Genetic association study of QT interval highlights role for calcium signaling pathways in myocardial repolarization

A full list of authors and affiliations appears at the end of the article.

Abstract

The QT interval, an electrocardiographic measure reflecting myocardial repolarization, is a heritable trait. QT prolongation is a risk factor for ventricular arrhythmias and sudden cardiac death (SCD) and could indicate the presence of the potentially lethal Mendelian Long QT Syndrome (LQTS). Using a genome-wide association and replication study in up to 100,000 individuals we identified 35 common variant QT interval loci, that collectively explain ∼8-10% of QT variation and highlight the importance of calcium regulation in myocardial repolarization. Rare variant analysis of 6 novel QT loci in 298 unrelated LQTS probands identified coding variants not found in controls but of uncertain causality and therefore requiring validation. Several newly identified loci encode for proteins that physically interact with other recognized repolarization proteins. Our integration of common variant association, expression and orthogonal protein-protein interaction screens provides new insights into cardiac electrophysiology and identifies novel candidate genes for ventricular arrhythmias, LQTS, and SCD.

Keywords

genome-wide association study; QT interval; Long QT Syndrome; sudden cardiac death; myocardial repolarization; arrhythmias

Introduction

Prolongation or shortening of the QT interval on the electrocardiogram are non-invasive markers of delayed or accelerated myocardial repolarization, increased risk of sudden cardiac death (SCD) and fatal arrhythmia as a side effect of medication therapy. Mendelian Long and Short QT Syndromes (LQTS, SQTS)[1] stem from mutations of strong effect (QT increase or decrease per mutation > ∼20-100 msec) in genes encoding ion channels or channel-interacting proteins. In unselected community-based individuals, variation in

continuous QT interval is normally distributed (ranging from 380 to 460 msec), with heritability estimates of 30-40%[2]. Common genetic variants that are associated individually with modest ($\approx$1-4 msec/allele) increments in QT interval duration have been detected through candidate gene and genome-wide association studies (GWAS) in large sample sizes including the QTGEN[3] and QTSCD[4] consortia, as well as others[5-9].

Several loci have been discovered independently in both genome-wide linkage studies of Mendelian LQTS families and GWAS of QT interval duration in unselected populations, including those harboring *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNJ2*, highlighting the value of both approaches and the overlap of common and rare variant loci. To date, hundreds of rare mutations in 13 LQTS-susceptibility genes have been reported, with 75% of LQTS stemming from *KCNQ1* (LQT1), *KCNH2* (LQT2) and *SCN5A* (LQT3) mutations, < 5% due to LQT4-13 and $\sim$20% remaining genetically elusive. Identification of the causal genes underlying QT interval variation in the general population has been more challenging.

Here, the QT Interval-International GWAS Consortium (QT-IGC) performed an expanded meta-analysis of GWAS in 76,061 individuals of European ancestry with targeted genotyping in up to 33,316 additional individuals, completed mutational analysis in probands with genetically elusive LQTS, determined whether QT-associated SNPs have effects on gene expression in various tissues (eQTLs) or on other human phenotypes, and further annotated QT-associated genes using protein-protein interaction analyses.

GWAS identifies 22 novel QT loci

Clinical characteristics of 31 study cohorts of European ancestry who contributed to the stage 1 GWAS are shown in Supplementary Table 1. All studies excluded individuals with atrial fibrillation, QRS duration greater than 120 msec, bundle branch block, or intraventricular conduction delay, and when available electronic pacemaker use or QT-altering medication use. In each cohort, QT interval duration adjusted for age, sex, RR interval (inverse heart rate), and principal components of genetic ancestry was tested for association with 2.5 million directly genotyped or imputed SNPs under an additive genetic model (Supplementary Table 2 and 3). We performed inverse variance weighted meta-analysis on the GWAS results from 76,061 individuals, and observed only modest over-dispersion of the test statistics given the sample size ($\lambda_{GC} = 1.076$, Supplementary Figure 1a). Exclusion of SNPs within 500kb of the sentinel SNP at genome-wide significant loci (some identified only after incorporation of replication genotyping; see below) did not significantly attenuate the excess of low p-values, consistent with a polygenic model of QT interval variation[10] ($\lambda_{GC} = 1.069$, Supplementary Figure 1b).

At an interim meta-analysis of GWAS results, SNPs were selected for two forms of replication. First, a set of the top 35 independent SNPs (one per locus) were selected for targeted replication genotyping in as many as 31,962 individuals of European ancestry (Supplementary Table 1) on a variety of platforms (Supplementary Table 2). Second, a set of $\sim$5,000 LD-pruned ($r^2 > 0.2$) SNPs with nominal evidence of association with QT interval (P 0.015) were included in a custom genotyping array (Metabochip) and genotyped in 1,354 individuals (Supplementary Note, Supplementary Tables 1, 4, 5)[11].

Meta-analysis of all GWAS and replication genotyping results in up to 103,331 individuals (Supplementary Note) identified a total of 35 genome-wide significant ($P < 5\times10^{-8}$) loci, of which 22 were novel and 13 have been reported previously[3-5,9] (Table 1, Figure 1, Supplementary Table 6). Some SNPs initially selected for replication genotyping were not ultimately the most significant SNP at a locus (Supplementary Table 7). Many loci had evidence of multiple independent signals of association based on having low LD ($r^2 < 0.05$) to other genome-wide significant SNPs, with a total of 68 independent SNPs at 35 loci (Supplementary Note, Supplementary Table 8a, Supplementary Figure 2).

Association of QT SNPs in individuals of African Ancestry

We examined the association of 67 of these SNPs in 13,105 individuals of African ancestry in the CARe-COGENT consortium[12] (one SNP was poorly imputed due to low minor allele frequency, MAF). Despite the limited power due to smaller sample size, 10 SNPs at 9 loci were significantly associated with QT interval ($P < 0.0007 = 0.05/67$) in the same direction as in QT-IGC (Supplementary Table 9). The SNP direction of effect was concordant between European- and African-derived samples for 51/67 SNPs (binomial $P = 5\times10^{-5}$) and effects were highly correlated (r = 0.60, $P = 9\times10^{-10}$, Supplementary Table 9). These findings are consistent with the hypothesis that a majority of common variants are associated with QT interval in both ancestral populations.

Variants with additional non-QT effects

Because heart rate (HR) is a strong determinant of unadjusted QT interval ($r^2 \sim 0.5\text{-}0.8$), we examined the 68 independent SNPs at 35 QT loci in a HR GWAS in 92,355 individuals of European or Indian Asian ancestry[13]. Among the 35 loci examined, we found significant association with HR of 5 SNPs at the 4 loci including *PLN*, *FEN1—FADS2*, *ATP2A2*, and *SCN5A-SCN10A* ($p < 0.0007 = 0.05/68$, Supplementary Table 10). Arguing against inadequate heart rate adjustment as the source of association of QT variants with HR was the modest correlation of QT effects (in models adjusting for RR interval, inverse heart rate) and HR effects for QT associated SNPs ($r^2 = 0.16$). Only 38 of 68 of the SNP effects on HR showed the inverse relationship that has been well established between QT interval duration and HR (binomial $p = 0.20$). In ARIC (n = 8,524), we found no evidence that QT-SNP associations were altered with additional adjustment for RR^2, RR^3, $RR^{1/2}$ or $RR^{1/3}$. In total, these findings favor independent pleiotropic effects of the SNPs on heart rate and QT interval.

QRS prolongation due to bundle branch block can result in delayed myocardial repolarization and QT prolongation, hence our exclusion of individuals with prolonged QRS duration/bundle branch block (Supplementary Note). We examined QT interval SNPs in a published GWAS of QRS duration ($N = 40,407$), which reflects electrical impulse propagation in the cardiac ventricles[14]. Among the 68 QT-associated SNPs, 15 were significantly associated with QRS duration (p < 0.0007) at 8 loci (Supplementary Table 10)[7,14]. Because QRS duration is a subinterval of QT interval on the ECG, it is perhaps not surprising that some QT-prolonging variants are also positively associated with QRS duration. However, the significant genetic effects show concordant ($N = 6$) as well as

discordant effects (N = 9).Across all SNPs there was no significant excess of concordant vs discordant effects (37 vs 31, binomial p = 0.27) or significant correlation of effect sizes (r^2 = 0.03, p = 0.18, Supplementary Table 10). Collectively, these findings suggest that while the fundamental electrophysiologic mechanisms underlying the SNP-QT relationships for some SNPs may be shared, many involve cell-type-specific effects and that a consistent general relationship between SNP effects on QT interval and QRS duration does not hold.

Examination of the NHGRI GWAS database (Supplementary Note) revealed additional associations of our QT associated SNPs (or their close proxies with r^2 > 0.8) at *SCN5A-SCN10A* with PR interval[15], at *MKL2* with age of menarche[16] and at *FEN1-FADS2* with high-density lipoprotein cholesterol, triglycerides[17], n-3 fatty acids[18], fasting plasma glucose and HOMA-B[19] and alkaline phosphatase[20] (Supplementary Table 11), which may point to novel repolarization mechanisms or simply reflect independent (pleiotropic) effects of the same genetic variation in different tissues.

Functional annotation of associated variants

Because common variants that code for changes in protein structure have an increased potential to be causal, we investigated the presence of coding variants among QT-associated loci using 1000 Genomes Project data (CEU).Among 68 total genome-wide significant SNPs at 35 loci, there were 5 loci in which the index or a highly correlated SNP was nonsynonymous.(Supplementary Table 8b).While most loci have multiple genes in associated intervals (Supplementary Figure 2), the genes that harbor genome-wide significant missense SNPs highly correlated with the top SNP are high-priority candidates to underlie the QT interval association at those loci.

Since non-coding variants may influence gene expression, we examined the index SNPs or proxies (r^2 > 0.8) at the 68 SNPs at 35 loci in publicly available eQTL datasets from diverse tissues. Twelve QT interval loci are associated with variable expression of at least one gene in one or more tissues with high correlation (r^2 > 0.8) between the top QT SNP and the top eQTL SNP (Supplementary Note, Table 1, Supplementary Table 12). QT-associated SNPs are associated with expression of the nearest gene at loci including *ANKRD9*, *ATP1B1*, *CNOT1*, *FADS1*, *LIG3*, and *TCEA3*. The eQTL data help point to specific genes at these loci as a potential source of the repolarization association signal, presumably through regulatory variation. However, some loci are associated with expression of multiple genes. We did not observe a significant signature of eQTLs among the QT loci that implicate a specific tissue or cell type in an atlas of human and mouse expression (P>0.01, Supplementary Note)[21].

Because genetic variants that influence gene expression may do so in a cell-type specific manner, we examined the association of QT-associated SNPs with gene expression in a collection of 313 left ventricular biopsy samples in the MAGNet consortium. This collection included samples from the hearts of individuals transplanted for heart failure (n=177) or healthy hearts from potential donors (n=136) that were ultimately not used (Supplementary Note). We examined 63 of the 68 QT-associated SNPs that were well imputed from genome-wide genotyping in relation to cis-expression of all genes within 1Mb of each SNP.

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After adjusting for age, sex, study site and presence of heart failure, 9 SNPs at 8 loci were significantly associated with one of 9 transcripts (one SNP was associated with 2 transcripts), correcting for multiple testing ($P < 4.4 \times 10^{-5} = 0.05/1,146$ SNP-transcript associations, Supplementary Note, Table 2). After adjustment for the best eSNP, the QT SNP association became non-significant (all P 0.01) for 8 of the 9 SNP-transcript associations, consistent with a potentially causal effect for these 8 eQTLs. Inclusion of interaction terms for heart failure status did not alter the results (data not shown). In sum, these findings highlight several genes that are plausibly modulated by the QT SNP (or a correlated variant) and thus high priority targets for further experimental work.

Lastly, because genetic variation that influences gene expression may act through modulation of enhancers, we examined data available from the NIH Roadmap Epigenomics Program[22]. We specifically focused on transcriptional enhancer elements marked by combinations of histone modifications (specifically presence of H3K4me1 and absence of H3K4me3), as emerging evidence indicates that variants associated with complex traits preferentially reside in these non-coding regulatory regions and can affect gene expression[23,24]. We tested whether lead QT interval-associated SNPs or highly correlated variants ($r^2 > 0.8$) overlapped enhancers in adult left ventricular tissue. Of 68 lead (or correlated) SNPs, 34 overlapped a left ventricular enhancer, a substantially greater proportion than randomly sampled sets of matched SNPs (z-score = 9.45, $P \ll 10^{-200}$ Supplementary Note, Supplementary Figure 3). These findings highlight specific SNPs at QT-associated loci that can be prioritized for experimental follow-up (Supplementary Table 13).

We also examined the association results for over-representation of specific pathways in QT loci compared to the genome as a whole using GO, KEGG, Panther, and Ingenuity gene set annotations (Supplementary Note). Three of the top 10 pathways in this analysis are specifically involved in calcium processes including: "regulation of the force of heart contraction" ($P = 1 \times 10^{-4}$), "cation transport" (5×10^{-4}), "cellular calcium ion homeostasis" (1×10^{-3}, Supplementary Note, Supplementary Table 14)[25]. These signals were confirmed by a secondary analysis, in which we matched the 68 QT-associated SNPs to randomly selected genome-wide SNPs to calculate statistical significance (Supplementary Note). Three GO terms involving "ion transport" were significantly enriched for QT associations ($P < 0.00044$) as well as a gene set based on having a cardiac phenotype in knockout mice ($P < 0.00025$, Supplementary Note).

Population variation in QT interval explained

The common variants at the 12 previously published common variant loci from the QTGEN and QTSCD consortia explained 3-6% of variation in QT interval, after accounting for effects of age, sex and heart rate[3,4]. The top SNPs at the additional novel loci increase the variance explained to 5.5-7.0%, while all 68 independent SNPs at the 35 loci explain 7.6-9.9% of variance (Supplementary Tables 15 and 16). A recent heritability analysis found that 21% of overall variance (>50% of heritable variation) in QT interval is explained by common autosomal SNPs captured on contemporary genome-wide genotyping arrays[10]. Because the current study was focused on identifying *bona fide* associations of specific loci,

rather than explaining overall variance, we set a stringent p-value threshold for identifying individual SNPs. Larger studies are likely to continue to identify additional novel QT loci, as well as additional independent signals of association at the 35 loci described here.

LQTS proband mutation screening

Common variant loci found in the current and prior studies include 5 genes previously established to cause monogenic LQTS (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNJ2*). Given the co-existence of common QT variants at loci with established rare coding mutations in LQTS disease genes, we hypothesized that some of the novel QT loci may likewise contain previously unrecognized Mendelian LQTS genes. We selected on the basis of statistical significance, proximity to the signal of association, absence of multiple nearby genes in the associated interval and known cardiac expression or involvement in ion flux, 6 genes (*ATP2A2*, *CAV1*, *CAV2*, *SLC8A1*, *SRL*, and *TRPM7*) from 5 novel loci for coding mutation screening. We studied 298 unrelated individuals with clinically diagnosed LQTS on the basis of the Schwartz score, but genotype negative for the canonical LQTS1-3 causative genes, for rare exonic or splice site sequence variants in these 6 genes (Supplementary Note, Supplementary Table 17). We identified 13 amino acid-altering variants present in cases but not in 300 controls of the same continental ancestry (Table 3). Of these, 11 were not observed in ∼6,800 individuals whole-exome sequenced by the Exome Sequencing Project (ESP), or included on the Exome Chip array; several are predicted to be disruptive to protein function (Supplementary Note, Table 3).

Of the 13 amino-acid altering variants, two mutations in *ATP2A2* (p.Ile276fsX281) and *TRPM7* (p.Ile19fsX59) result in frame shifts and premature truncation of the corresponding protein product. The *ATP2A2* mutation was detected in a 6-year old girl with LQTS on the basis of a QTc of 492 msec without symptoms. The proband's mother carried the mutant allele and had borderline QTc prolongation and T wave abnormalities; the proband's father lacked the mutation and had a normal QTc. The *TRPM7* mutation was detected in a 14-year old girl with LQTS on the basis of a QTc of 500 msec without symptoms. The mutation was found in the proband's mother and brother, both of whom had a normal QTc, and was absent in the proband's father who had a normal QTc. Whether or not these two loss-of-function alleles contribute to LQTS pathogenesis in these individuals cannot be determined from these observations alone.

Protein-protein interaction networks

We next sought evidence that proteins encoded by genes at common variant loci interact physically with known myocardial repolarization proteins. We have constructed a protein-protein interaction network from the InWeb database (Supplementary Note)[26]. Using the DAPPLE algorithm[27] we seeded the network with the first 12 known Mendelian LQTS genes and seven loci harboring previously-identified common QT variants (but not known Mendelian genes)[3,4]. Consistent with the known relationships among several of the Mendelian genes, significant interconnectivity was observed ($P = 0.0006$ for direct connections, $P = 0.008$ for indirect connections, Supplementary Note). We thus identified 606 proteins interacting directly with the seed proteins and investigated whether these

protein-protein interactions could help identify candidate genes within any of the 22 novel loci identified in the current study. We found 8 interactors from 7 novel loci (*ATP2A2*, *CAV1*, *CAV2*, *PRKCA*, *SLC8A1, ATXN1, ETF1, SGOL2*), representing significant enrichment compared to the null expectation (hypergeometric P = 0.03, Supplementary Note, Supplementary Table 18). We hypothesized that the other proteins interacting directly with the seed network may nonetheless be enriched for association, even if not genome-wide significant. We assigned association scores to all interacting proteins (except those in the 35 loci already identified) and tested for enrichment of association in those genes compared to all genes in the genome from non-associated regions. We found that interacting proteins were more associated than chance expectation (rank-sum P = 0.00012), suggesting that they include true associations yet to be discovered (Supplementary Figure 4, Supplementary Note). The protein interaction network analysis suggests that interactors of Mendelian LQTS genes are functionally involved in QT interval duration.

This conclusion is further supported by *in vivo* data presented in an accompanying paper by Lundby *et al.* (in press, Nature Methods). We immunoprecipitated proteins encoded by the Mendelian LQTS genes, *KCNQ1*, *KCNH2*, *CACNA1C*, *CAV3* and *SNTA1* from murine cardiac tissue and identified proteins they interact with by high performance orbitrap tandem mass spectrometry. We found proteins encoded by 12 genes from 10 loci identified by our GWAS that physically interact with proteins encoded by the 5 Mendelian LQTS genes, a significant enrichment compared to random expectation ($P = 1 \times 10^{-6}$ using permutation), including *ATP2A2* (SERCA2a), *SRL* (sarcalumenin), which regulates SERCA2a in cardiomyocytes[28-30], *CAV1* (caveolin 1), *PLN* (phospholamban), which also regulates SERCA2a, and ATP1B1 (Table 1). Molecular interactions of proteins encoded by genes at QT-associated loci with known mediators of the currents underlying myocardial repolarization strongly implicates these genes, and not others in the relevant associated intervals, as the causal genes underlying the QT interval association.

Discussion

Altogether, our integrated analysis of genomic, transcriptomic and proteomic data highlight calcium signaling as playing an important role in myocardial repolarization, the cellular process that underlies the QT interval, derangement of which is arrhythmogenic (see detailed description of genes at several loci in Supplementary Note).

Electrical activation and relaxation of the ventricular myocyte on average once per second requires the interplay of multiple coordinated ion channel fluxes. Cellular depolarization begins with Na^+ influx and is sustained by Ca^{2+} influx, which triggers Ca^{2+} release from the sarcoplasmic reticulum leading to myocardial contraction (Supplementary Figure 5). Prolonged inward (depolarizing) Ca^{2+} current during the plateau phase of the cardiac action potential leads to delays in ventricular myocyte repolarization, a subsequent prolonged QT interval on electrocardiogram, and a highly arrhythmogenic and potentially lethal substrate. In fact, gain-of-function mutations in the L-type Ca^{2+} channel lead to the highly arrhythmogenic Timothy syndrome (LQT8) that is associated with extremely prolonged QT intervals[31].

Normal myocyte repolarization results from efflux of potassium and less so Ca^{2+}; Ca^{2+} is actively taken up by the sarcoplasmic reticulum to halt myocardial contraction. The Na^+ that enters the myocyte is counterbalanced by an active Na^+/K^+ ATPase (a beta subunit of which is encoded by *ATP1B1*, at a common variant QT locus). The Ca^{2+} that enters the myocyte is counterbalanced by a Na^+/Ca^{2+} exchanger (NCX1, encoded by *SLC8A1*) to ensure net even cation balance, at the expense of a net depolarizing effect (potentially prolonging repolarization). Disruption of this delicate cation balance, and in particular Ca^{2+} homeostasis, can have a profound impact on action potential duration, formation of early afterdepolarizations (EADs), and triggered activity, leading to potentially lethal arrhythmias including torsade de pointes and ventricular fibrillation. In fact, administration of an inhibitor of the Na^+/Ca^{2+} exchanger is associated with reduced arrhythmia and shortened action potential duration in models of LQTS[32] and heart failure[33] and its over-expression delays myocardial repolarization and leads to ventricular arrhythmias[34].

ATP2A2 encodes the SERCA2a cardiac sarcoplasmic reticulum calcium pump and by alternative splicing a ubiquitously expressed SERCA2b calcium pump (Supplementary Figure 5). The protein is negatively regulated by phospholamban (*PLN*), also a QT-interval associated locus[3,4,6]. In turn, *PLN* is negatively regulated by *PRKCA*, a gene in a newly discovered QT-interval associated locus[35]. SERCA2a is responsible for Ca^{2+} sequestration by the cardiac sarcoplasmic reticulum and its dysregulation is implicated in heart failure due to the centrality of calcium cycling to excitation-contraction coupling. Dominant SERCA2 mutations are a cause of keratosis follicularis Darier-White disease (OMIM #124200)[36]. No study that we are aware of has described electrocardiographic or other cardiac changes in affected humans but detailed investigation of heterozygous Serca2 (+/-) mice show a reduction in Serca2 protein by about a third with deficits in myocardial relaxation and contractility, and a reduced Ca^{2+} transient by haploinsufficiency[37] as well as upregulation of transient receptor potential canonical 1 (TRPC1) channel[38]. Moreover, overexpression of SERCA2a in a rat model of heart failure demonstrated a substantial reduction in arrhythmias[39].

TRPM7 encodes the widely expressed transient receptor channel melastatin 7 protein, a 6 transmembrane molecule which is Mg^{2+} and Ca^{2+} permeable and has protein kinase function[40,41]. The *touchtone/nutria* zebrafish TRPM7 mutant demonstrates defective skeletogenesis, kidney stones[42] and abnormal melanophores[43]. *Trpm7* (-/-) deletion in mice is embryonic lethal; targeted deletion disrupts normal thymogenesis[40]. Targeted cardiac deletion in cultured embryonic ventricular myocytes leads to down-regulation of several genes involved in calcium cycling, including *SERCA2a*[44]. In migrating human embryonic lung fibroblasts, TRPM7 mediates transduction of mechanical stretch into calcium influx underlying calcium flickers (focally high intracellular calcium microdomains), involved in steering cell migration[45]. In human atrial fibroblasts, atrial fibrillation is associated with increased TRPM7-mediated Ca^{2+} influx while *TRPM7* knockdown results in loss of spontaneous Ca^{2+} influx[46]. More recently, targeted Trpm7 deletion in mice has been shown to result in lethal cardiomyopathy in early cardiogenesis; cardiomyopathy, delayed repolarization and heart block in mid cardiogenesis; and no recognizable phenotype in late cardiogenesis[47]. In total, this prior work raises the possibility that TRPM7 in humans leads

to altered myocardial repolarization through developmental differences or through ongoing functional effects in adulthood, potentially involving calcium signaling.

Potassium flux has long been recognized through rare mutations underlying LQTS as a critical effector of myocardial repolarization. Ca^{2+} has been recognized as a central mediator in excitation-contraction coupling. However, our studies of common and rare genetic variation now place Ca^{2+} as a central modulator of repolarization given the role of the proteins encoded by the Mendelian Timothy syndrome gene (LQT8) *CACNA1C*, as well as the following genes at common variant QT interval loci: *ATP2A2*, *PLN*, *PRKCA* and *SRL* and *SLC8A1*. How the Mg^{2+}/Ca^{2+} channel TRPM7 might contribute to repolarization is unclear but its involvement in Ca^{2+} flickers[45] suggests a potential role in localized Ca^{2+} fluxes or indirect effects on Ca^{2+}-sensitive potassium channels or the Na^+/Ca^{2+} exchanger.

Much work will be needed to understand the normal physiologic contribution to repolarization of these Ca^{2+}-regulating proteins, as well as the pathophysiologic consequences arising from their derangements. While anti-arrhythmic agents targeting the I_{Kr} (LQT2/*KCNH2*) channel have a relatively limited contribution to clinical management of some arrhythmias due to their propensity to cause other arrhythmias, targeting the newly identified proteins that contribute to myocardial repolarization could potentially treat some arrhythmias without pro-arrhythmia. Conversely, existing therapies that inadvertently target some of the newly discovered proteins could in fact contribute to arrhythmogenesis.

We have identified 22 novel QT interval loci, bringing to a total 35 common variant loci. We have used diverse approaches to highlight specific genes at these loci likely to mediate the repolarization effects. While we cannot say with certainty which gene underlies the QT trait at every locus, these complementary experiments represent a quantum leap in our understanding of this critical electrophysiologic process. The elucidation of fundamental mechanisms of arrhythmogenesis promises to expose new approaches to predict and prevent death from lethal ventricular arrhythmias in the general population.

Online Methods

Study cohorts

Cohorts for QT interval association analyses included individuals largely with population- or community-based ascertainment and a few with case-control sampling for traits not strongly associated with QT interval. Mandatory exclusions included presence of atrial fibrillation or a trial flutter, and presence of QRS duration > 120 msec or presence of right or left bundle branch block. Optional exclusions included use of QT-altering medications, presence of a pacemaker or implantable cardioverter defibrillator, or pregnancy. All studies were reviewed by local ethics committees and all participants provided informed consent.

Genotyping, imputation, quality control

GWAS studies used a variety of genome-wide genotyping arrays. All studies used hidden Markov model approaches to impute genotypes at unmeasured HapMap SNPs so that a common set of 2.5M SNPs were available across all discovery samples. Monomorphic SNPs and SNPs with beta estimates larger than 100,000 were removed from all results. Cohort-

specific SNP filters on minimum minor allele frequency, imputation quality metric, call rate and Hardy-Weinberg equilibrium p-value were selected to minimize any test statistic distortion of the quantile-quantile plot or genomic inflation factor (λ). Replication genotyping was performed using a variety of arrays.

Association analyses, meta-analysis

Genomic control was applied to genome-wide results from each cohort prior to meta-analysis. Meta-analyses were performed in parallel at two analytic sites using MANTEL[3] or METAL[48] using inverse variance weighted, fixed effects meta-analysis. Genome-wide significance was set at $P < 5\times10^{-8}$, a threshold accounting for the effective number of independent common variant tests in the genome of European-derived populations.[49]

Expression in cardiac samples

Samples of cardiac tissue were acquired from patients in the Myocardial Applied Genomics Network. Left ventricular free-wall tissue was harvested at the time of cardiac surgery from subjects with heart failure undergoing transplantation or from unused donor hearts. DNA samples were genotyped using the Affymetrix 6.0 genome-wide array and RNA expression measured using the Affymetrix Genechip ST1.2 array. Imputation to SNP genotypes in 1000 Genomes was performed. Analyses were restricted to samples with genetically inferred European ancestry. SNP genotype was tested for association with log2 transformed expression level, after adjustment for age, sex, study site, disease status and batch. Association of each QT-associated SNP with all transcripts within 1Mb of the SNP was examined for 63 QT-associated SNPs (5 SNPs were not available due to poor imputation). SNP-transcript associations meeting experiment-wide significance ($P < 4.4\times10^{-5} = 0.05/1,146$ tests) were examined after additional adjustment for the best cis eSNP for the transcript in question. We inferred that the SNP-transcript association could explain the SNP-QT association when the SNP-QT association was substantially attenuated after additional adjustment for the best cis eSNP.

Cardiac enhancer analyses

Enhancer annotations were generated by integrating combinations of histone modifications obtained from the Roadmap Epigenomics project using ChromHMM[23,50]. We identified SNPs in LD ($r^2 > 0.8$) with each of the 68 QT interval-associated loci using genotype data from the 1000 Genomes Project (CEU population) and computed overlap with ChromHMM-annotated enhancer elements in the left ventricle tissue sample (BC Left Ventricle N41) in the NIH Roadmap Epigenomics Program[22] using the intersect BED command in BED Tools (v2.12.0). To assess significance of the overlap, we compared the set of SNPs at 68 QT interval-associated loci against 100,000 sets of randomly sampled control SNPs. Control SNPs were chosen from the Affymetrix 660W genotyping array and were matched for size of the LD block (+/- 5 SNPs), MAF of the lead SNP (+/- 0.1) and distance to the nearest gene (+/- 25 kb if outside a gene).

LQTS mutation analysis

A cohort of 298 unrelated, LQT1-3 mutation negative patients with LQTS [191 females (64%), average age= 27 ± 20 years, average QTc = 529 ± 58 ms], who satisfied the case inclusion criteria of QTc 480 msec (n= 261, 86%) or Schwartz score[51] 3.0 (n=298, 100%), was derived from 7 international congenital LQTS recruitment centers [l'Institut du Thorax, Nantes, France (n=91), Mayo Clinic, Rochester, Minnesota, United States (n=72), University of Pavia, Pavia, Italy (n=38), Academic Medical Centre, Amsterdam, Netherlands (n=30), The Hospital for Sick Children, Toronto, Ontario, Canada (n=24), Munich Medical International GmbH, München, Germany (n=23), and St. George's Hospital, London, England (n=20)]. Of the 265 patients with a documented clinical history, 175 (66%) were symptomatic with 1 LQTS-related cardiac event (i.e. syncope or cardiac arrest). Six genes (*ATP2A2*, *CAV1*, *CAV2*, *SLC8A1*, *SRL*, *TRPM7*), derived from 5 genome-wide significant novel loci, were selected for comprehensive open-reading frame/splice-site mutation analysis. These 6 candidate genes were chosen based on nominal statistical significance, proximity to the signal of association, absence of multiple nearby genes in the associated interval, and known cardiac expression or involvement in ion channel macromolecular complexes. For each gene, mutational analysis was performed using either direct Sanger-based DNA sequencing of all patient samples or using an intermediate mutation detection platform (i.e. denaturing high performance liquid chromatography [DHPLC]) followed by direct DNA sequencing of only samples showing an aberrant DHPLC elution profile.

Protein-protein interaction in silico analyses

We used a public database of protein-protein interactions[26]. This database contains 428,430 interactions, 169,810 of which are high-confidence interactions across 12,793 proteins. All human interactions were pooled and interactions in orthologous protein pairs passing a strict threshold for orthology were included. Each interaction was assigned a probabilistic score based on the neighborhood of the interaction, the scale of the experiment in which the interaction was reported and the number of different publications in which the interaction had been sited. We used a published algorithm called DAPPLE (Disease Association Protein-Protein Link Evaluator) to build and analyze a network of seed genes[27]. We seeded the network with 12 known Mendelian LQTS proteins (KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, CAV3, SNTA1, KCNJ2, CACNA1C, ANK2, AKAP9, SCN4B) as well as genes from 7 previously associated common variant QT loci[3,4]. We considered direct connections among the seed proteins as well as indirect connections through other proteins, filtering on connections between proteins from different loci. DAPPLE evaluates the significance of the network and individual proteins within it by comparing it to 10,000 random, matched networks that are generated using a within-degree node-label permutation[27]. We considered the ability of protein-protein interactions to identify proteins newly associated in the QT-IGC meta-analysis. We translated the novel loci into genes, identifying 124 genes in total, 85 of which were in the In Web database.

Supplementary Material

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Authors

Dan E. Arking[1,*], Sara L. Pulit[2,3,4,*], Lia Crotti[5,6,7], Pim van der Harst[8,9], Patricia B. Munroe[10,11], Tamara T. Koopmann[12], Nona Sotoodehnia[13,14], Elizabeth J. Rossin[3,15,16], Michael Morley[17], Xinchen Wang[18,19,20], Andrew D. Johnson[21], Alicia Lundby[3,22,23], Daníel F. Gudbjartsson[24], Peter A. Noseworthy[2,3,25], Mark Eijgelsheim[26], Yuki Bradford[27], Kirill V. Tarasov[28], Marcus Dörr[29,30], Martina Müller-Nurasyid[31,32,33,34,35], Annukka M. Lahtinen[36,37], Ilja M. Nolte[38], Albert Vernon Smith[39,40], Joshua C. Bis[13], Aaron Isaacs[41], Stephen J. Newhouse[10], Daniel S. Evans[42], Wendy S. Post[43,44], Daryl Waggott[45], Leo-Pekka Lyytikäinen[46], Andrew A. Hicks[47], Lewin Eisele[48], David Ellinghaus[49], Caroline Hayward[50], Pau Navarro[50], Sheila Ulivi[51], Toshiko Tanaka[52], David J. Tester[53,54], Stéphanie Chatel[55,56], Stefan Gustafsson[57,58], Meena Kumari[59], Richard W. Morris[60], Åsa T. Naluai[61,62], Sandosh Padmanabhan[63], Alexander Kluttig[64], Bernhard Strohmer[65], Andrie G. Panayiotou[66,67], Maria Torres[68], Michael Knoflach[69], Jaroslav A. Hubacek[70], Kamil Slowikowski[71,72], Soumya Raychaudhuri[3,71,73,74,75], Runjun D. Kumar[76,77], Tamara B. Harris[78], Lenore J. Launer[78], Alan R. Shuldiner[79,80,81], Alvaro Alonso[82], Joel S. Bader[83], Georg Ehret[1], Hailiang Huang[3,15,16], W.H. Linda Kao[44], James B. Strait[28,52], Peter W. Macfarlane[84], Morris Brown[85], Mark J. Caulfield[10], Nilesh J. Samani[86], Florian Kronenberg[87], Johann Willeit[69], CARe Consortium[88], COGENT Consortium[88], J. Gustav Smith[2,3,25,89], Karin H. Greiser[64,90], Henriette Meyer zu Schwabedissen[91], Karl Werdan[92], Massimo Carella[93], Leopoldo Zelante[93], Susan R. Heckbert[13,94], Bruce M. Psaty[13,94,95,96,97], Jerome I. Rotter[98], Ivana Kolcic[99], Ozren Polašek[99], Alan F. Wright[50], Maura Griffin[100], Mark J. Daly[3,15], DCCT/EDIC[88], David O. Arnar[101], Hilma Hólm[24], Unnur Thorsteinsdottir[24], eMERGE consortium[88], Joshua C. Denny[102,103], Dan M. Roden[103,104,105], Rebecca L. Zuvich[27], Valur Emilsson[39], Andrew S. Plump[106], Martin G. Larson[21,107,108], Christopher J. O'Donnell[21,109], Xiaoyan Yin[21,107], Marco Bobbo[110], Adamo P. D'Adamo[51,111], Annamaria Iorio[110], Gianfranco Sinagra[110], Angel Carracedo[68,112,113], Steven R. Cummings[42], Michael A. Nalls[114], Antti Jula[115], Kimmo K. Kontula[116], Annukka Marjamaa[36,37], Lasse Oikarinen[117], Markus Perola[118,119,120], Kimmo Porthan[117], Raimund Erbel[121], Per Hoffmann[122,123,124,125], Karl-Heinz Jöckel[48], Hagen Kälsch[121], Markus M. Nöthen[122,123], HRGEN consortium[88], Marcel den Hoed[58,126], Ruth J.F. Loos[126,127,128], Dag S. Thelle[129,130], Christian Gieger[33], Thomas Meitinger[35,131,132], Siegfried Perz[133], Annette Peters[35,134], Hanna Prucha[135,136], Moritz F. Sinner[31], Melanie Waldenberger[132], Rudolf A. de Boer[8], Lude Franke[9], Pieter A. van der Vleuten[8,9], Britt Maria Beckmann[31], Eimo Martens[31,137], Abdennasser Bardai[12], Nynke Hofman[138], Arthur A.M. Wilde[12,139], Elijah R. Behr[140], Chrysoula Dalageorgou[141], John R. Giudicessi[54], Argelia Medeiros-Domingo[54], Julien Barc[56], Florence Kyndt[55,56], Vincent Probst[55,56], Alice Ghidoni[5,6], Roberto Insolia[5,6], Robert M. Hamilton[142,143], Stephen W. Scherer[144], Jeffrey Brandimarto[17], Kenneth Margulies[17], Christine E. Moravec[17], Greco M. Fabiola Del[47], Christian Fuchsberger[145], Jeffrey R. O'Connell[79,80], Wai K. Lee[63], Graham C.M. Watt[146], Harry Campbell[147], Sarah H. Wild[147], Nour E. El

Mokhtari[148], Norbert Frey[149], Folkert W. Asselbergs[150,151,152], Irene Mateo Leach[8], Gerjan Navis[153], Maarten P. van den Berg[8], Dirk J. van Veldhuisen[8], Manolis Kellis[18,19], Bouwe P. Krijthe[26,154], Oscar H. Franco[26,154], Albert Hofman[26,154], Jan A. Kors[155], André G. Uitterlinden[26,154,156], Jacqueline C.M. Witteman[26,154], Lyudmyla Kedenko[157], Claudia Lamina[87], Ben A. Oostra[26], Gonçalo R. Abecasis[145], Edward G. Lakatta[28], Antonella Mulas[158], Marco Orrú[158], David Schlessinger[159], Manuela Uda[158], Marcello R.P. Markus[160], Uwe Völker[30,161], Harold Snieder[38], Timothy D. Spector[162], Johan Ärnlöv[58,163], Lars Lind[164], Johan Sundström[164], Ann-Christine Syvänen[58], Mika Kivimaki[59], Mika Kähönen[165], Nina Mononen[46], Olli T. Raitakari[166,167], Jorma S. Viikari[168], Vera Adamkova[70], Stefan Kiechl[69], Maria Brion[68,169], Andrew N. Nicolaides[67,100], Bernhard Paulweber[157], Johannes Haerting[64], Anna F. Dominiczak[63], Fredrik Nyberg[130,170], Peter H. Whincup[171], Aroon Hingorani[59], Jean-Jacques Schott[55,56], Connie R. Bezzina[12], Erik Ingelsson[58,172], Luigi Ferrucci[52], Paolo Gasparini[51,111], James F. Wilson[147], Igor Rudan[147], Andre Franke[49], Thomas W. Mühleisen[122,123,173], Peter P. Pramstaller[47,174,175], Terho J. Lehtimäki[46], Andrew D. Paterson[176], Afshin Parsa[79,80], Yongmei Liu[177], Cornelia van Duijn[26], David S. Siscovick[13,94,97], Vilmundur Gudnason[39,40], Yalda Jamshidi[178], Veikko Salomaa[115], Stephan B. Felix[29,30], Serena Sanna[158], Marylyn D. Ritchie[179], Bruno H. Stricker[26,154,155,156,180], Kari Stefansson[24,40], Laurie A. Boyer[20], Thomas P. Cappola[17], Jesper V. Olsen[22], Kasper Lage[3,15,22,181,182], Peter J. Schwartz[183], Stefan Kääb[31,35], Aravinda Chakravarti[1], Michael J. Ackerman[53,54,184,*], Arne Pfeufer[47,131,185,*], Paul I.W. de Bakker[3,4,16,71,186,*], and Christopher Newton-Cheh[2,3,16,25,*]

Affiliations

[1]Center for Complex Disease Genomics, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA [2]Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA 02114, USA [3]Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA 02139, USA [4]Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands [5]Department of Molecular Medicine, Section of Cardiology, University of Pavia, Pavia, Italy [6]Department of Cardiology, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy [7]Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany [8]Department of Cardiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands [9]Department of Genetics, University of Groningen, University Medical Center Groningen, 9700 RB Groningen, The Netherlands [10]Clinical Pharmacology, William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, London EC1M 6BQ, UK [11]Barts and the London Genome Centre, William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, London EC1M 6BQ, UK [12]Heart Failure Research Center, Department of Clinical and Experimental Cardiology, Academic Medical Center, Amsterdam, The Netherlands [13]Cardiovascular Health Research Unit, Department of Medicine,

University of Washington, Seattle, USA [14]Cardiology Division, University of
Washington, Seattle, WA, USA [15]Analytic and Translational Genetics Unit,
Massachusetts General Hospital, Boston, MA, 02114, USA [16]Harvard Medical
School, Boston, MA 02115, USA [17]Perelman School of Medicine, University of
Pennsylvania, Philadelphia PA, USA [18]Broad Institute of MIT and Harvard,
Cambridge, MA USA [19]Computer Science and Artificial Intelligence Laboratory,
Massachusetts Institute of Technology, Cambridge, MA 02139, USA [20]Department
of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue,
Cambridge, MA 02139 USA [21]NHLBI's Framingham Heart Study, Framingham, MA,
01702, USA [22]Novo Nordisk Foundation Center for Protein Research, Faculty of
Health Sciences, University of Copenhagen, Blegdamsvej 3b, Copenhagen,
Denmark [23]The Danish National Research Foundation Centre for Cardiac
Arrhythmia, University of Copenhagen, Blegdamsvej 3b, DK-2200 Copenhagen,
Denmark [24]deCODE genetics, Sturlugata 8, 101 Reykjavik, Iceland
[25]Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA,
USA 02114 [26]Department of Epidemiology, Erasmus Medical Center, P.O. Box
2040, 3000 CA, Rotterdam, The Netherlands [27]Center for Human Genetics
Research, Vanderbilt University School of Medicine, Nashville, TN, 37232, USA
[28]Laboratory of Cardiovascular Sciences, Human Cardiovascular Studies Unit,
National Institute on Aging, National Institutes of Health, Baltimore, Maryland,
21224 [29]Department of Internal Medicine B, University Medicine Greifswald, 17475
Greifswald, Germany [30]DZHK (German Center for Cardiovascular Research),
partner site Greifswald, Germany [31]Department of Medicine I, University Hospital
Munich, Ludwig-Maximilians-University, Munich, Germany [32]Institute of Medical
Informatics, Biometry and Epidemiology, Chair of Epidemiology, Ludwig-
Maximilians-Universität, Munich, Germany [33]Institute of Genetic Epidemiology,
Helmholtz Zentrum Munich, German Research Center for Environmental Health,
Neuherberg, Germany [34]Institute of Medical Informatics, Biometry and
Epidemiology, Chair of Genetic Epidemiology, Ludwig-Maximilians-Universität,
Munich, Germany [35]DZHK (German Centre for Cardiovascular Research), partner
site Munich Heart Alliance, Munich, Germany [36]Research Programs Unit, Molecular
Medicine, University of Helsinki, 63FI-00014, Helsinki, Finland [37]Department of
Medicine, Helsinki University Central Hospital, FI-00029 HUS, Helsinki, Finland
[38]Department of Epidemiology, University of Groningen, University MedicalCenter
Groningen, Groningen, The Netherlands [39]Icelandic Heart Association, Holtasmari
1, IS-201 Kopavogur, Iceland [40]Faculty of Medicine, University of Iceland, 101
Reykjavik, Iceland [41]Genetic Epidemiology Unit, Dept. of Epidemiology, Erasmus
University Medical Center, 3000CA Rotterdam, the Netherlands [42]California Pacific
Medical Center Research Institute, San Francisco, CA 94107, USA [43]Division of
Cardiology, Johns Hopkins University School of Medicine, Baltimore, MD, 21205,
USA [44]Department of Epidemiology, Johns Hopkins University, Bloomberg School
of Public Health, Baltimore, MD, 21205, USA [45]Informatics and Biocomputing
Platform, Ontario Institute for Cancer Research, Toronto, Canada, M5G 0A3
[46]Department of Clinical Chemistry, Fimlab Laboratories and University of Tampere

School of Medicine, P.O. Box 66, 33101 Tampere, Finland [47]Center for Biomedicine, European Academy Bozen/Bolzano (EURAC), Bolzano, Italy - Affiliated Institute of the University of Lübeck, Lübeck, Germany [48]Institute for Medical Informatics, Biometry and Epidemiology, University Hospital of Essen, University Duisburg-Essen, Essen, Germany [49]Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Schittenhelmstr. 12, 24105 Kiel, Germany [50]MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine,Western General Hospital, Edinburgh, EH4 2XU, United Kingdom [51]Institute for Maternal and Child Health - IRCCS "Burlo Garofolo" – Trieste, Trieste, Italy, 34137 [52]Translational Gerontology Branch, National Institute on Aging, Baltimore, MD, 21225, USA [53]Division of Pediatric Cardiology, Department of Pediatrics, Mayo Clinic, Rochester, MN, USA 55905 [54]Windland Smith Rice Sudden Death Genomics Laboratory, Department of Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA 55905 [55]l'institut du thorax, CHU de Nantes, Université de Nantes, Nantes, France, F-44000 [56]l'institut du thorax, INSERM UMR1087, CNRS UMR 6291, Université de Nantes, Nantes, France, F-44000 [57]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Box 281, SE-171 77 Stockholm, Sweden [58]Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden [59]Department of Epidemiology and Public Health, University College London, 1-19 Torrington Place, London, WC1E 6BT [60]University College London, Dept of Primary Care & Population Health, Royal Free Campus, London NW3 2PF, UK [61]Department of Medical and Clinical Genetics, The Sahlgrenska Academy at the University of Gothenburg, 405 30 Gothenburg, Sweden [62]The Biobanking and Molecular Resource Infrastructure of Sweden (BBMRI), Gothenburg, Sweden [63]BHF Glasgow Cardiovascular Research Centre, Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, G12 8TA, UK [64]Institute of Medical Epidemiology, Biostatistics, and Informatics, Martin-Luther-University Halle-Wittenberg, 06112 Halle (Saale), Germany [65]Second Department of Internal Medicine, Paracelsus Medical University/Salzburger Landeskliniken, 5020 Salzburg, Austria [66]Cyprus International Institute for Environmental and Public Health in association with the Harvard School of Public Health, Cyprus University of Technology, Limassol, Cyprus [67]Cyprus Cardiovascular and Educational Research trust, Nicosia, 2368, Cyprus [68]Grupo de Medicina Xenómica, Centro Nacional de Genotipado-ISCIII, IDIS, Universidade de Santiago de Compostela [69]Department of Neurology, Innsbruck Medical University, Anichstraβe 35, A-6020 Innsbruck, Austria [70]Centre for Experimental Medicine, Institute for Clinical and Experimental medicine, Videnska 1958/9, Prague 4, 14021, Czech Republic [71]Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA [72]Harvard Bioinformatics and Integrative Genomics, Boston, Massachusetts, 02115 USA [73]Partners HealthCare Center for Personalized Genetic Medicine, Boston, Massachusetts, USA [74]Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital, Boston,

Massachusetts, USA [75]Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK [76]Computational and Systems Biology Program, Division of Biology and Biomedical Sciences, Washington University in St. Louis, St. Louis Missouri [77]Division of Oncology, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri [78]Laboratory of Epidemiology, Demography and Biometry, National Institute on Aging, Bethesda, MD 20892, USA [79]Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland, 21201, USA [80]Program for Personalized and Genomic Medicine, University of Maryland, Baltimore, Maryland, 21201, USA [81]Geriatric Research and Education Clinical Center, Veterans Administration Medical Center, Baltimore, Maryland, USA [82]Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota, United States of America [83]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21218, USA [84]Electrocardiology, University of Glasgow Institute of Cardiovascular and Medical Sciences, Royal Infirmary, Glasgow G31 2ER [85]Clinical Pharmacology, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK [86]Dept of Cardiovascular Science, University of Leicester, Glenfield Hospital, Groby Road, Leicester, LE3 9QP, UK [87]Division of Genetic Epidemiology, Innsbruck Medical University, 6020 Innsbruck, Austria [88]Full lists of members and affiliations appear in the Supplementary Note [89]Department of Cardiology, Lund University, Lund, Sweden [90]German Cancer Research Centre, Division of Cancer Epidemiology, 69120 Heidelberg, Germany [91]Department of Pharmacology, Ernst-Moritz-Arndt-University of Greifswald, 17487 Greifswald, Germany [92]Department of Medicine III, Medical Faculty, Martin-Luther-University Halle-Wittenberg, 06112 Halle (Saale), Germany [93]Medical Genetics Unit, Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy [94]Department of Epidemiology, University of Washington, Seattle, WA, USA [95]Department of Health Services, University of Washington, Seattle, WA, USA [96]Group Health Research Institute, Group Health Cooperative, Seattle, WA [97]Department of Medicine, University of Washington, Seattle, WA, USA [98]Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA, USA [99]Department of Public Health, Faculty of Medicine, University of Split, Soltanska 2, 21000 Split, Croatia [100]Vascular Screening and Diagnostic Centre, London, WB1 7BZ, UK [101]Division of Cardiology, Department of Medicine, Landspitali University Hospital, 101 Reykjavik, Iceland [102]Department of Biomedical Informatics, Vanderbilt University School of Medicine, Nashville, TN, 37232, USA [103]Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, 37232, USA [104]Department of Pharmacology, Vanderbilt University, Nashville, TN, 37232, USA [105]The Office of Personalized Medicine, Vanderbilt University, Nashville, TN, 37232, USA [106]Sanofi Research & Development, Paris, France [107]Department of Biostatistics, Boston University School of Public Health, Boston, MA, 02118, USA [108]Department of Mathematics and Statistics, Boston University, Boston, MA, 02115, USA [109]Cardiology Division, Massachusetts General Hospital, MA, 02114, USA [110]Cardiovascular Department, Ospedali Riuniti and University of Trieste,

Trieste, Italy, 34149 [111]University of Trieste, Trieste, Italy, 34137 [112]Fundación Publica Galega de Medicina Xenómica, SERGAS [113]Center of Excellence in Genomic Medicine Research, King Abdulaziz University Jeddah, KSA [114]Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD, 20892, USA [115]Chronic Disease Epidemiology and Prevention Unit, National Institute for Health and Welfare, PO Box 30, FI-00271 Helsinki, Finland [116]Department of Medicine, University of Helsinki, FI-00290 Helsinki, Finland [117]Division of Cardiology, Department of Medicine, Helsinki University Central Hospital, FI-00029 HUS, Helsinki, Finland [118]Public Health Genomics Unit, National Institute for Health and Welfare, PO Box 30, FI-00271 Helsinki, Finland [119]Institute for Molecular Medicine Finland FIMM, PO Box 20, FI-00014 University of Helsinki, Helsinki, Finland [120]Estonian Genome Center, University of Tartu, Tartu, Estonia [121]Department of Cardiology, University Hospital of Essen, University Duisburg-Essen, Essen, Germany [122]Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany [123]Institute of Human Genetics, University of Bonn, Bonn, Germany [124]Division of Medical Genetics, University Hospital Basel, Switzerland [125]Department of Biomedicine, University of Basel, Switzerland [126]MRC Epidemiology Unit, University of Cambridge, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK [127]The Mindich Child Health and Development Institute, The Icahn School of Medicine at Mount Sinai, New York, NY 10069 [128]The Charles Bronfman Institute for Personalized Medicine, The Icahn School of Medicine at Mount Sinai, New York, NY 10069 [129]Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, 0317 Oslo, Norway [130]Department of Public Health and Community Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, 40530 Gothenburg, Sweden [131]Institute of Human Genetics, Technische Universität München, Munich 81675, Germany [132]Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany [133]Institute for Biological and Medical Imaging, Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany [134]Institute of Epidemiology II, Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany [135]CK-CARE: Christine Kühne - Center for Allergy Research and Education [136]Department of Dermatology and Allergy, Technische Universität München, Munich, Germany [137]Department of Medicine, Hospital of Friedberg, Friedberg, Germany [138]Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands [139]Princess Al-Jawhara Al-Brahim Centre of Excellence in Research of Hereditary Disorders, Jeddah, Kingdom of Saudi Arabia [140]Cardiovascular and Cell Sciences Institute, St George's University of London, London UK [141]Biomedical Sciences, St Georges's University of London, Cranmer Terrace, London, SW17 0RE, UK [142]The Labatt Family Heart CentreThe Hospital for Sick Children, Toronto, Ontario Canada M5G 1X8 [143]Department of Pediatrics, The Hospital for Sick Children, Toronto, Ontario Canada M5G 1X8 [144]The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Ontario Canada M5G 1X8 [145]Center for Statistical Genetics,

Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA [146]General Practice and Primary Care, University of Glasgow, 1 Horselethill Road, Glasgow G12 9LX, UK [147]Centre for Population Health Sciences, University of Edinburgh, Teviot Place, Edinburgh, EH8 9AG, Edinburgh, Scotland [148]Biobank PopGen, Institute of Experimental Medicine, Christian-Albrechts-University of Kiel, 24105 Kiel, Germany [149]Department of Internal Medicine III, University Medical Center Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany [150]Durrer Center for Cardiogenetic Research, ICIN-Netherlands Heart Institute, Utrecht, The Netherlands [151]Department of Cardiology, Division Heart and Lungs, University Medical Centre Utrecht, Utrecht, The Netherlands [152]Institute of Cardiovascular Science, faculty of Population Health Sciences, University College London, London, United Kingdom [153]Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands [154]Netherlands Consortium for Healthy Aging (NCHA), The Netherlands [155]Department of Medical Informatics, Erasmus Medical Center, P.O. Box 2040, 3000CA, Rotterdam, The Netherlands [156]Department of Internal Medicine, Erasmus Medical Center, P.O. Box 2040, 3000CA, Rotterdam, The Netherlands [157]First Department of Internal Medicine, Paracelsus Medical University/Salzburger Landeskliniken, 5020 Salzburg, Austria [158]Istituto di Ricerca Genetica e Biomedica, CNR, Monserrato, 09042 Cagliari, Italy [159]Laboratory of Genetics, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA [160]Institute for Community Medicine, University Medicine Greifswald, 17487 Greifswald, Germany [161]Interfaculty Institute for Genetics and Functional Genomics, Ernst-Moritz-Arndt-University Greifswald, 17487 Greifswald, Germany [162]Department of Twin Research and Genetic Epidemiology, King's College London, London, United Kingdom [163]School of Health and Social Sciences, Dalarna University Falun, Sweden [164]Department of Medical Sciences, Uppsala University, Akademiska sjukhuset, SE-751 85 Uppsala, Sweden [165]Department of Clinical Physiology, Tampere University Hospital and University of Tampere School of Medicine, P.O. Box 2000, Tampere 33521, Finland [166]Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, P.O. Box 52, Turku 20521, Finland [167]Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, P.O. Box 52, Turku 20521, Finland [168]Department of Medicine, Turku University Hospital and University of Turku, P.O. Box 52, Turku 20521, Finland [169]Xenética de enfermidades cardiovasculares e oftalmolóxicas, IDIS. Complexo Hospitalario Universitario de Santiago-SERGAS. Red de Investigacion Cardiovascular [170]Global Epidemiology, AstraZeneca R&D, 431 83 Mölndal, Sweden [171]Division of Population Health Sciences & Education, St George's University of London, London SW17 0RE, UK [172]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, United Kingdom [173]Institute of Neuroscience and Medicine (INM-1), Structural and Functional Organization of the Brain, Genomic Imaging, Research Centre Juelich, D-52425 Juelich, Germany [174]Department of Neurology, University of Lübeck, 23538 Lübeck, Germany [175]Department of Neurology, General Central Hospital, 39100 Bolzano,

Italy [176]Genetics and Genome Biology Program,The Hospital for Sick Children Research Institute, Toronto, Ontario, M5G 1X8 , Canada [177]Department of Epidemiology and Prevention, Division of Public Health Sciences, Wake Forest University, Medical Center Boulevard, Winston-Salem, NC 27157, USA [178]Human Genetics Research Centre, St. George's University of London, London, SW17 0RE, United Kingdom [179]Center for Systems Genomics, Pennsylvania State University, University Park, PA, 16802, USA [180]Inspectorate of Health Care, P.O. BOX 16119, 2500 BC , The Hague, The Netherlands [181]Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, 2800 Lyngby, Denmark [182]Pediatric Surgical Research Laboratories, MassGeneral Hospital for Children, Massachusetts General Hospital, 02114 Boston, MA, USA [183]Center for Cardiac Arrhythmias of Genetic Origin, IRCCS Istituto Auxologico Italiano, Milan, Italy [184]Division of Cardiovascular Diseases, Department of Medicine, Mayo Clinic, Rochester, MN, USA 55905 [185]Institute for Bioinformatics and Systems Biology, Helmholtz Zentrum München Germany [186]Department of Epidemiology, University Medical Center Utrecht, Utrecht, The Netherlands

Acknowledgments

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Author contributions

Author contributions are indicated by cohort/group. All co-authors revised and approved the manuscript.

Writing Group. C.N.-C. takes overall responsibility for the QT-IGC study. The study design was developed by M.J.A., D.E.A., A.C., L.C., P.I.W.d.B., T.T.K., P.B.M., C.N.-C., A.P., S.L.P., P.J.S., N.S. in consultation with the respective study groups. The manuscript was written by C.N.-C. The manuscript was critically revised in detail by members of the writing team before circulation to all co-authors.

GWAS cohorts. AGES: Phenotyping: V.G. Data analysis: A.V.S. Oversight: T.B.H., L.J.L., V.G. **Amish studies**: Clinical data collection, genotyping and oversight: B.D.M., A.R.S. EKG data collection: W.P. Analysis: A.Parsa, J.R.O. Interpretation: A.Parsa, W.S.P.

ARIC: Study design: A.A., D.E.A, A.C., W.H.L.K. Analyses: D.E.A., J.S.B., A.C., G.E., H.H. Steering: D.E.A, A.C. Writing: D.E.A., A.C. **BLSA:** Analysis: T.T. Phenotype collection: J.B.S. Overall project supervision: L.F. **BRIGHT:** Phenotyping: M.B., M.J.C., P.W.M., P.B.M., N.J.S. Genotyping: P.B.M., S.J.N. Analysis: S.J.N. Overall study supervision: M.B., M.J.C., P.B.M., N.J.S. **Carlantino**: Sample/data collection: M.C., L.Z. Overall study supervision: P.G.. Data collection/statistical analysis: S.U. **CHS**: Study design: J.B., S.R.H., B.P., N.S. Data collection: S.R.H., B.P., D.S.S. Genotyping: J.I.R. Analysis, interpretation: J.B., N.S. Supervision of analyses: B.P. Funding for GWAS: B.P. **Croatia-Korcula& Croatia-Split**: GWAS analysis: C.H. Data collection, phenotype measurement, data entry, and field work supervision: I.K., O.P. Study design, funding: I.R., A.F.W. **DCCT/EDIC:** Analyses: D.W. Supervision of analyses: A.D.P. **deCODE:** Data collection: D.O.A., H.H. Study design: K.S., U.T., H.H., D.F.G., D.O.A. Data alignment, imputation and statistical analysis: D.F.G. Additional analysis, interpretation of results: K.S., U.T., H.H., D.F.G., D.O.A. **eMERGE:** Data curation, GWAS analysis: R.L.Z., Y.B. Supervision of QC/analysis of dataset: M.D.R. Study conception, analysis framework: D.R. Algorithm for case ascertainment: J.C.D. **ERF**: Analysis: A.I. Data acquisition: C.v.D., A.I., B.A.O., J.A.K., A.G.U. Overall study PIs: C.v.D., B.A.O. **FHS**: Analysis plan development: C.N.-C., C.O.D., P.A.N., M.G.L. GWAS analysis: X.Y.Y., M.G.L. Wrote manuscript: C.N.-C. Secured funding: C.N.-C., C.J.O. **FVG:** Data collection: M.B. Primary analysis: A.I. Statistical analysis: A.P.d'A. Overall study supervision: G.S. **Health2000**: Data analysis, replication genotyping, QC: A.M.L. Primary data analysis: A.Marjamaa. Phenotyping, including ECGs: A.J. Electrocardiographic measurements: K.P. GWAS and replication genotyping: M.P. Design of ECG study, analysis, interpretation: L.O. Genetic data collection, analysis: K.K.K. PI, supervision: V.S. **HealthABC**: Data collection, supervision: S.R.C., Y.L. Data analysis: D.S.E., M.A.N. **HNR**: Data collection: H.K. Data generation: H.K., T.W.M. Genetic data generation: M.N.N., P.H., T.W.M. Data analysis: L.E., P.H., T.W.M., M.N.N. Overall study design, PIs: R.E., K.-H.J. **KORA-F3/S4**: Overall QT project supervision: A.Pfeufer. Genotyping oversight: T.M. ECG collection, measurement and interpretation: M.F.S., S.P., B-M.B, E.M. Primary genetic analysis: M.M.-N. Interpretation of results: A.Pfeufer, S.Kaab, T.M., M.W. Overall study PI: A.Peters. **LifeLines:** Phenotyping: R.A.d.B., P.A.v.d.V. Genotyping: L.F. Analyses: I.M.N. and L.F. **MICROS**: Sample recruitment, overall study PI: P.P. Study supervision, genotyping, data coordination: A.A.H. Data analysis: F.D.G., C.F. **ORCADES**: Phenotype collection: S.H.W. Genotype generation: H.C., J.F.W. GWAS analysis: P.N. Raised funding: J.F.W. Overall study supervision: J.F.W. **PopGen**: Recruitment, phenotyping: N.E.E.M., N.F. Genotyping, data preparation: A.F. Data preparation, analysis: D.E. **PREVEND:** Phenotyping: M.P.v.d.B., D.J.v.V, G.N. Genotyping, data-analysis: F.W.A., I.M.L., P.v.d.H. Obtained funding: G.N., D.J.v.V., F.W.A., P.v.d.H. **Rotterdam Study-I and II**: Study concept, design: M.E., B.H.C.S. Data acquisition: M.E., B.P.K., J.A.K., A.H., J.C.M.W., B.H.C.S., A.G.U. Statistical analysis: M.E. Interpretation: M.E., B.H.C.S. Obtained funding: A.H., J.C.M.W., A.G.U., B.H.C.S. Study supervision: B.H.C.S. **SardiNIA**: Phenotyping: M.O. Genotyping, data analysis: G.A., E.G.L., A.Mulas, M.O., S.S., D.S., K.V.T., and M.U. Overall study supervision, PIs: D.S., M.U. **SHIP**: Data acquisition: M.D., M.R.P.M., U.V., S.B.F. Statistical analysis: U.V., M.D., M.R.P.M. Interpretation: U.V., M.D., S.B.F. Obtained funding: S.B.F., U.V. **TwinsUK:** Study concept, design: H.S., Y.J. Data

acquisition: T.D.S. Statistical analysis, interpretation: I.M.N., H.S., Y.J. Obtained funding: Y.J., T.D.S. **Young Finns Study**: Data collection: T.L., O.R., M.Kähönen, J.V. Genotyping: T.L., N.M. Genotyping: T.L. Phenotype preparation: O.R., M.Kähönen, J.V. Analysis: T.L., O.R., M.Kähönen, J.V., L.-P.-L. Obtained funding: T.L., O.R., M.Kähönen, J.V.

Directly genotyped SNP replication cohorts. (Author contributions for cohorts that contributed to both GWAS and replication genotyping are shown under the GWAS entry above) **BRHS**: Analysis: R.W.M. Custodian of genetic resource: R.W.M., P.H.W. Data collection for genetic resource: P.H.W. Development of genetic resource: A.D.H. Overall study supervision, PIs: P.H.W., R.W.M. ECG analyses: P.W.M. **Bruneck:** Data analysis, interpretation, writing: S.K. DNA preparation: F.K., C.L. ECG measurement, database: M.Knoflauch. Supervision, funding, administration, PI: J.W. **Carla**: Study concept, design: K.H.G., K.W. Genotyping: H.M.z.S. Supervision: K.W. Study design, analysis: A.K. Study concept, supervision, PI: J.H. **Cyprus**: Study concept, funding, supervision, analysis: A.N. Data acquisition, analysis, interpretation: M.G. Genetic, biochemical data acquisition, statistical analysis: A.G.P. **Czech Post-MONICA**: Data collection, submission: J.A.H., V.A. **Galicia**: Cohort collection: M.B. Study design: M.B. Genotyping platform management: A.C. Genotyping: M.T. Analysis: M.T. Interpretation: M.B., A.C. Financial support: A.C. **Intergene**: Genotyping, data analysis, epidemiology expertise: F.N. Genotyping, genetic expertise: A.T.N. Study design, data collection, disease area knowledge: D.S.T. **MIDSPAN Family Study**: Data acquisition, statistical analysis, interpretation: S.P. Genotyping: W.K.L. Overall study supervision, data collection, funding: A.F.D., G.C.M.W. **PIVUS**: Genotyping: A.-C.S. Phenotyping: L.L., J.A., J.S. Data analysis: S.G. Overall supervision, PI: E.I. **SAPHIR**: DNA preparation: F.K., C.L. Data collection: L.K., B.P., B.S. Data analysis: L.K., F.K., C.L., B.P., B.S. Study design, PI: B.P. **ULSAM:** Genotyping: A.-C.S. Phenotyping: L.L., J.A., J.S. Data analysis: S.G. Overall supervision, PI: E.I. **Whitehall II**: Data collection, submission: M.Kumari. Overall supervision: M.Kivimaki. Funding: A.H.

Meta-analysis of GWAS + **replication**. D.E.A. and S.L.P. independently performed quality control and meta-analysis of GWAS and replication association results. P.I.W.d.B., A.P. and C.N.-C. supervised the analyses.

Non-QT trait lookups. CARe-COGENT: Meta-analysis, lookup: J.G.S. **HRGEN:** Meta-analysis, lookup: M.d.H. Overall study supervision: R.J.F.L. **QRS GWAS:** Study supervision: N.S. Meta-analysis: D.E.A., P.I.W.d.B. Results lookup: S.L.P.

eQTL Analyses. Dataset acquisition: A.S.P., V.E. Analysis, interpretation: A.D.J. Cell-type enrichment tests: S.R.

Left ventricle eSNP analyses. Overall supervision: T.P.C. Recruitment, sample collection: K.M., C.M. Sample processing, expression analysis: J.B. Statistical analysis: M.M.

Left ventricle enhancer analyses. Analysis: X.W. Overall supervision: L.A.B., M.Kellis

Mouse knockout. Enrichment tests: K.S.

DAPPLE analysis. Concept, design, analysis: E.J.R. conceived, designed and performed the DAPPLE analyses. Supervision: K.L., M.J.D.

LQTS mutation screening. Amsterdam: SLC8A1 Sequencing: T.K.T. Clinical data collection: A.B., N.H., A.A.M.W. Study supervision: C.R.B., A.A.M.W. **London**: Recruitment, phenotyping, strategy: E.R.B. Screening for mutations in LQT1,2,3 and sample management: C.D. **Mayo**: LQTS cohort characteristic organization: D.J.T. TRPM7 mutation analysis, interpretation: D.J.T., A.M.D., J.R.G. Patient collection, study design, data review, overall supervision: M.J.A. **Munich:** Study oversight: S.Kääb, A.Pfeufer. Patient collection: B.M.B., E.M. **Nantes**: Scientific management: J-J.S Clinical, genetic information collection: S.C. ATP2A2 sequencing: S.C. Screening for mutations in LQT1,2,3, and clinical data collection: J.B. LQTS gene diagnosis management: F.K. Patient enrollment: V.P. **Pavia**: Patient collection, patient selection, molecular screening supervision: L.C., P.J.S. SRL mutation screening: A.G., R.I. **Toronto**: Identification of LQTS patients free of LQT1,2,3 mutations: R.M.H. Program co-development: S.W.S.

Immunoprecipitation experiments. Proteomic experiments, analysis:A.L. Overall study supervision: J.V.O.
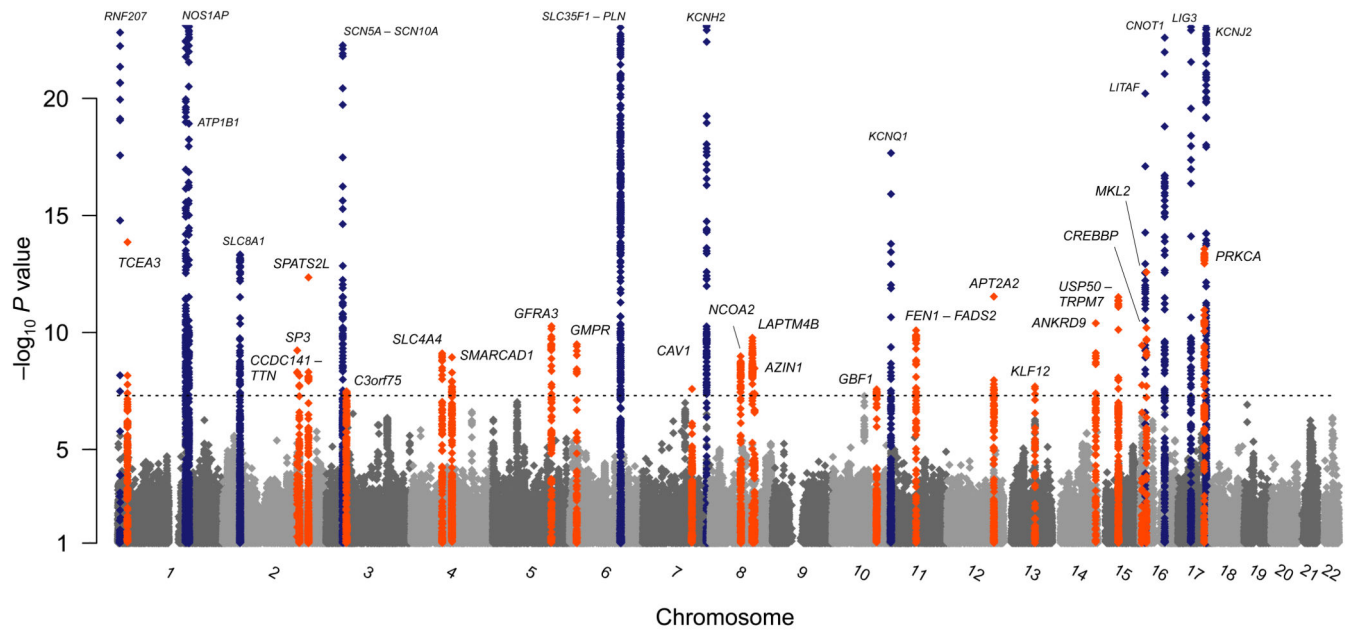
Figure 1.
Genome-wide association results for GWAS meta, annotated with gene names. Shown are association results from meta-analysis of QT interval GWAS in 76,198 individuals of European ancestry across 22 autosomes. Loci meeting $P < 5\times10^{-8}$ upon meta-analysis with replication data are annotated for novel (large font) and previously reported (small font) loci. Nearest genes are used for annotation but the causal gene at any given locus is unknown.

Table 1

Common genetic variants at loci associated with QT interval ($P < 5×10^{-8}$) on meta-analysis of GWAS+replication results (Supplementary Table 6). N is the effective number of samples contributing to the signal. For a given SNP, the effective sample size is the sum of the product of the cohort-specific sample size and imputation quality (ranging from 0 to 1). Function (Fxn) shown for coding variants with $r^2 = 1$ to sentinel SNP or proxy with $1.0 > r^2 > 0.8$ (~p) to the sentinel SNP. Expression quantitative trait loci (eQTL) transcripts are shown if associated at $P < 5×10^{-8}$ with sentinel SNPs or their close proxies ($r^2 > 0.8$, Supplementary Table 12, bolded if eQTL found in left ventricle for the sentinel SNP). Protein-protein interactor (PPI) relationships for nearby genes to genes in loci previously established to influence myocardial repolarization are provided (Supplementary Table 17). Interactors from immunoprecipitation (IP) experiments are shown from murine cardiac tissue using 5 baits (K1=KCNQ1, K2=KCNH2, CV=CAV3, CA=CACNA1C, S1=SNTA1) with protein identified in parentheses if different from the nearest gene listed.Loci at which a SNP (index or secondary) or a close proxy (r2>0.8) falls in a left ventricular enhancer are marked with I or S. Brackets indicate annotations for secondary signals of association (Supplementary Table 8a).

Nearest gene	SNP	chr	position hg18	coded/noncoded allele	coded allele freq	N	effect msec (SE)	P	LQTS gene locus	Fxn	eQTL transcript	PPI interactor known QT loci	IP interactor	LV enhancer
Previously discovered loci														
RNF207	rs846111	1p36	6,201,957	C/G	0.28	47,041	1.73 (0.13)	$7×10^{-40}$		G603A				
NOS1AP	rs12143842	1q23	160,300,514	T/C	0.24	75,053	3.50 (0.11)	$1×10^{-213}$						S
ATP1B1	rs10919070	1q24	167,365,661	C/A	0.13	75,707	-1.68 (0.14)	$1×10^{-31}$		intron	*ATP1B1,NME7*		ATP1B1-K1, ATP1B1-K2, ATP1B1-CA, ATP1B1-CV	I,S
SLC8A1	rs12997023	2p22	40,606,486	C/T	0.05	70,311	-1.69 (0.22)	$5×10^{-14}$				ANK2,CAV3		I,S
SCN5A-SCN10A	rs6793245	3p22	38,574,041	A/G	0.32	73,697	-1.12 (0.10)	$4×10^{-27}$	LQT3	intron		SCN5A-SNTA1		S
SLC35F1-PLN	rs11153730	6q22	118,774,215	T/C	0.50	74,932	-1.65 (0.10)	$2×10^{-67}$					PLN-CV, PLN-CA	S
KCNH2	rs2072413	7q36	150,278,902	T/C	0.27	65,331	-1.68 (0.11)	$1×10^{-49}$	LQT2, SQT1	intron		KCNE1		S
KCNQ1	rs7122937	11p15	2,443,126	T/C	0.19	72,978	1.93 (0.12)	$1×10^{-54}$	LQT1, SQT2	intron	*C11ORF21,PHEMX,TSPAN32*	KCNE1,KCNH2		1
LITAF	rs735951	16p13	11,601,037	A/G	0.46	62,994	-1.15 (0.10)	$2×10^{-28}$			*LITAF*			S
CNOT1	rs246196	16q21	57,131,754	C/T	0.26	76,513	-1.73 (0.11)	$2×10^{-57}$		intron	*NDRG4,CNOT1*		GOT2-CV, GOT2-K1	I,S
LIG3	rs1052536	17q12	30,355,688	C/T	0.53	75,961	0.98 (0.10)	$6×10^{-25}$		3'UTR	*LIG3,CCT6B*		UNC45B-K1, UNC45B-CV	1
KCNJ2	rs1396515	17q24	65,942,588	C/G	0.52	77,058	-0.98 (0.09)	$2×10^{-25}$	LQT7, SQT3					S
KCNE1	rs1805128	21q22	34,743,550	T/C	0.01	20,061	7.42 (0.85)	$2×10^{-18}$	LQT5	D85N		KCNQ1,KCNH2		
Novel loci														
TCEA3	rs2298632	1p36	23,583,062	T/C	0.50	83,031	0.70 (0.09)	$1×10^{-14}$		intron	*TCEA3*			
SP3	rs938291	2q31.1	174,450,854	G/C	0.39	101,902	0.53 (0.09)	$6×10^{-10}$						
TTN-CCDC141	rs7561149	2q31.2	179,398,101	C/T	0.42	85,299	-0.52 (0.09)	$7×10^{-9}$					CCDC141-CV,TTN-K2, TTN-CV	1
SPATS2L	rs295140	2q33	200,868,944	T/C	0.42	103,331	0.57 (0.09)	$2×10^{-11}$			*SPATS2L*	SGOL2-SCN5A		1
C3ORF75	rs17784882	3p21	47,519,007	A/C	0.40	76,184	-0.54 (0.10)	$3×10^{-8}$		intron	*KLHL18,PTPN23, SCAP,SETD2*		MYL3-CA	1
SLC4A4	rs2363719	4q13	72,357,080	A/G	0.11	70,821	0.97 (0.16)	$8×10^{-10}$		intron				1

Nearest gene	SNP	chr	position hg18	coded/noncoded allele	coded allele freq	N	effect msec (SE)	P	LQTS gene locus	Fxn	eQTL transcript	PPI interactor known QT loci	IP interactor	LV enhancer
SMARCAD1	rs3857067	4q22	95,245,457	A/T	0.46	101,382	-0.51 (0.08)	1×10^{-9}						
GFRA3	rs10040989	5q31	137,601,624	A/G	0.13	87,942	-0.85 (0.13)	5×10^{-11}			*FAM13B*	ETF1-RPL22		
GMPR	rs7765828	6p22	16,402,701	G/C	0.40	93,262	0.55 (0.09)	3×10^{-10}		intron (F256I-p)	*ATXN1*	ATXN1-ACOT7,ATXN1-KCNAB2		1
CAV1	rs9920	7q31	115,987,328	C/T	0.09	102,060	0.79 (0.14)	3×10^{-8}		3' UTR		CAV-ATP1B1, CAV2-ATP1B1	CAV1-CA, CAV1-S1, CAV1-CV, CAV2-CV	
NCOA2	rs16936870	8q13	71,351,896	A/T	0.10	74,196	0.99 (0.16)	1×10^{-9}		intron				1
LAPTM4B	rs11779860	8q22.1	98,919,506	C/T	0.47	73,404	-0.61 (0.10)	2×10^{-10}		intron				1
AZIN1	rs1961102	8q22.3	104,002,021	T/C	0.33	82,677	0.57 (0.10)	3×10^{-9}						
GBF1	rs2485376	10q24	104,039,996	A/G	0.39	70,552	-0.56 (0.10)	3×10^{-8}		intron			ACTR1A-CV	1
FEN1-FADS2	rs174583	11q12	61,366,326	T/C	0.34	100,900	-0.57 (0.09)	8×10^{-11}		intron	*FADS1, FADS2, FADS3*			1
ATP2A2	rs3026445	12q24	109,207,586	C/T	0.36	95,768	0.62 (0.09)	3×10^{-12}		intron	*VPS29, GPN3, ARPC3, C12ORF24*	PLN	ATP2A2-CV, ATP2A2-CA	1
KLF12	rs728926	13q22	73,411,123	T/C	0.36	69,219	0.57 (0.10)	2×10^{-8}		intron	*KLF12*			1
ANKRD9	rs2273905	14q32	102,044,752	T/C	0.35	83,532	0.61 (0.09)	4×10^{-11}		5' UTR	*ANKRD9*			1
USP50-TRPM7	rs3105593	15q21	48,632,310	T/C	0.45	77,240	0.66 (0.10)	3×10^{-12}						
CREBBP	rs1296720	16p13.3	3,813,643	C/A	0.20	59,812	0.83 (0.13)	4×10^{-10}		intron			CV-TRAP1	
MKL2	rs246185	16p13.12	14,302,933	C/T	0.34	77,411	0.72 (0.10)	3×10^{-13}						
PRKCA	rs9892651	17q24	61,734,255	C/T	0.43	74,683	-0.74 (0.10)	3×10^{-14}		intron	*PRKCA*	CACNA1C,KCNE1		1

Table 2

Association of QT SNPs with gene expression in human left ventricle

Shown are QT SNPs associated with expression of a transcript within 1Mb at experiment-wide significance (Supplementary Note). For each transcript, the best eSNP for that transcript is shown. The QT SNP association with transcript is shown with and without adjustment for the best eSNP for that transcript. QT SNPs and transcripts are bolded if the QT SNP and best eSNP are highly correlated ($r^2 > 0.8$), show attenuation of association in conditional models and show comparable strength of association with QT interval for both the QT SNP and best eSNP.

QT SNP	chr	position	transcript	best eSNP for transcript	r^2 between QT SNP & eSNP	direction of eSNP effect for QT increasing allele	Transcript association of QT SNP (P)	Transcript association of QT SNP with adjustment for best eSNP (P)	attenuated significance	QT association of QT SNP (P)	QT association of best eSNP (P)
rs17457880	1	160,434,778	*FCGR2B*	rs17457880	same	↑	1×10^{-5}	0.99	YES	3×10^{-10}	same
rs17457880	1	160,434,778	*FCGR3A*	rs9727076	0.00	↑	1×10^{-7}	9×10^{-9}	NO	3×10^{-10}	NA
rs295140	2	200,868,944	*SPATS2L*	rs295113	0.53	→	8×10^{-7}	0.74	YES	4×10^{-13}	0.76
rs174583	11	61,366,326	*FADS2*	rs174548	0.80	→	6×10^{-8}	0.94	YES	1×10^{-10}	8×10^{-8}
rs3026445	12	109,207,586	*VPS29*	rs6606686	0.86	↑	1×10^{-6}	0.84	YES	1×10^{-8}	2×10^{-7}
rs728926	13	73,411,123	*KLF12*	rs1886512	0.93	↑	4×10^{-5}	0.25	YES	2×10^{-8}	4×10^{-8}
rs735951	16	11,601,037	*LITAF*	rs7187498	0.93	↑	4×10^{-13}	0.62	YES	2×10^{-28}	NA
rs246196	16	57,131,754	*SETD6*	rs42945	0.30	↑	4×10^{-7}	0.20	YES	2×10^{-57}	9×10^{-22}
rs9892651	17	61,734,255	*PRKCA*	rs11658550	0.97	↑	2×10^{-41}	0.22	YES	3×10^{-14}	NA

Table 3

Candidate gene mutational screening

6 genes (*ATP2A2, CAV1, CAV2, SLC8A1, SRL, TRPM7*) at 5 loci were screened for amino-acid altering variants in 298 LQTS cases and compared to >300 same-ancestry controls, presence on an exome chip array designed from exome sequencing of >12,000 multi-racial samples (number of alternate alleles shown) and in the Exome Sequencing Project (alternate allele counts per total number of individuals shown). Predicted function by Polyphen2 (BENign, POSSibly damaging or PROBably damaging) or SIFT (TOLerated, DAMaging) is also indicated. See Supplementary Material for details.

gene	position hg19	exon	nucleotide change	amino acid change	# cases	in controls (yes/no)	alt alleles in Exome Chip	in ESP	PolyPhen/SIFT
ATP2A2	chr12:110,734,419	5	c.340_A>G	p.Asn114Asp	1	no	no	no	BEN/TOL
ATP2A2	**chr12:110,765,553 - 110,765,554**	**8**	**c.826_827insA**	**p.Ile276fsX281**	**1**	**no**	**no**	**no**	**STOP**
SLC8A1	chr2:40,656,318	1	c.1104_C>T	p.Ala368Val	1	no	no	24/5379	PROB/TOL
SLC8A1	chr2:40,397,450	6	c.2009_C>T	p.Pro670Leu	1	no	no	no	BEN/DAM
SLC8A1	chr2:40,342,664	10	c.2651_T>G	p.Val884Gly	1	no	no	no	PROB/DAM
SRL	chr16:4,256,990	2	c.1177_G>T	p.Gly393Cys	1	no	no	no	PROB/DAM
SRL	chr16:4,256,754	2	c.1409_G>A	p.Arg470Lys	1	no	no	no	BEN/TOL
SRL	chr16:4,256,384	7	c.2566_C>T	p.Arg856Cys	1	no	no	1/4915	PROB/TOL
TRPM7	**chr15:50,955,189**	**2**	**c.58_INS_A**	**p.Ile19fsX59**	**1**	**no**	**no**	**no**	**STOP**
TRPM7	chr15:50,935,731	5	c.341_A>T	p.Asp114Val	1	no	no	no	PROB/DAM
TRPM7	chr15:50,884,537	26	c.3895_A>C	p.Ser1299Arg	1	no	no	no	BEN/TOL
TRPM7	chr15:50,884,406	26	c.4026_A>T	p.Glu1342Asp	2	no	no	no	BEN/TOL
TRPM7	chr15:50,884,280	26	c.4152_A>T	p.Leu1384Phe	1	no	no	no	POSS/TOL