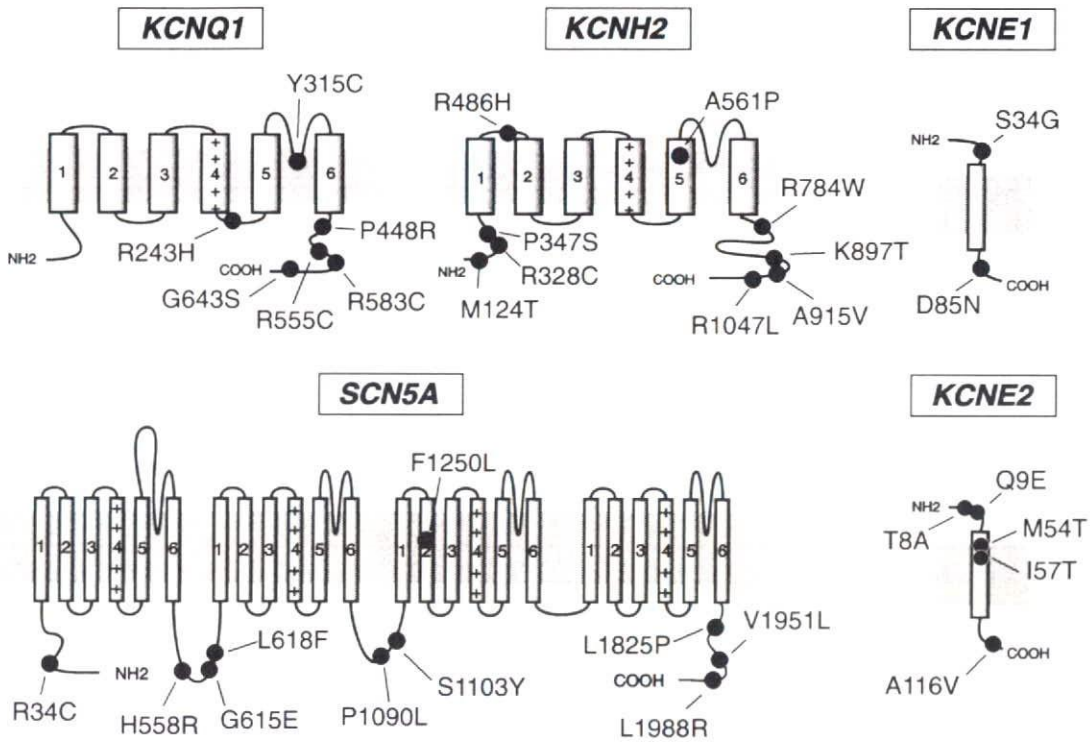

Fig 2. Mutations and non-synonymous single nucleotide polymorphisms (SNPs) of 5 long QT syndrome (LQTS) genes identified in drug-induced LQTS. Putative transmembrane domains of the 5 LQTS genes are shown with boxes and the locations of the SNPs are indicated with filled circles. Location of the *SCN5A* mutation, L1825P, is also shown.

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, antipsychotics, and antiarrhythmics[9] 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[10]

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 I_{Kr} channel genes, but also in I_{Ks} or sodium channel genes in patients with drug-induced TdP. In fact, we found a novel *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[11] Liu et al recently explained the mechanism of QT prolongation and TdP caused by an I_{Kr} blocker in a patient with the *SCN5A* mutation, L1825P[12] 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 (*KCNQ1*, *KCNH2*, and *SCN5A*)[13] 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.[14] 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.[15]

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[16] (responsible for isolated cardiac conduction defects) and M1766L[17] (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.[18] 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),[19] 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.[20] R555C appeared to represent a forme fruste phenotype, a factor favoring acquired LQT syndrome. Sesti et al identified a SNP of *KCNE2* T8A in a patient with sulfamethoxazole (SMZ)-associated LQTS (allelic frequency of 1.6% of the control population).[21] 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 I_{Ks} current density in vitro.[22] 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 bradyarrhythmias, 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.[23] In a LQT2 family with the *KCNH2* mutation A1116V, K897T is a genetic modifier that exaggerates the I_{Kr} reduction caused by A1116V; thus, only the individuals carrying both A1116V and K897T manifest QT prolongation.[24] 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.[25] More recently, a large LD-based SNP association study of LQTS genes showed that K897T is significantly associated with a shorter QTc interval.[26]

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.[27] 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.[27,28] 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.

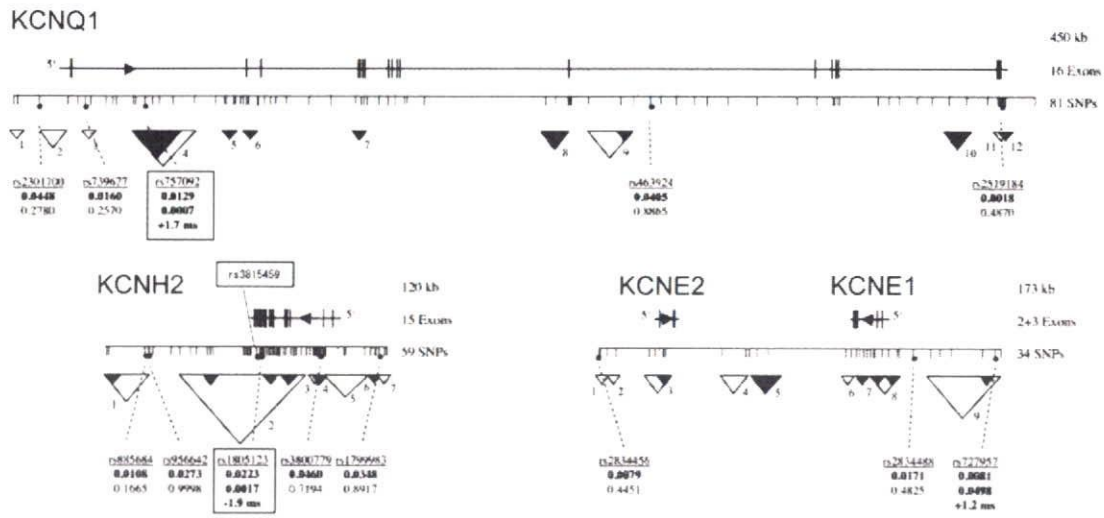

Fig 3. Genomic structure, linkage disequilibrium (LD)-structure, and genotyped single nucleotide polymorphisms (SNPs) in the investigated gene regions.[26] 174 SNPs genotyped in the screening sample are denoted as (|). 13 SNPs genotyped in the confirmation sample are marked by ●. The regions of LD structure are marked by D'-based haplotype block boundaries ( ) and by neighboring SNPs exceeding r^2-values of 0.5 (▼). For SNPs genotyped in both samples, p-values for association with QTc_RAS in the screening (Top) and in the confirmation sample (Bottom) and for associated SNPs the effect of 1 minor allele on QTc_RAS in the entire sample is given.

Table 2 Genetic Polymorphisms

	0	*1*	*2*	*QTc_RAS±SD*	*From total sample (n=3,966), n*
KCNQ1 rs757092	*GG*	*GA*	*AA*		
KCNH2 rs1805123 (K879T)	*CC*	*CA*	*AA*		
KCNH2 rs3815459	*GG*	*GA*	*AA*		
QT-prolongation score					
0				*412.7±13.4*	*79*
1				*415.5±16.9*	*462*
2				*416.6±16.9*	*1,021*
3				*418.3±17.8*	*1,132*
4				*419.3±16.9*	*641*
5				*423.2±19.4*	*135*

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; $p<0.00005$).

Genetic Factors Determining Cardiac Repolarization

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.

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,[31] 7 further loci, including 4 relevant K channel genes, have been mapped.[32] The *KCNQ1* mutation, S140G, was identified in Chinese familial AF, and the heterologously expressed S140G channel revealed gain-of-function properties on the I_{Ks} current, in contrast to the loss-of-function effects of the *KCNQ1* mutations previously described in LQT1 patients.[33] In addition to monogenic diseases, Lai et al reported a non-synonymous SNP (G38S) of *KCNE* as a risk factor for AF susceptibility.[34] 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.[35]

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.[36] 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 channels elicited robust Na current, despite showing altered gating properties, and the subcellular distribution was nearly normal (data not shown). Unfortunately, such contradictions of 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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The Common Long-QT Syndrome Mutation *KCNQ1*/A341V Causes Unusually Severe Clinical Manifestations in Patients With Different Ethnic Backgrounds

Toward a Mutation-Specific Risk Stratification

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Background—The impressive clinical heterogeneity of the long-QT syndrome (LQTS) remains partially unexplained. In a South African (SA) founder population, we identified a common LQTS type 1 (LQT1)–causing mutation (*KCNQ1*-A341V) associated with high clinical severity. We tested whether the arrhythmic risk was caused directly by A341V or by its presence in the specific ethnic setting of the SA families.

Methods and Results—Seventy-eight patients, all with a single *KCNQ1*-A341V mutation, from 21 families and 8 countries were compared with 166 SA patients with A341V and with 205 non-A341V LQT1 patients. In the 2 A341V populations (SA and non-SA), the probability of a first event through 40 years of age was similar (76% and 82%), and the QTc was 484±42 versus 485±45 ms (P=NS). Compared with the 205 non-A341V patients with the same median follow-up (30 versus 32 years), the 244 A341V patients were more likely to have cardiac events (75% versus 24%), were younger at first event (6 versus 11 years), and had a longer QTc (485±43 versus 465±38 ms) (all $P<0.001$). Arrhythmic risk remained higher ($P<0.0001$) even when the A341V patients were compared with non-A341V patients with mutations either localized to transmembrane domains or exhibiting a dominant-negative effect. A341V patients had more events despite β-blocker therapy.

Conclusions—The hot spot *KCNQ1*-A341V predicts high clinical severity independently of the ethnic origin of the families. This higher risk of cardiac events also persists when compared with LQT1 patients with either transmembrane or dominant-negative mutations. The identification of this high-risk mutation and possibly others may improve the risk stratification and management of LQTS. **(*Circulation*. 2007;116:2366-2375.)**

Key Words: arrhythmia ■ death, sudden ■ genetics ■ long-QT syndrome ■ risk factors

Heterogeneity of clinical manifestations is a well-known feature among patients affected by the long-QT syndrome (LQTS). The extent of this phenomenon became evident with the first large survey of LQTS as indicated by the presence within the same families of symptomatic and asymptomatic affected family members.[1] It was, however, only in the molecular era that scientific attempts were initiated to explain this puzzling clinical observation that also carries implications for patient management.

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The identification of the 3 main genes for LQTS prompted, within a few years, a series of relevant observations. On the basis of a relatively small number of genotyped patients, it

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was first suggested that LQTS type 3 (LQT3) was associated with less frequent but more lethal events.[2] Subsequently, in a larger number of genotyped families, it was shown that patients with either LQT2 or LQT3 were more likely to develop cardiac symptoms, but largely because of the higher incidence of LQT1 patients having a normal resting QTc (ie, <440 ms).[3] The shift of focus from the genes to the actual position of the various mutations within a given gene was the consequence of a study by Moss et al[4] that called attention to the fact that LQT2 patients with mutations in the pore region had a higher risk for cardiac events. However, the possibility that a discrete mutation could be associated with significantly higher risk for life-threatening cardiac events has so far remained unexplored or unproven, in part because the vast majority of LQTS-causing mutations are private, family-specific mutations. In LQTS, relatively few so-called mutational hot spots exist.

In 2005, we reported an LQT1-causing mutation, *KCNQ1*-A341V, in a South African (SA) founder population that was associated with unusual clinical severity.[5] Originated from a Dutchman who traveled to South Africa in 1670, this founder mutation comprises 22 *KCNQ1*-A341V genotype–positive SA families.[5] Although we assumed that this unexpected clinical phenotype was caused directly by this particular missense mutation, we could not exclude the possibility that the clinical severity was mediated not by the *KCNQ1*-A341V mutation per se but by some other probably genetic or epigenetic factors present in these families all living in South Africa for >300 years.

To answer this question and to determine whether the high arrhythmic risk observed in the SA families was indeed due solely to the *KCNQ1*-A341V mutation, one of relatively few "hot spot" missense mutations, we performed the present study on non-SA patients with LQT1 secondary to A341V.

Methods

Study Population

The study population was obtained through an international collaborative project involving 10 centers from 8 countries worldwide (Finland, France, Germany, Italy, Japan, the Netherlands, Russia, and the United States). Genetic and clinical data, collected on prespecified forms, included genotype status, demographic information, personal and family history of disease, type and timing of symptoms, ECG measurements, treatment, and response to therapy.

Data were recorded for a total of 84 patients from 24 unrelated, non-SA families harboring the *KCNQ1*-A341V mutation. Among them, 6 individuals from 3 families were compound heterozygotes (A341V plus an additional mutation on LQTS-related genes) and were excluded from analysis because individuals with 2 independent mutations are more likely to be symptomatic.[6,7]

A341V genotype–positive patients were classified as either symptomatic or asymptomatic on the basis of a previous experience of cardiac events (syncope, cardiac arrest [CA], sudden cardiac death [SCD]) as defined previously.[5] SCDs that occurred through 40 years of age in first-degree relatives and were judged to be LQTS-related according to an established policy[8] were assumed to have occurred in A341V mutation carriers and consequently were included, even in the absence of direct genotyping and/or ECG documentation.

Clinical Severity

The main objective of the study was to evaluate the clinical severity of LQTS among A341V genotype–positive patients with a heterogeneous ethnic background (non–SA-A341V) and to compare it with that of the SA founder population (SA-A341V) previously reported.[5] In addition, we compared the clinical course of all A341V patients with that of an LQT1 population derived from the LQTS database maintained at our institution in Pavia, Italy. As markers of clinical severity, we considered the proportion of symptomatic mutation carriers, the incidence of life-threatening arrhythmias, age at first cardiac event, QTc interval duration, and event-free survival by Kaplan-Meier cumulative estimates. The cumulative probability of a first event was considered, both for any event and for CA/SCD, before the institution of β-blocker therapy and through 40 years of age.

Furthermore, we took into account the disparity in the extent of genetic testing and clinical evaluation among the family members of the 2 A341V populations under study (non-SA and SA) because the SA pedigrees underwent extensive genetic testing. The inclusion of small nuclear families could have biased the results toward an overestimate of the clinical severity, so we also performed 3 different sensitivity analyses according to a priori established exclusion criteria to limit this potential selection bias. Specifically, all the analyses were repeated by (1) limiting the study population to 54 non-SA mutation carriers from 9 unrelated families and to 146 SA mutation carriers from 14 families with at least 4 affected individuals each; (2) excluding all probands, regardless of the number of affected individuals per family; and (3) combining these 2 criteria.

On the basis of recent findings that both transmembrane mutations[9] and dominant-negative functional mutations in *KCNQ1*[8] were associated with increased disease severity, we also considered the possible effect of the mutation site (transmembrane-spanning or pore-forming domains versus C- and N-terminal domains) and the possibility that the clinical severity of A341V might be a consequence of its dominant-negative nature. Therefore, we compared all A341V genotype–positive patients with the LQT1 population stratified for mutation site and the LQT1 patients with dominant-negative mutations.

Therapy

Data were collected on the administration and effectiveness of the treatment modalities applied to these LQTS patients: β-blockers, left cardiac sympathetic denervation, pacemaker, and implantable cardioverter-defibrillator. The assessment of the effectiveness of β-blockers was limited to those subjects with precise information on therapy and outcome and with at least 1 year of follow-up after initiation of treatment. To avoid the confounding role of possible comorbidities, we excluded from analysis those patients who started β-blocker therapy after 40 years of age. With the only exception of long-standing withdrawals (defined as a withdrawal of β-blocker therapy >1 week) or refusal of the prescribed β-blocker by the patient, all the events occurring during sporadic omission of the treatment were counted.

Statistical Analysis

The clinical characteristics of the genotyped groups were compared by Student *t* test or the Mann-Whitney *U* test as appropriate for continuous variables, which were expressed as mean and SD or as median and interquartile range (IQR). Categorical variables were presented as absolute and relative frequencies and compared by χ^2 test with Yates continuity correction. Event-free survival was described by Kaplan-Meier cumulative estimates, with comparisons performed by the log-rank test. Time from birth to first event through 40 years of age was considered both for any event and for CA/SCD. Survival analyses also were performed by gender. To represent the natural history of the disease and to avoid the confounding role of β-blockers, observations were censored at initiation of β-blocker therapy in survival analyses. Multivariate Cox proportional-hazards model was used to evaluate the significant and independent contribution of clinical and genetic factors to the risk of a first cardiac event. SPSS version 13 (SPSS Inc, Chicago, Ill) was used for computation. Values of $P<0.05$ (2 sided) were considered statistically significant.

Table 1. Clinical Characteristics of the Study Population and Comparison Between the 2 A341V Groups

	Non-SA-A341V Population	SA-A341V Population	*P*
Genotype-positive patients, n	78	166	...
Families, n	21	22	...
Female gender, n (%)	43 (55)	89 (54)	0.9
Symptomatic (any first event before 40 y of age), n (%)	53 (68)	131 (79)	0.09
Median age at onset, y (IQR)	6 (5–9)	6 (4–10)	0.82
CA/SCD, n (%)	19 (24)	55 (33)	0.21
SCD, n (%)	10 (13)	24 (14)	0.88
Asymptomatic, n (%)	25 (32)	35 (21)	...
≤15 y of age, n (%)	13 (17)	9 (5)	<0.01
ECG off β-blocker therapy, n	63	90	...
QTc, ms	484±42	485±45	0.89
≤440 ms, n (%)	5 (8)	11 (12)	0.56
≥500 ms, n (%)	15 (24)	30 (33)	0.27
Median follow-up, y (IQR)	21.5 (11–40)	33 (17–56)	0.001

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Clinical Severity

We report data on 78 *KCNQ1*-A341V genotype–positive patients originating from 21 worldwide families with a mean of 3.7±2.8 affected subjects per family.

Table 1 displays the clinical characteristics of this population. A slight but nonsignificant female gender predominance (55%) was present. During a median observation time of 21.5 years (IQR, 11 to 40 years) from birth to last contact, 53 patients (68%) became symptomatic before 40 years of age. Among these, 35 (45%) had syncope only, 9 (11.5%) had CA, and 10 (13%) suffered SCD. All the SCDs occurred while patients were off therapy; 7 occurred before 20 years of age. Exercise was the triggering factor for all (4 of 4) the episodes of witnessed SCD with available information on the circumstances associated with the terminal event. Overall, 19 patients (24%) suffered fatal or near-fatal events. Only 25 A341V patients (32%) were asymptomatic during the first 4 decades. Importantly, approximately half of these individuals are still ≤15 years of age and thus are too young to be considered truly asymptomatic with certainty because they are still at risk of a first cardiac event.

Table 1 also compares the occurrence of symptoms during follow-up from birth between the non-SA A341V patients and the SA-A341V population. The proportion of patients who experienced at least 1 cardiac event was not significantly different (68% in the non-SA population versus 79% in the SA population, $P=0.09$). However, the mean age at last contact was significantly different, with the non-SA population being younger (median, 21.5 years [IQR, 11 to 40 years] versus 33.5 years [IQR, 17 to 56 years]; $P=0.001$); furthermore, a higher number of asymptomatic subjects ≤15 years of age were in the non-SA population compared with the SA group (13 [17%] versus 9 [5%]; $P<0.01$). For this reason, the clinical status between the 2 groups also was compared following the exclusion of all A341V patients ≤15 years of age. The proportions of patients very likely to remain asymptomatic during comparable lengths of their clinical course remained small and very similar between the 2 A341V groups (25% and 19%, respectively, for non-SA versus SA group; $P=0.5$).

The median age at first event through 40 years of age was the same (6 years [IQR, 5 to 9 years] and 6 years [IQR, 4 to 10]; $P=0.82$), as was the incidence of LQTS-related fatal or near-fatal events (24% and 33%, respectively; $P=0.21$).

An ECG recorded in the absence of β-blocker therapy was available in 63 (81%) of the 78 non-SA A341V patients and in 90 (54%) of the 166 SA-A341V. Basal QTc was almost identical between the 2 groups (484±42 versus 485±45 ms, respectively; $P=0.89$). The QTc was ≤440 ms for 8% and 12% ($P=0.56$) of the 2 populations, respectively, whereas 24% and 33% had a QTc ≥500 ms ($P=0.27$).

Kaplan-Meier curves describing the cumulative survival to any first cardiac event (syncope, CA, SCD) before the institution of β-blocker therapy and through 40 years of age are shown for the entire non-SA population compared with the SA cohort in Figure 1. The median survival time (ie, the time by which at least 50% of the population has already had a first cardiac event) was 8 and 9 years, respectively (all together, 8 years; 95% confidence interval, 6.9 to 9.1). By 5 years of age, the cumulative event-free survival was 76% and 70%, respectively; by 10 years of age, it dropped to 38% and 35%. By the end of the observation period, no significant difference in survival was observed (24% versus 18%, $P=0.25$). However, because a slight trend toward a lower probability of a first cardiac event after 10 years of age was observed in the non-SA population, we also focused on those patients who had no cardiac events until 10 years of age and who were followed up through 40 years of age. Once again, no significant difference existed in event-free survival be-

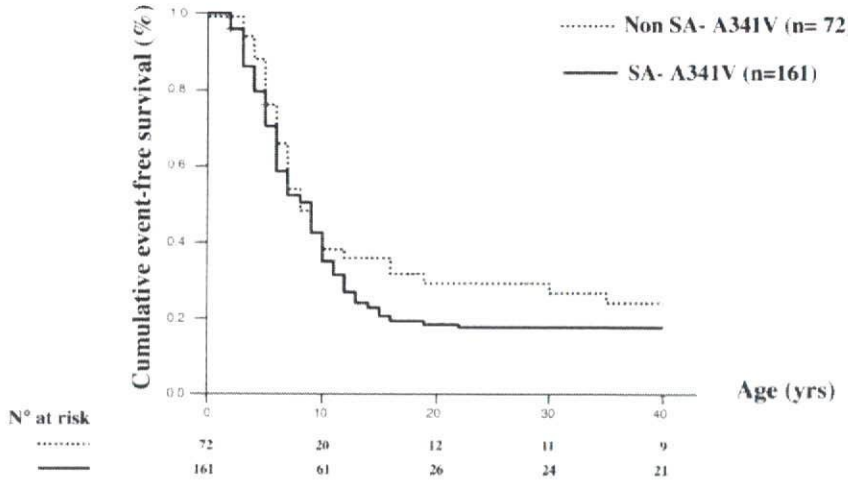

Figure 1. Unadjusted Kaplan-Meier estimate of the cumulative event-free survival in the non-SA and SA-A341V groups. Any cardiac event (syncope, CA, or LQTS-related SCD), whichever occurred first, was considered from birth through 40 years of age and before β-blocker therapy. Numbers at risk are indicated.

tween the 2 populations (data not shown; $P=0.11$). Notably, by 20 years of age, regardless of ethnic subgrouping, all A341V patients destined to become symptomatic had already experienced a first cardiac event, with very few events occurring after 20 years of age. No significant difference was observed between male and female patients among both the non-SA ($P=0.61$) and the SA A341V carriers ($P=0.19$).

When the end point for the comparison of the cumulative survival was limited to CA/SCD (Figure 2), Kaplan-Meier curves described an almost identical pattern between the 2 A341V populations. By 40 years of age, the cumulative probability for combined fatal/near-fatal events was 35% and 31%, respectively ($P=0.93$). The 3 sensitivity analyses confirmed the results reported above, and no significant differences were observed between the SA and non-SA populations.

Comparison Between A341V and Non-A341V LQT1 Populations

Because the SA and non-SA populations showed no significant difference in any of the markers of severity analyzed, all patients genotype positive for A341V were combined to compare the clinical expression of this specific mutation with that of a genetically heterogeneous non-A341V LQT1 group derived from our own LQTS database in Pavia (Table 2). The LQT1 A341V population (n=244) had a significantly greater percentage of symptomatic patients, earlier age at first cardiac event, higher incidence of life-threatening arrhythmias, more prolonged mean QTc, lower frequency of silent mutation carriers, and twice the proportion of subjects with a QTc ≥500 ms compared with the non-A341V LQT1 group (n=205).

When the combined A341V population was plotted against the LQT1 non-A341V group, a significant difference in the cumulative event-free survival emerged in that by 40 years of age, 80% of the A341V population (SA and non-SA) but only 30% of the LQT1 non-A341V group had already experienced a first cardiac ($P<0.0001$; Figure 3). A multivariate Cox model adjusted for gender and QTc showed that A341V patients were at higher risk of a first cardiac event compared with the LQT1 non-A341V group, with a hazard ratio of 4

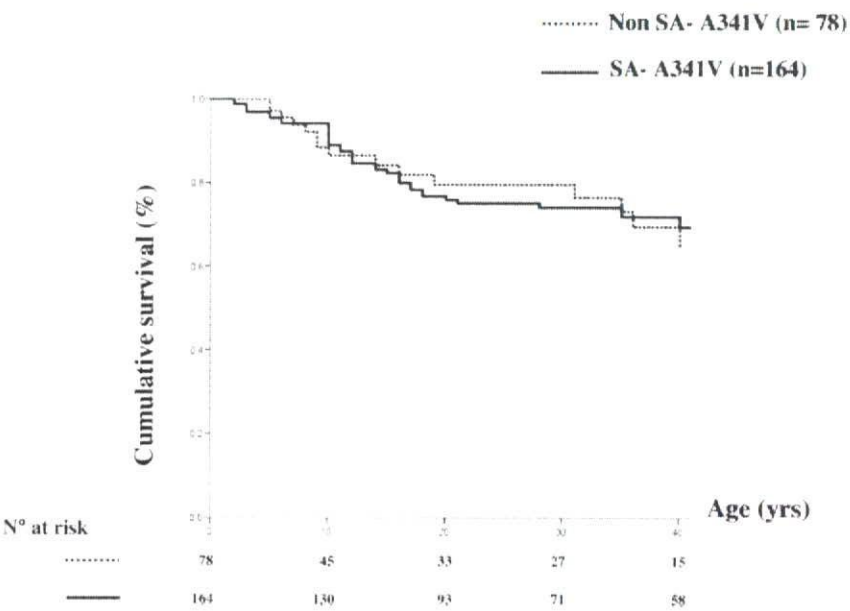

Figure 2. Unadjusted Kaplan-Meier estimate of the cumulative survival in the non-SA and SA-A341V groups. Only life-threatening cardiac events (CA or LQTS-related SCD) were considered from birth through 40 years of age and before β-blocker therapy. The SA group comprises 164 patients because of the lack of precise information on the exact time of the event in relation with therapy in 2 subjects. Numbers at risk are indicated.

Table 2. Clinical Characteristics of the Entire A341V Population and Comparison With a LQT1 Non-A341V Group

	All A341V	LQT1 Non-A341V	*P*
Genotype-positive patients, n	244	205	...
Female gender, n (%)	132 (54)	122 (59.5)	0.29
Symptomatic (any first event before 40 y of age), n (%)	184 (75)	49 (24)	<0.001
Median age at onset, y (IQR)	6 (5–10)	11 (4–17)	0.001
CA/SCD, n (%)	74 (30)	14 (7)	<0.001
ECG, n (%)	153 (63)	190 (93)	...
QTc, ms	485±43	465±38	<0.001
≤440 ms, n (%)	16 (10.5)	45 (24)	0.002
≥500 ms, n (%)	45 (29)	26 (14)	0.001
Median follow-up, y (IQR)	30 (15–51)	32 (14–46)	0.35

(95% confidence interval, 2.7 to 5.8; $P<0.001$). QTc was a significant and independent ($P=0.004$) predictor of cardiac events with a 6% increase in risk for each 10-ms increase in QTc. This pattern was confirmed when the comparison with the LQT1 population was performed according to the specific intragenic site of mutations and their functional effect. *KCNQ1*-A341V was associated with a much higher probability of experiencing a first cardiac event compared with the group comprising all other LQT1 non-A341V mutations, regardless of their being located in the transmembrane domain or in the C- and N-terminal regions of the protein ($P<0.0001$; Figure 4).

We then compared our 2 A341V populations with the non-A341V group comprising only mutations with a dominant-negative effect functionally demonstrated (Figure 5). Even in this case, patients with the dominant-negative A341V mutation had a significantly higher probability of becoming symptomatic than patients with other dominant-negative LQT1-causing mutations ($P<0.0001$). We also wanted to compare the A341V mutation with another dominant-negative mutation (*KCNQ1*-G314S) producing a significantly greater ($P<0.05$) loss in repolarizing current (~55% versus 70%)[5] and found that the probability of experiencing a first cardiac event was still significantly higher for A341V ($P=0.03$; Figure 6).

β-Blocker Therapy

For 67 of the 78 non-SA A341V patients (86%), adequate information on therapy and outcome was available. Of them, 34 (51%) received β-blocker therapy and fulfilled the prespecified criteria for the evaluation of the response to treatment. Their median age at initiation of therapy was 7.5 years (IQR, 6 to 27 years).

During a median observation time on β-blocker therapy of 7.5 years (IQR, 5 to 11 years), 14 A341V genotype–positive patients (41%) suffered at least 1 cardiac event, including 3 CAs but no SCD. Six patients also received an implantable cardioverter-defibrillator, and 1 of them received appropriate shocks. Thus, life-threatening events on β-blocker therapy occurred in 4 of 34 LQT1 patients with A341V (12%).

When the same inclusion criteria for analysis were applied to the SA group, it was observed that 70 of 150 patients (47%) were on β-blocker therapy, with a median age at initiation of therapy of 10 years (IQR, 4 to 18 years). During a median follow-up on β-blocker therapy of 12.5 years (IQR, 6.5 to 22.5 years), 34 of 70 carriers (49%) suffered at least 1

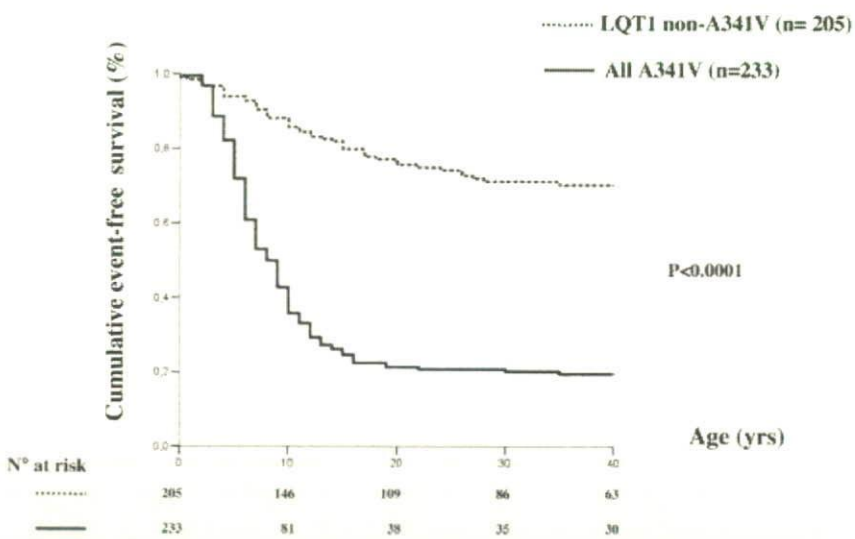

Figure 3. Unadjusted Kaplan-Meier estimate of the cumulative event-free survival (any first event) in the whole (non-SA+SA) A341V population plotted vs the LQT1 non-A341V group. Any cardiac event, whichever occurred first, was considered from birth through 40 years of age and before β-blocker therapy. Numbers at risk are indicated.

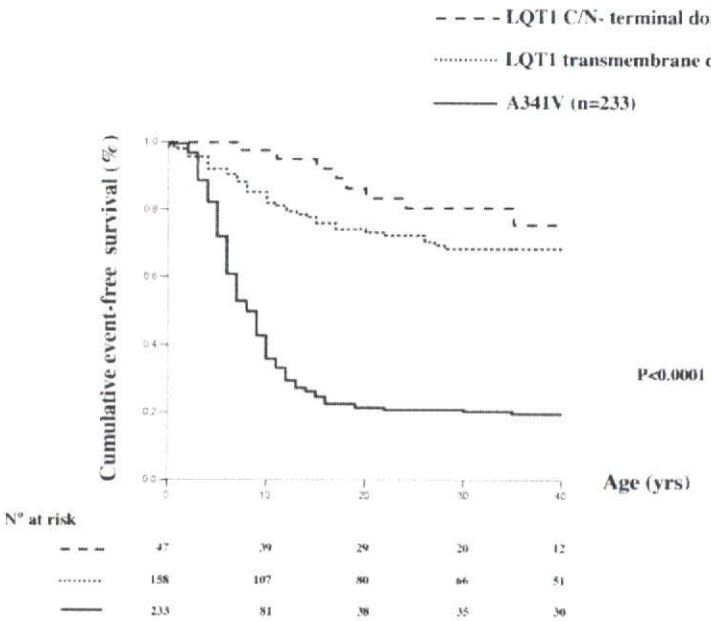

Figure 4. Unadjusted Kaplan-Meier estimate of the cumulative event-free survival (any first event) in the entire A341V and in the LQT1 non-A341V groups according to specific mutation site. Any cardiac event, whichever occurred first, was considered from birth through 40 years of age and before β-blocker therapy. Numbers at risk are indicated.

cardiac event, including 15 CAs and 5 SCDs, for a total of 20 life-threatening events on therapy (29%).

Among the 104 patients with A341V who were on β-blocker therapy, 19 (18%) life-threatening events occurred (18 CA and 1 implantable cardioverter-defibrillator shock) and 5 SCDs (5%). In comparison, among the 76 non-A341V assessable patients, a 7% incidence was shown of any cardiac event while on β-blockers; of note, no CAs and only 1 SCD (1%) occurred.

Discussion

We previously reported that *KCNQ1*-A341V, a mutation with a mild dominant-negative effect,[5] was associated with an unusually severe clinical phenotype in an SA founder population.[5] To determine whether this clinical severity was specific to the SA families or was related directly to the A341V mutation per se, we have collected data on A341V mutation carriers from 21 unrelated families originating from different parts of the world and having a different ethnic background.

We assume that the A341V mutation arose independently in different and unrelated families for 2 main reasons. First, this mutation was found in families living for centuries in very different parts of the world. Second, this mutation occurs in the context of a CpG dinucleotide, a known molecular hot spot for transition mutations.[10]

The major findings of the present study are that (1) the hot spot A341V on the *KCNQ1* gene is indeed associated with an unusual clinical severity independently of the origin of the families, (2) patients with this mutation are at higher risk for cardiac events compared with a more general LQT1 population, and (3) this clinical phenotype is not fully explained by the biophysical properties of the mutation. This evidence should now be taken into account in the risk stratification

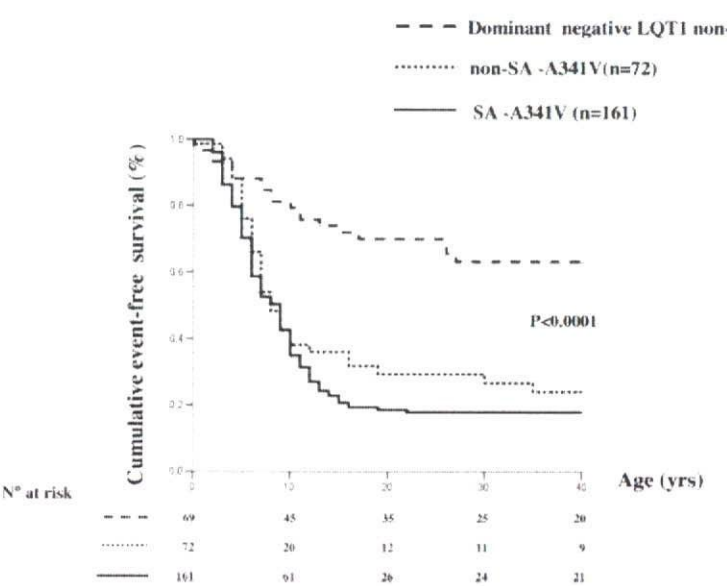

Figure 5. Unadjusted Kaplan-Meier estimate of the cumulative event-free survival (any first event) only in patients with LQT1 secondary to dominant-negative *KCNQ1* mutations; the 2 A341V groups are plotted vs the LQT1 non-A341V group. Any cardiac event, whichever occurred first, was considered from birth through 40 years of age and before β-blocker therapy. Numbers at risk are indicated.

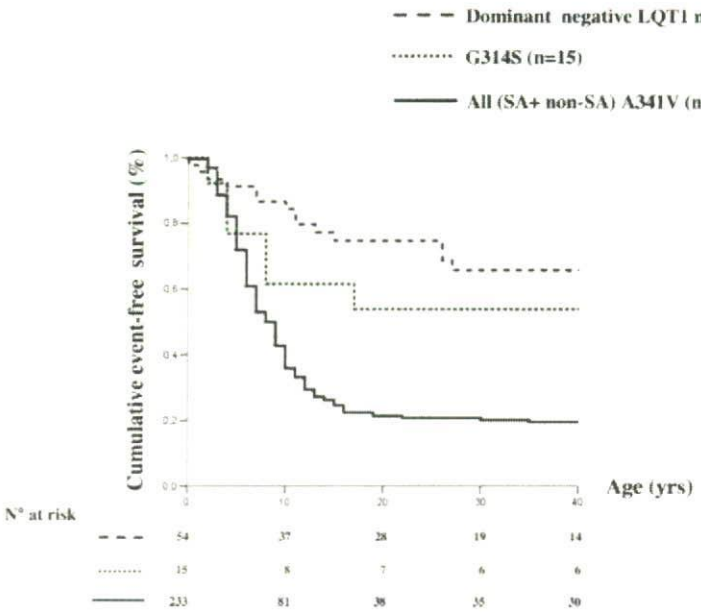

Figure 6. Unadjusted Kaplan-Meier estimate of the cumulative event-free survival (any first event) among patients with a mild (A341V) and strong (G314S) dominant-negative mutation. The entire A341V population (mild dominant-negative effect) is compared with a G314S population (strong dominant-negative effect) and the LQT1 non-A341V group. Numbers at risk are indicated.

process. We also unexpectedly found that recurrences of cardiac events despite β-blocker therapy were more frequent among *KCNQ1*-A341V patients than among LQT1 patients without this specific mutation.[11–15] Accordingly, we recommend careful follow-up and management of the A341V patients.

Mutation Site, Functional Effects, and Clinical Severity

Risk stratification for LQTS is important for the therapeutic decision-making process, especially when dealing with young asymptomatic patients, but despite significant progress compared with 20 to 30 years ago,[1,16] it is still in a developmental phase. In 2003, a risk stratification approach was proposed[3] that was based on gender, genotype, and degree of QT prolongation. However, this approach could not take into account the by-then only initial evidence that within the same genetic subgroup, important differences in the phenotypic manifestations of the disease may reflect the specific site of the mutations.

The first reports in this area came in 1997 by Donger et al[17] and in 2001 by Piippo et al[18] who called attention to the fact that the *KCNQ1*-R555C and *KCNQ1*-G589D mutations, respectively, both located in the C-terminal region, were associated with a somewhat less severe clinical phenotype. In 2002, Moss et al,[4] in a relatively large collaborative study, indicated that LQT2 patients with a mutation in the pore region of KCNH2 were at higher risk for cardiac events compared with patients with a mutation on the same gene but in different regions of the protein. This was followed in 2003 and 2004 by 2 studies[9,19] on the clinical impact of mutation site in LQT1 patients that reached opposite conclusions, thus complicating the attainment of a uniform interpretation.

Zareba et al[19] reported on 294 LQT1 patients from the International LQTS Registry[20] who had been classified into 3 groups according to their mutation site (prepore, pore, postpore) and found no significant differences in clinical presentation, ECG parameters, and cardiac events. Relevant here is the fact that in this cohort, *KCNQ1*-A341V, considered a pore mutation, represented only 6% (6 of 101 cases) of the entire "pore-region" population.

Shimizu et al[9] reported on 95 LQT1 Japanese patients from 37 different families who were classified according to the mutations being part of the transmembrane or of the C-terminal regions. Their main finding was a statistically significant greater risk of cardiac events for patients with mutations in the transmembrane region. Relevant here is the fact that in this investigation, at variance with the Zareba et al study, *KCNQ1*-A341V represented an impressive 29% (19 of 66 cases) of the entire "transmembrane" population.

We believe that an important contributing factor to the apparently very different results reported by Zareba et al and Shimizu et al lies in the large and significantly different representation of *KCNQ1*-A341V in their 2 reports (6% versus 29%; $P<0.001$). The striking clinical severity of this mutation, demonstrated in the present study, is probably sufficient to explain the more severe clinical picture associated with the Shimizu et al transmembrane mutations that included *KCNQ1*-A341V. Indeed, when following the same classification used by Shimizu et al, we divided our non-A341V LQT1 population according to the mutation site (transmembrane domain versus N and C terminal) and still observed a large difference between both these LQT1 genetic subgroups and the entire A341V population ($P<0.0001$).

Very recently, Moss et al[8] demonstrated in 600 LQT1 patients that both the transmembrane location of the mutations and their dominant-negative effect are independent risk factors for cardiac events. Accordingly, we took into consideration the biophysical properties of *KCNQ1*-A341V to verify whether they could explain our findings.

Initially, A341V had been regarded as a simple loss-of-function mutation without dominant-negative effect.[21,22] Later, Brink et al[5] demonstrated that this mutation was associated with a mild dominant-negative effect with a loss in

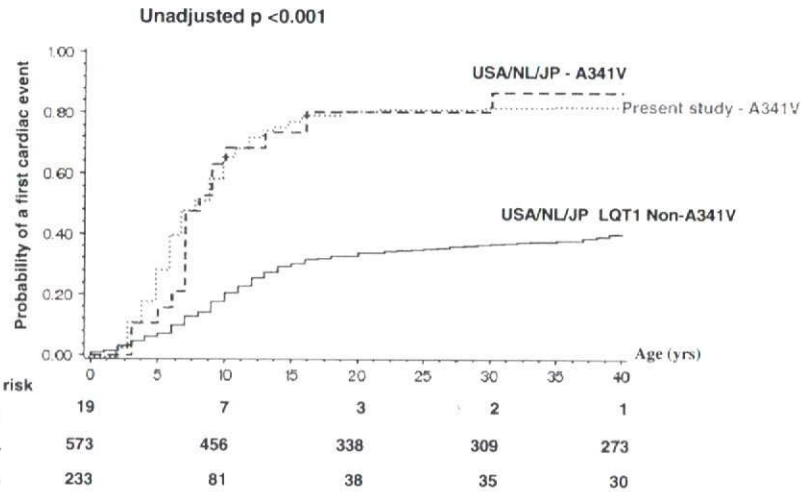

Figure 7. Unadjusted Kaplan-Meier estimate of the cumulative probability of a cardiac event (syncope, CA, or LQTS-related SCD, whichever occurred first) in the LQT1 population from the US-Netherlands-Japan collaborative study.[8] Carriers of A341V mutation are compared with all the other LQT1 non-A341V patients. Superimposed is the curve representing the cumulative probability of a first cardiac event in the entire (SA + non-SA) A341V population from the present study. Numbers at risk are indicated.

repolarizing current slightly exceeding 50% when coexpressed with wild type. When A341V was compared with our non-A341V LQT1 mutations with a dominant-negative effect, it was evident that A341V was associated with a significantly higher arrhythmic risk. Furthermore, when A341V was compared with a stronger dominant-negative mutation, G314S, that produced a loss of current of ≈70%,[5] the pattern indicating a higher risk among patients with A341V was again documented.

Because our own non-A341V population appeared to be somewhat less symptomatic than other LQT1 populations previously reported, for the sake of safety, we also made a comparison with the largest non-A341V population available to us, namely the 573 patients who were part of the recent study by Moss et al.[8] Figure 7 shows Kaplan-Meier curves for these 573 patients, for the 19 A341V patients from the same study, and for our own 233 A341V patients. Two important points become apparent. The first is that the probability of arrhythmic symptoms is twice as large (80% versus 40%; $P<0.0001$) among the A341V compared with the non-A341V patients. The second is the very impressive and practically identical Kaplan-Meier curves of the 19 A341V patients studied by Moss et al[8] and of the 233 A341V patients from our study.

These data conclusively demonstrate the striking clinical severity associated with the A341V mutation and, at variance with a major recent publication,[8] prove that cellular electrophysiological studies cannot always predict the clinical phenotype. Indeed, in the A341V patients, neither the location (transmembrane) nor the functional consequence of the mutation (dominant-negative effect) fully explains the unusually high clinical severity. We surmise that the current biophysical assessments of the electrophysiological effects of LQTS-causing mutations do not provide the whole gamut of information necessary to make a complete genotype-phenotype correlation.

Response to β-Blocker Therapy

In agreement with the evidence that among LQT1 patients, most cardiac events occur under conditions of increased sympathetic activity,[12] treatment with β-blockers is extremely effective in these LQTS patients who represent the largest genetic subtype.[11–15] Indeed, in LQT1 study populations with a percentage of symptomatic patients between 50% and 70%, the combined incidence of CA and SCD during rather long follow-up periods is only 1%.[13,15]

We were therefore surprised by observing what appears to be a rather incomplete protection for patients with A341V. A degree of caution is necessary in the interpretation of these data for which we do not have a ready explanation. It seems appropriate, however, to assess these patients very carefully with frequent follow-up visits to ensure that β-blockers are administered at full dose and to stress the importance of compliance. In addition, with QTc duration factored in as a known risk factor, the responsible physicians should be ready to consider the additional preventive steps represented by left cardiac sympathetic denervation[23] and by implantable cardioverter-defibrillators.

A341V Patients

The present data on a uniquely large population of patients carrying the same genetic defect (*KCNQ1*-A341V) demonstrate that within LQTS patients, mutation-specific behaviors exist independently of different genetic backgrounds and ethnicities. When we compared the clinical severity present in the SA and in the non-SA A341V population, we found that it was very similar. The sensitivity analyses, performed by excluding the probands and by including only those families with at least 4 affected individuals, confirmed these findings. Therefore, all A341V genotype–positive patients (n=244) were compared with a genetically heterogeneous LQT1 non-A341V population (n=205) and were shown to be more likely to have longer QT intervals, to suffer more arrhythmic events, and to be somewhat less protected by β-blockers from life-threatening events. Clearly, they represent a group at much higher risk compared with other LQT1 patients.

Study Limitations

The study had 2 potential limitations that we tried to obviate. In general, the SA families are larger than the non-SA families. For this reason, we performed sensitivity analyses that confirmed the validity of the data. The study of the SA

families goes back many more years and includes periods when the data collection cannot be accurately verified. Accordingly, we have excluded from the analysis of β-blocker therapy those older patients for whom precise information on dosage, compliance, and severity of the cardiac events could not be obtained with sufficient reliability.

Conclusions

The present study provides the largest data set on patients affected by LQTS who carry the exact same mutation. The data unequivocally show that *KCNQ1*-A341V is a mutation associated with unusual clinical severity. This finding, together with the recent evidence that genetically mediated neural control of heart rate may modulate arrhythmic risk in LQT1 patients,[24] begins to unravel the old and puzzling observation of the large heterogeneity in the clinical manifestations of LQTS. We do not believe that this mutation is unique in its clinical phenotype, and we believe that other mutations, more likely to be located in functionally important areas probably within the transmembrane region and close to the pore or in the S4 domain, confer a risk for life-threatening arrhythmias higher than that associated with other mutations. Thus, one can envision not only genotype-specific treatment algorithms but even mutation-specific considerations.

We were able to document these features because of the observations in the large SA founder population and because A341V is a relatively common LQT1-causing mutation. This has allowed us to pull together an adequate number of patients with this mutation from different parts of the world and to confirm the initial observation.[5] The severity of other specific mutations has probably escaped notice so far because they are less common and therefore their clinical impact has been lost within the large series of patients with more frequent mild mutations. The clinical message from our study is that in the future attention should be paid to families with a high percentage of symptomatic individuals and that, once the disease-causing mutations have been identified, collaborative studies similar to ours should be undertaken to test the possibility of identifying other clinically severe mutations. This will contribute to the development of a more accurate risk stratification grid for patients affected by LQTS.

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Disclosure

Dr Ackerman is a consultant for PGxHealth with respect to their FAMILION genetic test for cardiac channel mutations. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

The impressive clinical heterogeneity characteristic of the long-QT syndrome (LQTS) remains puzzling and hinders accurate risk stratification and targeted management. In a South African founder population, we identified a common LQTS type 1 (LQT1)–causing mutation (*KCNQ1*-A341V) associated with high clinical severity. We have now tested whether the arrhythmic risk was caused directly by A341V or by its presence in the specific ethnic setting of the South African families. We compared 78 patients from 10 countries, all with a single *KCNQ1*-A341V mutation, with 166 South African patients with A341V and 2 different populations of non-A341V LQT1 patients. In the 2 A341V populations, the probability of a first event before 40 years of age was similar (76% and 82%), and the QTc was similar. Compared with the LQT1 non-A341V patients, the A341V subjects were significantly more likely to have cardiac events, to be younger at first event, and to have a longer QTc. Arrhythmic risk remained higher even when the A341V group was compared with 573 LQT1 non-A341V patients. Thus, the hot spot *KCNQ1*-A341V predicts high clinical severity independently of the ethnic origin of the families. Neither the location (transmembrane) nor the functional consequence of the mutation (dominant-negative effect) fully explains the clinical phenotype. The identification of this high-risk mutation and possibly others may improve risk stratification and management of LQTS.

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Circ J 2008; **72:** 1926–1936

Clinical Impact of Genetic Studies in Lethal Inherited Cardiac Arrhythmias

Wataru Shimizu, MD

Over the past decade, molecular genetic studies have established a link between a number of inherited cardiac arrhythmias, including congenital long QT syndrome (LQTS) and Brugada syndrome (BrS), and mutations in genes encoding for ion channels or other membrane components. Twelve forms of LQTS have been identified in 50–70% of clinically affected patients. Genotype–phenotype correlations have been rigorously investigated in LQT1, LQT2 and LQT3 syndromes, which constitute more than 90% of genotyped LQTS patients, enabling stratification of risk and effective treatment of genotyped patients. Genotype-specific triggers for both the cardiac events and the clinical course have been reported, and genotype-specific therapy has been already introduced. More recently, mutation site-specific differences in the clinical phenotype have been reported in LQT1 and LQT2 patients, indicating the possibility of mutation site-specific management or treatment. In contrast, only one-third of BrS patients can be genotyped, and data on genotype–phenotype relationships in clinical studies are limited. A Haplotype B consisting of 6 individual DNA polymorphisms within the proximal promoter region of the *SCN5A* gene was recently identified only in Asians (frequency 22%). Individuals with Haplotype B show significantly longer duration of both PQ and QRS than those without Haplotype B, indicating that Haplotype B likely contributes to the higher incidence of BrS in Asian populations. (*Circ J* 2008; **72:** 1926–1936)

Key Words: Brugada syndrome; Genotype; Ion channel; Long QT syndrome; Sudden death

Advances in molecular genetic studies since the late 1990s have established a link between a number of lethal inherited cardiac arrhythmias and mutations in genes encoding for ion channels or other membrane components.[1–11] Most inherited cardiac arrhythmias have been linked to ion channelopathies giving rise to primary electrical diseases, including congenital and acquired long QT syndrome (LQTS),[1,2] Brugada syndrome (BrS),[3] progressive cardiac conduction defect (Lenegre disease),[4] catecholaminergic polymorphic ventricular tachycardia (CPVT),[5,6] arrhythmogenic right ventricular cardiomyopathy,[7] familial atrial fibrillation[8] familial sick sinus syndrome,[9,10] and short QT syndrome[11] (Table1). Among these primary electrical diseases, congenital LQTS is the Rosetta stone for understanding the genetic basis of inherited cardiac arrhythmias,[12] because the responsible mutations can be identified in multiple genes encoding different ion channels or membrane adaptor in approximately 50–70% of clinically affected patients. Similarly, causative mutations can be detected in the ryanodine receptor (*RyR2*) gene or calsequestrine gene in more than 60% of clinically diagnosed CPVT patients.[13] BrS is another common inherited cardiac arrhythmia syndrome, and responsible mutations have been identified in 6 genes; however, only one-third of patients with BrS can be genotyped. Responsible mutations have been identified much less in other inherited cardiac arrhythmias, so genetic screening is much more challenging. This review focuses on the recent progress in molecular genetic studies and their clinical impact on the inherited cardiac arrhythmias, congenital LQTS and BrS.

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Congenital LQTS

Prolonged QT interval and polymorphic ventricular tachycardia, known as torsades de pointes (TdP), recorded on an electrocardiogram (ECG) are trademarks of congenital LQTS (Fig 1).[12,14] The clinical diagnosis of congenital LQTS is mainly based on the corrected (QTc) interval at rest, and cardiac events such as syncope, aborted cardiac arrest and sudden cardiac death because of TdP.[14] However, the ECG diagnosis at rest has long been reported to miss some patients affected by congenital LQTS, as evidenced

Table 1 Genotype of Inherited Cardiac Arrhythmias

Congenital LQTS	*Romano-Ward*	*(1995-) CLQT 1-12*
	JLN	*(1997-) JLN 1, 2*
Acquired LQTS		*(1997-) ALQTS 1-2*
BrS		*(1998-) BrS 1-2*
PCCD		*(1999-) PCCD 1*
CPVT		*(2001-) CPVT 1, 2*
Familial SSS		*(2003-) SSS 1, 2*
Familial AF		*(2003-) AF 1-5*
ARVC		*(2004-) ARVC 1-5*
SQTS		*(2004-) SQTS 1-5*

LQTS, long QT syndrome; JLN, Jervell & Lange-Nielsen; BrS, Bugada syndrome; PCCD, progressive cardiac conduction defect; CPVT, catecholaminergic polymorphic ventricular tachycardia; SSS, sick sinus syndrome; AF, atrial fibrillation; ARVC, arrhythmogenic right ventricular cardiomyopathy; SQTS, short QT syndrome.

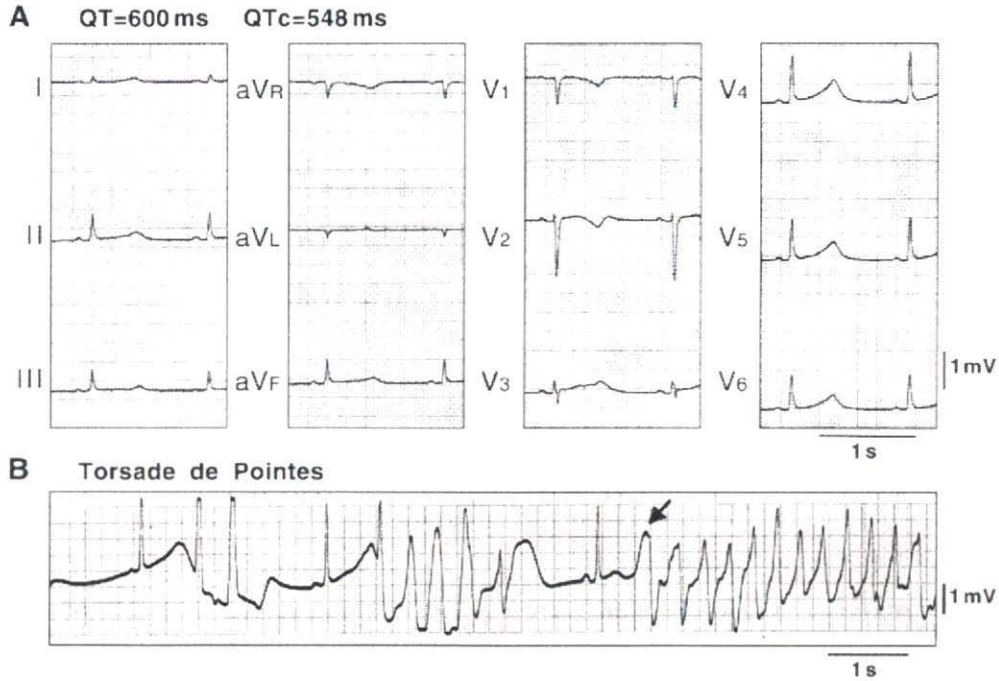

Fig 1. Twelve-lead electrocardiogram and torsades de pointes (TdP) in a patient with LQT2 syndrome. (A) Remarkable QT prolongation (corrected QT (QTc) interval=548 ms) and a low amplitude T wave with a notched configuration are seen. (B) TdP was induced following the typical short–long–short initiating sequence.

Table 2 Defect of Ion Channel or Membrane Adaptor Responsible for Congenital LQTS

Loci	*Chromosome*	*Gene*	*Ion channel*
Romano-Ward syndrome			
LQT1	*11 (11p15.5)*	*KCNQ1*	I_{Ks}
LQT2	*7 (7q35-36)*	*KCNH2*	I_{Kr}
LQT3	*3 (3p21-23)*	*SCN5A*	I_{Na}
LQT4	*4 (4q25-27)*	*Ankyrin-B*	*Na-K ATPase*, $I_{Na\ Ca}$
LQT5	*21 (21q22.1-q22.2)*	*KCNE1*	I_{Ks}
LQT6	*21 (21q22.1-q22.2)*	*KCNE2*	I_{Kr}
LQT7	*17 (17q23.1-24.2)*	*KCNJ2*	I_{K1}
LQT8	*12 (12p13.3)*	*CACNA1C*	$I_{Ca\text{-}L}$
LQT9	*3 (3p25)*	*CAV3*	I_{Na}
LQT10	*11 (11q23.3)*	*SCN4B*	I_{Na}
LQT11	*7 (7q21-q22)*	*AKAP-9*	I_{Ks}
LQT12	*20 (20q11.2)*	*SNTA1*	I_{Na}
JLN syndrome			
JLN1	*11 (11p15.5)*	*KCNQ1 (homozygous)*	I_{Ks}
JLN2	*21 (21q22.1-q22.2)*	*KCNE1 (homozygous)*	I_{Ks}

Abbreviations see in Table 1.

by syncopal events occurring among family members with a "normal" QT interval;[15] therefore, provocative testing using catecholamine infusion or exercise was developed to unmask concealed forms of congenital LQTS, before genetic screening became available.[16–20]

Genotype in Congenital LQTS

Because familial forms of congenital LQTS have long been recognized, a genetic background (inheritance) has long been expected. Since the first 2 genes responsible for LQTS were identified in 1995,[21,22] molecular genetic studies have revealed 12 forms of Romano-Ward-type congenital LQTS caused by mutations in the genes of the potassium, sodium and calcium channels or the membrane adapter located on chromosomes 3, 4, 7, 11, 12, 17, 20 and 21 (Table2).[23–31] Mutations in *KCNQ1* and *KCNE1*, the α and β subunits of the potassium channel gene, are responsible for defects (loss of function) in the slowly activating component of the delayed rectifier potassium current (I_{Ks}) underlying the LQT1 and LQT5 forms of LQTS.[32,33] Mutations in *KCNH2* and *KCNE2* cause defects in the rapidly activating component of the delayed rectifier potassium current (I_{Kr}), which is responsible for the LQT2 and LQT6 forms.[21,34] Mutations in *SCN5A*, the gene that encodes the α subunit of the sodium channel, result in an increase (gain of function) in the late sodium current (I_{Na}), which is responsible for LQT3.[22] Mutations in *KCNJ2* encoding the inward rectifier potassium current (I_{K1}) underlie Andersen's

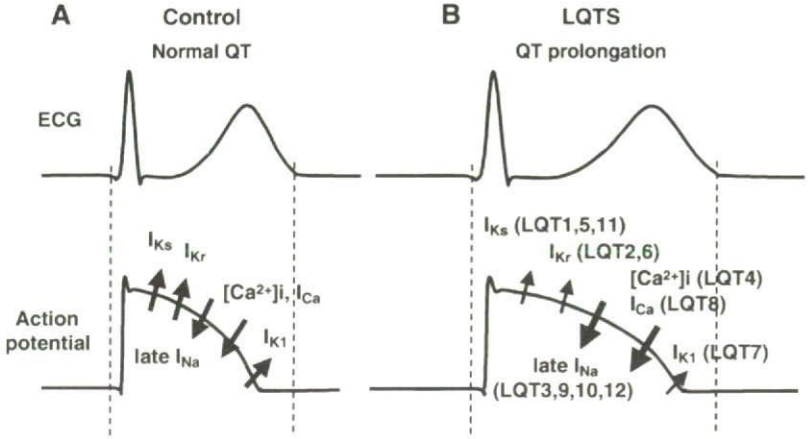

Fig 2. Ionic mechanism of QT prolongation in the LQT1 to LQT12 forms. Decreases in the outward potassium currents (I_{Ks}, I_{Kr}, I_{K1}) or increases in the inward sodium or calcium current (late I_{Na}, $I_{Ca\text{-}L}$) prolong the action potential duration, resulting in prolongation of the QT interval in all 12 genetic forms. ECG, electrocardiogram.

syndrome (LQT7), in which QT prolongation and ventricular arrhythmias are accompanied by periodic paralysis and dysmorphic features.[24] A mutation in *Ankyrin-B*, a member of a family of versatile membrane adapters, produces intracellular calcium overload, which underlies LQT4 syndrome and is associated with sinus bradycardia and paroxysmal atrial fibrillation, in addition to QT prolongation.[25] A mutation in *CACNA1C* is reported to be responsible for the defect in the L-type calcium current ($I_{Ca\text{-}L}$) underlying the LQT8 form, an arrhythmia disorder associated with dysfunction in multiple organ systems, including congenital heart disease, syndactyly, immune deficiency, and autism.[26] *CAV3* encoding caveolin-3 and *SCN4B* encoding *NavB4*, an auxiliary subunit of the cardiac sodium channel, are also reported to be associated with the LQT9 and LQT10 forms, respectively. Mutations in both genes result in a gain of function of late I_{Na}, thus causing an LQT3-like phenotype.[27,28] *AKAP-9* encoding Yotiao, which assembles *KCNQ1*, is reported to be linked to the LQT11 form.[29] Most recently, we and others reported mutations in a cytoskeletal protein syntrophin-α1 (*SNTA1*), which interacts with the cardiac sodium channel, thus resulting in an LQT3-like phenotype (LQT12).[30,31] At least some cases of sudden infant death syndrome (SIDS) are attributable to congenital LQTS.[35] Mutations in *SCN5A*,[35,36] *CAV3*,[37] *KCNQ1*[38] and *KCNH2*[38] are reported to be associated with SIDS. As a common mechanism, decreases in the outward potassium currents (I_{Ks}, I_{Kr}, I_{K1}) or increases in the inward sodium or calcium current (late I_{Na}, $I_{Ca\text{-}L}$) prolong the action potential duration (APD), resulting in prolongation of the QT interval, a common phenotype in LQTS in all 12 genetic forms (Fig 2). Among the 12 forms, the LQT1 and LQT2 syndromes are the most common genetic variants, and each accounts for approximately 40% of genotyped patients; LQT3 syndrome accounts for approximately 10% of genotyped patients.[23] Because the LQT1, LQT2 and LQT3 syndromes constitute more than 90% of genotyped patients with LQTS, the genotype–phenotype correlation has been rigorously investigated, and enables stratification of risk and effective treatment of genotyped patients with these 3 major forms.[23]

Autosomal recessive forms (JLN1 and JLN2) of the Jervell & Lange-Nielsen syndrome are associated with neurosensory deafness and generally more prominent QT prolongation and more severe ventricular arrhythmias compared with the autosomal dominant forms of the Romano-Ward syndrome.[39] JLN1 and JLN2 are reported to be responsible for homozygous or compound heterozygous mutations in the *KCNQ1* and/or *KCNE1* genes. Approximately 8% of LQTS patients carries homozygous or compound heterozygous mutations in 2 LQTS-causing genes, and are reported to have longer QTc and 3.5-fold more risk of cardiac arrest.[40] On the other hand, an autosomal recessive form of LQT1 syndrome without neurosensory deafness was reported by Priori et al.[41] Some single mutations in the *SCN5A* gene are reported to cause multiple phenotypes, such as BrS, sick sinus syndrome, and conduction disease, in addition to the LQT3 phenotype.[42,43]

Genotype–Phenotype Relationships in Congenital LQTS

T-Wave Morphology on the ECG A series of experimental studies using arterially-perfused canine wedge preparations have revealed that intrinsic transmural electrical heterogeneity of ventricular repolarization from the epicardial, mid-myocardial to the endocardial cells contributes to ST-T morphology and the QT interval on ECG, especially in the left precordial ($V_{4\text{–}6}$) leads, which are thought to reflect the potentials of the left ventricular anterolateral wall.[44–46] Under normal conditions, repolarization of the epicardial action potential occurs first, coinciding with the peak of the normal T wave, whereas repolarization of the longest action potential in the mid-myocardial layer coincides with the end of the T wave.[44] Repolarization of endocardial cells usually occurs between repolarization of the epicardial and mid-myocardial cells.[44] The amplified transmural electrical heterogeneity of ventricular repolarization associated with differential modification of ionic currents in each cell type, which is caused by mutations in each LQTS gene, results in genotype-specific T-wave morphology on the ECG.[44,45] Moss et al first proposed genotype-specific T-wave morphology in genotyped patients with the LQT1, LQT2 and LQT3 forms in 1995.[47] Broad-based, prolonged T waves are more commonly observed in LQT1 syndrome; low-amplitude T waves with a notched or bifurcated configuration are more frequently observed in LQT2; and late-appearing T waves with a prolonged isoelectric ST-segment are more specific in LQT3 syndrome. The genotype-specific T-wave pattern was further evaluated by Zhang et al in 2000, and numerous exceptions are reported for all 3 genotypes.[48]

Natural History Zareba et al suggested a higher cumulative probability of cardiac events in LQT1 and LQT2 patients than in LQT3 patients.[49] More than 50% of patients