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# Identification of a *KCNQ1* Polymorphism Acting as a Protective Modifier against Arrhythmic Risk in Long QT Syndrome

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## Abstract

**Background—**Long-QT Syndrome (LQTS) is characterized by such striking clinical heterogeneity, that even among family members carrying the same mutation, clinical outcome can range between sudden death to no symptoms. We investigated the role of genetic variants as modifiers of risk for cardiac events in LQTS patients.

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**Methods and Results—**In a matched case-control study including 112 LQTS patient duos from France, Italy and Japan, 25 polymorphisms were genotyped based on either their association with QTc duration in healthy populations or on their role in adrenergic responses. The duos were composed of two relatives harboring the same heterozygous *KCNQ1* or *KCNH2* mutation; one with cardiac events and one asymptomatic and untreated. The findings were then validated in two independent founder populations totaling 174 symptomatic and 162 asymptomatic LQTS patients, and a meta-analysis was performed. The *KCNQ1* rs2074238 T-allele was significantly associated with a decreased risk of symptoms 0.34 [0.19 – 0.61] ($p<0.0002$) and with shorter QTc ($p<0.0001$) in the combined discovery and replication cohorts.

**Conclusions—**We provide evidence that the *KCNQ1* rs2074238 polymorphism is an independent risk modifier with the minor T-allele conferring protection against cardiac events in LQTS patients. This finding is a step toward a novel approach for risk stratification in LQTS patients.

### Keywords

genetics; association studies; long QT syndrome; risk factor; polymorphism; ion channel

## Introduction

Long-QT syndrome (LQTS) is an uncommon hereditary cardiac disease characterized by delay in ventricular repolarization leading to a prolongation of the QT interval on ECG. The most frequent forms are autosomal dominant. The majority of genotype-positive patients are carriers of private heterozygous mutations in the genes *KCNQ1* (LQT1, 40–50%) and *KCNH2* (LQT2, 35–45%) encoding the alpha subunits of potassium channels[1].

One of the most puzzling features of LQTS is the heterogeneity of clinical manifestations within family members who carry the same mutation[1]. The phenotypic differences have been explained so far in terms of variable penetrance[2,3] but this is too vague an explanation. Accordingly, the presence of common genetic variants (polymorphisms) acting as modifiers and imprecisely but commonly referred to as “modifier genes” has been postulated.

While many mutation carriers are symptomatic, with syncope, cardiac arrest (CA) or sudden cardiac death (SCD), others remain asymptomatic even without treatment. The inter-familial phenotypic variability can be associated with the nature of the mutations (allelic heterogeneity) and the disease-causing genes (locus heterogeneity). However, variable disease expression is observed even among patients carrying an identical mutation[2, 3]. This intra-familial variability, which cannot be explained by mutational heterogeneity, emphasizes the important role of modifying and triggering factors in the clinical phenotype[4–8].

For LQTS patients, risk of cardiac events (CEs) is significantly correlated with the extent of the QT interval prolongation[9,10]. Due to its dependence on heart rate, the QT interval needs to be corrected by heart rate (QTc). QTc is a complex quantitative trait with estimated genetic heritability of approximately 30–50%[11]. Recent genome wide association studies (GWAS)[12–14] and other investigations[15,16] have shown that genetic variants in different genes, including those coding for ionic channels (*KCNQ1*, *KCNH2*, *KCNE1*, *KCNJ2* and *SCN5A*), or the neuronal nitric oxide synthase 1 regulator, CAPON (*NOS1AP*), can modulate QTc in healthy populations.

Individual sensitivity to sympathetic activation is another risk factor for CEs and, especially for LQT1 patients, most CEs occur under physical and emotional stress[17]. It has been suggested that functional polymorphisms in genes encoding adrenergic receptors (*ADRB1*,

*ADRB2* and *ADRA2C*) may contribute to an increasing arrhythmic risk in Finnish and South African LQT1 founder populations[5,7,18].

It was on this background that we postulated that single nucleotide polymorphisms (SNPs) influencing either QTc duration or adrenergic responses might contribute to explain the striking clinical heterogeneity observed in LQTS, even within patients harboring the same mutation. Accordingly, we have assessed a cohort of patients from France, Italy and Japan, with known heterozygous LQT1 or LQT2 mutations, to address whether polymorphisms act as genetic modifiers of clinical severity, by either increasing or reducing the risk of CE. As most of the previous studies were performed in Caucasians, we also investigated whether the same polymorphisms were involved in Caucasians and Japanese LQTS patients.

To validate the results obtained in this cohort, we selected the SNPs associated with arrhythmic risk or QTc duration for replication in two additional independent and well described founder populations from South Africa and Finland[19,20].

## Methods

### Study population and inclusion criteria

**Discovery cohort—**This study involved patients with clinically and molecularly diagnosed long-QT syndrome (LQT1 or LQT2) and represented a collaborative project comprising French, Italian and Japanese referral centers. LQTS probands, mainly symptomatic patients, and their relatives, were referred to our laboratories for genetic evaluation and enrolment in our study. The smallest pedigrees are nuclear families with first-degree relatives, which included the LQTS parent and his or her LQTS offspring. The largest kindreds are families with several generations. From the clinical data of LQTS families, we selected duos composed of two relatives harboring the same LQT1 or LQT2 heterozygous mutation; one of whom experienced CEs whereas the other was asymptomatic. One family with two heterozygous mutations was included in the study since both symptomatic and asymptomatic patients were carriers of both mutations.

Patients had undergone a clinical evaluation and cardiovascular examination, including a 12-lead ECG and 24-h Holter recording. Long-QT syndrome was diagnosed on the basis of a Schwartz score ≥3.5[21] and a positive molecular screening. CEs were syncope (fainting spells with temporary but complete loss of consciousness), aborted cardiac arrest (requiring resuscitation) and SCD. Symptomatic patients are considered those who experienced one or more CEs before age 35. Patients with a first event after age 35 or with a drug-induced event were excluded from the study. Most LQTS mutation carriers, even when still asymptomatic, are currently treated with beta-blockers; given their overall high efficacy in preventing CE[22], it is impossible to know whether these individuals would have remained asymptomatic without therapy. Accordingly, we included in the study only asymptomatic mutation carriers older than 35 years and without therapy or treated after age 35. Only families composed of patients symptomatic and asymptomatic according to these criteria were included. When several family members were available, we selected those most closely related, who would be expected to have fewer genetic differences. Moreover, when it was possible, we selected family members of the same sex. Thus, we selected 112 duos. Population characteristics (age, gender, ethnicity, ECG parameters, treatment and history of CE) have been collected (Table 1). In total there were 56 French, 15 Italian and 41 Japanese duos. All patients and family members taking part in this study gave their informed consent for the genetic study which was approved by the ethics committee.

## Replication populations

Two independent LQT1 founder populations were used to validate the findings obtained in the discovery cohort. Founder populations, characterized by a single ancestor affected by LQTS and by a large number of individuals and families who are all related to the ancestor and thereby carry the same disease-causing mutation, represent a powerful human model for studying the role of "modifier genes" in LQTS[23]. As differences in clinical severity cannot be attributed to different underlying mutations, the most likely contributing factors are "modifier genes". The South African *KCNQ1*-Ala341Val (SA-LQT1) founder population is characterized by unusual clinical severity, with 79% symptomatic mutation-carriers and a mean age at first CEs of six years. Furthermore 17% of the symptomatic subjects suffered cardiac arrest/sudden cardiac death[19]. The genotyped population was composed of 111 symptomatic and 41 asymptomatic LQTS patients.

The Finnish *KCNQ1*-Gly589Asp (Fin-LQT1) founder population represents the most common autosomal dominant LQTS allele in Finland with 80 multigenerational families[20]. At variance with the SA-LQT1 population, only 30% of the mutation carriers were symptomatic for CEs[20]. There were 63 symptomatic and 121 asymptomatic genotyped LQTS patients in this population.

These founder populations were previously described in detail[19,20] and the definition of CEs, symptomatic or asymptomatic status, was essentially the same as that of the discovery cohort.

## Selection and genotyping of studied polymorphisms

On the basis of recently reported association studies, we selected 25 polymorphisms described as influencing either QTc in healthy populations (21 SNPs) or adrenergic responses (2 SNPs and 2 deletions). We chose polymorphisms that gave the most robust and reproducible results and that were not in strong pairwise linkage disequilibrium (LD) ($r^2$<0.6) in European populations, based on data available in the literature and databases (Table 2 and Supplemental Table 1). SNPs found to be associated with clinical status or QTc in the discovery cohort were then investigated in two additional independent replication populations.

Polymorphisms were genotyped in the discovery cohort by using either HRM (High Resolution Melting) with an unlabeled probe, Tm shift primers, FRET (Fluorescence Resonance Energy Transfer), or Taqman assays (Applied Biosystems) on a LightCycler® 480 System (Roche), or by sequencing on a ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). All selected polymorphisms were also directly sequenced in a subset of 23 DNA samples. Genotyping by any of these methods and by direct sequencing yielded identical results. Six internal controls with known genotypes were systematically introduced into each 96-well plate. In the SA-LQT1 population, polymorphisms were genotyped by Taqman assay on 7900 HT Fast Real Time PCR System (Applied Biosystem) and in a subset of samples direct sequencing was performed as well, with the same results. In the Finnish LQT1 population, genotyping was performed using the Sequenom iPLEX Gold assay (MALDI-TOF mass spectrometry, MassARRAY Analyzer Compact, Sequenom Inc.). Primers and methods used for genotyping are listed in Supplemental Tables 2 and 3.

## Statistical analysis

Deviation from the Hardy-Weinberg equilibrium (HWE) was evaluated by an exact test separately in symptomatic and asymptomatic patients. We calculated pairwise LD statistics ($r^2$) using the Haploview© program. Allele frequencies were estimated by gene-counting separately in each population and sub-group (asymptomatic and symptomatic). We

normalized QTc by normal quantile transformation and used this z-transformed variable for analysis[24]. As QTc is influenced by age[25] and gender[26], we included these two covariates in the standard linear regression model to generate residuals. Next, we used these multivariate-adjusted residuals for the z-transformed variable to test for potential association with polymorphisms, clinical status (symptomatic versus asymptomatic) or severity of CEs (CA and/or SCD versus syncope). We also adjusted for ethnicity (Caucasian or Japanese) and we used residuals (QTc adjusted for age and gender) as co-variable to test for association between SNP and clinical status whenever appropriate. Comparison of age at the first CEs among symptomatic subgroups was performed by unpaired Student's t-test and log-rank test. Logistic regression was used to test the effect of QTc on severity of CEs among symptomatic patients. The association of polymorphisms with the binary clinical status, i.e. asymptomatic versus symptomatic, or with the quantitative QTc trait was assessed by use of the generalized estimating equations (GEE) technique to account for correlation among related individuals[27]. In these GEE analyses, the so-called "working independence matrix" was used. For the binary trait analysis in the discovery cohort, the GEE results were compared to those obtained from a conditional logistic regression analysis that dealt with the matched "case-control" design of our family duos study. Both methods yielded similar results (data not shown). In the founder populations, GEE results obtained from the "working independence matrix" were compared to those obtained from a working correlation matrix derived from the kinship coefficients between individuals. Both analyses led to comparable results (data not shown). Only results derived from the "working independence matrix" were reported thereafter.

Because of the size of our cohort, association analyses were conducted under the dominant model. Odds Ratios (OR) with 95% confidence intervals were calculated. One-sided p-values were reported under the hypotheses that polymorphisms which prolong QTc or increase response to adrenergic stress are associated with a higher risk of CEs, and that polymorphisms which diminish QTc or decrease response to adrenergic stress are associated with a lower risk of CEs. P-values were corrected for multiple testing using the Bonferroni procedure and those lower than $2.10^{-3}$ (=0.05/25) were considered statistically significant in the discovery cohort. The GEE method was also used to perform analyses in the two founder populations. Results obtained in each population were combined in a meta-analysis. Based on the different design of the three populations studied, random-effect meta-analysis relying on the inverse-variance weighting was conducted. Homogeneity of associations across the discovery cohort and the two replication populations was evaluated using the Cochran's Q test method. All analyses were carried out using R statistical software version 2.12.1 except where indicated.

## Results

### Study discovery population

A total of 60 LQT1 (53.6%) and 51 LQT2 (45.5%) duos, as well as one family with two heterozygous mutations (LQT1 and LQT2), were included (Table 1). There were 107 males (47.8%) and 117 females (52.2%). The mean QTc interval was longer in females (483±44 vs. 470±49 ms, p=0.01). The mean QTc interval of Caucasian (French and Italian) and Japanese patients was almost identical (477±43 and 476±53 ms, p=0.59). Symptomatic patients had a mean age at the first CEs of 13±7 years, and 11.6% suffered CA and/or SCD (n=13) whereas the remaining 88.4% had only syncope (n=99).

The mean age at the first CEs was lower in males than in females (11±6 vs. 15±7 years, p<0.0007 for t-test and p<0.0002 for log-rank test) and in LQT1 than in LQT2 patients (12±7 vs. 16±7 years, p<0.003 for t-test and p=0.01 for log-rank test) as illustrated by the Kaplan-Meier curves (Supplemental Figure 1).

### Association between QTc and clinical status in the discovery population

As expected, QTc was longer in symptomatic than in asymptomatic LQTS mutation carriers (491±49 vs. 462±40 ms; p<0.007). Patients who experienced CA or SCD were prone to have a QTc longer than other symptomatic mutation carriers (501±58 vs. 489±48 ms) but the association failed to reach statistical significance (p=0.12).

### Polymorphism studies in the discovery population

We observed a weak deviation from HWE for rs10494366 in Caucasian patients (p=0.02 for asymptomatic and p=0.04 for symptomatic patients) and for four polymorphisms in the Japanese asymptomatic group (p=0.02 for rs1805124, p=0.03 for rs17779747 and rs10919071, and p=0.04 for rs3778873). However, after Bonferroni correction for multiple testing, these deviations were no longer statistically significant.

### Polymorphism frequencies and linkage disequilibrium

Allele frequencies for all studied polymorphisms in Caucasian and Japanese LQTS patients are shown in Supplemental Table 4. As reported in databases, allele frequencies of some polymorphisms were quite different between ethnic groups (Supplemental Tables 1 and 4). As expected from our SNP selection, no strong pairwise LD between polymorphisms was observed in Caucasian or Japanese populations (Supplemental Figure 2).

### Association between polymorphisms and clinical status

Allele frequency distributions were similar between the symptomatic and the asymptomatic groups except for the *KCNQ1* rs2074238 polymorphism (Supplemental Table 4). Indeed, the *KCNQ1* rs2074238-T allele was significantly less frequent in symptomatic than in asymptomatic LQTS patients (0.04 vs. 0.10 in Caucasians, p<0.002) and associated with a protective Odds Ratio (OR) of 0.38 [0.19–0.73] (Table 3 and Supplemental Tables 4 and 5). This effect, present in the European patients, was not observed in the Japanese cohort as this SNP was not polymorphic.

### Association between polymorphisms and QTc

None of the studied SNPs demonstrated significant (at the Bonferroni corrected threshold) association with QTc duration (data not shown). Nevertheless, two SNPs, rs12029454 and rs2074238, showed suggestive evidence of association with p<0.05. The strongest association (p=0.006) with QTc duration was observed for the *NOS1AP* rs12029454 where carriers of the A allele had a longer QTc than non-carriers (484±50 vs. 471±44 ms) (Table 4 and Supplemental Table 6). Additionally, the *KCNQ1* rs2074238 T-allele tended to be associated with a shorter QTc than non T-carriers (464±35 vs. 479±44 ms, p=0.04) (Table 4). Of note, after adjustment for QTc, the effect of rs2074238 on the risk of CEs was barely modified (OR=0.36 [0.17–0.78], p=0.005). These two SNPs were then passed to the replication study.

### Replication study

We further investigated the associations observed for *KCNQ1* rs2074238 with CEs and QTc and that of *NOS1AP* rs12029454 with QTc in two additional independent founder populations, one from South Africa (SA) and the other from Finland (Fin).

In the SA-LQT1 founder population, the *KCNQ1* rs2074238 T-allele was less frequent in symptomatic than in asymptomatic individuals (0.01 vs. 0.06) and associated with a protective OR of 0.20 [0.09–0.44], p<0.0001 (Table 3). This association was consistent with what observed in the discovery samples. The same trend of association was observed in the Finnish population (OR=0.78 [0.26 – 2.34]) even though it did not reach statistical

significance (Table 3). The meta-analysis of the three studies suggested moderate[28] ($I^2$=0.51) but not significant (p=0.13) heterogeneity in the *KCNQ1* rs2074238 association with CEs risk. The resulting overall association was significant (OR=0.36 [0.18 – 0.71], p<0.002).

The association of rs2074238 with QTc was replicated in the two additional populations. In both South African and Finns, carriers of the rs2074238 T-allele demonstrated shorter QTc than non-carriers (467±23 vs. 488±43 ms, p=0.008and 445±31 vs. 465±34, p=0.003, respectively) (Table 4). The meta-analysis of these two populations showed significant association (p<0.0002), and furthermore, performing meta-analysis of these two populations together with the discovery samples demonstrated an overall statistical evidence of p<0.0001 for the association of rs2074238 with QTc, without evidence for heterogeneity ($I^2$=0, p=0.78).

After adjustment for QTc, the combined OR for CEs associated with the rs2074238 T-allele derived from the meta-analysis of the three studies was 0.34 [0.19 – 0.61] (p<0.0002).

In both SA-LQT1 and Finnish replication samples, carriers of the minor *NOS1AP* rs12029454-A allele tended to have longer QTc compared to non-carriers, 501±63 vs. 483±37 and 468±37 vs. 459±32, respectively, consistent with what observed in our discovery cohort, and the meta-analysis of the two replication populations led to a p value of 0.05 (Table 4). When the meta-analysis was performed on the three populations, there was no evidence for heterogeneity ($I^2$=0, p=0.87) while there was an association of rs12029454 with QTc (p<0.002).

## Discussion

The present study provides strong evidence that the *KCNQ1* rs2074238-T allele is associated with a decreased risk for cardiac events among patients affected by LQTS. This SNP had been previously identified as modulating QTc duration in healthy individuals, Europeans or of European descent[13,29,30], which is also the case in our LQTS population.

The correct identification, within LQTS family members carrying the same mutation, of those at higher or lower risk for life-threatening CEs is still a daunting problem for the physicians involved. The rs2074238 SNP could contribute to explain some of the puzzling phenotypic variability among LQTS patients and might improve the cardiac risk prediction already provided by QTc, gender, age or type of disease-causing mutation[9,10,31–33]. This finding may thus impact on clinical management and, together with the SNPs recently reported to increase arrhythmic risk[5–8,34,35], represents a significant step marking the progressive transition from gene-specific[9], to mutation-specific[31,32], and now toward SNP-specific risk stratification: a truly exciting evolution of genotype-phenotype correlation studies.

### Genetic findings

The association between rs2074238 and CEs, which was restricted to Caucasian patients in the discovery cohort, remained significant after correction for multiple testing and was replicated in the SA-LQT1 founder population, as well as in the meta-analysis. The same trend of association was observed in the Fin-LQT1 population, but failed to reach the 0.05 significant threshold. The lack of replication in this population could have been predicted because of the expected “ceiling effect”, i.e. if the disease-causing mutation is associated with few events it becomes very difficult to demonstrate a statistically significant protective effect, unless thousands of patients are studied. As this SNP was not polymorphic in the Japanese LQTS patients the association with CEs in a non-Caucasian population could not

be tested. Due to the low prevalence of this SNP among our LQT2 patients it seems prudent to say that this protective effect is primarily related to the LQT1 patients. Since the rs2074238 T-allele has a frequency of 6.5% in Caucasians, it is predicted to influence phenotypic differences in less than 10% of LQTS patients.

In addition to its clear protective effect (i.e. lower arrhythmic risk), we observed that the minor *KCNQ1* rs2074238-T allele was associated with shorter QTc in the three investigated cohorts. We also showed that its protective effect on cardiac event risk was not only due to its impact on QTc duration.

There are insufficient data available to postulate a mechanism underlying our findings. One reasonable consideration stems from the fact that *KCNQ1* rs2074238 (c.386+18089C>T) is located in intron 1 which shows only modest linkage disequilibrium ($r^2<0.50$) with known *KCNQ1* SNPs[36]; the Encyclopedia of DNA Elements (ENCODE) project[37] and the RegulomeDB variant classification[38] provide strong support for a functional role for rs2074238 in regulating *KCNQ1* expression. Further experimental data, including quantitative expression analyses, are needed to test this possibility and to clarify the underlying mechanism(s). After confirmation of a functional role for rs2074238 in regulating *KCNQ1* expression, it would be of interest to assess whether this effect could depend on whether the rs2074238-T allele is on the same haplotype as the LQT1 mutated allele (cis-effect) or associated with the non-mutated allele (trans-effect), as recently reported for SNPs located in *KCNQ1* 3′UTR[35].

We found another SNP, *NOS1AP* rs12029454, known for influencing QTc in the general population, which showed suggestive evidence of association with longer QTc duration in our LQTS discovery and validation cohorts. The importance of NOS1AP in modulating the QTc is well recognized in healthy subjects[12–14,29,30], as well as in LQTS patients[8,34].

## Study design

To eliminate the otherwise unavoidable bias introduced by studying different LQTS mutations with varying severity, we used two different approaches: 1) the use of duos sharing the same disease-causing mutations; 2) the use of founder populations. The second approach has already been validated in previous studies. Indeed, in the SA-LQT1 population carrying the *KCNQ1*-A341V founder mutation, we demonstrated that some common polymorphisms in *NOS1AP* increased the clinical severity and QTc duration[8]. Similarly, rs1805128 in *KCNE1* has been associated with QTc prolongation in 712 LQT1 or LQT2 Finnish founder mutation carriers derived from 126 LQTS families[39]. These results were observed in ethnically isolated founder populations from South Africa and Finland, an unusual situation given that most LQT1 or LQT2 patients carry private mutations. Studies on mixed populations are therefore also important to determine whether the modifying effect of certain SNPs could apply to different LQTS mutations, and not be restricted to a specific one. Such a study on three *NOS1AP* SNPs was conducted in 901 Caucasian LQTS patients from 520 families[34] and essentially confirmed the results reported in the South African LQTS founder population[8]. Thus, findings in founder populations, which are particularly suitable to identify modifier genes, can be translated to the general LQTS population with a reasonable degree of confidence.

This is the first study to test the effect of a large number of polymorphisms on phenotypic variability and QTc interval duration in LQTS patients selected for representing both asymptomatic and symptomatic carriers of the same mutation within each family, thus constituting a matched case-control study. In this way we avoided the confounding effects of genetic and allelic heterogeneity that is present whenever a study involves variable numbers

of patients with multiple disease-causing mutations, each associated with different cardiac risk.

### Comparison with published data

Of the several polymorphisms previously shown to affect QTc duration in large healthy populations[12–16,29,30] only two had a similar effect in our LQTS cohorts. This should not be surprising, because while a modest prolonging effect could be easily demonstrable in individuals with a normal QTc, such a small change is likely to be overshadowed by the major effect of the underlying disease-causing mutation. In other words, a 5–10 ms prolongation which may be noticed in populations with a normal QT interval and a modest standard deviation (usually around 20 ms) would be totally lost in populations, like ours, with much longer mean QTc and a much greater standard deviation (around 45 ms). Consequently, when powers were calculated using QUANTO software (http://hydra.usc.edu/gxe) to reproduce the effect of the various SNPs on QT prolongation, they ranged from 7 to 21% in the discovery cohort (Supplemental Table 7).

The SNPs within the adrenergic receptors genes *ADRB1* (rs1801252 and rs1801253)[5,7], *ADRA2C* (rs61767072)[5], and rs1805128 on *KCNE1*[39], previously associated with arrhythmic risk in LQTS patients, were not validated in the present cohort. However, the rare T-allele of *KCNE1* rs1805128 tended to be more frequent in Caucasian symptomatic patients (0.05 vs. 0.01; OR=7.66 [1.21–48.31], p=0.02) as previously observed (Supplemental Table 5). Among *NOS1AP* SNP**s**, only rs12029454 was associated with QT prolongation, while no association was identified with cardiac events in the discovery cohort. Thus, we did not replicate the previously reported association with the occurrence of cardiac events for rs4657139[8,34], rs16847548[8] (in strong linkage disequilibrium with rs12143842 in Europeans, $r^2>0.8$) or rs10494366[34] This lack of statistical replication could be due to the size of the population, the rarity of some variants, and thus insufficient power (Supplemental Table 7), but also to populations specificities, differences in genetic or environmental background. Differences in the clinical phenotypes could also contribute. For instance, the findings on the adrenergic receptor genes were obtained in patients selected on the basis of QTc changes during an exercise stress test[7] and on the distribution of baroreflex sensitivity[5]; while for the *KCNE1* polymorphism the association identified was limited to male patients[39]. It is likely that the effect of several SNPs on patient QT interval or cardiac risk are gene, mutation or allele-dependent [9,31,32,35]. In addition, the risk is dependent on the ratio of SNP alleles with opposing effects, for example reducing or favoring QT prolongation, and more complex stratifications should be performed in the future when those SNPs with the greatest effects will be progressively validated.

## Conclusions

The present demonstration of the existence of a common genetic variant capable of decreasing the risk for life-threatening arrhythmias among patients affected by the long QT syndrome represents a significant step in the never ending quest for a more thorough understanding of the complex relationship between genotype and phenotype.

Much progress has been made during the last 10–15 years and now our new findings suggest that we are moving toward a phase in which risk stratification will be further refined by identifying a cluster of polymorphisms capable of modifying the mutation-specific risk in either direction. This phase will require the identification of several other polymorphisms modifying clinical outcome. The impact on clinical management will not be immediate, but there is little doubt that a significant refinement in the strategy for risk stratification for LQTS is well under way.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Table 1

Characteristics of all LQTS mutation carriers from the discovery cohort

| | Caucasian | Japanese | All |
|---|---|---|---|
| LQT1 | 84 (59.2%) | 36 (43.9%) | 120 (53.6%) |
| LQT2 | 56 (39.4%) | 46 (56.1%) | 102 (45.5%) |
| LQT1+LQT2 | 2 (1.4%) | - | 2 (0.9%) |
| Female | 74 (52.1%) | 43 (52.4%) | 117 (52.2%) |
| Symptomatic | 39 (52.7%) | 26 (60.5%) | 65 (55.6%) |
| Male | 68 (47.9%) | 39 (47.6%) | 107 (47.8%) |
| Symptomatic | 32 (47.1%) | 15 (38.5%) | 47 (43.9%) |
| Asymptomatic/Symptomatic gender | | | |
| Female/Female | 18 (25.4%) | 9 (22.0%) | 27 (24.1%) |
| Male/Male | 15 (21.1%) | 7 (17.1%) | 22 (19.6%) |
| Female/Male | 17 (23.9%) | 8 (19.5%) | 25 (22.3%) |
| Male/Female | 21 (29.6%) | 17 (41.5%) | 38 (33.9%) |
| Age at first ECG (years) | | | |
| Asymptomatic | 49 ± 12 | 48 ± 10 | 48 ± 11 |
| Symptomatic | 19 ± 12 | 18 ± 9 | 19 ± 11 |
| Cardiac events | | | |
| Syncope | 58 (81.7%) | 41 (100%) | 99 (88.4%) |
| Cardiac arrest and/or sudden cardiac death | 13 (18.3%) | - | 13 (11.6%) |
| Age at the time of the first cardiac event (years) | | | |
| All symptomatic patients | 14 ± 8 | 13 ± 5 | 13 ± 7 |
| Female | 17 ± 9 | 13 ± 5 | 15 ± 7 |
| Male | 11 ± 7 | 11 ± 5 | 11 ± 6 |
| LQT1 | 12 ± 8 | 11 ± 5 | 12 ± 7 |
| LQT2 | 17 ± 8 | 14 ± 5 | 16 ± 7 |
| QTc (ms) | | | |
| Female | 480 ± 39 | 502 ± 50 | 483 ± 44 |
| Male | 473 ± 47 | 496 ± 56 | 470 ± 49 |

Data presented as n (%) or mean ± SD

## Table 2

Selected polymorphisms

| Gene | rs number | Location | Amino acid change | Tested effect* |
|---|---|---|---|---|
| *ADRA2C* | rs61767072 | exon | p.Gly322_Pro325del | at risk |
| *ADRB1* | rs1801252 | exon | p.Ser49Gly | protective |
| *ADRB1* | rs1801253 | exon | p.Arg389Gly | protective |
| *ADRB2* | rs28365031 | exon | p.Glu307_Glu309dup | at risk |
| *ATP1B1* | rs10919071 | intron | - | protective |
| *KCNE1* | rs1805128 | exon | p.Asp85Asn | at risk |
| *KCNH2* | rs1805123 | exon | p.Lys897Thr | protective |
| *KCNH2* | rs3778873 | intron | - | protective |
| *KCNH2* | rs3807375 | intron | - | at risk |
| *KCNH2* | rs3815459 | intron | - | at risk |
| *KCNJ2* | rs17779747 | 3′downstream | - | protective |
| *KCNQ1* | rs12296050 | intron | - | at risk |
| *KCNQ1* | rs2074238 | intron | - | protective |
| *KCNQ1* | rs757092 | intron | - | at risk |
| *NOS1AP* | rs10494366 | intron | - | at risk |
| *NOS1AP* | rs12029454 | intron | - | at risk |
| *NOS1AP* | rs12143842 | 5′upstream | - | at risk |
| *NOS1AP* | rs16857031 | intron | - | at risk |
| *NOS1AP* | rs4657139 | 5′upstream | - | at risk |
| *NOS1AP* | rs4657178 | intron | - | at risk |
| *CNOT3* | rs36643 | 5′near gene | - | protective |
| *PLN* | rs11970286 | 5′upstream | - | at risk |
| *PLN* | rs12210810 | 5′upstream | - | protective |
| *SCN5A* | rs12053903 | intron | - | protective |
| *SCN5A* | rs1805124 | exon | p.His558Arg | at risk |

*Minor alleles were considered at risk alleles when they increased QTc and the risk of cardiac events, or enhanced adrenergic responses; whereas they were considered as protective alleles when they decreased QTc and risk of cardiac events, or reduced adrenergic responses according to previous studies. Minor alleles were defined according to allele frequencies in Europeans.

**Table 3**

Association between SNP and clinical status in LQTS mutation carriers

| Gene | SNP | Population | Genotypic status* | Asymptomatic | Symptomatic | p-value‖ | OR [95% CI] |
|---|---|---|---|---|---|---|---|
| *KCNQ1* | rs2074238 | Discovery† | CC | 98 (87.5%) | 106 (94.6%) | $1.98\ 10^{-3}$ | 0.38 [0.19–0.73] |
| | | | CT | 14 (12.5%) | 6 (5.4%) | | |
| | | South African | CC | 36 (87.8%) | 108 (97.3%) | $3.13\ 10^{-5}$ | 0.20 [0.09–0.44] |
| | | | CT | 5 (12.2%) | 3 (2.7%) | | |
| | | Finnish | CC | 109 (90.1%) | 58 (92.1%) | 0.33 | 0.78 [0.26–2.34] |
| | | | CT | 12 (9.9%) | 5 (7.9%) | | |
| | | Meta-analysis (replication‡)§ | | - | - | 0.07 | 0.37 [0.10–1.42] |
| | | Meta-analysis (Discovery and replication‡) | | - | - | $1.68\ 10^{-3}$ | 0.36 [0.18 – 0.71] |

*‘CC’ represents the major homozygous genotype and ‘CT’ represents the heterozygous genotype

†Pooled population (Caucasian and Japanese)

‡South African and Finnish

§heterogeneity $I^2$=0.75, p=0.05

‖not corrected for multiple tests

**Table 4**

QTc duration in LQTS mutation carriers according to replicated SNPs

| | | | AA* | | Aa and aa* | | |
|---|---|---|---|---|---|---|---|
| **Gene** | **SNP** | **Population** | **QTc (ms)** | **n** | **QTc (ms)** | **n** | **p-value**** |
| *KCNQ1* | rs2074238 | Discovery† | 479±44 | 120 | 464±35 | 18 | 0.04 |
| | | South African | 488±43 | 101 | 467±23 | 6 | $8.14\ 10^{-3}$ |
| | | Finnish | 465±34 | 152 | 445±31 | 15 | $2.95\ 10^{-3}$ |
| | | Meta-analysis (replication‡)§ | 474 ± 40 | 253 | 451 ± 30 | 21 | $1.37\ 10^{-4}$ |
| | | Meta-analysis (Discovery and replication‡) | 476 ± 41 | 373 | 457 ± 33 | 39 | $3.43\ 10^{-5}$ |
| *NOS1AP* | rs12029454 | Discovery‖ | 471±44 | 125 | 484±50 | 95 | $6.08\ 10^{-3}$ |
| | | South African | 483±37 | 88 | 501±63 | 20 | 0.18 |
| | | Finnish | 459±32 | 97 | 468±37 | 70 | 0.08 |
| | | Meta-analysis (replication‡)# | 470±36 | 185 | 475±46 | 90 | 0.05 |
| | | Meta-analysis (Discovery and replication‡) | 471±40 | 310 | 483±42 | 185 | $1.46\ 10^{-3}$ |

Data presented as mean ± SD

*'AA' represents the major homozygous genotype (corresponding to 'CC' for rs2074238 and 'GG' for rs12029454) and 'Aa and aa' represents the minor allele ('T' for rs2074238 and 'A' for rs12029454) in the heterozygous or homozygous state.

†Caucasian population (rs2074238 is not polymorphic in the Japanese population)

‡South African and Finnish

§Heterogeneity $I^2$=0, p=0.74

‖Pooled population (Caucasian and Japanese)

#Heterogeneity $I^2$=0, p=0.84

**not corrected for multiple tests