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Novel Loci Associated with PR Interval in a Genome-Wide Association Study of Ten African American Cohorts

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Abstract:

Background - The PR interval (PR) as measured by the resting, standard 12-lead electrocardiogram (ECG) reflects the duration of atrial/atrioventricular nodal depolarization. Substantial evidence exists for a genetic contribution to PR, including genome-wide association studies that have identified common genetic variants at nine loci influencing PR in populations of European and Asian descent. However, few studies have examined loci associated with PR in African Americans.

Methods and Results - We present results from the largest genome-wide association study to date of PR in 13,415 adults of African descent from ten cohorts. We tested for association between PR (ms) and approximately 2.8 million genotyped and imputed single nucleotide polymorphisms. Imputation was performed using HapMap 2 YRI and CEU panels. Study-specific results, adjusted for global ancestry and clinical correlates of PR, were meta-analyzed using the inverse variance method. Variation in genome-wide test statistic distributions was noted within studies (lambda range: 0.9–1.1), although not after genomic control correction was applied to the overall meta-analysis (lambda: 1.008). In addition to generalizing previously reported associations with *MEIS1, SCN5A, ARHGAP24, CAV1,* and *TBX5* to African American populations at the genome-wide significance level ($P<5.0\times10^{-8}$), we also identified a novel locus: *ITGA9*, located in a region previously implicated in *SCN5A* expression. The 3p21 region harboring *SCN5A* also contained two additional independent secondary signals influencing PR ($P<5.0\times10^{-8}$).

Conclusions - This study demonstrates the ability to map novel loci in African Americans as well as the generalizability of loci associated with PR across populations of African, European and Asian descent.

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Key words: electrocardiography; epidemiology; GWAS; single nucleotide polymorphism genetics; PR interval

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Introduction

The PR interval (PR) is an electrocardiographic measurement of atrial conduction spanning the onset of sinus depolarization through the atrioventricular node. PR is a predictor of incident atrial fibrillation,[1] a common cardiac arrhythmia,[2] and a potent risk factor for pacemaker implantation, heart failure, stroke, and all-cause mortality.[1, 3] Substantial evidence exists for a genetic contribution to PR. Family-based studies have estimated the heritability of PR at approximately 34%[4, 5] and rare sodium channel mutations associated with atrial cardiac conduction defects have been characterized.[6, 7] Recent genome-wide association (GWA) studies performed in populations of European and Asian descent have identified common polymorphisms at nine loci that are associated with variation in PR.[5, 8-11] For example, *ARHGAP24*, *CAV1*, *SCN10A,* and *TBX5* have been reported in at least two PR GWA studies.

To date, the majority of GWA studies examining PR were performed in populations of European or Asian descent. The exception is a report by Smith and colleagues (2011),[12] which generalized four previously described PR loci identified in European and Asian populations (*SCN5A*, *SCN10A*, *MEIS1*, and *TBX5*) to 6,247 African American participants from four cohorts. However, Smith and colleagues neither detected novel associations nor identified genome-wide significant associations with several previously replicated loci, including *ARHGAP24*, *CAV1* and *WNT11*.[9, 10] It is therefore unclear whether these loci are relevant in African Americans. Additionally, the increased genetic diversity in populations of African descent provides opportunities for the identification of novel variants influencing PR. Epidemiologic studies have also reported that PR is longer in individuals of African compared to European ancestry,[13, 14] which provides additional motivation for GWA studies of PR in populations of African descent.

To further characterize genetic determinants of PR in populations of African descent, we

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extended the earlier efforts of Smith et al.[12] by including GWA study data from six additional African American cohorts (7,168 additional participants). These results were meta-analyzed with those previously reported by Smith et al. to provide the largest GWA study of PR to date in populations of African ancestry.

Results

We performed a GWA analysis of PR in 13,415 adults of African descent from ten cohorts, including three studies from the Continental Origins and Genetic Epidemiology Network (COGENT)[15] and four studies from the Candidate-gene Association Resource (CARe) consortia.[16] Four of the ten studies were included in the earlier study by Smith et al.:[12] the Atherosclerosis Risk in Communities (ARIC) Study, the Cleveland Family Study (CFS), the Jackson Heart Study (JHS), and the Multi-Ethnic Study of Atherosclerosis (MESA). Variation in study size was noted across cohorts (range: 191 – 4,149 participants) and the largest contributing study was composed entirely of females (Table 1). Across studies, participants were predominantly female (72%), middle-aged (overall mean age: 58 years), obese (overall mean body mass index (BMI): 31 kg/m^2) and pre-hypertensive (overall mean systolic blood pressure (SBP): 130 mmHg). Modest evidence of test statistic inflation was noted for the family-based CFS (λ: 1.10) and JHS (λ: 1.08), although inflation was neither observed in the remaining studies (λ range: 0.95, 1.04) nor in the overall meta-analysis after genomic control was applied (λ: 1.008) (Supplemental Figure 1). A total of 2.8 million genotyped and imputed autosomal SNPs were available for analysis after applying genotyping and imputation quality control measures (Supplemental Table 1).

In the meta-analysis, 90 SNPs at six loci were associated with PR at the genome-wide significance threshold of $P < 5.0 \times 10^{-8}$ after applying genomic control (Figure 1, Table 2). The

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strongest primary PR signal ($P = 5.26 \times 10^{-43}$, primary signals defined as the locus-specific SNP with the lowest *P*-value), was observed for rs3922844 in *SCN5A* (effect allele frequency (AF) = 0.58), and corresponded to a 4.5 ms decrease in PR per copy of the C allele (Figure 2c). We also identified two independent secondary signals at *SCN5A/10A*, a region characterized by low patterns of linkage disequilibrium (LD) and multiple recombination peaks (Figure 2C); one in *SCN5A* and a second in *SCN10A*, which was located 14.3 kb downstream of the *SCN5A* primary signal (Table 2, secondary signals defined as the locus-specific SNP with the lowest genome-wide significant *P*-value after conditioning on primary signals and successive secondary signals). Estimates for the eight signals (six primary; two secondary) were generally consistent across cohorts (Supplemental Table 2), and there was little evidence of among-study heterogeneity (Cochran's Q $P \geq 0.05$). The primary signals also were robust to adjustment for local ancestry (Supplemental Table 3).

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Five of the loci associated with PR were previously identified in populations of European and Asian descent: *SCN5A*/*SCN10A, MEIS1, ARHGAP24, CAV1,* and *TBX5*. Of note, *SCN5A*/*SCN10A, MEIS1,* and *TBX5* were also reported by the earlier PR GWA study of African Americans.[12] The novel locus, *ITGA9*, was located on chromosome 3, greater than one Mb upstream from the primary *SCN5A* signal. Several genes resided nearby *ITGA9*, although only *ITGA9* and *C3orf35* harbored SNPs in strong to moderate LD with rs267567.

None of the primary or secondary signals reported here were the same as the index SNPs reported in populations of European or Asian ancestry. Although we identified both a primary and secondary *SCN5A* signal for PR, only one study of European ancestral populations identified *SCN5A*,[10] and this study reported an index SNP (rs11708996) that was monomorphic in HapMap YRI. The *SCN10A* SNP that we identified (rs6801957) was in low LD with both previously

identified *SCN10A* variants (rs6800541 and rs6795970 ($r^2 < 0.10$, HapMap YRI)), which were reported in European ancestral populations. The *MEIS1* index SNP rs3891585 was in moderate LD with the previously described variant rs11897119 ($r^2 = 0.62$, HapMap YRI). Of the two index SNPs reported for *ARHGAP24* in populations of European descent, rs7692808 was in high LD ($r^2 = 0.94$, HapMap YRI) and rs7660702 was in low LD ($r^2 = 0.22$, HapMap YRI) with our *ARHGAP24* primary signal. Both studies that previously identified *CAV1* as a PR-associated locus reported the rs3807989 variant; this SNP was in very high LD with the primary *CAV1* SNP presented herein ($r^2 = 1.0$, HapMap YRI). Finally, both GWA studies of PR that identified *TBX5* reported the variant rs1895582, which also was in high LD with our *TBX5* primary signal, rs1895585 ($r^2 = 0.84$, HapMap YRI).

We identified six loci associated with PR in populations of African descent, yet we were unable to confirm associations at genome-wide significance thresholds for three PR loci that were previously identified in individuals of European descent: *NKX2-5*, *WNT11*, and *SOX5*.[8] Although the previously reported chromosome 5 and 11 loci had high minor allele frequencies (MAF) across contributing studies, consistent directions of effect, and little evidence of heterogeneity, neither previously reported index SNP was associated with PR ($P > 0.01$) (Table 3). Of note, all SNPs residing within a 1 Mb region of these loci had *P*-values that exceeded 0.0009 (results not shown). Data for the previously reported *SOX5* index SNP were only available in six contributing studies and the mean estimated MAF was 0.03. The *SOX5* locus also was monomorphic in the HapMap YRI population and all *P*-values within 1 Mb of this locus exceeded 0.0002 (results not shown).

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Discussion

This GWA study and meta-analysis of ten cohorts represents the largest effort in populations of

African descent to identify genetic determinants of PR. By building on recent work from the CARe consortium,[12] we identify three additional loci associated with PR in African ancestral populations; *ARHGAP24, CAV1,* and *ITGA9*. The *ITGA9* locus represents a novel finding, having not been identified in any prior GWA studies of PR to date.

ITGA9 is located approximately 1.1 Mb upstream from *SCN5A* and encodes an alpha integrin, an integral membrane glycoprotein that mediates diverse functions including cell–cell and cell–matrix adhesion, proliferation, and apoptosis.[17, 18] *ITGA9* also has been associated with hypertension[19] and several cancers.[20-22] Although, *ITGA9* has not been previously implicated in atrioventricular conduction, the extended 3p22-24 region has been shown to harbor variants affecting *SCN5A* expression. It is therefore possible that *ITGA9* marks a distal *SCN5A* regulatory element.[23, 24] Interestingly, pathway analysis suggests a role for *ITGA9* in cation binding, hypertrophic cardiomyopathy, and dilated cardiomyopathy.[25] Expression QTL studies also have associated variation in *ITGA9* with *cis* expression data from monocytes[26] and lymphoblastoid cell lines.[27] However, the transferability of associations to cardiac myocyte and conduction tissue warrants further investigation.

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In addition to identifying *ITGA9* as a potential *cis*-regulator of *SCN5A*, we also reported three independent SNPs influencing PR at the 3p21 locus. The 3p21 locus harbors both *SCN5A* and *SCN10A*, which encode integral membrane proteins and tetrodotoxin-resistant voltage-gated sodium channel subunits. The $NA_v1.5$ sodium channel alpha-subunit (encoded by *SCN5A*) is the predominant alpha-subunit expressed in cardiac muscle, and is responsible for the initial upstroke of the action potential in an ECG.[28] *SCN5A* mutations are associated with Brugada syndrome, long-QT syndrome, dilated cardiomyopathy, cardiac conduction disease, idiopathic ventricular fibrillation and atrial fibrillation[28] and have been identified in GWA studies of the

QT[29, 30] and QRS intervals[31] in populations of European descent.

The $NA_v1.8$ sodium channel alpha-subunit (encoded by *SCN10A*) is characterized by a long-duration action potential and preservation of excitability during rapid and sustained stimulation.[32] Seven variants at 3p21 have been previously reported,[8, 10, 12] and by extending the work of Smith et al.,[12] we detected an additional independent signal at genome-wide significance levels. The presence of numerous independent signals at the 3p21 region in African Americans was previously reported by a *SCN5A* candidate gene study in approximately 3,000 JHS participants, who also contributed to this analysis.[33] By including nine additional studies, we validate the previous work by Jeff and colleagues at genome-wide significance levels and identify a neighboring genome-wide significant signal in *SCN10A*. The ability to identify multiple *SCN5A/SCN10A* signals may in part be attributable to the greater nucleotide diversity and lower LD in African populations, as 3p21 is characterized by low LD and high recombination.

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In addition to *SCN5A*, we generalized four additional PR loci to populations of African ancestry: *ARHGAP24*, *MEIS1*, *TBX5,* and *CAV1*, the latter of which was also detected by a GWA study of atrial fibrillation.[34] Yet, the importance of *NKX2-5*, *WNT11*, and *SOX5* in the genetic architecture of PR in African Americans is less clear. Although the "winner's curse" and inflated genetic effect estimates from initial discovery[35] may help explain the inconsistent results, another possibility is that our study was underpowered to detect these loci, especially for the *SOX5* locus. In addition, our analysis was conducted in populations that were predominantly female, obese and pre-hypertensive. The degree to which these characteristics influenced the results presented herein remains unclear.

Several limitations of the present study warrant further consideration in order to inform

future efforts examining the genetic architecture of PR. The first is study heterogeneity, a common limitation of meta-analyses. In our meta-analysis, studies used common measurement protocols for determining PR and its clinical correlates. In addition, statistical assessments of heterogeneity did not suggest large variation in SNP effects across studies. Another limitation is confounding, either from cryptic population stratification or unmeasured PR risk factors. For example, one potential confounder we were unable to consider was atrial size, given widespread unavailability of echocardiographs. However, we adjusted for BMI, height, and systolic blood pressure, the major contributors to left atrial size. Regarding the potential for bias from population substructure, we adjusted for principal components in study-specific regression models and applied genomic control. These approaches are standard in GWA studies, yet the potential for residual confounding to produce either false-negative or false-positive results remains challenging to determine on a genome-wide level. Finally, we were unable to independently replicate the association with *ITGA9* in an independent population given difficulties identifying additional studies of African American participants with ECG measures, extant genotype data, and overlapping analytical timelines. Although results from other ancestral population could provide confirmatory evidence of the association between PR and *ITGA9*, failure to replicate could simply reflect allelic heterogeneity.

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In summary, our results suggest that polymorphisms from six loci on five chromosomes are associated with PR in African Americans, including a novel signal in *ITGA9* that may function as a distal *SCN5A* regulatory element. Our expanded meta-analysis also demonstrates the ability to map novel genes in African Americans and the generalizability of genetic variants associated with PR across global populations. Future work to refine these signals is clearly warranted, including additional examination of the extended chromosome 3p region that harbors

SCN5A, SCN10A and *ITGA9*. GWA studies in other admixed populations, as well as fine-mapping efforts, would be especially useful for further characterization loci identified herein, as well as the identification of new genes influencing atrial arrhythmogenesis.

Materials and Methods

Study populations

A meta-analysis of ten studies was performed to investigate the genetic determinants of PR. Three cohorts were from COGENT including the Health, Aging, and Body Composition Study (Health ABC n=1,054), the Healthy Aging in Neighborhoods of Diversity across the Life Span Study (HANDLS, n=945), and the Women's Health Initiative (WHI, n=4,149), and four cohorts were available from the CARe consortium, including the ARIC study (n=2,391), the CFS (n=267), the JHS (n=1,962), and MESA (n=1,627). The Baltimore Longitudinal Study of Aging (BLSA, n=155), the Bogalusa Heart Study (BHS, n=191), and the Cardiovascular Health Study (CHS, n=674) were the remaining contributing studies. Additional information on the participating studies is provided in the Supplementary Material. All studies were approved by local ethics committees and all participants provided written informed consent.

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PR interval measurement

For each study, certified technicians digitally recorded resting, supine (or semi-recumbent), standard twelve-lead ECGs using comparable procedures for preparing participants, placing electrodes, recording, transmitting, processing and controlling quality (Supplemental Table 4). Participants with the following characteristics were excluded: poor quality ECG, extreme PR (320 ms≤ PR≤80 ms), documented history of atrial fibrillation/flutter, heart failure, myocardial infarction, pacemakers antedating ECG assessment, Wolff-Parkinson-White syndrome, and second/third degree heart block.

Genotype arrays and imputation

Genome-wide SNP genotyping was performed within each cohort using the Affymetrix or Illumina genotyping arrays (Supplemental Table 1). First -degree relatives were excluded in all studies except the family-based CFS and JHS. SNPs were excluded for genotyping call rate thresholds between <95% and <99% and MAF ≤ 1%, the determination of which was study-specific.

Imputation was performed for ~2.5 million autosomal SNPs based on a 1:1 ratio of the HapMap Phase 2 CEU and YRI populations (Supplemental Table 1). SNPs with imputation quality< 0.3 or inconsistent allele designations as per HapMap forward strands were excluded. In addition, SNPs not seen in > 2 studies were excluded from the meta-analyses. After exclusions, 2,845,108 genotyped and imputed SNPs were available.

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Statistical analysis

Each study, with the exception of CFS, performed GWA analysis for PR across approximately 2.5 million SNPs based on linear regression under an additive genetic model. The family-based CFS study was analyzed using linear mixed-effects models as implemented in the R GWAF package.[36] Specifically, the within pedigree random genetic effects were modeled using a kinship coefficient matrix, with each family having a different covariance pattern. The full N x N kinship variance covariance matrix was generated using the R kinship function within the GWAF software package, according to the algorithm of K. Lange.[37] Although the JHS has a limited number of related participants, extensive analyses suggested that results from linear regression or linear mixed effects models were concordant.[15] Therefore, JHS results are based on linear regression models unadjusted for family structure.

The association of each SNP with PR was adjusted for age, sex, height, BMI, systolic

blood pressure, RR interval, and study site, when appropriate, to maintain consistency with Smith et al.[12] All studies included principal components in linear models to adjust for variation in global ancestry (Supplemental Table 1).[38] Genotyped data were substituted for imputed data, when available. Individual study results were corrected by their respective genomic inflation factors (λ);[39] genomic inflation factors > 1 may indicate sample duplications, unknown or poorly specified familial relationships, a poorly calibrated test statistic, systematic technical bias, or gross population stratification.[40]

A fixed effects inverse variance meta-analysis was performed to combine beta coefficients and standard errors from study-level regression results for each SNP. Primary signals were defined as the locus-specific SNP with the lowest genome-wide significant *P*-value ($P < 5 \times 10^{-8}$). Between-study heterogeneity of results was assessed by Cochran's *Q* statistic. Meta-analyses were implemented in the software METAL[41] and were confirmed by an independent analyst.

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A two-stage strategy was used to identify secondary signals. First, LD pruning was performed using PLINK, whereby independent signals were defined as at least two genome-wide significant SNPs in low LD ($r^2 < 0.20$) in the same 1 Mb region. Next, each study performed a conditional analysis by adjusting for the most strongly associated SNP(s) at each locus with at least two bins, restricting to SNPs with *P*-values $< 5.0 \times 10^{-8}$. SNPs outside 1 Mb of the primary signal were not considered in conditional analyses because no loci exhibited LD patterns that extended beyond 1 Mb, and because conditioning on potential mediators may induce bias, the direction and magnitude of which are difficult to predict.[42] Results for secondary signals were presented after conditional adjustment that adjusted for locus-specific primary signals. Additional iterations adjusting for subsequent secondary signals as well as the primary signal

were performed in the WHI, HABC, HANDLS, and CHS cohorts (n=5,768, 43% of sample size) until no genome-wide significant associations remained.

As a sensitivity analysis, we assessed the impact of local ancestry by including SNP-specific local ancestry estimates as a covariate in models for genome-wide significant signals. Locus-specific ancestry (i.e. probabilities of whether an individual has 0, 1, or 2 alleles of African ancestry at each locus) was only available for directly genotyped SNPs and was estimated using a Hidden Markov Model and the local haplotype structure to detect transitions in ancestry along the genome.[43]

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Table 1. Characteristics of 13,415 African-American participants from ten cohort studies.[a]

Variable[b]	ARIC n=2,391	BLSA n=155	BHS n=191	CFS n=267	CHS n=674	HABC[c] n=1,054	HANDLS n=945	JHS n=1,962	MESA n=1,627	WHI[c] n=4,149
PR interval (ms)	172 ± 27	172 ± 25	161 ± 23	169 ± 26	172 ± 29	171 ± 28	162 ± 25	171 ± 26	171 ± 26	167 ± 25
RR interval (ms)	923 ± 150	957 ± 130	896 ± 149	903 ± 131	921 ± 158	931 ± 154	907 ± 154	949 ± 148	975 ± 155	915 ± 146
Age (years)	53.2 ± 8.8	64.4 ± 11.4	35.7 ± 4.8	44.3 ± 15.2	72.6 ± 5.5	73.4 ± 2.9	48.6 ± 9.0	49.3 ± 11.7	62.1 ± 10.1	61.6 ± 6.8
Female sex (%)	1,480 (62)	98 (63)	127 (66)	154 (58)	431 (64)	609 (58)	527 (56)	1,203 (61)	887 (55)	4,149 (100)
BMI (kg/m^2)	29.5 ± 6.1	28.3 ± 5.2	31.5 ± 8.7	34.5 ± 9.2	28.4 ± 5.5	28.5 ± 5.4	29.9 ± 8.1	32.3 ± 7.8	30.2 ± 5.9	31.6 ± 6.2
Systolic BP (mmHg)	128.1 ± 20.7	133.7 ± 15.6	124.3 ± 17.9	126.1 ± 14.4	146.2 ± 21.5	138.7 ± 22.0	120.8 ± 21.9	124.6 ± 17.8	131.6 ± 21.6	131.9 ± 17.3
Genomic inflation factor (λ)	1.023	0.969	0.989	1.099	1.043	1.014	0.947	1.079	1.008	1.010
% European ancestry[d]	15 (11, 22)	ND	18 (13, 21)	18 (13, 26)	24 (16, 36)	19 (12, 28)	16 (11, 22)	16 (12, 21)	19 (12, 30)	21 (13, 31)

[a]Sample sizes presented are the maximum number of participants with SNP data.
[b]Data are presented as mean (standard deviation) for continuous variables and percentages for categorical variables.
[c]The HABC and WHI studies replaced imputed data with genotyped data when available and therefore have a range of genotyped participants (HABC minimum = 939 participants; WHI minimum = 3,898 participants).
[d]Presented as median (25th percentile, 75th percentile)
ARIC, Atherosclerosis Risk in Communities; BLSA, Baltimore Longitudinal Study on Aging; BHS, Bogalusa Heart Study; CFS, Cleveland Family Study; CHS, Cardiovascular Health Study; HABC, The Health, Aging, and Body Composition Study; HANDLS, The Healthy Aging in Neighborhoods of Diversity across the Life Span Study; JHS, Jackson Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis; ND, not determined. WHI, Women's Health Initiative.

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Table 2. Summary of six primary and two secondary independent loci ($P < 5.0x10^{-8}$) obtained for PR in 13,415 African-American participants from ten cohort studies.

SNP	Gene	Chr	Position (Build 36)	Alleles[a]	Effect allele Frequency	Study-specific direction of β[b]	β (se)	*p*	p_{het}
Primary signals[c]									
rs3891585	*MEIS1*	2	66,610,480	A/G	0.43	+-++-+++++	2.13 (0.31)	1.42×10^{-11}	0.11
rs267567	*ITGA9*	3	37,549,028	A/G	0.18	+++-++++++	2.73 (0.41)	4.14×10^{-11}	0.54
rs3922844	*SCN5A*	3	38,599,257	T/C	0.58	----------	-4.54 (0.33)	5.26×10^{-43}	0.58
rs11732231	*ARHGAP24*	4	86,902,584	C/G	0.23	++++++++++	2.28 (0.39)	2.96×10^{-9}	0.30
rs11773845	*CAV1*	7	115,978,537	A/C	0.36	----------	-2.29 (0.33)	4.45×10^{-12}	0.53
rs1895585	*TBX5*	12	113,286,521	A/G	0.30	++-+++++++	3.19 (0.35)	1.36×10^{-19}	0.42
Secondary signals[d]									
rs6763048	*SCN5A*	3	38,656,398	A/G	0.73	+++++++++	2.62 (0.38)	3.75×10^{-12}	0.74
rs6801957	*SCN10A*	3	38,742,319	T/C	0.27	++++	3.36 (0.58)	9.11×10^{-9}	0.15

[a]Coded allele listed first.
[b]Study-specific direction of β estimates are listed in alphabetical order by study. The + and - symbols represent an increase and decrease, respectively, in the PR interval per copy of the minor allele.
[c]Defined as locus-specific SNP with the lowest P-value.
[d]Defined as significant SNPs after conditional analysis that adjusted for locus-specific primary signal. The conditional analysis for rs6801957 was performed in four cohorts (CHS, HABC, HANDLS and WHI) adjusting for successively less significant SNPs until no genome-wide significant SNPs remained.
Chr, chromosome; se, standard error; *p*, meta-analysis *p*-value; p_{het}, Cochran's *Q* heterogeneity *p*-value.

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Table 3. Associations between PR and three previously reported PR loci[10] that were not genome-wide significant in a meta-analysis of 13,415 African-American participants from ten cohort studiesi.

SNP	Gene	Chr	Position (Build 36)	Alleles[a]	Effect Allele Frequency	Study-specific direction of β[b]	β (se)	*p*	p_{het}
rs251253	*NKX2-5*	5	172,412,942	T/C	0.36	-++++++-++	0.77 (0.33)	1.84×10^{-2}	0.53
rs4944092	*WNT11*	11	75,587,267	A/G	0.57	++-+++++-+	0.41 (0.32)	2.05×10^{-1}	0.18
rs11047543	*SOX5*	12	24,679,606	A/G	0.03	-+???---+?	-2.49 (1.25)	4.57×10^{-2}	0.12

[a]Coded allele listed first.
[b]Study-specific direction of β estimates are listed in alphabetical order of the studies. The + and - symbols represent an increase and decrease, respectively, in the PR interval per copy of the minor allele. A "?" denotes studies that did not contribute to the SNP meta-analysis.
Chr, chromosome; se, standard error; *p*, meta-analysis *p*-value; p_{het}, Cochran's *Q* heterogeneity *p*-value.

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Figure Legends:

Figure 1. Manhattan plot of the association of SNPs with PR in a meta-analysis of ten African American cohorts. The x-axis represents the chromosomal position for each SNP, and the y-axis represents the -log10 *P*-value for association with PR, which is truncated at $1x10^{-23}$.

Figure 2. Regional association plots of six loci associated with PR interval in ten African American cohorts. SNP *P*-values (represented by circles) at each locus are shown on the $-\log_{10}$(*P*-value) scale as a function of chromosomal position. Strength of LD is indicated by the color category. Purple diamonds denotes the locus-specific primary signal. Recombination rate is plotted in the background and known genes are shown on the bottom of the plot. A) *MEIS1*; b) *ITGA9*; c) *SCN5A*; d) *ARHGAP24*; e) *CAV1*; f) *TBX5*.

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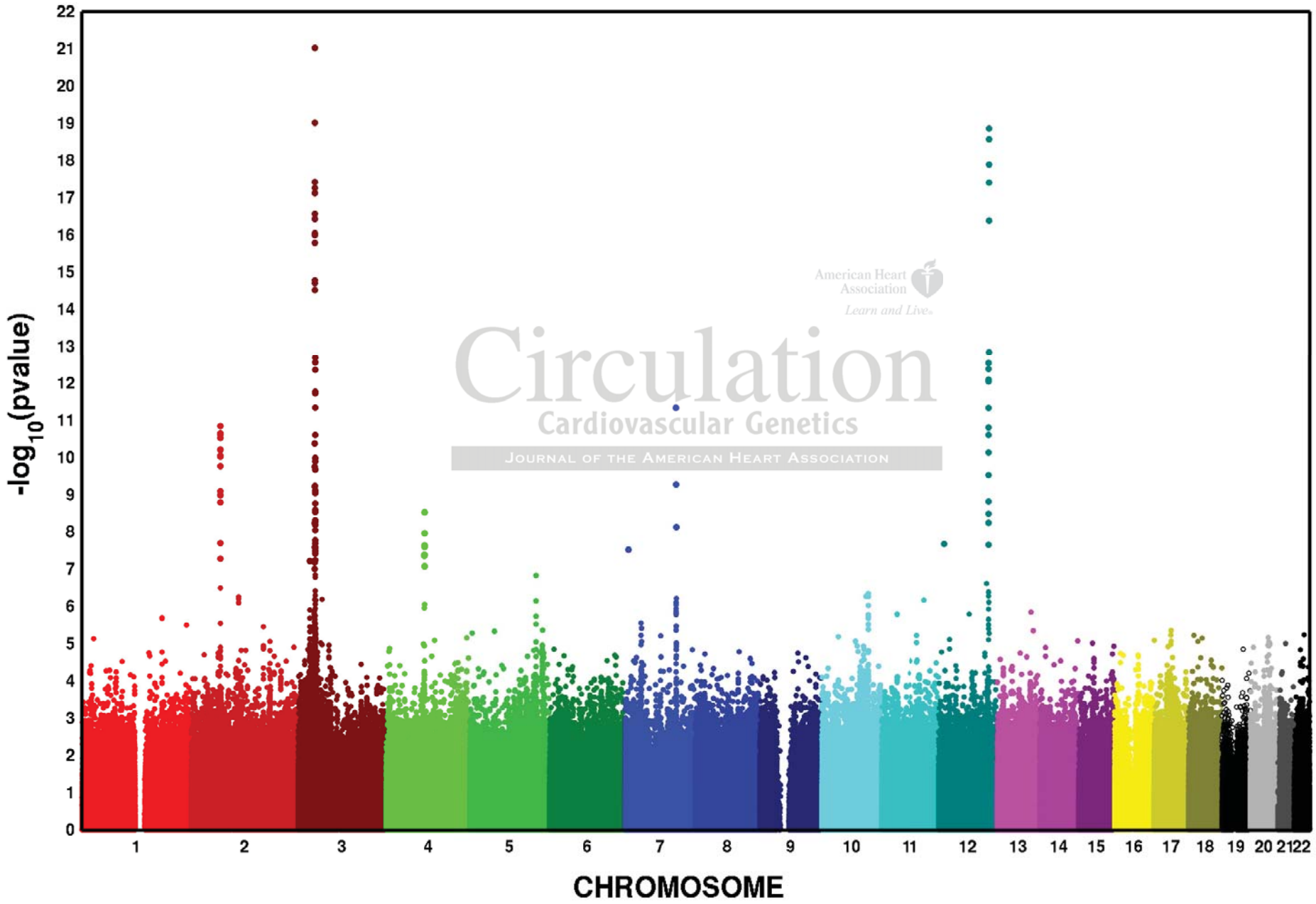

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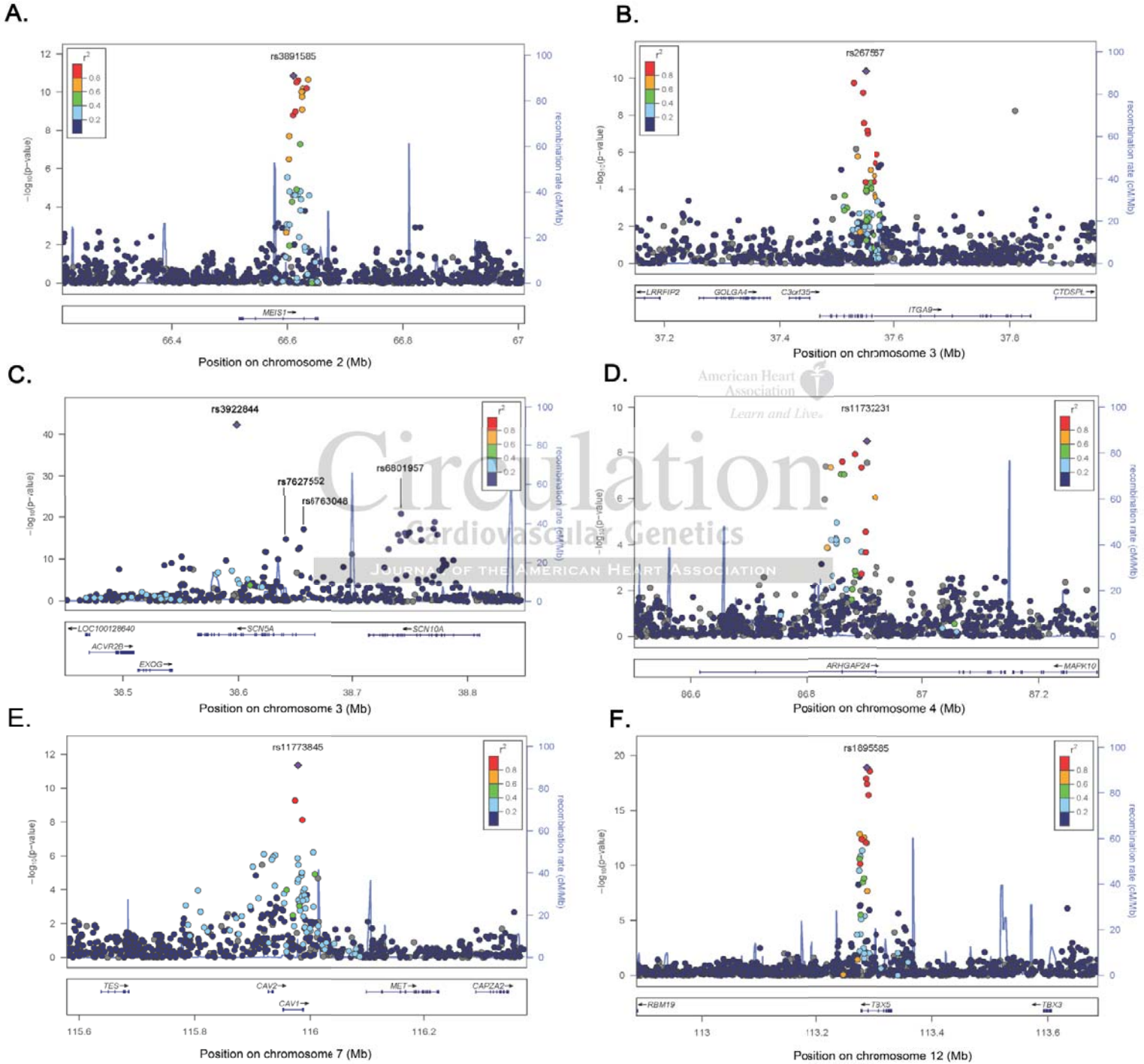

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SUPPLEMENTAL MATERIAL

The supporting information has the following sections in order:

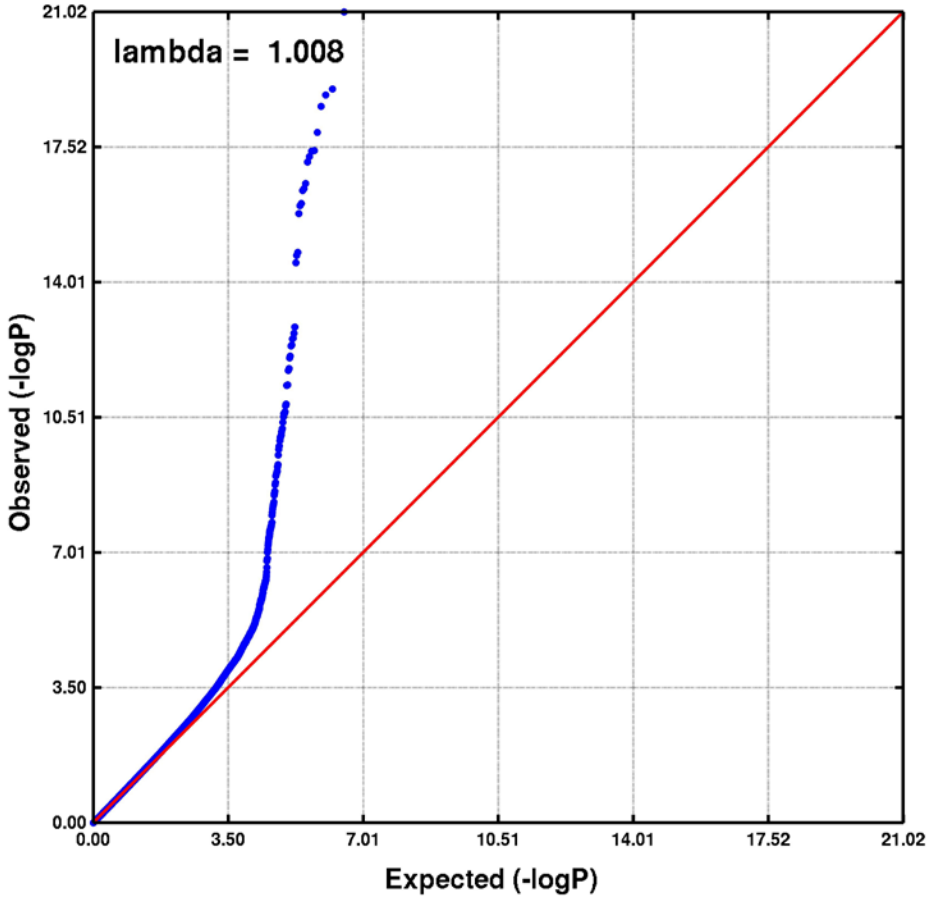
I. Supplemental Figure and Tables

II. Supplemental Materials and Methods

III. Supplemental References

I. Supplemental Figure and Tables

Supplemental Figure 1. Quantile-quantile plot showing the association of SNPs with PR interval in the meta-analysis of ten genome-wide association studies of African Americans. The red line represents the expected distribution of -log10 p-values under the null hypothesis. The blue dots represent the observed -log10 p-values.



Supplemental Table 1. Genotyping characteristics for the genome-wide association analyses of ten studies in the meta-analysis.[a]

Study	Genotyping array	Genotype calling algorithm	Study-specific filters				Imputation software	GWAS statistical analysis software	Imputation quality (Median: 25%, 75%)	N. autosomal SNPs passing QC	Method of PC estimation[e]
			Sample call rate	SNP call rate	SNP MAF[b]	HWE p-value[c]					
ARIC	Affymetrix 6.0	Birdseed	<95%	<90%	<1%	NA	MACH v1.16	PLINK	0.98 (0.91, 1.0)	796,384	EIGENSTRAT, 10 PCs
BLSA	Illumina 550K	BeadStudio	≤98.5%	<99%	<1%	$\leq 10^{-4}$	MACH 1.0	Merlin	0.95 (0.86, 1.0	501,704	EIGENSTRAT, 2 PCs
BHS	Illumina Human610 BeadChip, HumanCVD BeadChip	BeadStudio	<99%	<90%	NA	NA	MACH v1.0.16	PLINK	0.96 (0.89, 0.99)	608,756	LAMP, 10 PCs
CFS	Affymetrix 6.0	Birdseed	<95%	<90%	<1%	NA	MACH v1.16	R	0.99 (0.94, 1.0)	867,495	EIGENSTRAT, 10 PCs
CHS	Illumina HumanOmni1-Quad_v1 BeadChip system	Illumina Genome Studio	<95%	≤97%	NA	$<10^{-5}$	BEAGLE version 3.2.1	R	0.98 (0.94, 1.0)	963,248	R, 10 PCs
HABC	Illumina 1M	BeadStudio	<97%	<97%	<1%	$<10^{-6}$	MACH v1.16	R	0.97 (0.92, 1.0)	1,007,948	EIGENSTRAT, 2 PCs
HANDLS	Illumina 1M[d]	BeadStudio	≤95%	≤95%	≤1%	$\leq 10^{-7}$	MACH v1.16	R, MACH2QTL	0.98 (0.93, 1.0)	907,763	PLINKv1.06, 10 PCs
JHS	Affymetrix 6.0	Birdseed	<95%	<90%	<1%	NA	MACH v1.16	PLINK	0.98 (0.92, 1.0)	868,969	EIGENSTRAT, 10 PCs
MESA	Affymetrix 6.0	Birdseed	<95%	<90%	<1%	NA	MACH v1.16	PLINK	0.99 (0.93, 1.0)	881,666	EIGENSTRAT, 10 PCs
WHI	Affymetrix 6.0	Birdseed	<95%	<95%	<1%	$<10^{-6}$	MACH v1.0.16	PLINK, ProbABEL	0.97 (0.89, 1.0)	829,370	STRUCTURE, 4 PCs

[a]All studies used HapMap phase 2 release 22 build 36 and the HapMap Reference panel 1:1 CEU:YRI phase II.
[b]SNP MAF filter applied pre-imputation.
[c]Genotyped SNP results used to replace imputed SNP results were not filtered by HWE P-value.
[d]1024 HANDLS participants were successfully genotyped at the equivalent of Illumina 1M SNP coverage (709 samples using Illumina 1M and 1Mduo arrays, the remainder using a combination of 550K, 370K, 510S and 240S to equate the million SNP level of coverage).
[e]Software program used to estimate principal components (PC) and the number of top principal components used as covariates in the analysis.
ARIC, Atherosclerosis Risk in Communities; BLSA, Baltimore Longitudinal Study on Aging; BHS, Bogalusa Heart Study; CFS, Cleveland Family Study; CHS, Cardiovascular Health Study; HABC, The Health, Aging, and Body Composition Study; HANDLS, The Healthy Aging in Neighborhoods of Diversity across the Life Span Study; JHS, Jackson Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis; WHI, Women's Health Initiative.

Supplemental Table 2. Summary of study-specific independent genome-wide significant association results for PR interval obtained at 17 independent loci.

SNP	Nearest Gene	Chr	Alleles[a]	Effect Allele Frequency	β[b] (se)									
					ARIC	BLSA	BHS[c]	CFS	CHS	HABC	HANDLS	JHS	MESA	WHI
Primary signals[d]														
rs3891585	*MEIS1*	2	A/G	0.43	2.42 (0.78)	-1.37 (2.69)	0.97 (2.47)	3.51 (2.16)	-0.91 (1.44)	0.29 (1.21)	3.86 (1.11)	3.37 (0.83)	2.69 (0.88)	1.77 (0.54)
rs267567	*ITGA9*	3	A/G	0.18	2.72 (1.07)	6.61 (3.64)	3.01 (3.54)	-1.36 (2.79)	0.92 (1.93)	2.97 (1.57)	1.84 (1.51)	3.53 (1.11)	4.64 (1.17)	2.24 (0.68)
rs3922844	*SCN5A*	3	T/C	0.58	-5.08 (0.84)	-4.56 (3.00)	-4.09 (2.43)	-0.91 (2.46)	-5.35 (1.55)	-3.38 (1.18)	-4.06 (1.13)	-6.14 (0.86)	-4.57 (0.94)	-4.12 (0.57)
rs11732231	*ARHGAP4*	4	C/G	0.23	1.59 (1.00)	4.42 (3.14)	1.89 (3.18)	0.28 (2.89)	2.21 (1.84)	5.08 (1.43)	3.04 (1.31)	0.70 (1.01)	4.08 (1.13)	1.88 (0.64)
rs11773845	*CAV1*	7	A/C	0.36	-0.33 (0.83)	-1.34 (2.83)	-3.66 (2.48)	-4.01 (2.25)	-3.43 (1.62)	-2.29 (1.25)	-1.88 (1.18)	-2.51 (0.88)	-3.07 (0.95)	-2.64 (0.55)
rs1895585	*TBX5*	12	A/G	0.30	2.45 (0.89)	4.61 (3.07)	-0.42 (2.75)	2.78 (2.48)	5.37 (1.67)	4.57 (1.35)	1.08 (1.24)	4.04 (0.92)	2.90 (1.01)	3.36 (0.59)
Secondary signals[e]														
rs6763048	*SCN5A*	3	A/G	0.73	2.25 (0.93)	7.13 (3.26)	4.61 (2.91)	3.70 (2.6)	4.20 (1.84)	2.02 (1.42)	3.89 (1.38)	3.92 (0.94)	2.95 (1.06)	3.19 (0.64)
rs6801957	*SCN10A*	3	T/C	0.17	---	---	---	---	0.97 (2.13)	3.34 (1.69)	6.31 (1.52)	---	---	2.96 (0.72)

[a]Coded allele listed first.
[b]Beta (β) estimates represent the difference in PR interval (milliseconds) per copy of the minor allele, adjusted for the covariates in the model. ARIC, Atherosclerosis Risk in Communities; BLSA, Baltimore Longitudinal Study on Aging; BHS, Bogalusa Heart Study; CFS, Cleveland Family Study; CHS, Cardiovascular Health Study; HABC, The Health, Aging, and Body Composition Study; HANDLS, The Healthy Aging in Neighborhoods of Diversity across the Life Span Study; JHS, Jackson Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis; WHI, Women's Health Initiative.
[c]The Bogalusa Heart Study did not have information on two SNPs (rs10049479 and rs7627552).
[d]Defined as locus-specific SNP with the lowest P-value.
[e]Defined as significant SNPs after conditional analysis that adjusted for locus-specific primary signal. This approach was performed adjusting for successively less significant SNPs until no genome-wide significant SNPs were identified.
Chr, chromosome; se, standard error.

Supplemental Table 3. Local ancestry analyses of primary and secondary PR loci that reached genome-wide significance ($P < 5.0 \times 10^{-8}$) across up to seven studies of African American participants.*

					β (se)[b]								Pre-Local Ancestry Analysis	Post-Local Ancestry Analysis	
SNP	Nearest Gene	Chr	Alleles[a]	Effect Allele Frequency	ARIC	CFS	HABC	HANDLS	JHS	MESA	WHI	N	*p*	β (se)	*p*
Primary signals[c, d]															
rs3891585	*MEIS1*	2	A/G	0.43	2.48 (0.78)	3.49 (2.22)	-0.12 (1.19)	3.85 (1.11)	3.44 (0.83)	2.66 (0.88)	1.81 (0.54)	12,395	7.28×10^{-13}	2.36 (0.32)	3.57×10^{-13}
rs267567	*ITGA9*	3	A/G	0.18	1.86 (1.13)	-2.57 (3.02)	2.76 (1.61)	1.84 (1.50)	3.80 (1.21)	3.36 (1.25)	1.73 (0.76)	12,395	1.31×10^{-10}	2.26 (0.46)	7.61×10^{-07}
rs3922844	*SCN5A*	3	A/G	0.58	--	--	-3.00 (1.23)	-4.05 (1.13)	--	--	--	1,999	5.03×10^{-06}	-3.57 (0.49)	1.78×10^{-05}
rs11732231	*ARHGAP4*	4	C/G	0.23	--	--	4.00 (1.65)	3.10 (1.31)	--	--	--	1,999	4.25×10^{-05}	3.45 (1.03)	7.68×10^{-04}
rs11773845	*CAV1*	7	A/C	0.36	-0.35 (0.86)	-4.56 (2.34)	-2.69 (1.30)	-1.80 (1.17)	-2.40 (0.90)	-3.12 (0.97)	-3.19 (0.58)	12,395	8.46×10^{-11}	-2.45 (0.35)	3.09×10^{-12}
rs1895585	*TBX5*	12	A/G	0.30	--	--	4.56 (1.33)	1.23 (1.23)	--	--	3.39 (0.59)	6,148	2.00×10^{-10}	3.20 (0.49)	9.15×10^{-11}

[a]Coded allele listed first.
[b]Local ancestry estimates were not available for three studies (Baltimore Longitudinal Study of Aging, Bogalusa Heart Study, and Cardiovascular Health Study).
[c]Defined as locus-specific SNP with the lowest P-value.
[d]Secondary signals were not presented because no cohort had local ancestry estimates for all relevant *SCN5A*/*SCN10A* loci.
ARIC, Atherosclerosis Risk in Communities; CFS, Cleveland Family Study; CHS, Cardiovascular Health Study; HABC, The Health, Aging, and Body Composition Study; HANDLS, The Healthy Aging in Neighborhoods of Diversity across the Life Span Study; JHS, Jackson Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis; WHI, Women's Health Initiative.
Chr, chromosome; se, standard error; *p*, meta-analysis *p*-value.

Supplemental Table 4. Phenotype details for the ten studies in the meta-analysis.

Study	ECG machine	Measurement system
ARIC	Marquette MAC PC	Marquette 12SL
BLSA	GE Marquette MAC5000	Marquette 12SL
BHS	Marquette MAC PC	Marquette 12SL
CFS	Marquette MAC6	Marquette 12SL
CHS	Marquette MAC PC	Marquette 12SL
HABC	Marquette MAC PC	Marquette 12SL
HANDLS	GE Marquette MAC5500	Marquette 12SL
JHS	Marquette MAC PC	MC MEANS
MESA	Marquette MAC1200	Marquette 12SL
WHI	Marquette MAC PC	Marquette 12SL

ARIC, Atherosclerosis Risk in Communities; BLSA, Baltimore Longitudinal Study on Aging; BHS, Bogalusa Heart Study; CFS, Cleveland Family Study; CHS, Cardiovascular Health Study; HABC, The Health, Aging, and Body Composition Study; HANDLS, The Healthy Aging in Neighborhoods of Diversity across the Life Span Study; JHS, Jackson Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis; WHI, Women's Health Initiative.

II. Supplemental Materials and Methods - Description of Participating Studies

Atherosclerosis Risk in Communities Study (ARIC): The ARIC study is an ongoing, prospective population-based cohort designed to examine the etiology of cardiovascular and pulmonary disease, patterns of medical care, and disease variation over time (3). The study includes 15,792 participants selected using probability sampling from four United States communities (Forsyth County NC, Jackson MS, suburban Minneapolis MN, and Washington County MD). Between 1987 and 1989, the study enrolled participants aged 45–64 years. Standardized physical examinations and interviewer-administered questionnaires were conducted at baseline, and at three triennial follow-up examinations. Blood was drawn for DNA extraction and participants consented to genetic testing. After exclusions, 2,391 African Americans with genotype and phenotype data were included in the analysis. This study was approved by the institutional review board at each field center and all subjects provided written informed consent. This analysis was approved by the University of North Carolina at Chapel Hill School of Public Health Institutional Review Board.

Baltimore Longitudinal Study on Aging (BLSA): The BLSA study is a population-based study aimed to evaluate contributors of healthy aging in the older population residing predominantly in the Baltimore-Washington DC area (4, 5). Starting in 1958, participants were examined every one to four years, depending on their age, during visits to the NIA's Gerontology Research Center in Baltimore. Currently there are approximately 1,100 active participants enrolled in the study. Each man received an extensive interim medical and psychological history and physical examination at each visit. Blood samples were collected for DNA extraction, and genome-wide genotyping

was completed for 1,231 participants. After exclusions, 155 African Americans with genotype and phenotype data were included in the analysis. The BLSA has continuing approval from the Institutional Review Board (IRB) of the Johns Hopkins Bayview Medical Center, the Gerentology Research Center, and Medstar Research Institute. Informed consent was obtained from all participants.

Bogalusa Heart Study (BHS): The BHS is a long-term epidemiologic study of cardiovascular risk factors from birth through the age of 38 years in a biracial population with whites and African Americans. Between 1973 and 2008, nine cross-sectional surveys of children aged 4-17 years and 10 cross-sectional surveys of adults aged 18-48 years, who had been previously examined as children, were conducted for cardiovascular disease risk factor examinations in Bogalusa, Louisiana (6). In the ongoing Longitudinal Aging Study funded by NIH and NIA since 2000, there are 1,202 Caucasian and African American participants who have been examined 4-14 times from childhood to adulthood with DNA available for genotyping. After exclusions, 191 African Americans with genotype and phenotype data were included in the analysis. Study protocols were approved by the Institutional Review Board of the Tulane University Medical Center. Informed consent was obtained from all participants.

Cardiovascular Health Study (CHS): The CHS is a population-based, longitudinal cohort designed to study risk factors for cardiovascular disease in the elderly (7). Starting in 1989, 244 African American adults aged 65 years or older were sampled from four United States communities (Forsyth County, North Carolina; Pittsburgh, Pennsylvania;

Sacramento County, California; and Washington County, Maryland). Between 1992 and 1993, a second cohort of 687 African Americans was enrolled. Extensive physical and laboratory evaluations were performed at baseline to identify the presence and severity of CVD risk factors such as hypertension, hypercholesterolemia and glucose intolerance; subclinical disease such as carotid artery atherosclerosis, left ventricular enlargement, and transient ischemia; and clinically overt CVD. In 2010, genotyping was performed for 844 African-American participants who consented to genetic testing and had available DNA. After exclusions, 674 African Americans with genotype and phenotype data were included in the analysis. The institutional review board at each of the study sites approved the study protocols, and written informed consent was obtained from all participants.

Cleveland Family Study (CFS): The CFS is a family-based longitudinal study designed to study the risk factors for sleep apnea (8). Participants include first-degree or selected second-degree relatives of a proband with either laboratory diagnosed obstructive sleep apnea or neighborhood control of an affected proband. ECG recordings used for the present study were performed at the final exam cycle conducted in a Clinical Research Unit between 2001 and 2006. Families were selected for genotyping on the basis of genetic informativity, including multigenerational data or individuals from the extremes of the distribution of apnea phenotype (9, 10). The 632 African Americans with available DNA were genotyped as part of CARe. After exclusions, 267 African Americans with genotype and phenotype data were included in the analysis. The institutional review

board approved the study, and written informed consent was obtained from all participants.

The Health Aging and Body Composition (HABC): The Health ABC Study is a longitudinal study of the factors that contribute to incident disability and the decline in function of healthier older persons, with a particular emphasis on changes in body composition in old age (11, 12). Between 1997 and 1998, 3,075 adults aged 70-79 years were recruited using mass mailings with telephone follow-up. White individuals were identified through a random sample of Medicare-eligible residents and African American individuals were identified from two clinical centers in Pittsburgh, Pennsylvania, and Memphis, Tennessee. Participants had no reported difficulty in walking a quarter of a mile, climbing 10 steps without resting, or performing mobility-related activities of daily living. Exclusion criteria were any life-threatening condition, participation in any research study involving medications or modification of eating or exercise habits, plans to move from the geographical area within 3 years and difficulty in communicating with the study personnel or cognitive impairment. The key components of Health ABC included a baseline exam with blood specimen collection, annual follow-up clinical exams, and phone contacts every 6 months to identify major health events and document functional status between clinic visits. After exclusions, 1,054 African Americans with genotype and phenotype data were included in the analysis. The institutional review boards at both clinical centers approved the study, and written informed consent was obtained from all participants.

The Healthy Aging in Neighborhoods of Diversity across the Life Span Study (HANDLS): HANDLS is an interdisciplinary, community-based, prospective longitudinal epidemiologic study examining the influences of race and socioeconomic status (SES) on the development of age-related health disparities in overall longevity, cardiovascular disease, and cognitive decline among socioeconomically diverse African Americans and whites in Baltimore (13). A total of 3,722 (2200 African American and 1522 Caucasian) participants between 30 and 64 years of age were recruited as a fixed cohort of participants by household screenings from an area probability sample of Baltimore based on the 2000 Census. Data were collected in two separate phases. Phase 1 consisted of screening, recruitment, a household interview (assessing sociodemographic information and physiological and psychological chronic exposure) and a first 24 hour recall; phase 2 consisted of an in-depth examination in a mobile medical research vehicle (MRV) and included a second dietary assessment with a 24 hour recall, psychometric measures (e.g. for depressive symptoms and cognitive function), anthropometric and body composition measurements. Genotyping was performed for 1,024 participants who self-report as African Americans. After exclusions, 945 African Americans with genotype and phenotype data were included in the analysis. The study protocol was approved by the human subjects review boards at both MedStar Research Institute and the University of Delaware. All participants provided written informed consent.

Jackson Heart Study (JHS): The JHS is a prospective population-based study of the causes of the high prevalence of common complex diseases among African Americans

in the Jackson, Mississippi metropolitan area, including cardiovascular disease, type-2 diabetes, obesity, chronic kidney disease, and stroke (14). Between 2000 and 2004, 5,302 adult African American residents of a tri-county area near Jackson, Mississippi (Hinds County, Rankin County and Madison County) were recruited from four sources, including (1) randomly sampled households from a commercial listing; (2) ARIC participants; (3) a structured volunteer sample that was designed to mirror the eligible population; and (4) a nested family cohort. Unrelated participants were between 35 and 84 years old, and members of the family cohort were ≥21 years old when consent for genetic testing was obtained and blood was drawn for DNA extraction. ECG recordings and DNA extraction were performed at the baseline examination. Based on DNA availability, appropriate informed consent, and genotyping results that met quality control procedures, genotype data were available for 3,030 individuals, including 885 who were also ARIC participants. In the current study, JHS participants who were also enrolled in the ARIC study were analyzed with the ARIC dataset. After exclusions, 1,962 African Americans with genotype and phenotype data were included in the analysis. The institutional review board approved the study protocol, and written informed consent was obtained from all participants.

Multi-Ethnic Study of Atherosclerosis (MESA): MESA is a multicenter, population-based cohort initiated to investigate subclinical cardiovascular disease and the risk factors that predict progression to clinically overt cardiovascular disease (15). Between 2000 and 2002, the study enrolled 6,814 asymptomatic males and females aged 45–84 from six US field centers (Baltimore, MD; Chicago, IL; Los Angeles, CA; New York, NY; St. Paul,

MN; Winston-Salem, NC). Those with a history of CVD (defined as physician-diagnosed myocardial infarction, angina, heart failure, stroke, transient ischemic attack or history of invasive procedure for CVD) were excluded from participation. Approximately 28% of the sample was African American participants. ECG recordings and blood for DNA extraction were obtained at the baseline examination. After exclusions, 1,627 African Americans with genotype and phenotype data were included in the analysis. This study was approved by the institutional review boards of each study site, and written informed consent was obtained from all participants.

Women's Health Initiative (WHI): The WHI is a large and complex clinical investigation of strategies for the prevention and control of some of the most common causes of morbidity and mortality among postmenopausal women, including cancer, cardiovascular disease, and osteoporotic fractures. The WHI comprises both randomized clinical trials (CT) and an observational study (OS). This study is limited to WHI CT participants, as ECGs were not available for WHI OS participants. The WHI clinical trials were designed to allow randomized, controlled evaluation of estrogen with or without progestin treatment, calcium/vitamin D supplementation, and dietary modification on the risk of breast and colorectal cancer, cardiovascular disease, and bone fractures (16). Between 1993 and 1998, the trials enrolled 68,132 postmenopausal women aged 50–79 years who were followed at one of 75 US examination sites (including satellites, remote sites, and their changes in location). Women were ineligible if they had medical conditions predictive of survival time less than 3 years, if they were known to have conditions inconsistent with study participation and adherence, or if they

were active participants in another randomized, controlled trial. Those who remained eligible and interested were invited to follow-up examinations at 1, 3, 6, and 9 years. Of the CT and OS minority participants enrolled in WHI, the WHI SNP Health Association Resource (SHARe) GWA study project includes 12,157 (8,515 self-identified African American and 3,642 self-identified Hispanic) women who consented to genetic research. DNA was extracted by the Specimen Processing Laboratory at the Fred Hutchinson Cancer research Center (FHCRC) using specimens that were collected at enrollment. Specimens were stored at -80°C. After exclusions, 4,149 African Americans with genotype and phenotype data were included in the analysis. Study protocols and consent forms were approved by the Institutional Review Boards at all participating institutions.

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