Circulation
Cardiovascular Genetics

Sequencing of *SCN5A* Identifies Rare and Common Variants Associated With Cardiac Conduction: Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium

Jared W. Magnani, Jennifer A. Brody, Bram P. Prins, Dan E. Arking, Honghuang Lin, Xiaoyan Yin, Ching-Ti Liu, Alanna C. Morrison, Feng Zhang, Tim D. Spector, Alvaro Alonso, Joshua C. Bis, Susan R. Heckbert, Thomas Lumley, Colleen M. Sitlani, L. Adrienne Cupples, Steven A. Lubitz, Elsayed Z. Soliman, Sara L. Pulit, Christopher Newton-Cheh, Christopher J. O'Donnell, Patrick T. Ellinor, Emelia J. Benjamin, Donna M. Muzny, Richard A. Gibbs, Jireh Santibanez, Herman A. Taylor, Jerome I. Rotter, Leslie A. Lange, Bruce M. Psaty, Rebecca Jackson, Stephen S. Rich, Eric Boerwinkle, Yalda Jamshidi and Nona Sotoodehnia
for CHARGE Consortium, the NHLBI s Exome Sequencing Project (ESP), and the UK10K

Circ Cardiovasc Genet. 2014;7:365-373
doi: 10.1161/CIRCGENETICS.113.000098
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1942-325X. Online ISSN: 1942-3268

Sequencing of *SCN5A* Identifies Rare and Common Variants Associated With Cardiac Conduction: Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium

Jared W. Magnani, MD, MSc*; Jennifer A. Brody, BA*; Bram P. Prins, MSc; Dan E. Arking, PhD; Honghuang Lin, PhD; Xiaoyan Yin, PhD; Ching-Ti Liu, PhD; Alanna C. Morrison, PhD; Feng Zhang, PhD; Tim D. Spector, MD, MSc, FRCP; Alvaro Alonso, MD, PhD; Joshua C. Bis, PhD; Susan R. Heckbert, MD, PhD; Thomas Lumley, PhD; Colleen M. Sitlani, PhD; L. Adrienne Cupples, PhD; Steven A. Lubitz, MD, MPH; Elsayed Z. Soliman, MD, MSc, MS; Sara L. Pulit, BA; Christopher Newton-Cheh, MD, MPH; Christopher J. O'Donnell, MD; Patrick T. Ellinor, MD, PhD; Emelia J. Benjamin, MD, ScM; Donna M. Muzny, MS; Richard A. Gibbs, PhD; Jireh Santibanez; Herman A. Taylor, MD, MPH; Jerome I. Rotter, MD; Leslie A. Lange, PhD; Bruce M. Psaty, MD, PhD; Rebecca Jackson, MD; Stephen S. Rich, PhD; Eric Boerwinkle, PhD; Yalda Jamshidi, PhD; Nona Sotoodehnia, MD, MPH; for CHARGE Consortium, the NHLBI's Exome Sequencing Project (ESP), and the UK10K†

Background—The cardiac sodium channel *SCN5A* regulates atrioventricular and ventricular conduction. Genetic variants in this gene are associated with PR and QRS intervals. We sought to characterize further the contribution of rare and common coding variation in *SCN5A* to cardiac conduction.

Methods and Results—In Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Targeted Sequencing Study, we performed targeted exonic sequencing of *SCN5A* (n=3699, European ancestry individuals) and identified 4 common (minor allele frequency >1%) and 157 rare variants. Common and rare SCN5A coding variants were examined for association with PR and QRS intervals through meta-analysis of European ancestry participants from CHARGE, National Heart, Lung, and Blood Institute's Exome Sequencing Project (n=607), and the UK10K (n=1275) and by examining Exome Sequencing Project African ancestry participants (n=972). Rare coding *SCN5A* variants in aggregate were associated with PR interval in European and African ancestry participants (P=1.3×10^{-3}). Three common variants were associated with PR and QRS interval duration among European ancestry participants and one among African ancestry participants. These included 2 well-known missense variants: rs1805124 (H558R) was associated with PR and QRS shortening in European ancestry participants (P=6.25×10^{-4} and P=5.2×10^{-3}, respectively) and rs7626962 (S1102Y) was associated with PR shortening in those of African ancestry (P=2.82×10^{-3}). Among European ancestry participants, 2 novel synonymous variants, rs1805126 and rs6599230, were associated with cardiac conduction. Our top signal, rs1805126 was associated with PR and QRS lengthening (P=3.35×10^{-7} and P=2.69×10^{-4}, respectively) and rs6599230 was associated with PR shortening (P=2.67×10^{-5}).

Conclusions—By sequencing *SCN5A*, we identified novel common and rare coding variants associated with cardiac conduction. **(*Circ Cardiovasc Genet*. 2014;7:365-373.)**

Key Words: electrocardiography ■ genomics

The PR and QRS intervals are electrocardiographic measures of cardiac atrioventricular conduction. Community-based studies have identified associations between PR and QRS measurements and adverse cardiovascular outcomes. PR prolongation has been associated with risk of atrial fibrillation (AF), pacemaker implantation, heart failure, and all-cause

Received February 1, 2013; accepted March 23, 2014.

*Dr Magnani and J.Brody contributed equally to this work.

†A full list of ESP and UK10K consortium members are in the Data Supplement Material.

The Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.113.000098/-/DC1.

Correspondence to Jared W. Magnani, MD, MSc, Section of Cardiovascular Medicine, Boston University School of Medicine, 88 E. Newton St, Boston, MA 02118. E-mail jmagnani@bu.edu or Nona Sotoodehnia, MD, MPH, Cardiovascular Health Research Unit, University of Washington, Seattle, WA. E-mail nsotoo@uw.edu

mortality.[1–3] QRS prolongation has been associated with heart failure and cardiovascular mortality in clinical trial– and community-based cohorts.[4–8] Genome-wide association studies (GWAS) and candidate gene studies have identified common genetic variants in the cardiac sodium channel *SCN5A* gene to be associated with PR and QRS intervals among those of European and African ancestry.[9–14] Missense mutations in this gene have been associated with supraventricular and ventricular arrhythmias.[15]

The functional contributions of lower frequency and rare variants to PR and QRS intervals in the general population remain largely unknown. In the present study, we sought to (1) sequence the *SCN5A* gene to catalog coding variants in this gene; (2) examine the associations of rare *SCN5A* coding variants with PR and QRS intervals; and (3) identify novel associations of common and low-frequency coding variants, perhaps poorly tagged by GWAS, with cardiac conduction. To address these aims, we combined exonic sequencing of the *SCN5A* gene across multiple consortia: Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Targeted Sequencing Study, the National, Heart, Lung, and Blood Institute's Exome Sequencing Project (ESP), and the United Kingdom–based UK10K.

Methods

Study Samples: CHARGE

CHARGE conducted targeted sequencing on a sample of participants selected for their extremes of PR and QRS phenotypes from the Atherosclerosis Risk in Communities (ARIC) Study, the Cardiovascular Health Study (CHS), and the Framingham Heart Study (FHS). In all 3 cohorts, the PR and QRS phenotypes were ascertained from standardized applications of 12-lead ECG. ECG analysis and quantification of the PR and QRS phenotypes for the 3 cohorts have been presented elsewhere.[12,13,16]

The comprehensive methods for sequencing are presented by Lin et al in the accompanying article. In brief, 77 loci identified in prior GWAS were selected for sequencing at the Baylor College of Medicine Human Genome Sequencing Center. In total, 52 736 unique variants were identified using the SOLiD platform-based multiplexed sequencing protocol developed specifically for the CHARGE. SAMtools[17] was used for variant detection and calling. Individual variant calls that were >100 base pairs from the capture region, of low quality (phred-scaled base quality <30), with <2 reads of the alternate allele, or <10 reads overall were set to missing. Variant sites within a cohort failing any of the following criteria were removed: (1) allelic imbalance ratio that was >80% or less that 20%, (2) missingness rate >20%, (3) deviation from Hardy–Weinberg equilibrium with a $P<1\times10^{-5}$, or (4) reporting >1 alternative alleles. Too many variants within a short genomic interval can indicate regional sequencing errors or uncalled structural variants. Dense clusters of single nucleotide polymorphisms (SNPs), defined as ≥3 SNPs in a 10 bp window, were, therefore, removed. The present analysis is focused solely on the results of targeted exome sequencing of *SCN5A* and its association with the PR and QRS intervals.

For PR interval participant selection, individuals at the upper tail of the trait distribution were selected using a model using the ECG phenotype as the independent variable and age, sex, study center, height, and body mass index as dependent variables. For QRS duration selection, individuals were chosen at the upper tail of the distribution of the phenotype. For both phenotypes, 100 participants from ARIC, 50 from CHS, and 50 from FHS, with equal numbers of men and women, were selected for sequencing after exclusions. CHARGE participants were excluded from the analysis for the following reasons: nonwhite race, lacking PR or QRS measurement, prevalent AF, history of myocardial infarction or heart failure, pacemaker implantation, or use of class I or III antiarrhythmic medications. Individuals with QRS duration >120 ms were also excluded from the QRS analysis.

The examination of common variation in genotypes imputed to 1000 Genomes included all participants of European descent in ARIC, CHS, and FHS with available imputed genotypes. Details of the genotyping platform, quality control imputation, and reference panel are provided in the Methods in the Data Supplement.

Study Samples: ESP

ESP is designed to examine genomic associations with heart, lung, and blood diseases. Participants in ESP were selected from cohort studies by having extremes of quantitative phenotypes (low-density lipoprotein cholesterol, blood pressure, body mass index) or disease end points (eg, ischemic stroke, early onset myocardial infarction).[18] Library construction, exome capture, sequencing, mapping, calling, and filtering have been described elsewhere.[18,19] Briefly, deep (60-80X target depth) whole exome sequencing was performed at 2 genome centers using Illumina GAII or HiSeq2000 sequencers. Single-nucleotide variants were called using the UMAKE pipeline at University of Michigan, using *glfMultiples4* software that implements a maximum likelihood model and allowed all samples to be analyzed simultaneously. Reads were mapped using human reference (hg19) with Burrows–Wheeler Aligner[20] and summarized in BAM (.bam, binary version of sequence alignment data) files for joint calling input. All low-quality reads (phred-scaled mapping quality <20) and pair-end reads likely to be polymerase chain reaction duplicates were removed. Sites deemed to be false-positive were excluded from further analyses. Variant calls with a read depth <10× were set to missing. Variant sites were removed if the mean sample read depth across all samples was >500×, the variant deviated from race-specific Hardy–Weinberg equilibrium ($P<1\times10^{-7}$). A support vector machine classifier was used to separate likely true-positive and false-positive variant sites as described elsewhere.[21] Support vector machine filtering started by collecting a series of features related to quality of each SNV, including overall depth, fraction of samples with coverage, allelic imbalance, correlation of alternative alleles with strand and read position (strand and cycle bias), and inbreeding coefficient for each variant. SNVs that deviated significantly from expected values in ≥3 categories were flagged as likely false-positives when training the support vector machine filter. Multidimensional scaling was performed to validate European and African ancestry.

ESP participants were excluded for lacking PR or QRS measurement, pacemaker or defibrillator implantation, or prevalent AF. European ancestry ESP samples with available ECG measurements come from ARIC, CHS, FHS, Multi-Ethnic Study of Atherosclerosis (MESA), and the Women's Health Initiative (WHI). To avoid sample overlap with the CHARGE sample, ARIC, CHS, and FHS participants of European ancestry were excluded from the ESP analysis. In the present analysis, the ESP African ancestry sample consisted of participants from ARIC, CHS, the Jackson Heart Study, MESA, and WHI.

Study Samples: UK10K

UK10K (http://www.uk10k.org/) is a large-scale sequencing project based on collaboration between investigators at the Wellcome Trust Sanger Institute and clinical experts in genetic diseases. The aims were to associate genetic variation with phenotypic traits and identify rare variants contributing to disease in the TwinsUK Registry (http://www.twinsuk.ac.uk/), a cohort study investigating the genetic epidemiology of diverse traits and diseases in twins that has been described in detail.[12] Low-coverage whole-genome sequencing was performed at the Wellcome Trust Sanger Institute and the Beijing Genomics Institute using the Illumina HiSeq platform according to manufacturer's protocol. Variant calls were made using SAMtools/bcftools[17] by pooling the alignments from individual low-coverage BAM files. The Genome Analysis Toolkit[22] was used to filter sites (Variant Quality Score Recalibration)[23] and to model and calibrate the variants. The VQSLOD (log odds ratio of being a true variant) score for SNPs was set to −0.6804, setting the maximum truth sensitivity tranche to 99.5%.

Samples were excluded if there was a high overall discordance to SNP array data (>3%), if the heterozygosity rate was >3 SD from population mean, or if the mean read depth was <4×. To ensure only samples of European ancestry were included, the data set was pruned to the HapMap3 populations, followed by principal components analysis using EIGENSTRAT,[24] after which samples were removed that did not cluster to European ancestry. Hereafter were excluded related samples (identity by state >0.125, third degree relatedness) and checked zygosity in the sequence data against zygosity in GWA data using identity by state, removing cotwin samples (dizygotic and monozygotic). This procedure led to a final data set of 1754 complete sequences, with an overall read depth of 6.95×.

PR and QRS intervals were obtained in TwinsUK from standardized methods with automated measurement by the Cardiofax ECG-9020K (Nihon Kohden UK Ltd, Middlesex, UK). UK10K participants were excluded for non-European ancestry, missing the PR or QRS phenotypes, prevalent AF, or a history of pacemaker implantation.

Because we used exome sequencing from 3 different studies in our analysis, we compared the quality control metrics and calling approaches across the studies. In particular, rare variants are challenging to call consistently and may be spurious; we, therefore, characterized the quality of our variants in Table I in the Data Supplement, which includes number of variants called, TiTv ratio, and average depth of coverage across the 3 studies.

Statistical Analysis

Briefly, we categorized variation into 2 classes: rare (<1% minor allele frequency [MAF]) or common. Common variants were examined individually using linear regression in CHS, ARIC, ESP, and UK10K and linear mixed effect models in FHS to account for familial structure. Analyses for both PR and QRS intervals were adjusted for age, sex, height, body mass index, and cohort. Analyses in ESP were additionally adjusted for principal components, phenotype sampling group, and sequence center. In the CHARGE, analyses weighted by the sampling probabilities were conducted to obtain unbiased population effect estimates. We combined results using fixed-effects inverse variance–weighted meta-analysis of study-specific association estimates. We initially combined results from the 3 CHARGE cohorts. We then combined results from the CHARGE, ESP, and UK10K studies. Analysis of 1000 Genomes imputed data from ARIC, CHS, and FHS used the same adjustments and were combined with a fixed-effects inverse variance–weighted meta-analysis of the study-specific association estimates.

For each phenotype, we adjusted for multiple testing using a Bonferroni correction. Among those of European ancestry, 4 common coding SNPs were examined, and a meta-analytic $P<0.0125$ (0.05/4 variants) was deemed significant for each phenotype. For individuals of African ancestry, 10 SNPs were examined and a meta-analytic $P<0.005$ (0.05/10 variants) was deemed significant for each phenotype. Pairwise R^2 values were reported from the SNAP web interface using the 1000 Genomes project Pilot 1 data.[25]

Rare variation in the coding regions was jointly analyzed using the Sequence Kernel Association test (SKAT), which was adapted for a meta-analysis framework[26] as described in the Methods in the accompanying article. Unlike burden tests, the SKAT test does not assume a consistent direction of effect for all variants. There have been several reports of mutations in ion channel genes, some of which increase and some that decrease channel function;[27–29] hence, we select the SKAT omnidirectional test over simpler variant collapsing rare variant tests. Disease-causing mutations have been cataloged along the entire length of the sodium channel,[30] implying multiple or broad functional domains. We, therefore, determined a priori to include all coding rare variants along the length of *SCN5A* in a single, combined rare variant test. All SKAT tests were adjusted for age, sex, height, body mass index, and study-specific population variables. ESP analyses were additionally adjusted for principal components, phenotype sampling group, and sequence center. Consortium results were combined with a Fisher *P* value meta-analysis. We deemed the threshold for statistical significance as 0.05 for each phenotype.

All study participants provided informed consent. Institutional review board oversight and approval was performed by each of the participating studies.

Results

In total, the CHARGE population consisted of 3699 individuals of European ancestry from 3 community-based cohort studies (ARIC, n=1645; CHS, n=1021; FHS, n=1033). There were 1579 participants in the ESP samples, of whom 972 (61.6%) were of African ancestry. The UK10K study examined 1275 individuals of European ancestry. Mean PR interval in each study ranged from 152 ms to 171 ms, and mean QRS interval from 88 ms to 95 ms (Table 1). Cohort characteristics are described in Table 1.

SNP Catalog

Targeted exonic sequencing of *SCN5A* in 3699 CHARGE European descent participants identified 157 rare variants (3 nonsense variants, 4 intronic splice-site variants, 91 nonsynonymous SNPs, and 59 synonymous SNPs), as shown in Figure 1. Most of these rare variants were novel (n=134, 85%) compared with 1000 Genomes Pilot 1 data. Four common

Table 1. Phenotypic Characteristics of the Study Samples

	CHARGE			Extension Cohorts		African Ancestry
	ARIC	CHS	FHS	ESP	UK10K	ESP
Total n	1645	1021	1033	607	1275	972
Men, %	50.6	45.8	47.5	10.2	0	29.4
Age	54.5 (5.7)	72.2 (5.3)	38.2 (9.5)	63.4 (8.2)	54.6 (11.0)	58.3 (8.8)
BMI, kg/m^2	27.4 (5.8)	26.8 (5.2)	26.4 (6.3)	28.9 (5.6)	26 (4.6)	32.5 (9.0)
Height, cm	169.3 (9.6)	165.5 (9.3)	168.3 (9.7)	162.6 (7.6)	161.9 (6.2)	166.6 (8.9)
SBP, mm Hg	119.5 (19.0)	136.1 (22.7)	121.4 (16.9)	132.2 (22.7)	124.2 (42.6)	133.1 (22.5)
RR interval, ms	923.8 (137.5)	950.6 (158.5)	842.9 (159.0)	913.8 (145.0)	914.3 (145.2)	909.8 (158.9)
PR Interval, ms	164.1 (27.7)	170.9 (31.9)	152.5 (22.8)	160.5 (24.3)	159.3 (22.7)	168.1 (24.2)
QRS Interval, ms	91.9 (10.0)	90.3 (11.5)	88.7 (10.0)	88.9 (13.0)	87.4 (8.3)	91.4 (14.7)

Continuous variables are listed as mean (SD) and categorical as n (%). ARIC indicates Atherosclerosis Risk in Communities Study; BMI, body mass index; CHARGE, Cohorts for Heart and Aging Research in Genetic Epidemiology; CHS, Cardiovascular Health Study; ESP, Exome Sequencing Project; FHS, Framingham Heart Study; and SBP, systolic blood pressure.

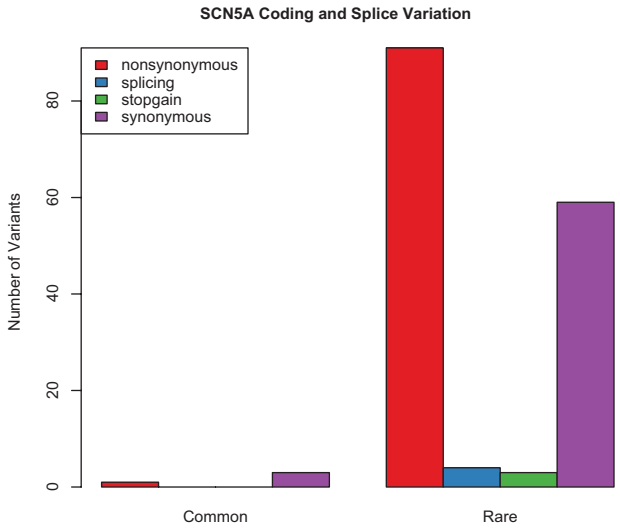

Figure 1. Distribution of SCN5A variants by function, stratified by common or rare (<1%) minor allele frequency.

(MAF >1%) coding variants (1 nonsynonymous, 3 synonymous variants) were identified. Although none of these common variants are novel, 2 were not present in HapMap2 and hence not investigated by prior large-scale GWAS efforts. Similarly, targeted sequencing among ESP and UK10K European ancestry individuals identified only these 4 common variants. Among 972 black participants in ESP, we identified 10 common variants (4 nonsynonymous, 6 synonymous) and 71 rare variants (40 nonsynonymous, 31 synonymous).

Rare Variant Analysis

We jointly analyzed the rare coding variation in *SCN5A* using SKAT for association with cardiac conduction. We found that rare variants in aggregate were associated with PR interval in both European (P=0.01) and African ancestry (P=0.01) participants separately and combined (meta-analytic $P=8.9\times10^{-3}$) but not with the QRS interval (Table 2 presents the gene-based test associations for the ECG phenotypes across the 3 studies). Among the individual rare variants, there was no clustering of more extreme effects within any specific functional regions of the gene including the pore-forming domains (Figure 2). The effect size for rare variants ranged from PR shortening of 60 ms to PR lengthening of 120 ms. We identified 3 singleton nonsense variants among those of European ancestry in the CHARGE analysis. Individuals with nonsense variants had PR intervals both shorter and longer than the mean PR interval (Figure 2). No nonsense variants were identified among the 972 black participants.

Common Variant Analysis

Across the 3 European ancestry studies (n=5581), 3 of the 4 common coding variants were associated with the PR interval (Table 3) and 2 with QRS duration (Table 4). The well-characterized nonsynonymous SNP (rs1805124, MAF 18.4%, H558R in the I-II loop of the *SCN5A* channel) was associated with shorter PR (β=−2.44 ms; $P=6.25\times10^{-4}$) and QRS (β=−0.77 ms; $P=5.20\times10^{-3}$) intervals in meta-analysis across the CHARGE, ESP, and UK10K studies (Tables 3 and 4). In addition to H558R, we identified 2 novel associations between common synonymous SNPs and cardiac conduction. The strongest association was with rs1805126 (D1818D, MAF 33.6%), which was associated with both PR (β=2.51 ms; $P=3.35\times10^{-7}$) and QRS (β=0.67 ms; $P=2.69\times10^{-4}$) interval prolongation in meta-analysis. The second novel synonymous SNP (rs6599230, A29A, MAF 21.9%) was associated with PR shortening (β=−2.40 ms; $P=2.67\times10^{-5}$). No significant heterogeneity of effect was detected across the cohorts for the common variants, P>0.05 for all comparisons (Tables 3 and 4). Although the 3 variants associated with cardiac conduction in our study were not in linkage disequilibrium (LD) with each other (R^2<0.06), 2 of the SNPs were in at least modest LD with previously identified PR or QRS SNPs. D1818D (rs1805126) was in high LD (0.78) with intronic SNP rs10865879, the top signal associated with PR and QRS intervals from prior GWAS studies.[12,13] H558R, rs1805124, was not in LD with the top index SNPs associated with PR or QRS in prior reports but in modest LD (R^2=0.21) with a secondary *SCN5A*-QRS signal (rs11710077).[12] By contrast, the novel synonymous SNP rs6599230 (A29A) was not in LD (R^2<0.05) with any previously identified independent SCN5A index signal from GWAS studies of cardiac atrioventricular or ventricular conduction and may represent a new independent association signal (Table 5).

To increase the sample size examined for common variants, we performed 1000 genomes imputation on GWAS data from 9374, 2833, and 7837 European descent individuals from ARIC, CHS, and FHS, respectively (Table II in the Data Supplement). Meta-analysis across the combined 20 044 individuals in ARIC, CHS, and FHS showed that all 3 of these coding SNPs were strongly associated with PR and QRS intervals (Table III in the Data Supplement).

Among blacks, we examined the association of 10 common coding variants (including the 4 identified among those of European ancestry), with PR and QRS intervals. The 3 SNPs associated with PR and QRS among European descent individuals were not associated among blacks. In addition to H558R (rs1805124), 3 other common missense SNPs were identified among blacks (Table 6). The missense variant rs7626962

Table 2. Gene-Based SKAT Results (*P* Values) for Rare Coding Variants (MAF <1.0%) in *SCN5A*

ECG Measure	CHARGE	ESP	UK10K	ESP African Ancestry	Meta-Analysis
PR	0.003	0.46	0.22	0.01	1.32E-03
QRS	0.87	0.24	0.39	0.64	0.55

CHARGE indicates Cohorts for Heart and Aging Research in Genetic Epidemiology; ESP, Exome Sequencing Project; MAF, minor allele frequency; and SKAT, Sequence Kernel Association test.

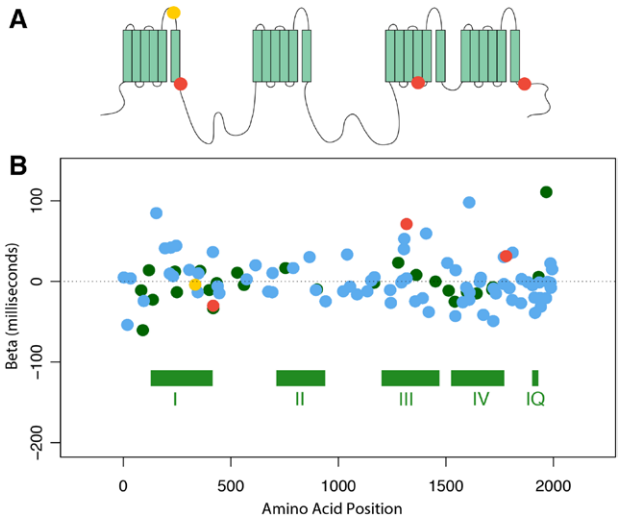

Figure 2. **A**, Schematic of Nav1.5, encoded by SCN5A, marking the position of nonsense and splice-site variants in relation to the pore-forming transmembrane segment domains. **B**, Effect estimates (ms) associated with each rare variant on the *y* axis and the amino acid position is on the *x* axis. Numerals I to IV refer to the pore-forming domains and IQ indicates the isoleucine and glutamine positions in the SCN5A sodium channel gene. Rare coding variants classified as synonymous (green), missense (blue), splice-site (orange), or nonsense (red).

S1102Y (MAF 5.1%) was associated with a 7.4 ms decrease in PR interval (P=2.8×10^{-03}) among blacks. This variant was not present in CHARGE sample of European ancestry participants. None of the 6 common synonymous variants identified were associated with PR or QRS intervals.

Discussion

We conducted targeted exonic sequencing of the *SCN5A* gene to identify rare and common variants and determine their association with the PR and QRS intervals. We combined sequencing data from 3 separate consortia—the CHARGE, NHLBI's ESP, and UK10K—and examined associations among those of European and African ancestry. Our approach facilitated a novel examination of rare and common coding variants of the cardiac *SCN5A* sodium channel and their relationships with highly accessible ECG measures of cardiac conduction. We identified novel common and rare coding variant associations with cardiac conduction. Identification of genetic variants may have important implications for understanding the genetics and heritability of cardiac arrhythmias.

Our investigation focused on genetic variants in coding regions of *SCN5A*, the predominant cardiac sodium channel gene, because of this gene's prominent role in cardiac depolarization and conduction.[31,32] The *SCN5A* gene is located on chromosome 3 (3p21), contains 28 exons, and encodes the $Na_v1.5$ pore-forming unit integral to the cardiac voltage-gated sodium channel.[33] Variable *SCN5A* transcript expression has diverse effects on sodium channel function.[34] Common variation in *SCN5A* has been associated with modest effects on cardiac conduction among those of European and African ancestries in several GWAS studies conducted by our group and others.[10,12,13] Rare or private mutations in *SCN5A* have been implicated in an array of conduction defects that include long-QT syndrome type 3,[35] Brugada syndrome,[36] atrial standstill,[37] and sinus node dysfunction.[38,39] In particular, mutations in *SCN5A* have been associated with pronounced conduction disease because of high-grade atrioventricular heart block.[40–42] Although previous studies have shown that common variants are associated with modest effects and rare or private mutations are associated with large effects in families with Mendelian disorders, this is the first study to show that the combined effect of rare variants in aggregate is associated with cardiac conduction in the general population.

Of the 4 common coding variants found among European ancestry individuals, we identified 2 novel synonymous associations (rs1805126 and rs6599230) with cardiac atrioventricular and ventricular conduction and validated the association of a previously identified and well-characterized missense variant (rs1805124, H558R). None of the common variant associations identified among those of European ancestry were found among blacks; however, the sample size among blacks was considerably smaller than among those of European ancestry, hence limiting power in this population. Sequencing among blacks did identify 4 common nonsynonymous and 6 common synonymous variants; one previously described missense variant (rs7626962, S1102Y) was associated with PR interval.

Two missense variants were associated with PR interval duration in our study. The common nonsynonymous SNP rs1805124 (H558R)[43] alters molecular electrophysiology in the presence of additional genetic mutations.[44,45] This SNP has

Table 3. Common Coding (MAF >1%) Variants Identified in the CHARGE, ESP, and UK10K Consortia and Their Association With the PR Interval

				CHARGE			ESP			UK10K			Meta-Analysis		
rsID	A1/A2	Function	Amino Acid*	CAF, %	Effect (SE)†	*P* Value	CAF, %	Effect (SE)†	*P* Value	CAF, %	Effect (SE)†	*P* Value	Effect (SE)†	*P* Value	Heterogeneity *P* Value
rs1805124	T/C	Nonsyn	H558R	18.4	−4.65 (1.24)	2.08E-05	24.4	−1.18 (1.56)	0.45	23.4	−1.42 (1.06)	0.18	−2.44 (0.71)	6.25E-04	0.09
rs1805126	A/G	Syn	D1818D	33.6	2.94 (0.64)	2.69E-09	33.5	2.9 (1.47)	0.05	34.4	1.51 (0.90)	0.09	2.51 (0.49)	3.35E-07	0.41
rs7430407	C/T	Syn	E1061E	13.9	0.11 (1.13)	0.89	12.3	−0.49 (2.13)	0.82	12.5	2.01 (1.34)	0.13	0.70 (0.80)	0.38	0.46
rs6599230	C/T	Syn	A29A	21.9	−2.08 (0.73)	0.02	19.9	−5.09 (1.73)	3.40E-03	20.4	−2.04 (1.08)	5.82E-02	−2.40 (0.57)	2.67E-05	0.26

A1 indicates reference allele; A2, coded allele; CAF, coded allele frequency; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; ESP, Exome Sequencing Project; MAF, minor allele frequency; Nonsyn, nonsynonymous; rsID, reference single nucleotide polymorphisms; and Syn, synonymous.

*Amino acid positions relative to NM_000335.4.

†Effect size measured in ms.

Table 4. Common Coding (MAF >1%) Variants Identified in the CHARGE, ESP, and UK10K Consortia and Their Association With the QRS Interval

				CHARGE			ESP			UK10K			Meta-Analysis		
rsID	A1/A2	Function	Amino Acid*	CAF, %	Effect† (SE)	*P* Value	CAF, %	Effect† (SE)	*P* Value	CAF, %	Effect† (SE)	*P* Value	Effect† (SE)	*P* Value	Heterogeneity *P* Value
rs1805124	T/C	Nonsyn	H558R	18.4	−0.04 (0.53)	0.75	24.4	−1.39 (0.58)	0.02	23.3	−0.89 (0.39)	0.02	−0.69 (0.31)	5.20E-03	0.21
rs1805126	A/G	Syn	D1818D	33.6	0.61 (0.24)	1.00E-04	33.5	0.43 (0.56)	0.45	34.3	0.87 (0.33)	0.01	0.69 (0.20)	2.69E-04	0.74
rs7430407	C/T	Syn	E1061E	13.9	0.01 (0.45)	0.85	12.3	−0.58 (0.81)	0.47	12.4	−0.06 (0.50)	0.90	0.15 (0.34)	0.74	0.81
rs6599230	C/T	Syn	A29A	21.9	−0.55 (0.27)	0.20	19.9	−1.16 (0.67)	0.08	20.6	0.22 (0.40)	0.58	−0.64 (0.25)	0.06	0.13

A1 indicates reference allele; A2, coded allele; CAF, coded allele frequency; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; ESP, Exome Sequencing Project; MAF, minor allele frequency; Nonsyn, nonsynonymous; rsID, reference single nucleotide polymorphisms; and Syn, synonymous.

*Amino acid positions relative to NM_000335.4.

†Effect size measured in ms.

been associated with PR and QRS in GWAS studies. The second missense SNP, a common variant of the *SCN5A* sodium channel gene (rs7626962, S1102Y), present among blacks but largely absent among those of European ancestry, has been associated with cardiac conduction and arrhythmias.[10,46] Electrophysiological studies have reported that the S1102Y variant of the cardiac sodium channel undergoes minimal kinetic shifts at baseline, but when exposed to other factors, such as cellular acidosis, late I_{Na} current is increased.

The 2 synonymous SNPs described in this article have not been previously associated with cardiac conduction. The mechanism by which either of these 2 SNPs may influence cardiac conduction is unknown and requires further investigation. The effects of the identified variants on cellular electrophysiology and their interactions with other mutations require investigation.

In meta-analysis conducted of GWAS data with 1000 genomes imputation from ARIC, CHS, and FHS, all 3 common coding variants were associated with an ≈2 ms alteration of PR duration and <1 ms of the QRS interval. Although the immediate clinical implications of these modest alterations are limited, the longer term contributions include enhancing genomic studies, identifying the missing heritability of genetic traits, and contributing to improved risk stratification. The PR and QRS intervals are easily acquired from the ECG and have a vast history of use in clinical care and research. Our research elucidates that multiple genomic variants may influence the durations of these measures of conduction. Next steps will include exploring genomic associations of rare and common variants with diseases such as AF, heart block, and cardiomyopathy.

Several limitations deserve consideration. First, genomic sequencing and analysis in CHARGE, ESP, and UK10K were all performed at distinct sites. Although differences in site sequencing technique, depth of coverage, variant calling methodologies, and quality control metrics may have contributed toward differential calling of sequenced variants, we anticipate such misclassifications to bias our findings toward the null. Furthermore, sequenced variants were analyzed within each study by SKAT and then meta-analyzed across the 3 studies. This meta-analysis approach to SKAT ensures that comparisons are made only within a single study, thereby ensuring that all study participants have the same sequencing approach and QC standards, and hence would not increase the type I error rate.

Second, the PR and QRS measures may have been altered by unrecognized clinical conditions or technical differences. We would expect, however, that such biases would again be nondifferential. Finally, although our study is the largest to examine sequence data for association with cardiac conduction, the meta-analytic sample size of 5581 European and 972 African ancestry individuals is too small to identify variants with modest associations.

In summary, by sequencing the coding region of an important gene in cardiac conduction, *SCN5A,* we have identified novel common coding variant associations with atrioventricular conduction in the general population. Importantly, this is the first study to show that the combined effect of rare variants

Table 5. Summary of Linkage Disequilibrium Between *SCN5A* SNPs and Those Identified in Prior PR and QRS Interval GWAS

	rs10865879 (PR and QRS GWAS Index SNPs)	rs11708996 (QRS GWAS Secondary SNP)	rs11710077 (QRS GWAS Secondary SNP)	rs1805124 (H558R)	rs1805126 (D1818D)	rs6599230 (A29A)
rs11708996	0.06					
rs11710077	0.07	0.04				
rs1805124	0.03	0.05	0.21			
rs1805126	0.78	0.04	0.10	0.04		
rs6599230	0.06	0.04	0.04	0.04	0.04	
rs7430407	0.03	0.01	0.02	0.00	0.06	0.00

Linkage disequilibrium is pairwise and reported as R^2, determined using 1000 Genomes project Pilot 1 data. GWAS indicates Genome-Wide Association Studies; and SNP, single nucleotide polymorphisms.

Table 6. Common Coding (MAF ≥1%) Variants Identified in the (n=972) ESP African Ancestry Sample and Their Association With the PR and QRS Interval

					PR		QRS	
rsID	A1/A2	Function	Amino Acid*	CAF, %	Effect (SE)†	*P* Value	Effect (SE)†	*P* Value
rs7626962	G/T	Nonsyn	S1102Y	5.20	−7.38 (2.46)	2.82E-03	2.1 (1.01)	0.04
rs1805124	T/C	Nonsyn	H558R	22.20	0.41 (1.41)	0.77	0.27 (0.57)	0.64
rs41313691	G/T	Nonsyn	S524Y	2.50	−2.66 (3.54)	0.45	−1.58 (1.4)	0.26
rs6791924	G/A	Nonsyn	R34C	8.50	−1.97 (1.95)	0.31	−0.72 (0.79)	0.36
rs13324293	G/A	Syn	I1947I	16.70	1.03 (1.5)	0.49	−0.43 (0.61)	0.49
rs1805126	A/G	Syn	D1818D	50.10	2.04 (1.3)	0.12	0.07 (0.53)	0.89
rs41315495	G/A	Syn	F1615F	14.90	−2.27 (1.53)	0.14	0.07 (0.62)	0.91
rs7430407	T/C	Syn	E1061E	67.70	3.53 (2.05)	0.09	−0.03 (0.82)	0.97
rs41313699	G/A	Syn	F434F	3.20	3.35 (3.2)	0.3	1.15 (1.32)	0.38
rs6599230	T/C	Syn	A29A	68.80	−0.6 (2.08)	0.77	−0.76 (0.84)	0.37

A1 indicates reference allele; A2, coded allele; CAF, coded allele frequency; ESP, Exome Sequencing Project; MAF, minor allele frequency; Nonsyn, nonsynonymous; rsID, reference single nucleotide polymorphisms; and Syn, synonymous.

*Amino acid positions relative to NM_000335.4.

†Effect size measured in ms.

in aggregate is associated with cardiac conduction in the general population. Our work provides insights into the genomic associations of cardiac conduction in individuals of European and African ancestry.

Acknowledgments

We wish to acknowledge the support of the National Heart, Lung, and Blood Institute (NHLBI) and the contributions of the research institutions, study investigators, field staff, and study participants in creating this resource for biomedical research. We acknowledge the Baylor Genome Center (National Institutes of Health grant U54 HG003273) for its contributions. Funding for GO Exome Sequencing Project was provided by NHLBI grants RC2 HL-103010 (HeartGO), RC2 HL-102923 (LungGO), and RC2 HL-102924 (WHISP). The exome sequencing was performed through NHLBI grants RC2 HL-102925 (BroadGO) and RC2 HL-102926 (SeattleGO). The Framingham Heart Study analysis was conducted using the Linux Clusters for Genetic Analysis computing resources at Boston University Medical Campus.

Sources of Funding

Funding support for building on Genome-wide association studies (GWAS) for National Heart, Lung, and Blood Institute (NHLBI) diseases: the US Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) Consortium Targeted Sequencing Study was provided by the National Institutes of Health (NIH) through the American Recovery and Reinvestment Act of 2009 (5RC2HL102419). Data for building on GWAS for NHLBI diseases: the US CHARGE Consortium was provided by Dr Boerwinkle on behalf of the Atherosclerosis Risk in Communities (ARIC) Study, Dr Cupples, principal investigator for the Framingham Heart Study, and Dr Psaty, principal investigator for the Cardiovascular Health Study. The ARIC Study is performed as a collaborative study supported by NHLBI contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN2682011000010C, HHSN2682011000011C, and HHSN2682011000012C). The Framingham Heart Study is conducted and supported by the NHLBI in collaboration with Boston University (contract No. N01-HC-25195) and its contract with Affymetrix, Inc, for genome-wide genotyping services (contract No. N02-HL-6-4278), for quality control by Framingham Heart Study investigators using genotypes in the SNP Health Association Resource (SHARe) project. This project was supported by 6R01-NS 17950NIH, 1RO1HL092577, 1R01 HL102214l, 1RC1HL101056, 5R21DA027021, 1RO1HL104156, K23HL11472, 1K24HL105780, and 1R03AG045075. Dr Magnani is further supported by a Boston University School of Medicine Department of Medicine Career Investment Award. A portion of this research was conducted using the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center. Cardiovascular Health Study (CHS): This CHS research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, and N01HC85086 and NHLBI grants HL080295, HL087652, and HL105756 with additional contribution from the National Institute of Neurological Disorders and Stroke. Additional support was provided through AG023629 from the National Institute on Aging. A full list of CHS investigators and institutions can be found at http://www.chs-nhlbi.org/pi.htm. UK10K: The UK10K project was funded by the Wellcome Trust award WT091310. Twins UK (TUK): TUK was funded by the Wellcome Trust and ENGAGE project grant agreement HEALTH-F4-2007–201413. The study also receives support from the Department of Health via the National Institute for Health Research (NIHR)-funded BioResource, Clinical Research Facility and Biomedical Research Centre based at Guy's and St. Thomas' NHS Foundation Trust in partnership with King's College London. Dr Spector is an NIHR senior Investigator and ERC Senior Researcher. Funding for the project was also provided by the British Heart Foundation grant PG/12/38/29615 (Dr Jamshidi).

Disclosures

B.M. Psaty serves on the DSMB of a clinical trial of a device funded by Zoll LifeCor and on the Steering Committee of the Yale Open Data Access Project funded by Medtronic. The other authors report no conflicts.

Appendix

From the NHLBI and Boston University's Framingham Heart Study, MA (J.W.M., H.L., X.Y., C.-T.L., A.C., C.N.-C., C.J.O., E.J.B.); Section of Cardiovascular Medicine (J.W.M., E.J.B.) and Section of Computational Biomedicine (H.L.), Boston University School of Medicine, MA; Department of Medicine, Cardiovascular Health Research Unit (J.A.B., J.C.B., S.R.H., C.M.S., B.M.P., N.S.), Department of Epidemiology (S.R.H., B.M.P.), Department of Health Services (B.M.P.), and Division of Cardiology (N.S.), University of Washington, Seattle; Human Genetics Research Centre, St. George's University of London, London, United

Kingdom (B.P.P., F.Z., Y.J.); McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD (D.E.A.); Department of Biostatistics, Boston University School of Public Health, MA (X.Y., C.-T.L., A.C.); Human Genetics Center, University of Texas Health Science Center, Houston (A.C.M., E.B.); Department of Twin Research and Genetic Epidemiology Unit, St. Thomas' Campus, King's College London, St. Thomas' Hospital, London, United Kingdom (F.Z., T.D.S.); Division of Epidemiology and Community Health, University of Minnesota, Minneapolis (A.A.);Department of Statistics, University of Auckland, Auckland, New Zealand (T.L.); Cardiovascular Research Center, Massachusetts General Hospital, Charlestown, MA (S.A.L., S.L.P., C.N.-C., P.T.E.); Cardiology Division, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston (S.A.L., C.N.-C., C.J.O., P.T.E.); Epidemiological Cardiology Research Center, Wake Forest University School of Medicine, Winston Salem, NC (E.Z.S.); Broad Institute, Cambridge, MA (S.L.P., C.N.-C.); Boston University Schools of Medicine and Public Health, MA (E.J.B.); Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX (D.M.M., R.A.G., J.S., E.B.); University of Mississippi Medical Center, Jackson, MI (H.A.T.); Institute for Translational Genomics and Populations Sciences, Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, Torrance, CA (J.I.R.); Department of Genetics, University of North Carolina, Chapel Hill (L.A.L.); Group Health Research Institute, Group Health Cooperative, Seattle, WA (B.M.P.); Department of Medicine, Wexner Medical Center, Ohio State University, Columbus (R.J.); and Center for Public Health Genomics, University of Virginia, Charlottesville (S.S.R.).

References

1. Cheng S, Keyes MJ, Larson MG, McCabe EL, Newton-Cheh C, Levy D, et al. Long-term outcomes in individuals with prolonged PR interval or first-degree atrioventricular block. *JAMA*. 2009;301:2571–2577.
2. Soliman EZ, Prineas RJ, Case LD, Zhang ZM, Goff DC Jr. Ethnic distribution of ECG predictors of atrial fibrillation and its impact on understanding the ethnic distribution of ischemic stroke in the Atherosclerosis Risk in Communities (ARIC) study. *Stroke*. 2009;40:1204–1211.
3. Magnani JW, Wang N, Nelson KP, Connelly S, Deo R, Rodondi N, et al; Health, Aging, and Body Composition Study. Electrocardiographic PR interval and adverse outcomes in older adults: the Health, Aging, and Body Composition study. *Circ Arrhythm Electrophysiol*. 2013;6:84–90.
4. Dhingra R, Pencina MJ, Wang TJ, Nam BH, Benjamin EJ, Levy D, et al. Electrocardiographic QRS duration and the risk of congestive heart failure: the Framingham Heart Study. *Hypertension*. 2006;47:861–867.
5. Morin DP, Oikarinen L, Viitasalo M, Toivonen L, Nieminen MS, Kjeldsen SE, et al. QRS duration predicts sudden cardiac death in hypertensive patients undergoing intensive medical therapy: the LIFE study. *Eur Heart J*. 2009;30:2908–2914.
6. Okin PM, Devereux RB, Kjeldsen SE, Edelman JM, Dahlöf B. Incidence of heart failure in relation to QRS duration during antihypertensive therapy: the LIFE study. *J Hypertens*. 2009;27:2271–2277.
7. Desai AD, Yaw TS, Yamazaki T, Kaykha A, Chun S, Froelicher VF. Prognostic significance of quantitative QRS duration. *Am J Med*. 2006;119:600–606.
8. Elhendy A, Hammill SC, Mahoney DW, Pellikka PA. Relation of QRS duration on the surface 12-lead electrocardiogram with mortality in patients with known or suspected coronary artery disease. *Am J Cardiol*. 2005;96:1082–1088.
9. Chambers JC, Zhao J, Terracciano CM, Bezzina CR, Zhang W, Kaba R, et al. Genetic variation in SCN10A influences cardiac conduction. *Nat Genet*. 2010;42:149–152.
10. Jeff JM, Brown-Gentry K, Buxbaum SG, Sarpong DF, Taylor HA, George AL Jr, et al. SCN5A variation is associated with electrocardiographic traits in the Jackson Heart Study. *Circ Cardiovasc Genet*. 2011;4:139–144.
11. Smith JG, Magnani JW, Palmer C, Meng YA, Soliman EZ, Musani SK, et al; Candidate-gene Association Resource (CARe) Consortium. Genome-wide association studies of the PR interval in African Americans. *PLoS Genet*. 2011;7:e1001304.
12. Sotoodehnia N, Isaacs A, de Bakker PI, Dörr M, Newton-Cheh C, Nolte IM, et al. Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction. *Nat Genet*. 2010;42:1068–1076.
13. Pfeufer A, van Noord C, Marciante KD, Arking DE, Larson MG, Smith AV, et al. Genome-wide association study of PR interval. *Nat Genet*. 2010;42:153–159.
14. Holm H, Gudbjartsson DF, Arnar DO, Thorleifsson G, Thorgeirsson G, Stefansdottir H, et al. Several common variants modulate heart rate, PR interval and QRS duration. *Nat Genet*. 2010;42:117–122.
15. McNair WP, Sinagra G, Taylor MR, Di Lenarda A, Ferguson DA, Salcedo EE, et al; Familial Cardiomyopathy Registry Research Group. SCN5A mutations associate with arrhythmic dilated cardiomyopathy and commonly localize to the voltage-sensing mechanism. *J Am Coll Cardiol*. 2011;57:2160–2168.
16. Alonso A, Krijthe BP, Aspelund T, Stepas KA, Pencina MJ, Moser CB, et al. Simple risk model predicts incidence of atrial fibrillation in a racially and geographically diverse population: the CHARGE-AF consortium. *J Am Heart Assoc*. 2013;2:e000102.
17. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al; 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25:2078–2079.
18. Tennessen JA, Bigham AW, O'Connor TD, Fu W, Kenny EE, Gravel S, et al; Broad GO; Seattle GO; NHLBI Exome Sequencing Project. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. *Science*. 2012;337:64–69.
19. Fu W, O'Connor TD, Jun G, Kang HM, Abecasis G, Leal SM, et al; NHLBI Exome Sequencing Project. Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants. *Nature*. 2013;493:216–220.
20. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2010;26:589–595.
21. Lange LA, Hu Y, Zhang H, Xue C, Schmidt EM, Tang ZZ, et al; NHLBI Grand Opportunity Exome Sequencing Project. Whole-exome sequencing identifies rare and low-frequency coding variants associated with LDL cholesterol. *Am J Hum Genet*. 2014;94:233–245.
22. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20:1297–1303.
23. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet*. 2011;43:491–498.
24. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006;38:904–909.
25. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics*. 2008;24:2938–2939.
26. Wu MC, Lee S, Cai T, Li Y, Boehnke M, Lin X. Rare-variant association testing for sequencing data with the sequence kernel association test. *Am J Hum Genet*. 2011;89:82–93.
27. Bellocq C, van Ginneken AC, Bezzina CR, Alders M, Escande D, Mannens MM, et al. Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. *Circulation*. 2004;109:2394–2397.
28. Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, et al. Sudden death associated with short-QT syndrome linked to mutations in HERG. *Circulation*. 2004;109:30–35.
29. Nishio Y, Makiyama T, Itoh H, Sakaguchi T, Ohno S, Gong YZ, et al. D85N, a KCNE1 polymorphism, is a disease-causing gene variant in long QT syndrome. *J Am Coll Cardiol*. 2009;54:812–819.
30. Ruan Y, Liu N, Priori SG. Sodium channel mutations and arrhythmias. *Nat Rev Cardiol*. 2009;6:337–348.
31. Remme CA, Wilde AA, Bezzina CR. Cardiac sodium channel overlap syndromes: different faces of SCN5A mutations. *Trends Cardiovasc Med*. 2008;18:78–87.
32. Bezzina C, Veldkamp MW, van Den Berg MP, Postma AV, Rook MB, Viersma JW, et al. A single Na(+) channel mutation causing both long-QT and Brugada syndromes. *Circ Res*. 1999;85:1206–1213.
33. Wang Q, Li Z, Shen J, Keating MT. Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. *Genomics*. 1996;34:9–16.
34. Rook MB, Evers MM, Vos MA, Bierhuizen MF. Biology of cardiac sodium channel Nav1.5 expression. *Cardiovasc Res*. 2012;93:12–23.
35. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell*. 1995;80:805–811.
36. Kapplinger JD, Tester DJ, Alders M, Benito B, Berthet M, Brugada J, et al. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. *Heart Rhythm*. 2010;7:33–46.
37. Groenewegen WA, Firouzi M, Bezzina CR, Vliex S, van Langen IM, Sandkuijl L, et al. A cardiac sodium channel mutation cosegregates

with a rare connexin40 genotype in familial atrial standstill. *Circ Res*. 2003;92:14–22.
38. Benson DW, Wang DW, Dyment M, Knilans TK, Fish FA, Strieper MJ, et al. Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (SCN5A). *J Clin Invest*. 2003;112:1019–1028.
39. Gui J, Wang T, Jones RP, Trump D, Zimmer T, Lei M. Multiple loss-of-function mechanisms contribute to SCN5A-related familial sick sinus syndrome. *PLoS One*. 2010;5:e10985.
40. Holst AG, Liang B, Jespersen T, Bundgaard H, Haunso S, Svendsen JH, et al. Sick sinus syndrome, progressive cardiac conduction disease, atrial flutter and ventricular tachycardia caused by a novel SCN5A mutation. *Cardiology*. 2010;115:311–316.
41. Watanabe H, Yang T, Stroud DM, Lowe JS, Harris L, Atack TC, et al. Striking *In vivo* phenotype of a disease-associated human SCN5A mutation producing minimal changes *in vitro*. *Circulation*. 2011;124:1001–1011.
42. Vorobiof G, Kroening D, Hall B, Brugada R, Huang D. Brugada syndrome with marked conduction disease: dual implications of a SCN5A mutation. *Pacing Clin Electrophysiol*. 2008;31:630–634.
43. Yang P, Kanki H, Drolet B, Yang T, Wei J, Viswanathan PC, et al. Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. *Circulation*. 2002;105:1943–1948.
44. Cheng J, Morales A, Siegfried JD, Li D, Norton N, Song J, et al. SCN5A rare variants in familial dilated cardiomyopathy decrease peak sodium current depending on the common polymorphism H558R and common splice variant Q1077del. *Clin Transl Sci*. 2010;3:287–294.
45. Tan BH, Valdivia CR, Rok BA, Ye B, Ruwaldt KM, Tester DJ, et al. Common human SCN5A polymorphisms have altered electrophysiology when expressed in Q1077 splice variants. *Heart Rhythm*. 2005;2:741–747.
46. Splawski I, Timothy KW, Tateyama M, Clancy CE, Malhotra A, Beggs AH, et al. Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia. *Science*. 2002;297:1333–1336.