Identification of a KCNQ1 Polymorphism Acting as a Protective Modifier Against Arrhythmic Risk in Long-QT Syndrome

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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 and no symptoms. We investigated the role of genetic variants as modifiers of risk for cardiac events in patients with LQTS.

Methods and Results—In a matched case–control study including 112 patient duos with LQTS 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 2 relatives harboring the same heterogeneous KCNQ1 or KCNH2 mutation: 1 with cardiac events and 1 asymptomatic and untreated. The findings were then validated in 2 independent founder populations totaling 174 symptomatic and 162 asymptomatic patients with LQTS, 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 patients with LQTS. This finding is a step toward a novel approach for risk stratification in patients with LQTS. (Circ Cardiovasc Genet. 2013;6:354-361.)

Key Words: association studies ▪ genetics ▪ ion channel ▪ long-QT syndrome ▪ polymorphism ▪ risk factor

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, 25%–30%).

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and KCNH2 (LQT2, 35%–45%) encoding the α-subunits of potassium channels.1

**Clinical Perspective on p 361**

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.

Although many mutation carriers are symptomatic, with syncope, cardiac arrest (CA), or sudden cardiac death (SCD), others remain asymptomatic even without treatment. The interfamilial 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.4,5 This intrafamilial variability, which cannot be explained by mutational heterogeneity, emphasizes the important role of modifying and triggering factors in the clinical phenotype.4,6

For patients with LQTS, risk of cardiac events (CEs) is significantly correlated with the extent of the QT interval prolongation.9,10 Because of 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 ≈30% to 50%.11 Recent genome-wide association studies12–14 and other investigations15,16 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 (SA) 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 patients of European descent, we also investigated whether the same polymorphisms were involved in European and Japanese patients with LQTS.

To validate the results obtained in this cohort, we selected the SNPs associated with arrhythmic risk or QTc duration for replication in 2 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 LQT1 (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 parent with LQTS and one or her offspring with LQTS. The largest kindreds are families with several generations. From the clinical data of families with LQTS, we selected duos composed of 2 relatives harboring the same LQT1 or LQT2 heterogeneous mutation, one of whom experienced CEs, the other of whom was asymptomatic. One family with 2 heterozygous mutations was included in the study because 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. LQTS was diagnosed on the basis of a Schwartz score ≥3.5 and a positive molecular screening. CEs were syncope (fainting spells with temporary but complete loss of consciousness), aborted CA (requiring resuscitation), and SCD. Symptomatic patients are considered those who experienced one or more CEs before 35 years of age. Patients with a first event after 35 years of age or with a drug-induced event were excluded from the study. Most LQTS mutation carriers, even when still asymptomatic, are currently treated with β-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 >35 years of age and without therapy or treated after 35 years of age. 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, sex, 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 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.21 As differences in clinical severity cannot be attributed to different underlying mutations, the most likely contributing factors are modifier genes. The SA 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 6 years. Furthermore, 17% of the symptomatic subjects experienced CA/SCD.20 The genotyped population was composed of 111 symptomatic and 41 asymptomatic patients with LQTS.

The Finnish KCNQ1-Gly589Asp (Finnish-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 patients with LQTS 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.
Polymorphisms were genotyped in the discovery cohort either by sequencing on a ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). All selected polymorphisms were also directly sequenced by using ready-made primers, fluorescence resonance energy transfer, or Taqman assays (Applied Biosystems). Single nucleotide polymorphisms were confirmed by direct sequencing yielded identical results. Six intergenic regions were genotyped by Taqman assay on 7900 HT Fast Real Time PCR System (Applied Biosystems), and in a subset of samples, direct sequencing was performed as well, with the same results. In the Finnish patients was almost identical (477±43 and 476±53 ms; P=0.01). The mean QTc interval of European (French and Italian) and Japanese patients was 473±47 ms (496±56 ms).

**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; Table I in the online-only Data Supplement). SNPs found to be associated with clinical status or QTc in the discovery cohort were then investigated in 2 additional independent replication populations.

Polymorphisms were genotyped in the discovery cohort either by using high-resolution melting with an unlabeled probe, Taqman primers, 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 intergenic regions 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 Biosystems), 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 Tables II and III in the online-only Data Supplement.

**Statistical Analysis**

Deviation from the Hardy–Weinberg equilibrium 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 subgroup (asymptomatic and symptomatic). We normalized QTc by normal quantile transformation and used this z-transformed variable for analysis. As QTc is influenced by age and sex, we included these 2 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 SCD versus syncope). We also adjusted for ethnicity (Europeans or Japanese), and we used residuals (QTc adjusted for age and sex) as covariates 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 \( t \) 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, that is, asymptomatic versus symptomatic, or with the quantitative QTc trait was assessed by use of the generalized estimating equation (GEE) technique to account for correlation among related individuals. 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 with 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 with 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 (ORs) with 95% confidence intervals were calculated. One-sided \( P \) values were reported under the hypotheses that polymorphisms that prolong QTc or increase response to adrenergic stress are associated with a higher risk of CEs, and polymorphisms that 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 2x10\(^{-5}\) (=0.05/25) were considered statistically significant in the discovery cohort. The GEE method was also used to perform analyses in the 2 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 2 replication populations was evaluated using the Cochran’s \( Q \) test method. All analyses were performed 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 1 family with 2 heterozygous mutations (LQT1 and LQT2), were included (Table 1). There were 107 men (47.8%) and 117 women (52.2%). The mean QTc interval was longer in women (483±44 versus 470±49 ms; \( P=0.01 \)). The mean QTc interval of European (French and Italian) and Japanese patients was almost identical (477±43 and 476±53 ms;
Polymorphism Studies in the Discovery Population

We observed a weak deviation from Hardy–Weinberg equilibrium for rs10494366 in European 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; \(P=0.04\) for rs3778873). However, after Bonferroni correction for multiple testing, these deviations were no longer statistically significant.

Polymorphism Frequencies and LD

Allele frequencies for all studied polymorphisms in European and Japanese patients with LQTS are shown in Table IV in the online-only Data Supplement. As reported in databases, allele frequencies of some polymorphisms were quite different between ethnic groups (Tables I and IV in the online-only Data Supplement). As expected from our SNP selection, no strong pairwise LD between polymorphisms was observed in European or Japanese populations (Figure II in the online-only Data Supplement).

Association Between Polymorphisms and Clinical Status

Allele frequency distributions were similar between the symptomatic and asymptomatic groups except for the \(KCNQ1\) rs2074238 polymorphism (Table IV in the online-only Data Supplement). Indeed, the \(KCNQ1\) rs2074238-T allele was significantly less frequent in symptomatic than in asymptomatic LQTS patients (0.04 versus 0.10 in Europeans; \(P<0.002\)) and associated with a protective OR of 0.38 (0.19–0.73; Table 3 and Tables IV and V in the online-only Data Supplement). 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, 2 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 versus 471±44 ms; Table 4 and Table VI in the online-only Data Supplement). Additionally, the \(KCNQ1\) rs2074238 T-allele tended to be associated with a shorter QTc than non-T-carriers (464±35 versus 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 2 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 2 additional independent founder populations, 1 from South Africa and the other from Finland.

In the SA-LQT1 founder population, the \(KCNQ1\) rs2074238 T-allele was less frequent in symptomatic than in asymptomatic individuals (0.01 versus 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 that observed in the discovery samples. The same trend of association was observed in the

### Table 2. Selected Polymorphisms

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs number</th>
<th>Location</th>
<th>Amino Acid Change</th>
<th>Tested Effect*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADR2C</td>
<td>rs61767072</td>
<td>Exon</td>
<td>p.Gly322_Pro325del</td>
<td>At risk</td>
</tr>
<tr>
<td>ADRB1</td>
<td>rs1801252</td>
<td>Exon</td>
<td>p.Ser496Gly</td>
<td>Protective</td>
</tr>
<tr>
<td>ADRB1</td>
<td>rs1801253</td>
<td>Exon</td>
<td>p.Arg389Gly</td>
<td>Protective</td>
</tr>
<tr>
<td>ADRB2</td>
<td>rs28365031</td>
<td>Exon</td>
<td>p.Glu307_Glu309dup</td>
<td>At risk</td>
</tr>
<tr>
<td>ATP1B1</td>
<td>rs10919071</td>
<td>Intron</td>
<td>...</td>
<td>Protective</td>
</tr>
<tr>
<td>KCNE1</td>
<td>rs1805128</td>
<td>Exon</td>
<td>p.Asp85Asn</td>
<td>At risk</td>
</tr>
<tr>
<td>KCNH2</td>
<td>rs1805123</td>
<td>Exon</td>
<td>p.Lys897Thr</td>
<td>Protective</td>
</tr>
<tr>
<td>KCNH2</td>
<td>rs3778873</td>
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<td>...</td>
<td>At risk</td>
</tr>
<tr>
<td>KCNH2</td>
<td>rs3807375</td>
<td>Intron</td>
<td>...</td>
<td>At risk</td>
</tr>
<tr>
<td>KCNH2</td>
<td>rs3815459</td>
<td>Intron</td>
<td>...</td>
<td>At risk</td>
</tr>
<tr>
<td>KCNJ2</td>
<td>rs1777947</td>
<td>3’-Downstream</td>
<td>...</td>
<td>Protective</td>
</tr>
<tr>
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<td>Intron</td>
<td>...</td>
<td>At risk</td>
</tr>
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<td>rs2074238</td>
<td>Intron</td>
<td>...</td>
<td>Protective</td>
</tr>
<tr>
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<td>Intron</td>
<td>...</td>
<td>At risk</td>
</tr>
<tr>
<td>NOS1AP</td>
<td>rs10494366</td>
<td>Intron</td>
<td>...</td>
<td>At risk</td>
</tr>
<tr>
<td>NOS1AP</td>
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<td>Intron</td>
<td>...</td>
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</tr>
<tr>
<td>NOS1AP</td>
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<td>5’-Upstream</td>
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<td>At risk</td>
</tr>
<tr>
<td>NOS1AP</td>
<td>rs16857031</td>
<td>Intron</td>
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<td>At risk</td>
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<tr>
<td>NOS1AP</td>
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<td>5’-Upstream</td>
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<td>rs4657178</td>
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<td>Protective</td>
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</tr>
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</tr>
<tr>
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<td>Protective</td>
</tr>
<tr>
<td>SCN5A</td>
<td>rs1805124</td>
<td>Exon</td>
<td>p.His558Arg</td>
<td>At risk</td>
</tr>
</tbody>
</table>

*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 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.

\(P=0.59\). Symptomatic patients had a mean age at the first CE of 13±7 years, and 11.6% experienced CA and SCD (n=13), whereas the remaining 88.4% had only syncope (n=99).

The mean age at the first CE was lower in men than in women (11±6 versus 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 versus 16±7 years; \(P<0.003\) for \(t\) test and \(P=0.01\) for log-rank test) as illustrated by the Kaplan–Meier curves (Figure I in the online-only Data Supplement).

Association Between QTc and Clinical Status in the Discovery Population

As expected, QTc was longer in symptomatic than in asymptomatic individuals (491±49 versus 462±40 ms; \(P<0.007\)). Patients who experienced CA or SCD were prone to have a QTc longer than in other symptomatic mutation carriers (501±58 versus 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 Hardy–Weinberg equilibrium for rs10494366 in European patients (\(P=0.02\) for
Finnish population (OR, 0.78 [0.26–2.34]), although it did not reach statistical significance (Table 3). The meta-analysis of the 3 studies suggested moderate ($I^2=0.51$) but not significant ($P=0.13$) heterogeneity in the $KCNQ1$ rs2074238 association with CE 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 2 additional populations. In both SA and Finnish, carriers of the rs2074238 T-allele demonstrated shorter QTc than noncarriers (467±23 versus 488±43 ms, $P=0.008$; 445±31 versus 465±34 ms, $P=0.003$, respectively) (Table 4). The meta-analysis of these 2 populations showed significant association ($P<0.0002$). Furthermore, performing meta-analysis of these 2 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 3 studies was 0.34 (0.19–0.61; $P<0.0002$).

### Table 3. Association Between SNP and Clinical Status in LQTS Mutation Carriers

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Population</th>
<th>Genotypic Status</th>
<th>Asymptomatic</th>
<th>Symptomatic</th>
<th>$P$ Value$§$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$KCNQ1$</td>
<td>rs2074238</td>
<td>Discovery*</td>
<td>CC</td>
<td>98 (87.5%)</td>
<td>106 (94.6%)</td>
<td>1.98×10$^{-3}$</td>
<td>0.38 (0.19–0.73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CT</td>
<td>14 (12.5%)</td>
<td>6 (5.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>South African</td>
<td>CC</td>
<td>36 (67.8%)</td>
<td>108 (97.3%)</td>
<td>3.13×10$^{-4}$</td>
<td>0.20 (0.09–0.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CT</td>
<td>5 (12.2%)</td>
<td>3 (2.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Finnish</td>
<td>CC</td>
<td>109 (90.1%)</td>
<td>58 (92.1%)</td>
<td>0.33</td>
<td>0.78 (0.26–2.34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CT</td>
<td>12 (9.9%)</td>
<td>5 (7.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meta-analysis (replication)$†$</td>
<td>...</td>
<td>...</td>
<td>0.07</td>
<td>0.37 (0.10–1.42)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meta-analysis (discovery and replication)$‡$</td>
<td>...</td>
<td>...</td>
<td>1.68×10$^{-2}$</td>
<td>0.36 (0.18–0.71)</td>
<td></td>
</tr>
</tbody>
</table>

CC represents the major homozygous genotype; CI, confidence interval; CT, the heterozygous genotype; LQTS, long-QT syndrome; and SNP, single-nucleotide polymorphism.

*Pooled population (European and Japanese).
†South African and Finnish.
‡Heterogeneity: $F=0.75$, $P=0.05$.
§Not corrected for multiple tests.

### Table 4. QTc Duration in LQTS Mutation Carriers According to Replicated SNPs

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

Data presented as mean±SD. AA represents the major homozygous genotype (corresponding to CC for rs2074238 and GG for rs12029454); Aa and aa, the minor allele (T for rs2074238 and A for rs12029454) in the heterozygous or homozygous state. LQTS indicates long-QT syndrome; and SNP, single-nucleotide polymorphism.

*European population (rs2074238 is not polymorphic in the Japanese population).
†South African and Finnish.
‡Heterogeneity: $F=0$, $P=0.74$.
§Pooled population (European and Japanese).
‖Heterogeneity: $F=0$, $P=0.84$.
**Not corrected for multiple tests.
In both SA-LQT1 and Finnish replication samples, carriers of the minor NOS1AP rs12029454 A-allele tended to have longer QTc compared with noncarriers (501±63 versus 483±37 and 468±37 versus 459±32, respectively), consistent with that observed in our discovery cohort, and the meta-analysis of the 2 replication populations led to a P value of 0.05 (Table 4). When the meta-analysis was performed on the 3 populations, there was no evidence for heterogeneity (F=0; P=0.87), although 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 CEs 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 patients with LQTS and might improve the cardiac risk prediction already provided by QTc, sex, age, or type of disease-phenotype correlation studies.

Genetic Findings

The association between rs2074238 and CEs, which was restricted to European 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 Finnish-LQT1 population but failed to reach the 0.05 significance threshold. The lack of replication in this population could have been predicted because of the expected ceiling significance of association with longer QTc duration 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 2 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.9 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 3 NOS1AP SNPs was conducted in 901 LQTS patients of European descent from 520 families,34 and essentially confirmed the results reported in the SA-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.

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 LD (r²<0.50) with known KCNQ1 SNPs35; the Encyclopedia of DNA Elements project36 and the RegulomeDB variant classification37 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 nonmutated allele (trans-effect), as recently reported for SNPs located in KCNQ1 3′-untranslated region.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

Duchateau et al

KCNQ1 SNP Lowers Arrhythmic Risk in LQTS

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Comparison With Published Data

Of the several polymorphisms previously shown to affect QTc duration in large healthy populations,12-16,29,30 only 2 had a similar effect in our LQTS cohorts. This should not be surprising because, although 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- to 10-ms prolongation, which may be noticed in populations with a normal QT interval, and a modest standard deviation (usually ±20 ms) would be totally lost in populations, like ours, with much longer mean QTc and a much greater SD (±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 (Table VII in the online-only Data Supplement).

The SNPs within the adrenergic receptor genes ADRB1 (rs1801252 and rs1801253),27 ADR2C (rs61767072),27 and rs1805128 on KCN1,29 previously associated with arrhythmia risk in LQTS patients, were not validated in the present cohort. However, the rare T-allele of KCN1 tended to be more frequent in European symptomatic patients (0.05 versus 0.01; OR, 7.66 [1.21–48.31]; P = 0.02) as previously observed (Table V in the online-only Data Supplement). Among NOS1AP SNPs, only rs12029454 was associated with QT prolongation, although no association was identified with CEs in the discovery cohort. Thus, we did not replicate the previously reported association with the occurrence of CEs for rs4657139,8,34 rs168475488 (in strong LD with rs12143842 previously reported association with the occurrence of CEs in the discovery cohort. Thus, we did not replicate the

References


**CLINICAL PERSPECTIVE**

The striking heterogeneity of the clinical manifestations present even among family members carrying the same mutation is 1 of the most puzzling features of long-QT syndrome. We and others have postulated a major role for common genetic variants acting as modifiers. Here, we performed a case–control study in 112 patient duos with long-QT syndrome by genotyping 25 polymorphisms associated mostly with QTc duration and then validated the findings in 2 independent founder populations. We identified a KCNQ1 single-nucleotide polymorphism significantly associated with a decreased risk for cardiac events in the combined discovery and replication cohorts. The identification of a protective modifier represents an important step toward single-nucleotide polymorphism–specific risk stratification. On the one hand, it contributes to a better understanding of the complex relationship between genotype and phenotype; on the other, it represents 1 more step toward the necessary identification of a cluster of polymorphisms capable of modifying risk in either direction.
Identification of a KCNQ1 Polymorphism Acting as a Protective Modifier Against Arrhythmic Risk in Long-QT Syndrome

Sabine Duchatelet, Lia Crotti, Rachel A. Peat, Isabelle Denjoy, Hideki Itoh, Myriam Berthet, Seiko Ohno, Véronique Fressart, Maria Cristina Monti, Cristina Crocamo, Matteo Pedrazzini, Federica Dagradi, Alessandro Vicentini, Didier Klug, Paul A. Brink, Althea Goosen, Heikki Swan, Lauri Toivonen, Annukka M. Lahtinen, Kimmo Kontula, Wataru Shimizu, Minoru Horie, Alfred L. George, Jr, David-Alexandre Trégouët, Pascale Guicheney and Peter J. Schwartz

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# SUPPLEMENTAL MATERIAL

## Supplemental Tables

### Supplemental Table 1. Selected polymorphisms

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs number</th>
<th>References</th>
<th>Minor allele frequency in European (1000Genomes)</th>
<th>Minor allele frequency in East Asian (1000Genomes)</th>
<th>Minor allele frequency in European (Hapmap)</th>
<th>Minor allele frequency in Japanese (Hapmap)</th>
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<td>ADRA2C</td>
<td>rs61767072</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>0.273</td>
<td>-</td>
<td>0</td>
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<td>rs1801253</td>
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<td>0.308</td>
<td>0.151</td>
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<td>ADRB2</td>
<td>rs28365031</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td>rs10919071</td>
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<td>0.125</td>
<td>0.023</td>
<td>0.129</td>
<td>-</td>
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<td>rs1805128</td>
<td>5-9</td>
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<td>0.239</td>
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<td>0.170</td>
<td>0.302</td>
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<td>0.359</td>
<td>0.762</td>
<td>0.376</td>
<td>0.805</td>
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<td>KCNH2</td>
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<td>-</td>
<td>0.807</td>
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<td>0.310</td>
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<td>4, 9, 13, 15-22</td>
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<td>0.318</td>
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<td>0.119</td>
<td>0.205</td>
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<td>0.696</td>
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<td>0.500</td>
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<td>PLN</td>
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<td>0.183</td>
<td>0.103</td>
<td>0.179</td>
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</table>

Minor alleles were defined according to alleles frequencies in Europeans.
Supplemental Table 2. Methods and primers used for genotyping

**A. FRET**

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<thead>
<tr>
<th>Gene</th>
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<th>Forward primer</th>
<th>Reverse primer</th>
<th>Anchor probe (Red640)</th>
<th>Sensor probe</th>
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<tbody>
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<td>rs1805123</td>
<td>CCCGGCAGTACGGAGTTA</td>
<td>AAGGTCTGAGGCCTGGGTA</td>
<td>CGCCTGCCGAAAGGACAACCGT</td>
<td>GCCTCACCCGTCGCGT</td>
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<tr>
<td>NOS1AP</td>
<td>rs1202945*</td>
<td>TATTATTCATTTAACACGGGGTG</td>
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SNP allelic variants are bolded

* in the discovery cohort

**B. HRM with an unlabeled probe**

<table>
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SNP allelic variants are bolded

**C. Sequencing**

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<td>rs61767072</td>
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**D. Taqman**

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<td>10919071</td>
<td>C__1264222_10</td>
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<td>KCNH2</td>
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<td>Gene</td>
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<td>Applied Biosystems Taqman Assay</td>
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† in the discovery and the replication populations  
‡ in the replication populations

E. **Tm shift primers**

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**Supplemental Table 4.** Polymorphism allele frequencies in Caucasian and Japanese LQTS patients from the discovery cohort

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|--------|---------------|-----------|----------------|---------|
|        |               | AS        | Del | Ins | Del | All | Ins | Del | Ins | Del |
| ADRA2C | rs61767072    | 0.92      | 0.08 | 0.95 | 0.05 | 0.92 | 0.08 | 0.95 | 0.05 |
| ADRA2C | rs1801252     | 0.89      | 0.11 | 0.90 | 0.10 | 0.88 | 0.12 | 0.85 | 0.15 |
| ADRA2C | rs1801253     | 0.73      | 0.27 | 0.77 | 0.23 | 0.68 | 0.32 | 0.79 | 0.21 |
| ADRA2C | rs28365031    | 0.76      | 0.24 | 0.67 | 0.33 | 0.77 | 0.23 | 0.62 | 0.38 |
| ATP1B1 | rs10919071    | 0.87      | 0.13 | 0.90 | 0.10 | 0.87 | 0.13 | 0.92 | 0.08 |
| KCNE1  | rs1805128     | 0.99      | 0.01 | 0.98 | 0.02 | 0.95 | 0.05 | 1.00 | 0.00 |
| KCNE1  | rs1805123     | 0.78      | 0.22 | 0.96 | 0.04 | 0.81 | 0.19 | 0.99 | 0.01 |
| KCNE1  | rs3778873     | 0.80      | 0.20 | 0.98 | 0.02 | 0.80 | 0.20 | 0.98 | 0.02 |
| KCNE1  | rs3807375     | 0.59      | 0.41 | 0.17 | 0.83 | 0.61 | 0.39 | 0.18 | 0.82 |
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Asymptomatic patients (AS)

Symptomatic patients (S)
Supplemental Table 5. Polymorphism genotypes in Caucasian and Japanese LQTS patients from the discovery cohort

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<td>9</td>
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<tr>
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<tr>
<td>CNOT3</td>
<td>rs36643</td>
<td>TT</td>
<td>TC+CC</td>
</tr>
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<td>49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.94 [0.57-1.54]</td>
<td>21</td>
</tr>
<tr>
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</tr>
<tr>
<td>PLN</td>
<td>rs11970286</td>
<td>CC</td>
<td>CT+TT</td>
</tr>
<tr>
<td></td>
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<td>23</td>
<td>36</td>
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<tr>
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<td></td>
<td>1.22 [0.72-2.08]</td>
<td>24</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>PLN</td>
<td>rs12210810</td>
<td>GG</td>
<td>GC+CC</td>
</tr>
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<td></td>
<td></td>
<td>64</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.27 [0.05-1.38]</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCN5A</td>
<td>rs12053903</td>
<td>AA</td>
<td>AG+GG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.33 [0.73-2.42]</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCN5A</td>
<td>rs1805124</td>
<td>AA</td>
<td>AG+GG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.84 [0.48-1.44]</td>
<td>36</td>
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</table>

Asymptomatic patients (AS)
Symptomatic patients (S)
Insertion (I)
Deletion (D)
**Supplemental Table 6.** QTc duration in Caucasian and Japanese LQTS patients from the discovery cohort presented by rs12029454 genotype

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>AA</th>
<th>Aa and aa</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>QTc (ms)</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOS1AP</td>
<td>rs12029454</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>474 ± 43</td>
<td>97</td>
<td>485 ± 43</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Japanese</td>
<td>463 ± 49</td>
<td>28</td>
<td>483 ± 54</td>
<td>54</td>
</tr>
</tbody>
</table>
Supplemental Table 7. Power to detect previously reported effect on cardiac events or QTc in LQTS patients or healthy populations in our discovery cohort

A. Power to detect previously reported effect on cardiac events in LQTS patients in our discovery cohort

<table>
<thead>
<tr>
<th>SNP</th>
<th>Previously reported effect</th>
<th>Power $^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10494366</td>
<td>1.42 Tomas et al., 2010</td>
<td>30%</td>
</tr>
<tr>
<td>rs12143842</td>
<td>1.40 Crotti et al., 2009</td>
<td>35%</td>
</tr>
<tr>
<td>rs4657139</td>
<td>1.38 Tomas et al., 2010</td>
<td>27%</td>
</tr>
<tr>
<td></td>
<td>1.80 Crotti et al., 2009</td>
<td>58%</td>
</tr>
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</table>

B. Power to detect previously reported effect on QTc in LQTS patients in our discovery cohort

<table>
<thead>
<tr>
<th>SNP</th>
<th>Previously reported effect</th>
<th>Power $^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12143842</td>
<td>8 ms Tomas et al., 2010</td>
<td>39%</td>
</tr>
<tr>
<td>rs4657139</td>
<td>7 ms Tomas et al., 2010</td>
<td>27%</td>
</tr>
</tbody>
</table>

C. Power to detect previously reported effect on QTc in healthy populations in our discovery cohort

<table>
<thead>
<tr>
<th>SNP</th>
<th>Power $^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10919071</td>
<td>9-12%</td>
</tr>
<tr>
<td>rs1805128</td>
<td>14-17%</td>
</tr>
<tr>
<td>rs1805123</td>
<td>9-12%</td>
</tr>
<tr>
<td>rs3778873</td>
<td>9%</td>
</tr>
<tr>
<td>rs3807375</td>
<td>7-13%</td>
</tr>
<tr>
<td>rs3815459</td>
<td>8%</td>
</tr>
<tr>
<td>rs17779747</td>
<td>7%</td>
</tr>
<tr>
<td>rs12296050</td>
<td>8-9%</td>
</tr>
<tr>
<td>rs2074238$^\delta$</td>
<td>14-19%</td>
</tr>
<tr>
<td>rs757092</td>
<td>9%</td>
</tr>
<tr>
<td>rs10494366</td>
<td>9-14%</td>
</tr>
<tr>
<td>rs12029454</td>
<td>15%</td>
</tr>
<tr>
<td>rs12143842</td>
<td>13-21%</td>
</tr>
<tr>
<td>rs16857031</td>
<td>13%</td>
</tr>
<tr>
<td>SNP</td>
<td>Power‡</td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
</tr>
<tr>
<td>rs4657139</td>
<td>15%</td>
</tr>
<tr>
<td>rs4657178</td>
<td>10%</td>
</tr>
<tr>
<td>rs36643</td>
<td>7%</td>
</tr>
<tr>
<td>rs11970286</td>
<td>8%</td>
</tr>
<tr>
<td>rs12210810†</td>
<td>7-8%</td>
</tr>
<tr>
<td>rs12053903</td>
<td>8%</td>
</tr>
<tr>
<td>rs1805124</td>
<td>8%</td>
</tr>
</tbody>
</table>

* Effect reported for rs16847548 which is in strong linkage disequilibrium with rs12143842 (r²>0.8 in Europeans)
† Using QUANTO program, we calculated the power to detect previously reported effect, at a significance level of 0.05, under a dominant model, with a prevalence of 35%, according to allele frequencies in our discovery population and for 112 duos constituting our matched case-control study.
‡ Using QUANTO program, we calculated the power to detect previously reported effect, at a significance level of 0.05, under a dominant model, with a prevalence of 35%, according to allele frequencies in our discovery population and for 224 individuals constituting our LQTS cohort
§ Power was calculated according to allele frequencies in Caucasian discovery population and for 142 individuals constituting our Caucasian matched case-control study (SNPs non polymorphic in Japanese)
Supplemental Figures

Supplemental Figure 1.

A

B
Supplemental Figure 2.

A.  
**KCNH2**

- rs1805123
- rs3807375
- rs3778873

**KCNQ1**

- rs12296050
- rs757092

**NOS1AP**

- rs12143842
- rs10494366
- rs16857031
- rs12029454
- rs4657178

B.  

**KCNH2**

- rs1805123
- rs3807375
- rs3778873

**KCNQ1**

- rs12296050
- rs757092

**NOS1AP**

- rs12143842
- rs10494366
- rs16857031
- rs12029454
- rs4657178

$r^2$ colors:
- [0.9, 1]
- [0.8, 0.9]
- [0.7, 0.8]
- [0.6, 0.7]
- [0.5, 0.6]
- [0.4, 0.5]
- [0.3, 0.4]
- [0.2, 0.3]
- [0.1, 0.2]
- [0.0, 0.1]
Supplemental Figures Legends

**Supplemental Figure 1.** Kaplan-Meier curves of survival free from cardiac events among the symptomatic group from the discovery cohort.

The age-related probability of not experiencing a first cardiac event, with birth used as time of origin, was plotted using the Kaplan-Meier method in the symptomatic population by genetic locus (A) or sex (B).

**Supplemental Figure 2.** Pairwise LD between polymorphisms in LQTS patients from the discovery cohort

A. Pairwise LD calculated from asymptomatic Caucasian patients

SNPs in *ADRB1, PLN* or *SCN5A* have $r^2<0.1$

B. Pairwise LD calculated from asymptomatic Japanese patients

SNPs in *ADRB1, PLN* or *SCN5A* have $r^2<0.1$
Supplemental References


