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Transethnic Genome-Wide Association Study Provides Insights in the Genetic Architecture and Heritability of Long QT Syndrome

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BACKGROUND: Long QT syndrome (LQTS) is a rare genetic disorder and a major preventable cause of sudden cardiac death in the young. A causal rare genetic variant with large effect size is identified in up to 80% of probands (genotype positive) and cascade family screening shows incomplete penetrance of genetic variants. Furthermore, a proportion of cases meeting diagnostic criteria for LQTS remain genetically elusive despite genetic testing of established genes (genotype negative). These observations raise the possibility that common genetic variants with small effect size contribute to the clinical picture of LQTS. This study aimed to characterize and quantify the contribution of common genetic variation to LQTS disease susceptibility.

METHODS: We conducted genome-wide association studies followed by transethnic meta-analysis in 1656 unrelated patients with LQTS of European or Japanese ancestry and 9890 controls to identify susceptibility single nucleotide polymorphisms. We estimated the common variant heritability of LQTS and tested the genetic correlation between LQTS susceptibility and other cardiac traits. Furthermore, we tested the aggregate effect of the 68 single nucleotide polymorphisms previously associated with the QT-interval in the general population using a polygenic risk score.

RESULTS: Genome-wide association analysis identified 3 loci associated with LQTS at genome-wide statistical significance ($P<5\times10^{-8}$) near *NOS1AP*, *KCNQ1*, and *KLF12,* and 1 missense variant in *KCNE1* (p.Asp85Asn) at the suggestive threshold ($P<10^{-6}$). Heritability analyses showed that ≈15% of variance in overall LQTS susceptibility was attributable to common genetic variation (h^2_{SNP} 0.148; standard error 0.019). LQTS susceptibility showed a strong genome-wide genetic correlation with the QT-interval in the general population (r_g=0.40; $P=3.2\times10^{-3}$). The polygenic risk score comprising common variants previously associated with the QT-interval in the general population was greater in LQTS cases compared with controls ($P<10^{-13}$), and it is notable that, among patients with LQTS, this polygenic risk score was greater in patients who were genotype negative compared with those who were genotype positive ($P<0.005$).

CONCLUSIONS: This work establishes an important role for common genetic variation in susceptibility to LQTS. We demonstrate overlap between genetic control of the QT-interval in the general population and genetic factors contributing to LQTS susceptibility. Using polygenic risk score analyses aggregating common genetic variants that modulate the QT-interval in the general population, we provide evidence for a polygenic architecture in genotype negative LQTS.

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The full author list is available on page 334.

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Clinical Perspective

What Is New?

- A genome-wide association study in long QT syndrome (LQTS) patients establishes and quantifies the role of common genetic variation in susceptibility to LQTS.
- Genetic overlap exists between control of QT-interval in the general population and susceptibility to LQTS.
- Polygenic risk score analyses based on common genetic variants that modulate the QT-interval in the general population provide evidence for a polygenic architecture in LQTS patients that remains genetically elusive despite genetic testing of established genes (ie, genotype negative).

What Are the Clinical Applications?

- These findings enhance the understanding of the genetic basis of LQTS and underscore the genetic relationship between QT-interval in the general population and susceptibility to LQTS.
- Increasing burden of QT-prolonging common variants is associated with higher susceptibility for LQTS.
- Polygenicity in genotype negative LQTS patients implies that risk is not primarily attributable to 1 genetic factor inherited from 1 of the biological parents as is the case for autosomal dominant LQTS.
- Future clinical utility of genetic testing based on polygenic inheritance necessitates the availability of polygenic risk scores with high discriminative capacity.

Long QT syndrome (LQTS) is a rare inherited disorder of ventricular repolarization characterized by prolongation of the QT interval on the ECG.[1,2] LQTS has a prevalence of approximately 1 in 2500, and is a major and often preventable cause of sudden cardiac death in the young.[3,4] Multiple genes have been implicated in LQTS and clinical genetic testing is now performed to identify causative rare genetic variants.[5] Disease-causing variants (ie, mutations) in the 3 major LQTS genes (ie, *KCNQ1* [LQT1], *KCNH2* [LQT2], and *SCN5A* [LQT3]), account for up to 80% of LQTS cases overall and >95% of genotype positive LQTS.[2]

Studies in families with multiple mutation carriers have shown that disease penetrance (proportion of carriers that manifest with a prolonged QT-interval) can be low,[6–8] and that among those with disease manifestations, there can be broad variability in the types of symptoms and severity thereof (variable expression).[2,6–8] These observations suggest that, like other Mendelian disorders, allocating the disease in the individual patient exclusively to a rare variant at a single locus (ie, monogenetic) might be an oversimplification of biological phenomena. It is likely that a combination of genetic and nongenetic modifying factors underlies this clinical variability. A comprehensive knowledge of such risk factors that affect penetrance and expressivity of disease-causing variants in LQTS will improve the predictive accuracy of genetic testing in the individual patient and enable personalized clinical interventions. While many clinical risk factors such as sex, hypokalemia, or bradyarrhythmia, have been implicated as modulators of the clinical manifestations of LQTS,[9] modulatory genetic factors remain largely unexplored with the exception of a few proof-of-concept studies using a candidate gene approach.[10–14]

Besides variability in disease manifestations among carriers of pathogenic variants, an outstanding issue in LQTS is the fact that in ≈20% of patients, an underlying causal rare genetic variant remains unidentified after extensive panel-based genetic testing.[15] This complicates cascade screening in families and the presymptomatic identification of affected relatives. Although a small proportion of such patients with genotype negative LQTS could have a yet unknown Mendelian defect, another possibility is that a more complex inheritance pattern underlies the disorder in a subset of these patients.

Previous work has shown that a genome-wide association study (GWAS) comparing cases of a rare arrhythmia syndrome with unaffected controls can define modulators of disease susceptibility and suggest a polygenic etiology.[16] We report here a GWAS in ≈1700 unrelated patients with LQTS, of European or Japanese ancestry, identifying common genetic variants implicated in LQTS disease susceptibility, and providing a quantification of the contribution of common genetic variants to LQTS predisposition. Using polygenic risk score analyses aggregating common genetic variants that modulate the QT-interval in the general population, we provide evidence for a polygenic architecture in genotype negative LQTS.

METHODS

The summary statistics generated in this study are available from the corresponding author on request or on the Cardiovascular Disease Knowledge Portal (http://www.broadcvdi.org/).

Study Population

We established an international consortium allowing recruitment of 1781 unrelated patients with LQTS: 1344 cases of European ancestry from 23 referral centers in Europe, New Zealand, and North America, as well as 437 patients of East Asian ancestry from 4 referral centers in Japan (Table I in the Data Supplement). Included unrelated individuals were probands (97%) except when DNA was not available, in which case 1 other affected family member was included instead. Included patients had a clinical diagnosis of LQTS[5] and were classified as "genotype positive" if they carried a single rare variant in 1 of the 3 established major LQTS genes (*KCNQ1* [LQT1], *KCNH2* [LQT2] and *SCN5A* [LQT3]), or "genotype

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negative" if no rare variant was identified in genes unequivocally associated with nonsyndromic LQTS (*KCNQ1*, *KCNH2*, *SCN5A*, *CALM1-3*, and *TRDN*).[17–19] A rare variant was defined as a protein sequence altering (ie, missense, nonsense, frameshift deletion, in-frame deletion, large deletion, and duplication) or splice-site variant with an allele frequency $<1\times10^{-4}$ in the Genome Aggregation Database.[20–22] Genetic testing and variant curation as per the American College of Medical Genetics and Genomics and Association of Molecular Pathology guidelines[23] was conducted as described in the Methods section in the Data Supplement. All subjects or their guardians provided informed consent, and the study was approved by the appropriate ethical review boards.

Phenotypic Characterization and Measurement of the QT-Interval

Clinical data were collected at each of the participating centers. We collected a baseline ECG for each patient, preferably not during β-blocker use. The QT-interval duration was measured as previously described (Figure I in the Data Supplement, and Methods in the Data Supplement).[24] In genotype negative patients, a LQTS diagnosis was additionally curated by 2 clinicians (NL, RT) and in case of uncertainty, 2 senior LQTS experts (AAW, PJS) were consulted. As per international guidelines,[5] we only included genotype negative patients with a LQTS risk score≥3.5 or with a resting QTc≥500ms in repeated 12-lead ECGs, in the absence of a secondary cause for QT prolongation.

Genome-Wide Array Genotyping, Quality Control, and Imputation

We performed genome-wide genotyping for all European cases on the Illumina HumanOmniExpress array and for all Japanese cases on the Illumina Global Screening Array. Genotypic data of 8219 control individuals of European ancestry and 1671 individuals of Japanese ancestry were obtained from different cohorts (Table II in the Data Supplement). Quality control,[25] imputation and association analysis were performed separately in the European and Japanese datasets. All genetic variants were mapped to and reported using Genome Reference Consortium Human genome build 37.

After quality control (see Methods in the Data Supplement for details), we performed genome-wide imputation using Eagle2 phasing, Minimac3 and the Haplotype reference consortium (HRCr1.1) panel implemented on the Michigan Imputation Server for both the European and Japanese datasets.[26] After imputation, only single nucleotide polymorphisms (SNPs) with minor allele frequency >0.01 and a Minimac3 imputation score of R^2>0.3 were included in further analyses.

Genome-Wide Association Analysis

We performed genome-wide association analyses to assess the role of common variants in LQTS susceptibility (case–control) and severity (QTc within the cases). Case–control association of alternate allele dosage with LQTS was performed using logistic regression correcting for genotypic principal components 1 to 10. Quantitative trait analyses for QTc were conducted using a linear regression model correcting for age, β-blocker use at ECG, LQTS type (*KCNQ1* [LQT1], *KCNH2* [LQT2], *SCN5A* [LQT3], or genotype negative), sex, and principal components 1 to 10. Genome-wide association analyses were carried out separately for the European and Japanese LQTS cohorts, followed by meta-analysis using an inverse variance weighted fixed effect model, implemented in METAL (version 2011-03-25).[27] Genome-wide statistical significance and suggestive thresholds were set to $P<5\times10^{-8}$ and $P<1\times10^{-6}$, respectively. Summary statistics were uploaded to FUMA (Functional Mapping and Annotation of GWAS) for generation of Manhattan, quantile-quantile, and regional association plots for risk loci.[28]

Survival Analyses

Time to life-threatening arrhythmic events (LAE) survival analyses were performed in the LQTS cases. Follow-up started at birth and stopped at the date of a documented LAE, the last visit, or the 41st birthday, whichever came first. LAE were defined as out of hospital cardiac arrest, hemodynamically unstable ventricular tachycardia/ventricular fibrillation, or appropriate implantable cardioverter-defibrillator therapy. The effect of genotype positive versus genotype negative status was estimated using Cox proportional hazards regression with/without adjustment for classic risk factors (ie, sex and QTc≥500 ms). To examine possible differences in effect of these well-recognized risk factors in genotype positive and genotype negative LQTS cases, interactions between these risk factors and genotype status were included in the model. In addition, puberty and a sex × puberty interaction were included to model the modifying effect of puberty on the effect of sex. Puberty was included as time-varying covariate and the age of puberty was set at 16 years in both sexes (ie, during the follow-up period before the age of 16, puberty was coded as 0, whereas puberty was coded as 1 during the remainder of the follow-up period). Kaplan Meier curves were created to illustrate the cumulative event free survival and log rank tests were used to compare the survival curves.

Polygenic Risk Scores

For all cases and controls, we calculated a weighted QT polygenic risk score (PRS_{QT}) comprising 68 SNPs that had been associated with the QT-interval in the general population at genome-wide statistical significance, in a study primarily including Europeans.[29] All 68 SNPs were included in the European dataset analyses whereas only 60/68 SNPs were well-imputed and included in the Japanese dataset analyses (Table III in the Data Supplement). PRS_{QT} was calculated by multiplying the alternate allele dosage by the associated effect size (β) in the published QT GWAS for each of the 68 SNPs. Then, the PRS_{QT} was normalized to a mean of 0 and standard deviation of 1. We used logistic regression to test for association of PRS_{QT} with case–control status, correcting for principal components 1 to 10. We also used *P* value thresholding and R^2 pruning with *P* values of 5×10^{-8}, 1×10^{-5}, 1×10^{-4}, 1×10^{-3}, and 1×10^{-2} and R^2 of 0.2 and 0.1 on summary statistics from a European[29] and Japanese[30] descent general population QT-interval GWAS. The resulting 10 models were used to calculate a European and Japanese PRS_{QT}. The association of PRS_{QT} with LQTS was assessed using a logistic regression for the European and Japanese cases separately. The best model was selected based on the maximal C-statistic, as recently

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performed.[31] No other covariate was used to avoid model overfitting.

The odds ratios (ORs) associated with quartile 2, 3, and 4 were calculated using the first PRS_{QT} quartile as the reference. The association of PRS_{QT} and known QT predictors with QTc was performed using a univariable linear regression followed by multivariable analysis, including in the final model only those variables with a $P<0.05$ in the univariable analyses. The association of PRS_{QT} quartiles with time to LAEs was assessed using Cox proportional hazards regression with/without adjustment for classic risk factors. Association analyses of PRS_{QT} with case–control status, QTc, and time to LAE were performed separately in the European and Japanese datasets, followed by a fixed-effects model meta-analysis.

Common Variant Heritability

We used the generalized restricted maximum likelihood (GREML) approach of GCTA (GCTA-GREML)[32] to estimate how much of the variance in LQTS susceptibility could be attributed to common genetic variants (SNP-based heritability, h^2_{SNP}). Before heritability estimation, we conducted additional stringent genetic quality control, as previously suggested (Methods in the Data Supplement).[33] We estimated the SNP-heritability on the liability scale assuming a 0.04% prevalence with principal components 1-10 as covariates.[1] We assessed the robustness of heritability estimates from GCTA-GREML using the GREML and phenotype-correlation genotype-correlation regression[34] analyses implemented in LDAK.[35] We estimated h^2_{SNP} in the overall LQTS and genotype positive LQTS dataset in the both European and Japanese ancestries. Because of small sample size we were not able to estimate h^2_{SNP} in genotype negative patients with LQTS using the approaches implemented in GCTA or LDAK.

Genetic Correlation With Other Traits

We used bivariate linkage disequilibrium score regression[36] to evaluate the genetic correlation between LQTS susceptibility (as obtained in the European descent case–control GWAS) and other cardiac electric traits,[2] namely PR, QRS, QT, heart rate (HR) at rest, HR in response to exercise and recovery, and atrial fibrillation (see Methods in the Data Supplement for origin of summary statistics). We used Bonferroni correction to account for multiple testing (P=0.05/7=0.0071). We did not constrain the bivariate regression intercepts in any of these analyses given the potential for (modest) sample overlap and population stratification.

RESULTS

Clinical Characteristics of the Case Cohort

Demographic and clinical characteristics of the unrelated LQTS cases are presented in Table IV in the Data Supplement separately for the European and Japanese datasets and in Table 1 for the combined cohort. We included a total of 1781 unrelated patients with LQTS of European (n=1344, mean QTc±SD: 484±48ms) and Japanese descent (n=437, QTc: 485±49ms). A total of 1584 cases (89%) were genotype positive, carrying a rare variant in *KCNQ1* (LQT1, n=800), *KCNH2* (LQT2, n=661), or *SCN5A* (LQT3, n=123), while in 197 (11%) no disease causing variant was identified (ie, genotype negative) despite extensive genetic testing.

Table 1. Clinical Characteristics of All Unrelated LQTS Cases

Parameter	Genotype Positive (n=1584)	Genotype Negative (n=197)
Male, n (%)	584/1584 (37)	76/197 (39)
QTc mean±SD, ms	482±47	500±52
Genotype, n (%)		
KCNQ1	800/1584 (50)	—
KCNH2	661/1584 (42)	—
SCN5A	123/1584 (7.8)	—
Syncope, n (%)	722/1584 (46)	75/197 (38)
LAE (OHCA or VF/VT) before age 41, n (%)	262/1578 (17)	33/196 (17)
Age at first LAE, median [IQR]	21 [13–29]	26 [16–35]
Treatment during follow-up, n (%)		
Beta-blocker	1169/1487 (79)	124/168 (74)
ICD	277/1562 (18)	38/172 (22)
PM	50/1565 (3.2)	11/171 (6.4)
LCSD	29/1583 (1.8)	1/171 (0.6)
Family history of SCD <50 yr of age, n (%)	323/1409 (23)	24/189 (13)

ICD indicates implantable cardioverter-defibrillator; IQR, interquartile range; LAE, life-threatening arrhythmic event; LCSD, left cardiac sympathetic denervation; OHCA, out-of-hospital cardiac arrest; PM, pacemaker; SCD, sudden cardiac death, SD, standard deviation; and VF/VT, hemodynamically unstable ventricular fibrillation/tachycardia

The mean QTc interval in genotype negative cases was higher in comparison with genotype positive ones (500±52ms vs 482±47ms, $P=2\times10^{-5}$) and in genotype negative cases a family history of sudden cardiac death at <50 years of age in 1st and 2nd degree relatives was less frequent compared with genotype positive ones (12.7% vs 22.9%, P=0.001). Of the 1584 genotype positive cases, 1333 (84%) carried a pathogenic or likely-pathogenic variant according to American College of Medical Genetics and Genomics and Association of Molecular Pathology guidelines, and the remainder had a variant of unknown significance. The QTc did not significantly differ between carriers of variants of unknown significance and those with a pathogenic or likely-pathogenic variant (P=0.9).

In total, 429 cases (24%) had an LAE at a median age of 28 years (interquartile range, 17 to 46 years), with 295 cases (17%) having such an event by age 40. LAE-free survival did not significantly differ between genotype negative and positive cases (P=0.8) or between European and Japanese cases (P=0.053; Figure 1). In a multivariable Cox proportional hazard model, male sex (OR 1.9; P=0.004), QTc>500ms (OR 1.8; $P=4\times10^{-6}$) and Japanese ancestry (OR 1.4; P=0.03) were independent risk factors for LAE (Table V in the Data Supplement). We found a significant sex-puberty interaction ($P=1\times10^{-6}$), where males were at higher risk of LAE in the prepubertal years but lower risk thereafter (Figure II in the Data

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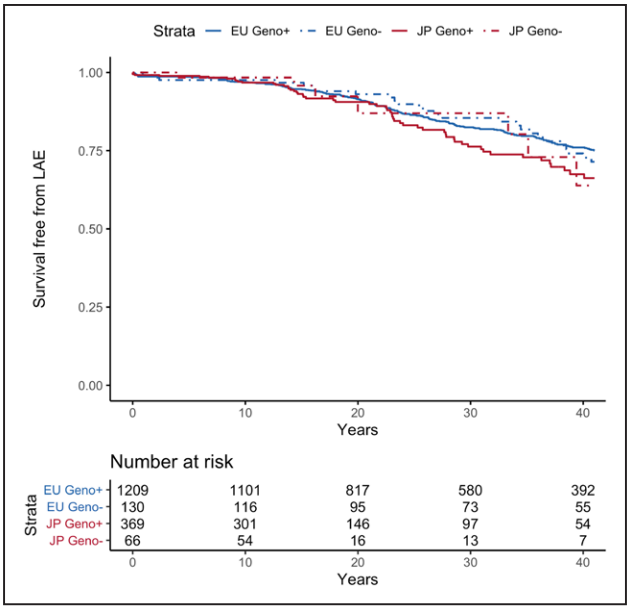

Figure 1. Kaplan-Meier life-threatening arrhythmic event–free survival curves stratified by ancestry.
EU indicates European LQTS cases; Geno-, genotype negative LQTS cases; Geno+, genotype positive LQTS cases; JP, Japanese LQTS cases; LAE, life-threatening arrhythmic event (defined as the composite of out of hospital cardiac arrest or hemodynamically unstable ventricular tachycardia/arrhythmia; and LQTS, long QT syndrome. Log-rank test P=0.3.

Supplement). The effect of the conventional risk factors sex ($P_{interaction}$=0.3) and QTc≥500ms ($P_{interaction}$=0.7) did not differ between genotype positive and genotype negative cases. Genotype (*KCNQ1*, *KCNH2*, *SCN5A*, or negative) significantly affected time to LAE (log-rank test P<0.001; Figure III in the Data Supplement). Cases with a rare variant in *KCNQ1* had a lower risk of LAE compared with *KCNH2*, *SCN5A*, and genotype negative ones (P<0.01 for all comparisons). None of the other post hoc pairwise comparisons reached statistical significance. Time to LAE did not differ between cases with a variant of unknown significance and those with pathogenic or likely-pathogenic variant (Figure IV in the Data Supplement).

Case–Control GWAS

We conducted a case–control GWAS separately in European (1238 cases vs 8219 controls, genomic test inflation (λ)=1.024) and Japanese (418 cases vs 1617 controls, λ=1.034) cases using ancestry-matched controls (Figures V and VI in the Data Supplement), followed by transethnic meta-analysis (λ=1.028). This uncovered 3 loci reaching the threshold for genome-wide statistical significance (Table 2, Figure 2, and Figures VII and VIII in the Data Supplement). The most significant association was obtained for rs12143842 (OR=1.32 [95% CI, 1.21–1.42]; P=1.09×10^{-11}) located upstream of *NOS1AP* (Figure VIIIA in the Data Supplement). The lead SNP at the second locus was located in an intron of *KCNQ1* (rs179405, OR=1.38 [95% CI, 1.23–1.54]; P=1.92×10^{-8}; Figure VIIIB in the Data Supplement). At the third locus, the lead SNP, rs17061696 (OR=1.25 [95% CI, 1.15–1.35]; P=4.33×10^{-8}), was located in an intron of *KLF12* (Figure VIIIC in the Data Supplement). All 3 loci had been previously associated with the QT-interval duration, a measure of myocardial repolarization on the ECG, in the general population (Table 1).[29] The low-frequency missense variant in *KCNE1*, p.Asp85Asn (rs1805128, OR=2.78 [95% CI, 1.67–3.90]; P=5.31×10^{-7}; Figure VIIID in the Data Supplement) reached the suggestive statistical significance threshold in the European case–control analysis. This variant, which is rare and not well imputed in the Japanese dataset (minor allele frequency=0.001; R^2<0.3), has the largest reported effect size among the 68 independent SNPs (hereafter referred to as QT-SNPs) previously associated with QT-interval in the general population (7.4ms increase per minor allele).[29] Of note, The KCNE1-p.Asp85Asn variant had a more pronounced effect in genotype negative (OR=7.64 [95% CI, 3.66–15.95]; P=5.99×10^{-8}) than in genotype positive LQTS (OR=2.28 [95% CI, 1.46–3.54]; P=2.59×10^{-4}).

Genetic Overlap Between LQTS and QT-Interval in the General Population

The identification of SNPs previously associated with QT-interval in the general population is in line with the fact that QT-interval prolongation on the ECG (representing prolonged cardiac repolarization) is the central intermediate phenotype underlying LQTS. In fact, 23 of the 68 QT-SNPs previously associated with QT-interval in the general population, were associated with LQTS at nominal significance (ie, P<0.05), while only 4 would be expected under the null hypothesis (Table VI in the Data Supplement). We observed a strong positive correlation between the effect that each of the 68 QT-SNPs had on the QT-interval in the general population[29] and the risk they conferred for LQTS in the current study. This effect was consistent across both the European (Figure 3A; R^2=0.67; P=2.04×10^{-17}) and the Japanese (Figure 3B; R^2=0.52; P=1.43×10^{-10}) datasets. Overlap between genetic risk for LQTS and genetic determinants of the QT-interval in the general population[29] was further demonstrated by genome-wide bivariate linkage disequilibrium score regression,[36] which detected a significant positive genetic correlation (r_g=0.40, SE=0.14; P=3.2×10^{-3}) between these phenotypes. No significant correlation was found for other cardiac electric traits (Figure IX in the Data Supplement).

Analysis of PRS_{QT} in LQTS Disease Susceptibility

We then tested the aggregate effect of the 68 QT-SNPs (PRS_{QT}) on susceptibility to LQTS by means of PRS analysis (Table III in the Data Supplement). PRS_{QT} was

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Table 2. Significant Loci in LQTS Case–Control GWAS

					Meta-Analysis			European			Japanese			
Lead SNP	GRCh37	Alternative Allele	Reference Allele	Closest Gene	OR	95%CI	*P*	AAF (Controls /Cases)	OR	*P*	AAF (Controls /Cases)	OR	*P*	Effect on QT (ms)*
rs12143842	1:162033890	T	C	*NOS1AP*	1.31	1.21–1.42	1.09E−11	0.26/0.32	1.29	7.34E−08	0.38/0.47	1.41	2.13E−05	3.5
rs179405	11:2525395	A	G	*KCNQ1*	1.38	1.23–1.54	1.92E−08	0.14/0.17	1.34	4.03E−06	0.10/0.14	1.63	5.42E−04	1.9†
rs17061696	13:74511991	C	G	*KLF12*	1.25	1.15–1.35	4.33E−08	0.37/0.43	1.27	8.91E−08	0.19/0.21	1.16	1.43E−01	0.58

AAF indicates alternative allele frequency; GRCh37, genomic position on build GRCh37; and OR, odds ratio per alternative allele.

*QT increase (in ms) per alternative allele in the general population.

†The lead SNP at the *KCNQ1* locus (rs179405) is in linkage disequilibrium with rs7122937 (R^2=0.497) which had been previously associated with QT-interval in the general population (1.9 ms increase per risk allele).

significantly associated with a diagnosis of LQTS in the European set, the Japanese set, and in the meta-analysis of both datasets (Figure 4A and 4C; Table 3; meta-analysis β=0.34, SE=0.03; P=1.1×10^{-38}, heterogeneity P=0.15). Similar results were obtained when we excluded common variants located at the known Mendelian LQTS loci from the PRS (Table VII in the Data Supplement). Ten different PRS derived by the pruning and thresholding method on summary statistics from the European descent general population QT-interval GWAS did not significantly outperform the PRS_{QT} in discriminating case–control status (Table VIIIA in the Data Supplement). Similarly, Japanese ancestry-specific PRS derived from summary statistics of a small Japanese

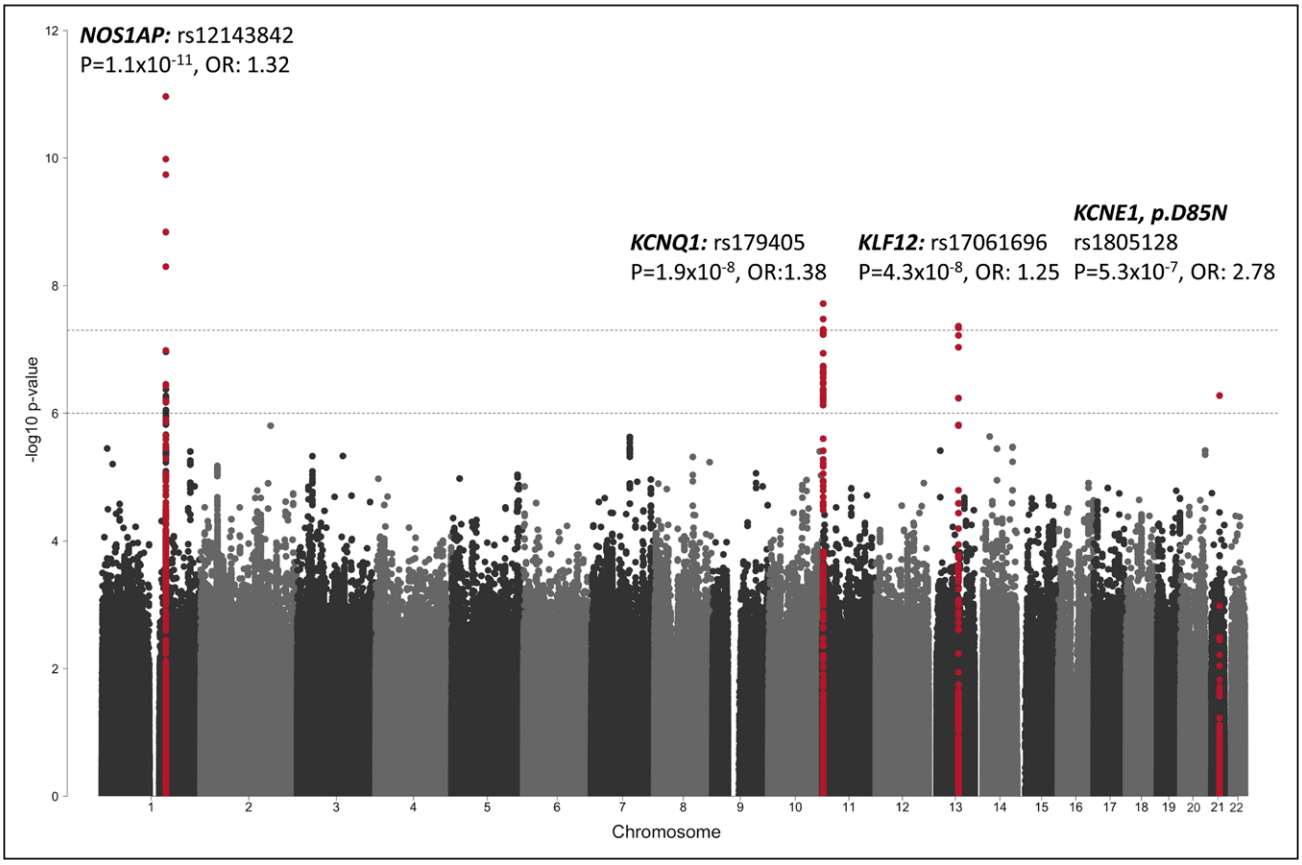

Figure 2. Manhattan plot of long QT syndrome case–control meta-analysis.
Manhattan plot displaying the base-pair position of each of the tested single nucleotide polymorphisms (SNPs; each dot represents an individual SNP) along the chromosomes on the *x* axis and the corresponding −log10 transformed association *P* value on the *y* axis. The association *P* values from the meta-analysis of the 2 genome-wide association studies conducted separately in European and Japanese cases and controls, respectively, are displayed. The upper and lower dashed lines indicate the genome-wide significance (P<5×10^{-8}) and suggestive significance (P<1×10^{-6}) thresholds, respectively. SNPs at genomic regions that reached the genome-wide or suggestive significance thresholds, are marked in red, whereas SNPs from other regions are marked in black or grey. The association for variant rs1805128 (*KCNE1*:p.Asp85Asn) is solely driven by the European analysis because it is not well imputed and rare (R^2<0.3. minor allele frequency=0.001) in the Japanese dataset.

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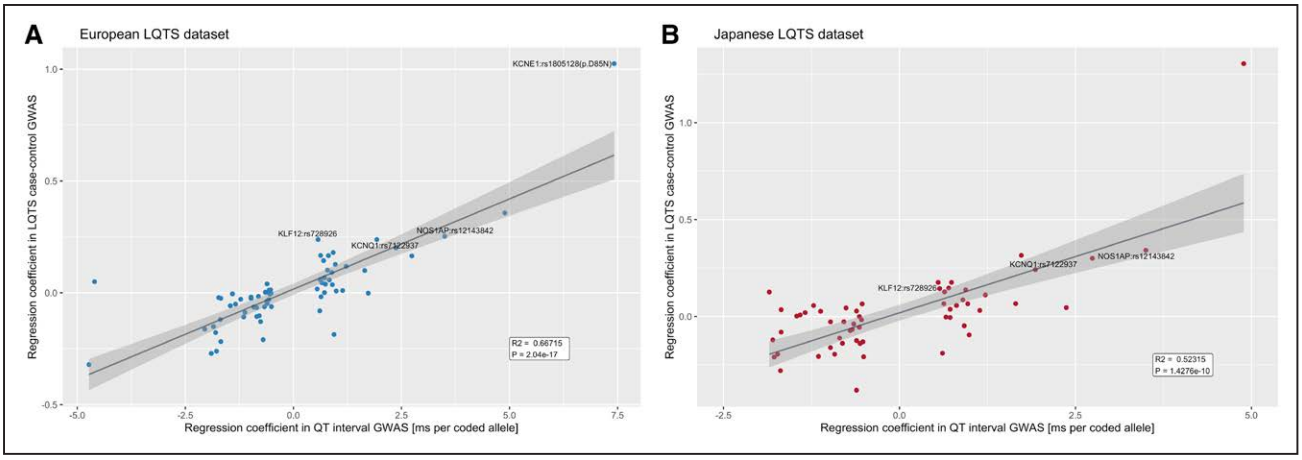

Figure 3. Correlation of effect size of QT-associated single nucleotide polymorphisms with their effect size in long QT syndrome genome-wide association study.

The *x* axis represents the effect estimates from the QT-interval genome-wide association study (GWAS) conducted in the general population (milliseconds per alternative allele) and the *y* axis the effect of each of these QT-interval associated alleles on disease risk of long QT syndrome (LQTS; [Ln(OR)]) in the European (**A**) and Japanese (**B**) datasets. All 68 SNPs associated with QT in the general population were assessed in Europeans, whereas 60 SNPs were properly imputed in the Japanese dataset. In the LQTS-GWAS meta-analysis, 23/68 SNPs previously associated with the QT in the general population reached nominal significance (see Table VI in the Data Supplement). Loci that reached genome-wide significance in the LQTS case–control transethnic meta-analysis *NOS1AP*-rs12143842, *KCNQ1*-rs179405, *KLF12*-rs728926, and *KCNE1*-rs1805128 are identified with text.

QT-interval GWAS[30] had less discriminative accuracy in the Japanese case–control dataset compared with the European-derived $PRS_{QT,}$ likely because of the small size of the Japanese QT-interval GWAS (Table VIIIB in the Data Supplement).

We next explored whether the genetic architecture of genotype negative patients (ie, those lacking a rare variant after extensive genetic testing of the established LQTS disease genes) differed from that of genotype positive patients. This was done by comparing PRS_{QT} between both groups, uncovering a significantly higher PRS_{QT} in genotype negative patients, pointing to a more prominent role for common variants in disease susceptibility in these patients. This effect was consistently observed in both the European (P=5.1×10^{-6}, Figure 4B) and the Japanese (P=2.0×10^{-3}, Figure 4D) datasets (Table 3). Similar results were obtained in a sensitivity analysis correcting for QT-interval, ensuring that enrichment of QT prolonging alleles in the genotype negative patients was not driven by differences in QT-interval (P=7.4×10^{-5} in Europeans; P=2.6×10^{-3} in Japanese, Table 3). These associations remained statistically significant when we restricted the analysis to patients with a pathogenic or likely-pathogenic variant according to American College of Medical Genetics and Genomics and Association of Molecular Pathology guidelines (ie, excluding cases with a rare variant of unknown significance; Methods in the Data Supplement and Tables IX and X in the Data Supplement). Increasing PRS_{QT} quartiles were associated with a significantly higher disease susceptibility for genotype negative LQTS compared with the lowest quartile (Figure 5; Table XI in the Data Supplement). It is notable that, using a PRS_{QT} percentile threshold of 80, 90, and 95, individuals above the threshold compared with those below have an OR (95%CI) of 2.9 (2.2–4.0), 4.1 (2.9–5.8), and 5.7 (3.9–8.4), respectively, for genotype negative LQTS. Of interest, the higher PRS_{QT} in genotype negative patients compared with genotype positive patients was reflected by the larger difference in PRS_{QT} between genotype negative patients versus controls (Table 3; meta-analysis β=0.735, SE=0.074; P=2.24×10^{-23}) compared with genotype positive versus controls (Table 3; meta-analysis β=0.294, SE=0.028; P=1.09×10^{-25}).

Common Variant Heritability of LQTS

To evaluate the proportion of variance in LQTS susceptibility explained by common genetic variants (h^2_{SNP}) we used GCTA-GREML.[32,37] Assuming a disease prevalence of 0.04%,[1] the SNP heritability estimate on the liability scale was h^2_{SNP}=0.148 (SE=0.019 [95% CI, 0.111–0.185]; P=5.0×10^{-18}) in the overall European LQTS dataset. h^2_{SNP} was similar when the analysis was restricted to genotype positive patients with LQTS. Similar results were also observed in the Japanese dataset and when using the phenotype-correlation genotype-correlation regression[34] and the GREML estimation implemented in LDAK,[35] as well as when we restricted h^2_{SNP} analyses to only patients with a pathogenic or likely-pathogenic variant (Table XII in the Data Supplement).

Association Analyses of Single SNPs and PRS_{QT} With LQTS Severity

To identify genetic modifiers of disease severity we conducted a GWAS for QT-interval within the LQTS cases which did not uncover any genome-wide significant

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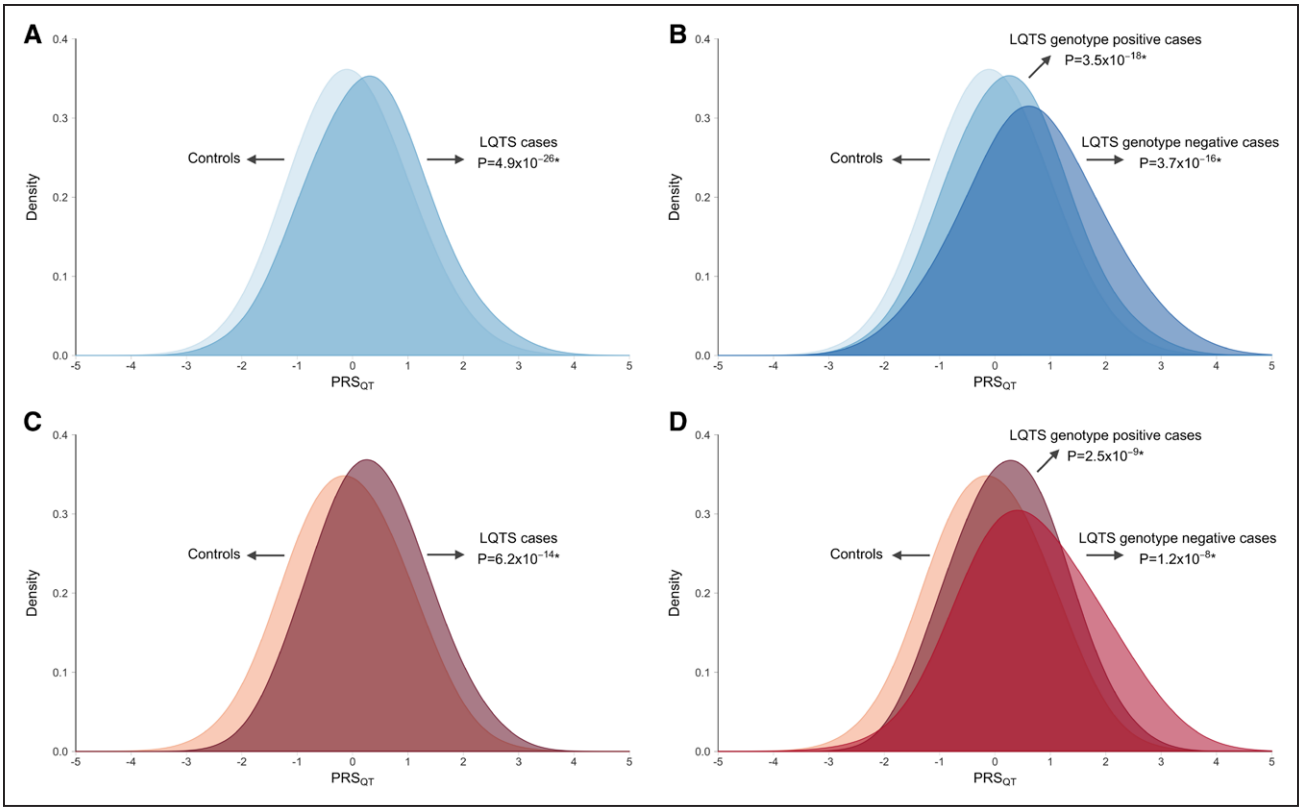

Figure 4. Distribution of QT polygenic score in controls, long QT syndrome, and genotype positive and negative subgroups.
The *x* axis represents the QT polygenic score (PRS_{QT}) in the European (**A** and **B**; blue) and Japanese (**C** and **D**; red) long QT syndrome (LQTS) case–control datasets. In **A** and **C**, all LQTS cases are grouped regardless of whether they are genotype positive or negative, whereas in **B** and **C**, cases have been stratified in genotype positive and negative LQTS subgroups. PRS_{QT} was normalized to a mean of 0 and standard deviation of 1. Reported *P* values refer to the effect of PRS_{QT} in a logistic regression correcting for the first 10 principal components. *Refers to case–control association. Comparison of PRS_{QT} between genotype negative versus genotype positive LQTS uncovered a significantly higher PRS_{QT} in genotype negative patients. This effect was consistently observed in both the European ($P=5.1\times10^{-6}$) and the Japanese ($P=2.0\times10^{-3}$) patients (Table 3).

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loci (Figure X in the Data Supplement). None of the 68 SNPs previously associated with QTc in the general population[29] showed association with QTc after Bonferroni correction. PRS_{QT} showed a weak positive correlation with QTc in the European cases (correlation coefficient [r]=0.06; *P*=0.042; Figure XI in the Data Supplement). In a multivariable linear regression model including clinical covariates associated with QTc (age at ECG recording, LQTS type, and sex), PRS_{QT} was not significantly associated with QTc (Table XIII in the Data Supplement). Similarly, in a subanalysis restricted to probands (comprising 97% of the total of unrelated LQTS cases) using the multivariable linear regression model, PRS_{QT} was not significantly associated with QTc (data not shown). In exploratory subgroup analyses, PRS_{QT} was independently associated with QTc in *KCNH2* rare variant carriers but not in *KCNQ1* rare variant carriers (Table XIII in the Data Supplement). This result was not replicated in the Japanese LQTS dataset. PRS_{QT} was not significantly associated with time to LAE in neither Europeans nor Japanese cases (Figure XII in the Data Supplement).

DISCUSSION

Our findings establish an important role for common genetic variation in LQTS susceptibility and support a complex (polygenic) architecture in genotype negative LQTS. Case–control GWAS identified 3 genome-wide significant risk loci near *NOS1AP*, *KCNQ1*, and *KLF12*. Heritability analysis demonstrated that ≈15% of LQTS disease liability is attributable to common genetic variation. PRS analysis testing the aggregate effect of SNPs previously associated with QT-interval in the general population (PRS_{QT}) identified a higher PRS_{QT} in LQTS cases compared with controls and higher PRS_{QT} in genotype negative versus genotype positive LQTS.

Shared Genetics of LQTS and QT-Interval in the General Population

The case–control GWAS uncovered 3 genetic LQTS susceptibility loci at genome-wide statistical significance near *NOS1AP*, *KCNQ1*, and *KLF12*, and 1 missense variant in *KCNE1* at the suggestive threshold (Figure 2).

Table 3. Association of QT Polygenic Score With Long QT Syndrome

Association Analysis of PRS_{QT}	European				Japanese				Meta-analysis			
	n	β	SE	P	n	β	SE	P	n	β	SE	*P*
All LQTS vs Controls	1238/ 8219	0.322	0.030	4.93E−26	418/ 1671	0.412	0.055	6.16E−14	1656/9890	0.343	0.0263	1.08E−38
Genotype positive LQTS vs Controls	1115/ 8219	0.277	0.032	3.47E−18	356/ 1671	0.348	0.058	2.52E−09	1471/ 9890	0.294	0.028	1.09E−25
Genotype negative LQTS vs Controls	123/ 8219	0.733	0.090	3.74E−16	62/ 1671	0.740	0.129	1.19E−08	185/9890	0.735	0.0738	2.24E−23
Genotype negative vs Genotype positive LQTS	123/ 1115	0.447	0.098	5.05E−06	62/356	0.401	0.129	2.01E−03	185/1471	0.430	0.078	3.54E−08
Genotype negative vs Genotype positive LQTS*	123/ 1115	0.409	0.103	7.36E−05	62/356	0.393	0.130	2.62E−03	185/1471	0.403	0.0807	6.05E−07

β indicates regression coefficient; n, sample size (cases/controls); *P*, *P*-value; PRS_{QT}, QT polygenic score; and SE, standard error.
*Correcting for QTc.

The association of SNPs at *KCNQ1* points to the involvement of common variants acting alongside rare variants in these genes in mediating disease susceptibility, akin to what was previously reported for common and rare variation in and around the *SCN5A* gene in Brugada syndrome.[16] All 4 risk loci had been previously implicated in genetic control of the QT-interval by GWAS in the general population.[29] For the 68 SNPs associated with QT-interval in the general population, we noted a strong positive correlation between their effect on QT-interval (obtained in the general population) and their OR for LQTS susceptibility, indicating, as expected, that the larger the effect a SNP has on the QT-interval, the more it increases LQTS susceptibility (Figure 3). The strong genetic correlation between LQTS susceptibility and QT-interval in the general population provides quantitative support for genetic overlap (Figure IX in the Data Supplement).

The association with the highest effect in the case–control GWAS was found for the p.Asp85Asn missense variant in *KCNE1* (rs1805128). This variant increased susceptibility for LQTS in the overall cohort but had a more prominent effect in genotype negative LQTS with an OR of ≈7 versus an OR of 2 in genotype positive patients. This variant has an allele frequency of ≈1.2% in non-Finnish Europeans and ≈0.5% in East Asians, and has the largest effect size on the QT in the general (European descent) population (7.42 ms per minor [Asn] allele).[29] It has been shown to be enriched in patients with drug-induced torsades de pointes.[38]

Genetic Architecture of Genotype Positive LQTS

LQTS has traditionally been viewed as a monogenic disorder mostly attributed to a rare variant with a drastic effect on ion channel function. We now demonstrate that a considerable extent (≈15%) of disease liability is attributable to common genetic variation. In genotype positive LQTS families, where the penetrance of pathogenic variants may be low for certain variants,[8] the contribution of common variants to disease susceptibility may also contribute to variable disease penetrance. It has been well established that LQTS probands have a longer QT-interval and greater arrhythmic risk compared with family members carrying the same variant.[7,13,39] This observed increased penetrance in probands may result from a greater burden of common QT-prolonging variants compared with other, less-severely affected, or unaffected mutation-carriers. However, because this study comprised only unrelated patients, this remains to be determined. Whether the PRS_{QT} could discriminate between affected versus unaffected mutation carrier family members is intuitively appealing but remains to be formally demonstrated.

Genotype Negative LQTS, A Polygenic Subtype of LQTS?

PRS analysis, testing the aggregate effect of SNPs previously associated with QT-interval in the general population (PRS_{QT}), identified a higher PRS_{QT} in genotype negative versus genotype positive patients. This observation points to genotype negative LQTS, comprising ≈10% of patients with LQTS, as a polygenic subtype of the disorder where the underlying etiology involves, at least in part, a high burden of common QT prolonging alleles. As such, genetic susceptibility in genotype negative patients may not be determined to a large extent by 1 strong genetic factor as occurs in genotype positive patients but results from the accumulation of multiple variants (polygenic inheritance). The lower rate of family history of sudden cardiac death in genotype negative patients with LQTS is in line with polygenic inheritance. Our observations corroborate findings in other heritable phenotypes, such as familial hypercholesterolemia, where patients without a disease-causing variant in the *LDLR*, *APOB*, and *PCSK9* genes have a higher PRS based on low-density lipoprotein modulating variants in comparison with those with rare familial hypercholesterolemia causing genetic variants.[40] As such, the accumulation of multiple discrete common variants may confer

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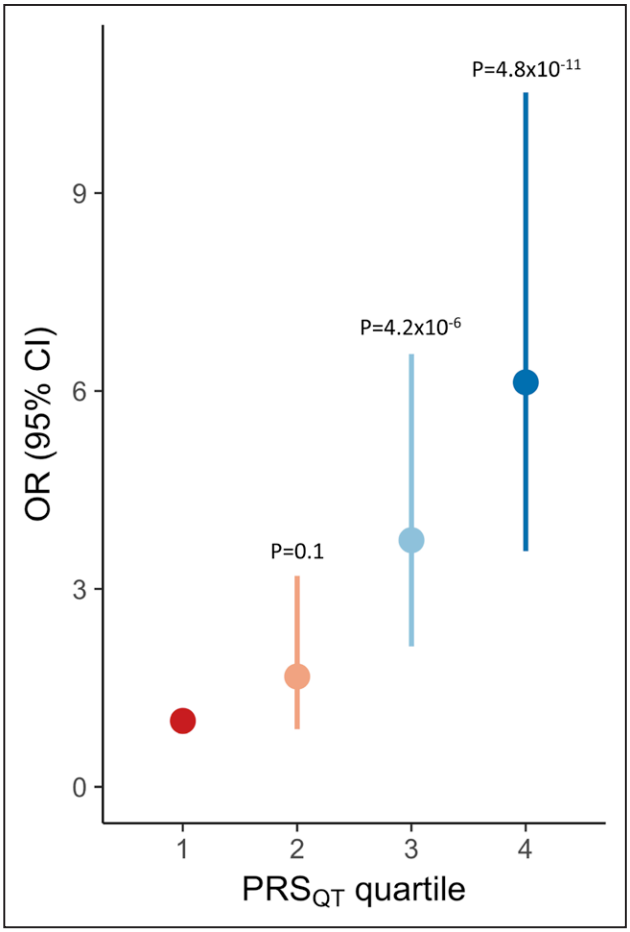

Figure 5. Increasing long QT syndrome risk with increasing QT polygenic score quartiles.
Odds ratio (OR) for genotype negative long QT syndrome (LQTS; filled circles) and 95% CI (vertical bars) associated with each QT polygenic score (PRS_{QT}) quartile taking the first PRS_{QT} quartile as the reference. Data shown correspond to a meta-analysis of effects computed separately in the European and Japanese datasets. *P* values refer to comparison of each quartile against the first quartile.

risk similar to a monogenic mutation. This was recently demonstrated for common disorders such as coronary artery disease and atrial fibrillation, where individuals at the upper extreme of the PRS distribution had a risk of developing the disease reportedly comparable with carriers of a monogenic mutation.[31] The overlap in the PRS_{QT} distributions among genotype negative LQTS cases and controls (Figure 4) suggests that other factors are involved, possibly including low-frequency genetic variants with intermediate effect sizes as well as other common variants with smaller effect sizes.

In addition to providing insight into the genetic architecture of genotype negative LQTS, we here also describe for the first time the natural history in these patients. All ≈200 genotype negative patients with LQTS met diagnostic criteria for definite LQTS (ie, QTc>500ms or LQTS score≥3.5) and underwent sequencing of the unequivocal nonsyndromic LQTS genes. Genotype negative patients with LQTS had a higher QTc in comparison with patients with LQT1–3 but similar event-free survival as their genotype positive counterparts (Figure 1). The effect of established clinical risk factors, for example sex and QTc-duration, did not significantly differ between genotype positive and negative (no interaction effect) suggesting they may also be used to stratify risk of events in genotype negative LQTS.

Common Variants Do Not Contribute to LQTS Severity Within Probands

We sought to identify genetic modifiers of LQTS. In contrast to the case–control GWAS, GWAS for QTc and arrhythmic events within the unrelated LQTS cases did not uncover any genome-wide significant locus. PRS_{QT} was also not significantly associated with QTc nor with the occurrence of events. At first glance, this may seem contradictory to previous studies in LQTS that demonstrated a modulatory effect of SNPs at *NOS1AP* on the QTc and arrhythmic events,[10,11,13] as well as a study in the general population that showed a modulatory effect of PRS derived from previous GWAS on QT-interval.[41] For example, a study we previously conducted in patients with LQT2 uncovered strong associations with large effect sizes (>12 ms/allele) for SNPs at *NOS1AP*.[13] An important difference however, is that the current study did not include family members but only 1 patient per family (97% probands), whereas the previous studies considered both probands and genotype positive relatives. Conceptually, inclusion of both probands and relatives results in greater variation in QTc and is thus expected to increase statistical power for detection of modulatory effects. Moreover, the different rare variants in the patients we studied here are associated with biophysical defects of varying severity. As such, they are also expected to contribute to interindividual variability which is difficult to account for. For instance, patients with LQT2 with pore-region variants are known to be more severely affected than other patients with LQT2.[42] Indeed, in a subanalysis, restricted to European patients with LQT2, where we accounted for the mutation location (pore versus nonpore), we detected an association of PRS_{QT} with QTc. In sum, our data show that common variants do not affect disease severity across all probands studied. Further studies are needed to explore their predictive role in family members.

Potential Clinical Implications

In genotype negative LQTS, disease susceptibility estimation for relatives does not follow a Mendelian pattern. In our cohort, a positive family history of sudden cardiac death was less often observed in genotype negative individuals compared with genotype positive ones, suggesting that risk for family members in genotype negative patients may be lower. Polygenicity in genotype negative individuals implies that risk is not

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primarily attributable to 1 genetic factor inherited from 1 of the biological parents as is the case for autosomal dominant LQTS. In such cases, cascade screening may necessitate clinical evaluation of both maternal and paternal family members. Future clinical utility of genetic testing based on polygenic inheritance necessitates the availability of polygenic risk scores with high discriminative capacity. The discriminative capacity of a PRS based on QT modulating SNPs is expected to improve as knowledge concerning variants that modulate the QT-interval become known, for example through larger GWAS studies, or by combining it with nongenetic modifiers. In a recent study, a PRS based on 61 QT-SNPs (a subset of the 68 QT modulating SNPs included in the PRS_{QT} used herein) explained a substantial proportion of QT-interval response to QT-prolonging drugs in a trial of 3 QT-prolonging drugs conducted in healthy individuals, as well as risk of torsade de pointes in a case–control study.[43] This provides further support to a liability threshold model whereby multiple factors, genetic and nongenetic, impact on cardiac repolarization and determine arrhythmic risk. In this respect, calculation of PRS_{QT} for the purpose of preventive avoidance of QT-prolonging drugs may be desirable for relatives of genotype negative LQTS. It is clear that further studies are needed to address how testing for polygenic susceptibility may become clinically useful.

Study Limitations

Although in genotype negative patients with LQTS we performed sequencing of the coding region of nonsyndromic LQTS genes, this may have missed copy number variation or disease-causing variants in the noncoding region[44] of established genes as well as mutations in yet unknown disease genes. This may have blunted the differences between genotype negative and genotype positive patients and thus would not affect the study conclusions. Despite being the largest international dataset of unrelated patients with LQTS published to date, the study had limited statistical power to detect lower effect associations at GWAS significance threshold. The prespecified design of meta-analyzing European and Japanese GWAS may also miss disease loci with differences in haplotype structure among European and East Asian chromosomes. Nonetheless, GWAS in separate ancestries did not detect any association at GWAS threshold. Last, studies in larger patient sets are required to further refine our understanding of the genetic architecture underlying LQTS in genotype negative patients.

Conclusions

This work establishes an important role for common genetic variation in susceptibility to LQTS. Common genetic variation affecting the QT-interval in the general population contributes to disease susceptibility in both genotype positive and genotype negative LQTS. The role of common variants is predominant in genotype negative LQTS, suggesting that the latter may constitute a polygenic form of LQTS. Increasing burden of QT-prolonging common variants (eg, PRS_{QT}) is associated with higher susceptibility for LQTS but is not associated with disease severity within LQTS probands. Further studies are needed to assess the role of polygenic risk within LQTS families.

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Supplemental Materials

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