Transethnic genome-wide association study provides insights in the genetic architecture and heritability of long QT syndrome

Running title: Genome-wide association study in long QT syndrome

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ABSTRACT

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 (GWAS) followed by transethnic meta-analysis in 1,656 unrelated LQTS patients of European or Japanese ancestry and 9,890 controls to identify susceptibility single nucleotide polymorphisms (SNPs). We estimated the SNP heritability (h^2_{SNP}) of LQTS and tested the genetic correlation between LQTS susceptibility and other cardiac traits. Furthermore, we tested the aggregate effect of the 68 SNPs previously associated with QTc in the general population using a polygenic risk score (PRS_{QT}).

Results: Genome-wide association analysis identified three loci associated with LQTS at genome-wide statistical significance ($P<5\text{x}10^{-8}$) near *NOS1AP*, *KCNQ1* and *KLF12,* and one 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 [SE] 0.019). LQTS susceptibility showed a strong genome-wide genetic correlation with the QT interval in the general population ($r_g=0.40$, $P=3.2\text{x}10^{-3}$). PRS_{QT} was greater in LQTS cases compared to controls ($P<10^{-13}$), and notably, among LQTS patients PRS_{QT} was greater in genotype negative compared to genotype positive patients ($P<0.005$).

Conclusion: 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.

Key words: long QT syndrome, genome-wide association study, polygenic risk score, heritability, QT-interval

Non-standard Abbreviations and Acronyms

ACMG - American College of Medical Genetics and Genomics

AMP - Association of Molecular Pathology

GWAS - Genome-wide association study

HRC - Haplotype reference consortium

gnomAD - Genome Aggregation Database

LAE - Life-threatening arrhythmic event

LQTS - Long QT syndrome

MAF - Minor allele frequency

PRS - Polygenic risk score

QC - Quality control

SCD - Sudden cardiac death

SNP - Single nucleotide polymorphisms

VUS - Variant of unknown significance

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 remain genetically elusive despite genetic testing of established genes (i.e. genotype negative).

What are the clinical implications?

- 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 one genetic factor inherited from one 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.

INTRODUCTION

Long QT syndrome (LQTS) is a rare inherited disorder of ventricular repolarization characterized by prolongation of the QT interval on the electrocardiogram (ECG).[1,2] LQTS has a prevalence of approximately 1 in 2500, and is a major and often preventable cause of sudden cardiac death (SCD) 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 (i.e. mutations) in the three major LQTS genes, i.e. *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 (i.e. monogenetic) might be an oversimplification of biological phenomena. It is likely that a combination of genetic and non-genetic 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, hypokalaemia, 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 pre-symptomatic identification of affected relatives.

Although a small proportion of such genotype negative LQTS patients 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 genome-wide association study (GWAS) comparing cases of a rare arrhythmia syndrome to unaffected controls can define modulators of disease susceptibility and suggest a polygenic etiology.[16] We report here a GWAS in ~1,700 unrelated LQTS patients 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 upon request.

Study population

We established an international consortium allowing recruitment of 1,781 unrelated LQTS patients: 1,344 cases of European ancestry from 23 referral centres in Europe, New Zealand and North America, as well as 437 patients of East Asian ancestry from 4 referral centres in Japan (**Table I in the Supplement**). Included unrelated individuals were probands (97%) except when DNA was not available, in which case one other affected family member was included instead. Included LQTS patients had a clinical diagnosis of LQTS[5] and were classified as "genotype positive" if they carried a single rare variant in one of the three established major LQTS genes [*KCNQ1* (LQT1), *KCNH2* (LQT2) and *SCN5A* (LQT3)], or "genotype negative" if no rare variant was identified in genes unequivocally associated with non-syndromic LQTS (*KCNQ1*, *KCNH2*, *SCN5A*, *CALM1-3* and *TRDN*).[17–19] A rare variant was defined as a protein sequence altering (i.e. 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 (gnomAD).[20–22] Genetic testing and variant curation as per the American College of Medical Genetics and Genomics and Association of Molecular Pathology (ACMG/AMP) guidelines[23] was conducted as in the **Supplementary Methods**. 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 centres. We collected a baseline ECG for each patient, preferably not during beta-blocker use. The QT-interval duration was measured as previously described (**Figure I in the Supplement, Supplementary**

Methods).[24] In genotype negative patients, a LQTS diagnosis was additionally curated by 2 clinicians (NL, RT) and in case of uncertainty, two 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 8,219 control individuals of European ancestry and 1,671 individuals of Japanese ancestry were obtained from different cohorts (**Table II in the Supplement**). Quality control (QC)[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 (GRCh37).

After QC (see **Supplementary Methods** 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 MAF>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 PC 1-10. Quantitative trait analyses for QTc were conducted using a linear regression model correcting for age, beta-blocker use at ECG, LQTS type ([*KCNQ1 (LQT1)*, *KCNH2 (LQT2)* and *SCN5A (LQT3),* or genotype negative), sex, and PC 1-10. 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 $P<5x10^{-8}$ and $P<1x10^{-6}$, 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 document LAE, the last visit or the 41st birthday, whichever came first. LAE were defined as out of hospital cardiac arrest (OHCA) or hemodynamically unstable ventricular tachycardia/ventricular fibrillation (VT/VF) or appropriate implantable cardioverter-defibrillator (ICD) therapy. The effect of genotype positive versus genotype negative status was estimated using Cox proportional hazards regression with/without adjustment for classic risk factors, i.e. sex and $QTc \geq 500$ 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 (i.e. during the follow up period prior to 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 (PRS)

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 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 PC 1-10. We also used P-value thresholding and R2 pruning with P-values of $5x10^{-8}$, $1x10^{-5}$, $1x10^{-4}$ and $1x10^{-3}$, $1x10^{-2}$ and R2 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 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 life-threatening arrhythmic events (LAE) 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[32] to estimate how much of the variance in LQTS susceptibility could be attributed to common genetic variants (SNP-based heritability, h^2_{SNP}). Prior to heritability estimation, we conducted additional stringent genetic QC, as previously suggested (**see Supplementary Methods**).[33] We estimated the SNP-heritability on the liability scale assuming a 0.04% prevalence with

PC1-10 as covariates.[1] We assessed the robustness of heritability estimates from GCTA-GREML using the generalized restricted maximum likelihood (REML) and PCGC (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 LQTS patients using the approaches implemented in GCTA or LDAK.

Genetic correlation with other traits

We used bivariate LD score regression[36] to evaluate the genetic correlation between LQTS susceptibility (as obtained in the European descent case-control GWAS) and other cardiac electrical traits[2], namely PR, QRS, QT, heart rate (HR) at rest, HR in response to exercise and recovery, and atrial fibrillation (AF) (see **Supplementary Methods** 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 of the Supplement** separately for the European and Japanese datasets and in **Table 1** for the combined cohort. We included a total of 1,781 unrelated LQTS patients of European (n=1,344, mean QTc ± standard deviation (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 (i.e. genotype negative) despite extensive genetic testing.

The mean QTc interval in genotype negative cases was higher in comparison to genotype positive ones (500±52ms vs. 482±47ms, $P=2x10^{-5}$) and in genotype negative cases a family history of SCD <50 years in 1st and 2nd degree relatives was less frequent compared to genotype positive ones (12.7% vs. 22.9%, P=0.001). Of the 1584 genotype positive cases, 1333 (84%) carried an ACMG pathogenic or likely-pathogenic variant and the remainder had a VUS. The QTc did not significantly differ between carriers of VUS and those with a pathogenic or likely pathogenic variant (P=0.9).

In total, 429 cases (24%) had a life-threatening arrhythmic event (LAE) at a median age of 28 [17-46] 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; $P=4x10^{-6}$) and Japanese ancestry (OR 1.4; P=0.03) were independent risk factors for LAE (**Table V in the Supplement**). We found a significant sex-puberty interaction ($P=1x10^{-6}$), where males were at higher risk of LAE in the pre-pubertal years but lower risk thereafter (**Figure II in the 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 (Logrank test $P<0.001$; **Figure III in the Supplement**). Cases with a rare variant in *KCNQ1* had a lower risk of LAE compared to *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 VUS and those with pathogenic or likely pathogenic variant (**Figure IV in the Supplement**).

Case-control GWAS

We conducted a case-control GWAS separately in European (1,238 cases vs. 8,219 controls, genomic test inflation (λ) =1.024) and Japanese (418 cases vs. 1,617 controls, λ=1.034) cases using ancestry-matched controls (**Figure V and VI in the 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, Figure VII and VIII in the Supplement**). The most significant association was obtained for rs12143842 (OR=1.32, 95% confidence interval (CI): 1.21-1.42, $P=1.09\times10^{-11}$) located upstream of *NOS1AP* (**Figure VIII-a in the 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, $P=1.92\times10^{-8}$ (**Figure VIII-b in the Supplement**). At the third locus, the lead SNP, rs17061696 (OR=1.25, 95%CI: 1.15-1.35, $P=4.33\times10^{-8}$), was located in an intron of *KLF12* (**Figure VIII-c in the Supplement**). All three loci had been previously associated with the QT-interval duration, a measure of myocardial repolarization on the electrocardiogram (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, $P=5.31\times10^{-7}$, **Figure VIII-d in the 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 (MAF=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=5.99x10^{-8}) than in genotype positive LQTS (OR=2.28, 95%-CI: 1.46-3.54, P=2.59x10^{-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 (i.e. P<0.05), while only 4 would be expected under the null hypothesis (**Table VI in the 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=2.04x10^{-17}) and the Japanese (**Figure 3b**; R^2=0.52; P=1.43x10^{-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 LD score regression[36], which detected a significant positive genetic correlation (r_g=0.40, SE=0.14, P=3.2x10^{-4}) between these phenotypes. No significant correlation was found for other cardiac electrical traits (**Figure IX in the 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 polygenic risk score (PRS) analysis (**Table III in the Supplement**). PRS_{QT} was 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=1.1x10^{-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 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 VIII-a in the Supplement**). Similarly, Japanese ancestry-specific PRS derived from summary statistics of a small Japanese QT-interval GWAS[30] had less discriminative accuracy in the Japanese case-control dataset compared to the European-derived $PRS_{QT,}$ likely due to the small size of the Japanese QT-interval GWAS (**Table VIII-b in the Supplement**).

We next explored whether the genetic architecture of genotype negative patients (i.e. 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 ($P=5.1x10^{-6}$, **Figure 4b**) and the Japanese ($P=2.0x10^{-3}$, **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 ($P=7.4x10^{-5}$ in Europeans; $P=2.6x10^{-3}$ 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 (ACMG/AMP) guidelines (i.e. excluding cases with a rare variant of unknown significance (VUS); **Supplementary Methods**, **Table IX and X in the Supplement**). Increasing PRS_{QT} quartiles were associated with a significantly higher disease susceptibility for genotype negative LQTS compared to the lowest quartile (**Figure 5**, **Table XI in the Supplement**). Notably, using a PRS_{QT} percentile threshold of 80, 90 and 95, individuals above the threshold compared to 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 to genotype positive patients was reflected by the larger difference in PRS_{QT} between genotype negative patients vs. controls (**Table 3;** meta-analysis β=0.735, SE=0.074, P=2.24x10^{-23}) compared to genotype positive vs. controls (**Table 3;** meta-analysis β=0.294, SE=0.028, P=1.09x10^{-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 the generalized restricted maximum likelihood (GREML) approach of GCTA.[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=5.0x10^{-18}) in the overall European LQTS dataset. h^2_{SNP} was similar when the analysis was restricted to genotype positive LQTS patients. Similar results were also observed in the Japanese dataset and when using the PCGC (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 Supplement**).

Association of single SNPs and $\mathrm{PRS}_{\mathrm{QT}}$ 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 loci (**Figure X in the Supplement**). None of the 68 SNPs previously associated with QTc in the general population[29] showed association with QTc after Bonferroni correction. $\mathrm{PRS}_{\mathrm{QT}}$ showed a weak positive correlation with QTc in the European cases (correlation coefficient [r]=0.06; P=0.042; **Figure XI in the Supplement**). In a multivariable linear regression model including clinical covariates associated with QTc (age at ECG recording, LQTS type and sex), $\mathrm{PRS}_{\mathrm{QT}}$ was not significantly associated with QTc (**Table XIII in the Supplement**). Similarly, in a sub-analysis restricted to probands (comprising 97% of the total of unrelated LQTS cases) using the multivariable linear regression model, $\mathrm{PRS}_{\mathrm{QT}}$ was not significantly associated with QTc (**data not shown**). In exploratory subgroup analyses, $\mathrm{PRS}_{\mathrm{QT}}$ was independently associated with QTc in *KCNH2* rare variant carriers but not in *KCNQ1* rare variant carriers (**Table XIII in**

the 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 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. Polygenic risk score 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 to controls and higher PRS_{QT} in genotype negative vs. genotype positive LQTS.

Shared genetics of LQTS and QT interval in the general population

The case-control GWAS uncovered three genetic LQTS susceptibility loci at genome-wide statistical significance near *NOS1AP*, *KCNQ1* and *KLF12*, and one missense variant in *KCNE1* at the suggestive threshold (**Figure 2**). 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 odds ratio 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 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 odds ratio of ~7 (versus an odds ratio 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 seen 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 to 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 to other, less-severely affected or unaffected mutation-carriers. However, since this study was comprised of only unrelated patients, this remains to be determined. Whether the PRS_{QT} could discriminate between affected vs. unaffected mutation carrier family members is intuitive appealing but remains to be formally demonstrated.

Genotype negative LQTS: A polygenic subtype of LQTS?

Polygenic risk score 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 vs. genotype positive patients. This observation points to genotype negative LQTS, comprising ~10% of LQTS patients, 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 one 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 SCD in genotype negative LQTS patients is in line with polygenic inheritance. Our observations corroborate findings in other heritable phenotypes, such as familial hypercholesterolemia (FH), where patients without a disease-causing variant in the *LDLR*, *APOB* and *PCSK9* genes have a higher PRS based on LDL modulating variants in comparison to those with rare FH causing genetic variants.[40] As such, the accumulation of multiple discrete common variants may confer 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 to 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 LQTS patients met diagnostic criteria for definite LQTS (i.e. QTc>500ms or LQTS score ≥3.5) and underwent sequencing of the unequivocal non-syndromic LQTS genes. Patients with genotype negative LQTS had a higher QTc in comparison to LQT1-3 patients but similar event-free survival as their genotype positive counterparts (**Figure 1**). The effect of established clinical risk factors, i.e. 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 prior GWAS on QT-interval.[41] For example, a study we previously conducted in LQT2 patients 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 one 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, LQT2 patients with pore-region variants are known to be more severely affected than other LQT2 patients.[42] Indeed, in a sub-analysis, restricted to European LQT2 patients, where we accounted for the mutation location (pore versus non-pore), 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 SCD was less often observed in genotype negative individuals compared to 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 primarily attributable to one genetic factor inherited from one 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 non-genetic modifiers. In a recent study, a PRS based on 61 QT SNPs (a subset of the 68 QT modulating SNPs included in the $\mathrm{PRS}_{\mathrm{QT}}$ 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 non-genetic, impact on cardiac repolarization and determine arrhythmic risk. In this respect, calculation of $\mathrm{PRS}_{\mathrm{QT}}$ for the purpose of preventive avoidance of QT-prolonging drugs may be desirable for relatives of genotype negative LQTS. Clearly, further studies are needed to address how testing for polygenic susceptibility may become clinically useful.

Study limitations

Although in genotype negative LQTS patients we performed sequencing of the coding region of non-syndromic LQTS genes, this may have missed copy number variation or disease-causing variants in the non-coding 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 patients and thus would not affect the study conclusions. Despite being the largest international dataset of unrelated LQTS patients published to date, the study had limited statistical power to detect lower effect associations at GWAS significance threshold. The prespecified design of meta-analysing 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. Finally, studies in larger patient sets are required to further refine our understanding of the genetic architecture underlying LQTS in genotype negative patients.

Conclusion

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 (e.g. 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

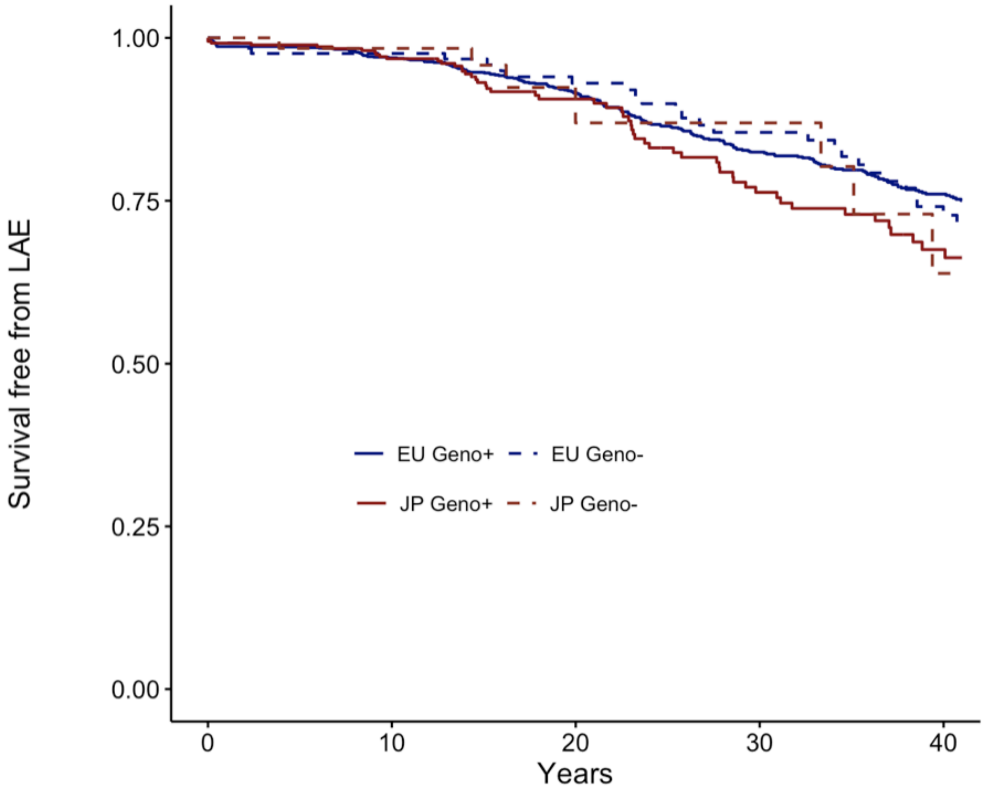
Expanded Methods

Online-only Figures I-XII

Supplemental Excel File with Supplementary Table I-XIII

References [45–57] (45-57)

Figure 1 | Kaplan-Meier LAE-free survival curves stratified by ancestry



Number at risk

Strata	0	10	20	30	40
EU Geno+	1209	1101	817	580	392
EU Geno-	130	116	95	73	55
JP Geno+	369	301	146	97	54
JP Geno-	66	54	16	13	7

Years

Figure 1 | Kaplan-Meier LAE-free survival curves stratified by ancestry. EU and JP refer to European and Japanese LQTS cases, respectively. Geno+ and Geno- refer to genotype positive and genotype negative LQTS cases, respectively. LAE, life-threatening arrhythmic event defined as the composite of out of hospital cardiac arrest or hemodynamically unstable ventricular tachycardia/fibrillation. Logrank test P=0.3.

Figure 2 | Manhattan plot of LQTS case-control meta-analysis

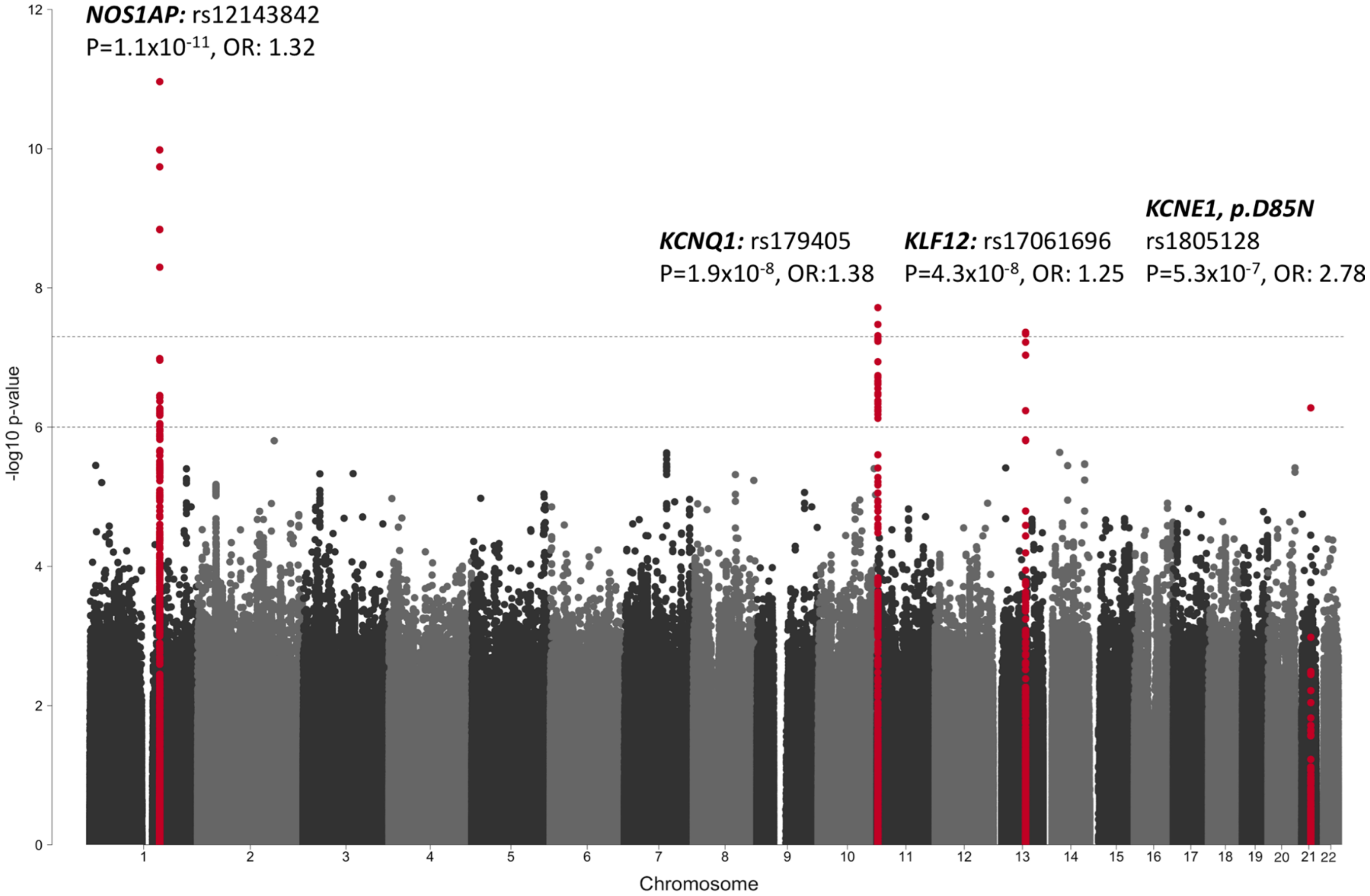
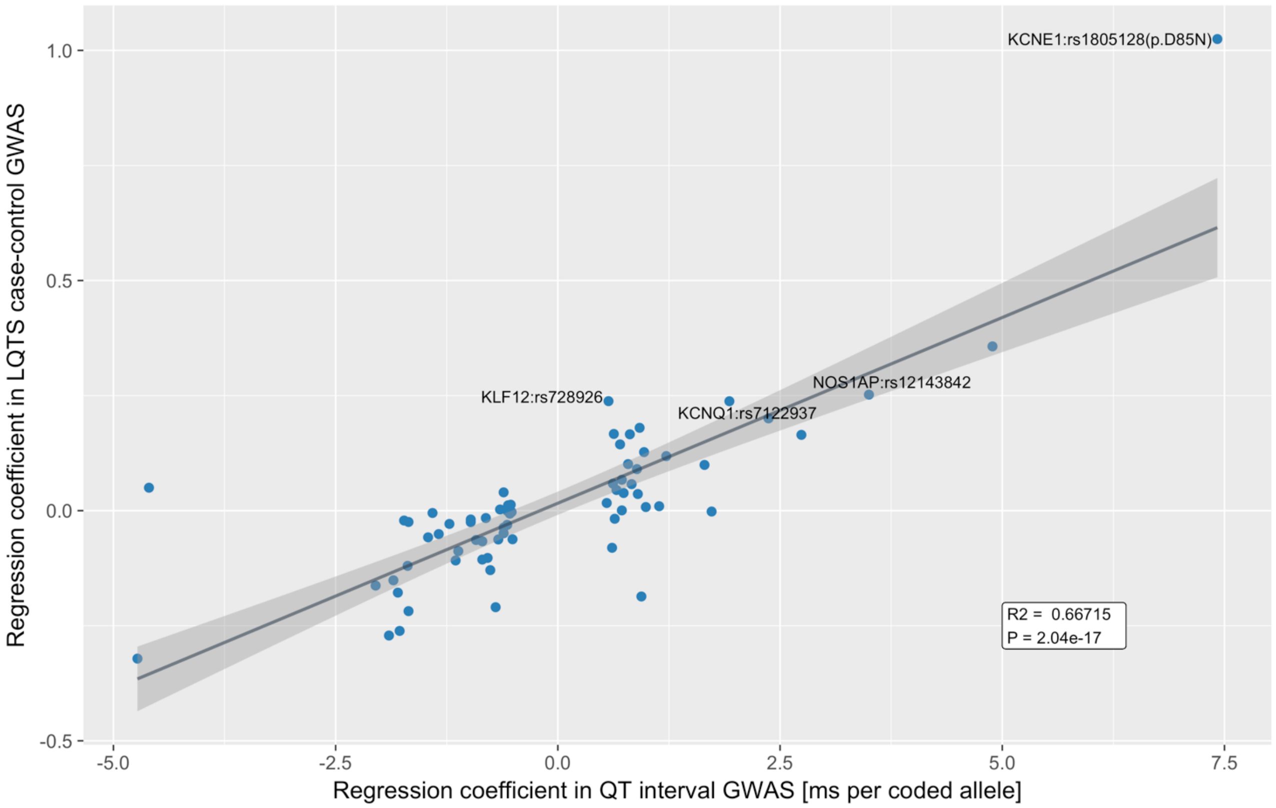


Figure 2 | Manhattan plot of LQTS case-control meta-analysis. Manhattan plot displaying the base-pair position of each of the tested SNPs (each dot represents an individual SNP) along the chromosomes on the *x* axis and the corresponding -log10 transformed association *P* value on the *y* axis. The association P-values from the meta-analysis of the two GWAS conducted separately in European and Japanese cases and controls, respectively, are displayed. The upper and lower dashed lines indicate the genome-wide significance ($P<5x10^{-8}$) and suggestive significance ($P<1x10^{-6}$) 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 since it is not well imputed and rare ($R^2<0.3$. MAF=0.001) in the Japanese dataset.

Figure 3 | Correlation of effect size of QT-associated SNPs with their effect size in LQTS GWAS

A. European LQTS dataset



B. Japanese LQTS dataset

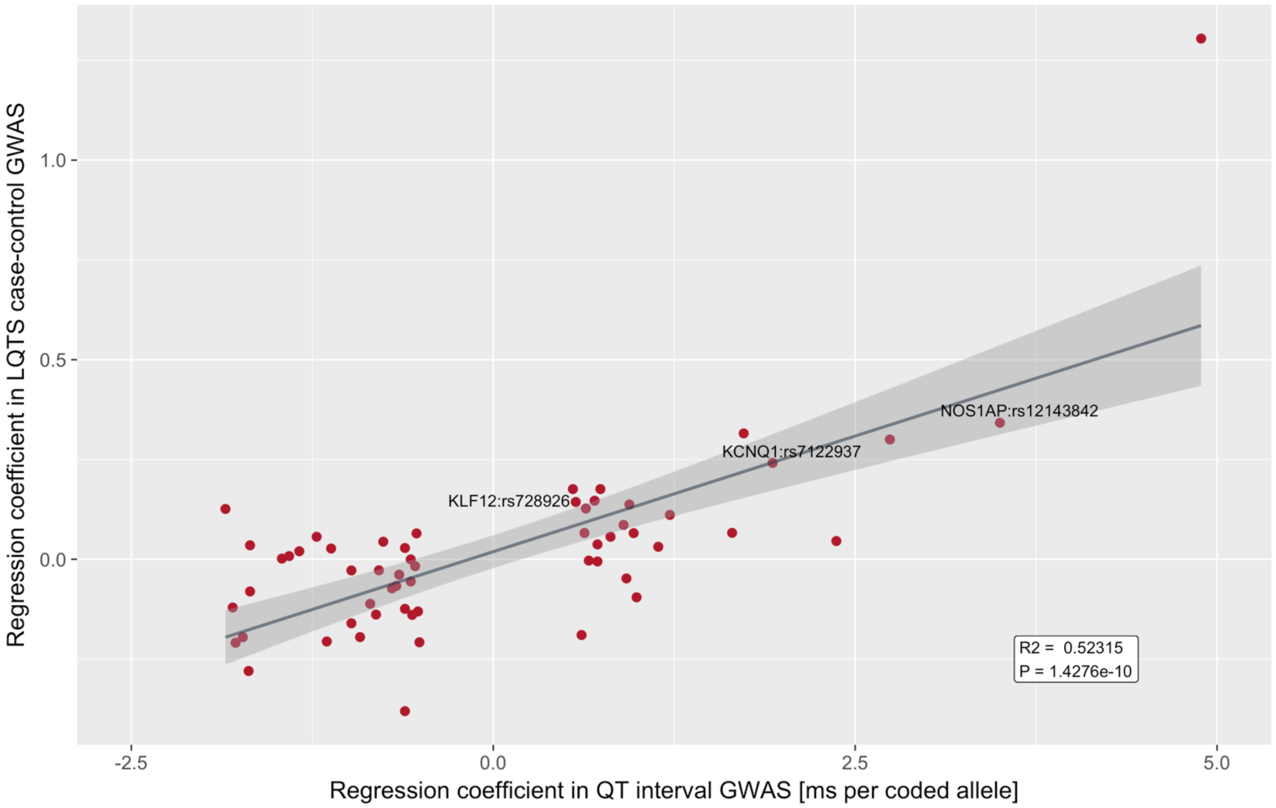


Figure 3 | Correlation of effect size of QT-associated SNPs with their effect size in LQTS GWAS. The x-axis represents the effect estimates from the QT-interval GWAS conducted in the general population (milliseconds per alternative allele) and the y-axis the effect of each of these QT-interval associated alleles on disease risk of LQTS [Ln(OR)] in the European (**A, blue**) and Japanese (**B, red**) 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 **Supplementary Table 6**). Loci that reached genome-wide significance in the LQTS cases-control transethnic meta-analysis (*NOS1AP*-rs12143842, *KCNQ1*-rs179405 and *KLF12*-rs728926) and *KCNE1*-rs1805128 are identified with text.

Figure 4 | Distribution of PRS_{QT} in controls, all LQTS and genotype positive and negative subgroups

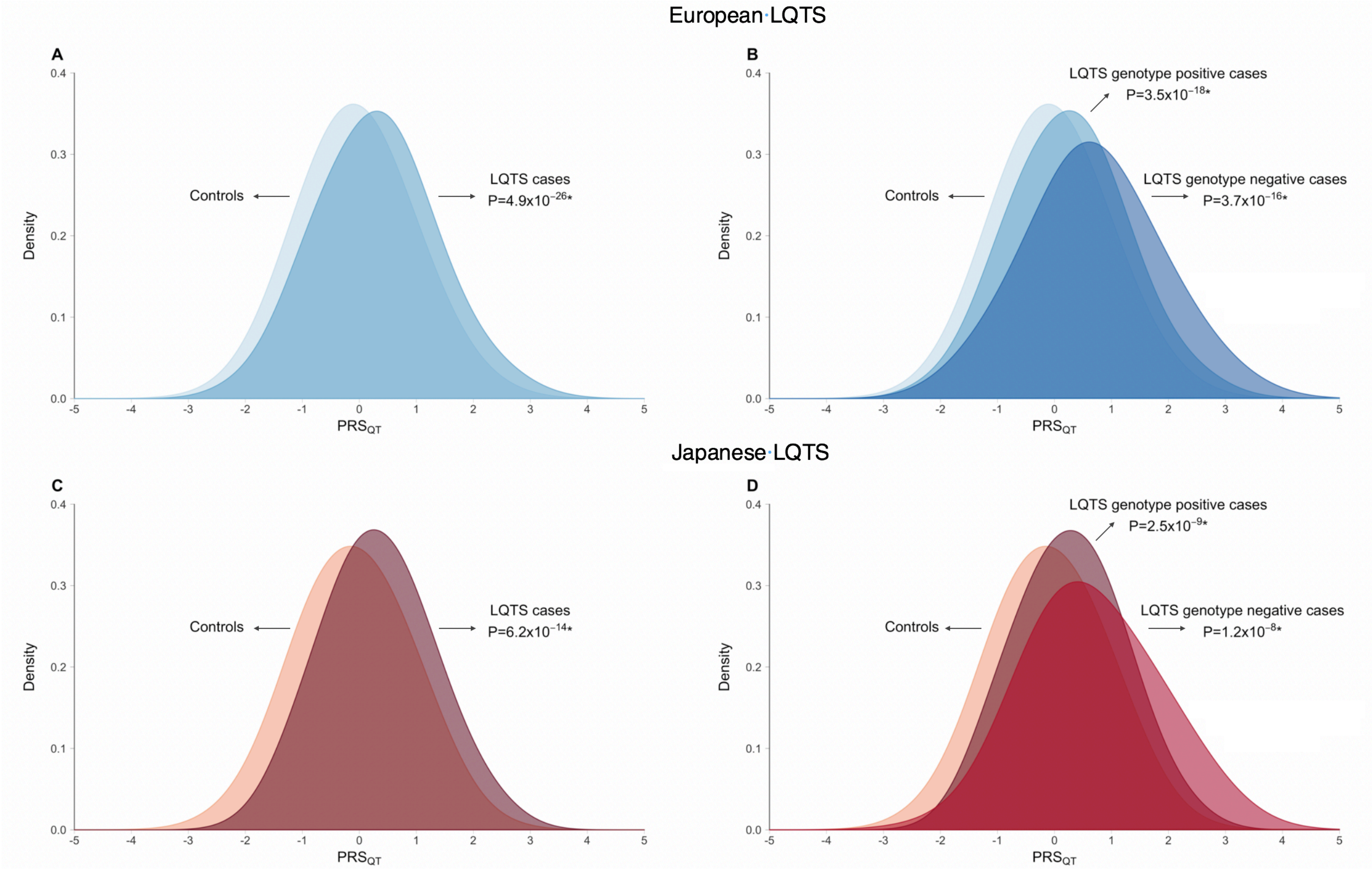


Figure 4 | Distribution of PRS_{QT} in controls, LQTS and genotype positive and negative subgroups. The X-axis represents the QT polygenic score (PRS_{QT}) in the European (**A-B**, blue) and Japanese (**C-D**, red) LQTS case-control datasets. In **A and C**, all LQTS cases are grouped regardless of 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 vs. genotype positive LQTS uncovered a significantly higher PRS_{QT} in genotype negative patients. This effect was consistently observed in both the European ($P=5.1\times10^{-6}$, **Figure 4b**) and the Japanese ($P=2.0\times10^{-3}$, **Figure 4d**) patients (**Table 3**).

Figure 5 | Increasing genotype negative LQTS risk with increasing PRS_{QT} quartiles

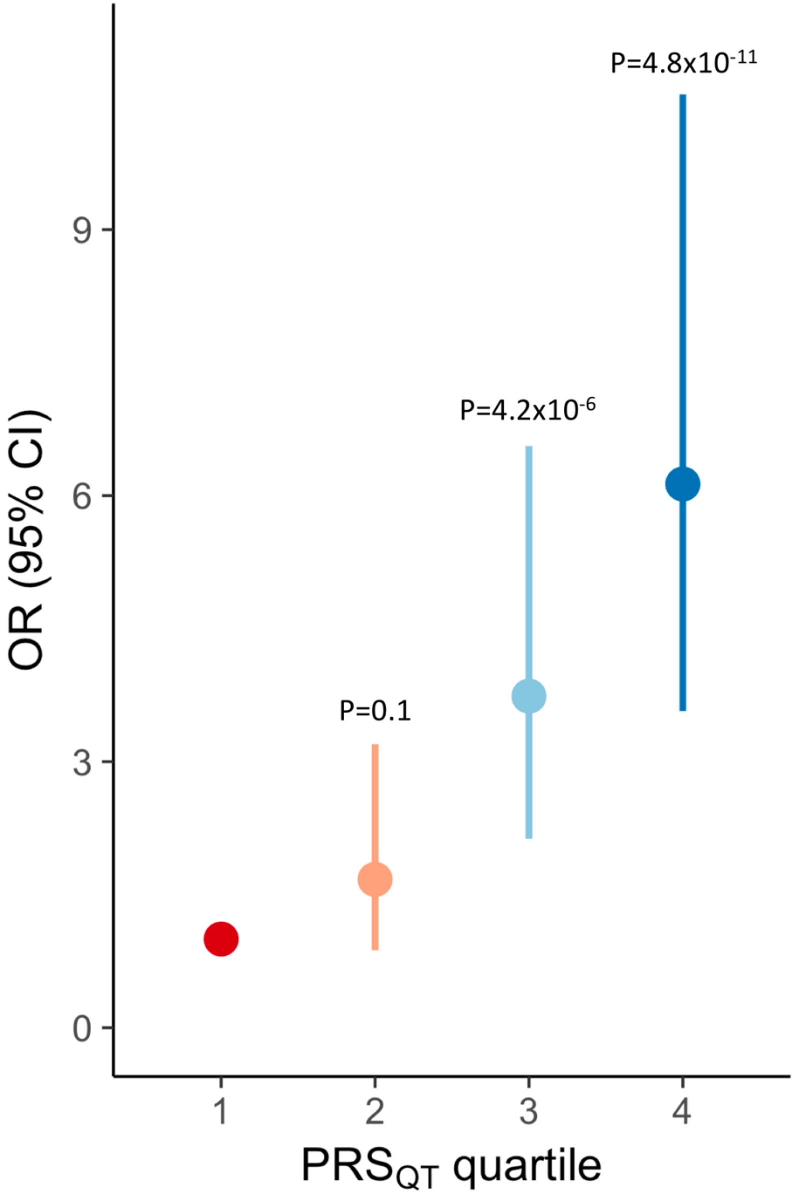


Figure 5 | Increasing LQTS risk with increasing $\mathrm{PRS_{QT}}$ quartiles. Odds ratio (OR) for genotype negative LQTS (filled circles) and 95% confidence intervals (vertical bars) associated with each $\mathrm{PRS_{QT}}$ quartile taking the first $\mathrm{PRS_{QT}}$ quartile as the reference. Data 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.

Table 1 | Clinical characteristics of all unrelated LQTS cases

Parameter	**Genotype positive (n=1584)**	**Genotype negative (n=197)**
Male, no. (%)	584/1584 (37)	76/197 (39)
QTc mean ± SD, ms	482±47	500±52
Genotype, no. (%)		
KCNQ1	800/1584 (50)	-
KCNH2	661/1584 (42)	-
SCN5A	123/1584 (7.8)	-
Syncope, no. (%)	722/1584 (46)	75/197 (38)
LAE (OHCA or VF/VT) before age 41, no. (%)	262/1578 (17)	33/196 (17)
Age at first LAE, median [IQR]	21 [13-29]	26 [16-35]
Treatment during follow-up, no. (%)		
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 years of age, no. (%)	323/1409 (23)	24/189 (13)

Table legend:

ICD, 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; VF/VT, hemodynamically unstable ventricular fibrillation/tachycardia

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

Table legend: 95%CI, 95% confidence interval; AAF, alternative allele frequency; GRCh37, genomic position on build GRCh37; 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 (LD) with rs7122937 (R2=0.497) which had been previously associated with QT-interval in the general population (1.9 ms increase per risk allele).

Table 3 | Association of PRS$_{QT}$ with LQTS

	European				**Japanese**				**Meta-analysis**			
Association analysis of PRS$_{QT}$	**n**	$\boldsymbol{\beta}$	**SE**	**P**	**n**	$\boldsymbol{\beta}$	**SE**	**P**	**n**	$\boldsymbol{\beta}$	**SE**	**P**
All LQTS vs. Controls	1,238/8,219	0.322	0.030	4.93E-26	418/1,671	0.412	0.055	6.16E-14	1,656/9,890	0.343	0.0263	1.08E-38
Genotype positive LQTS vs. Controls	1,115/8,219	0.277	0.032	3.47E-18	356/1,671	0.348	0.058	2.52E-09	1,471/9,890	0.294	0.028	1.09E-25
Genotype negative LQTS vs. Controls	123/8,219	0.733	0.090	3.74E-16	62/1,671	0.740	0.129	1.19E-08	185/9,890	0.735	0.0738	2.24E-23
Genotype negative vs. Genotype positive LQTS	123/1,115	0.447	0.098	5.05E-06	62/356	0.401	0.129	2.01E-03	185/1,471	0.430	0.078	3.54E-08
Genotype negative vs. Genotype positive LQTS*	123/1,115	0.409	0.103	7.36E-05	62/356	0.393	0.130	2.62E-03	185/1,471	0.403	0.0807	6.05E-07

Table legend: β, regression coefficient; n, sample size (cases/controls); P, P value; SE, standard error; *Correcting for QTc

URLs

LDAK: http://dougspeed.com/ldak

LD score regression: https://github.com/bulik/ldsc

FUMA: http://fuma.ctglab.nl

PLINK: https://www.cog-genomics.org/plink2

METAL: https://genome.sph.umich.edu/wiki/METAL

Michigan Imputation Server: https://imputationserver.sph.umich.edu

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