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Sequencing of *SCN5A* identifies rare and common variants associated with cardiac conduction

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Abstract

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 further characterize the contribution of rare and common coding variation in *SCN5A* to cardiac conduction.

Methods and Results—In the Cohorts for Heart and Aging Research in Genomic Epidemiology Targeted Sequencing Study (CHARGE), 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, NHLBI's Exome Sequencing Project (ESP, n=607) and the UK10K (n=1275) and by examining ESP African-ancestry participants (N=972). Rare coding *SCN5A* variants in aggregate were associated with PR interval in European and African-ancestry participants ($P=1.3\times10^{-3}$). Three common variants were associated with PR and/or QRS interval duration among European-ancestry participants and one among African-ancestry participants. These included two well-known missense variants; rs1805124 (H558R) was associated with PR and QRS shortening in European-ancestry participants ($P=6.25\times10^{-4}$ and $P=5.2\times10^{-3}$ respectively) and rs7626962 (S1102Y) was associated with PR shortening in those of African ancestry ($P=2.82\times10^{-3}$). Among European-ancestry participants, two novel synonymous variants, rs1805126 and rs6599230, were associated with cardiac conduction. Our top signal, rs1805126 was associated with PR and QRS lengthening ($P=3.35\times10^{-7}$ and $P=2.69\times10^{-4}$ respectively), and rs6599230 was associated with PR shortening ($P=2.67\times10^{-5}$).

Conclusions—By sequencing *SCN5A*, we identified novel common and rare coding variants associated with cardiac conduction.

Keywords

PR interval; QRS interval; genetics; sequencing; cohort

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 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: the Cohorts for Heart and Aging Research in Genomic Epidemiology Targeted Sequencing Study (CHARGE), 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 three cohorts the PR and QRS phenotypes were ascertained from standardized applications of 12-lead electrocardiograms (ECG). ECG analysis and quantification of the PR and QRS phenotypes for the three cohorts have been presented elsewhere.[12,13,16]

The comprehensive methods for sequencing are presented by Lin et al. in the accompanying manuscript. 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 fewer than 2 reads of the alternate allele or fewer than 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 greater than 80% or less that 20%; (2) missingness rate greater than 20%; (3) deviation from Hardy-Weinberg Equilibrium (HWE) with a p value of $< 1\times10^{-5}$; 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 SNPs, defined as 3 or more SNPs in a 10bp 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 employing 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 following exclusions. CHARGE participants were excluded from the analysis for the following: non-white race; lacking PR or QRS measurement; prevalent atrial fibrillation; 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 Supplemental methods.

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 endpoints (e.g., ischemic stroke, early onset myocardial infarction).[18] Library construction, exome capture, sequencing, mapping, calling and filtering have been described elsewhere.[18,19] Briefly, deep (60–80× target depth) whole exome sequencing was performed at two genome centers using Illumina GAII or HiSeq2000 sequencers. SNVs were called using the UMAKE pipeline at University of Michigan, utilizing *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 files for joint calling input. All low quality reads (Phred-scaled mapping quality < 20) and pair-end reads likely to be PCR 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 HWE ($p<1\times10^{-7}$). A support vector machine (SVM) classifier was used to separate likely true positive and false-positive variant sites as described elsewhere.[21] SVM 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 three or more categories were flagged as likely false positives when training the SVM filter. Multidimensional scaling was performed in order to validated 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, VQSR)[23] and to model and calibrate the variants. The VQSLOD 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 larger than 3SD from population mean or if the mean read depth was below 4×. To ensure only samples of European ancestry were included, the dataset 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 (IBD > 0.125, third degree relatedness) and checked zygosity in the sequence data against zygosity in GWA data using identity by state, removing co-twin samples (dizygotic and monozygotic). This procedure led to a final dataset 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 atrial fibrillation, or a history of pacemaker implantation.

As 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 Supplemental Table 1, which includes number of variants called, TiTv ratio and average depth of coverage across the 3 studies.

Statistical analysis

Briefly, we categorized variation into two 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, four common coding single nucleotide polymorphisms (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 accompanying methods paper. 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's 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 three 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 to 1000 Genomes Pilot 1 data. Four common (MAF>1%) coding variants (1 nonsynonymous, 3 synonymous variants) were identified. While 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 African ancestry 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 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 60ms to PR lengthening of 120ms. We identified 3 singleton nonsense variants among those of European ancestry in the CHARGE. 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 African ancestry participants.

Common Variant Analysis

Across the three European ancestry studies (n=5581), three of the four common coding variants were associated with the PR interval (Table 3) and two 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 (beta= −2.44 ms; $P=6.25\times10^{-4}$) and QRS (beta= −0.77ms; $P=5.20\times10^{-3}$) intervals in meta-analysis across the CHARGE, ESP, and UK10K studies (Table 3 and 4). In addition to H558R, we identified two 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 (beta=2.51 ms, $P=3.35\times10^{-7}$) and QRS (beta=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 (beta= −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). While the three variants associated with cardiac conduction in our study were not in LD with each other ($R^2<0.06$), two 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 (r2<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 (Supplemental Table 2). Meta-analysis across the combined 20,044 individuals in ARIC, CHS, and FHS showed that all three of these coding SNPs were strongly associated with PR and QRS intervals (Supplemental Table 3).

Among African Americans, we examined the association of 10 common coding variants (including the 4 identified among those of European ancestry), with PR and QRS intervals. The three SNPs associated with PR and QRS among European-descent individuals were not associated among African Americans. In addition to H558R (rs1805124), 3 other common missense SNPs were identified among African Americans (Table 6). The missense variant rs7626962 S1102Y (MAF 5.1%) was associated with a 7.4 ms decrease in PR interval (P=2.8×10^{-03}) among African Americans. 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 three 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 relations 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 a number of 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 due to high-grade atrioventricular heart block.[40–42] While 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 four common coding variants found among European ancestry individuals, we identified two 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 African Americans; however, the sample size among African Americans was considerably smaller than among those of European ancestry, hence limiting power in this population. Sequencing among African Americans 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 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 African Americans but largely absent among those of European ancestry, has been associated with cardiac conduction and arrhythmias.[10,46] Electrophysiologic 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 two synonymous SNPs described in this manuscript have not been previously associated with cardiac conduction. The mechanism by which either of these two 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 three common coding variants were associated with an approximate 2 ms alteration of PR duration and <1 ms of the QRS interval. While 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 atrial fibrillation, heart block, and cardiomyopathy.

Several limitations deserve consideration. First, genomic sequencing and analysis in CHARGE, ESP, and UK10K were all performed at distinct sites. While differences in site sequencing technique, depth of coverage, variant calling methodologies, and quality control metrics may have contributed towards differential calling of sequenced variants, we anticipate such misclassifications to bias our findings towards the null. Furthermore,

sequenced variants were analyzed within each study by SKAT and then meta-analyzed across the three 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 non-differential. 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 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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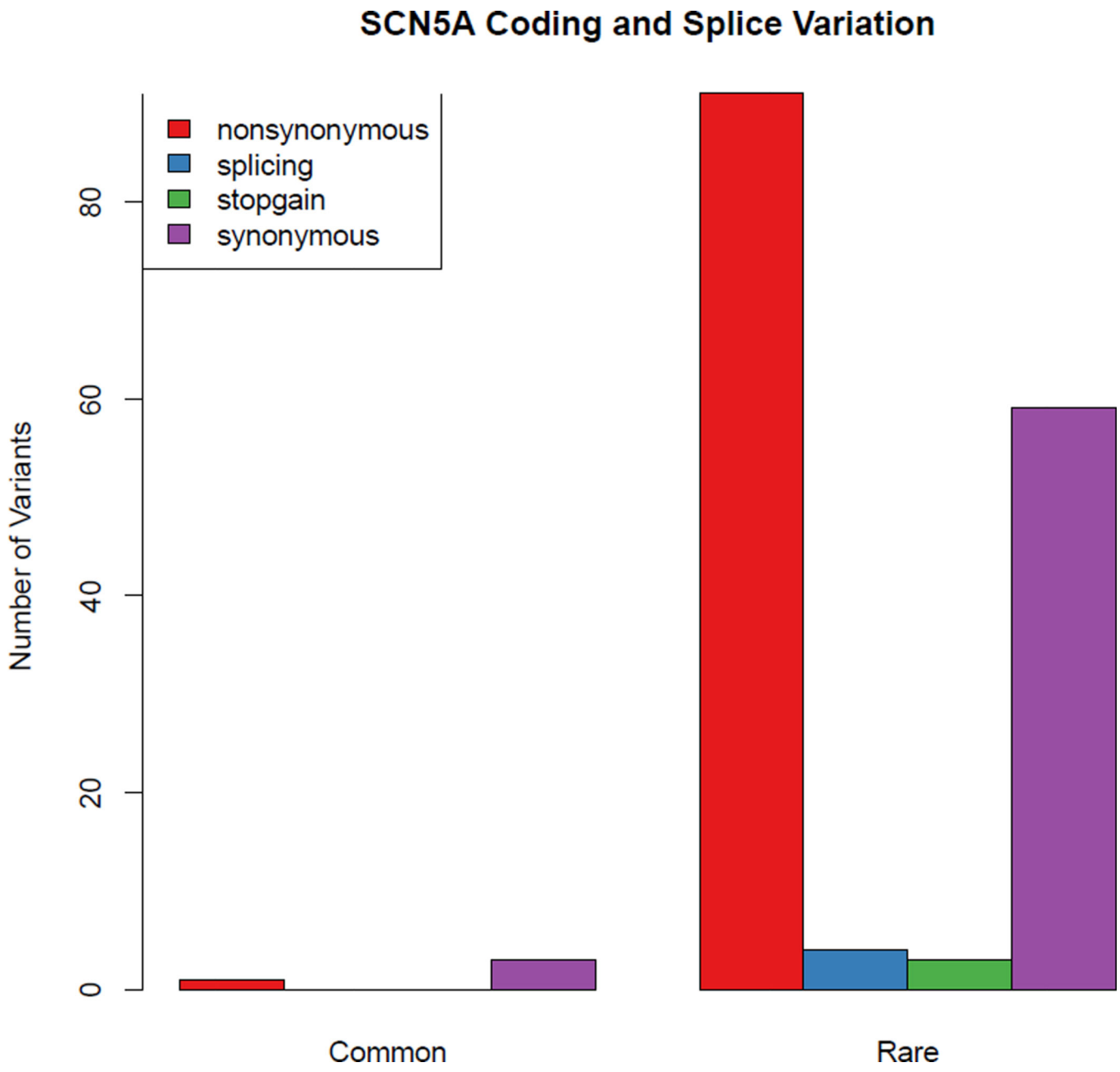

Figure 1.
Distribution of SCN5A variants by function, stratified by common or rare (<1%) minor allele frequency.

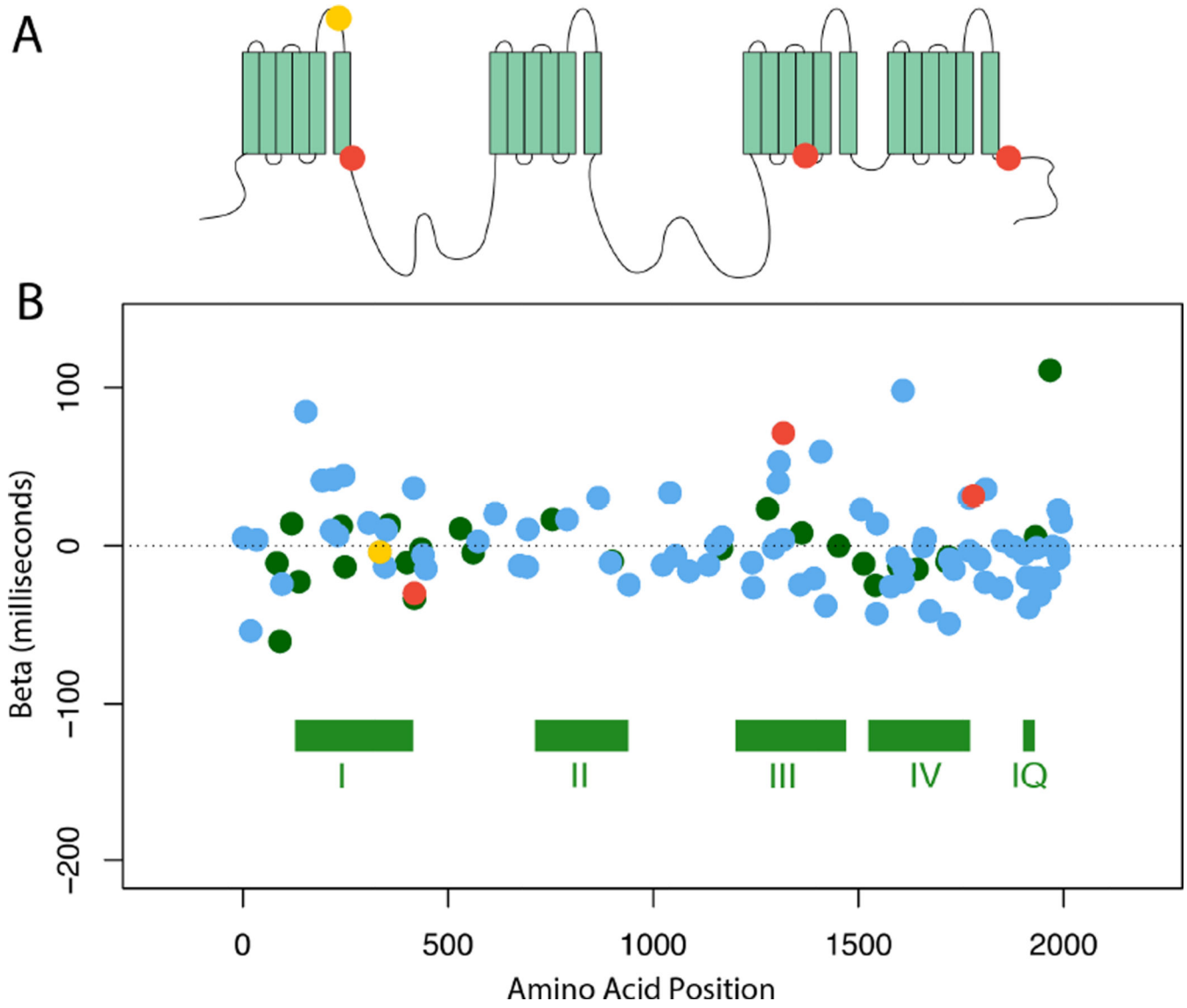

Figure 2.

Panel A is 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. Panel B shows the effect estimates (ms) associated with each rare variant on the y-axis and the amino acid position is on the x-axis. Numerals I-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).

Table 1

Phenotypic characteristics of the study samples.

	CHARGE			Extension Cohorts		African American
	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 (%).

CHARGE indicates Cohorts for Heart and Aging Research in Genetic Epidemiology Targeted Sequencing Study; ARIC, Atherosclerosis Risk in Communities Study; CHS, Cardiovascular Health Study; FHS, Framingham Heart Study; ESP, Exome Sequencing Project; EA, European ancestry; AA, African Ancestry; BMI, body mass index; SBP, systolic blood pressure.

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

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***	**CAF**	**Effect (SE)†**	***P***	**CAF**	**Effect (SE)†**	***P***	**Effect (SE)†**	***P***	***Hetero-geneity P***
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

CHARGE indicates Cohorts for Heart and Aging Research in Genomic Epidemiology; ESP, Exome Sequencing Project; rsID, reference SNP; A1 reference allele; A2 coded allele; nonysyn, nonsynonymous; syn, synonymous; CAF, coded allele frequency; SE, standard error; *P*, *P*-value.

*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***	**CAF**	**Effect† (SE)**	***P***	**CAF**	**Effect† (SE)**	***P***	**Effect† (SE)**	***P***	***Hetero-geneity P***
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)	.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

CHARGE indicates Cohorts for Heart and Aging Research in Genomic Epidemiology; ESP, Exome Sequencing Project; rsID, reference SNP; A1 reference allele; A2 coded allele; nonysyn, nonsynonymous; syn, synonymous; CAF, coded allele frequency; SE, standard error; P, P-value.

*Amino acid positions relative to NM_000335.4;

†Effect size measured in milliseconds.

Table 5

Summary of linkage disequilibrium between *SCN5A* SNPs and those identified in prior PR and QRS interval genome-wide association studies.

	rs10865879 (PR & 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 are pairwise and reported as R^2, determined using 1000 Genomes project Pilot 1 data.

Table 6

Common coding (MAF≥1%) variants identified in the (n=972) ESP African American sample and their association with the PR and QRS interval.

					PR		QRS	
rsID	**A1/A2**	**Function**	**Amino Acid***	**CAF**	**Effect (SE)†**	***P***	**Effect (SE)†**	***P***
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

ESP indicates Exome Sequencing Project; rsID, reference SNP; A1, reference allele; A2, coded allele; nonsyn, nonsynonymous; syn, synonymous; CAF, coded allele frequency; SE, standard error; *P*, *P*-value.

*Amino acid positions relative to NM_000335.4;

†Effect size measured in milliseconds.