

Common variants at ten loci modulate the QT interval duration in the QTSCD Study

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The QT interval, a measure of cardiac repolarization, predisposes to ventricular arrhythmias and sudden cardiac death (SCD) when prolonged or shortened. A common variant in *NOS1AP* is known to influence repolarization. We analyze genome-wide data from five population-based cohorts (ARIC, KORA, SardiNIA, GenNOVA and HNR) with a total of 15,842 individuals of European ancestry, to confirm the *NOS1AP* association and identify nine additional loci at $P < 5 \times 10^{-8}$. Four loci map near the monogenic long-QT syndrome genes *KCNQ1*, *KCNH2*, *SCN5A* and *KCNJ2*. Two other loci include *ATP1B1* and *PLN*, genes with established electrophysiological function, whereas three map to *RNF207*, near *LITAF* and within *NDRG4-GINS3-SETD6-CNOT1*, respectively, all of which have not previously been implicated in cardiac electrophysiology. These results, together with an accompanying paper from the QTGEN consortium, identify new candidate genes for ventricular arrhythmias and SCD.

The lack of serologic biomarkers to predict ventricular tachycardia, ventricular fibrillation and sudden cardiac death has made genome-wide searches for common genetic variants influencing these traits extremely important¹. Studies of the QT interval from the electrocardiogram (EKG), which captures major temporal and spatial aspects of the repolarization process, are particularly attractive as its prolongation or shortening reflects alterations of cardiac repolarization known to trigger ventricular tachycardia and ventricular fibrillation and predispose to SCD². Importantly, QT interval prolongation has been associated with increased cardiovascular mortality in individuals

with heart disease³, as well as in the general population⁴. Furthermore, mutations in genes associated with long- and short-QT syndromes (LQTS, SQTs) markedly increase the odds of SCD⁵.

Previous genetic analyses of QT interval have largely relied on family-based studies focused on rare mendelian QT syndromes. These have resulted in the identification of eleven genes in which mutations cause prolongation or shortening of the QT interval and SCD. Recently, in an initial genome-wide association study (GWAS) in the KORA community-based cohort, an association between common genetic variants in the *NOS1AP* (*CAPON*) gene and the

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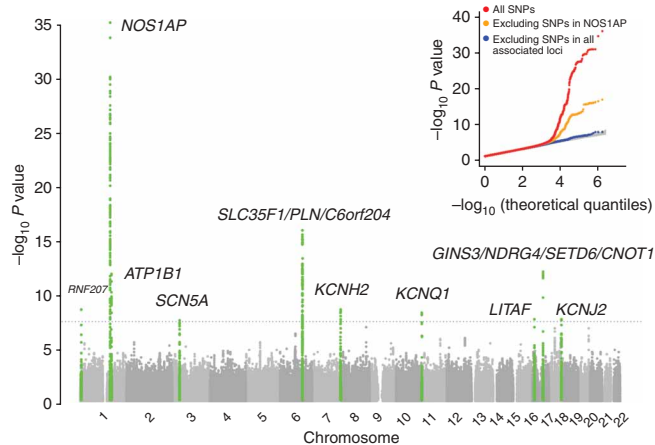


Figure 1 Manhattan and quantile-quantile plots of genome-wide association analyses. Genome-wide association results were combined across all studies by inverse variance weighting. The blue dotted line marks the threshold for genome-wide significance (5×10^{-8}). SNPs within loci exceeding this threshold are highlighted in green. The upper right panel shows the quantile-quantile plot, where the red line corresponds to all test values, the orange line corresponds to results after excluding SNPs at *NOS1AP* and the blue line to results after excluding SNPs at all associated loci (highlighted in green in the Manhattan plot). The gray area corresponds to the 90% confidence region of the null distribution of *P* values (generated from 100 simulations).

QT interval was identified⁶. This association highlights the importance of the nitric oxide synthase pathway in myocardial function and has now been replicated in several studies^{7–9}. Genetic variants at *NOS1AP* explain $\sim 1\%$ of the variance in QT interval, and

common genetic variants in *NOS1AP* are also associated with SCD in populations of European ancestry¹⁰.

In this study, we carried out GWAS in five population-based cohorts from Europe and the United States (ARIC¹¹, SardiNIA¹², KORA¹³, GenNOVA¹⁴ and Heinz Nixdorf Recall (HNR)¹⁵) (Supplementary Table 1 online). In all the participating studies all individuals studied and all analyses on their samples were done according to the Helsinki declarations and were approved by the local medical ethics and institutional review committees. All participants gave signed informed consent. We used preexisting genome-wide SNP data of

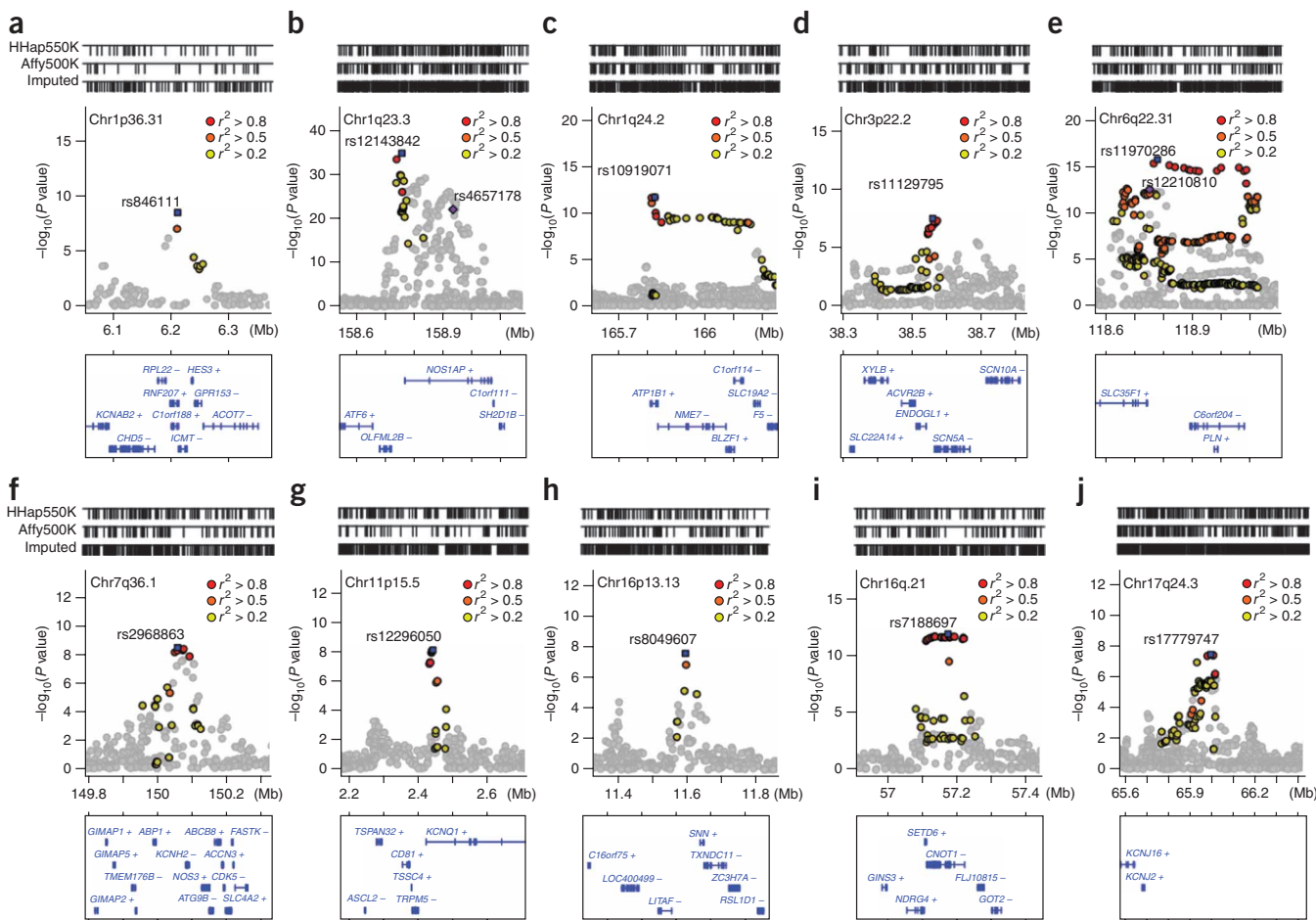


Figure 2 Association results at each significant locus. (a–j) The gene locus is from left to right: *RNF207*, *NOS1AP*, *ATP1B1*, *SCN5A*, *SLC35F1*-*PLN*-*C6orf204*, *KCNH2*, *KCNQ1*, *LITAF*, *GINS3*-*NDRG4*-*SETD6*-*CNOT1* and *KCNJ2*. Each panel spans ± 500 kb around each SNP except for panel A (300 kb), panel B, panel C, panel E (600 kb) and panel J (800 kb). At the top of each panel, the location and density of SNPs in the Illumina HumanHap 550K, Affymetrix 500K chips, and imputed SNPs are shown. The SNPs are colored according to their linkage disequilibrium with the leading variant, which is highlighted with a blue square. SNPs representing independent signals from the leading variant are highlighted with a purple diamond. Gene transcripts are annotated in the lower box, + or -, indicating the direction of transcription. In panel F, some gene names are omitted for clarity.

Table 1 Genome-wide significant variants associated with the QT interval

Locus information							QTSCD consortium results					Replication data from QTGEN consortium					Meta-analysis	
Locus	SNP	Chr	Position (build 35)	Gene related position	Coded/noncoded allele	Method	<i>n</i>	RSQR	Freq. coded allele	Beta (ms)	s.e. (ms)	<i>P</i> value	<i>N</i> (eff)	Freq. coded allele	Beta (ms)	s.e. (ms)	<i>P</i> value	<i>P</i> value
<i>RNF207</i> ^a	rs846111	1	6,213,636	3' UTR	C/G	I	8,624	0.8827	0.290	1.4893	0.2523	3.56e-09	6,192	0.286	2.140	0.368	4.69e-09	3.69e-16
<i>NOS1AP</i>	rs12143842	1	158,765,548	-5.7 kb	T/C	GT	15,854	0.9573	0.240	2.8793	0.2315	1.63e-35	12,709	0.286	3.771	0.263	2.26e-45	1.88e-78
<i>ATP1B1</i>	rs10919071	1	165,831,141	Intron 6	A/G	GT	15,854	0.9877	0.867	2.0469	0.2915	2.18e-12	13,602	0.882	1.405	0.342	3.94e-05	1.20e-15
<i>SCN5A</i>	rs11129795	3	38,564,167	3' UTR	A/G	I	15,854	0.9719	0.233	-1.2696	0.2306	3.67e-08	12,894	0.250	-1.387	0.268	2.33e-07	4.52e-14
<i>PLN</i>	rs11970286	6	118,787,067	-189 kb	T/C	GT	15,854	0.9551	0.441	1.6426	0.1997	1.97e-16	12,619	0.460	1.386	0.228	2.37e-09	2.35e-24
<i>KCNH2</i>	rs2968863	7	150,060,785	-18.9 kb	T/C	I	15,854	0.9753	0.290	-1.3491	0.2289	3.79e-09	12,802	0.225	-1.403	0.263	6.14e-10	1.96e-15
<i>KCNQ1</i>	rs12296050	11	2,445,918	Intron 1	T/C	I	15,854	0.9741	0.200	1.4412	0.2503	8.52e-09	12,690	0.218	1.877	0.298	2.01e-16	2.80e-17
<i>LITAF</i>	rs8049607	16	11,599,254	-11 kb	T/C	I	15,854	0.7093	0.458	1.2469	0.2248	2.91e-08	10,078	0.494	1.456	0.263	1.90e-08	5.78e-15
<i>NDRG4</i>	rs7188697	16	57,179,679	+74.6 kb	A/G	I	15,854	0.9258	0.744	1.6639	0.2344	1.25e-12	12,506	0.719	1.982	0.263	2.72e-15	6.78e-25
<i>KCNJ2</i>	rs17779747	17	66,006,600	+319 kb	T/G	I	15,854	0.9483	0.351	-1.1555	0.2093	3.36e-08	12,440	0.346	-1.024	0.248	3.46e-05	6.02e-12

This table lists the most significantly associated SNP at the ten identified loci. In each locus, at least one marker exceeds the genome-wide significant threshold of 5×10^{-8} . Beta coefficient and standard error are relative to the regression model when using QT residuals as dependent variable. Meta-analysis with data from the QTGEN consortium³⁴ supports the evidence of association at each locus. 'Method' indicates whether a SNP has been directly genotyped on one of the array platforms (GT) or whether its genotypes have been imputed in all of the samples (I). A more complete list including at least one genotyped SNP from each of the ten loci can be found in **Supplementary Table 4**. RSQR (sometimes also termed OEvvar) denotes the average of the ratio of the observed variance of imputed allele counts to their expectation based on estimated allele frequencies weighted for all samples from QTSCD, which indicates deviation from Hardy-Weinberg equilibrium and quality of imputation. SNPs data were only included if RSQR exceeded a value of 0.3 in the respective study. *N*(eff) denotes our estimate of the effective number of analyzed individuals in QTGEN.

^aFor SNP rs846111 in *RNF207* SardiNIA and Kora F3, results were not used because of RSQR < 0.3.

15,842 individuals randomly ascertained within these cohorts to identify additional genes modulating the QT interval. Cohort descriptions are detailed in **Supplementary Methods** online. Samples had been genotyped using either Affymetrix Gene Chip Human Mapping Array Set 6.0 (ARIC, KORA S4) or 500K (KORA F3, SardiNIA) and Illumina HumHap300v2 (GenNOVA) or HumanHap 550v3 BeadChips (KORA S4, HNR): genotyping details and SNP quality control filters for each study are summarized in **Supplementary Table 2** online. To facilitate comparison of results across studies, we imputed HapMap SNPs in all study participants using the HapMap CEU sample as a reference (HapMap release 21)¹⁶. We excluded individuals with atrial fibrillation, pacer and/or defibrillator implants, prolonged QRS intervals (>120 ms) indicating bundle branch blocks or other conduction disorders, as well as pregnant women. Heart rate (RR interval), age and sex were included as covariates and adjusted for in all analyses (**Supplementary Table 3a** online). The standard deviation of the adjusted QT interval meta-analyzed across all studies was ± 17.8 ms.

RESULTS

Meta-analysis of GWAS studies of QT interval

Results for the consequent genome-wide analysis are summarized in **Figures 1** and **2**. The genomic control factor (λ) for this analysis was 1.016, indicating that unmodeled relatedness and population structure had no appreciable impact on our results¹⁷. The quantile-quantile plot in **Figure 1** (inset) shows a clear excess of extreme *P* values, indicating the presence of true associations. After having identified ten main association signals across the genome ($P < 5 \times 10^{-8}$) we carried out a second round of genome-wide analysis after adjusting for the main signals. This round led to the identification of independent secondary signals in two of the ten loci ($P < 5 \times 10^{-8}$).

Replication of association in the *NOS1AP* locus

Consistent with a previous GWAS study⁶, the strongest main association signal maps to the *NOS1AP* locus. There, the most significant association was at SNP rs12143842 ($P = 1.62 \times 10^{-35}$, **Figs. 1** and **2b**, **Table 1**, and **Supplementary Table 4** online). In the *NOS1AP* locus we

identified an independent secondary signal at rs4657178 which was in low linkage disequilibrium (LD) to the main signal ($r^2 = 0.001$ to rs12143842 in HapMap CEU, $P = 1.02 \times 10^{-22}$ before and 9.98×10^{-13} after adjustment for main association signals, **Table 2**) supporting the notion of at least two common QT-modifying variants at that locus. The SNP rs10494366, indicating the most significant association signal in the previous report⁶, was in moderate LD with the main signal, and to a lesser degree also with the secondary signal (**Table 3**). After adjustment for the ten main association signals a significant reduction in the association with this SNP occurs, indicating that it does not represent an independent tertiary signal but rather a marker for the main signal at rs12143842.

After excluding SNPs in a 1-Mb region surrounding *NOS1AP*, we still observed a clear excess of small *P* values distributed across several genetic loci (**Fig. 1**, inset), indicating the presence of additional genomic loci associated with the QT interval. Overall, nine additional loci show association at $P < 5 \times 10^{-8}$, corresponding to genome-wide significance at 5% after adjustment for ~ 1 million independent tests, the estimated multiple testing burden of HapMap SNPs in samples of European ancestry¹⁶.

Identification of association signals at four LQTS-related genes

Notably, four of these associations map in or near genes known to harbor both LQTS- and SQTs-causing mutations: *KCNQ1* (LQT1, SQT2, 11p15.5, rs12296050, $P = 8.52 \times 10^{-9}$), *KCNH2* (LQT2, SQT1, 7q36.1, rs2968863, $P = 3.79 \times 10^{-9}$), *KCNJ2* (LQT7, SQT3, 17q24.3, rs17779747, $P = 3.36 \times 10^{-8}$) and *SCN5A* (LQT3, Brugada syndrome, 3p22.2, rs11129795 with $P = 3.67 \times 10^{-8}$). *SCN5A* encodes a sodium channel, and, in previous studies, the nonsynonymous variant S1103Y with allele frequency 8% only in individuals of African ancestry has been associated with increased risk of arrhythmias¹⁸. The three other genes encode potassium channel α -subunits in the cardiomyocyte plasma membrane.

Previous candidate gene analyses provided evidence of association between common variants and QT interval for three of these loci: *KCNQ1*, *KCNH2* and *SCN5A*. We investigated the previously identified

Table 2 Independent secondary genome-wide significant association signals at the identified loci

Locus information		QTSCD consortium results										Replication data from QTGEN consortium				Meta-analysis			
Locus	Second SNP	r^2 to main signal	Chr.	Position (build 35)	Gene related			Method	RSQR	Freq.				N(eff)	Freq.			P	
					position	Coded allele	Noncoded allele			coded allele	Beta (ms)	s.e. (ms)	P value		coded allele	Beta (ms)	s.e. (ms)		P value
<i>NOS1AP</i>	rs4657178	0.001	1	158,942,268	Intron 2	T	C	GT	1.00	0.33	2.19	0.22	1.02e-22	13,884	0.26	2.19	0.26	2.92e-17	6.74e-33
Results after adjustment for the ten main SNPs:											1.55	0.22	9.98e-13						
<i>PLN</i>	rs12210810	0.067	6	118,759,897	-216 kb	C	G	I	0.92	0.06	-3.13	0.43	3.24e-13	12,005	0.06	-2.39	0.53	4.86e-06	1.83e-17
Results after adjustment for the ten main SNPs:											-2.29	0.42	4.50e-08						

This table lists independent genome-wide significant secondary association signals at the identified loci. A locus was defined as the region ± 500 kb around the main hit SNP. Association results are given without (white) and with (gray) adjusting QT interval for the ten main identified association signals. Two signals exceed the genome-wide significance level even after this adjustment. All independent SNPs are in moderate to low LD ($r^2 < 0.1$) with the main hits. A more detailed list including six further signals with $P < 1 \times 10^{-3}$ can be found in **Supplementary Table 5**. This list includes a second signal identified in *KCNH2* (rs3778873, $P = 5.07 \times 10^{-8}$ before and $P = 7.90 \times 10^{-5}$ after adjustment), which is in LD to previously identified second signals in *KCNH2* from two candidate gene studies, rs3815459 ($r^2 = 0.61$) (ref. 20) and rs3807375 ($r^2 = 0.35$) (ref. 21). Please see **Table 3** for a more detailed analysis of previously published QT associations from candidate genes. LD data are from HapMap.

variants with respect to our data on the basis of their surrounding LD patterns and effect sizes before and after adjustment for the main signals (**Table 3** and **Supplementary Fig. 1** online). The nonsynonymous coding *KCNH2* variant K897T (rs1805123)^{19–21}, the variant in intron 1 of *KCNQ1* (rs757092)²⁰ and the noncoding *SCN5A* variant D1819D (rs1805126)²² all seem to be in LD with the main association signals we detected. After adjustment for the main signals, with which they were in moderate to high LD, these SNPs were no longer significantly associated. In contrast, the signal at the *SCN5A* variant H558R (rs1805124)²² did not reach genome-wide significance in our data ($P = 4.06 \times 10^{-3}$) but remained almost unchanged after adjustment, suggesting a small but independent association signal. Additionally, the previously published secondary association signals in *KCNH2* show independent but slightly less than genome-wide significant signals in our data: rs3815459 (ref. 20) ($P = 5.07 \times 10^{-8}$) and rs3807375 (ref. 21) ($P = 4.68 \times 10^{-7}$) (**Table 3**). Both seem to be in LD with the strongest secondary signal we identified in *KCNH2* at rs3778873 bordering genome-wide significance ($P = 5.07 \times 10^{-8}$ before and $P = 7.90 \times 10^{-5}$ after adjustment for main signals) (**Supplementary Table 5** online).

These results emphasize that in many loci we are likely to identify genes with an allelic series comprising both common variants influencing QT interval with modest effects (~ 3 – 6 ms per locus in this study) in healthy volunteers as well as rare variants with a more marked effect (> 100 ms) in individuals with a genetic syndrome²³.

In the 17q24.3 region the strongest association signal was missed in previous candidate gene studies probably because the associated signal at SNP rs17779747 maps ~ 300 kb away from the *KCNJ2* gene. Although the LD block it resides on extends toward the gene, SNP rs17779747 is not in high LD with any common variant within the *KCNJ2* coding sequence, the strongest being to the synonymous coding rs173135 (L382L, $r^2 = 0.014$). *KCNJ2* remains the best prior candidate in the region as mutations in it are known to cause Andersen syndrome (MIM170390), a condition characterized by periodic paralysis, dysmorphic features and cardiac ventricular arrhythmias triggered by repolarization disturbances. Alternatively, other genes from the region, including the nearby paralog *KCNJ16*, may have a role²⁴.

Association with two myocardial ATPase-related genes

We identified two new loci with genes encoding proteins with well-established myocardial electrophysiological functions: in chromosome 1q24.2 the strongest signal was within *ATP1B1* (Na^+/K^+ ATPase beta

subunit 1, rs10919071, $P = 2.18 \times 10^{-12}$, **Fig. 2c**). *ATP1B1* encodes a transmembrane protein that has a crucial role in the maintenance of Na^+ and K^+ gradients across membranes, thus regulating electrical excitability of muscles, and may also be involved in the regulation of blood pressure²⁵. These data make *ATP1B1* a strong functional candidate, although *NME7*, *BLZF1*, *C1orf114* and *SLC19A2* cannot be excluded without functional validation.

In chromosome 6q22.31 a broad association signal covers *PLN* (phospholamban), *SLC35F1* and *C6orf204*, with the strongest signal at the intergenic SNP rs11970286 ($P = 1.96 \times 10^{-16}$, **Fig. 2e**). *PLN* is the strongest regional candidate, as it is a regulator of the sarcoplasmic reticulum Ca^{2+} ATPase (encoded by *ATP2A2*, also known as *SERCA2*) responsible for diastolic lowering of the cytoplasmic Ca^{2+} concentration. Mutations in this gene have previously been associated with inherited cardiomyopathies and congestive heart failure²⁶. Neither *SLC35F1* nor *C6orf204* has previously been shown to have a functional role in the myocardium. In the 6q22.31 region we identified an independent second variant (rs12210810, intergenic, $P = 3.24 \times 10^{-13}$ before and $P = 4.50 \times 10^{-8}$ after adjustment for main signals). The low LD to the main signal ($r^2 = 0.067$) and the fact that the rare allele at this second signal was associated with shorter QT intervals strengthen the assumption of two independent causal variants in or near *PLN*. At each of these two loci, there were no additional compelling biological candidates that we could identify.

Association signals in three previously unrecognized loci

At the remaining three loci discovered, there was no obvious biological candidate. One of these loci in chromosome 1p36.31 overlaps *LITAF* (rs8049607, $P = 2.90 \times 10^{-8}$, **Fig. 2h**), which encodes a DNA-binding protein thought to have a role in the regulation of *TNFA* expression. Mutations in this gene have previously been implicated in Charcot-Marie-Tooth type 1C neuropathy and are sometimes associated with reduced nerve conduction velocity²⁷. Another candidate gene in the region is *TXNDC11*, which encodes thioredoxin domain-containing protein 11 and harbors a nonsynonymous SNP in weak LD with the leading variant (V756L, rs3190321, $r^2 = 0.022$), as well as *SNN*, encoding stannin (rs8191288, $r^2 = 0.022$). A second newly identified locus was in chromosome 1p36.31, with a nonsynonymous coding SNP in *RNF207* as the main signal (G603A, rs846111, $P = 3.56 \times 10^{-9}$, **Fig. 2a**). Its best proxy, rs709209, is another nonsynonymous SNP in the same gene (N573S, $r^2 = 0.673$). *RNF207* encodes a RING-type zinc-finger protein of unknown function. Other regional

Table 3 Association signals of previously identified QT-modifying variants

Locus	Previously identified		Refs.	tagSNP for previously identified SNP if not genotyped or imputed (r^2) ^a	Best regional hit identified in our study (r^2) ^a	Position Chr (build 35) ^a	Gene-related position ^a	Coded allele ^b	Noncoded allele ^b	RSQR	Freq.				
	SNP	Alias									coded allele	Beta (ms) ^c	s.e. (ms) ^c	<i>P</i> value	
<i>NOS1AP</i>	rs10494366	–	(6)	–	rs12143842 (0.11)	1 158,817,343	Intron 1	T	G	0.9819	–2.1977	0.2041	4.90e–27		
					rs4657178 (0.04)										
					Results after adjustment for the ten main SNPs:								–0.4141	0.1992	3.77e–02
<i>SCN5A</i>	rs1805124	H558R	(22)	–	rs11129795 (0.02)	3 38,620,424	Exon 12	T	C	0.9364	–0.6727	0.2341	4.06e–03		
					Results after adjustment for the ten main SNPs:								–0.6577	0.2287	4.03e–03
<i>SCN5A</i>	rs1805126	D1819D	(22)	–	rs11129795 (0.72)	3 38,567,410	Exon 28	A	G	0.9684	1.109	0.2093	1.17e–07		
					Results after adjustment for the ten main SNPs:								0.0513	0.2044	8.02e–01
<i>KCNH2</i>	rs1805123	K897T	(19–21)	rs2968863 (0.89) ^c	rs2968863 (0.89) ^c	7 150,060,785	Exon 11	T	C	0.9753	0.290	–1.3491	0.2289	3.79e–09	
					Results after adjustment for the ten main SNPs:								–0.0040	0.2240	9.86e–01
<i>KCNH2</i>	rs3815459	(2nd hit)	(20)	rs3778873 (0.61) ^c	rs2968863 (0.08) ^c	7 150,082,042	Intron 13	C	G	0.7185	0.219	–1.769	0.3247	5.07e–08	
					Results after adjustment for the ten main SNPs:								–1.2475	0.3160	7.90e–05
<i>KCNH2</i>	rs3807375	(2nd hit)	(21)	–	rs2968863 (0.20)	7 150,104,858	Intron 2	T	C	0.8226	1.0668	0.2117	4.68e–07		
					Results after adjustment for the ten main SNPs:								0.6299	0.2063	2.27e–03
<i>KCNQ1</i>	rs757092	–	(20)	–	rs12296050 (0.20)	11 2,455,754	Intron 1	A	G	0.9918	–0.4276	0.2026	3.48e–02		
					Results after adjustment for the ten main SNPs:								0.0130	0.1984	9.48e–01
<i>KCNE1</i>	rs1805127	G38S	(35,36)	–	–	21 34,743,691	Exon 1	T	C	0.9200	–0.0989	0.2126	6.42e–01		
					Results after adjustment for the ten main SNPs:								–0.2320	0.2076	2.64e–01
<i>KCNE1</i>	rs1805128	D85N	(35)	–	–	21 34,743,550	Exon 1	T	C	0.4585	1.0139	0.7582	1.81e–01		
					Results after adjustment for the ten main SNPs:								1.2523	0.7360	8.88e–02
<i>KCNE1</i>	rs727957	–	(20)	–	–	21 34,801,942	+55 kb	T	G	0.8381	0.3805	0.2537	1.34e–01		
					Results after adjustment for the ten main SNPs:								0.3842	0.2481	1.22e–01

We compared association results identified in QTSCD to previously published association signals. The previously identified signal in *NOS1AP* (rs10494366) (ref. 6) is in weak to moderate LD to both the first (rs12143842, $r^2 = 0.11$) as well as the secondary association signal identified in our study (rs4657178, $r^2 = 0.04$). Data after adjustment (gray line) indicate its contribution to be almost entirely accounted for by rs12143842. In *SCN5A*, the synonymous SNP D1819D (rs1805126, ref. 22) drops in signal by adjustment, indicating that it is accounted for by the main *SCN5A* hit. In contrast, the nonsynonymous *SCN5A* SNP H558R has a signal that is not genome-wide significant but holds up after adjustment. H558R may therefore be a weak but independent signal in *SCN5A*. The signal identified in *KCNH2* (rs2968863) is in LD to the previously identified nonsynonymous SNP *KCNH2*-K897T (rs1803123, refs. 19–21) and accounts for its association signal. Two intronic SNPs have previously been published to represent second independent signals in *KCNH2*: rs3815459 (ref. 20) (tagged by rs3778873) and rs3807375 (ref. 21). Both are in LD ($r^2 = 0.45^*$) and retain most of their association signal after adjusting, thus supporting the existence of a second signal in *KCNH2*. In addition, the genome-wide analysis of SNPs after adjustment for main signals also identifies rs3778873 as a potential second hit ($P = 7.9 \times 10^{-5}$) (Supplementary Table 5). The signal identified in intron 1 of *KCNQ1* (rs12296050) is in LD to previously identified SNP rs757092 ($r^2 = 0.18$) (ref. 20). SNP *KCNE1*-D85N (rs1805128) was imputed with low quality only in our consortium (RSQR = 0.459). r^2 data are obtained from HapMap (build 22) unless indicated. ^aValue for previously identified SNP. ^bFor previously identified SNP or tagSNP for previously identified SNP. ^cData derived from the genotyping of $n = 702$ individuals of the KORA S4 population as described in ref. 20. In this population, the LD relations in candidate genes *KCNQ1* and *KCNH2* are also detailed in Supplementary Figure 1.

candidate genes are *GPR153* (rs4908542, intronic, $r^2 = 0.445$), *CHD5* (rs12754299, $r^2 = 0.021$), *ICMT* (rs846108, $r^2 = 0.020$), *HES2* (rs932402, $r^2 = 0.018$) and the gene for the shaker-related potassium channel *KCNAB2* (rs2294934, $r^2 = 0.028$). None of the SNPs in the neighboring genes were coding and none of the genes have been previously implicated in myocardial pathology.

The third identified locus maps to chromosome 16q21, the strongest signal being an intronic SNP within the *CNOT1* gene (rs7188697, $P = 1.25 \times 10^{-12}$, Fig. 2i), encoding a subunit of the CCR4-NOT transcription complex. This locus is highly conserved and syntenic in many vertebrates and contains other potentially causal genes such as *SETD6*, encoding SET domain-containing protein 6 (rs37036, $r^2 = 0.920$), *NDRG4*, encoding vascular smooth muscle cell-associated protein 8 (SMAP-8) (rs40186, $r^2 = 0.468$), and *GINS3*, encoding GINS complex subunit 3 (rs8054945, $r^2 = 0.041$). A recent genetic screen in zebrafish mutants of resistance and sensitization to dofetilide, a drug causing atrioventricular blocks, uncovered a *GINS3* mutation that modifies cardiac repolarization (personal communication, D. Milan, Massachusetts General Hospital and Harvard Medical School). *NDRG4*, a gene known to be expressed in the heart during zebrafish development that regulates proliferation and growth of cardiomyocytes²⁸, is also a

plausible candidate. Human *NDRG4* has three alternative promoters, all of them containing consensus binding sequences for the Tbx5 transcription factor²⁹, which is known to operate in conjunction with Nkx2.5 in human heart development³⁰. Additionally, a combined effect of several genes may exist given the high degree of conserved synteny in that locus. For all the loci we identified, but particularly for these last three, more experimental evidence will be required to identify likely functional mechanisms. This could include the sequencing of genes in individuals with unexplained long-QT syndrome and functional screens in model organisms.

Investigation of explained variance and sex-specific effects

The nine newly identified main association signals identified here increase the proportion of explained variance in heart rate-, sex- and age-adjusted QT interval from 1.0% by the *NOS1AP* SNP rs12143842 signal alone to 3.3% in a meta-analysis across all studies; a more comprehensive analysis of all variation at these loci, including secondary signals, is likely to increase this quantity further. In addition, there are likely rarer variants at each of these genes that modulate QT intervals to a greater extent but have been missed in our screen for common variants, as has been observed for high-density-lipoprotein cholesterol levels³¹.

Table 4 Sex- and age-specific association results for replicated loci

Sex specific					Females			Males			Comparing effects	
Locus	SNP	Signal	Coded allele	Noncoded allele	Effect	s.e.	<i>P</i> value	Effect	s.e.	<i>P</i> value	Δ (females-males)	<i>P</i> value
<i>RNF207</i>	rs846111		C	G	1.193	0.363	1.02e-03	1.519	0.340	7.72e-06	-0.326	0.512
<i>NOS1AP</i>	rs12143842		T	C	3.077	0.325	2.91e-21	2.087	0.318	5.26e-11	0.990	0.030
<i>NOS1AP</i>	rs4657178	2nd	T	C	2.356	0.319	1.40e-13	1.633	0.301	6.01e-08	0.723	0.099
<i>ATP1B1</i>	rs10919071		A	G	1.683	0.414	4.79e-05	1.923	0.394	1.04e-06	-0.240	0.675
<i>SCN5A</i>	rs11129795		A	G	-1.198	0.328	2.59e-04	-0.981	0.312	1.66e-03	-0.217	0.632
<i>PLN</i>	rs11970286		T	C	1.649	0.287	8.59e-09	1.429	0.268	9.66e-08	0.220	0.575
<i>PLN</i>	rs12210810	2nd	C	G	-2.787	0.609	4.70e-06	-3.060	0.581	1.40e-07	0.273	0.746
<i>KCNH2</i>	rs2968863		T	C	-1.194	0.325	2.40e-04	-1.135	0.309	2.42e-04	-0.059	0.895
<i>KCNQ1</i>	rs12296050		T	C	1.611	0.359	7.01e-06	1.149	0.338	6.69e-04	0.462	0.348
<i>LITAF</i>	rs8049607		T	C	1.049	0.317	9.26e-04	1.462	0.306	1.72e-06	-0.413	0.348
<i>NDRG4</i>	rs7188697		A	G	1.225	0.333	2.30e-04	1.676	0.318	1.38e-07	-0.451	0.327
<i>KCNJ2</i>	rs17779747		T	G	-0.928	0.298	1.85e-03	-1.239	0.283	1.21e-05	0.312	0.448

Age specific					Young (age ≤ 50 y)			Old (age > 50 y)			Comparing effects	
Locus	SNP	Signal	Coded allele	Noncoded allele	Effect	s.e.	<i>P</i> value	Effect	s.e.	<i>P</i> value	Δ (old-young)	<i>P</i> value
<i>RNF207</i>	rs846111		C	G	2.283	0.413	3.24e-08	1.006	0.313	1.32e-03	-1.277	0.014
<i>NOS1AP</i>	rs12143842		T	C	2.503	0.359	3.27e-12	2.526	0.294	8.67e-18	0.023	0.961
<i>NOS1AP</i>	rs4657178	2nd	T	C	2.373	0.357	3.03e-11	2.035	0.284	7.07e-13	-0.338	0.458
<i>ATP1B1</i>	rs10919071		A	G	1.489	0.455	1.06e-03	2.127	0.370	8.58e-09	0.638	0.276
<i>SCN5A</i>	rs11129795		A	G	-0.863	0.368	1.89e-02	-1.261	0.289	1.27e-05	-0.399	0.394
<i>PLN</i>	rs11970286		T	C	1.198	0.319	1.73e-04	1.872	0.250	7.60e-14	0.673	0.097
<i>PLN</i>	rs12210810	2nd	C	G	-1.741	0.672	9.56e-03	-3.550	0.544	7.02e-11	-1.809	0.036
<i>KCNH2</i>	rs2968863		T	C	-1.169	0.357	1.04e-03	-1.079	0.291	2.05e-04	0.090	0.846
<i>KCNQ1</i>	rs12296050		T	C	1.314	0.407	1.24e-03	1.299	0.312	3.08e-05	-0.015	0.977
<i>LITAF</i>	rs8049607		T	C	1.100	0.357	2.07e-03	1.274	0.281	5.80e-06	0.174	0.701
<i>NDRG4</i>	rs7188697		A	G	1.396	0.372	1.75e-04	1.479	0.296	5.67e-07	0.083	0.861
<i>KCNJ2</i>	rs17779747		T	G	-0.441	0.332	1.85e-01	-1.226	0.263	3.12e-06	-0.785	0.064

This table summarizes the combined association results for sex- and age-specific effects. Young and old subgroups were defined as age ≤ 50 y and age > 50 y. None of the effects was significant after adjustment for multiple testing requiring $P < 0.002$.

Variation in demographic factors such as age and sex also contributes to the QT interval phenotype³². We carried out association analysis for males and females separately, and for younger and older subgroups of individuals (stratified by age ≤ 50 y). As shown in **Table 4**, the main signal at *NOS1AP* shows a stronger effect in females, supporting a recently published study that demonstrated a sex-specific effect for SNP rs10494366 (refs. 9,33). In addition, we observed data supporting the possibility of age-specific effects at *RNF207* and *PLN*. In *RNF207* the QT-prolonging effect of the rare C allele of rs846111 is 1.277 ms larger in young individuals ($P = 0.014$), whereas in *PLN* the QT-shortening effect of the rare C allele of rs12210810 is 1.809 ms larger in older individuals ($P = 0.036$). We replicated the previously published sex-specific effect at *NOS1AP*; no other age- or sex-specific effects remained significant after adjustment for the number of performed tests.

Comparison of results with an independently conducted GWAS study

Finally, our analyses should be compared with the results of an independent yet similar GWAS by the QTGEN consortium³⁴ that adds further confidence in these results. All the loci that reach $P < 5 \times 10^{-8}$ in our screen also show evidence for association in their study of 13,685 individuals (each with $P < 1 \times 10^{-4}$). Furthermore, our data

provides evidence for association with the QT interval at chromosome 17q12 near the genes *LIG3* and *RFFL* (rs2074518, $P = 3.03 \times 10^{-6}$), a locus that exceeded the genome-wide significance threshold in the QTGEN consortium. Owing to reduced genotyping coverage of the *KCEN1* locus as compared to QTGEN, our data do not support the association with the nonsynonymous D85N variant in *KCNE1* (refs. 20,22,34–36).

Evaluation of a QT score

To assess the cumulative effect of the ten main QT-associated SNPs, we summed up the number of QT interval-prolonging alleles for each participant in the studies of unrelated individuals ($N = 10,563$) (**Supplementary Methods**). Overall, we observed a 1.53 ± 0.08 ms ($P = 1.79 \times 10^{-88}$) increase in the mean QT interval for each additional QT-prolonging allele, with a difference of 18.1 ms between individuals with a QT score of ≤ 6 or ≥ 16 (**Fig. 3**). The 58% of individuals with a score of ≥ 11 had an OR of 1.49 for prolonged QT using clinical thresholds (males ≥ 440 ms, females ≥ 450 ms) when compared with the 42% of individuals with a score of ≤ 10 (95% CI = 1.27–1.76, $P = 1.54 \times 10^{-6}$). Further at the extremes, the ~8% of individuals with a score of ≥ 14 had an OR of 2.52 for prolonged QT when compared to the ~10% individuals with a score ≤ 8 (95% CI = 1.74–3.66, $P = 4.83 \times 10^{-7}$).

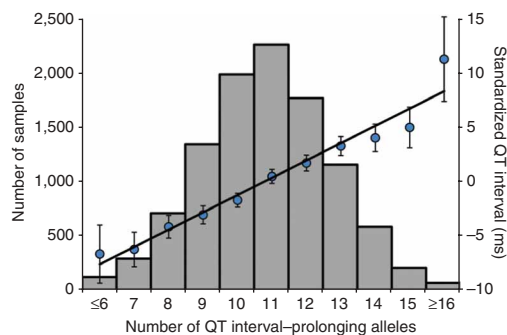


Figure 3 Combined effect of the QT interval-prolonging alleles in the studies of unrelated individuals. Individuals were classified by counting their number of QT-prolonging alleles in all ten identified markers (max score 20). Dosages for the QT-prolonging allele as calculated by MACH1 were added and then rounded to the nearest integer. Gray bars indicate the number of individuals in each score class, blue dots indicate the mean QT interval for each class, and the black line is the linear regression through these points.

DISCUSSION

Our results illustrate the power of GWAS to identify common variants both at loci previously unsuspected of involvement in cardiovascular function as well as at loci with a documented role in the regulation of QT interval. Although the statistical evidence of these associations is compelling mostly owing to the size of current GWAS, we are aware of limitations of this approach. As population stratification may confound our association findings, we have calculated a genome-wide F_{st} statistic between populations³⁷. This indicates an overall F_{st} statistic of 0.004 between all cohorts, comparatively minor relative to the value of 0.12 obtained by the International HapMap Project when comparing Europeans, Africans and Asians (**Supplementary Table 6** online). We report cohort-specific results in **Supplementary Table 7** online.

Also we did not account for some known covariates of QT interval, as these either did not contribute significantly to the model fit (**Supplementary Table 3b**) or were not uniformly available across studies and the increase of explained variance of QT interval was small at the population level (such as for example, for underlying cardiac pathologies, serum potassium levels and medication use) (**Supplementary Table 3c**). We may potentially have missed some association signals especially in the presence of interaction. As most complex genetic associations identified today perform well in log linear models³⁸ and as the accompanying study³⁴ did not identify any loci that our study would dismiss, we do not consider this limitation a major one. A post hoc analysis of the ten main association signals identified in a model fitting additional covariates indicated no major improvement in model fit (**Supplementary Table 8** online): six out of the ten loci slightly gained in significance level attained, whereas four loci decreased. Other limitations include the overestimation of effect sizes in initial discoveries due to the ‘winners curse’ phenomenon and the inability of the association approach to identify underlying genes or mechanisms in the regions of association signals.

The identification of causal genes and mechanisms at each of the loci remains a major task. This may be performed by functional experiments, but genetic studies are integral to scrutinize loci for causal mutations and establish pathways that regulate myocardial function. Promising genetic methods include SNP fine mapping at each locus at a resolution higher than that of the HapMap, and resequencing the target locus in individuals from the extremes of the QT interval distribution and in individuals diagnosed with long-QT syndrome in order to identify additional common as well as rare

variants that may reveal the causal genes. The approach of establishing an allelic series at a given locus including both common and rare variants seems particularly promising. Four of ten loci we identified by association do overlap with genes known to harbor mutations in LQTS and SQTs. Loci showing allelic series have also been identified in genome-wide association studies of lipid levels³⁹, height⁴⁰ and uric acid⁴¹. In the future, it may be worthwhile to include all the loci identified here in sequencing efforts in cases of long- and short-QT syndromes and in subjects where these syndromes are induced after exposure to specific medications. More immediately, our results point to a specific set of loci that are associated with QT interval and provide further targets for molecular studies of susceptibility to QT-triggered ventricular arrhythmias, sudden cardiac death and cardiovascular function in general.

METHODS

Summary. For a description of methods used, see **Supplementary Methods**.

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

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Designed the scientific rationale of the work: A.P., S.S., D.E.A., R.J.P., S.S.N., A.A.H., D.S., T.M., M.U., J.C., S.K., G.R.A., A.C. Obtained funding: A.P., R.E.,

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