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

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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=5.2×10⁻⁴, respectively) and rs6599230 was associated with PR shortening (P=2.67×10⁻⁵). Among European ancestry participants, 2 novel synonymous variants, rs1805126 and rs7626962 (S1102Y) was associated with PR shortening in those of African ancestry (P=2.82×10⁻³). 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=5.2×10⁻⁴, 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

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mortality.\textsuperscript{1,3} QRS prolongation has been associated with heart failure and cardiovascular mortality in clinical trial– and community-based cohorts.\textsuperscript{4,5} 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.\textsuperscript{6–11} Missense mutations in this gene have been associated with supraventricular and ventricular arrhythmias.\textsuperscript{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.\textsuperscript{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, 52736 unique variant sites were identified using the SOLiD platform-based multiplexed sequencing protocol developed specifically for the CHARGE. SAMTools\textsuperscript{21} 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 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 myocardi al 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).\textsuperscript{14} Library construction, exome capture, sequencing, mapping, calling, and filtering have been described elsewhere.\textsuperscript{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 glm/Multiples4 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\textsuperscript{22} 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 <10x were set to missing. Variant sites were removed if the mean sample read depth across all samples was >500x, the variant deviated from race-specific Hardy–Weinberg equilibrium (P<1×10\textsuperscript{−6}), a support vector machine classifier was used to separate likely true-positive and false-positive variant sites as described elsewhere.\textsuperscript{23} 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.\textsuperscript{24} 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\textsuperscript{25} by pooling the alignments from individual low-coverage BAM files. The Genome Analysis Toolkit\textsuperscript{26} was used to filter sites (Variant Quality Score Recalibration)\textsuperscript{27} 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 <4x. 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, 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 6.95x.

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 multiplicity 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. Pairwise R² values were reported from the SNAP web interface using the 1000 Genomes project Pilot 1 data.

Rare variation in the coding regions was jointly analyzed using the Sequence Kernel Association test (SKAT), which was adapted for a meta-analysis framework 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; 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, 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 non-synonymous 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>
<thead>
<tr>
<th>Phenotypic Characteristics of the Study Samples</th>
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<tr>
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<tr>
<td>Total n</td>
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<tr>
<td>Men, %</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
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<tr>
<td>Height, cm</td>
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<tr>
<td>SBP, mmHg</td>
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<tr>
<td>RR interval, ms</td>
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<tr>
<td>PR interval, ms</td>
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<tr>
<td>QRS interval, ms</td>
</tr>
</tbody>
</table>

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.
Among the individual rare variants, there was no clustering of test associations for the ECG phenotypes across the 3 studies. PR intervals both shorter and longer than the mean PR interval ms to PR lengthening of 120 ms. We identified 3 singleton nonsense variants among those of European ancestry in the 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=3.35×10⁻⁷) and QRS (β=0.67 ms; P=2.69×10⁻⁵) 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=2.67×10⁻⁵). 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 (R²<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 intrinsic 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 (R²=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 (Table II in the Data Supplement). Meta-analysis across the combined 2044 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 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 3 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).

**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=6.25×10⁻⁴) and QRS (β=−0.77 ms; P=5.20×10⁻⁴) intervals in meta-analysis across the CHARGE, ESP, and UK10K studies (Tables 3 and 4). Among 972 African American participants in ESP, we identified 10 common variants (4 nonsynonymous, 6 synonymous) and 71 rare variants (40 nonsynonymous, 31 synonymous). Among 972 African American participants, we examined the association of 3 SNPs associated with PR and QRS among European descent individuals in ARIC, CHS, and FHS showed that all 3 of these coding SNPs were strongly associated with PR and QRS intervals (Table 4).

**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=8.9×10⁻⁴) 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 African American participants.

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

<table>
<thead>
<tr>
<th>ECG Measure</th>
<th>CHARGE</th>
<th>ESP</th>
<th>UK10K</th>
<th>ESP African Ancestry</th>
<th>Meta-Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td>0.003</td>
<td>0.46</td>
<td>0.22</td>
<td>0.01</td>
<td>1.32E-03</td>
</tr>
<tr>
<td>QRS</td>
<td>0.87</td>
<td>0.24</td>
<td>0.39</td>
<td>0.64</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Table 2 indicates Cohorts for Heart and Aging Research in Genetic Epidemiology; ESP, Exome Sequencing Project; MAF, minor allele frequency; and SKAT, Sequence Kernel Association test.
Table 3. Common Coding (MAF >1%) Variants Identified in the CHARGE, ESP, and UK10K Consortia and Their Association With the PR Interval

<table>
<thead>
<tr>
<th>rsID</th>
<th>A1/A2 Function</th>
<th>Amino Acid*</th>
<th>CHARGE CAF, % Effect (SE)†</th>
<th>P Value</th>
<th>ESP CAF, % Effect (SE)†</th>
<th>P Value</th>
<th>UK10K CAF, % Effect (SE)†</th>
<th>P Value</th>
<th>Meta-Analysis Effect (SE)†</th>
<th>P Value</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1805124</td>
<td>T/C</td>
<td>Nonsyn</td>
<td>18.4 −4.65 (1.24) 2.08E-05</td>
<td>0.45</td>
<td>24.4 −1.18 (1.56)</td>
<td>0.45</td>
<td>23.4 −1.42 (1.06)</td>
<td>0.18</td>
<td>−2.44 (0.71) 6.25E-04</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>rs1805126</td>
<td>A/G</td>
<td>Syn</td>
<td>33.6 2.94 (0.64) 2.69E-09</td>
<td>0.05</td>
<td>33.5 2.9 (1.47)</td>
<td>0.05</td>
<td>34.4 1.51 (0.90)</td>
<td>0.09</td>
<td>2.51 (0.49) 3.35E-07</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>rs7430407</td>
<td>C/T</td>
<td>Syn</td>
<td>13.9 0.11 (1.13) 0.89</td>
<td>0.82</td>
<td>12.3 −0.49 (2.13)</td>
<td>0.82</td>
<td>12.5 2.01 (1.34)</td>
<td>0.13</td>
<td>0.70 (0.80) 0.38</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>rs6599230</td>
<td>C/T</td>
<td>A29A</td>
<td>21.9 −2.08 (0.73)</td>
<td>0.02</td>
<td>19.9 −5.09 (1.73)</td>
<td>0.02</td>
<td>20.4 −2.04 (1.08)</td>
<td>0.24</td>
<td>−2.40 (0.57) 2.67E-05</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

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.
rs1805124 (H558R)\(^{41}\) alters molecular electrophysiology in the presence of additional genetic mutations.\(^{34,45}\) This SNP has been associated with PR and QRS in GWAS studies. The second missense SNP, a common variant of the \textit{SCN5A} sodium channel gene (rs7626962, S1102Y), present among African ancestry individuals is too small to identify variants with modest associations.\(^{10,46}\) 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 project Pilot 1 data. GWAS indicates Genome-Wide Association Studies; and SNP, single nucleotide polymorphisms.

### Table 4.

<table>
<thead>
<tr>
<th>rsID</th>
<th>A1/A2 Function Amino Acid*</th>
<th>AF CHARGE</th>
<th>Effect</th>
<th>P Value CHARGE</th>
<th>AF ESP</th>
<th>Effect</th>
<th>P Value ESP</th>
<th>AF UK1K</th>
<th>Effect</th>
<th>P Value UK1K</th>
<th>Meta-Analysis</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1805124</td>
<td>T/C</td>
<td>Nonysyn</td>
<td>H558R</td>
<td>18.4</td>
<td>0.04</td>
<td>0.53</td>
<td>0.75</td>
<td>24.4</td>
<td>−1.39</td>
<td>0.58</td>
<td>0.02</td>
<td>23.3</td>
</tr>
<tr>
<td>rs1805126</td>
<td>A/G</td>
<td>Syn</td>
<td>D1818D</td>
<td>33.6</td>
<td>0.61</td>
<td>0.25</td>
<td>1.00E-04</td>
<td>33.5</td>
<td>0.43</td>
<td>0.56</td>
<td>0.45</td>
<td>34.3</td>
</tr>
<tr>
<td>rs7430407</td>
<td>C/T</td>
<td>Syn</td>
<td>E1061E</td>
<td>13.9</td>
<td>0.01</td>
<td>0.45</td>
<td>0.85</td>
<td>12.3</td>
<td>−0.58</td>
<td>0.81</td>
<td>0.47</td>
<td>12.4</td>
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<tr>
<td>rs6599230</td>
<td>C/T</td>
<td>Syn</td>
<td>A29A</td>
<td>21.9</td>
<td>−0.55</td>
<td>0.27</td>
<td>0.20</td>
<td>19.9</td>
<td>−1.16</td>
<td>0.67</td>
<td>0.08</td>
<td>20.6</td>
</tr>
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</table>

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

* Amino acid positions relative to NM_000335.4.

† Effect size measured in ms.

rs1805124 (H558R)\(^{41}\) alters molecular electrophysiology in the presence of additional genetic mutations.\(^{34,45}\) This SNP has been associated with PR and QRS in GWAS studies. The second missense SNP, a common variant of the \textit{SCN5A} sodium channel gene (rs7626962, S1102Y), present among African ancestry individuals is too small to identify variants with modest associations.\(^{10,46}\) 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 project Pilot 1 data. GWAS indicates Genome-Wide Association Studies; and SNP, single nucleotide polymorphisms.

### Table 5.

<table>
<thead>
<tr>
<th>rsID</th>
<th>PR and QRS GWAS Index SNPs</th>
<th>(PR and QRS GWAS) Index SNPs</th>
<th>(QRS GWAS) Secondary SNP</th>
<th>(QRS GWAS) Secondary SNP</th>
<th>(QRS GWAS) Secondary SNP</th>
<th>(PR and QRS GWAS) Index SNPs</th>
<th>(QRS GWAS) Secondary SNP</th>
<th>(QRS GWAS) Secondary SNP</th>
<th>(QRS GWAS) Secondary SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10865879</td>
<td>0.06</td>
<td>0.07</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>rs11708996</td>
<td>0.03</td>
<td>0.05</td>
<td>0.21</td>
<td>0.10</td>
<td>0.04</td>
<td>0.06</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>rs11710077</td>
<td>0.78</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>rs1805124</td>
<td>0.06</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>rs1805126</td>
<td>0.06</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>rs6599230</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.00</td>
<td>0.06</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.00</td>
</tr>
</tbody>
</table>

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.
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.

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 Framingham Heart Study analysis was conducted using the Linux Clusters for Genetic Analysis computing resources at Boston University Medical Campus.

Sources of Funding

Dr Sotoodehnia is supported by RO1 HL111089, RO1 HL092111, RO1 HL088456, AND RO1 HL116747. Funding support for building on Genome-wide association studies (GWAS) for National Heart, Lung, and Blood Institute (NHLBI) diseases: the US Cohorts for 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 (HHSN268201000005C, HHSN268201000006C, HHSN268201100007C, HCSN26820100008C, HCSN268201100009C, HCSN268201100010C, HCSN268201100011C, and HCSN268201100012C). 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 Affymax, 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, 1R01HL092577, 1R01 HL1022141, 1RC1HL101056, 5R21DA027021, 1R01HL104156, K23HL11472, 1K24HL105780, and IR03AG045075. 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., N.C.-N., 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

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

<table>
<thead>
<tr>
<th>rsID</th>
<th>A1/A2</th>
<th>Function</th>
<th>Amino Acid*</th>
<th>CAF, %</th>
<th>Effect (SE)†</th>
<th>P Value</th>
<th>Effect (SE)†</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7626962</td>
<td>G/T</td>
<td>Nonsyn</td>
<td>S1102Y</td>
<td>5.20</td>
<td>−7.38 (2.46)</td>
<td>2.82E-03</td>
<td>2.1 (1.01)</td>
<td>0.04</td>
</tr>
<tr>
<td>rs1805124</td>
<td>T/C</td>
<td>Nonsyn</td>
<td>H558R</td>
<td>22.20</td>
<td>0.41 (1.41)</td>
<td>0.77</td>
<td>0.27 (0.57)</td>
<td>0.64</td>
</tr>
<tr>
<td>rs41313691</td>
<td>G/T</td>
<td>Nonsyn</td>
<td>S524Y</td>
<td>2.50</td>
<td>−2.66 (3.54)</td>
<td>0.45</td>
<td>−1.58 (1.4)</td>
<td>0.26</td>
</tr>
<tr>
<td>rs6791924</td>
<td>G/A</td>
<td>Nonsyn</td>
<td>R34C</td>
<td>8.50</td>
<td>−1.97 (1.95)</td>
<td>0.31</td>
<td>−0.72 (0.79)</td>
<td>0.36</td>
</tr>
<tr>
<td>rs13324293</td>
<td>G/A</td>
<td>Syn</td>
<td>I1947I</td>
<td>16.70</td>
<td>1.03 (1.5)</td>
<td>0.49</td>
<td>−0.43 (0.61)</td>
<td>0.49</td>
</tr>
<tr>
<td>rs1805126</td>
<td>A/G</td>
<td>Syn</td>
<td>D1818D</td>
<td>50.10</td>
<td>2.04 (1.3)</td>
<td>0.12</td>
<td>0.07 (0.53)</td>
<td>0.89</td>
</tr>
<tr>
<td>rs41315495</td>
<td>G/A</td>
<td>Syn</td>
<td>F1615F</td>
<td>14.90</td>
<td>−2.27 (1.53)</td>
<td>0.14</td>
<td>0.07 (0.62)</td>
<td>0.91</td>
</tr>
<tr>
<td>rs7430407</td>
<td>T/C</td>
<td>Syn</td>
<td>E1061E</td>
<td>67.70</td>
<td>3.35 (2.05)</td>
<td>0.09</td>
<td>−0.03 (0.82)</td>
<td>0.97</td>
</tr>
<tr>
<td>rs41313699</td>
<td>G/A</td>
<td>Syn</td>
<td>F434F</td>
<td>3.20</td>
<td>3.35 (3.2)</td>
<td>0.3</td>
<td>1.15 (1.32)</td>
<td>0.38</td>
</tr>
<tr>
<td>rs6599230</td>
<td>T/C</td>
<td>Syn</td>
<td>A29A</td>
<td>68.80</td>
<td>−0.06 (2.08)</td>
<td>0.77</td>
<td>−0.76 (0.84)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

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.
References


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


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## SUPPLEMENTAL MATERIAL

### Supplemental Table 1. Comparison of Quality control metrics across the 3 studies (CHARGE, ESP, UK10K).*

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Novel†</th>
<th>Novel Rare‡</th>
<th>Novel Common‡</th>
<th>Known Rare‡</th>
<th>Known Common‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHARGE</td>
<td>3,675</td>
<td>82%</td>
<td>82%</td>
<td>0.1%</td>
<td>18%</td>
<td>13%</td>
</tr>
<tr>
<td>ESP</td>
<td>1,005,248</td>
<td>87%</td>
<td>85%</td>
<td>1.8%</td>
<td>13%</td>
<td>8%</td>
</tr>
<tr>
<td>UK10K</td>
<td>510,969</td>
<td>82%</td>
<td>81%</td>
<td>1%</td>
<td>18%</td>
<td>5%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Novel†</th>
<th>Novel Rare‡</th>
<th>Novel Common‡</th>
<th>Known Rare‡</th>
<th>Known Common‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TiTv†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHARGE</td>
<td>3.03</td>
<td>2.85</td>
<td>2.86</td>
<td>0.67</td>
<td>4.09</td>
<td>4.19</td>
</tr>
<tr>
<td>ESP</td>
<td>3.08</td>
<td>3.04</td>
<td>3.04</td>
<td>3.05</td>
<td>3.34</td>
<td>3.27</td>
</tr>
<tr>
<td>UK10K</td>
<td>2.99</td>
<td>2.95</td>
<td>2.96</td>
<td>2.32</td>
<td>3.21</td>
<td>3.57</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Novel†</th>
<th>Novel Rare‡</th>
<th>Novel Common‡</th>
<th>Known Rare‡</th>
<th>Known Common‡</th>
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<tbody>
<tr>
<td><strong>Mean Depth‡</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHARGE</td>
<td>39.78</td>
<td>39.27</td>
<td>39.29</td>
<td>29.26</td>
<td>42.13</td>
<td>42.38</td>
</tr>
<tr>
<td>ESP</td>
<td>51.03</td>
<td>51.16</td>
<td>51.21</td>
<td>48.15</td>
<td>50.23</td>
<td>51.47</td>
</tr>
<tr>
<td>UK10K</td>
<td>7.02</td>
<td>7.04</td>
<td>7.06</td>
<td>5.83</td>
<td>6.89</td>
<td>7.16</td>
</tr>
</tbody>
</table>

CHARGE indicates Cohorts for Heart and Aging Research in Genomic Epidemiology; ESP, Exome Sequencing Project.

* Given that the limited number of variants identified in the SCN5A gene were not large enough to yield stable, accurate metrics, we provide summaries for all coding variants sequenced as part of each parent study. Because these metrics (e.g. TiTv) differs between coding and non-coding regions of the genome, we provide metrics limited to coding regions only.

†Novel defined as not present in 1000G 10-08.

‡Rare is <1% and common ≥1%.
**Supplemental Table 2.** Characteristics of the 1000 Genomes validation cohorts.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ARIC PR Interval</th>
<th>ARIC QRS Interval</th>
<th>CHS</th>
<th>FHS PR Interval</th>
<th>FHS QRS Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EA</td>
<td>AA</td>
<td>EA</td>
<td>AA</td>
<td>EA</td>
</tr>
<tr>
<td>N</td>
<td>8038</td>
<td>2464</td>
<td>8040</td>
<td>2467</td>
<td>2833</td>
</tr>
<tr>
<td>Age (mean±SD), years</td>
<td>54.0 (5.7)</td>
<td>53.2 (5.7)</td>
<td>54.0</td>
<td>53.2 (5.7)</td>
<td>72.1 (5.2)</td>
</tr>
<tr>
<td>Female sex, N (%)</td>
<td>54.2</td>
<td>62.8</td>
<td>54.2</td>
<td>62.8</td>
<td>1781 (62.9)</td>
</tr>
<tr>
<td>Height (mean±SD), m</td>
<td>1.69 (.09)</td>
<td>1.68 (.09)</td>
<td>1.69</td>
<td>1.68 (.09)</td>
<td>1.64 (0.09)</td>
</tr>
<tr>
<td>Body mass index (mean±SD), kg/m²</td>
<td>26.8 (4.7)</td>
<td>29.5 (5.9)</td>
<td>26.8</td>
<td>29.5 (5.9)</td>
<td>26.2 (4.5)</td>
</tr>
<tr>
<td>PR interval (mean±SD), msec</td>
<td>160.2 (23.1)</td>
<td>171.5 (27.0)</td>
<td>--</td>
<td>--</td>
<td>166.6 (27.9)</td>
</tr>
<tr>
<td>QRS interval (mean±SD), msec</td>
<td>--</td>
<td>--</td>
<td>91.1</td>
<td>90.0 (9.7)</td>
<td>88.3 (10.1)</td>
</tr>
</tbody>
</table>

*FHS additionally adjusted for cohort. EA indicates European ancestry; AA, African ancestry.*
Supplemental Table 3. Common coding (MAF>1%) variants and their association with the PR and QRS intervals in 1000 Genomes meta-analysis.

<table>
<thead>
<tr>
<th>rsID</th>
<th>A1/A2</th>
<th>Function</th>
<th>Amino Acid*</th>
<th>CAF</th>
<th>PR Effect (SE)†</th>
<th>P</th>
<th>QRS Effect† (SE)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1805124</td>
<td>T/C</td>
<td>nonsyn</td>
<td>H558R</td>
<td>23.40%</td>
<td>-1.90 (0.28)</td>
<td>1.82E-11</td>
<td>-0.89 (0.12)</td>
<td>3.06E-16</td>
</tr>
<tr>
<td>rs1805126</td>
<td>A/G</td>
<td>syn</td>
<td>D1818D</td>
<td>34.30%</td>
<td>1.85 (0.25)</td>
<td>2.84E-13</td>
<td>0.69 (0.11)</td>
<td>6.46E-11</td>
</tr>
<tr>
<td>rs7430407</td>
<td>C/T</td>
<td>syn</td>
<td>E1061E</td>
<td>10.70%</td>
<td>-0.97 (0.39)</td>
<td>0.012</td>
<td>0.10 (0.16)</td>
<td>0.89</td>
</tr>
<tr>
<td>rs6599230</td>
<td>C/T</td>
<td>syn</td>
<td>A29A</td>
<td>20.30%</td>
<td>-2.23 (0.31)</td>
<td>1.11E-12</td>
<td>-0.57 (0.13)</td>
<td>4.95E-07</td>
</tr>
</tbody>
</table>

rsID indicates 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 ms.
Supplemental methods for 1000 Genomes

Atherosclerosis Risk in Communities (ARIC) Study

ARIC African American Genotyping and Imputation:
In ARIC, genotyping was performed at the Broad Institute using the Affymetrix 6.0 array. Genotypes were called using Birdseed software. For genotyping, participants were excluded if they had a call rate <95% or if their genotype was discordant with known sex or finger-printing genotyping (to identify possible sample swaps occurring in the lab). Genotyping was successful in 3207 persons, and 336 individuals were subsequently removed for being first-degree relatives, genetic outliers, and/or not matching existing genotype data, resulting in 2871 individuals available for imputation. The following exclusions were applied to identify a final set of 806,416 autosomal SNPs used for imputation: call rate < 95%, HWE P < 10^{-5}, MAF < 1%. Imputation was performed in two steps: (1) pre-phasing with ShapeIt (v1.r532), followed by (2) imputation with IMPUTE2. Phasing with ShapeIt that was run with the parameters: --states-phase 200. Final imputations using IMPUTE2 included the reference panel: 1,000 Genomes haplotypes -- Phase I integrated variant set release (v3) in NCBI build 37 (hg19) in chunks of size 5 Mb. All 1092 individuals were used for the imputation from the reference panel.

ARIC European American Genotyping and Imputation:
In ARIC, genotyping was performed at the Broad Institute using the Affymetrix 6.0 array. Genotypes were called using Birdseed software. For genotyping, participants were excluded if they had a call rate <95% or if their genotype was discordant with known sex or finger-printing genotyping (to identify possible sample swaps occurring in the lab). Genotyping was successful in 9747 persons, and 591 individuals were subsequently removed for being first-degree relatives, genetic outliers, and/or not matching existing genotype data, resulting in 9156 individuals available for imputation. The following exclusions were applied to identify a final set of 669,450 autosomal SNPs used for imputation: call rate < 95%, HWE P < 10^{-5}, MAF < 1%. Imputation was performed in two steps: (1) pre-phasing with ShapeIt (v1.r532), followed by (2) imputation with IMPUTE2. Phasing with ShapeIt that was run with the parameters: --states-phase 200. Final imputations using IMPUTE2 included the reference panel: 1,000 Genomes haplotypes -- Phase I integrated variant set release (v3) in NCBI build 37 (hg19) in chunks of size 5 Mb. All 1092 individuals were used for the imputation from the reference panel.

Cardiovascular Health Study (CHS)

CHS African American Genotyping and Imputation:
In CHS, genotyping was performed at the General Clinical Research Center's Phenotyping/Genotyping Laboratory at Cedars-Sinai Medical Center using the Illumina HumanOmni1-Quad_v1 BeadChip system. Genotypes were called using the Illumina GenomeStudio software. For genotyping, participants were excluded if they had a call rate ≤95% or if their genotype was discordant with known sex or prior genotyping (to identify possible sample swaps). Genotyping was attempted in 844 participants, and was successful in 823 persons; the latter constitute the CHS sample for this study. The following exclusions were applied to identify a final set of 963,248 SNPs (940,567 autosomal & 22,681 X): call rate < 97%, HWE P< 10^{-5}, > 1 duplicate error or Mendelian inconsistency (for reference CEPH trios), heterozygote frequency =0. IMPUTE version 2.2.2 was used to perform imputation for the CHS African-American participants (chromosomes 1-23) using the 1,000 Phase I integrated variant set based on the phased haplotypes released March 2012 (v3).

CHS European American Genotyping and Imputation:
Genotyping was performed at the General Clinical Research Center's Phenotyping/Genotyping Laboratory at Cedars-Sinai Medical Center using the Illumina 370CNV BeadChip system. Genotypes were called using the Illumina GenomeStudio software. The following exclusions were applied to identify a final set of 306,655 autosomal SNPs: call rate < 97%, HWE P < 10^{-5}, > 2 duplicate errors or Mendelian inconsistencies (for reference CEPH trios), heterozygote frequency =0, SNP not found in HapMap. Cleaned genotypes from the Illumina CNV370 were merged with genotypes from ITMAT-Broad-CARe (IBC) Illumina iSELECT chip. The IBC chip contained 46,423 markers on a subset of 2861 participants. The genotypes from the two chips were merged into a final set of 359,592 unique SNPs and then updated to hg19 position using plink. MaCH was used to pre-phase the genotypes. The phased genotypes were then imputed into a reference panel of 1092 individual of multiple ethnicities from the Phase1 version3 haplotypes of Thousand Genomes project using minimac (release stamp 2012-11-16).

**Framingham Heart Study.**

Genotyping was performed in FHS at the Affymetrix (Santa Clara, CA) using Affymetrix GeneChip Human mapping 500K array plus an additional Affymetrix 50K supplemental array (HuGeneFocused50K). 412,053 out of a total number of 549,781 genotyped SNPs were used as input to the MACH program for imputation. A total of 137,728 genotyped SNPs were removed based on the following filtering criteria: HWE p-value <10^{-6}, call rate < 96.9%, minor allele frequency < 0.01, mapping incorrectly from Build 36 to Build 37 locations, missing physical location information, Mendelian errors >1000, outside autosomal and X chromosomes, duplication. The phased genotypes were then imputed into a cosmopolitan reference panel of 1092 individuals of multiple ethnicities from the Phase 1 version3 haplotypes of 1000 Genomes project using minimac.
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ESP Cohorts
21Acute Lung Injury (ALI), 22Atherosclerosis Risk in Communities (ARIC), 23Cardiovascular Health Study (CHS), 24Chronic Obstructive Pulmonary Disease (COPDGene), 25Coronary Artery Risk Development in Young Adults (CARDIA), 26Cystic Fibrosis (CF), 27Early Pseudomonas Infection Control (EPIC), 28Framingham Heart Study (FHS), 29Jackson Heart Study (JHS), 30Lung Health Study (LHS), 31Multi-Ethnic Study of Atherosclerosis (MESA), 32Pulmonary Arterial Hypertension (PAH), 33Severe Asthma Research Program (SARP), 34Women's Health Initiative (WHI)

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