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Common genetic variation near the Connexin-43 gene is associated with resting heart rate in African Americans: A Genome-wide Association Study of 13,372 participants

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Abstract

Background: Genome-wide association studies have identified several genetic loci associated with variation in resting heart rate in European and Asian populations. No study has evaluated genetic variants associated with heart rate in African Americans.

Objective: We sought to identify novel genetic variants associated with resting heart rate in African Americans.

Methods: Ten cohort studies participating in the CARe and COGENT consortia performed genome-wide genotyping of single nucleotide polymorphisms (SNPs) and imputed 2,954,965 SNPs using HapMap YRI and CEU panels in 13,372 participants of African ancestry. Each study measured the RR interval (ms) from ten-second resting 12-lead ECGs and estimated RR-SNP associations using covariate-adjusted linear regression. Random-effects meta-analysis was used to combine cohort-specific measures of association and identify genome-wide significant loci ($p \leq 2.5 \times 10^{-8}$).

Results: Fourteen SNPs on chromosome 6q22 exceeded the genome-wide significance threshold. The most significant association was for rs9320841 (+13 ms per minor allele, $p=4.98 \times 10^{-15}$). This SNP was approximately 350 kb downstream of *GJA1,* a locus previously identified as harboring SNPs associated with heart rate in Europeans. Adjustment for rs9320841 also attenuated the association between the remaining 13 SNPs in this region and heart rate. In addition, SNPs in *MYH6*, which have been identified in European GWAS, were associated with similar changes in the resting heart rate as this population of African Americans.

Conclusion: An intergenic region downstream of *GJA1,* the gene encoding Connexin-43, the major protein of the human myocardial gap junction and an intragenic region within *MYH6* are associated with variation in resting heart rate in African Americans as well as in populations of European and Asian origin.

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Key Words: African Americans; heart rate; single nucleotide polymorphisms; meta-analysis

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Glossary of Abbreviations

SNPs	single nucleotide polymorphisms
ECG	electrocardiogram
GWAS	genome-wide association study
BMI	body mass index

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Introduction

Multiple studies have found that an elevated resting heart rate is associated with mortality risk[1-5] including that attributable to sudden cardiac death[6] and cardiovascular disease.[7] These findings suggest that function of the sinus node, the dominant pacemaker in the heart, and the autonomic nervous system are associated with adverse clinical outcomes.

Although non-genetic influences of nodal and autonomic function are well known,[8] genetic factors account for 26% to 32% of the variation in resting heart rate in populations of European and Asian ancestry.[9-11] Genome-wide association studies (GWAS) conducted in populations of European and Asian ancestry have recently identified single nucleotide polymorphisms (SNPs) associated with resting heart rate at several loci including *GJA1* on chromosome 6, *MYH6* on chromosome 14, *CD34* on chromosome 1, and *GPR133* on chromosome 12.[12-15] To the best of our knowledge, however, no study has evaluated the association of genetic variants with heart rate among populations of African descent. Such populations have greater genetic diversity compared to those of European and Asian origin, which may facilitate identification of additional associated loci.[16-18] It is also unclear whether loci identified in populations of European and Asian ancestry are relevant in populations of African descent.

In an attempt to identify new loci and evaluate existing, known associations, we examined the association of genetic variants with resting heart rate as measured by the RR interval on the electrocardiogram (ECG) among 10 African American cohort studies participating in the Candidate-gene Association Resource (CARe) and the Continental Origins and Genetic Epidemiology Network (COGENT) ECG consortia.

Methods

Study Populations

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The CARe[19] and COGENT[20] consortia included 13,372 self-reported African Americans meeting inclusion criteria. The participants originated in ten cohort studies: the Atherosclerosis Risk in Communities study (ARIC, n=2,391); Baltimore Longitudinal Study of Aging (BLSA, n=155); Bogalusa Heart Study (BHS, n=148); Cardiovascular Health Study (CHS, n=674); Cleveland Family Study (CFS, n=267); 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); Jackson Heart Study (JHS, n=1,962), Multi-Ethnic Study of Atherosclerosis (MESA, n=1,627); and Women's Health Initiative clinical trials (WHI, n=4,149). Additional information is provided in the Supplemental Methods, including cohort-specific genotype and imputation quality control methods (see supplements 1 and 2). Participants with missing covariates, poor quality ECGs, pacemakers or implantable cardioverter-defibrillators, paroxysmal or persistent atrial fibrillation, heart failure, myocardial infarction, 2nd or 3rd degree atrioventricular block, and extremes of heart rate (>100 or <50 beats per minute) were excluded. Participants on medications altering nodal or atrioventricular conduction (beta blockers, non-dihydropyridine calcium channel blockers, digoxin, type I or III antiarrhythmics) also were excluded.

The study was approved by the Institutional Review Boards at each participating center. Written informed consent was obtained from all participants.

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ECG Recordings

A standard ten-second, resting ECG was obtained and recorded digitally on all participants from the ten cohorts included in this analysis. Standard 12-lead positions were recorded at baseline in all cohort studies using a Marquette MAC PC, MAC6 or MAC1200 ECG machine system (GE Healthcare, Milwaukee, WI, USA). RR interval (ms) was measured electronically as the unit-corrected inverse of heart rate

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(beat/minute). All ECGs were processed automatically using GE Marquette 12-SL version 2001 running under GE Magellan Research Work station or MC Means. The ECG software is FDA approved. Heart rate was calculated from the median RR interval during the 10-second recording. Since ECG recordings were simultaneous in all 12 leads, the rate was not affected by the lead from which the RR interval was recorded. The automated nature of calculating heart rate from the median RR interval ensures the highest repeatability with no inter- or intra- observer variability. Poor quality ECGs were excluded by software algorithms. As an added quality control measure, all ECGs were visually checked.

After a filtering process that results in signal conditioning and averaging, the program generates a median complex. All QRSs of the same shape are aligned in time and the interval measurements depend upon the proper identification of fiduciary points, which are determined from an analysis of all 12 leads simultaneously. The intervals are then measured according to published standards.[21]

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Genotyping and quality control

Genome-wide SNP genotyping was performed within each cohort using genotyping arrays from Affymetrix or Illumina (supplement 2). Studies underwent similar quality control procedures (specific details in the supplemental materials). DNA samples with an array-wide genotyping success rate <95% were excluded. Autosomal heterozygosity rates were estimated to identify and exclude samples with poor DNA quality or contamination. Duplicated or contaminated samples were identified from identity by descent estimates and excluded. In addition, SNPs with a genotyping success rate < 90% per SNP within each cohort, SNPs that map to multiple locations, SNPs where missingness could be predicted from surrounding haplotypes and SNPs

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associated with chemistry plates were excluded. African ancestry was confirmed through either principal components[22] or multidimensional scaling analyses. Population-based (i.e. non-family-based) studies utilized identity-by-descent estimates to exclude cryptically related individuals. Subsequent identical SNP filters after imputation and GWAS analyses were applied to summary statistics at the meta-analysis level.

Imputation and quality control

SNP imputation was performed in each cohort to facilitate the combination of results from different genotyping platforms and to increase genotype coverage. Genotyped SNPs passing quality control metrics described above and reference haplotypes from HapMap Phase 2 (release 22 on NCBI build 36) were utilized to impute ~ 2.5 million SNPs using MACH v1.16[23] or BEAGLE. Untyped SNPs were imputed using a 1:1 ratio of CEU:YRI HapMap reference haplotypes based on consistency across other CARe-COGENT studies. Imputed SNPs were excluded if imputation quality was below 0.30 as reported by MACH or BEAGLE.

Statistical Analysis

GWAS analysis was performed in either PLINK (ARIC, BHS, CHS, JHS, WHI), R (HANDLS, Health ABC, MESA), ProbABEL (WHI) or MERLIN (BLSA) using linear regression with an additive genetic model based on allelic dosages accounting for imputation uncertainty. The family-based CFS study was analyzed using linear mixed-effects models as implemented in the GWAF package for R.[24] Pedigrees for CFS were confirmed using identity by state (IBS) or IBD estimates from PREST-Plus (http://www.utstat.utoronto.ca/sun/Software/Prest/). Previously published analyses indicated that inclusion of related individuals from the JHS family-based sub-cohort had little effect on P-value inflation.[20] As a result, these related individuals were included in

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the present analysis. Eigenvectors were used to adjust for global ancestry in population substructures. Principal components were used to adjust for global ancestry in population stratification.

Cohort-specific genome-wide association was examined on a SNP-by-SNP basis using simple linear models regressing RR (ms) on allele dosage, age, sex, body mass index (BMI), global measures of African ancestry, and when relevant, study site. Cohort-specific SNP association estimates were combined using fixed- and random-effects meta-analysis, the latter to examine potential effects of among-cohort heterogeneity on the combined estimates and the extent to which it can support qualitative inference to other African American populations. Given evidence of greater genetic and geographical diversity across African American cohorts compared to Europeans and initial evidence of heterogeneity across studies, random-effects estimates, which have wider 95% confidence intervals than fixed-effects estimates, were reported in the current meta-analysis. Genomic control methods were applied when study-specific and combined distributions of test statistics suggested early departure from the null (lambda value > 1). Genomic inflation factors were evaluated in each cohort before the random-effects meta-analysis and in the combined results.[25] We calculated X^2 tests of homogeneity (Cochran's Q) using METAL and I^2 estimates with R. Prior to conducting meta-analyses, SNP results with a minor allele frequency <0.01 or imputation quality scores <0.3 were excluded. In addition, SNPs not seen in > 2 studies were excluded from the meta-analyses.

To confirm that the random-effects model was not overly conservative, standard fixed-effects meta-analyses were conducted on SNP association estimates for each cohort using METAL (and incorporating genomic control at the meta-analysis level). For the meta-analysis, we pre-specified a genome-wide significance threshold of 2.5×10^{-8} as suggested for populations of African ancestry,[26] accounting for ~2 million independent

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common variant tests. Other polymorphisms that were detected at the same locus as the initial SNP were subsequently analyzed in conditional regression models to assess statistical independence. Finally, SNPs that have been identified in prior GWAS but not in the discovery phase of our analysis were evaluated using a less stringent threshold. Specifically, we evaluated thirteen genome-wide significant SNPs described by prior RR GWAS in individuals of European and Asian ancestry[13-15] using a significance level of 3.85×10^{-3} (Bonferroni corrected p-value calculated as 0.05/13).

Results

This GWAS of the RR interval included 13,372 adults of African descent from ten cohort studies. Each study contributed a widely varying number of participants (range 148 to 4,149). The ARIC, JHS and WHI studies accounted for the majority of participants in this analysis: 8,502 (64%) of the 13,372. On average, the study population was middle-aged (mean 56.5 [range 35 to 73 years]), obese (mean BMI 30.8 kg/m^2), and 71% were women.

Genomic inflation was minimal in most studies and modest in the family-based CFS (lambda 1.070) and JHS (1.071) (Table 1). Specifically, the lambda estimates from the random-effects meta-analysis did not suggest inflation of the test statistic (0.868), and the secondary fixed-effects modeling did not show a significant departure from null expectations (lambda 1.017) (Figures 1A and 1B).

A total of 2,954,965 SNPs were incorporated into this meta-analysis after data quality control. Fourteen SNPs at a largely intergenic region on chromosome 6q22 (Figure 2) reached genome-wide significance. The most significant association at this locus was for rs9320841 (+13 ms per minor allele, SE 1.7 ms, random effects $P=4.98 \times 10^{-15}$). This SNP is located in a noncoding region, 350kb downstream from *GJA1* and 64 kb upstream from *HMGB3P18*. The magnitude and direction of the association was

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similar across most cohorts ($P_{heterogeneity}$ = 0.45) as shown in Figure 3. None of the other thirteen SNPs in this region were independent variants associated with resting heart rate. The results for the regional association plot at the *GJA1* locus are depicted in Figure 4. This plot covers 1000 kb of the genomic region associated with the *GJA1* locus and demonstrates strong linkage disequilibrium with other SNPs in this gene cluster that were associated with variations in heart rate. Adjustment for rs9320841, however, eliminated the significance of these additional SNPs.

We also evaluated a series of SNPs from the chromosome 6q22 locus that were identified in prior European and Asian GWAS. Both rs9398652 and rs12110693 in the 6q22 locus were associated with the RR interval that were similar to estimates reported in prior studies of Asian[14] and European[13] populations; however, only rs9398652 reached genome-wide significance in the current meta-analysis. The rs9398652 SNP was approximately 30 kb downstream and in high linkage disequilibrium with the leading SNP from the present study (rs9320841; CEU r^2 1.00; YRI r^2 0.81). In addition, rs12110693 was also in strong linkage disequilibrium with rs9320841 (rs9320841; CEU r^2 1.00; YRI r^2 0.76) (Table 2). The final reported SNP from the 6q22 locus, rs11154022, did not reach genome-wide significance, was the greatest distance from rs9320841 (approximately 365 kb upstream) and not in LD with it (CEU r^2 0.01; YRI r^2 0.01).

Other variants that were identified from prior European and Asian GWAS were also tested (Table 2). The 2 SNPs that have been previously identified at the *MYH6* locus (rs452036 and rs365990) were associated with resting heart rate in African Americans using the replication threshold (3.85×10^{-3}). These variants are associated with a similar increase in the sinus cycle length across Europeans, Asians and African Americans. We were unable to confirm associations for several previously published loci at replication thresholds: *CD46* on chromosome 1, *SLC35F1* on chromosome 6, *SLC12A9* and *UfSp1* on chromosome 7, *FADS1* on chromosome 11, an intergenic

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region on chromosome 12, *GPR133* on chromosome 12, and *MYH7* on chromosome 14 (Table 2). These findings were consistent across the different cohorts analyzed through the CARe-COGENT consortium (Figure 5). Further evaluation of these loci (the 1 Mb regions, 500 kb upstream and downstream of the SNPs in Table 2) did not identify any other genome-wide significant RR-SNP associations despite having adequate power (power >0.8).

Discussion

In a large GWAS of African Americans, we generalized a previously reported association between a variant on chromosome 6q22.31 and resting heart rate to a population of African descent. The present findings suggest that rs9320841, which is located in an intergenic region 350 kb downstream from *GJA1,* is the leading SNP at this locus associated with heart rate. In addition, rs9320841 is in high LD with other intergenic SNPs from this region previously associated with heart rate in GWAS in populations of European and Asian ancestry.[13 14]

Multiple studies including the current report have demonstrated intergenic SNPs in proximity to rs9320841 that are associated with variation in heart rate among individuals of Asian, European and African ancestry. The closest putative transcript to rs9320841 on chromosome 6q22.31 is *HMGB3P18*, which has no known function. However, *GJA1,* which is approximately 350 kb upstream of this SNP, encodes connexin 43, the main cardiac gap junction channel that is found throughout the heart and is responsible for intercellular conductance in the atria and ventricles.[27] Connexin 43 is expressed abundantly in the atria and permits the node to conduct impulses to the surrounding muscle.[28] Experimental models have demonstrated that deletion of various gap junction subunits results in a sick sinus syndrome phenotype with bradycardia, sinus dysrhythmia and sinus node exit block.[29, 30] As a result, these intergenic variants in the

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6q22 locus, which are in close proximity to *GJA1* and have been identified across different populations, may reduce sinus automaticity.

Although rs9320841 and previously identified 6q22.31 loci are 300-500 kb away from and in low LD with SNPs in *GJA1,* recent studies suggest that variations in intergenic regions may regulate transcription factor binding and chromatin modification.[31] Functional and translational studies focused on this intergenic region on chromosome 6q22 will be required to understand its potential effect on *GJA1*.

In the portion of our study that restricted the analysis to previously identified variants, we observed an association between two SNPs located within the *MYH6* gene and resting heart rate. *MYH6* encodes one of the myosin heavy chain subunits in the cardiac sarcomere and is a major component of the cardiac contractile system. In addition, *MYH6* encodes a cardiac-specific microRNA, miR-208a, which is a key regulatory molecule that is necessary for normal cardiac conduction.[32] Specifically, miR-208a regulates expression of connexin 40, a gap junction protein that is implicated in sinus automaticity and cardiac arrhythmias.[29, 30, 33] As a result, changes in the *MYH6* genetic architecture could alter micro RNA production, gap junction formation and sinus node function. Prior GWAS in European populations have identified common variants in this gene to be associated with resting heart rate[13, 15] and rare variants, located 0.3 to 4.4 kb from these SNPs, to be associated with sick sinus syndrome.[34] Although the SNPs at the *MYH6* locus were not identified in the discovery phase of our analysis at genome-wide significance thresholds ($p<2.5x10^{-8}$), the similar magnitude and direction of the point estimates in our analysis suggest that the *MYH6* gene affects sinus node automaticity in diverse populations.

While we were unable to replicate associations for other previously published loci at a threshold level of 3.85×10^{-3} (0.05/13), the similar magnitude and direction of the point estimates suggest consistency across ancestries. Specifically, *SLC12A9* and

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UfSp1 on chromosome 7 and the *MYH7* region on chromosome 14 had effects on heart rate similar to those described by prior studies. Compared to individuals of European ancestry, however, African Americans have greater genetic diversity,[18] which may lower the frequency of a particular allele and subsequently reduce the statistical likelihood of detecting an effect on the RR interval. In addition, linkage disequilibrium is commonly lower in African Americans[35] and subsequently reduces the likelihood that a common SNP is in linkage disequilibrium with a causal variant. Further, these analyses were conducted in a population that was predominantly female, middle-aged and overweight. This demographic profile differs from that of prior studies and may have influenced the results.

A common limitation of meta-analyses is among-study phenotype heterogeneity; however, the current study followed similar electrocardiographic and clinical protocols when measuring heart rate and its correlates. In addition, the statistical assessment of heterogeneity did not suggest large variation in SNP effects across studies. Moreover, the random-effects meta-analysis of these effects was weighted for both their within- *and* among-study variation. Another limitation of GWAS is potential for population stratification, including confounding by ancestry. However, we attempted to minimize bias from population structure by excluding participants of non-African ancestry, adjusting for principal components in study-specific regression models, and applying genomic control methods.

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Conclusion

In summary, the genome-wide significance of an association linking resting heart rate and the *GJA1* locus previously described in European and Asian populations has now been generalized to African Americans. In addition, this analysis has replicated associations initially discovered in Europeans between common variants within the

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MYH6 gene and a reduction in heart rate to an African American population. Generalizability across global populations and biological plausibility of the heart rate - *GJA1* and heart rate – *MYH6* associations highlight the potential importance of these loci in the intrinsic (nodal and myocardial) determination of resting heart rate.

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Table 1 Description of contributing African American cohort studies

Cohort Study	n	Age (y)	Male (%)	BMI (kg/m^2)	HR (beat/min)	RR (ms)	λ
ARIC	2391	53.3 (5.8)	39	29.4 (6.1)	67 (10)	896	1.023
BLSA	155	64.4 (11.4)	37	28.3 (5.2)	63 (8)	952	1.050
BHS	148	35.7 (4.8)	33	31.7 (8.9)	68 (11)	882	1.004
CHS	674	72.8 (5.6)	35	28.4 (5.5)	67 (11)	896	1.005
CFS	267	42.7 (14.9)	43	34.4 (9.3)	68.6 (9)	875	1.070
Health ABC	1054	73.4 (2.9)	45	28.1 (5.3)	66 (8)	909	0.996
HANDLS	945	48.5 (9.0)	44	29.9 (8.1)	67 (11)	896	1.007
JHS	1962	49.3 (11.8)	37	32.4 (7.8)	66 (10)	909	1.071
MESA	1627	61.5 (10.1)	46	30.2 (5.9)	65 (9)	923	1.003
WHI	4149	61.7 (6.9)	0	31.6 (6.2)	66 (8)	909	1.017
All Studies*	13372	56.5	29	30.8	66.3	906	1.029

Mean (standard deviation) is tabulated for age, body mass index (BMI) and heart rate (HR). ARIC, Atherosclerosis Risk in Communities study; BLSA, Baltimore Longitudinal Study on Aging; BHS, Bogalusa Heart Study; CHS, Cardiovascular Health Study; CFS, Cleveland Family Study; Health ABC, Health Aging and Body Composition; HANDLS, 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 clinical trials.

*Sum (n), % (male) and weighted mean (age; BMI; HR; RR, λ) across studies.

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Table 2 Analysis of the SNPs reaching genome-wide significance in previous European and Asian studies

						Random-Effects Analysis		Fixed-Effects Analysis			Previous studies		
SNP	**Chr**	**Locus**	**Position (Build 36)**	**Minor/Major Allele**	**MAF**	**β (SE)**	**P**	**β (SE)**	**P**	**I^2**	**Reference**	**β (SE)**	**P from publication**
rs12731740	1q32	*CD46, C1orf132, CD34*	206091443	T/C	0.03	-6.4 (8.2)	1.0	-6.3 (8.2)	0.45	65.6	Cho 2009	-14.0 (2.3)	2.9×10^{-9}
rs12110693	6q22	*GJA1, HMGB3P18*	122199969	A/G	0.49	-11.4 (1.6)	1.4×10^{-7}	-12.4 (1.7)	2.0×10^{-13}	6.4	Cho 2009	-8.6 (1.4)	1.6×10^{-9}
rs9398652	6q22	*GJA1, HMGB3P18*	122187733	A/C	0.49	-12.8 (1.7)	1.1×10^{-13}	-12.7 (1.7)	6.8×10^{-14}	2.6	Eijgelsheim 2010	-12.6 (1.6)	7.7×10^{-16}
rs11154022	6q22	*GJA1, HMGB3P18*	121790241	A/G	0.13	4.0 (2.8)	0.2	3.9 (2.8)	0.2	0	Eijgelsheim 2010	5.8 (1.1)	3.5×10^{-8}
rs281868	6q22	*SLC35F1*	118680754	A/G	0.44	1.3 (1.8)	0.4	1.4 (1.8)	0.4	0	Eijgelsheim 2010	-6.3 (1.0)	1.5×10^{-10}
rs314370	7q22	*SLC12A9*	100291144	C/T	0.06	-9.4 (3.9)	0.01	-9.4 (3.9)	0.02	0	Eijgelsheim 2010	-7.6 (1.2)	2.3×10^{-10}
rs12666989	7q22	*UfSp1*	100324690	C/G	0.068	-9.4 (3.6)	0.007	-9.4 (3.6)	0.008	0	Eijgelsheim 2010	-7.0 (1.2)	9.4×10^{-9}
rs174547	11q12	*FADS1*	61327359	C/T	0.09	-5.1 (3.0)	0.1	-4.2 (3.2)	0.2	8.01	Eijgelsheim 2010	-6.2 (1.0)	8.2×10^{-10}
rs17287293	12p12	*Intergenic*	24662145	G/A	0.05	1.36 (1.2)	0.8	-3.2 (4.4)	0.5	37.7	Eijgelsheim 2010	8.6 (1.3)	5.7×10^{-11}
rs885389	12q24	*GPR133*	130187715	A/G	0.34	2.2 (1.6)	0.3	1.1 (1.8)	0.6	0	Marroni 2009	-14.0 (2.5)	3.9×10^{-8}
rs452036	14q12	*MYH6*	22935725	G/A	0.38	9.5 (2.0)	1.8×10^{-4}	9.6 (2.0)	7.8×10^{-7}	30.9	Eijgelsheim 2010	7.8 (1.0)	8.1×10^{-15}
rs365990	14q12	*MYH6*	22931651	A/G	0.38	9.8 (2.0)	7.7×10^{-5}	9.8 (2.0)	8.9×10^{-7}	26.5	Eijgelsheim 2010 Holm 2010	7.7 (1.0)	5.4×10^{-14}
rs223116	14q12	*MYH7, NDNG*	23046850	G/A	0.23	4.3 (2.2)	0.05	4.3 (2.2)	0.05	0	Eijgelsheim 2010	7.4 (1.3)	1.1×10^{-8}

β (SE) = difference in RR interval duration per minor allele (standard error), in milliseconds. Chr = chromosome. MAF = minor allele frequency. P = P value.

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Figure Legend

Figure 1. QQ plots of meta-analysis utilizing either random effects (1A) or fixed effects (1B) modeling. The x-axis marks the expected values and the left-hand y-axis marks the observed values. A line originating from the origin and having a slope of 1 is depicted in red.

Figure 2: Manhattan plot of RR associations for all SNPs. The P-values from random-effects meta-analysis of 2,954,965 successfully imputed or genotyped SNPs in ≥ 2 cohorts. Red points=SNPs with $P<2.5 \times 10^{-8}$ (considered genome-wide significant). Orange points = SNPs with P values ranging from less than 1×10^{-5} to 2.5×10^{-8}. Regions containing red points were considered genome-wide significant. SNP=single nucleotide polymorphism.

Figure 3: Forest Plot depicting the effect (beta coefficient) of rs9320841 on RR in milliseconds per allele (95% confidence interval) across the individual cohort studies and overall using random-effects modeling ($I^2=0$).

Figure 4: Regional association plots for the RR interval plotted using P- values estimated from 13,372 African Americans from ten studies. Positions are from NCBI build 36. Linkage disequilibrium and recombination rates are estimated from HapMap phase II data. SNPs are represented by circles. The large blue diamond is the SNP with the lowest P- value. The circle color represents correlation with the top SNP: blue indicates weak correlation, and red indicates strong correlation. Recombination rate is plotted in the background and known genes in the region are shown at the bottom of the plot.

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Figure 5: Forest Plot depicting the effect in milliseconds per allele of SNPs achieving genome-wide significance in European and Asian studies across the individual African American cohorts.

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