Supplemental Material

Supplemental Methods:

CHARGE-TSS Sample Selection. The Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) Consortium is a consortium of population- and community-based cohorts designed to facilitate studies of genetic epidemiology.[1] The CHARGE-TSS is a case-cohort study design examining the relation of 14 phenotypic traits to genomic sequencing. Loci were identified by GWAS conducted by the CHARGE consortium and in the present targeted sequence study, CHARGE served as the discovery cohort. CHARGE-TSS included a random sample, stratified by sex, of at least 1000 Atherosclerosis Risk in Communities (ARIC) Study, 500 Cardiovascular Health Study (CHS), and 500 Framingham Heart Study (FHS) participants who were of European ancestry. In each cohort, investigators selected an additional sample of individuals from the extremes of the phenotype distribution of several cardio-metabolic traits. Two of those extreme sample groups were selected on the basis of PR or 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 likewise chosen at the upper tail of the distribution of the phenotype residuals. Individuals with a QRS interval > 120ms were excluded from selection. All residuals were created separately by gender and an even number of men and women were selected. In total, 200 participants from the extreme distributions of each the PR and QRS intervals (ARIC N=100, CHS N=50 and FHS N=50) were selected. All ECG parameter values were derived from automatic calculations on individual ECGs. Cohort participants were ineligible for case selection if they met any of the following exclusion criteria: non-white race; no available PR or QRS measurement; prevalent atrial fibrillation; history of myocardial infarction or heart failure; history of pacemaker implantation; and use of Class I or III antiarrhythmics. In total 3699 participants were used in this analysis, including individuals selected on the basis of extreme PR, QRS, or other cardio-metabolic traits and a cohort random sample.

Loci for targeted sequencing were identified by GWAS conducted by the CHARGE consortium for a number of phenotypes. The present analysis focuses on targeted sequencing of regions of the *SCN10A* gene

for PR and QRS intervals. Sequencing was performed at a single core facility (Human Genome Sequencing Center at Baylor College of Medicine, Houston, Texas). Sequencing was conducted on the SOLiD platform and read alignment by employing the BFAST algorithm.[1] Data were merged in order to yield a comprehensive read for each sample. Sample reads were converted into pileup data files using SAMtools.[2] After filtering, variants were annotated for function by ANNOVAR.[3]

Replication and Haplotype Study Sample: Exome Sequencing Project

The NHLBI Exome Sequencing Project (ESP), a parallel exome sequencing consortium, served as the replication cohort. ESP participants are of European or African ancestry and selected from cohort and case-control studies in order to examine genomic associations of heart, lung and blood disease.[4] Participants in ESP were categorized by extremes of quantitative phenotypes (low-density lipoprotein cholesterol, blood pressure, body mass index (BMI)) and three disease endpoints (ischemic stroke, chronic obstructive pulmonary disease and early onset myocardial infarction). In addition, the participating cohort studies contributed a large, carefully phenotyped reference group for sequencing. ESP participants were excluded from this analysis if they did not have measured PR or QRS intervals, had a history of pacemaker or defibrillator implantation, or had prevalent AF. Studies with genotyping ESP and with PR and QRS interval comprised five large US based cohorts: two biracial cohorts of European and African-descent individuals (ARIC, CHS), one cohort of predominantly African-Americans (JHS), one cohort of predominantly European-descent individuals (FHS), and one multi-ethnic cohort (Multi-Ethnic Study of Atherosclerosis (MESA)); as well as the Women's Health Initiative (WHI), a multi-ethnic US population-based study of post-menopausal women. Because ARIC, CHS, and FHS contributed to ESP, participants of European ancestry from these cohorts were excluded from the ESP association analysis described here in order to preclude participation of the same individual in both the discovery and replication cohorts. In total, our replication sample (ESP-Replication) included 607 ESP participants of European ancestry and 972 African American participants. Haplotypes were derived from all

ESP participants, including those from ARIC, CHS and FHS, thus increasing the ESP-Haplotype sample to 4,306 participants of European ancestry and 972 African American participants.

Whole exome sequencing was performed at two genome centers using Illumina GAII or HiSeq2000 sequencers. All samples were jointly processed for variant detection and quality control. Sequencing and quality control of the ESP exomes has been described in detail elsewhere.[5,6]

Extension Study Sample: CHARGE Exome chip cohorts

To increase the sample size, we examined genotype data from the entire ARIC, CHS and FHS cohorts. CHARGE cohort studies were genotyped using the Illumina HumanExome v1.0 array. Genotypes from the CHARGE cohorts were jointly called and underwent extensive quality control at the University of Texas Health Science Center at Houston.[7] Cohort participants were excluded from analyses using the same criteria as the sequence analyses: non-white race; no available PR or QRS measurement; prevalent atrial fibrillation; history of myocardial infarction or heart failure; history of pacemaker implantation; and use of Class I or III antiarrhythmics. Participants with a QRS interval >120ms were excluded from the QRS interval analyses. A total of 20,666 European ancestry participants (CHARGE-Exome sample) were analyzed.

Statistical analysis of common and rare variants

We categorized allelic variation into two classes: rare (< 1.0% minor allele frequency [MAF]) or common (≥1% MAF). Common variants were examined individually using linear regression except in FHS, where a linear mixed effect models was used to account for familial structure. Analyses for both PR and QRS intervals were adjusted for age, sex, height, BMI, and study-specific population variables. In order to obtain unbiased population effect estimates in the discovery cohorts, analyses weighted by the sampling probabilities were also conducted. We performed fixed-effect inverse variance weighted meta-analysis of study-specific association results to combine results. For our most significant gene-wide signal, we used a conservative significance threshold of $p < 5x10^{-6}$. For missense variant analyses, we used a Bonferroni correction for the number of tests (2 phenotypes, PR, QRS, and 7 SNPs), 0.05/14. Secondarily, we ran multivariable analyses in

ESP for each of the seven missense *SCN10A* variants adjusting for all common missense variants in the adjacent sodium channel genes, *SCN5A* (rs1805124) and *SCN11A* (rs72869687, rs33985936, rs13059805) to determine independence of signal.

Rare variation in the region was jointly analyzed using the Sequence Kernel Association Test (SKAT), which was adapted for a meta-analysis framework.[8,9] Each study provided single variant z-statistics from score tests as well as genotype covariance matrices. The score statistics were meta-analyzed and combined into a weighted sum, where the weights upweight variants with lower MAFs. The non-standard sampling distribution for the test statistic is estimated using the eigenvalues of a sample-size weighted average of the per-study genotype covariance matrices. For replication of the rare variant test in ESP, the SKAT software provided by the authors of the method was used.[9] All SKAT tests were likewise adjusted for age, sex, height, BMI and study specific population variables. All analyses were performed by the R software packages (www.r-project.org/).

Haplotype Analysis

Genotypes of both European and African descent populations from ESP were phased separately by race using PHASE.[10] PHASE estimates of haplotype frequency were reported. Since each individual is diploid, two best estimated haplotypes are reported for each participant. Mean PR intervals per haplotype were estimated by averaging the PR values for each person with the haplotype. Individuals that were homozygous for a haplotype would contribute a PR value for each of their haplotypes.

Haplotype association testing was performed with HAPSTAT using an additive model, which implies that having two copies of a causal haplotype has twice the effect on PR as compared to having a single copy. The wild-type (WT) haplotype was used as the reference group.[11,12] The same exclusions and adjustments as used in the single SNP genotype association testing were applied. Individuals of both races are included in the association analyses and the analyses were adjusted for race.

Methods for Gene Expression Analysis

We performed an expression quantitative trait loci (eQTL) analysis using the most significant 5 SNPs at the *SCN10A* locus associated with PR or QRS intervals in this study or in prior GWAS efforts of PR or QRS intervals (rs10428132, rs6795970, rs6801957, rs6800541, rs6599250). Given that these variants are in high linkage disequilibrium (LD), we used a p-value threshold of 0.05 to assess significance. We examined eQTL associations from three sources: Cleveland Clinic study (left atrial appendage), University of Pennsylvania study (right and left atrium and left ventricle),[13] and Massachusetts General Hospital (left atrial appendage). Through publically available eQTL datasets, we secondarily examined eQTL for a variety of other tissue types, many of which are not known to express either *SCN5A* or *SCN10A*.

Cleveland Clinic study

In the Cleveland Clinic study, human left atrial appendage tissue was obtained with consent from 230 European-American patients undergoing cardiac surgery. Use of discarded surgical tissue was approved by the Institutional Review Board of the Cleveland Clinic. Total RNA was extracted using Trizol. Genome-wide RNA levels were measured using Illumina HT12 v.3 microarrays. RNA expression levels were background corrected, log2-transformed, quantile-normalized and batch adjusted. These subjects were genotyped using Illumina Hap550 and Hap650 arrays.

University of Pennsylvania

In the Penn study, right (n=52) and left atrial tissue (n=53) was obtained with consent from subjects undergoing heart transplantation or from unused organ donors. Cold cardioplegia was used prior to cardiac explantation and the lateral walls of the atria were dissection and frozen. Total RNA was extracted using Trizol and Genome-wide RNA levels were measured using Affymetrix HU 133A arrays. Genotypes were

obtained from Affymetrix 6.0 platform and all subjects were genetic European Americans by MDS analysis of genotypes. Data were processed and analyzed as described above for the Cleveland Clinic samples.

Samples of cardiac tissue were acquired from individuals in the Myocardial Applied Genomics Network. Left ventricular free-wall tissue was collected at the time of cardiac surgery from subjects with heart failure undergoing transplantation or from unused donor hearts. DNA samples were genotyped using the Affymetrix 6.0 genome-wide array, and RNA expression was measured using the Affymetrix Genechip ST1.2 array. Imputation to SNP genotypes in the 1000 Genomes Project was performed. Analyses were restricted to 313 samples with genetically inferred European ancestry. SNP genotype was tested for association with log2-transformed expression levels, after adjustment for age, sex, study site, disease status and batch.

Massachusetts General Hospital

A total of 121 human left atrial samples (LA) were obtained during cardiac surgery for valvular heart disease (n=83) or cardiac transplantation (n=27) at Massachusetts General Hospital (MGH). Normal LA tissue was also obtained from the National Disease Research Interchange repository (n=11). The sample collection was approved by the Institutional Review Board at Massachusetts General Hospital.

For DNA extraction, the tissues were digested in 200ul DirectPCR solution (VIAGEN Biotech Inc, Los Angeles, CA) containing about 80 ug of Proteinase K followed by phenol: chloroform: isoamyl alcohol extraction and ethanol precipitation. Genotype at rs6800541 was determined using custom designed TaqMan probe (Life Technologies, Carsbad, CA). DNA amplification and genotype analysis was done using iTaq Universal Probes Supermix (Bio-Rad) on CFX384 Real-Time System.

For RNA extraction, the tissues were homogenized in 1 ml of TRIZOL® Reagent (Life Technologies, Carlsbad, CA). After addition of chloroform and centrifugation aqueous phase was then collected, and mixed with an equal amount of 100% ethanol and purified on the RNeasy column according manufacturer's instructions. The reverse transcription reaction was performed using iScript kit (Bio-Rad Laboratories,

Hercules, CA) according to manufacturer's instructions. We then performed qPCR using iTaq SYBR-green reagent (Bio-Rad) on CFX384 Real-Time System. Primer pairs were validated with standard curve and single peak on the melting curve, primer efficiency assessed with serial dilutions of the sample. Conditions for qPCR were 50°C for 2 min, 95°C for 10 min, 95°C for 15 sec, 60°C for 1 min (last two steps was repeated for 40 times) followed by the melting curve assessment. Cq values were exported and relative expression compared to the housekeeping genes, TBP and HPRT, was calculated. The primer sequences are listed in **Supplemental Table 5**.

Human *SCN10A* clone, mutagenesis and expression

The human *SCN10A* alpha subunit cDNA built-in a pCMV6-XL5 vector was purchased from OriGene. The *SCN10A* DII-DIII linker variants/haplotypes (IV, PT, VA, LP, VA+LP, IV+VA and IV+VA+LP) were generated using the QuikChange lightning (Agilent) PCR mutagenesis kit using appropriately designed mutagenic primers. *SCN10A* wild type and variant/haplotype cDNA clones were validated by sequencing. The *SCN10A* constructs were expressed in a Neuroblastoma 2a (N2a) cell line derived from mouse (ATCC, catalog number CCL-131). N2a cells were used because these neuronal cells expressed functional ion channels when transfected with SCN10A constructs. The N2a cells were maintained in Eagle's Minimum Essential Medium with 10% fetal bovine serum (ATCC) and 5% penicillin/streptomycin at 37°C with 5% CO_2 and 95% O2. The N2a cells were seeded on cover slips in 35mm culture dishes and transiently transfected with 1 µg *SCN10A* (wild type or variant) and 0.6 µg GFP using Fugene 6 (Promega) according to manufacturer instructions. GFP positive cells were identified 48 hours after transfection and were used for electrophysiology studies.

Patch Clamp Electrophysiology and Data Analysis

Wild type and variant/haplotype Nav1.8 currents were measured using the whole-cell patch clamp recording technique at room temperature. Borosilicate patch pipettes were pulled using P1000 (Sutter) with tip resistances ranging from 2-4 MΩ when filled with internal solution containing (in mM), 130 CsCl, 10 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 11 EGTA, 5 MgATP adjusted to pH 7.2 with CsOH. The N2a cells were continuously

perfused with an external solution containing (in mM), 130 NaCl, 5 CsCl, 2 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, 5 glucose and adjusted to pH 7.4 with CsOH. Tetrodotoxin (TTX, 150 nM) was used to block endogenous sodium currents. In non-transfected N2a cells, the above solutions with TTX abolished all endogenous currents. In the whole cell configuration, TTX was perfused onto cells for four minutes and recordings were made after 5 minutes. The current recordings were acquired with the Axopatch 200B amplifier and filtered at 5 kHz using online P/4 protocol to subtract linear leak along with 80% series resistance and whole-cell capacitance compensation. Data were acquired and analyzed using pClamp9 and SigmaPlot 12.

The steady-state voltage dependent activation curves were determined from a holding potential of -100 mV and stepping to a range of depolarizing pulses from -70 mV to +60 mV for 50 ms in 10 mV increments. Conductance-voltage relationships were plotted using the peak current with the equation: $G_{Na} = I_{Na}/(V-E_{Na})$, and then fitted with a Boltzmann equation (G_{NA}/G_{NAmax}= min + max/(1 + exp-$(V-V_{1/2}/k)$). Steady-state inactivation curves were determined from a holding current of -100 mV, stepping to a range of depolarizing pre-pulses from -100 mV to -10 mV for 500 ms and then using a single test pulse at +20 mV for 50 ms to measure current availability. Currents elicited were normalized to maximum peak current at -100 mV and fit with a Boltzmann equation. The late sodium current was determined from a holding current of -100 mV and then pulsing to +20 mV for 475 ms. The percent late sodium current was measured by dividing the steady-state inactivated current at the end of the 475 ms pulse by the peak current at +20 mV. Recovery from inactivation was determined by an initial test pulse to +20 mV for 475 ms, then stepping to -100 mV for a variable duration ranging from 0.5 ms to 1 s followed by a final test pulse to +20 mV. The recovery from inactivation was fit with a double exponential. Open state inactivation was determined for voltages from -10 mV to +40 mV by fitting the currents with a single exponential .The data are presented as mean ± s.e. and a student's t-test was used to determine a significant difference (P<0.05).

Immunohistochemistry

Explanted human hearts that were not used for transplant were obtained from Lifeline of Ohio in accordance with The Ohio State University Institutional Review Board, as published previously.[14] Explanted

hearts were cardioplegically arrested and cooled to 4^0C from the time of aortic cross-clamp in the operating room and during transport and dissection. Right atrial tissue was isolated and then flash frozen in Optimal Cutting Temperature (OCT) media, and stored at -80^0C until use. Tissue from three different specimens were used in this study: 921821, 394176, and 219852.

Samples were cryosectioned and Masson trichrome staining was performed (Sigma) for morphology and localization. Adjacent sections were selected, thawed to room temperature, and fixed in 4% paraformaldehyde for 5 minutes. Sections were then washed in PBS 3 times for 5 minutes each. Following fixation, sections were permeabilized with 0.1% Triton X-100 for 15 minutes, washed 3 times with PBS for 5 minutes each, then blocked with 10% normal goat serum for 30 minutes. Tissue sections were then incubated with the primary antibody overnight at 4^0C (anti-Nav 1.8, Abcam AB66743, at a concentration of 1:200 and anti-Cx43, Invitrogen 35-5000, concentration 1:200). The following morning, the samples were washed with PBS 3 times for 5 minutes each, then incubated with the secondary antibody (anti-rabbit AlexaFluor 546 1:500 and anti-mouse AlexaFluor 488 1:500), for 2 hours at room temperature while protected from light, followed by 5 PBS washes for 5 minutes each, then mounted with Vectashield mounting medium containing DAPI.

Immunocytochemistry

To verify the SCN 10A antibody efficacy and specificity for SCN 10A over SCN 5A, N2A cells were co-transfected with GFP and either the WT SCN10A construct or SCN 5A constructs as described above. After 48 hours of incubation, coverslips of transfected N2A cells were fixed in 4% formaldehyde for 15 minutes and then washed twice in PBS. The coverslips were then blocked with 5% nonfat dry milk in 0.1% Triton X-100 for 2 hours at room temperature on a rotator. Coverslips were then washed twice with PBS and incubated overnight with the primary antibody (anti-Nav 1.8, Abcam AB 66743, concentration 1:200, or anti-Nav1.5, Alomone ASC-005, 1:200) in PBS with 1% bovine serum albumin. The following day, coverslips were washed with PBS 3 times, then incubated with secondary antibody (anti-rabbit AlexaFluor 546 1:500) for 1.5 hours at

room temperature, followed by two washes of PBS witn 0.2% Tween, and then washed with PBS twice. Coverslips were then mounted slides with DAPI Pro-Gold Anti-Fade medium (Molecular Probes).

Supplemental Results for Gene Expression Analyses

Atrial tissue: We examined atrial eQTL associations from three sources: Cleveland Clinic study, University of Pennsylvania study,[2] and Massachusetts General Hospital (**Supplemental Table 5**). In the Cleveland Clinic study, we found no association between *SCN5A* mRNA expression and rs6800541 genotype in the left atrial appendage samples from 230 individuals (p=0.74, **Figure S2**). Findings were similar for the other SNPs examined, rs10428132, rs6795970, and rs6801957, which are all in high LD with rs6800541. *SCN10A* was not reliably detectable due to low expression. On the basis of both Illumina HT12 microarray analysis and RNAseq, *SCN10A* mRNA is much less abundant than that of *SCN5A* in human left atrial appendage samples from Cleveland. Median (log_2scale) abundance of *SCN10A* was -0.40, while that of *SCN5A* was 4.41, as detected with the Illumina_1694956 probe. Similarly, there was ~1000x greater abundance of *SCN5A* than *SCN10A* using RNAseq. In the University of Pennsylvania study, we found no evidence of an association between SCN5A mRNA expression and rs6800541 genotype in right (n=52) or left (n=53) atrial tissue samples (p>0.05 for both).[2] In the MGH study, we found no association between rs6800541 genotype and *SCN5A* (p=0.71) or *SCN10A* (p=0.14) expression in left atrial tissue samples from 121 individuals (**Supplemental Figure 2**).

Ventricular tissue: In the Penn Study, there was no evidence of an association between *SCN5A* mRNA expression and rs10428132 genotype in the left ventricular samples from 313 individuals (p>0.05). There was a trend towards an association between SCN5A expression and rs6599250 (a proxy for rs10428132 with an R2=1.0, P=0.08). We also examined the results from the Gene-Tissue Expression (GTEx) Portal and found no association between SCN5A expression in 83 left ventricular samples and the following SNPs: rs10428132, rs6795970, rs6801957, rs6800541, rs6599250 (http://www.gtexportal.org/home/ accessed 1/30/2017).

Figure S1

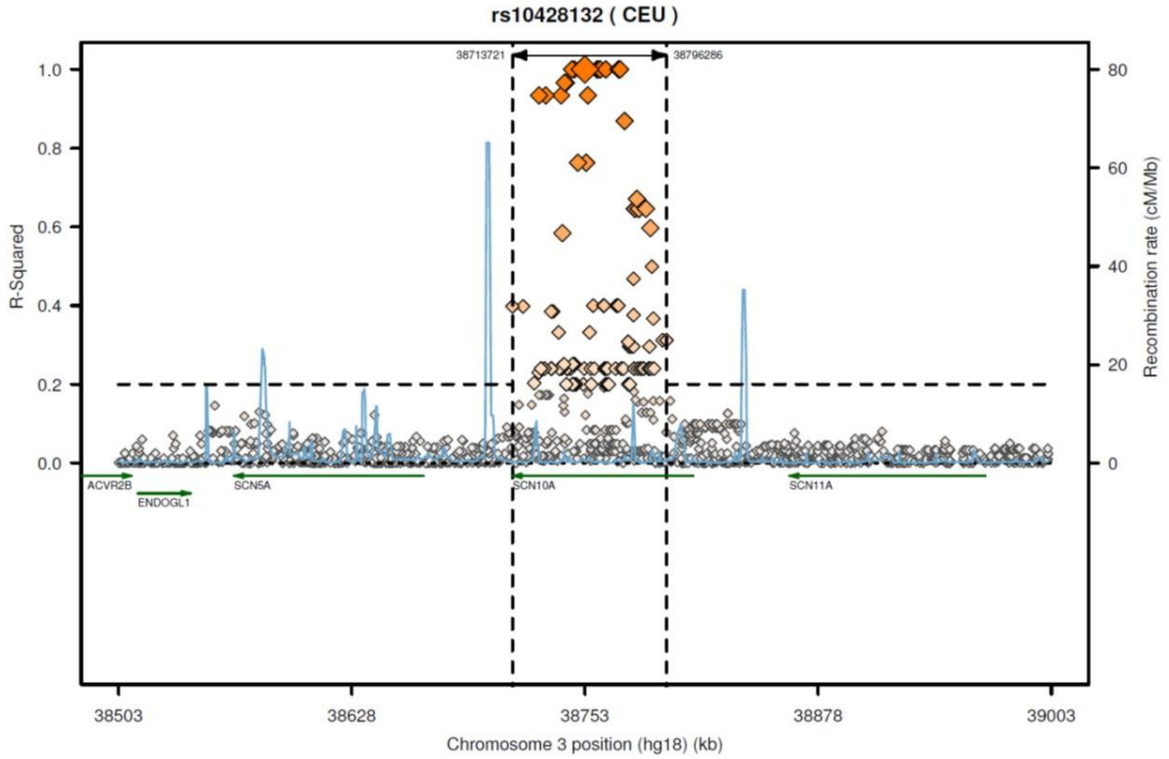

Figure S1. LD plot of top *SCN10A* variant associated with PR interval. Linkage Disequilibrium (LD) association plot of the top *SCN10A* single nucleotide polymorphism, rs10428132, identified from the *SCN10A* sequencing data associated with PR interval. The plot shows that only common variants in *SCN10A* are in LD with rs10428132. All other common variants in neighboring loci, in particular, *SCN5A* and *SCN11A* are not associated with the top *SCN10A* variant, rs10428132.

Figure S2

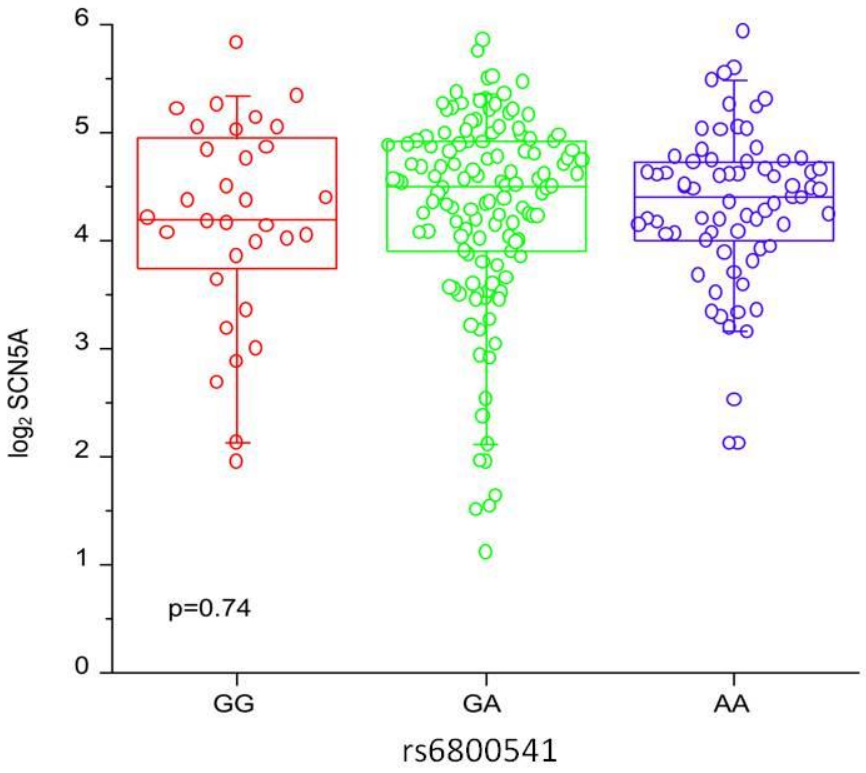

Figure S2. eQTL Analysis. Box-whisker plots show an eQTL analysis of left atrial appendage *SCN5A* and *SCN10A* mRNA expression vs. rs6800541 genotype in a sample of 230 individuals of European ancestry from the Cleveland Clinic. Findings were similar for the other SNPs examined, rs10428132, rs6795970, and rs6801957, which are all in high LD with rs6800541. No association was seen between genotype and *SCN5A* expression. *SCN10A* was not reliably detectable due to low expression. The mRNA was quantified using Illumina HT12 v.3 expression arrays. Expression values were batch corrected, natural log-transformed, baseline corrected and quantile normalized. The lower and upper bounds of the box represent the 25th and 75th percentiles, with the mid-line showing the median value. The whiskers are plotted at the 5th and 95th percentile values. Gene expression was assessed by ANOVA and significance was determined using an F-statistic.

Figure S3

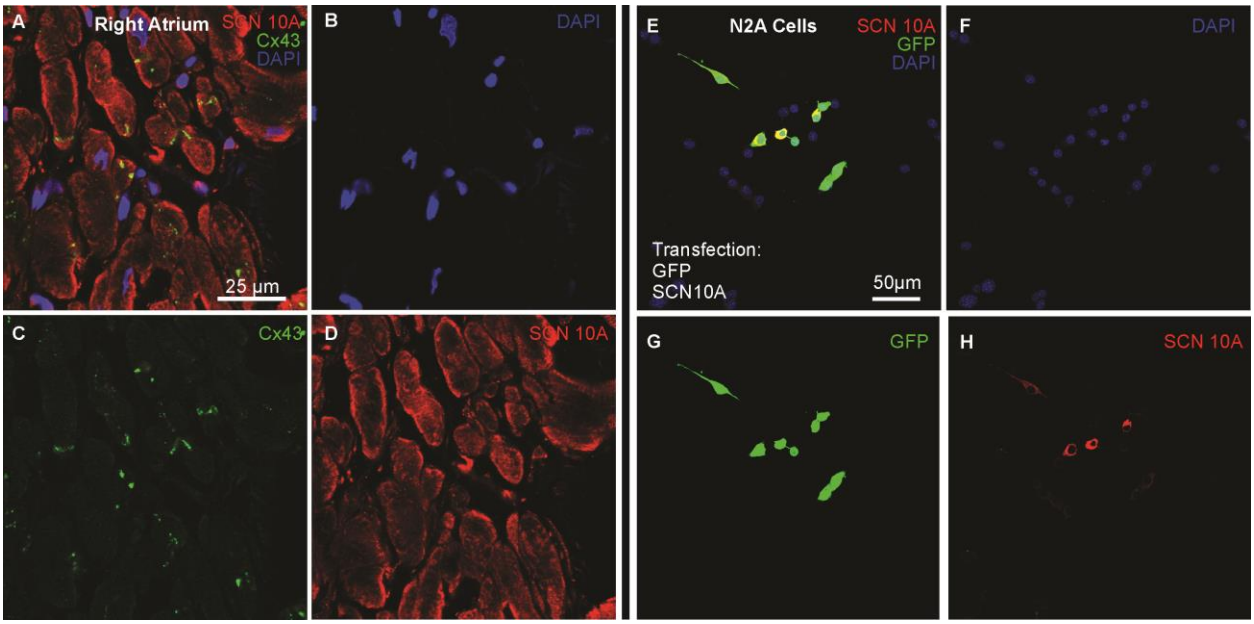

Figure S3. ***SCN10A*** **expression in human right atrium. Panels A-D:** Immunohistochemistry of right atrial tissue stained with anti-*SCN10A*, anti-Cx43, and DAPI demonstrates *SCN10A* expression in myocytes, with increased expression noted in the membrane at cell edges. **Panels E-F:** Verification of anti-*SCN10A* antibody efficacy in N2A cells co-transfected with separate constructs for GFP and WT *SCN10A*. GFP transfection reflects the transfection efficiency in N2A cells and confirms that transfection was successful in some cells. Intense red fluorescence from the *SCN10A* antibody in a similar cell subset confirms expression of *SCN10A* expression in these cells and the *SCN10A* antibody efficacy.

Figure S4

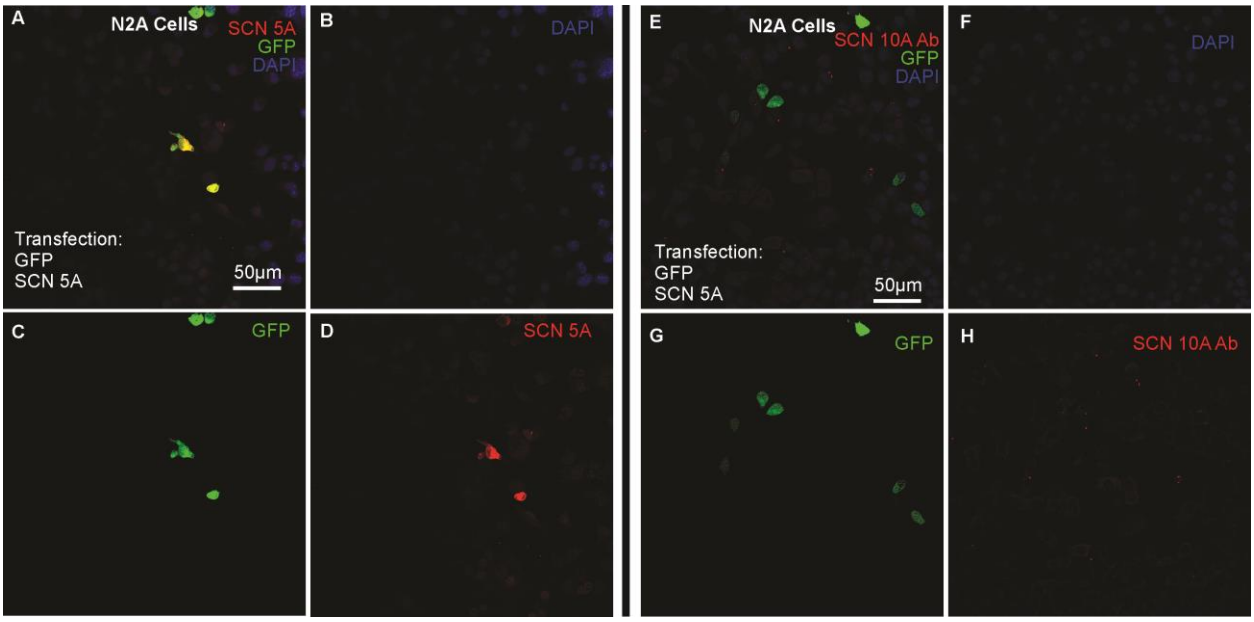

Figure S4: Confirming *SCN10A* antibody specificity. Panels A-D: N2A cells were co-transfected with separate GFP and *SCN5A* constructs. GFP expression confirms that transfection was successful, and intense red fluorescence in a subset of these cells confirms *SCN5A* expression. **Panels E-H:** N2A cells co-transfected with separate constructs of GFP and *SCN5A*, similar to Panels A-D. These cells were then treated with the *SCN10A* antibody. Lack of red fluorescence suggests that there is no appreciable binding of the *SCN10A* antibody to *SCN5A*.

Figure S5

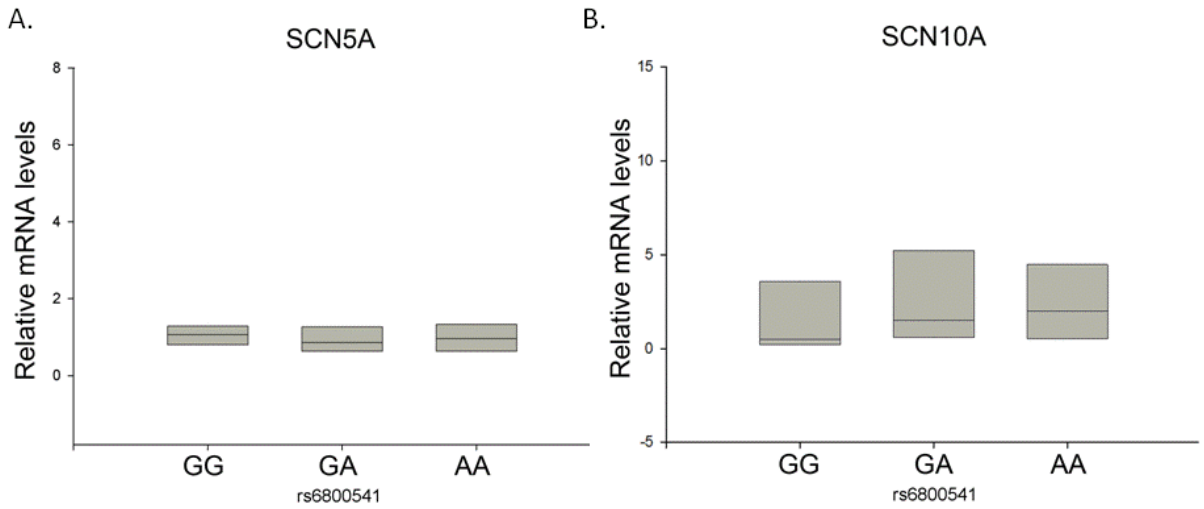

Figure S5. *SCN5A* and *SCN10A* expression analysis associated with rs6800541 from left atrial appendage. Box plots show a quantitative PCR analysis from left atrial appendage of (**A**) *SCN5A* and (**B**) *SCN10A* expression versus rs6800541, from 121 individuals of European Ancestry from MGH. There was no association between genotype and *SCN5A* (p=0.71) or *SCN10A* (p=0.14) expression.

Figure S6

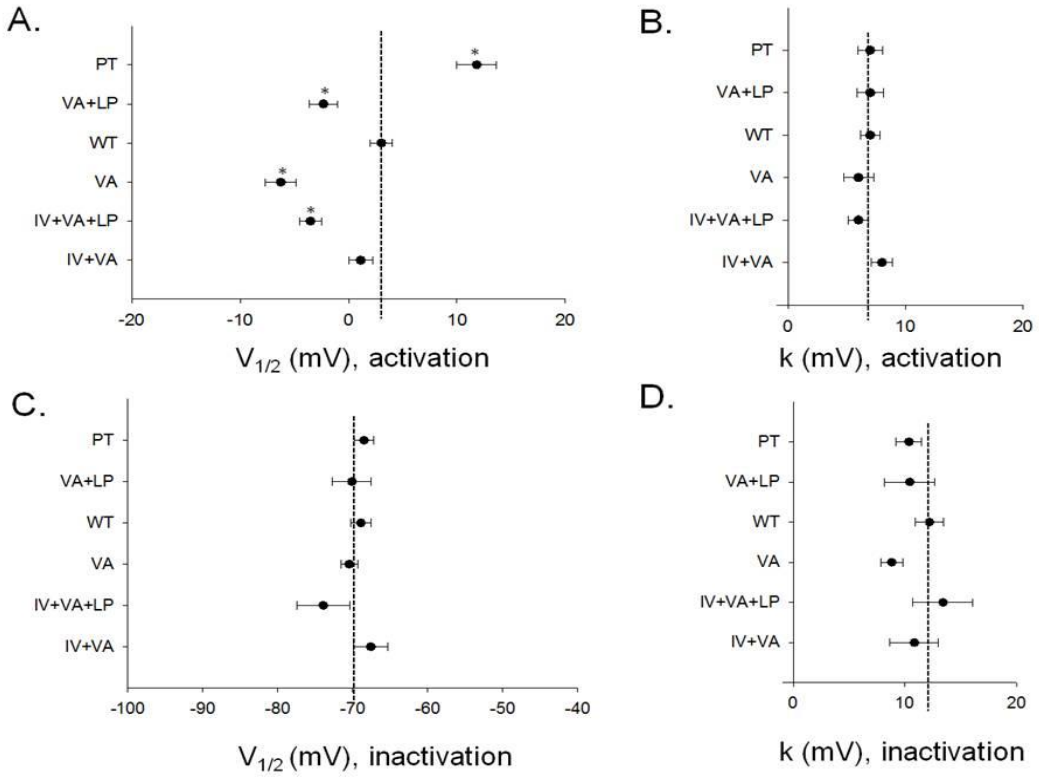

Figure S6. Summary plots of activation and inactivation parameters of Nav1.8 haplotype channels. A, B) Summary plots of **channel activation** properties for $V_{1/2}$ and slope (k) for each haplotype generated from the fitted curves shown in figure 2B (see methods). The haplotypes are vertically plotted on the y-axis by their PR interval with shortest on the bottom and longest at the top. The $V_{1/2}$ of channel activation had a modest relationship with haplotype. The $V_{1/2}$ for PT was significantly left shifted and VA, VA+LP, and IV+VA+LP were both right shifted compared to wild type (WT). IV+VA was not significantly different from WT. The slopes for the haplotype variants were not significantly different from WT. **C, D)** Summary plots of channel inactivation properties for $V_{1/2}$ and slope (k) for each haplotype generated from the fitted curves shown in figure 3C (see methods). The $V_{1/2}$ and slope for the haplotype variants were not significantly different from WT. The asterisks represent a significant difference compared to WT ($p < 0.05$).

Figure S7

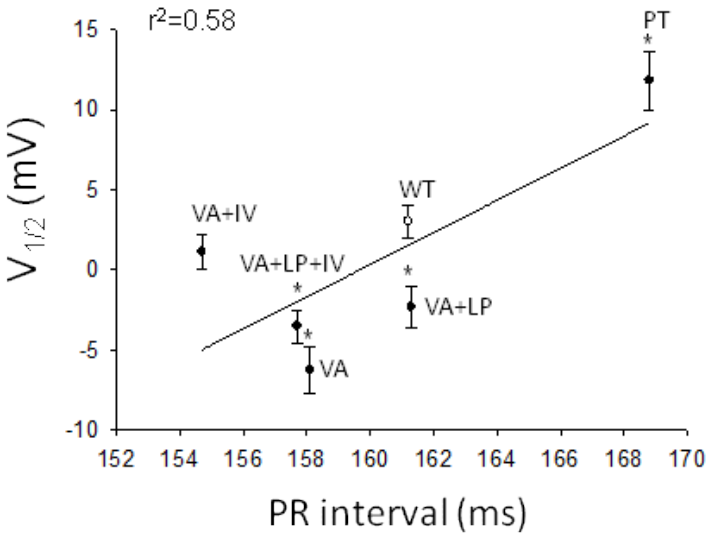

Figure S7. Correlation plot for midpoint of activation versus haplotype. The midpoint of activation ($V_{1/2}$) for each haplotype is plotted against PR interval duration. The data were fitted with a straight line which gave a $r^2 = 0.58$, *P* = 0.08. The asterisks represent a significant difference from wild type (t-test, $p < 0.05$)

Figure S8

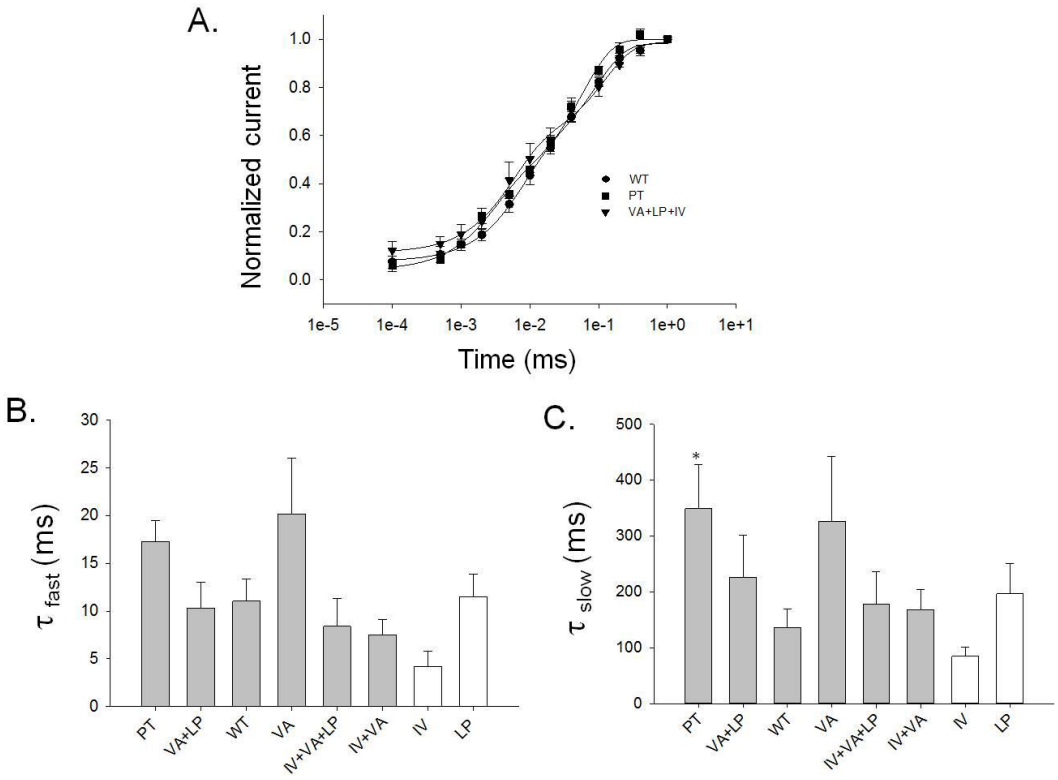

Figure S8. Effect of haplotype and variant channels on the recovery of inactivation. **A)** Plot of recovery from inactivation for wild type (WT, circles), PT (squares) and IV+VA+LP (inverted triangles), x-axis is interpulse duration at -100 mV (log scale). The smooth lines represent fits with a double exponential function. **B, C)** Plots of fast and slow time constants, respectively, for the all haplotypes (grey bars) and the two single variants which did not occur in isolation in the study populations (white bars). The slow time constant for PT was significantly different from WT (*P*= 0.03). The slow and fast time constants for the other haplotypes and variants were not significantly different from wild type (p>0.05).

Figure S9

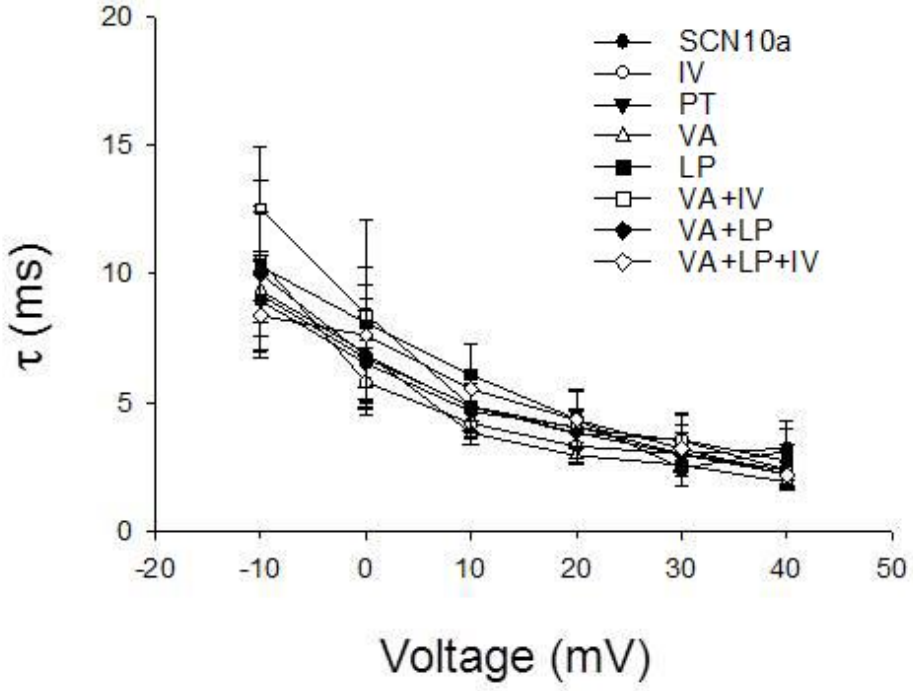

Figure S9. No difference in the rate of open state inactivation between haplotype and variant channels. Plots of the time course of open state inactivation, time constant versus test voltage (-10, 0, 10, 20, 30, 40 mV), for wild type and haplotype variants (filled symbols), and the two single variants that did not occur in isolation in the study population (open symbols). Current traces were fitted with a single exponential for each test voltage. The time course of open state inactivation for each haplotype and the two variants were not significantly different from wild type ($p>0.05$).

Table S1. Cohort Characteristics.

	CHARGE Targeted Sequencing			Exome Sequencing Project			CHARGE ExomeChip Genotyping		
Study	**ARIC**	**CHS**	**FHS**	**WHI**	**MESA**	**African Americans**	**ARIC**	**CHS**	**FHS**
Number	1645	1021	1033	525	82	972	9828	3256	7582
Ancestry	EA	EA	EA	EA	EA	AA	EA	EA	EA
Sex, men (%)	50.6	45.8	47.5	0	75.6	29.4	46.1	40.4	45.2
Age, years	54.5 (5.7)	72.2 (5.3)	38.2 (9.5)	63.9 (7.9)	60.7 (9.6)	58.3 (8.8)	54.1 (5.7)	72.4 (5.4)	39.3 (9.8)
PR interval, ms	164 (28)	171 (32)	153 (23)	160 (25)	163 (23)	168 (24)	160 (23)	167 (28)	152 (22)
QRS interval, ms	92 (10)	90 (12)	89 (10)	88 (13)	95 (12)	91 (15)	91 (9)	88 (10)	87 (9)
RR interval, ms	924 (138)	951 (159)	843 (159)	160 (25)	163 (23)	910 (159)	928 (136)	955 (151)	920 (171)
Body mass index, kg/m^2	27.4 (5.8)	26.8 (5.2)	26.4 (6.3)	29.0 (5.8)	28.1 (4.3)	32.5 (9.0)	26.9 (4.7)	26.7 (4.4)	26.0 (5.0)
Systolic blood pressure, mm Hg	120 (19)	136 (23)	121 (17)	133 (23)	127 (23)	133 (22.5)	118 (17)	135 (21)	119 (15)

Mean (standard deviation) unless otherwise indicated.
Abbreviations: EA, European American; AA, African American

Table S2. Complete list of variants identified at the *SCN10A* locus.

Please see the attached excel file entitled “Table S2.xlsx”. In worksheet “Coding” a list of coding variants identified in *SCN10A* is provided. Variants are categorized according to functional effect and PolyPhen-2, SIFT, Phylop and LRT predictions are provided. In the worksheet “Non-coding”, a list of non-coding variants ordered by position is provided.

Table S3. Coding variation summary

A.

	Common	Rare
nonsynonymous	7	121
splicing;nonsynonymous	0	4
splicing	0	2
stopgain	0	6
synonymous	10	54

B.

		P-value
Synonymous/ Nonsynonymous		4.56×10^{-02}
Missense Predictions	PolyPhen-2	1.45×10^{-02}
	LRT	7.23×10^{-03}
	PhyloP	1.29×10^{-05}
	SIFT	1.65×10^{-03}

Table S3. Coding variation summary. A) Functional class of coding variant identified in CHARGE sequencing. We identified 327 common (MAF>1%) variants in the gene, of which 7 were missense and 10 were synonymous. Of 2882 rare variants, 327 were putatively functional (missense, nonsense, or splice). Four of the variants in splice sites fell on the exon side of the splice junction and also resulted in amino acid changes. Insertion / deletions were not assessed. **B)** Nonsynonymous variants tended to have a lower minor allele frequency than synonymous variants. Rarer variants were more likely to be associated with adverse bioinformatic predictions of function and conservation.

Table S4. Common *SCN10A* Variant Associations with the PR and QRS intervals (EA=European Americans, AA=African Americans)

			CHARGE Targeted Sequencing (EA, n=3699)			Exome Sequencing Project (EA, n=607)		Exome Sequencing Project (AA, n=972)			CHARGE Exome Chip Project (EA, n=20666)		
SNP ID	**Amino acid substitution**	**A1/A2**	**Freq* (%)**	**β (SE) [msec]**	***P* value**	**β (SE) [msec]**	***P* value**	**Freq† (%)**	**β (SE) [msec]**	***P* value**	**Freq‡ (%)**	**β (SE) [msec]**	***P* value**
PR interval													
rs57326399	I962V	T/C	25.2	-2.22 (0.94)	$6.7x10^{-3}$	-2.83 (1.54)	0.07	10.2	0.01 (1.83)	0.99	25.8	-1.93 (0.25)	$9.75x10^{-15}$
rs73062575	P1045T	G/T	2.4	5.36 (2.03)	$6.8x10^{-4}$	12.69 (3.70)	$6.5x10^{-4}$	0.6	10.79 (6.98)	0.12	2.7	3.4 (0.68)	$4.87x10^{-7}$
rs6795970	V1073A	A/G	58.3	-3.94 (0.67)	$8.9x10^{-12}$	-5.13 (1.43)	$3.6x10^{-4}$	90.2	-2.14 (1.89)	0.26	60.2	-3.31 (0.22)	$2.73x10^{-49}$
rs12632942	L1092P	A/G	23.6	-2.12 (0.74)	$5.2x10^{-4}$	-2.75 (1.56)	0.08	13.9	2.06 (1.59)	0.19	26.0	-1.69 (0.25)	$1.02x10^{-11}$
rs74717885	I206M	T/C	1.3	-6.74 (3.03)	0.12	-1.19 (5.48)	0.83	0.2	NA		1.3	-1.87 (2.86)	0.51
rs7630989	S509P	A/G	2.7	-4.75 (2.00)	0.20	-2.03 (4.46)	0.65	20.9	-1.6 (1.31)	0.22	3.1	-2.23 (1.92)	0.25
rs77804526	V1697I	C/T	1.0	-5.60 (3.44)	0.80	1.40 (5.95)	0.82	0.2	NA		1.3	-1.51 (3.0)	0.61
rs6800541	PR GWAS	C/T	58.6	-4.15 (0.66)	$4.0x10^{-14}$								
rs6801957	QRS GWAS	T/C	58.3	-4.06 (0.66)	$3.0x10^{-14}$								
rs10428132	PR, This Study	T/G	57.8	-4.17 (0.66)	$1.5x10^{-14}$								
rs6599251	QRS, This Study	G/T	53.2	-3.40 (0.65)	$6.5x10^{-12}$								

QRS interval													
rs57326399	I962V	T/C	25.2	-0.63 (0.37)	0.079	-0.09 (0.59)	0.88	10.4	-0.41 (0.73)	0.58	26.0	-0.55 (0.1)	$2.4x10^{-8}$
rs73062575	P1045T	G/T	2.4	0.71 (0.80)	0.043	1.68 (1.40)	0.23	0.5	2.24 (3.01)	0.46	2.7	0.9 (0.27)	$8.1x10^{-4}$
rs6795970	V1073A	A/G	58.3	-0.89 (0.25)	$3.2x10^{-4}$	-0.16 (0.59)	0.79	90.7	0.27 (0.77)	0.72	60.3	-0.67 (0.09)	$5.7x10^{-14}$
rs12632942	L1092P	A/G	23.6	-0.60 (0.28)	0.058	0.37 (0.55)	0.5	14.1	-0.57 (0.64)	0.37	25.8	-0.44 (0.1)	$8.8x10^{-6}$
rs74717885	I206M	T/C	1.3	-2.29 (1.12)	0.31	0.12 (2.06)	0.95	0.2	NA				
rs7630989	S509P	A/G	2.7	-0.85 (0.76)	0.45	0.27 (1.71)	0.87	20.8	0.36 (0.53)	0.5			
rs77804526	V1697I	C/T	1.0	-3.49 (1.35)	0.10	-0.96 (2.23)	0.67	0.2	NA				
rs6800541	PR GWAS	C/T	58.6	-0.91 (0.25)	$4.6x10^{-4}$								
rs6801957	QRS GWAS	T/C	58.3	-0.82 (0.25)	$5.7x10^{-4}$								
rs10428132	PR This Study	T/G	57.8	-0.78 (0.25)	$2.0x10^{-3}$								
rs6599251	QRS This Study	G/T	53.2	-0.73 (0.25)	$1.2x10^{-4}$								

*Frequency in CHARGE Targeted Sequencing Study population, †Frequency in ESP African American population,‡ Frequency in Exome Chip population

Abbreviations: EA, European American; AA, African American; Freq, coded allele frequency; A1 non-coded allele, A2 coded allele

Table S5. Correlation (r^2) between SNPs previously reported to be associated with PR and QRS interval duration with each other (rs6801957 and rs6800541, respectively), with the most significant intronic variants for PR and QRS intervals reported in this manuscript (rs10428132 and rs6599251, respectively), with the four coding variants associated with cardiac conduction identified by this study (rs57326399, rs73062575, rs6795970, rs12632942), and with the 3 coding variants identified in this study but not associated with PR or QRS intervals (rs74717885, rs7630989, rs77804526)**.**

	rs10428132	rs6599251	rs6801957	rs6800541	rs57326399	rs73062575	rs6795970	rs12632942	rs74717885	rs7630989	rs77804526
	This study PR, intronic	This study QRS, intronic	Prior QRS GWAS, intronic	Prior PR GWAS, intronic	I962V	(P1045T)	(V1073A)	(L1092P)	(I206M)	(S509P)	(V1697I)
rs10428132	1	0.86	0.92	0.97	0.21	0.04	0.83	0.21	0.01	0.02	0.01
rs6599251		1	0.79	0.83	0.25	0.04	0.72	0.25	0.02	0.03	0.01
rs6801957			1	0.88	0.25	0.03	0.87	0.24	0	0.01	0
rs6800541				1	0.2	0.04	0.85	0.21	0.01	0.02	0.01
rs57326399					1	0.01	0.21	0.76	0	0.01	0
rs73062575						1	0.03	0.02	0	0	0
rs6795970							1	0.21	0	0.01	0
rs12632942								1	0	0.01	0
rs74717885									1	0.23	0.69
rs7630989										1	0.23
rs77804526											1

Table S6. Study samples and tissue sources for eQTL analyses

Study	Tissue source	Number of samples	Reference
Cleveland Clinic eQTL	Left atrial appendage (European ancestry)	230	-
	Left atrial appendage (African Americans)	34	-
Penn eQTL	Left atrium	53	-
	Right atrium	52	-
	Left ventricle	313	
MGH	Left atrial appendage	121	
Framingham Heart Study SABRe project	Whole blood	2446	
eQTL Browser and GTEx Browser:			
Dimas_fibroQTL	Fibroblasts	75	[15]
Dimas_lymphoQTL	LCLs	75	[15]
Dimas_TcellsQTL	T-cells	75	[15]
Montgomery_transcriptQTL	LCLs	60	[16]
Montgomery_exonQTL	LCLs	60	[16]
Myers_eqtl	Brain cortex	279	[17]
Pickrell_eqtl	LCLs	69	[18]
Pickrell_sqtl	LCLs	69	[18]
Schadt_eqtl	Liver	427	[19]
Stranger_eqtl	LCLs	210	[20]
Veyrieras_PP_eqtl	LCLs	210	[21]
Veytieras_PVALUE_eqtl	LCLs	210	[21]
Zeller_eqtl	Monocytes	1490	[22]
Gibbs_cerebellum	Brain Cerebellum	143	[23]
Gibbs_fontal_cortex	Brain Frontal Cortex	143	[23]
Gibbs_temporal_cortex	Brain Temporal Cortex	144	[23]
Gibbs_pons	Brain Pons	142	[23]
LCL: lymphoblastoid cell lines			

Table S7. Quantitative PCR primer pairs for detection of SCN5A and SCN10A in left atrial appendage samples from MGH.

Primer name	**Sequence**
SCN5A-forward	5’- CTGACCTCACCATCACTATGTG -3’
SCN5A-reverse	5’- GCTGTGAAAATCCCTGTGAAG -3’
SCN10A-forward	5’- TTGCTGAGGGTGAATCTGATC -3’
SCN10A-reverse	5’- CACACCTCTCGACTTGCTG -3’
TBP-forward	5’-GAATATAATCCCAAGCGGTTTG-3’
TBP-reverse	5’-ACTTCACATCACAGCTCCCC-3’
HPRT-forward	5’-ATCAGACTGAAGAGCTATTGTAATGACCA-3’
HPRT-reverse	5’-TGGCTTATATCCAACACTTCGTG-3’

Table S8. PR interval conditioned analysis of *SCN10A* common variants in ESP with *SCN5A* and *SCN11A* common variants

				PR Interval		Conditioned PR Interval*	
SNP ID	**Amino acid substitution**	**A1/A2**	**Freq (%)**	**β (SE) [msec]**	***P* value**	**β (SE) [msec]**	***P* value**
rs57326399	I962V	T/C	25.2	-2.83 (1.54)	0.07	-2.61 (1.63)	0.11
rs73062575	P1045T	G/T	2.4	12.69 (3.70)	$6.5x10^{-4}$	12.6 (3.76)	8.6x10-4
rs6795970	V1073A	A/G	58.3	-5.13 (1.43)	$3.6x10^{-4}$	-5.18 (1.46)	4.1x10-4
rs12632942	L1092P	A/G	23.6	-2.75 (1.56)	0.08	-2.56 (1.64)	0.12
rs74717885	I206M	T/C	1.3	-1.19 (5.48)	0.83	-1.45 (5.53)	0.79
rs7630989	S509P	A/G	2.7	-2.03 (4.46)	0.65	-2.07 (4.48)	0.64
rs77804526	V1697I	C/T	1.0	1.40 (5.95)	0.82	-1.49 (6.01)	0.80

*PR interval conditioned on all common nonsynonymous SNPs in adjacent sodium channels *SCN5A* (rs1805124, MAF=24%) and *SCN11A* (rs72869687, MAF=1.1%; rs33985936, MAF=25%; rs13059805, MAF=2.6%)

Abbreviations: Freq, coded allele frequency; A1 non-coded allele, A2 coded allele

Table S9. Haplotype associations with QRS interval

						European Americans		African Americans		QRS Association (EA and AA)
Haplotype Name	**Variants in Haplotype**	**Alleles***				**Freq†**	**QRS interval‡**	**Freq†**	**QRS interval‡**	**P-value**
IV+VA	I962V+V1073A	**V**	P	**A**	L	1.7 (.24)	89.86 (1.50)	0 (.16)	NA	0.71
IV+VA+LP	I962V+V1073A+L1092P	**V**	P	**A**	**P**	25.6 (.83)	88.58 (0.34)	10 (.68)	88.54 (0.73)	0.13
VA	V1073A	I	P	**A**	L	32.2 (.89)	88.50 (0.32)	75.9 (.97)	88.82 (0.76)	0.84
WT	WT	I	P	V	L	35.9 (.91)	88.65 (0.30)	9.2 (.66)	88.66 (0.73)	Reference
VA+LP	V1073A+L1092P	I	P	**A**	**P**	1.4 (.21)	89.60 (1.75)	4 (.45)	88.32 (1.12)	0.64
PT	P1045T	I	**T**	V	L	3.1 (.32)	90.31 (1.11)	0.6 (.18)	89.20 (4.20)	0.53

*Specific alleles listed in order from 5' end of gene for the four variants: I962V, P1045T, V1073A, L1092P; †% Frequency (SE); ‡Mean, msec (SE)

Table S10. Expression of *SCN10A*/Nav1.8 channels in cardiac tissue.

	Atria	Purkinje Fibers	Ventricle	Neuronal
Transcript	Present: H[24], M[24,25]	Present: M[26]	Present: H[24], M[24–26] Absent: M[27]	N/A
Protein	Present: H[28] Absent: M[29]	N/A	Absent: M[29]	Present: H[28], M[29], C[30]
$I_{Na1.8}$ late	Absent: M[29]	N/A	Present: M[25,27], R[25] Absent: M[29]	Present: M[29]

H, M, C, and R refer to human, mouse, canine, and rabbit, respectively. N/A: no applicable data available

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