Common and Rare Coding Genetic Variation Underlying the Electrocardiographic PR Interval

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Abstract

Background— Electrical conduction from the cardiac sinoatrial node to the ventricles is critical for normal heart function. Genome-wide association studies (GWAS) have identified more than a dozen common genetic loci that are associated with PR interval. However, it is unclear whether rare and low-frequency variants also contribute to PR interval heritability.

Methods and Results—We performed large-scale meta-analysis of the PR interval that included 83,367 participants of European ancestry and 9,436 of African ancestry. The Illumina HumanExome BeadChip examined both common and rare variants. We identified 31 genetic loci that were significantly associated with PR interval after Bonferroni correction ($P<1.2x10^{-6}$), including 11 novel loci that have not been reported previously. Many of these loci are involved in heart morphogenesis. In gene-based analysis, we found that multiple rare variants at *MYH6* ($P = 5.9x10^{-11}$) and *SCN5A* ($P=1.1x10^{-7}$) were associated with PR interval. *SCN5A* locus also was implicated in the common variant analysis, whereas *MYH6* was a novel locus.

Conclusion—We identified common variants at 11 novel loci and rare variants within two gene regions that were significantly associated with PR interval. Our findings provide novel insights to the current understanding of atrioventricular conduction, which is critical for cardiac activity and an important determinant of health.

Key Words: Electrocardiogram ■ PR interval ■ genetics ■ exome chip ■ epidemiology

Introduction

Electrical conduction from the cardiac sinoatrial node to the ventricles is critical for normal heart function. Abnormalities of atrioventricular conduction can cause significant morbidity, and have been associated with atrial fibrillation (AF),[1,2] need for pacemaker implantation,[2] cardiac malformations, and sudden death.[3,4] Conduction from the sinus node through the atria, atrioventricular node, and His-Purkinje fibers is readily evaluated from surface electrocardiogram (ECG), by measurement of the duration of PR interval. Despite the critical role that the cardiac conduction system plays in cardiac physiology and disease, the formation and regulation of the conduction system remains incompletely understood.

Recent data indicate that cardiac conduction measurements are heritable[5-7] and have a genetic basis.[8-11] To date, genetic studies of PR interval have been relatively modest-sized largely European-ancestry samples, and have implicated cardiac expressed ion channels, cardiac developmental transcription factors, signaling molecules, as well as novel pathways not previously known to be involved in cardiac conduction processes. Nevertheless, existing studies have focused on the role of common and predominantly noncoding genetic variants, which account for only a modest proportion of trait heritability.[6]

To better understand the biological and potential clinical implications of genetic variation underlying cardiac conduction, there is a need to examine both common and rare variation underlying atrioventricular conduction in large, well-powered, multiethnic studies. Moreover, assessment of genetic variation that alters protein coding has the potential to more directly implicate genes involved in processes critical to cardiac conduction. We therefore sought to examine PR interval duration in relation to predominantly coding genetic variants, in large, multi-ethnic analyses using the exome chip.

Methods

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results, subject to Data Use/Sharing Agreements adopted by individual participating cohorts. The summary results from the current manuscript are available at the Broad Cardiovascular Disease Knowledge Portal (www.broadcvdi.org).

Study participants

The current project included participants of European ancestry (EA) from 22 studies: Age, Gene/Environment Susceptibility Study (AGES); Atherosclerosis Risk in Communities study (ARIC); British Genetics of Hypertension (BRIGHT); Massachusetts General Hospital Cardiology and Metabolic Patient cohort (CAMP); Cardiovascular Health Study (CHS); Erasmus Rucphen Family Study (ERF); Framingham Heart Study (FHS); Genes for Cerebral Hemorrhage on Anticoagulation (GOCHA); Genetic Regulation of Arterial Pressure In Humans in the Community (GRAPHIC); INTER99; Cooperative Health Research in the Region Augsburg (KORA); CROATIA-Korcula (KORCULA); LifeLines Cohort Study (LifeLines); Multi-Ethnic Study of Atherosclerosis (MESA); The Netherlands Epidemiology of Obesity (NEO); Rotterdam Study (RS); Generation Scotland: Scottish Family Health Study (GS:SFHS); Study of Health in Pomerania (SHIP); TwinsUK; Utrecht Health Project (UHP); Women's Health Initiative (WHI); and Young Finns Study (YFS).

In addition, we included participants of African ancestry (AA) from five studies. These studies included ARIC, CHS, Jackson Heart Study (JHS), MESA and WHI.

Institutional Review Boards or Ethics Committees approved study procedures at each contributing site. All participants provided written informed consent to participate in genetic research.

Measurement of PR interval

PR interval duration, in milliseconds, was measured from the onset of the P wave to the onset of the QRS interval for each cohort. The following exclusions were applied: extreme PR values (≤ 80 ms or ≥

320 ms); second or third degree heart block; atrial fibrillation on baseline ECG; history of myocardial infarction, heart failure, or Wolff–Parkinson–White syndrome; pacemaker placement; use of class I or III blocking medications (ATC code prefix C01B); digoxin use (ATC code C01AA05) or pregnancy.

Genotyping

Genotyping was performed independently in each study using the Illumina Human Exome BeadChip (v1.0, 1.1, or 1.2). Data were called and cleaned according to CHARGE ExomeChip best practices.[12] Detailed information for each study regarding genotyping platforms, variant calling, and quality control metrics is shown in **Supplementary Table 1**. All studies used the same set of reference alleles to recode variants to ensure consistency.

Statistical analyses

Prior to association analysis, PR interval was first adjusted for covariates by taking residuals from a linear regression of PR on age, sex, height, body mass index, and RR interval. Each cohort additionally adjusted as necessary for cohort-specific variables, such as clinic sites, family structure, and population structure. To reduce sensitivity to extreme PR values, the residuals were inverse-normal transformed and used as the outcome for association testing.

Because single-marker based analyses typically have low power to identify associations between rare variants and traits, we separated the analysis for common and rare variants based on minor allele frequency (MAF). Common variants were defined as those with MAF≥1%, and the remaining variants were defined as rare variants (MAF<1%). For each of the common variants, we evaluated its association with the transformed PR interval, and accounted for multiple testing by Bonferroni correction ($P < 0.05/42075=1.2x10^{-6}$). For the rare variants, we restricted analyses to nonsynonymous or splicing variants with MAF <1%, because such variants are more likely to be functional than synonymous or

more common variants. As we expect some rare variants may act in the same or opposite directions even in the same gene region,[13] we used a modified version of the Sequence Kernel Association Test (SKAT),[14] which avoids problems of signals cancelling out each other in burden test results. Many gene regions had few or no rare nonsynonymous or splicing variants. Monomorphic variants from each study also were reported in the cohort level results as they were used for the cumulative MAF computations in gene-based tests. Gene regions with a cumulative MAF of rare variants <1% were excluded, resulting in 5,761 gene regions that were tested (see results below). Therefore, Bonferroni-corrected significance threshold for our gene-based tests was $P<0.05/5,761=8.7x10^{-6}$. In secondary analyses, we limited the analysis to damaging variants, defined as nonsense variants or variants predicted to be damaging by PolyPhen-2 [15] or SIFT.[16]

Analyses were performed using the "prepScores" function of the "seqMeta" R package. Family-based studies implemented the "kins" option in "prepScores" to specify kinship matrices. Each study provided single variant z-statistics from score tests, as well as genotype covariance matrices, which were then combined by fixed effects meta-analysis. The heterogeneity across studies was assessed by the Cochran's Q, which is a non-parametric statistical test defined as the weighted sum of squared differences between individual study effects and the pooled effect. We performed both race stratified and race combined meta-analyses, and the race combined results were used for the remaining sections unless stated otherwise.

Comparison with genetic loci associated with AF and P-wave indices (PWI)

We also compared genetic loci associated with PR interval with those associated with AF and PWI to see if there are any shared genetic mechanisms. "AF loci" were identified by a recent exome chip analysis that included 22,806 AF cases and 132,612 referents.[17] "PWI loci" were identified from a meta-analysis

of P-wave duration and P-wave terminal force that included 44,456 participants.[18] In addition, for each of the top variants associated with PR, we also examined its association with AF and PWI.

Examine potential function of PR-related variants for gene expression, regulation and biological pathways

Pathway analysis was performed by MAGENTA[19] with default settings. The summary result for the common variants was used as the input, and significant pathways were defined as those with a false discovery rate (FDR)[20] <0.05. The implication of genetic variants on cardiac gene expression (eQTL analysis) was performed by querying the GTEx database.[21] At each PR-related locus, we identified the top variant and its neighboring variants that were within 500kb and in linkage disequilibrium with the top variant ($r^2 \geq 0.5$). Four heart and vascular tissues were queried, including artery aorta, artery coronary, atrial appendage and heart left ventricle. Significant eQTLs were defined as those with FDR<0.05. Regulatory regions were downloaded from the ENCODE Project[22] and the NIH Roadmap Epigenomics Program.[23] Four tracks were created: 1) included all 98 cell types from Roadmap epigenomics H3K27ac sites; 2) included only four heart tissues (aorta, right atrium, left ventricle, right ventricle) from Roadmap epigenomics H3K27ac sites; 3) included all 125 cell lines from ENCODE DNaseHS sites; 4) included only three heart-derived cell lines (cardiac fibroblasts, atrial fibroblasts, cardiac myocytes). The enrichment of PR-related loci in regulatory regions was examined by the "VSE" R package.[24] For comparison, we randomly created 1,000 variant sets with MAF values and LD structures similar to those seen for PR-related loci.

Results

The current analyses included a total of 92,803 individuals from 27 cohorts, with 83,367 individuals from 22 studies of European ancestry and 9,436 individuals from 5 studies of African ancestry. Clinical characteristics of the study participants are in **Table 1**.

Identification of 31 loci associated with PR interval

A total of 42,075 common variants were analyzed (MAF ≥ 1%). As shown in **Figure 1** and **Table 2**, 31 loci were significantly associated with PR interval after Bonferroni correction ($P < 1.2x10^{-6}$), including 22 loci that reached the conventional genome-wide significance threshold ($P < 5x10^{-8}$). The results of the random effects meta-analysis were similar to those of the fixed effects analysis (**Supplementary Table 2**). The most significant locus was tagged by rs6795970 ($P= 4.0x10^{-240}$), a missense variant in *SCN10A*, which encodes a sodium channel that has been associated previously with the PR interval (r^2=0.97 with the top SNP rs6599250 reported previously).[8] Highly associated variants clustered in the linker region between the second and third domains of *SCN10A* (**Figure 2**). The top variants at 12 loci are missense variants. In addition, the top variants at 4 loci (including 3 novel loci) are low-frequency variants (1% < MAF < 5%), illustrating the power of exome chip analyses to identify low-frequency coding associations. Detailed information of the nearest gene to each genome wide significant locus is given in **Supplementary Table 3**.

We then examined the associations between these top PR variants with AF and electrocardiographic PWI. Eight out of 31 PR loci identified in our analysis were associated with AF after Bonferroni correction ($P<0.05/31=1.6x10^{-3}$), consistent with some shared mechanisms between the regulation of PR interval and AF. Variants in *SCN10A* most significantly associated with PR interval were also significantly associated with AF (**Supplementary Table 4**). Among PR-related SNPs, rs60632610 at the *SYNPO2L* locus

was most significantly associated with AF (Odds ratio: 1.90 (0.87-0.93), P=1.5x10^{-10}). **Supplementary Figure 1** shows the overlap among loci associated with PR interval, AF, and PWI.

We also performed a sensitivity analysis that separated samples of European and African ancestry. As shown in **Supplementary Table 5** and **Supplementary Figure 2**, all of the 31 loci except rs17391905 at the 1p32.3 locus (P = 2.6x10^{-6}) were also significant in the analysis of European-only samples. **Supplementary Table 6** and **Supplementary Figure 3** show the result for the analysis of African ancestry-only samples. Three loci were significant: *SCN5A* (rs3922844), *SCN10A* (rs6795970), and *TBX5* (rs883079) after Bonferroni correction; $P < 1.3 \times 10^{-6}$. All three loci were also significant in the analysis of European-only samples. The result from each individual study is shown in **Supplementary Table 7**.

Rare variations in *MYH6* and *SCN5A* are associated with PR interval

We next examined the association between PR interval and rare variants (MAF<1%) in gene regions. Variation in two gene regions, *MYH6* (P = 5.9x10^{-11}) and *SCN5A* (P = 1.1x10^{-7}), was associated with PR interval (**Table 3**). **Supplementary Tables 8 and 9** show the association of each rare variant within *MYH6* and *SCN5A* with PR interval, respectively. *MYH6* encodes a cardiac myosin heavy chain subunit, and *SCN5A* encodes the major cardiac sodium channel and was previously found to be associated with PR interval.[8] *MYH6* was also recently found to associate with PWI.[18] We also performed an ancestry-stratified analysis in the same way as the combined analysis. The same two gene regions were significant using data from European samples alone (P = 4.1x10^{-12} and 8.3x10^{-7} for *MYH6* and *SCN5A*, respectively). These two genes did not reach the significance cutoff in African samples (P = 0.03 and 0.01 for *MYH6* and *SCN5A*, respectively). Two other genes, *HEATR2* (P = 2.2x10^{-6}) and *THRAP3* (P = 4.2x10^{-6}), were significantly associated in African samples alone. However, in the combined analysis, these two genes were not significant (P=0.02 and 0.06 for *HEATR2* and *THRAP3*, respectively), probably due to a low cumulative allele frequency.

In our secondary analysis of pooled samples, we analyzed only damaging variants, defined as nonsense mutations or alternations predicted to be damaging by PolyPhen-2[15] or SIFT.[16] Three genes reached the significance cutoff (P<0.05/2030=2.5x10^{-5}), including *GORASP1* (P=1.1x10^{-5}), *NEBL* (P=1.9x10^{-5}), and *SCN5A* (P=2.2x10^{-5}) (**Supplementary Table 10**).

Expression quantitative trait loci (eQTL) analysis

We also performed eQTL analysis to determine if any of the novel PR-related variants were associated with cardiac gene expression using data from GTEx.[21] Eight loci were associated with expression of at least one gene in the atrial appendage, left ventricle, coronary artery, or aorta, suggesting the importance of these loci in the regulation of gene expression in heart or vascular tissues (**Supplementary Table 11**).

Enrichment of PR-related variants in regulatory regions

We examined involvement of PR-related variants in regulatory function. As shown in **Supplementary Figure 4**, PR-related variants were significantly enriched in regulatory regions in both primary heart tissues (P_{adj}=3.7x10^{-9}) and heart-derived cell lines (P_{adj}=0.002), but not in all tissues (P_{adj}>0.05). The observed enrichment suggested involvement of these loci in tissue-specific regulatory functions. In addition, the variants also tended to locate within evolutionarily conserved regions (P_{adj}=2.8x10^{-5} for primates and 6.4x10^{-5} for mammals).

Enrichment of PR-related variants in biological pathways

We examined the enrichment of PR-related variants in biological pathways by MAGENTA.[19] **Supplementary Table 12** shows the top pathways identified. The most significant pathway was heart morphogenesis (P=3.6x10^{-5}, FDR=0.049), suggesting that many PR-related genes might be involved in

cardiac development. The pathway was only the significant pathway after correction for multiple testing (FDR<0.05).

Discussion

We conducted a large-scale analysis of the genetic determinants of atrioventricular conduction in 92 803 individuals by studying the electrocardiographic PR interval. In total, we observed 31 genetic loci that were associated with atrioventricular conduction, 11 of which are novel. In aggregate, the results implicate loci containing genes encoding ion channels in the heart, sarcomeric proteins, cardiac transcription factors, and other proteins with unknown cardiac function. Our findings provide new insights to the current understanding of atrioventricular conduction, which is critical for cardiac function.

Interestingly, rare variants in *SCN5A* and *MYH6* were associated with PR interval. A missense mutation (D1275N) in *SCN5A* has previously been reported in a large family with multiple members affected by dilated cardiomyopathy, conduction disorder, and arrhythmia.[25] The mutation, together several other mutations within the same gene, has also been associated with dilated cardiomyopathy,[26] atrial fibrillation,[27] and long-QT syndrome.[28-31] Rare mutations within *MYH6* were associated with sick sinus syndrome,[28] congenital heart defects,[32] and atrial septal defects.[33]

Our observations support and extend prior analyses of cardiac conduction. Most previous genome-wide association studies involved the study of common genetic variation in smaller samples of up to 28,517 individuals.[8,10,11] In keeping with those prior studies, we again observed that *SCN10A* is the most prominent gene involved in atrioventricular conduction. Our recent GWAS based on 105K samples corroborates many of our current findings.[34] However, our current study had greater power than those earlier analyses for assessment of rare coding variation.

Our study has two major implications. First, our results underscore the utility of assessing coding variation as an efficient way to identify functional molecular domains. In particular, our findings provide

insights into the functional topology of *SCN10A.* The *SCN10A* sodium channel gene is widely expressed in the nervous system and heart,[21] but it has only recently been implicated in cardiac conduction[8,34-36] and arrhythmias such as AF[35] and Brugada syndrome.[37] *SCN10A* encodes an alpha subunit (with six transmembrane spanning regions), which forms tetrameric, voltage gated sodium channels responsible for the Nav 1.8 late sodium channel current.[38,39] We found a collection of amino acid substitutions in the linker region between the second and third domains of *SCN10A* that were associated with PR duration (**Figure 2**). Variants in this linker region that were associated with the PR interval also were associated with AF, suggesting that function of this domain may have important clinical implications.

Prior work on the homologous *SCN5A* cardiac sodium channel gene -- which is also a cardiac conduction locus -- indicates that this linker region is critical for sodium channel inactivation. Sodium influx is predominantly responsible for cardiomyocyte depolarization. Moreover, channel inactivation is essential for restoration of the hyperpolarized state needed for cyclic cardiomyocyte depolarization and contraction. Therefore, variations in this linker region might be involved in Nav 1.8 inactivation. Other data are necessary to identify relationships among variation in the linker region, the late sodium channel current, and channel inactivation in both healthy and diseased states.

Together with previously discovered susceptibility genes, our findings implicate genes in different functional classes that regulate atrioventricular conduction such as ion channels and cardiac transcription factors. In many cases, anomalies in these genes have been found to cause human cardiac diseases, such as congenital heart defects, primary cardiac conduction abnormalities, and syndromes predisposing to sudden cardiac death (**Supplementary Table 3**). Interestingly, some of the genes are not expressed (in high abundance) in the right atrial appendage or the left ventricle, according to existing data sets -- although most are active in the heart (**Supplementary Table 13**). Atrioventricular nodal conduction also can be influenced by external tone from the autonomic nervous system. Therefore,

further work is necessary to determine the mechanisms by which identified genes that are not expressed in the heart influence the PR interval.

We acknowledge several limitations of our study. Because PR interval was measured across many cohorts, it is possible that there is some heterogeneity that would diminish our power to detect modest associations. We excluded individuals with extreme values of PR interval, which might have been gleaned from large variations in cardiac conduction. We also performed inverse normal transformation on the raw PR interval to reduce the heterogeneity, which on the other hand might reduce the interpretability. Although we performed single-variant and gene-based tests, we did not examine the association of haplotype patterns with PR interval, so it is unclear if there are any haplotypes that might be associated with PR interval. Most of the genetic variants analyzed were in exons. Therefore the effects of variants within regulatory regions were not investigated. We note that the variants identified may not be causally related to the studied phenotypes (PR interval, AF, and PWI), but may be in LD with causal variants. We anticipate that future increases in sample size with additional replications and more comprehensive genotyping platforms, such as denser SNP arrays or genome sequencing, will help address these limitations.

In conclusion, we studied genetic variants associated with PR interval duration and identified 31 common loci -- including 11 that were novel -- and two rare variant regions. Our findings greatly expand our knowledge of the genes that underlie atrioventricular conduction in the heart.

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Table 1. Clinical characteristics of the participating studies

Ancestry	Study	Total N	Men, N (%)	Age, yrs, mean	PR interval, ms, mean± SD	RR interval, ms, mean± SD	BMI, kg/m2, mean ± SD	Height, cm, mean ± SD	SBP, mmHg, mean ± SD	Beta blockers (%)	Diuretics (%)	Calcium antagonists* (%)
European ancestry	AGES	2052	742 (36.2)	75.9±5.4	170.5±26.8	895±129	27.0±4.4	166±9	143±20	635 (31.0)	ND	108 (5.3)
	ARIC	9828	4528 (46.1)	54.1±5.7	160.3±23.3	928±136	26.9±4.7	169±9	118±17	789 (8.0)	1085 (11.0)	176 (1.8)
	BRIGHT	841	324 (38.9)	57.6±10.7	161.1±19.9	960±169	27.5±3.8	166±9	153±24	248 (29.5)	260 (30.9)	18 (2.1)
	CAMP	2493	1394 (55.9)	60.7±11.6	163.0±26.8	926±166	28.5±5.8	171±10	ND	Excluded	ND	Excluded
	CHS	3247	1313 (40.4)	72.4±5.4	167.8±28.2	956±151	26.4± 4.4	165±9	136±21	366 (11.3)	750 (23.1)	206 (6.3)
	ERF	982	447 (45.5)	48.2±14.3	152.9±23.2	982±159	26.9±4.6	168±10	140±20	3 (0.3)	133 (13.5)	25 (2.5)
	FHS	7580	3428 (45.2)	39.3±9.8	152.0±22.1	910±175	26.0±5.0	169±10	119±15	Excluded	ND	Excluded
	GOCHA	355	161 (45.4)	73.2±8.2	167.6±27.7	913±173	26.1±4.6	169±10	N/A	Excluded	ND	ND
	GRAPHIC	1755	893 (50.9)	39.1±14.5	153.0±24.0	934±145	26.1±4.6	171±9	128±19	39 (2.2)	ND	ND
	INTER99	5836	2843 (48.7)	46.1±7.9	158.2±22.4	921±150	26.3±4.6	172±9	130±18	ND	ND	ND
	KORA	2617	1247 (47.6)	48.3±13.0	162.1±22.2	944±149	26.9±4.4	168±9	127±19	199 (7.6)	152 (5.8)	13 (0.5)
	KORCULA	293	106 (36.2)	55.0±13.4	159.8± 24.0	929±127	28.0± 4.3	168± 9	139± 14	8 (2.7)	3 (1.0)	6 (2.0)
	LifeLines	1934	781 (40.3)	45.2±13.1	156.7±24.7	896±145	25.9±4.5	175±9	122±16	64 (3.3)	39 (2.0)	23 (1.2)
	MESA	2455	1171 (47.7)	62.8±10.2	164.7±25.2	1047±158	27.8±5.1	169±10	123±21	Excluded	ND	Excluded
	NEO	5782	2717 (47.0)	55.9±5.9	164.5±23.4	940±151	30.0±4.8	174±10	133±17	Excluded	ND	ND
	RS	2358	1086 (46.1)	68.6±8.1	168.2±24.7	871±144	26.3±3.6	168± 9	ND	293 (12.4)	ND	ND
	GS:SFHS	9168	3786 (41.3)	52.0±13.6	164.1± 24.9	886±146	26.9± 5.1	168± 10	134± 18	192 (2.1)	ND	ND
	SHIP	6493	2608 (40.2)	49.2±15.3	158.5±23.3	897±146	27.5±5.0	170±9	131±20	ND	ND	ND
	TwinsUK	465	32 (6.9)	52.3±11.7	159.6±22.6	923±148	26.8±5.4	163±7	119±16	ND	ND	ND
	UHP	1735	779 (44.9)	39.1±13.0	155.9±22.5	950±151	24.9±3.9	175±10	125±17	69 (4.6)	32 (1.8)	18 (1.0)
	WHI	13252	0 (0)	66.0±6.5	161.4±24.0	921±138	28.7±5.6	162±6	130±18	735 (5.5)	1715 (13.3)	1230 (9.3)
	YFS	1846	824 (44.6)	41.9±5.0	156.2±22.6	1028±165	26.4±4.9	172±9	119±14	38 (2.1)	24 (1.3)	1 (0.1)
African ancestry	ARIC	3366	1291 (38.4)	53.3±5.8	171.2±26.8	929±151	29.4±6.1	168±9	128±22	315 (9.4)	717 (21.3)	222 (6.6)
	CHS	627	232 (37.0)	72.4±5.5	170.2±28.1	918±161	28.4± 5.5	165±9	142± 22	54 (8.6)	217 (34.6)	60 (9.6)
	JHS	2220	833 (37.5)	52.7±12.5	172.7±27.3	956±150	31.4±6.4	169±9	126±18	Excluded	ND	Excluded
	MESA	1565	718 (45.9)	62.3±10.0	170.9±26.3	1050±172	30.2±5.9	168±10	132±21	Excluded	ND	Excluded
	WHI	1658	0 (0)	64.6±6.4	167.1± 24.8	921±148	31.1±5.8	162±7	134±17	87 (5.2)	393 (23.7)	341 (20.6)

Exclusion criteria are given in **Supplementary Table 1**. SBP, systolic blood pressure; BMI, body mass index; ND, not determined; SD, standard deviation; *Non-dihydropyridine calcium antagonists.

Table 2. Common variants significantly associated with PR interval from meta-analysis of all studies

SNP	Locus	Closest gene	Function	Coding allele	CAF*	Beta	SE	*P* value	Number of studies‡	Prolong or shorten PR interval	Novel locus
rs6795970	3p22.2	*SCN10A*	Missense	A	0.37	0.1705	0.0052	$4.0x10^{-240}$	27	Prolong	
rs3922844	3p22.2	*SCN5A*	Intronic	A	0.34	-0.1069	0.0053	$9.3x10^{-90}$	26	Shorten	
rs3807989	7q31.2	*CAV1*	Intronic	A	0.43	0.0908	0.0050	$3.0x10^{-74}$	27	Prolong	
rs7660702	4q21.23	*ARHGAP24*	Intronic	C	0.33	-0.0921	0.0053	$1.2x10^{-68}$	27	Shorten	
rs17287293	12p12.1	*LINC00477*	Intergenic	G	0.14	-0.1084	0.0071	$1.9x10^{-52}$	27	Shorten	
rs11897119	2p14	*MEIS1*	Intronic	C	0.39	0.0566	0.0055	$4.2x10^{-25}$	25	Prolong	
rs1896312	12q24.21	*TBX3*	Intergenic	G	0.28	0.0564	0.0055	$8.7x10^{-25}$	26	Prolong	
rs883079	12q24.21	*TBX5*	3'UTR	G	0.29	0.0550	0.0054	$4.5x10^{-24}$	26	Prolong	
rs116202356	3p22.2	*DLEC1*	Missense	A	0.02	-0.1953	0.0199	$1.0x10^{-22}$	27	Shorten	
rs251253	5q35.1	*CREBRF*	Intergenic	G	0.42	-0.0439	0.0051	$4.7x10^{-18}$	26	Shorten	
rs11153730	6q22.31	*SLC35F1*	Intergenic	C	0.47	-0.0420	0.0049	$9.5x10^{-18}$	27	Shorten	Novel
rs35658696	5q21.1	*PAM*	Missense	G	0.04	0.0956	0.0119	$8.5x10^{-16}$	27	Prolong	
rs2070492	3p22.2	*SLC22A14*	Missense	T	0.10	0.0624	0.0083	$4.0x10^{-14}$	27	Prolong	Novel
rs2585897	13q12.11	*XPO4*	Intronic	A	0.17	0.0471	0.0064	$2.8x10^{-13}$	27	Prolong	
rs2042995	2q31.2	*TTN*	Missense	C	0.26	0.0375	0.0057	$4.3x10^{-11}$	27	Prolong	
rs4399693	2p25.1	*ID2*	Intergenic	A	0.34	0.0374	0.0058	$9.1x10^{-11}$	25	Prolong	
rs41306688	13q34	*ADPRHL1*	Missense	C	0.03	0.1002	0.0173	$7.4x10^{-9}$	22	Prolong	Novel
rs4745	1q22	*EFNA1*	Missense	T	0.49	0.0299	0.0053	$1.2x10^{-8}$	26	Prolong	
rs11078078	17p12	*LINC00670*	Intronic	A	0.40	0.0281	0.0050	$2.2x10^{-8}$	27	Prolong	
rs60632610	10q22.2	*SYNPO2L*	Missense	T	0.15	-0.0371	0.0068	$4.5x10^{-8}$	27	Shorten	Novel
rs11848785	14q24.2	*SIPA1L1*	Intronic	G	0.24	0.0317	0.0058	$4.6x10^{-8}$	27	Prolong	
rs3733414	4q35.2	*FAT1*	Missense	A	0.38	0.0280	0.0051	$4.8x10^{-8}$	27	Prolong	
rs17362588	2q31.2	*CCDC141*	Missense	A	0.08	-0.0491	0.0090	$5.5x10^{-8}$	27	Shorten	Novel
rs2296172	1p34.3	*MACF1*	Missense	G	0.20	0.0326	0.0061	$1.1x10^{-7}$	27	Prolong	Novel
rs9398652	6q22.31	*GJA1*	Intergenic	A	0.14	0.0390	0.0074	$1.3x10^{-7}$	26	Prolong	Novel
rs442177	4q22.1	*AFF1*	Intronic	C	0.42	-0.0262	0.0050	$1.8x10^{-7}$	26	Shorten	Novel
rs7002002	8q24.3	*PLEC*	Missense	A	0.38	-0.0272	0.0052	$2.1x10^{-7}$	25	Shorten	Novel
rs1768208	3p22.1	*MOBP*	Intron	T	0.25	0.0288	0.0057	$3.6x10^{-7}$	27	Prolong	Novel
rs2119788	4q34.1	*HAND2*	Intergenic	C	0.52	-0.0246	0.0049	$5.6x10^{-7}$	27	Shorten	Novel
rs17391905†	1p32.3	*C1orf185*	Intergenic	G	0.03	-0.0694	0.0142	$9.6x10^{-7}$	27	Shorten	
rs524295	10q24.1	*ALDH18A1*	Intergenic	A	0.40	-0.0261	0.0053	$9.7x10^{-7}$	26	Shorten	

*Coding allele frequency

†SNP was not significant if African participants were excluded.

‡Some variants did not reach pass the quality filtering in respective studies and thus were excluded.

Table 3. Top 10 gene regions associated with PR interval by the SKAT test*

Gene	*P* value	Qmeta†	CMAF‡	#Variants	Number of studies with at leat one rare variant	Average number of variants in each study
MYH6	**5.9×10^{-11}**	**23537340**	**0.0215**	**32**	**27**	**12**
SCN5A	**1.1×10^{-7}**	**16604843**	**0.0289**	**35**	**27**	**13**
GORASP1	1.3×10^{-5}	14361252	0.0308	16	27	6
NEBL	1.9×10^{-5}	11787699	0.0309	36	27	11
TRIML2	1.2×10^{-4}	10173978	0.0223	23	27	10
SLC22A11	1.5×10^{-4}	6539656	0.0136	11	27	6
MTRF1	2.8×10^{-4}	9073098	0.0235	10	26	3
CD36	3.5×10^{-4}	8001777	0.0156	28	27	9
CAPRIN2	3.7×10^{-4}	6886375	0.0169	15	27	7
PIK3R6	6.0×10^{-4}	9763336	0.0316	23	26	8

*****The analysis included only nonsynonymous and splice site rare variants (MAF<1%) within the gene regions

†Qmeta: The SKAT Q-statistic, defined as $\sum_{j=1}^{n} w_j S_j$, where w_j is the weight, and S_j is the squared score.

‡CMAF: Cumulative minor allele frequency; SKAT: Sequence Kernel Association Test

The significance level for gene-based tests after Bonferroni correction was $P<0.05/5759=8.7\times10^{-6}$; the two genes that reached this significant cutoff are highlighted in bold font.

Figure Legends

Figure 1. Manhattan plot showing the association between common variants and PR interval from combined ancestry analysis. The x-axis represents the chromosomal position for each SNP, and the y-axis represents the $-\log_{10}$(p-value) of the association with PR interval. The dashed line represents the genome-wide significance cutoff of 5×10^{-8}, and the blue line represents the Bonferroni *P*-value cutoff of 1.3×10^{-6}. Black color represents known loci, whereas red color represents novel loci.

Figure 2. Diagram of sodium voltage-gated channel alpha subunit 10 (SCN10A). Each yellow circle represents a genetic variant with a *P*-value less than the significance cutoff (1.2×10^{-6}). Each red circle represents a genetic variant with a *P*-value greater than the significance cutoff, but less than 0.05.