ORIGINAL ARTICLE

ExomeChip-Wide Analysis of 95 626 Individuals Identifies 10 Novel Loci Associated With QT and JT Intervals

See Editorial by Bos and Pereira

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BACKGROUND: QT interval, measured through a standard ECG, captures the time it takes for the cardiac ventricles to depolarize and repolarize. JT interval is the component of the QT interval that reflects ventricular repolarization alone. Prolonged QT interval has been linked to higher risk of sudden cardiac arrest.

METHODS AND RESULTS: We performed an ExomeChip-wide analysis for both QT and JT intervals, including 209 449 variants, both common and rare, in 17 341 genes from the Illumina Infinium HumanExome BeadChip. We identified 10 loci that modulate QT and JT interval duration that have not been previously reported in the literature using single-variant statistical models in a meta-analysis of 95 626 individuals from 23 cohorts (comprised 83 884 European ancestry individuals, 9610 blacks, 1382 Hispanics, and 750 Asians). This brings the total number of ventricular repolarization associated loci to 45. In addition, our approach of using coding variants has highlighted the role of 17 specific genes for involvement in ventricular repolarization, 7 of which are in novel loci.

CONCLUSIONS: Our analyses show a role for myocyte internal structure and interconnections in modulating QT interval duration, adding to previous known roles of potassium, sodium, and calcium ion regulation, as well as autonomic control. We anticipate that these discoveries will open new paths to the goal of making novel remedies for the prevention of lethal ventricular arrhythmias and sudden cardiac arrest.

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Clinical Perspective

Prolonged QT interval has been associated with increased risk of sudden cardiac arrest, a major cause of mortality, with between 180 000 and 450 000 cases of sudden cardiac arrest in the United States of America annually. Because the vast majority of sudden cardiac arrest occurs in the absence of clinical features that would bring a victim to medical attention, identifying additional risk factors and dissecting the pathogenesis of disease are of high importance. In this study, we conduct ExomeChip-wide analyses in 95 626 population-based multiethnic individuals to interrogate the role of a largely unstudied class of variation on ventricular repolarization in the population—coding single nucleotide variants. These variants fill in the gap between the extremely rare large-effect coding variants that result in the Mendelian long- and short-QT syndromes and the common small-effect largely noncoding variation identified through genome-wide association studies. The focus on exons and coding variants has an added benefit of directly implicating genes. Our approach of focusing on coding variants and both QT and JT intervals measures has identified 10 novel loci associated with ventricular repolarization and has implicated 17 specific genes, 7 of which are in novel loci. Our analyses show a role for myocyte internal structure and interconnections in modulating QT interval duration, adding to previous known roles of potassium, sodium, and calcium ion regulation, as well as autonomic control. We anticipate that these discoveries will open new paths to the goal of making novel remedies for the prevention of lethal ventricular arrhythmias and sudden cardiac arrest.

Prolonged QT interval has been associated with increased risk of sudden cardiac arrest (SCA), a major cause of mortality, with between 180 000 and 450 000 cases of SCA in the United States of America annually.[1] Because the vast majority of SCA occurs in the absence of clinical features that would bring a victim to medical attention,[2] identifying additional risk factors and dissecting the pathogenesis of disease are of high importance.

Heritability estimates of QT interval are between 30% and 40%, indicating that genetic variants play a large role in modulating QT interval in the general population.[3] Mendelian syndromes of QT interval (long- and short-QT syndrome), which lead to increased risk of cardiac arrhythmias and SCA, occur in ≈1 in 2000 individuals and are caused by variants in ion channels or their interacting proteins.[4] Previous candidate gene and genome-wide association studies (GWAS), largely screening common noncoding variants, have identified 35 loci containing variants that modestly influence QT interval, the largest of these studies, the QT Interval International GWAS Consortium (QT-IGC),[5] included a discovery population of 76 061 European ancestry individuals.

In this study, we conduct ExomeChip-wide analyses in population-based samples to interrogate the role of a largely unstudied class of variation on ventricular repolarization in the population—coding single nucleotide variants (SNVs). These variants fill in the gap between the extremely rare large-effect coding variants that result in the Mendelian long- and short-QT syndromes and the common small-effect largely noncoding variation identified through GWAS. The focus on exons and coding variants has an added benefit of directly implicating genes. By contrast, noncoding variation typically implicates a region of the genome, often containing multiple genes, and therefore requiring extensive functional experiments to implicate a specific gene. Furthermore, in this study, we examine both QT and JT interval to more comprehensively examine ventricular repolarization. We have previously observed that variation in specific loci can influence ventricular depolarization and repolarization in a concordant fashion.[5,6]

We performed a meta-analysis of 23 cohorts including 95 626 multiethnic individuals comprised 83 884 European ancestry individuals, 9610 blacks, 1382 Hispanics, and 750 Asian individuals (Table I in the in the Data Supplement). Each individual was genotyped for 191 740 coding SNVs in 17 341 genes using the Illumina Infinium HumanExome BeadChip (ExomeChip), along with 17 709 noncoding SNVs of known importance from previous GWAS and variants tiling across the genome. These variants were chosen by evaluating ≈12 000 exome sequences for coding variants that appeared in at least 3 individuals.

METHODS

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results, subject to Data Use/Sharing Agreements adopted by individual participating cohorts. GWAS summary results will be available through the CHARGE Consortium Summary Results webpage available at dbGaP (phs000930).

This study was approved by local institutional review boards, and all participating subjects gave informed consent (detailed ethics statements in the Data Supplement).

SNV Association Tests and Meta-Analysis

Detailed methods are provided in the Data Supplement. Briefly, all cohorts excluded individuals with QRS intervals ≥120 ms, heart rate <40 beats per minute or >120 beats per

minute , left or right bundle branch block, atrial fibrillation on baseline ECG, Wolff–Parkinson–White syndrome, pacemaker, use of class I or class III blocking medication, or pregnant. Clinical characteristics summary statistics for each cohort are provided in Table I in the Data Supplement.

SNV effect size estimates are calculated via standard inverse variance–weighted meta-analysis of results provided by each cohort from a linear association model with QT/JT as the dependent variable, including covariates age, sex, RR interval (inverse heart rate), height, body mass index, and cohort-specific adjustments (principal components, clinic, family structure). Significance is similarly calculated by inverse variance–weighted meta-analysis; however, instead of raw QT/JT as the dependent variable in the linear regression, an inverse rank normal transformation is performed (details in the Data Supplement). These 2 models are used in tandem to avoid *P* value inflation from the analysis of the rare variants on the ExomeChip while maintaining the easy interpretation of effect sizes in milliseconds. The main analysis included all ethnic groups meta-analyzed together. SNVs with minor allele count <10 were excluded from the meta-analysis. SNVs were considered statistically significant if they exceeded the Bonferroni correction threshold of $P<2\times10^{-7}$.

Use of Functional Variants to Implicate Individual Genes Using Genome-Wide Significance

Genome-wide significance (GwiS) uses a greedy forward selection algorithm to identify independent genetic effects within a given gene/locus.[7] A locus was defined by the SNV with the most significant association±1 megabase. GWiS was run on European-only summary statistics from 22 cohorts (QT, n=83 884; JT, n=80 330), with linkage disequilibrium (LD) estimated from the merged ExomeChip and HapMap-imputed Atherosclerosis Risk in Communities (ARIC) European ancestry data set (n=9537; Data Supplement). An attempt to replace GWiS identified noncoding variants with equivalent coding variants ($r^2>0.8$) did not yield any substitutions.

RESULTS

QT Interval ExomeChip Analysis Identifies 6 Novel Loci

Meta-analysis identified SNVs in 25 loci associated with QT interval at ExomeChip-wide significance ($P<2\times10^{-7}$; Figure I in the Data Supplement). Of these, 19 loci were previously associated with QT interval, and 6 loci were novel (Table 1). At 4 of these novel loci (*PM20D1*, *SLC4A3*, *CASR*, and *NRAP*), the top hit is a nonsynonymous variant. For the 2 novel loci where the index SNV is a noncoding variant, no genes in these loci harbored coding SNVs associated with QT interval. Analyses stratified by ethnicity found similar effect sizes between European ancestry individuals and blacks and same general direction of effects in the much smaller Hispanics (n=1382) and Chinese (n=750) cohorts (Table II and Figure II in the Data Supplement).

Nineteen of the 25 loci associated with QT interval at ExomeChip-wide significance in our study had been associated with QT interval in prior European ancestry GWAS studies (Table 2, **P* value). Table 2 detail the 35 known QT loci identified from prior GWAS of European ancestry individuals. Of the 14 previously identified loci for which the most significant SNV in our current study is a coding variant (Table 2, A), 3 loci reached ExomeChip-wide significance in our study (**P* value). Of the 21 previously identified loci for which the most significant SNV in our study is a noncoding variant not in LD ($r^2>0.8$) with a nearby coding variant, 16 loci exceeded the significance threshold in our study (Table 2, B, **P* value). For 5 of these 16 loci where the top signal was a noncoding SNV, they nonetheless harbored coding variants in ≥1 nearby genes that also reached ExomeChip-wide significance (Table II in the Data Supplement).

Table 1. Six Novel Loci Associated With QT Interval

Nearby Gene	SNV	Chr	Coded/ Noncoded Allele	CAF	Effect in ms (SE)	*P* Value	Function	Gene(s) With Independent Coding Variation	DEPICT Implicated Gene(s)	eQTL
PM20D1	rs1361754	1	G/A	0.511	0.47 (0.08)	1E-09	Nonsynonymous	*PM20D1*		*PM20D1,* NUCKS1, RAB7L1,* SLC41A1*
SLC4A3	rs55910611	2	A/G	0.006	–3.06 (0.61)	2E-07	Nonsynonymous	*SLC4A3*		
CASR	rs1801725	3	T/G	0.126	–0.58 (0.12)	4E-08	Nonsynonymous	*CASR*		*CSTA*
ZNF37A	rs4934956	10	T/C	0.497	0.58 (0.10)	2E-10	Intergenic			
NRAP	rs3189030	10	A/G	0.299	–0.48 (0.09)	4E-08	Nonsynonymous	*NRAP*	*NRAP*	*CASP7**
GOSR2	rs17608766	17	C/T	0.123	0.72 (0.12)	3E-09	UTR3			*RPRML*

Significance was determined from analysis of inverse rank normal transformed residuals to avoid *P* value inflation from the analysis of rare variants. Effect size estimates in milliseconds (ms) are reported from untransformed analyses. n=95 626 number of samples. DEPICT[9] genes pass FDR <5% cutoff. Expression quantitative trait loci (eQTL) genes are pulled from the Genotype-Tissue Expression portal[10,11] using the representative SNV and GWiS independent SNVs. CAF indicates coded allele frequency; DEPICT, Data-driven Expression-Prioritized Integration for Complex Traits; FDR, false discovery rate; GwiS, genome-wide significance; SNV, single-nucleotide variants; and UTR3, three prime untranslated region.

*Gene if the eQTL is in the left ventricle.

Table 2. Thirty-Five Loci Previously Associated With QT Interval

Nearby Gene	SNV	Chr	Coded/ Noncoded Allele	CAF	Effect in ms (SE)	*P* Value	Function	QT-IGC Implicated Gene(s)	Gene(s) With Independent Coding Variation	DEPICT Implicated Gene(s)	eQTL
A, Known QT loci with coding variant as top SNV											
RNF207	rs709209	1	G/A	0.379	1.23 (0.09)	1E-48*	Nonsynonymous	*RNF207(c)*	*RNF207*	*RNF207*	*GPR153*
SP3	rs1047640	2	C/T	0.120	0.60 (0.12)	3E-06	Nonsynonymous		*SP3*		
TTN-CCDC141	rs72648998	2	T/C	0.054	1.00 (0.18)	3E-09*	Nonsynonymous	*CCDC141(i), TTN(i)*	*TTN*		*FKBP7, PRKRA*
SPATS2L	rs192861441	2	A/G	0.004	−2.22 (0.67)	3E-04	Nonsynonymous	*SPATS2L(t), SGOL2(p)*			
C3ORF75	rs2276853	3	G/A	0.411	−0.36 (0.08)	2E-05	Nonsynonymous	*KLHL18(t), PTPN23(t), SCAP(t), SETD2(t), MYL3(i)*		*NBEAL2*	*NBEAL2, PTPN23, SCAP*
SMARCAD1	rs7439869	4	T/C	0.378	0.41 (0.08)	8E-07	Nonsynonymous		*SMARCAD1*		
GMPR	rs1042391	6	T/A	0.551	−0.42 (0.09)	3E-06	Nonsynonymous	*GMPR(c), ATXN1(tp)*	*GMPR*		*GMPR*
KCNH2	rs1805123	7	G/T	0.214	−1.47 (0.10)	7E-51*	Nonsynonymous	*KCNH2(p)*	*KCNH2*†		*KCNH2*
LAPTM4B	rs17831160	8	A/G	0.030	−0.64 (0.24)	3E-03	Nonsynonymous				
AZIN1	rs143025416	8	A/G	0.001	4.90 (1.55)	2E-03	Nonsynonymous				
GBF1	rs143226354	10	T/C	8.89E-05	14.18 (4.66)	4E-03	Splicing/ nonsynonymous	*ACTR1A(i)*			
ATP2A2	rs11068997	12	A/G	0.040	−0.94 (0.21)	4E-07	Nonsynonymous	*VPS29(t), GPN3(t), ARPC3(t), C12ORF24(t), ATP2A2(pi)*	*GIT2, TCTN1*	*ATP2A2, PPTC7*	
USP50-TRPM7	rs8042919	15	A/G	0.097	−0.57 (0.14)	4E-05	Nonsynonymous				*SPPL2A, AP4E1, USP50*
CREBBP	rs143903106	16	T/G	0.001	4.10 (1.46)	5E-03	Nonsynonymous	*TRAP1(i)*			
B, Known QT loci with noncoding variant as top SNV											
TCEA3	rs1077514	1	G/A	0.179	−0.58 (0.11)	4E-08*	Intronic	*TCEA3(t)*			*TCEA3,‡ ASAP3*
NOS1AP	rs12143842	1	T/C	0.240	3.18 (0.10)	3E-255*	Intergenic				
ATP1B1	rs10919071	1	G/A	0.115	−1.37 (0.13)	3E-30*	Intronic	*ATP1B1(ti), NME7(t)*			*NME7*
SLC8A1	rs2540226	2	T/G	0.482	0.24 (0.08)	2E-03	Intergenic	*SLC8A1(p)*			*THUMPD2*
SCN5A-SCN10A	rs12053903	3	C/T	0.379	−0.88 (0.09)	1E-26*	Intronic	*SCN5A(p)*	*SCN10A*	*SCN5A*	*SNORA6, SCN5A*§
SLC4A4	rs7689609	4	C/T	0.212	0.64 (0.12)	4E-08*	Intronic				
GFRA3	rs4835768	5	G/A	0.485	0.34 (0.08)	7E-05	Intergenic	*FAM13B(t), ETF1(p)*		*MYOT, FAM13B*	
SLC35F1-PLN	rs11153730	6	C/T	0.467	1.41 (0.08)	5E-74*	Intergenic	*PLN(i)*		*PLN*	*SSXP10*
CAV1	rs3807989	7	A/G	0.429	0.54 (0.08)	4E-12*	Intronic	*CAV1(pi), CAV2(pi)*			*AC002066.1*
NCOA2	rs2926707	8	G/T	0.348	0.31 (0.09)	3E-04	Intronic				
KCNQ1	rs2074238	11	T/C	0.074	−3.58 (0.16)	8E-130*	Intronic	*C11ORF21(t), PHEMX(t), TSPAN32(t), KCNQ1(p)*	*KCNQ1*	*KCNQ1*	
FEN1-FADS2	rs1535	11	G/A	0.325	−0.48 (0.09)	8E-10*	Intronic	*FADS1(t), FADS2(t), FADS3(t)*			*FAD2,‡ FADS1, TMEM258*
KLF12	rs1886512	13	A/T	0.381	0.57 (0.09)	2E-10*	Intronic	*KLF12(t)*			*KLF12*‡
ANKRD9	rs11704	14	C/G	0.291	0.35 (0.09)	7E-05	UTR3	*ANKRD9(t)*		*ANKRD9*	*ZNF839*

(*Continued*)

Table 2. Continued

Nearby Gene	SNV	Chr	Coded/ Noncoded Allele	CAF	Effect in ms (SE)	*P* Value	Function	QT-IGC Implicated Gene(s)	Gene(s) With Independent Coding Variation	DEPICT Implicated Gene(s)	eQTL
LITAF	rs8049607	16	T/C	0.503	1.05 (0.08)	8E-44*	Intergenic	*LITAF(t)*			*LITAF*‡
MKL2	rs30208	16	T/C	0.501	0.45 (0.08)	2E-09*	Intergenic				
CNOT1	rs7188697	16	G/A	0.247	−1.57 (0.10)	4E-63*	Intronic	*NDRG4(t), CNOT1(t), GOT2(i)*			*SETD6, NDRG4*
LIG3	rs2074518	17	A/G	0.428	−0.79 (0.08)	2E-21*	Intronic	*LIG3(t), CCT6B(t), UNC45B(i)*			*LIG3,‡ CCT6B, RFFL,‡ RP5-837J1.2*
PRKCA	rs9912468	17	G/C	0.417	−0.68 (0.08)	2E-15*	Intronic	*PRKCA(t)*			*PRKCA*‡
KCNJ2	rs17779747	17	T/G	0.304	−1.08 (0.09)	3E-37*	Intergenic				
KCNE1	rs727957	21	T/G	0.168	0.48 (0.11)	3E-05	Intronic	*KCNE1(cp)*			

A section lists the 14 (of 35) previously identified loci (QT-IGC study of European ancestry individuals[5]) for which the most significant SNV in our current study is a coding variant. Because of the design of the Exome Chip with a focus on coding variants, only select intronic or intergenic SNVs were interrogated, and therefore not all QT-IGC SNVs were examined. B section lists the 21 previously identified loci for which the most significant SNV in our study is a noncoding variant not in LD (r^2>0.8) with a nearby coding variant. Significance was determined from analysis of inverse rank normal transformed residuals to avoid *P* value inflation from the analysis of rare variants. Effect size estimates in milliseconds (ms) are reported from untransformed analyses. n=95 626 number of samples. Within the QT-IGC Implicated Gene(s) column, evidence for the gene is c, coding variant; t, eQTL transcript; p, in silico protein-protein interactor; i, immunoprecipitation interactor. DEPICT[9] genes pass FDR<5% cutoff. Expression quantitative trait loci (eQTL) genes are pulled from the Genotype-Tissue Expression portal[10,11] using the representative SNV and GWiS independent SNVs. CAF indicates coded allele frequency; DEPICT, Data-driven Expression-Prioritized Integration for Complex Traits; FDR, false discovery rate; GwiS, genome-wide significance; QT-IGC, QT Interval International GWAS Consortium, and SNV, single nucleotide variants.

**P* value if significantly associated after Bonferroni correction, $P<2\times10^{-7}$.

‡Gene if the eQTL is in the left ventricle.

§GWiS independent SNV rs9851724 used to identify eQTL.

†Conditional analyses in ARIC contradict this result, see text for details.

JT Interval Association Identifies 4 Novel Loci

Although ventricular depolarization and repolarization are often coregulated, this is not universally true. Therefore, to more specifically examine ventricular repolarization, we also investigated genetic associations with JT interval, defined mathematically by subtracting the QRS interval (ventricular depolarization and conduction) from the QT interval, which primarily reflects ventricular repolarization.[8] Among the 15 590 ARIC participants, the correlations (r^2) among the intervals were 0.84 for QT and JT; 0.02 for QRS and JT; and 0.08 for QT and QRS. We analyzed JT interval as described above for QT interval while adding QRS interval as an additional covariate to further remove the effect of ventricular depolarization on the analysis. Thirty coding variants in 14 loci were associated with JT interval (Table III and Figure III in the Data Supplement). Four of these 14 loci were not identified as QT interval loci (Table 3). Three of these 4 novel repolarization loci had index SNVs that were coding variants: *SENP2*, *SLC12A7*, and *NACA*. The SNV rs9470361 (near *CDKN1A*) has previously been associated with QRS interval with an effect size estimate in the opposite direction (Table 3). Indeed, for 3 of these loci (*SENP2*, *CDKN1A*, and *NACA*), where an association was found with JT but not with QT interval, the index SNVs were significantly associated with QRS duration but with effect estimates in the opposite direction (Table 3). Hence, at these loci, variants that prolong the QRS interval (depolarization) shorten the JT interval (repolarization). Analyses run stratified by ethnicity found similar effect sizes between European ancestry individuals and blacks (Table III in the Data Supplement).

Use of Coding Variants to Implicate Specific Genes

Leveraging information from nominally significant coding SNVs, we sought to implicate causative genes in each locus by demonstrating that putatively functional coding variants are associated with ventricular repolarization independently of noncoding SNVs. We have previously[5] shown that several QT loci contain multiple independent genetic effects, including some loci harboring multiple significant coding variants (Tables II and III in the Data Supplement). Thus, even if not the top hit at a locus, putative functional SNVs can still implicate a specific gene at a locus. We used the GWiS[7] algorithm to determine the number of independent effects in all 45 ventricular repolarization associated loci from Tables 1 through 3 and to identify the SNV that best represents each independent effect in European ancestry individuals (n=83 884; Table IV in the Data Supplement). The *SCN5A-SCN10A* locus is a particularly illustrative example of the use of this approach. Although coding variants in *DLEC1*, *SCN5A*, and *SCN10A* are each ExomeChip-wide significant, after using GWiS, the

Table 3. Four Novel Loci Associated With JT Interval

Nearby Gene	SNV	Chr	Coded/ Noncoded Allele	CAF	JT Effect in ms (SE)	JT *P* Value	QRS Effect in ms (SE)	QRS *P* Value	Function	Gene(s) With Independent Coding Variation	DEPICT Implicated Gene(s)	eQTL
SENP2	rs6762208	3	A/C	0.358	0.44 (0.08)	2E-07*	−0.31 (0.05)	3.45E-12*	Nonsynonymous	*SENP2*		
SLC12A7	rs737154	5	C/T	0.500	−0.40 (0.08)	2E-07*	0.07 (0.04)	8.84E-02	Splicing/ synonymous	*SLC12A7*		*NKD2*
CDKN1A	rs9470361	6	A/G	0.249	−0.76 (0.09)	2E-15*	0.84 (0.05)	1.21E-63*	Intergenic			
NACA	rs2926743	12	A/G	0.252	0.53 (0.09)	6E-08*	−0.32 (0.05)	9.40E-11*	Nonsynonymous	*NACA*	*RBMS2*	

Significance was determined from analysis of inverse rank normal transformed residuals to avoid *P* value inflation from the analysis of rare variants. Effect size estimates in milliseconds (ms) are reported from untransformed analyses. QRS interval association summary data for these 4 variants were contributed by our coauthors Drs Prins, Jamshidi, and Arking from ExomeChip analyses they are running as a part of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Electrocardiogram (EKG) working group. n=95 626 samples for JT interval association and n=85 593 samples for QRS interval association. DEPICT[9] genes pass FDR<5% cutoff. Expression quantitative trait loci (eQTL) genes are pulled from the Genotype-Tissue Expression portal[10,11] using the representative SNV and GWiS independent SNVs. CAF indicates coded allele frequency; DEPICT, Data-driven Expression-Prioritized Integration for Complex Traits; FDR, false discovery rate; GwiS, genome-wide significance; and SNV, single nucleotide variants.

**P* value if significantly associated after Bonferroni correction, $P<2\times10^{-7}$.

†Gene if the eQTL is in the left ventricle.

signal coming from the coding variants in *DLEC1* and *SCN5A* is explained by noncoding variants, and only the *SCN10A* coding variant signal remains (Table V in the Data Supplement). In the Gene(s) with independent coding variation column in Tables 1 through 3, we list the 17 genes in 16 loci that have an independent effect represented by a coding variant.

For the loci listed in Table 2 B, such as the *SCN5A-SCN10A* locus, where intronic and intergenic variants were included in the analyses, the independent associations in coding SNVs identified by GWiS are independent of the noncoding variants in the region. This analysis implicates 2 genes for involvement in cardiac repolarization among those of European descent: *SCN10A* and *KCNQ1*. For the novel loci in Table 1 where a coding SNV is the most significant association in our study, it is unlikely that noncoding variants of importance are present in those loci because the loci were not found during the QT-IGC efforts, a study of similar sample size.

In contrast, for the 14 previously identified QT loci where the top SNV in our study was a coding variant (Table 2, A), the GWiS findings are less conclusive because intronic and intergenic SNVs were largely not examined in these regions. Therefore, to determine whether the associated coding variants are independently associated with QT interval and hence implicate a causal gene, or alternatively, are associated simply because of LD with a more strongly associated noncoding variant not genotyped with the ExomeChip, we performed additional analyses in a subset of the data set, ARIC, that includes both the QT-IGC top SNV, as well as the top SNV, from the current study. We performed conditional analyses at the 7 loci in Table 2, A where significant associations were identified by GWiS (the remaining 7 loci did not have any SNVs identified as significant by GWiS after accounting for multiple testing), by including both the QT-IGC and ExomeChip variants in the same regression model in the ARIC Europeans data set (n=9537; Table VI in the Data Supplement). Conditional analyses demonstrate that the coding variant in *SP3* is independent of the top noncoding SNV at this locus discovered from QT-IGC, implicating this gene in QT interval modulation. For *GMPR*, the coding variant is in almost perfect linkage disequilibrium with the noncoding QT-IGC variant (r^2=0.99 in ARIC), suggesting that the coding variant may be the causal variant explaining the QT-IGC signal. For a third locus, *RNF207,* although conditional analysis suggested that the QT-IGC SNV accounts for the association at this locus, both the top QT-IGC SNV as well as the top SNV from this study are coding variants in high LD, thus implicating the *RNF207* gene in myocardial repolarization. For the remaining 4 loci, 1 coding variant is associated because of the stronger noncoding QT-IGC signal (*KCNH2*); 2 were not properly tested because of no effect in ARIC of the ExomeChip variant (*ATP2A2*) or the QT-IGC variant (*TTN*), although there was low LD (r^2<0.04) between the coding and noncoding variants, suggesting independence; and 1 was unclear (*SMARCAD1*), as putting both SNVs in the model significantly altered the β estimates for both SNVs.

In Silico Analyses to Implicate Causal Genes

To further decode the role these loci might play in regulating ventricular repolarization, Data-driven Expression-Prioritized Integration for Complex Traits[9] was used to investigate whether identified loci contain genes from functional annotated gene sets/pathways. Included in Tables 1 through 3 in the DEPICT Implicated Gene(s) column is a list of genes with a false discovery rate <5%. Furthermore, we looked up each of the Tables 1 through 3 SNVs in the Genotype-Tissue Expression Portal to identify single-tissue expression quantitative trait loci[10,11] (left ventricle expression quantitative trait loci, represented by footnote symbols in tables). Findings for

Data-driven Expression-Prioritized Integration for Complex Traits and expression quantitative trait loci analyses are largely consistent with those genes identified because of harboring significant coding variants and help clarify the causative gene.

DISCUSSION

Our approach of focusing on coding variants and both QT and JT intervals has identified 10 novel loci associated with ventricular repolarization and has implicated 17 specific genes, 7 of which are in novel loci. Previous studies have implicated roles for potassium ion regulation, sodium ion regulation, calcium ion regulation, and autonomic control of QT interval,[12] and our results provide support for each of these pathways. *SLC12A7* (*KCC4*), which is highly expressed in the left ventricle,[10,11] is a potassium chloride cotransporter involved in potassium efflux.[13] *CASR* is a G protein–coupled receptor that maintains circulating calcium ion homeostasis via parathyroid hormone secretion in the parathyroid and kidney tubule ion handling.[14]

In addition to previously implicated pathways, our analyses highlight a role for genes involved in generating the physical force of contraction inside of cardiomyocytes and for conducting electric signal between cardiomyocytes across the heart. Pathway enrichment analyses using Data-driven Expression-Prioritized Integration for Complex Traits (detailed methods in the Data Supplement) identified the GO category GO:0005916, which comprised the genes that code for fascia adherens, the structure that links myofibrils between cardiomyocytes, and contains N-cadherin. *NRAP*, found to have a significant independent coding variant, likely anchors terminal actin filaments of myofibrils to other protein complexes beneath the sarcolemma[15,16] and is expressed exclusively in skeletal muscle and heart.[10,11] skNAC (skeletal *NACA*) knockout mice, a muscle-specific isoform of *NACA*, which was found to have a significant independent coding variant, die between embryonic days 10.5 and 12.5 because of cardiac defects, showing interventricular septal defects and a thin myocardial wall.[17] With these 3 points of evidence combined with the previously known locus and GWiS-implicated gene, *TTN*, a clear class of genes emerge that influence ventricular repolarization through their effect on myocyte structure.

It is important to note that the intercalated disc, which is the interface between cardiomyocytes, contains fascia adherens, desmosomes, and gap junctions, the last of which is known to play a role in ion-mediated relaying of action potentials between cardiomyocytes and, in combination with the gene *NOS1AP*, has been implicated as regulating QT interval.[18] In contrast, we implicate a nonion-dependent structural/mechanical interconnect between cardiomyocytes mediated by the fascia adherens.

By looking specifically at ventricular repolarization (JT interval) without the influence of depolarization (QRS interval), we detected additional loci related to ventricular repolarization while teasing apart the differential regulation of the various phases of ventricular conduction. Our current results are consistent with our prior findings that variation in some loci influence ventricular depolarization and repolarization in a concordant fashion, others influence depolarization and repolarization in a discordant fashion, and still other loci are associated with one phenotype and not the other.[5,6] Although ventricular depolarization and repolarization are often coregulated, the difference in genetic effect indicates this is not universally true. Several limitations should be noted. First, we did not have an additional sample to perform replication studies although results were consistent across the diverse cohorts included in our study (Figures IV–XIII in the Data Supplement). Second, correlation of effect sizes was weak between the European ancestry and Hispanic and Asian populations, limiting extrapolation of findings to these populations.

In summary, we have identified 10 loci newly associated with ventricular repolarization. This brings the total number of ventricular repolarization–associated loci to 45. In addition, we have directly implicated 17 specific genes contained in these loci as likely affecting ventricular repolarization and outlined a class of genes that mechanically control QT interval. These new discoveries will likely allow for the development of novel vectors for the prevention of lethal ventricular arrhythmias and SCA.

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FOOTNOTES

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