Novel Loci Associated With PR Interval in a Genome-Wide Association Study of 10 African American Cohorts

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Background—The PR interval, as measured by the resting, standard 12-lead 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 9 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 10 cohorts. We tested for association between PR (ms) and ≈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 (λ range: 0.9–1.1), although not after genomic control correction was applied to the overall meta-analysis (λ: 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 2 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. **(*Circ Cardiovasc Genet*. 2012;5:639-646.)**

Key Words: electrocardiography ■ epidemiology ■ genome-wide association study ■ PR interval ■ single-nucleotide polymorphism genetics

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 ≈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

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polymorphisms at 9 loci that are associated with variation in PR.[5,8–11] For example, *ARHGAP24*, *CAV1*, *SCN10A,* and *TBX5* have been reported in at least 2 PR GWA studies.

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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 et al (2011),[12] which generalized 4 previously described PR loci identified in European and Asian populations (*SCN5A*, *SCN10A*, *MEIS1*, and *TBX5*) to 6247 African American participants from 4 cohorts. However, Smith et al neither detected novel associations nor identified significant genome-wide 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. Epidemiological studies have also reported that PR is longer in individuals of African compared with 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 extended the earlier efforts of Smith et al[12] by including GWA study data from 6 additional African American cohorts (7168 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 10 cohorts, including 3 studies from the Continental Origins and Genetic Epidemiology Network[15] and 4 studies from the Candidate-Gene Association Resource consortia.[16] Four of the 10 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: 191–4149 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: 31 kg/m^2), and prehypertensive (overall mean systolic blood pressure: 130 mm Hg). Modest evidence of test statistic inflation was noted for the family-based CFS (λ: 1.10) and JHS (λ: 1.08), although inflation was observed in neither the remaining studies (λ range: 0.95, 1.04) nor in the overall meta-analysis after genomic control was applied (λ: 1.008) (online-only Data Supplement Figure I). A total of 2.8 million genotyped and imputed autosomal single-nucleotide polymorphisms (SNPs) were available for analysis after applying genotyping and imputation quality control measures (online-only Data Supplement Table I).

In the meta-analysis, 90 SNPs at 6 loci were associated with PR at the genome-wide significance threshold of $P<5.0\times10^{-8}$ after applying genomic control (Figure 1, Table 2). The strongest primary PR signal ($P=5.26\times10^{-43}$, primary signals defined as the locus-specific SNP with the lowest probability value) was observed for rs3922844 in *SCN5A* (effect allele frequency=0.58) and corresponded to a 4.5-ms decrease in PR per copy of the C allele (Figure 2C). We also identified 2 independent secondary signals at *SCN5A/10A*, a region characterized by low patterns of linkage disequilibrium (LD) and multiple recombination peaks (Figure 2C); 1 in *SCN5A* and the other 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 probability value after conditioning on primary signals and successive secondary signals). Estimates for the 8 signals (6 primary, 2 secondary) were generally consistent across cohorts (online-only Data Supplement Table II), and there was little evidence of among-study heterogeneity

Table 1. Characteristics of 13 415 African American Participants From 10 Cohort Studies*

Variable†	ARIC n=2391	BLSA n=155	BHS n=191	CFS n=267	CHS n=674	HABC‡ n=1054	HANDLS n=945	JHS n=1962	MESA n=1627	WHI‡ n=4149
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, y	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, %	1480 (62)	98 (63)	127 (66)	154 (58)	431 (64)	609 (58)	527 (56)	1203 (61)	887 (55)	4149 (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, mm Hg	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§	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)

ARIC indicates Atherosclerosis Risk in Communities; BLSA, Baltimore Longitudinal Study on Aging; BHS, Bogalusa Heart Study; BMI, body mass index; BP, blood pressure; 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; PR, PR interval; RR, RR interval; and WHI, Women's Health Initiative.

*Sample sizes presented are the maximum number of participants with single-nucleotide polymorphism data.

†Data are presented as mean (SD) for categorical variables, unless otherwise indicated.

‡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 = 3898 participants).

§Presented as median (25th percentile, 75th percentile).

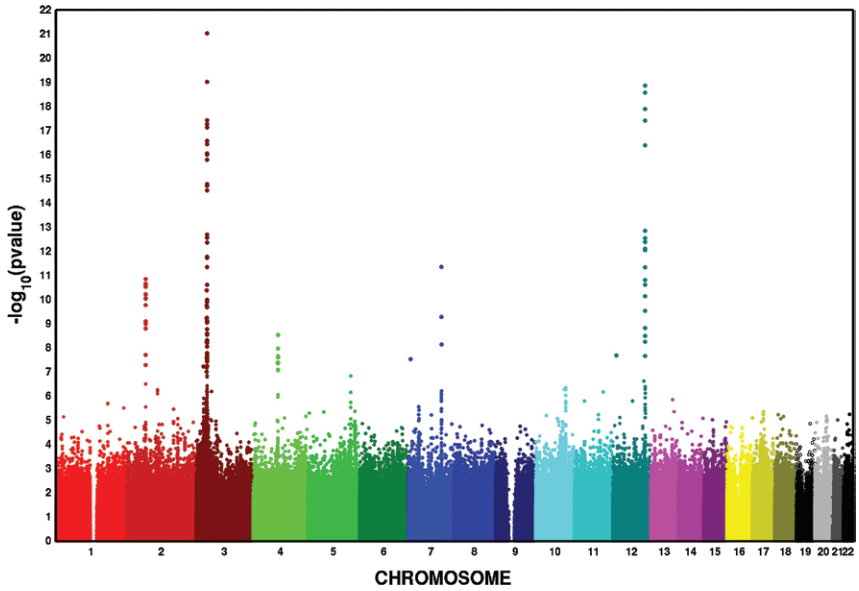

Figure 1. Manhattan plot of the association of single-nucleotide polymorphisms with PR interval in a meta-analysis of 10 African American cohorts. The *x*-axis represents the chromosomal position for each single-nucleotide polymorphism, and the y-axis represents the log10 probability value for association with PR interval, which is truncated at 1×10^{-23}.

(Cochran's *Q* $P\geq0.05$). The primary signals also were robust to adjustment for local ancestry (online-only Data Supplement Table III).

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, >1 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 was the same as the index SNPs reported in populations of European or Asian ancestry. Although we identified both primary and secondary *SCN5A* signal for PR, only 1 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 2 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).

Table 2. Summary of 6 Primary and 2 Secondary Independent Loci ($P<5.0\times10^{-8}$) Obtained for PR Interval in 13 415 African Americans Participants From 10 Cohort Studies

SNP	Gene	Chr	Position (Build 36)	Alleles*	Effect Allele Frequency	Study-Specific Direction of β†	β (SE)	*P*	P_{het}
Primary signals‡									
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§									
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

SNP indicates single-nucleotide polymorphism; Chr, chromosome; PR, PR interval; and P_{het}, Cochran's Q heterogeneity *P* value.

*Coded allele listed first.

†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 per copy of the minor allele.

‡Defined as locus-specific SNP with the lowest *P* value.

§Defined as significant SNPs after conditional analysis that adjusted for locus-specific primary signal. The conditional analysis for rs6801957 was performed in 4 cohorts (CHS, HABC, HANDLS, and WHI) adjusting for successively less significant SNPs until no genome-wide significant SNPs remained.

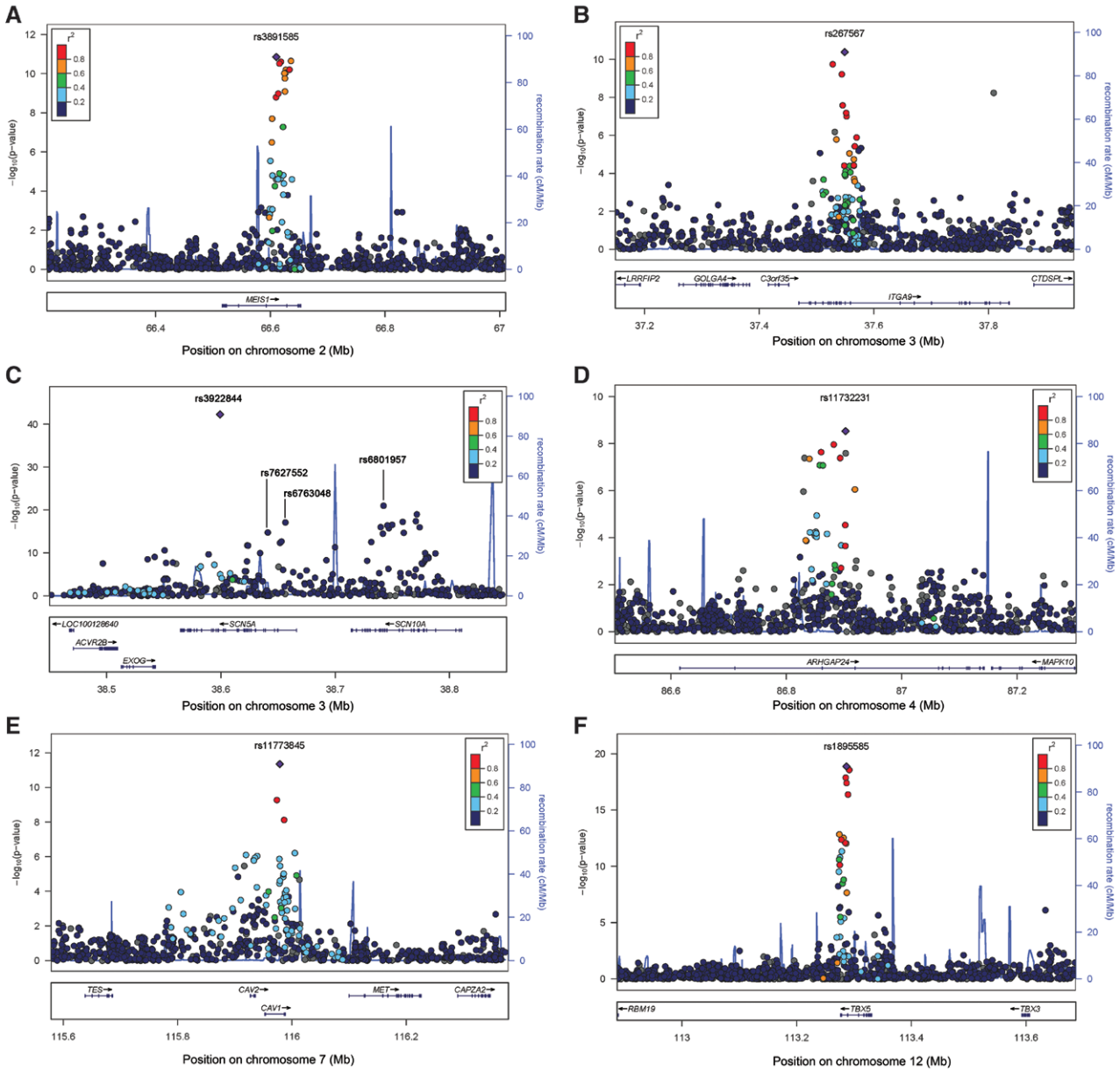

Figure 2. Regional association plots of 6 loci associated with PR interval (PR) in 10 African American cohorts. Single-nucleotide polymorphism (SNP) probability values (represented by circles) at each locus are shown on the $-\log_{10}$(probability value) scale as a function of chromosomal position. Strength of linkage disequilibrium (LD) is indicated by the color category. Purple diamonds denote 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*.

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 6 loci associated with PR in populations of African descent, yet we were unable to confirm associations at genome-wide significance thresholds for 3 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 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 probability values >0.0009 (results not shown). Data for the previously reported *SOX5* index SNP were only available in 6 contributing studies, and the mean estimated minor allele frequency was 0.03. The *SOX5* locus also was monomorphic in the HapMap YRI population, and all probability values within 1 Mb of this locus was >0.0002 (results not shown).

Discussion

This GWA study and meta-analysis of 10 cohorts represents the largest effort in populations of African descent to identify genetic determinants of PR. By building on recent work from the Candidate-Gene Association Resource consortium,[12] we identify 3 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 previous GWA studies of PR to date.

ITGA9 is located ≈1.1 Mb upstream from *SCN5A* and encodes an α 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 quantitative trait loci 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.

In addition to identifying *ITGA9* as a potential *cis* regulator of *SCN5A*, we also reported 3 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 α-subunit (encoded by *SCN5A*) is the predominant α 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 α 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] 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 ≈3000 JHS participants, who also contributed to this analysis.[33] By including 9 additional studies, we validate the previous work by Jeff et al 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.

In addition to *SCN5A*, we generalized 4 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 prehypertensive. The degree to which these characteristics influenced the results presented herein remains unclear.

Several limitations of the present study warrant further consideration to inform future efforts examining the genetic architecture of PR. First, 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, 1 potential confounder we were unable to consider was atrial size, given widespread unavailability of echocardiographs. However, we adjusted for body mass index, 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 analytic 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.

In summary, our results suggest that polymorphisms from 6 loci on 5 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

Table 3. Associations Between PR Interval and 3 Previously Reported PR Loci[10] That Were Not Genome-Wide Significant in a Meta-Analysis of 13 415 African American Participants From 10 Cohort Studies

SNP	Gene	Chr	Position (Build 36)	Alleles*	Effect Allele Frequency	Study-Specific Direction of β†	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

SNP indicates single-nucleotide polymorphism; Chr, chromosome; PR interval (PR); and P_{het}, Cochran's Q heterogeneity *P* value.

*Coded allele listed first.

†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 per copy of the minor allele. A "?" denotes studies that did not contribute to the SNP meta-analysis.

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 of loci identified herein, as well as the identification of new genes influencing atrial arrhythmogenesis.

Materials and Methods

Study Populations

A meta-analysis of 10 studies was performed to investigate the genetic determinants of PR. Three cohorts were from Continental Origins and Genetic Epidemiology Network, including the Health, Aging, and Body Composition (HABC) study (n=1054), the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study (n=945), and the Women's Health Initiative (WHI) (n=4149), and 4 cohorts were available from the Candidate-Gene Association Resource consortium, including the ARIC study (n=2391), the CFS (n=267), the JHS (n=1,962), and MESA (n=1627). The Baltimore Longitudinal Study of Aging (BLSA) (n=155), the Bogalusa Heart Study (BSA) (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 online-only Data Supplement. All studies were approved by local ethics committees, and all participants provided written informed consent.

PR Interval Measurement

For each study, certified technicians digitally recorded resting, supine (or semirecumbent), standard 12-lead ECGs using comparable procedures for preparing participants, placing electrodes, recording, transmitting, processing, and controlling quality (online-only Data Supplement Table IV). 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 (online-only Data Supplement Table I). 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 minor allele frequencies ≤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 (online-only Data Supplement Table I). 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.

Statistical Analysis

Each study, with the exception of CFS, performed GWA analysis for PR across ≈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×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, body mass index, 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 (online-only Data Supplement Table I).[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-effect inverse variance meta-analysis was performed to combine β coefficients and SEs from study-level regression results for each SNP. Primary signals were defined as the locus-specific SNP with the lowest genome-wide significant probability value ($P<5\times10^{-8}$). 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.

A 2-stage strategy was used to identify secondary signals. First, LD pruning was performed using PLINK (PLINK v1.07. http://pngu.mgh.harvard.edu/purcell/plink/), whereby independent signals were defined as at least 2 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 SNPs at each locus with at least 2 bins, restricting to SNPs with probability values $<5.0\times10^{-8}$. 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 the WHI, HABC, HANDLS, and CHS cohorts (n=5768, 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 (ie, 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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Appendix

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CLINICAL PERSPECTIVE

The PR interval, a potent risk factor for arrhythmia, pacemaker implantation, heart failure, stroke, and all-cause mortality, is influenced by many factors, including common and rare genetic variants. Recent genome-wide association studies performed in populations of European and Asian descent have identified several common genetic variants associated with PR. However, limited data exist on loci associated with PR in African Americans. One exception is a PR genome-wide association study by Smith et al including 6247 participants from 4 cohorts. Here, we extend this meta-analysis by including an additional 7168 participants from 6 cohorts to identify novel loci influencing PR in African Americans. In addition to generalizing 4 PR loci in *ARHGAP24*, *MEIS1*, *TBX5*, and *CAV1* to populations of African ancestry, we identified 1 novel signal in *ITGA9* and 2 additional independent and genome-wide significant secondary signals in the 3p21 region that harbors *SCN5A* and *SCN10A*. Our findings highlight the ability to map novel loci in African Americans; the generalizability of loci associated with PR across populations of African, European, and Asian descent; and the new mechanistic insights on biological processes underlying PR.