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Common variants at ten loci influence QT interval duration in the QTGEN Study

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QT interval duration, reflecting myocardial repolarization on the electrocardiogram, is a heritable risk factor for sudden cardiac death and drug-induced arrhythmias. We conducted a meta-analysis of three genome-wide association studies in 13,685 individuals of European ancestry from the Framingham Heart Study, the Rotterdam Study and the Cardiovascular Health Study, as part of the QTGEN consortium. We observed associations at $P < 5 \times 10^{-8}$ with variants in *NOS1AP, KCNQ1, KCNE1, KCNH2* and *SCN5A*, known to be involved in myocardial repolarization and mendelian long-QT syndromes. Associations were found at five newly identified loci, including 16q21 near *NDRG4* and *GINS3*, 6q22 near *PLN*, 1p36 near *RNF207*, 16p13 near *LITAF* and 17q12 near *LIG3* and *RFFL*. Collectively, the 14 independent variants at these 10 loci explain 5.4–6.5% of the variation in QT interval. These results, together with an accompanying paper, offer insights into myocardial repolarization and suggest candidate genes that could predispose to sudden cardiac death and drug-induced arrhythmias.

Sudden cardiac death (SCD) and drug-induced ventricular arrhythmia, a key barrier to drug development, are poorly predicted[1]. Prolongation of electrocardiographic QT interval duration, a measure of myocardial repolarization time, is a risk factor for drug-induced arrhythmias and SCD. Continuous QT interval duration is heritable[2] ($h^2 \approx 0.35$) and has multiple environmental and genetic contributors, although there has been limited characterization of genetic variation within populations[3]. Congenital long- and short-QT syndromes of ventricular arrhythmias and SCD due to extremes of QT interval duration are often caused by mutations with large effect sizes, commonly in genes encoding ion channels involved in myocardial repolarization. These mutations are typically private to specific families and individually explain little of the population variation in QT interval duration or SCD risk[4]. The few common variants in candidate genes associated with QT interval duration thus far reported[5–9] leave much of its heritability unexplained. Genome-wide association studies can be used to identify genetic variants that typically confer modest effect sizes for quantitative complex traits such as QT interval duration. We completed a meta-analysis of three genome-wide association studies of QT interval duration in 13,685 self-identified white individuals of European ancestry drawn from three prospective cohort studies: the Framingham Heart Study (FHS, $n = 7{,}650$), the Rotterdam Study (RS, $n = 4{,}606$) and the first and second rounds of genotyping in the Cardiovascular Health Study (CHS, $n = 1{,}429$)[10].

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Table 1 Clinical characteristics by cohort and by sex

	Framingham Heart Study		Rotterdam Study		Cardiovascular Health Study	
	Men $n = 3,440$	Women $n = 4,210$	Men $n = 1,854$	Women $n = 2,752$	Men $n = 802$	Women $n = 627$
Age (y)	40 (10)	40 (11)	68 (8)	69 (9)	73 (6)	73 (5)
Body mass index	27 (4)	25 (5)	26 (3)	27 (4)	26 (3)	26 (5)
Hypertension	23%	14%	34%	41%	54%	60%
Diabetes	2.3%	1.6%	10%	10%	16%	11%
Raw QT (msec)	390 (35)	391 (38)	398 (29)	399 (29)	417 (35)	413 (31)
Heart rate (beats/minute)	67 (13)	72 (14)	68 (12)	71 (12)	66 (11)	69 (10)
RR interval (msec)	943 (167)	880 (164)	904 (155)	865 (140)	979 (167)	928 (140)
QTc (msec)[a]	404 (22)	419 (22)	421 (23)	431 (22)	423 (19)	431 (22)
Standard deviation of QT residuals (msec)[b]	17.1	17.3	18.3	17.9	17.3	17.3

[a]Bazett's correction for heart rate: $QTc = QT/\sqrt{(RR\ interval)}$. [b]Residuals are from sex-specific linear regression models adjusting for age and RR interval.

RESULTS

Overall QT interval GWAS results

The mean ages of the individuals in the FHS, RS and CHS cohorts were 40, 69 and 73 y, respectively. Additional clinical characteristics are shown in **Table 1**. QT interval measures were adjusted for age and RR interval (inverse heart rate) using cohort- and sex-specific linear regression, and the standardized residuals served as the phenotype for the association analysis. We started with a set of SNPs passing study-specific quality control filters: in FHS, 378,163 SNPs from the Affymetrix 500K chip + 50K gene-centered MIP; in RS, 512,349 SNPs from the Illumina 550K array; and in CHS, 332,946 SNPs from the Illumina 370CNV array. We imputed genotypes with reference to genotypes from HapMap CEU (see Methods)[11]. After quality control filtering, we used imputed genotypes from up to 2,543,686 SNPs to test for association in cohort-specific analyses. Genomic control was used to adjust for test-statistic inflation[12], which was minimal, with λ_{gc} ranging from 1.02 to 1.04. Cohort-specific quantile-quantile plots of *P*-value distributions are shown in **Supplementary Figure 1** online. Using inverse variance weights, we combined genomic-controlled association results under an additive model from the three cohorts in fixed-effects meta-analysis (overall $\lambda_{gc} = 1.012$). Nine loci were independently associated at a genome-wide $P < 5 \times 10^{-8}$ (**Table 2** and **Figs. 1** and **2**). An additional locus was borderline significant ($P = 8 \times 10^{-8}$), but was externally validated. Five of the ten associated loci are related to the myocardial repolarization genes previously known to be associated with QT interval duration in the general population or in mendelian conditions: *NOS1AP, KCNQ1, KCNH2, KCNE1* and *SCN5A*. We observed an excess number of associations compared with the expectation under the null. For a nominal $P < 10^{-5}$ we observed 568 associations compared with 25 expected under the null hypothesis ($P \ll 10^{-4}$, **Supplementary Table 1** online). This finding suggests that among the many false positives at less stringent statistical thresholds additional truly associated variants may exist.

We had the opportunity to compare our top results with the QTSCD Study, which included 15,854 individuals of European ancestry[13]. All associations but one were confirmed at two-sided $P < 0.05$ (**Table 2**).

Genes known to be involved in myocardial repolarization

At the *NOS1AP* locus, we observed the strongest association in the genome for rs12143842, 6 kb 5′ of *NOS1AP*, with 0.21 s.d. QT increase per minor allele copy (minor allele frequency, MAF = 0.26, $P = 8 \times 10^{-46}$, **Table 2** and **Fig. 2a**). All results are shown on the s.d. scale (1 s.d. $\approx$ 17.5 msec). Two additional independent signals were observed at rs12029454 (MAF = 0.15) and rs16857031 (MAF = 0.14) in intron 2 and intron 1, respectively, all with r^2 to each other <0.05 in HapMap and with $P < 0.05$ when entered into a single regression model in FHS and RS (CHS with a smaller sample is underpowered, **Supplementary Table 2** online). We have previously reported association in FHS and RS samples of rs10494366 at *NOS1AP* ($P = 5 \times 10^{-30}$ in the current report) and this association has been widely replicated[5,14–17]. This SNP is not significant in models adjusting for the three SNPs identified in the current study ($P > 0.05$) and it shows some degree of correlation to each of the three SNPs (to rs12143842, $r^2 = 0.46$–0.47 in FHS and RS and $r^2 = 0.11$ in HapMap CEU; to rs12029454, $r^2 = 0.17$ in RS and HapMap CEU; to rs16857031, $r^2 = 0.17$ in HapMap CEU)[18]. We conclude that there are three independent signals at the locus and that rs10494366 captures the association signal from at least one of these three SNPs.

We identified two common variants in intron 1 of *KCNQ1* that were associated with QT interval duration (**Table 2** and **Fig. 2b**). Rare mutations in *KCNQ1*, encoding a potassium channel involved in myocardial repolarization, have been associated with long-QT syndrome type 1 and short-QT syndrome type 2 (ref. 4). In this meta-analysis, SNP rs2074238 (MAF = 0.06) was associated with 0.47 s.d. shorter QT interval for each minor allele ($P = 3 \times 10^{-16}$) and rs12576239 (MAF = 0.13) was associated with 0.12 s.d. longer QT interval for each minor allele ($P = 2 \times 10^{-10}$). The two SNPs were independently associated with QT in models that included both SNPs ($P = 6 \times 10^{-5}$, $P = 1 \times 10^{-4}$, respectively, in FHS and $P = 3 \times 10^{-10}$, $P = 0.03$ in RS, **Supplementary Table 2**). Coupled with the low correlation of the two SNPs (HapMap CEU $r^2 = 0.009$, FHS $r^2 = 0.014$, RS $r^2 = 0.011$) these findings support two independent association signals at the locus. Pfeufer *et al.* previously reported association with QT interval of rs757092 (MAF = 0.38), which lies 3 kb away from rs12576239 in intron 1 and to which it is partially correlated ($r^2 = 0.31$ HapMap CEU) and 14 kb away from rs2074238, to which it is not correlated ($r^2 = 0.005$ HapMap CEU)[7]. We did not find supportive evidence of association of rs757092, which was well imputed, with QT interval in QTGEN ($P = 0.11$).

The SNP rs4725982, 3′ of *KCNH2*, was associated with QT interval (+0.09 s.d. per minor allele, MAF = 0.22, $P = 6 \times 10^{-9}$, **Table 2** and **Fig. 2f**). A second SNP at *KCNH2*, rs2968864, was associated with shorter QT interval duration for increasing minor allele count, but did not reach our prespecified genome-wide significance threshold for

Table 2 SNPs with evidence for independent association at ten loci with $P < 5 \times 10^{-8}$

SNP	Chr.	Function/gene	Other genes within 500 kb at newly identified loci	Coded allele	Allele freq.	QTGEN				QTSCD			Meta-analysis QTGEN + QTSCD		
						Effective sample size	β (s.d.)	s.e.m.	*P*	β (s.d.)	s.e.m.	*P*	β (s.d.)	s.e.m.	*P*
rs12143842	1q	Upstream *NOS1AP*		T	0.26	13,241	0.21	0.02	8×10^{-46}	0.16	0.01	5×10^{-36}	0.18	0.01	2×10^{-78}
rs12029454	19	Intron *NOS1AP*		A	0.15	12,172	0.21	0.02	6×10^{-28}	0.15	0.02	3×10^{-20}	0.17	0.01	3×10^{-45}
rs16857031	19	Intron *NOS1AP*		G	0.14	13,154	0.19	0.02	3×10^{-23}	0.12	0.02	1×10^{-14}	0.15	0.01	1×10^{-34}
rs2074238	11p	Intron *KCNQ1*		T	0.06	2,888	–0.47	0.06	3×10^{-16}	–0.33	0.14	0.02	–0.45	0.05	3×10^{-17}
rs37062	16q	Intron *CNOT1*	*GINS3, NDRG4, SLC38A7, GOT2*	G	0.24	13,440	–0.12	0.02	3×10^{-15}	–0.09	0.01	5×10^{-12}	–0.10	0.01	3×10^{-25}
rs11756438	6q	Intron *c6orf204*	*SLC35F1, PLN, ASF1A*	A	0.47	12,707	0.09	0.01	4×10^{-11}	0.08	0.01	2×10^{-12}	0.08	0.01	5×10^{-22}
rs12576239	11p	Intron *KCNQ1*		T	0.13	13,211	0.12	0.02	2×10^{-10}	0.08	0.02	3×10^{-7}	0.10	0.01	1×10^{-15}
rs846111	1p	3′ UTR *RNF207*	*NPHP4, CHDS, ACOT7, PLEKHG5, KLH21*	C	0.28	6,480	0.12	0.02	1×10^{-9}	0.08	0.01	4×10^{-9}	0.10	0.01	1×10^{-16}
rs4725982	7q	Downstream *KCNH2*		T	0.22	13,706	0.09	0.02	6×10^{-9}	0.08	0.01	1×10^{-8}	0.09	0.01	5×10^{-16}
rs8049607	16p	Upstream *LITAF*	*CLEC16A, SNN, ZC3H7A, TNFRSF17*	T	0.49	10,543	0.08	0.01	2×10^{-8}	0.07	0.01	4×10^{-8}	0.07	0.01	5×10^{-15}
rs1805128	21q	Missense *KCNE1*		A	0.010	7644	0.48	0.09	2×10^{-8}	–0.06	0.04	0.16	0.05	0.04	0.22
rs12053903	3p	Intron *SCN5A*		C	0.34	13,491	–0.08	0.01	3×10^{-8}	–0.06	0.01	6×10^{-8}	–0.07	0.01	1×10^{-14}
rs2074518	17q	Intron *LIG3*	*RFFL*	T	0.46	13,488	–0.07	0.01	8×10^{-8}	–0.05	0.01	7×10^{-6}	–0.06	0.01	6×10^{-12}
rs2968864	7q	Downstream *KCNH2*		C	0.25	12,932	–0.08	0.02	1×10^{-7}	–0.08	0.01	1×10^{-9}	–0.08	0.01	8×10^{-16}

A SNP at the *LIG3* locus met our significance threshold in an interim analysis and was confirmed in the QTSCD consortium study. Chromosomal positions and coded alleles are given relative to the forward strand of NCBI build 36. Effect sizes are shown as beta estimates from linear regression models for increasing copy of the coded allele and are on the standard deviation scale (1 s.d. ≈ 17.5 msec). A beta estimate of 0.08 s.d. is equivalent to a change in QT interval of 1.4 msec and an effect of 0.48 s.d. is equivalent to an 8.4 msec change. The effective sample size reflects the power relative to the total sample size of 13,685 with imputed data resulting from variation in imputation quality (see Methods). Selected genes from each locus are shown for reference. Results using the same coded allele from the QTSCD study (reported separately) and meta-analysis of the QTGEN and QTSCD studies using inverse variance weighting are shown ($n \leq 29,539$).

unselected genetic variants in the QTGEN samples (–0.08 s.d. per minor allele, MAF = 0.25, $P = 1 \times 10^{-7}$, **Table 2** and **Fig. 2f**). Rare mutations in *KCNH2*, encoding a potassium channel involved in myocardial repolarization and drug-induced arrhythmias, are known to underlie congenital long-QT syndrome type 2 and short-QT syndrome type 1 (ref. 4). The two SNPs were significant or nearly so when entered into a single regression model ($P = 4 \times 10^{-3}$, $P = 3 \times 10^{-4}$, respectively, in FHS and $P = 4 \times 10^{-3}$, $P = 0.16$, respectively, in RS, **Supplementary Table 2**). Coupled with the low correlation between the SNPs in HapMap CEU ($r^2 = 0.09$), the two SNPs thus seem to represent independent signals of association. The missense variant rs1805123 (K897T) has been associated with QT interval in most studies, including our own[6–9,19], and is perfectly correlated with rs2968864 ($r^2 = 1.0$ in FHS, data not shown), which is thus not a new finding[8]. An intronic SNP has been previously reported by Pfeufer *et al.* to be associated with QT interval (rs3815459), is poorly correlated with the currently reported rs4725982 or rs2968864/rs1805123 variants ($r^2 = 0.08$, $r^2 = 0.08$, respectively, in KORA, personal communication, A. Pfeufer, Technical University Munich) and could not be imputed because it is not represented in HapMap. Another variant previously reported by us (rs3807375)[8] has limited correlation with rs2968864 ($r^2 = 0.21$ HapMap CEU) and rs4725982 ($r^2 = 0.39$ HapMap CEU) and is not significant in models containing rs2968864 and rs4725982, suggesting that it does not represent an independent signal of association.

SNP rs1805128 was associated with QT interval duration (+0.48 s.d. per minor allele, MAF = 0.01, $P = 2 \times 10^{-8}$, **Table 2** and **Fig. 2h**). This SNP encodes a change from aspartate to asparagine at amino acid 85 (D85N) in *KCNE1*, encoding a potassium channel involved in myocardial repolarization in which rare mutations result in long-QT syndrome type 5 (ref. 4). D85N is poorly covered by the fixed genotyping arrays used here, but was included on the supplemental Affymetrix 50K array used in FHS, for which results are presented. Association of rs1805128 has been previously reported with extremes of QT interval duration in 398 individuals from the DESIR cohort ($P = 0.02$)[9], and by us in 4,487 CHS participants ($P = 0.003$)[20], and more recently in 5,043 individuals from the Health2000 study ($P = 4 \times 10^{-11}$)[21]. Notably, the D85N variant has also been related to drug-induced arrhythmia[22,23] and long-QT syndrome[24].

In the published literature, no common variants in *SCN5A* have been convincingly associated with QT interval duration in European-derived individuals. We observed an association of rs12053903 in intron 27 of *SCN5A* with QT interval (–0.08 s.d. per minor allele, MAF = 0.34, $P = 3 \times 10^{-8}$, **Table 2** and **Fig. 2i**). Rare mutations in *SCN5A*, encoding the cardiac sodium channel, result in long-QT syndrome type 3 (ref. 25). A common missense variant in *SCN5A*, S1102Y, is associated with QT prolongation, ventricular arrhythmias and sudden cardiac death in African Americans[25,26], but is nearly monomorphic (MAF < 0.01) in individuals of European ancestry.

The finding of nine associated common variants in five genes known to influence myocardial repolarization and cardiac arrhythmias at the top of our list of results, with eight achieving a stringent genome-wide significance threshold in our meta-analysis of three independent cohorts, gave us confidence in the validity of the five newly identified loci that exceeded this threshold.

QT interval associations with newly identified loci

The first newly identified locus on chromosome 16q21 near *NDRG4*, *SETD6*, *CNOT1*, *SLC38A7* and *GINS3* included SNP rs37062 (MAF = 0.24), which falls in intron 40 of *CNOT1*, a regulator of RNA transcription, and which was associated with 0.12 s.d. reduced QT

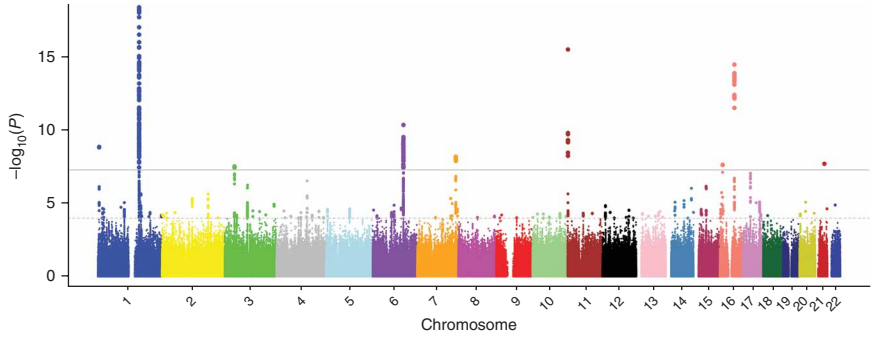

Figure 1 QT interval association results for 2,543,686 imputed SNPs in 13,685 individuals from three cohorts. Results are shown on the $-\log_{10}(P)$ scale and are truncated at $-\log_{10}(P) = 18$ for display purposes. The solid bar corresponds to the genome-wide significance threshold of 5×10^{-8}.

per minor allele ($P = 3 \times 10^{-15}$, **Table 2** and **Fig. 2c**). None of the nearby genes is known to modulate myocardial repolarization in humans, although recent experiments in zebrafish suggest potential candidates at the locus. Milan *et al.* tested zebrafish mutants previously generated by an insertional mutagenesis screen[27] for altered response to challenge with dofetilide, a QT–prolonging medication used in humans that prolongs cardiomyocyte action potential duration in humans and zebrafish. They found that a mutant with an insertion in intron 1 of GINS complex subunit 3 (GINS3) was resistant to the QT-prolonging effects of dofetilide (personal communication, D. Milan, Massachusetts General Hospital). The GINS complex is involved in the establishment of DNA replication forks. In humans, *GINS3* falls near the 16q21 interval associated with QT interval in our meta-analysis (127 kb from rs37062). In addition, a recent report on *NDRG4* (N-myc downstream-regulated gene family member 4, 56 kb from rs37062) in zebrafish observed expression restricted to

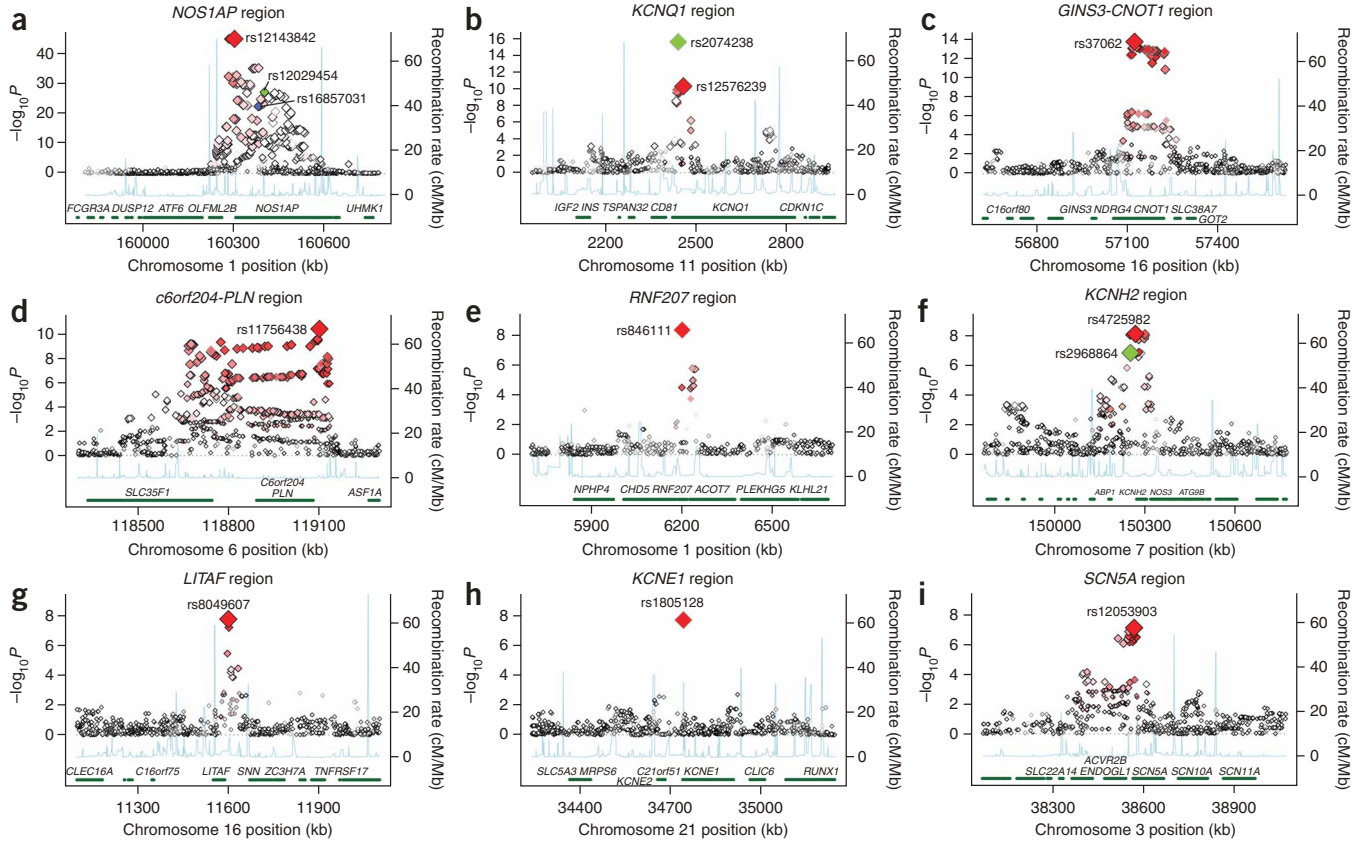

Figure 2 Regional association plots for one megabase surrounding each associated locus. Statistical significance of associated SNPs at each locus are shown on the $-\log(P)$ scale as a function of chromosomal position (NCBI build 36). The primary associated SNP at each locus is shown in red. The correlation of the primary SNP to other SNPs at the locus is shown on a scale from minimal (white) to maximal (bright red). The quality of imputation as assessed by the observed/expected variance on allele dosage is represented by the darkness of the diamond outline ranging from maximal (black) to minimal (light gray). Estimated recombination rates from HapMap and RefSeq annotations are shown. (**a**) 1q23.3 including *NOS1AP* with three independent associations, rs12143842 in red, rs12029454 in green and rs16857031 in blue. (**b**) 11p15.5 including *KCNQ1* with two independent associations, rs2074238 in green and rs12576239 in red. (**c**) 16q21 including *GINS3*, *NDRG4* and *CNOT1*. (**d**) 6q22.31 including *c6orf204* and *PLN*. (**e**) 1p36.31 including *RNF207*. (**f**) 7q36.1 including *KCNH2* with two independent associations, rs4725982 in red and rs2968864 in green. (**g**) 16p13.3 including *LITAF*. (**h**) 21q22.12 including *KCNE1*. (**i**) 3p22.2 including *SCN5A*. (**j**) 17q12 including *LIG3* and *RFFL*.

Table 3 QT interval association results of directly genotyped SNPs compared to imputed SNPs

	Imputed SNP genotype association				Directly genotyped SNP association				Sample
SNP	Beta (s.d.)	s.e.m.	*P*	*N*_effective	Beta (s.d.)	s.e.m.	*P*	*N*	
rs2074238	–0.47	0.06	1×10^{-13}	2,359	–0.31	0.03	6×10^{-23}	6,975	FHS + RS
rs846111	0.09	0.03	4×10^{-4}	3,709	0.10	0.02	2×10^{-7}	6,825	FHS + RS
rs8049607	0.09	0.02	1×10^{-6}	5,964	0.08	0.02	4×10^{-6}	6,921	FHS + RS
rs12576239	0.13	0.04	2×10^{-3}	2,560	0.13	0.04	1×10^{-3}	2,566	FHS only
rs4725982	0.10	0.03	3×10^{-3}	2,556	0.11	0.03	1×10^{-3}	2,556	FHS only
rs12053903	–0.04	0.03	0.17	2,385	–0.04	0.03	0.24	2,465	FHS only

Shown are meta-analysis of genotype–phenotype association results using imputed and directly genotyped SNPs in (i) a subset of the Framingham sample plus the entire Rotterdam Study ($n \leq 6{,}975$) or (ii) the Framingham subset only ($n \leq 2{,}566$). Three SNPs were genotyped only in the Framingham Heart Study (and did not specifically have low imputation quality). Effects are shown on the standard deviation (s.d.) scale. For SNPs that were less well imputed (small effective sample size) the increase in significance tracks with the fall in standard error and rise in effective sample size. *N*_effective is the sample size: (*N*) × (observed/expected variance); see Methods.

the central nervous system and the heart starting at 24 h postfertilization[28]. Morpholino knockdown of *NDRG4* was associated with hypoplastic hearts with pericardial edema, dilated atria, looping defects and slower heart rates compared to controls. Zebrafish respond to exposure to QT-prolonging drugs with heart rate slowing[29], although this may be a nonspecific finding in the *NDRG4* morphants. Although we cannot exclude a source of the association in the many other genes at this locus, *GINS3* and *NDRG4* are promising candidates for further work.

The second newly identified locus is on chromosome 6q22.31. SNP rs11756438 (MAF = 0.47) lies in an intron of a predicted gene of unknown function *c6orf204* and near *SLC35F1* and *PLN* and was associated with 0.09 s.d. higher QT interval per minor allele ($P = 4 \times 10^{-11}$, **Table 2** and **Fig. 2d**). Notably, *PLN* (122 kb away from this SNP) encodes phospholamban, an inhibitor of cardiac sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a). Phospholamban knockout mice show enhanced myocardial contractility in response to β-adrenergic agonists[30]. Cardiomyocyte dysregulation of Ca^{2+} handling due to increased phospholamban activity has been linked to dilated cardiomyopathy and heart failure in mouse models[31] and in a human family with cardiomyopathy and ventricular tachycardia[32]. Although more work will be required to localize the source of the signal of association reported here, it is of note that *NOS1AP* activates neuronal nitric oxide synthase 1 (ref. 5), a regulator of calcium cycling in the sarcoplasmic reticulum, and that rare variants in *CACNA1C*, encoding a subunit of the L-type voltage-dependent calcium channel, cause congenital long QT syndrome[33]. These observations suggest a unifying hypothesis that genetic variation influencing calcium cycling in cardiac myocytes influences repolarization and, when altered, contributes to arrhythmogenesis.

The third newly identified locus was on chromosome 1p36.31 near several genes including *CHD5*, *RPL22*, *RNF207*, *ICMT*, *HES3*, *GPR153* and *ACOT7*. The top SNP at the locus, rs846111 (MAF = 0.28), lies in the 3′ untranslated region of *RNF207* and was associated with 0.12 s.d. higher QT interval for each copy of the minor allele ($P = 1 \times 10^{-9}$, **Table 2** and **Fig. 2e**). *RNF207*, which encodes ring finger protein 207, is of unknown function, in a family of molecules involved generally in protein–protein interaction and ubiquitination.

The fourth newly identified locus was on chromosome 16p13.3 upstream of *LITAF*, encoding lipopolysaccharide-induced tumor necrosis factor. This gene has no known relationship to myocardial repolarization, but missense mutations in the gene have been related to Charcot-Marie-Tooth, a hereditary motor and sensory neuropathy[34]. The top SNP at the locus, rs8049607 (MAF = 0.49), was associated with 0.08 s.d. higher QT interval for each minor allele copy ($P = 2 \times 10^{-8}$, **Table 2** and **Fig. 2g**).

The fifth newly identified locus was on chromosome 17q12 near *LIG3* and *RFFL*. The top SNP at the locus, rs2074518 (MAF = 0.46) in intron 11 of *LIG3*, was associated with 0.07 lower QT interval for each minor allele copy ($P = 8 \times 10^{-8}$, **Table 2** and **Fig. 2j**). Although the result did not achieve genome-wide significance in our data alone, replication was observed in the QTSCD consortium samples ($P = 7 \times 10^{-6}$, joint $P = 6 \times 10^{-12}$). *LIG3* encodes DNA ligase III, is involved in DNA base-excision repair and is not an obvious candidate to modulate myocardial repolarization. The nearby gene *RFFL* encodes the rififylin protein and is involved in the endocytic recycling compartment.

Estimates of the coverage by the imputed SNPs of SNPs found in HapMap CEU at each of the newly identified loci are shown in **Supplementary Table 3** online.

Technical validation of poorly imputed SNPs

Because the imputation quality of individual SNPs varied owing to variation in coverage of SNPs by fixed genotyping arrays, we directly genotyped three sentinel SNPs in the entire RS sample and in a subset of the FHS sample (total $n \approx 7{,}000$), as well as three additional SNPs only in the FHS subset. For example, rs2074238 in intron 1 of *KCNQ1* had an effective sample size (see Methods) in 6,975 individuals examined of only 2,359 when accounting for the relatively low imputation quality in Framingham (observed/expected variance = 0.10) and Rotterdam (0.45). The SNP was filtered out in the CHS analysis by the imputation quality control thresholds. We compared the significance of imputed association results in the directly genotyped subsample to that of the direct genotyping results. Direct genotyping confirmed the association of rs2074238 with QT interval with a substantially stronger significance, consistent with the rise in effective sample size from 2,359 to 6,975 individuals ($P = 1 \times 10^{-13}$ imputed vs. $P = 6 \times 10^{-23}$ directly genotyped, **Table 3**). The appropriate filters to be applied to imputed genotypes of varying quality are a matter of debate, but it was certainly encouraging that no association based on imputed results failed to be supported by directly genotyped SNP results (**Table 3**). Including poorly imputed variants in analyses may be valuable even though they have substantially reduced power to detect truly associated variants.

Because of the strong effect of sex on QT interval variation, which explains approximately 5% of its variability, we tested for but observed no significant interaction of sex with the SNP–QT associations.

DISCUSSION

Examination of results for 14 SNPs at 10 loci identified by the QTGEN consortium in data from the QTSCD consortium demonstrated strong confirmation of results for 12 of the 14 associations

(**Table 2**)[13]. One SNP that only weakly replicated is the less common *KCNQ1* SNP rs2074238, which is poorly imputed from Affymetrix arrays used in QTSCD. The weak replication is likely due to the resultant reduction in power, given the strong association in our data ($P = 6 \times 10^{-23}$ upon direct genotyping in 6,975 individuals). A SNP that did not replicate is the low-frequency (MAF = 0.01) missense SNP rs1805128 (D85N) in *KCNE1*, which is poorly imputed from Affymetrix genotypes and is well replicated in external studies with direct genotyping[9,20,21]. Both SNPs thus had limited power to be replicated because of genotype imprecision from poor imputation quality. Additionally, genetic variants reaching genome-wide significance in the QTSCD consortium samples were strongly confirmed in the QTGEN consortium samples, including rs10919071 at a locus containing *ATP1B1* (QTGEN $P = 4 \times 10^{-5}$) and rs17779747 at a locus containing *KCNJ2*, a gene associated with long-QT syndrome (QTGEN $P = 4 \times 10^{-5}$).

In summary, the QTGEN meta-analysis of three genome-wide association studies detected nine common variants at five known candidate genes and an additional five common variants at loci not previously recognized to modulate myocardial repolarization. In total, these variants explain a substantial proportion of variation in QT interval (Framingham 5.4%, Rotterdam 6.5%, CHS 2.3%, **Supplementary Table 2**). These variants in aggregate explain more of QT interval variation than any other covariate (excluding heart rate) including female sex, a known risk factor for QT prolongation and drug-induced arrhythmia.

To assess the potential clinical impact of the genetic variants examined here, we constructed a QT genotype score using the allele copy number and the effect estimates for the 14 SNPs from our meta-analysis and tested the score in the FHS and RS samples (the larger samples). The top quintile of QT genotype score was associated with 9.7 msec and 12.4 msec higher Bazett-corrected QTc (the heart rate correction used in clinical settings) compared to the bottom quintile in FHS and RS samples ($P = 5 \times 10^{-28}$, $P = 1 \times 10^{-31}$, respectively). A prolonged QTc $\geq$450 msec in men and $\geq$470 msec in women has previously been shown to be associated with 2.5-fold increased hazard of sudden cardiac death in the Rotterdam Study[1]. The top quintile of QT genotype score was associated with odds ratios for prolonged QTc of 2.6 and 3.1 in FHS and RS ($P = 3 \times 10^{-5}$, $P = 6 \times 10^{-7}$, respectively, **Supplementary Table 4** online). The finding that the top 20% of genotype score in the population has a QTc increase compared to the bottom 20% in excess of the QT-prolonging effect of some drugs causing arrhythmias (as little as 8 msec), and that one of the SNPs (rs1805128 in *KCNE1*) is associated with the congenital long-QT syndrome[24] and drug-induced arrhythmias[22,23], further supports the clinical relevance of the genetic variants identified in the current report. Tests of the hypothesis that these variants, individually or in aggregate, contribute to risk of sudden cardiac death or drug-induced arrhythmias will require additional work in well-powered samples.

Although genetic effects have been thought to be weaker at older ages, marked associations were identified among these populations of middle aged and older adults. This may be expected for common variants of modest effects that elude negative selection and modulate traits in which environmental factors have only a modest role.

Additional fine mapping with direct genotyping to refine the signal of association and to identify the specific genes involved will be required. As illustrated by the number of common variants in genes associated with long- and short-QT syndromes, the spectrum of allele frequencies and effect sizes for the variants at many genes ranges from rare variants of strong effect underlying mendelian forms of disease to less common variants with intermediate effects to highly polymorphic variants with comparatively modest effects. Certainly, this study will have missed variants that have even more modest effects, that are poorly captured by fixed genotyping arrays including those in the minor allele frequency range 0.5–5%, and that because of random sampling variation failed to rise to the top of our results but might in an equally sized independent sample. Resequencing of these genes will be needed to fully characterize the allelic architecture of QT variation in the general population as well as their relevance to the approximately 25% of families with long-QT syndrome without recognized mutations in known genes and the great majority of those who die of sudden cardiac death in the general population without recognized genetic risks.

METHODS

Study samples. The QTGEN Study includes European-derived samples from three cohorts. The Framingham Heart Study (FHS) is a community-based, longitudinal cohort study comprising three generations of individuals in multigenerational pedigrees and additional unrelated individuals. The current study included individuals from generation 1 (eleventh examination), generation 2 (first examination) and generation 3 (first examination)[35–37]. The Rotterdam Study (RS) is a prospective population-based cohort study of chronic diseases begun in 1990 (refs. 38,39). The current RS study sample included data from one of four examination cycles at which the first eligible electrocardiogram was available for each individual. The Cardiovascular Health Study (CHS) is a prospective cohort study of risk factors for heart disease and stroke begun in 1989 and included 4,925 self-described white participants[40]. The CHS study sample used in this analysis included self-identified whites from the first two rounds of genotyping in a nested case-cohort study of myocardial infarction. Data on QT interval and covariates came from the baseline examination, at which prevalent cardiovascular disease was an exclusion criterion in the parent case-cohort study. All studies were approved by local institutional review boards and written informed consent was given.

Individuals were excluded for bundle branch block or QRS duration >120 msec, atrial fibrillation or flutter, pacemaker activity, or use of a QT-altering medication (not applied to FHS generation 3). After exclusions there were 7,650 FHS, 4,606 RS and 1,429 CHS individuals with phenotype and genotype data who contributed to genotype–phenotype association analyses.

QT measurement methods. In FHS, paper electrocardiograms were scanned and digital caliper measurements were made using proprietary software. In the Rotterdam Study, digital measurements of the QT interval were made using the Modular ECG Analysis System (MEANS)[41]. In the Cardiovascular Health Study, the electrocardiograms were recorded on MAC PC-DT ECG recorder (Marquette Electronics) machines and measurements of QT interval made using the Marquette 12SL algorithm (see **Supplementary Methods** online for full details).

Phenotype modeling. The overall strategy involved linear regression to adjust QT interval for effects of age, sex and RR interval (inverse heart rate) and residuals were used in genotype–phenotype association testing. In FHS, cohort-, sex- and cardiac cycle–specific regression models were created to adjust for age and RR interval. Residuals from these regression models were standardized to mean = 0, s.d. = 1 and then averaged across up to four cardiac cycles. The averaged residuals were then restandardized to mean = 0, s.d. = 1 in cohort- and sex-specific samples. These residuals were then used in genotype–phenotype association testing. In RS, sex-specific regression models were constructed to adjust for RR interval and age and generate residuals. In CHS, the adjustment for age, sex, RR interval and study site was performed in the genotype–phenotype association step (see below).

Genotyping. In FHS, genotyping was done using the Affymetrix 500K GeneChip array, called using the BRLMM algorithm[42], and a custom-designed gene-centric 50K MIP. In RS, genotyping was done using the Infinium II HumanHap550K Genotyping BeadChip version 3. In CHS, genotyping was done using the Illumina 370CNV BeadChip system. Associations of poorly imputed SNPs were validated by re-genotyping FHS samples using Sequenom

and RS samples using Taqman. See **Supplementary Methods** for cohort-specific genotyping details including filters.

Imputation. We imputed estimated allele dosage, defined as the expected number of copies of the minor allele (a fractional value between 0 and 2), of all autosomal SNPs using MACH[43] (HapMap CEU release 22, build 36) in FHS and RS and using BIMBAM[11] (release 21a, build 35) in CHS (see **Supplementary Methods** for details).

Genotype–phenotype association method. In FHS, standardized QT residuals were tested for association with imputed allele dosage under an additive genetic model using the linear mixed effects model of the kinship package in R to account for relatedness. In the Rotterdam Study, QT residuals were tested for association using MACH2QTL, which uses dosage value as a predictor in a linear regression framework[43]. In CHS, QT interval was linearly regressed on age, sex, clinic, RR-interval and SNP dosage using R. The regression was weighted to reflect case-cohort sampling probabilities.

Meta-analysis. The minor allele from HapMap CEU genotypes was used to define the coded allele in all analyses, regardless of frequency in individual cohorts. For an A/T SNP, the T allele is the 'coded allele' under the following coding: AA = 0, AT = 1, TT = 2. To implement genomic control, the λ_{GC} value was used to correct the standard error as follows, SE_corrected = SE $\times \sqrt{(\lambda_{GC})}$. Each effect estimate (beta) was standardized to the s.d. of the cohort-specific adjusted residuals to put all results on the s.d. scale. The ratio of the observed to the expected variance of the imputed allele frequency was used as the quality metric for the imputation of a given SNP[44]. To account for the difference in power, and thus the interpretation of resulting P values for each SNP, we created a variable N_effective which discounted the total sample size by imputation quality as follows: N_effective = N $\times$ (observed/expected variance). We used inverse variance–weighted fixed-effects meta-analysis of the beta estimates from linear regression as the primary meta-analysis method. Weighting by the square root of the N_effective resulted in very similar –log(P value) for results with $P < 0.01$ ($r = 0.9980$). The scripts developed for this project are freely available online. We a priori declared results significant at $P < 5 \times 10^{-8}$, based on estimates adjusting for all common variant tests in the human genome of individuals of European ancestry for a target genome-wide $\alpha = 0.05$ (ref. 45).

Exchange with QTSCD consortium. We submitted to the QTSCD consortium a list of our top SNP associations (one SNP per signal of association) and received QTSCD results for those SNPs. A reciprocal exchange was performed. We performed meta-analysis of the QTGEN meta-analysis results ($n = 13{,}685$) with the QTSCD results ($n = 15{,}854$) using inverse variance weights.

QT genotype score. A QT genotype score was calculated using the effect estimates from the QTGEN meta-analysis for the coded allele of each of 14 SNPs. The score, on the s.d. scale, was calculated as follows for each individual: QT genotype score = $\text{beta}_1 \times \text{allele\_copy\_number}_1 + \text{beta}_2 \times \text{allele\_copy\_number}_2 + \ldots \text{beta}_{14} \times \text{allele\_copy\_number}_{14}$

URLs. Meta-analysis scripts, http://www.broad.mit.edu/~debakker/meta.html; R project, http://www.r-project.org/.

Note: Supplementary information is available on the Nature Genetics website.

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