Genome-wide association study of heart rate and its variability in Hispanic/Latino cohorts

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BACKGROUND Although time–domain measures of heart rate variability (HRV) are used to estimate cardiac autonomic tone and disease risk in multiethnic populations, the genetic epidemiology of HRV in Hispanics/Latinos has not been characterized.

OBJECTIVE The purpose of this study was to conduct a genome-wide association study of heart rate (HR) and its variability in the Hispanic Community Health Study/Study of Latinos, Multi-Ethnic Study of Atherosclerosis, and Women's Health Initiative Hispanic SNP-Health Association Resource project (n = 13,767).

METHODS We estimated HR (bpm), standard deviation of normal-to-normal interbeat intervals (SDNN, ms), and root mean squared difference in successive, normal-to-normal interbeat intervals (RMSSD, ms) from resting, standard 12-lead ECGs. We estimated associations between each phenotype and 17 million genotyped or imputed single

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nucleotide polymorphisms (SNPs), accounting for relatedness and adjusting for age, sex, study site, and ancestry. Cohort-specific estimates were combined using fixed-effects, inverse-variance meta-analysis. We investigated replication for select SNPs exceeding genome-wide ($P < 5 \times 10^{-8}$) or suggestive ($P < 10^{-6}$) significance thresholds.

RESULTS Two genome-wide significant SNPs replicated in a European ancestry cohort, 1 one for RMSSD (rs4963772; chromosome 12) and another for SDNN (rs12982903; chromosome 19). A suggestive SNP for HR (rs236352; chromosome 6) replicated in an African-American cohort. Functional annotation of replicated SNPs in cardiac and neuronal tissues identified potentially causal variants and mechanisms.

CONCLUSION This first genome-wide association study of HRV and HR in Hispanics/Latinos underscores the potential for even modestly sized samples of non-European ancestry to inform the genetic epidemiology of complex traits.

KEYWORDS Epidemiology; Genetic association studies; Electrocardiogram; Autonomic nervous system; Ion channels/membrane transport

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Introduction

Elevated resting heart rate (HR) is associated with various cardiovascular diseases, including hypertension,[1] acute myocardial infarction,[2,3] and sudden cardiac death.[4] Even at rest, HR fluctuates cyclically because it is autonomically influenced by baroreflex tone, vagal outflow, neurohumoral rhythms, emotion, and other factors.[5] Cyclical HR fluctuation—termed heart rate variability (HRV)—is measured by time– and frequency–domain ECG metrics.[5] The relevance of these metrics for predicting morbidity and mortality independent of HR is well recognized.

Although HR and HRV have been used to estimate cardiac autonomic tone and disease risk in multiethnic populations, their genetic characterization remains incomplete despite substantial heritability.[6,7] The only HRV genome-wide association study (GWAS) reported to date was small (n = 747 related individuals) and used a lenient threshold for statistical significance ($P < 10^{-3}$).[8] Furthermore, because HR GWAS have been conducted in European ancestry,[9] African-American,[10] and Asian[11] populations, the relevance of the identified loci to other populations remains unknown. Cardiovascular genetics may be particularly important for Hispanics/Latinos[12,13] because of their disproportionate burden of cardiac problems. Failure to extend GWAS analyses to diverse populations can reduce their global relevance and represent missed opportunities for discoveries and biologic insights.[14] We report the first GWAS of HR and HRV in Hispanic/Latino populations, providing new information about cardiac autonomic phenotypes in an understudied population.

Methods

HR and HRV

Standard, 12-lead ECGs were digitally recorded from resting, supine, or semirecumbent participants using comparable procedures across cohorts (see Supplemental Methods).

Genotyping

The genotyping platforms and algorithms, single nucleotide polymorphism (SNP) inclusion criteria, quality control, and imputation software are given in Supplementary Table S1. Imputation was based on the 1000 Genomes phase 1 reference panel.[15] We tested only SNPs and no insertions/deletions.

Statistical analysis

GWAS scans of HR (bpm), root mean squared difference in successive, normal-to-normal interbeat intervals (RMSSD, ms), and standard deviation of normal-to-normal interbeat intervals (SDNN, ms) were performed separately for the Hispanic Community Health Study/Study of Latinos (HCHS/SOL), Women's Health Initiative (WHI) Hispanics, and Multi-Ethnic Study of Atherosclerosis (MESA) Hispanics. We combined results from these cohorts via inverse variance-weighted fixed-effects meta-analysis. The P value threshold for genome-wide statistical significance was 5×10^{-8} (the standard GWAS threshold[16]). $P < 10^{-6}$ was considered suggestive. Significant and suggestive loci were assessed in phase 2 only if the minor allele frequency was >1% and the SNP represented a novel locus. We attempted replication separately in African-American and European ancestry cohorts. No independent Hispanic/Latino cohorts were available. The P value threshold for replicating the 5 HRV SNPs was 0.05/10 = 0.005 (5 SNPs evaluated in African-American and European ancestry—10 hypothesis tests). Similarly, the P value threshold for replicating the 2 HR SNPs was 0.05/4 = 0.0125. Phase 2 used 1-sided P values.

We analyzed natural log of RMSSD and SDNN and untransformed HR. Analysis models adjusted for age, sex, body mass index (HR only), and appropriate study-specific covariates (e.g., principal components of ancestry; see Supplemental Methods). HCHS/SOL used mixed models accounting for genetic relatedness among participants and the study's complex sampling design.[17] We investigated genomic inflation using λ_{GC} and quantile–quantile plots of P values. Associated loci were visualized using LocusZoom.[18] Meta-analysis results are available on dbGaP (https://www.ncbi.nlm.nih.gov/gap; accession number phs000930).

Participants were excluded from analysis for atrial fibrillation, heart failure, angina, pacemaker implantation, ectopic beats, poor-quality ECG, too few intervals for calculating HRV, HR <40 or HR >120, or use of tricyclic antidepressant or antiarrhythmic medications. HR analyses also excluded participants taking beta-blockers. Jackson Heart Study (phase 2 cohort) also excluded participants taking digoxin or anticholinergic medications.

SNPs were excluded from phase 1 analysis if the effective count of the minor allele was <30 or imputation quality

Table 1 Characteristics of phase 1 discovery cohorts (Hispanic/Latino) and phase 2 replication cohorts (European and African-American) for GWAS of HRV phenotypes

Ancestry	Cohort	N	Age (years) [mean (SD)]	Female (%)	RMSSD (ms)	RMSSD λ_{GC}	SDNN (ms)	SDNN λ_{GC}
Hispanic/Latino	HCHS/SOL	10,830	45.2 (13.6)	59.4	36 (29)	1.02	30 (23)	1.02
	WHI	1525	59.7 (6.4)	100	22 (18)	1.02	20 (15)	1.01
	MESA	1412	61.4 (10.2)	51.7	26 (23)	0.97	22 (17)	0.97
European	WHI	4730	67.4 (6.2)	100	21 (21)	NA	19 (16)	NA
African-American	JHS	1428	59.7 (11.7)	61.6	31 (26)	NA	26 (20)	NA
	MESA	1480	62.2 (10.1)	54.1	33 (28)	NA	27 (20)	NA

was poor (RSQ $\leq$0.3). Effective count was defined as 2*MAF*(1 – MAF)*n*oevar, where oevar measures imputation quality.

We examined whether SNP associations with HR from GWAS in European ancestry generalize to Hispanics/Latinos.[9] The approach summarizes the evidence with an r value and rejects the generalization null hypothesis for r $<$0.05, controlling the false discovery rate at 5%.[19] We further investigated generalization with a figure comparing reported associations to Hispanic/Latino results.

We interrogated replicated loci to identify potentially causal variants. In brief, we assessed whether variants lie within putative regulatory regions identified from ChIP-Seq (chromatin immunoprecipitation followed by sequencing) signals (see Supplemental Methods). We prioritized SNPs within a putative promoter or enhancer that overlapped with a DNaseI hypersensitive site. To hypothesize likely modes of action for these potentially causal variants, we report expression quantitative trait locus (eQTL) targets and/or motifs disrupted by prioritized variants.

Results

The discovery study included 13,767 Hispanics/Latinos from 3 cohorts (13,184 for HR), with HCHS/SOL contributing 84% of participants. Phase 2 included a European ancestry cohort (4,730 participants for HRV traits; 7,073 for HR; females only) and African-American ancestry cohorts (2,908 for HRV traits and 4,771 for HR). Tables 1 and 2 describe the cohorts contributing data to either phase.

Genome-wide association analysis

Meta-analyses of the 3 phase 1 discovery cohorts yielded λ_{GC} values of 1.00, 1.00, and 1.01 for RMSSD, SDNN, and HR, respectively (see Supplementary Table S2).[20] These indices and quantile–quantile plots of *P* values raised no concerns for genomic inflation (Figure 1).

The GWAS scan of RMSSD yielded 2 genome-wide significant loci on chromosomes 12 and 19. The chromosome 19 locus overlapped the sole genome-wide significant locus for SDNN. The chromosome 12 locus approached genome-wide significance for SDNN ($P = 1.2 \times 10^{-7}$). HR analysis yielded 1 genome-wide significant locus on chromosome 14. All 3 traits yielded suggestive loci (Figure 1). We selected 5 HRV SNPs and 2 HR SNPs for phase 2 (Table 3 and Supplementary Table S3). We did not select the genome-wide significant SNP for HR because it overlaps a recognized HR locus.[9]

Both genome-wide significant HRV loci replicated in the European ancestry sample (Table 3; replication $P <.005$). Neither locus was statistically significant in the phase 2 African-American sample, in which power was lower. None of the 3 suggestive HRV loci was statistically significant in phase 2. Of these 3 SNPs, 2 had good imputation quality in phase 2 data, and 1 had modest imputation quality (see Supplementary Table S4).

Of the 2 suggestive HR SNPs, rs236352 on chromosome 6 was significantly associated with HR in the African-American phase 2 sample ($P <.0125$). The other suggestive HR SNP (rs17180489 on chromosome 14) was not significantly associated with HR in either phase 2 cohort. Variant rs17180489 had good imputation quality in phase 1 data but only modest quality in phase 2 (see Supplementary Table S4).

Figures 2 through 4 show regional association plots for the 3 SNPs that replicated in phase 2. Supplementary Figures S1 through S7 are plots for all selected SNPs. Figure 5 summarizes results for the 7 SNPs analyzed in both phases of the study for comparisons of effect sizes across ancestries.

Table 2 Characteristics of phase 1 discovery cohorts (Hispanic/Latino) and phase 2 replication cohorts for GWAS of HR

Ancestry	Cohort	N	Age (years)	Female (%)	HR (bpm) [mean (SD)]	BMI (kg/m^2)	λ_{GC}
Hispanic/Latino	HCHS/SOL	10,245	44.6 (13.5)	58.8	63 (9)	29.5 (5.9)	1.06
	WHI	1527	59.9 (6.4)	100	66 (10)	29.4 (5.5)	1.02
	MESA	1412	61.4(10.2)	51.7	63 (10)	29.5 (5.1)	1.00
European	WHI	7073	66.2 (6.6)	100	67 (10)	28.3 (5.6)	NA
African-American	JHS	1424	59.7 (11.7)	61.6	64 (10)	32.1 (7.3)	NA
	WHI	3347	60.7 (6.7)	100	68 (11)	31.5 (6.4)	NA

BMI = body mass index; GWAS = genome-wide association study; HCHS/SOL = Hispanic Community Health Study/Study of Latinos; HR = heart rate; HRV = heart rate variability; JHS = Jackson Heart Study; MESA = Multi-Ethnic Study of Atherosclerosis; WHI = Women's Health Initiative.

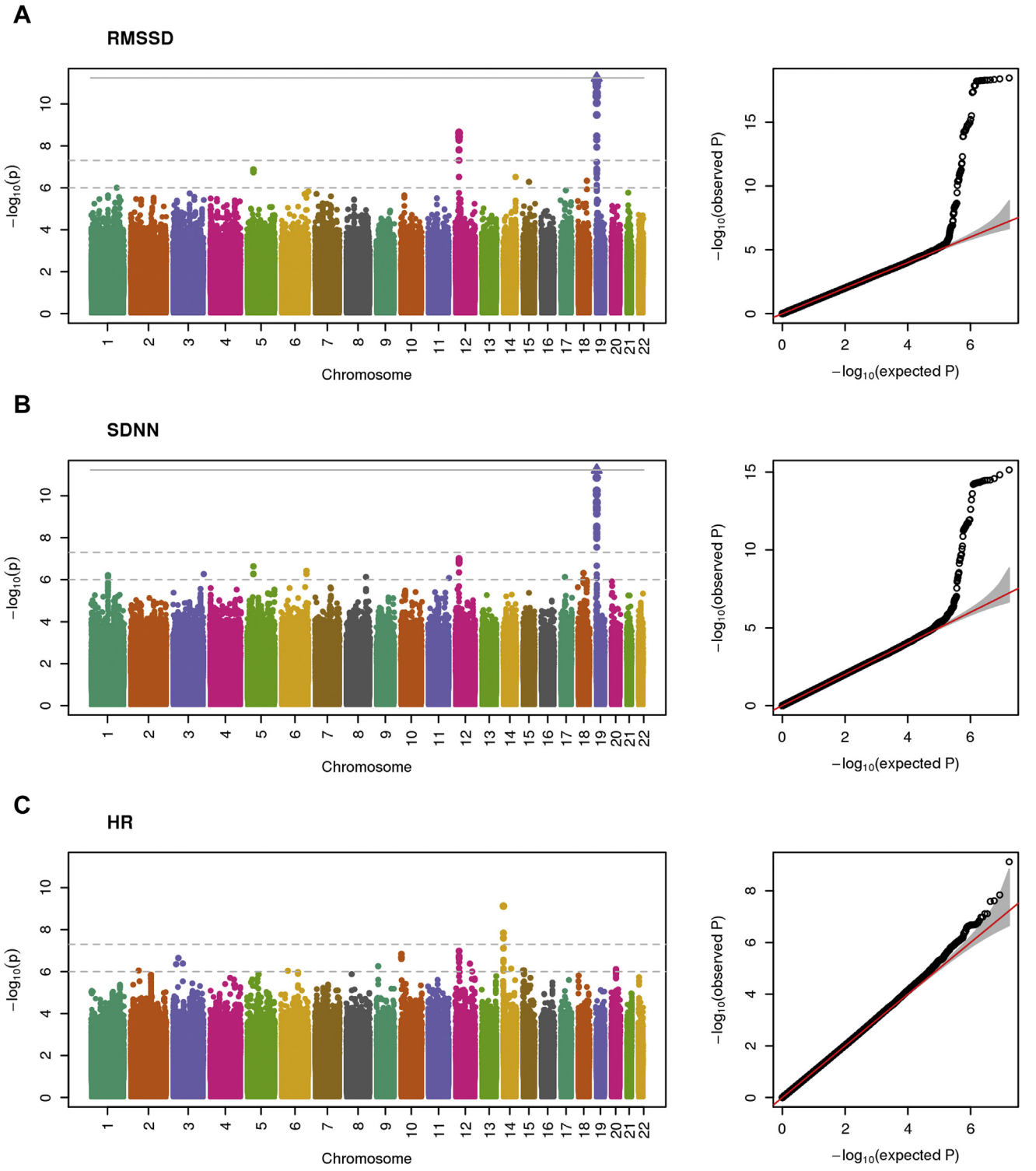

Figure 1 Manhattan and quantile–quantile plots for the meta-analyzed associations in Hispanics/Latinos for phenotypes RMSSD **(A),** SDNN **(B),** and HR **(C).** HR = heart rate; RMSSD = root mean squared difference in successive, normal-to-normal interbeat intervals; SDNN = standard deviation of normal-to-normal interbeat intervals.

The 2 replicated HRV SNPs explained 0.91% and 0.72% of the variability of RMSSD and SDNN, respectively, in HCHS/SOL. The chromosome 6 SNP explained 0.15% of the variability of HR, and 1.17% when combined with the previously reported 21 HR SNPs.[9]

We investigated associations of 4 candidate SNPs in genes *ADBR2* and *ADBR1* (see Supplementary Table S5). Genome-wide summary data from this investigation are publically available to support future genetic investigations.[21]

Table 3 SNPs selected for phase 2

SNP	Trait	CHR	Effect allele	Nearest gene	HA EAF	HA B	HA *P* value	EA EAF	EA B	EA *P* value	AA EAF	AA B	AA *P* value
RS4963772*	RMSSD	12	A	LINC00477	0.13–0.15	0.069	2×10^{-9}	0.15	0.053	**0.0046**	0.03	0.047	.2003
RS12982903*	SDNN	19	G	*FUT5*	0.93–0.94	0.129	7×10^{-16}	0.92	0.114	$\mathbf{2 \times 10^{-6}}$	0.96–0.97	−0.028	.6596
RS236352	HR	6	G	*PPIL1*	0.67–0.68	0.605	9×10^{-7}	0.66	0.226	0.0949	0.71–0.72	0.586	**.0067**
RS8009773	RMSSD	14	A	*C14orf177*	0.74–0.77	0.048	3×10^{-7}	0.84	0.012	0.2713	0.44–0.56	0.040	.0155
RS9428238	SDNN	1	C	*NHLH2*	0.57–0.61	0.039	6×10^{-7}	0.48	0.039	0.2434	0.72–0.75	0.009	.3258
RS149496015	SDNN	11	C	*OPCML*	0.98	0.193	8×10^{-7}	0.97	−0.053	0.8709	0.98–0.99	−0.050	.0433
RS17180489	HR	14	G	*RGS6*	0.90	1.200	7×10^{-7}	0.87	0.579	0.0730	0.94–0.96	−1.547	.9524

Two SNPs (*) were genome-wide significant in the Hispanic/Latino meta-analysis; all others were suggestive. The significance threshold for replication analysis is 0.005 for HRV SNPs and 0.0125 for HR SNPs. Bold *P* values indicate replication. The effect allele is the allele associated with higher trait values in phase 1. Figure 4 compares effect estimates across the 3 ancestry groups; Supplementary Table S3 includes additional SNP-level details.

AA = African American; CHR = Chromosome; EA = European ancestry; EAF = Effect allele frequency; HA = Hispanic ancestry; SNP = single nucleotide polymorphism.

Generalization analysis

We evaluated genome-wide significant loci reported in a previous GWAS of HR in European ancestry populations (N ≈ 181,000) for generalization to Hispanic/Latino populations. Eleven of the 21 reported HR SNPs generalized ($r < 0.05$), with effects similar to those in the original report (Figure 6). Confidence bounds for 9 of the 10 remaining SNPs show that the summary results for Hispanics/Latinos are consistent with effects observed in the European ancestry study, supporting the interpretation that the association discovered in European ancestry cohorts generalizes to Hispanics/Latinos. For a single SNP, rs2067615, the direction

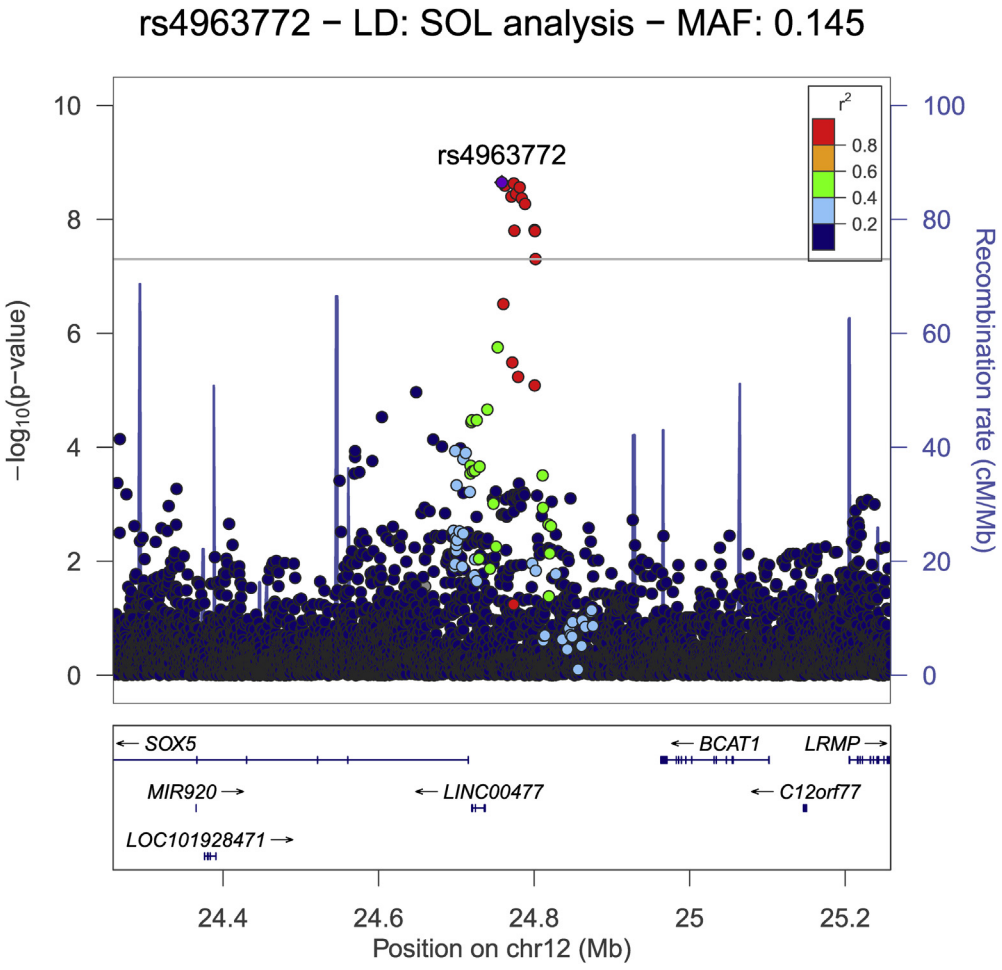

Figure 2 Regional association plot for the root mean squared difference in successive, normal-to-normal interbeat intervals (RMSSD) genome-wide significant locus rs4963772 on chromosome 12, which replicated in the European ancestry phase 2 cohort. –log10 *P* values from the Hispanic/Latino meta-analysis are plotted on the vertical axis, and chromosome position is on the horizontal axis. Linkage disequilibrium r^2 was estimated in the largest phase 1 cohort, Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Values of r^2 with respect to rs4963772 are displayed using color. Nearby genes are displayed under the horizontal axis.

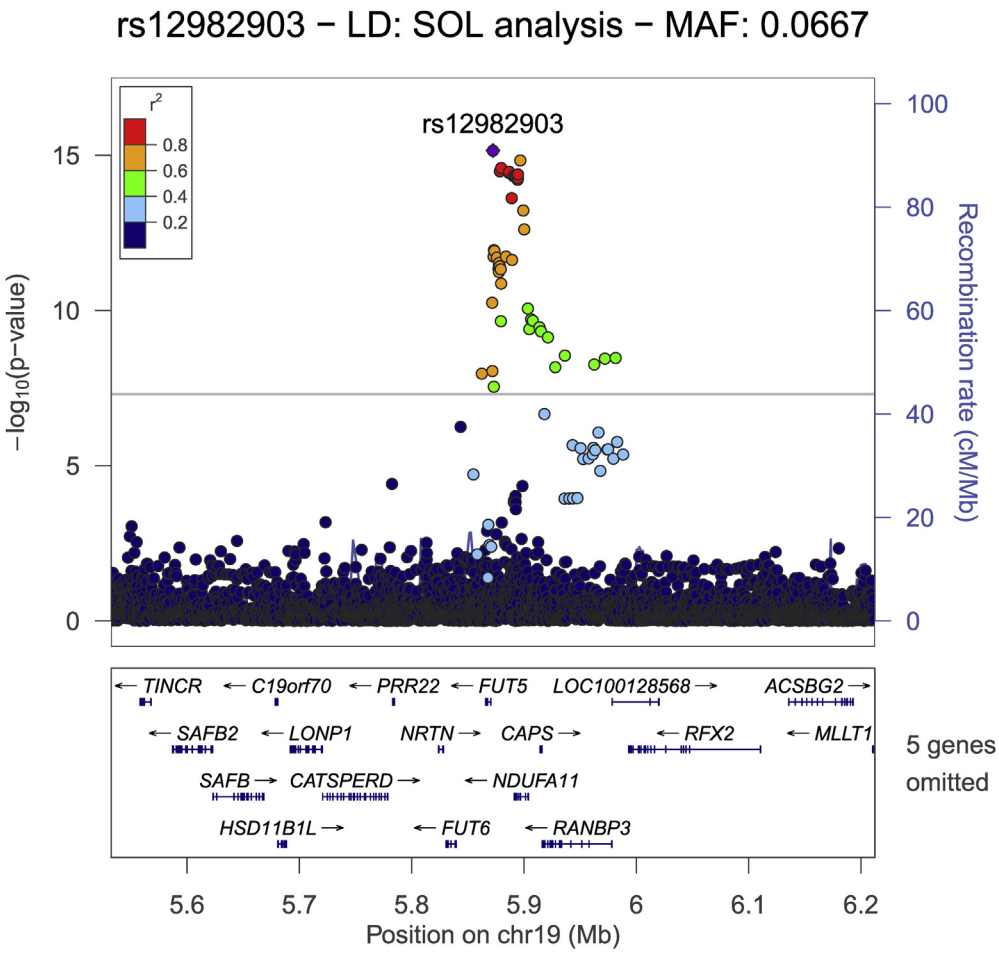

Figure 3 Regional association plot for the standard deviation of normal-to-normal interbeat intervals (SDNN) genome-wide significant locus rs12982903 on chromosome 19, which replicated in the European ancestry phase 2 cohort. –log10 *P* values from the Hispanic/Latino meta-analysis are plotted on the vertical axis, and chromosome position is on the horizontal axis. Linkage disequilibrium r^2 was estimated in Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Values of r^2 with respect to rs12982903 are displayed using color. Nearby genes are displayed under the horizontal axis. Significant single nucleotide polymorphisms span a region with several genes.

of the effect estimate in Hispanics/Latinos was opposite to that in the European ancestry study. Generalization analysis for this SNP is inconclusive due to wide confidence bounds. There is no comparable published GWAS of HRV for similar generalization analyses.

Discussion

There is growing attention to the lack of diversity in GWAS,[14,22] which reduces the relevance of medical genomics globally and results in missed opportunities to leverage diversity to improve biologic understanding. There are fewer extensively phenotyped epidemiologic cohorts of non-Europeans; investigators must be resourceful in addressing the lack of diversity in GWAS. Therefore, we used 3 well-characterized Hispanic/Latino cohorts, with independent replication in European ancestry and African-American cohorts. We found novel associations between SNPs and HR or HRV, despite modest sample sizes. The findings are the first of their kind in Hispanics/Latinos and provide insight into the biologic mechanisms underlying SDNN, RMSSD, and HR.

At the chromosome 19 locus, the lead SDNN SNP (rs12982903) is in linkage disequilibrium with several potentially causal variants ($r^2 \geq 0.8$ in HCHS/SOL), including (1) rs12980262; (2) rs12974440; (3) rs12974991; (4) rs12975210; and (5) rs17271904 (see Supplementary Figure 8). Variant rs12980262 is a missense alteration (g.5893047 G>A; c.557 C>T; p.Ala186Val) located in the last exon of *NDUFA11* (NADH:ubiquinone oxidoreductase subunit A11). The altered protein is an isoform of a subunit of the membrane-bound mitochondrial complex I (NADH-ubiquinol reductase in the electron transport chain). Furthermore, ChIP-Seq data suggest that this SNP lies within a putative enhancer active in the fetal heart, right ventricle, and right atrium (home of the sinoatrial node) or pacemaker. Thus, variant rs12980262 may influence HRV through altered mitochondrial electron transport and/or gene regulation.

Noncoding SNPs (2) through (5) lie within putative enhancers and are active in various cardiac tissues; they may also have regulatory roles. In particular, rs12974440, rs12974991, and rs12975210 overlap with a DNaseI hypersensitive site in several heart tissues and have been reported

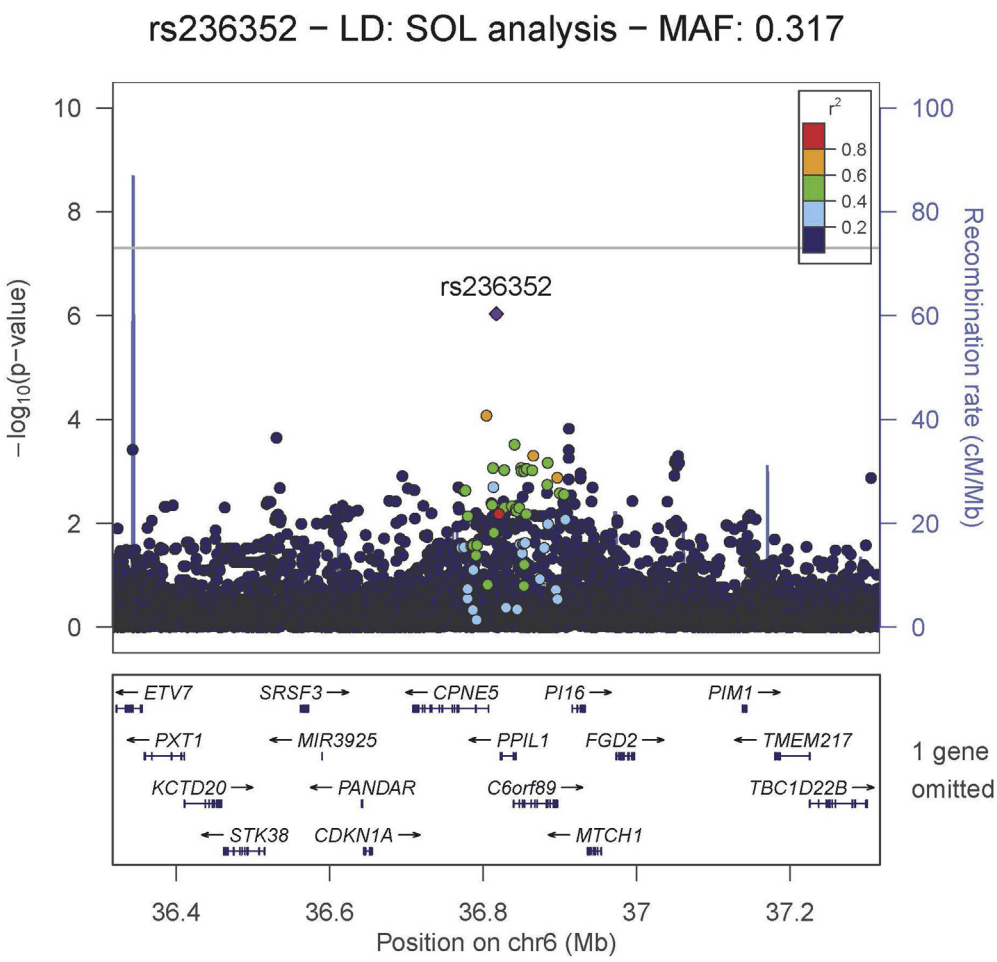

Figure 4 Regional association plot for the heart rate (HR) suggestive locus rs236352 on chromosome 6, which replicated in the African-American phase 2 cohort. –log10 *P* values from the Hispanic/Latino meta-analysis are plotted on the vertical axis, and chromosome 6 position is plotted on the horizontal axis. Linkage disequilibrium r^2 was estimated in Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Values of r^2 with respect to rs236352 are displayed using color in the plot. Nearby genes are displayed under the horizontal axis.

as eQTLs for downstream genes *RANBP3* (in whole blood) and *CAPS* (in whole blood and brain). In this context, *CAPS* is a potentially interesting enhancer target because it encodes a protein (calcyphosine) that binds calcium (Ca^{2+}) and appears to be up-regulated by cAMP and thyroid-stimulating hormone in the thyroid.[23] Although the exact function of calcyphosine remains unclear, thyroid effects on the heart are well recognized,[24] and the inward flow of Ca^{2+} through T-type Ca^{2+} channels (i_{Ca}) accelerates the autonomically controlled rate of phase 4 depolarization (and therefore discharge) of sinoatrial pacemaker cells in the right atrium.[25]

In addition to using epigenetic datasets in cardiac tissues, we examined data from neuronal progenitor cells. These progenitors give rise to tissues involved in the control of HR and its variability by the central and peripheral autonomic nervous systems. Three noncoding variants lie within putative enhancers in neuronal progenitor cells: rs17271904 (on chromosome 19; see Supplementary Figure 8) and variants rs17287293 and rs11047543 in high LD with the lead RMSSD SNP (rs4963772 on chromosome 12). These variants may influence HR via central and/or peripheral autonomic nervous pathways. Moreover, *KNOP1P1*, 20 kb upstream of rs4963772, has been associated with HR[9,26] and PR interval duration.[27,28]

The chromosome 6 HR SNP is within 200 kb of a locus that was suggestive in the large European ancestry HR GWAS (see Supplemental Table 5 in den Hoed et al[9]). The gene closest to our lead SNP, *PPIL1* (peptidylprolyl isomerase like 1), is proximal to a well-characterized QRS locus.[29–32] Noncoding lead HR SNP rs236352 and its proxy rs236349 are likely functionally relevant in cardiac tissues (see Supplementary Figure 8). Variant rs236352 lies within a predicted cardiac superenhancer.[33,34] The putative superenhancer overlaps a DNaseI hypersensitive site in cardiac tissues, ChIP-Seq peaks of RNA polymerase II, subunits of cohesion complexes (e.g., SMC3), and chromatin regulators (e.g., EP300), which are known to associate with superenhancers.[33,35] Plausible right atrial gene targets of the superenhancer that contains rs236352 are its eQTL targets, *CPNE5*, which encodes a Ca^{2+}-dependent, phospholipid-binding protein, and *PPIL1*. Both are expressed in the right atrium, although neither gene has

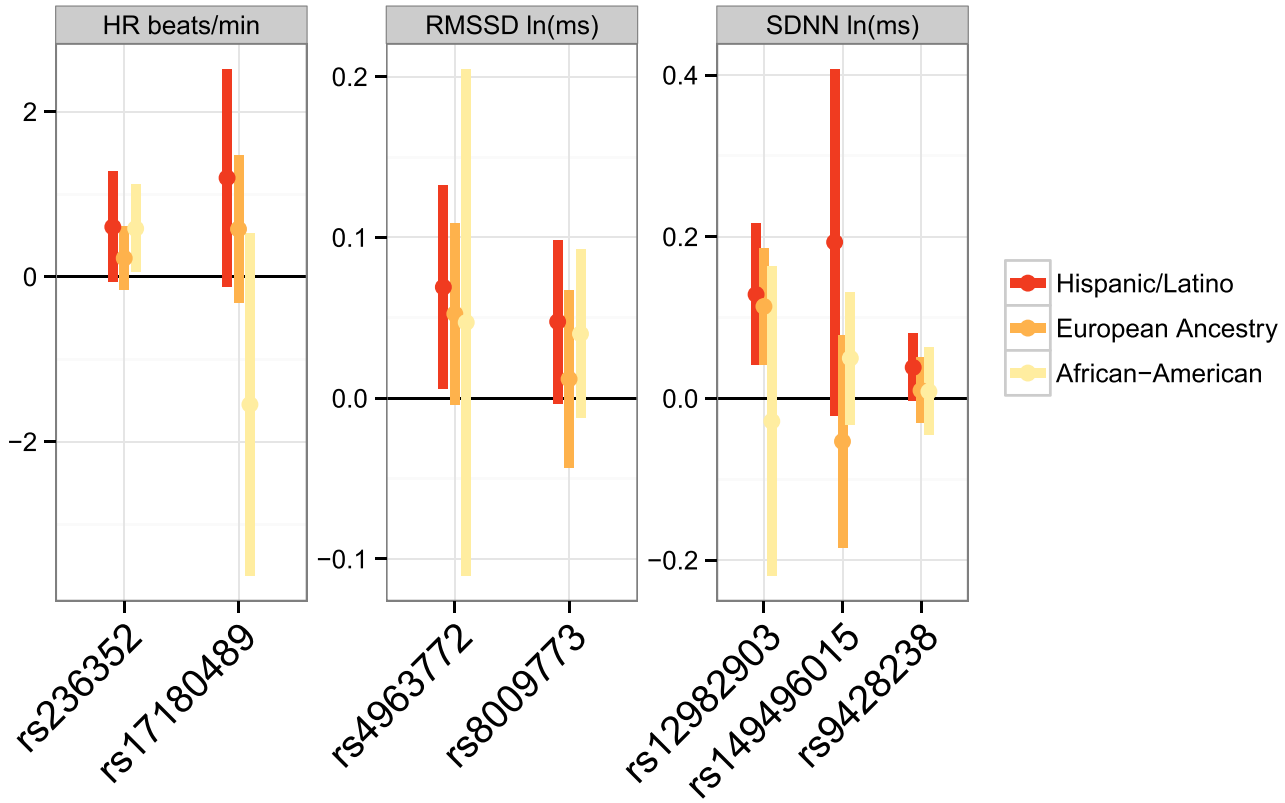

Figure 5 Comparison of associations (regression estimated β values) for the 7 single nucleotide polymorphisms selected for phase 2 replication analysis. Bars represent confidence intervals using an α level that incorporates an appropriate adjustment for multiplicity. For Hispanics/Latinos, $\alpha = 5 \times 10^{-8}$ for all confidence intervals. For European ancestry and African-Americans, $\alpha = 0.05/4$ for HR and $\alpha = 0.05/10$ for SDNN and RMSSD. Abbreviations as in Figure 1.

been identified as an eQTL in cardiac tissue. Interestingly, lead SNP rs236352 is predicted to disrupt the DNA binding motif of a T-box transcription factor that regulates *CPNE5* expression.[36] Moreover, a short stretch of unannotated RNA overlapping the *CPNE5* promoter is expressed differentially in the right (vs left) atrium, suggesting that the RNA may have a functional role in the right atrium. For example, if this unannotated RNA is an enhancer RNA associated with the superenhancer, then it could have a regulatory role in the right atrium.[37]

The mechanisms by which these replicated and epigenetically well characterized HR and HRV loci may exert effects in the right atrium are biologically plausible. However, functional evaluation is needed to confirm the postulated underlying mechanisms. Clinical implications remain unclear. Moreover, associations for the lead SNP at the chromosome 19 and 12 loci were not statistically significant in the phase 2 African-American sample. For the RMSSD–rs4963772 association, this can be explained by the lower replication sample size, lower minor allele frequency, and therefore lower power for detecting association with this phenotype measured with error. Although the extent of measurement error is somewhat lower for RMSSD than SDNN and in studies with 30- vs 10- second ECG recordings (MESA vs HCHS/SOL and WHI), the same can reasonably be said about the SDNN–rs12982903 association, for which low imputation quality may have further reduced power. Importantly, HRV and SNP measurement error should reduce statistical power but not introduce bias.

Because the European cohort in phase 2 included only females, HRV–SNP associations were not replicated in males. However, these SNPs were genome-wide significant in phase 1. There was no significant evidence that sex modifies the associations in the largest phase 1 cohort, HCHS/SOL ($P > .05$). However, the association signal of SNP rs4963772 (chromosome 12) was stronger in females than males in sex-stratified analyses.

Our GWAS discovered and replicated a novel HR-associated locus on chromosome 6. We are the first to detect this association, even though HR was analyzed in a European ancestry GWAS 6 times larger. Possible reasons include chance, gene–environment interaction, and population-specific variation.

Conclusion

HRV is an understudied phenotype, and the current finding of 2 genetic associations represents an advance in HRV genetics. In addition, we discovered a novel genetic association with HR, which replicated in African-Americans. Functional annotation analysis revealed plausible mechanisms for these associations. This novel discovery of genetic association for a well-studied phenotype, HR, argues for the importance of efforts to expand genetic association studies to populations of diverse ancestry.

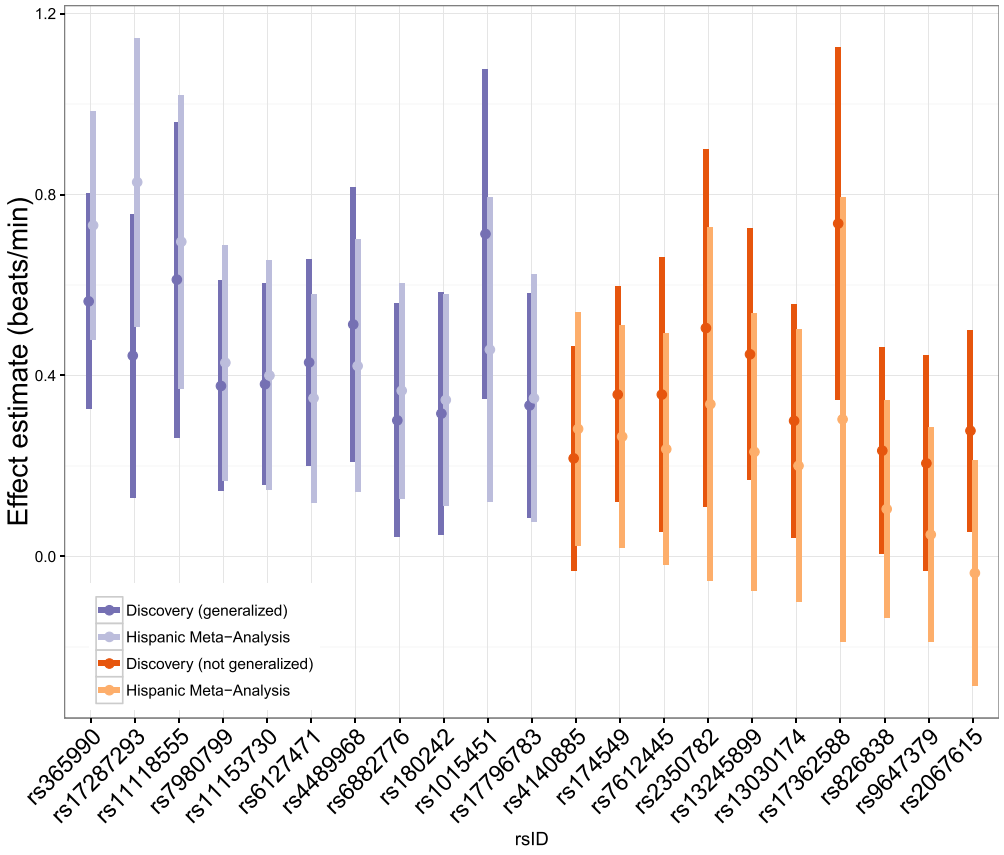

Figure 6 Generalization analysis of previously reported heart rate–associated SNPs. For each SNP, the left-hand darker bar shows a confidence interval ($\alpha = 5 \times 10^{-8}$) for the association (regression estimated β) as reported in den Hoed et al.[9] The right-hand lighter bar shows a confidence interval ($\alpha = 0.05/21$) for the association in the Hispanics/Latinos meta-analysis. *Purple bars* are for the 11 SNPs found to generalize to Hispanics/Latinos using the r value approach; *orange bars* are for remaining 10 SNPs. For the SNPs that generalized, there is evidence that the magnitude of association is similar in Hispanics/Latinos compared to European ancestry. In addition, for SNPs that did not generalize, there is no compelling evidence of a different (or null) association in Hispanics/Latinos compared to European ancestry populations. Point estimates for Hispanics/Latinos associations tend to be attenuated compared to estimates for European ancestry, as expected due to the "winner's curse."[38] For rs2067615, point estimates have opposite sign, but confidence intervals are wide and overlap. SNP = single nucleotide polymorphism.

Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.hrthm.2017.06.018.

References

1. Wannamethee G, Shaper AG. The association between heart rate and blood pressure, blood lipids and other cardiovascular risk factors. J Cardiovasc Risk 1994;1:223–230.
2. Johansen CD, Olsen RH, Pedersen LR, Kumarathurai P, Mouridsen MR, Binici Z, Intzilakis T, Køber L, Sajadieh A. Resting, night-time, and 24 h heart rate as markers of cardiovascular risk in middle-aged and elderly men and women with no apparent heart disease. Eur Heart J 2013;34:1732–1739.
3. Kannel WB, Kannel C, Paffenbarger RS, Cupples LA. Heart rate and cardiovascular mortality: the Framingham Study. Am Heart J 1987;113:1489–1494.
4. Shaper AG, Wannamethee G, Macfarlane PW, Walker M. Heart rate, ischaemic heart disease, and sudden cardiac death in middle-aged British men. Br Heart J 1993;70:49–55.
5. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Eur Heart J 1996;17:354–381.
6. Singh JP, Larson MG, O'Donnell CJ, Tsuji H, Evans JC, Levy D. Heritability of heart rate variability: the Framingham Heart Study. Circulation 1999;99:2251–2254.
7. Russell MW, Law I, Sholinsky P, Fabsitz RR. Heritability of ECG measurements in adult male twins. J Electrocardiol 1998;30(Suppl):64–68.
8. Newton-Cheh C, Guo CY, Wang TJ, O'donnell CJ, Levy D, Larson MG. Genome-wide association study of electrocardiographic and heart rate variability traits: the Framingham Heart Study. BMC Med Genet 2007;8(Suppl 1):S7.
9. den Hoed M, Eijgelsheim M, Esko T, et al. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. Nat Genet 2013;45:621–631.
10. Deo R, Nalls MA, Avery CL, et al. 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. Heart Rhythm 2013;10:401–408.
11. Cho YS, Go MJ, Kim YJ, et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet 2009;41:527–534.
12. Schroeder EB, Chambless LE, Liao D, Prineas RJ, Evans GW, Rosamond WD, Heiss G. Diabetes, glucose, insulin, and heart rate variability: the ARIC study. Diabetes Care 2005;28:668–674.
13. Meyer ML, Gotman NM, Soliman EZ, Whitsel EA, Arens R, Cai J, Daviglus ML, Denes P, González HM, Moreiras J, Talavera GA, Heiss G. Association of glucose homeostasis measures with heart rate variability among Hispanic/Latino adults without diabetes: the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Cardiovasc Diabetol 2016;15:45.
14. Bustamante CD, Burchard EG, DelaVega FM. Genomics for the world. Nature 2011;475:163–165.
15. Howie B, Marchini J, Stephens M. Genotype imputation with thousands of genomes. G3 (Bethesda) 2011;1:457–470.
16. Pe'er I, Yelensky R, Altshuler D, Daly MJ. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. Genet Epidemiol 2008;32:381–385.

17. Conomos MP, Laurie CA, Stilp AM, et al. Genetic diversity and association studies in US Hispanic/Latino populations: applications in the Hispanic Community Health Study/Study of Latinos. Am J Hum Genet 2016; 98:165–184.
18. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR, Willer CJ. LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics 2010;26:2336–2337.
19. Sofer T, Heller R, Bogomolov M, Avery CL, Graff M, North KE, Reiner AP, Thornton TA, Rice K, Benjamini Y, Laurie CC, Kerr KF. A powerful statistical framework for generalization testing in GWAS, with application to the HCHS/SOL. Genet Epidemiol 2017;41:251–258.
20. Devlin B, Roeder K. Genomic control for association studies. Biometrics 1999; 55:997–1004.
21. Rich SS, Wang ZY, Sturcke A, Ziyabari L, Feolo M, O'Donnell CJ, Rice K, Bis JC, Psaty BM. Rapid evaluation of phenotypes, SNPs and results through the dbGaP CHARGE Summary Results site. Nat Genet 2016;48:702–703.
22. Popejoy AB, Fullerton SM. Genomics is failing on diversity. Nature 2016; 538:161–164.
23. Shao W, Wang Q, Wang F, Jiang Y, Xu M, Xu J. Abnormal expression of calcyphosine is associated with poor prognosis and cell biology function in colorectal cancer. Onco Targets Ther 2016;9:477–487.
24. Polikar R, Burger AG, Scherrer U, Nicod P. The thyroid and the heart. Circulation 1993;87:1435–1441.
25. Choudhury M, Boyett MR, Morris GM. Biology of the sinus node and its disease. Arrhythm Electrophysiol Rev 2015;4:28–34.
26. Eijgelsheim M, Newton-Cheh C, Sotoodehnia N, et al. Genome-wide association analysis identifies multiple loci related to resting heart rate. Hum Mol Genet 2010; 19:3885–3894.
27. Pfeufer A, van Noord C, Marciante KD, et al. Genome-wide association study of PR interval. Nat Genet 2010;42:153–159.
28. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, Klemm A, Flicek P, Manolio T, Hindorff L, Parkinson H. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res 2014; 42:D1001–D1006.
29. Hong KW, Lim JE, Kim JW, Tabara Y, Ueshima H, Miki T, Matsuda F, Cho YS, Kim Y, Oh B. Identification of three novel genetic variations associated with electrocardiographic traits (QRS duration and PR interval) in East Asians. Hum Mol Genet 2014;23:6659–6667.
30. Ritchie MD, Denny JC, Zuvich RL, et al. Genome- and phenome-wide analyses of cardiac conduction identifies markers of arrhythmia risk. Circulation 2013; 127:1377–1385.
31. Sotoodehnia N, Isaacs A, de Bakker PI, et al. Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction. Nat Genet 2010;42:1068–1076.
32. Holm H, Gudbjartsson DF, Arnar DO, Thorleifsson G, Thorgeirsson G, Stefansdottir H, Gudjonsson SA, Jonasdottir A, Mathiesen EB, Njølstad I, Nyrnes A, Wilsgaard T, Hald EM, Hveem K, Stoltenberg C, Løchen ML, Kong A, Thorsteinsdottir U, Stefansson K. Several common variants modulate heart rate, PR interval and QRS duration. Nat Genet 2010;42:117–122.
33. Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-Andre V, Sigova AA, Hoke HA, Young RA. Super-enhancers in the control of cell identity and disease. Cell 2013;155:934–947.
34. Khan A, Zhang X. dbSUPER: a database of super-enhancers in mouse and human genome. Nucleic Acids Res 2016;44:D164–D171.
35. Kagey MH, Newman JJ, Bilodeau S, et al. Mediator and cohesin connect gene expression and chromatin architecture. Nature 2010;467:430–435.
36. Mori AD, Zhu Y, Vahora I, Nieman B, Koshiba-Takeuchi K, Davidson L, Pizard A, Seidman JG, Seidman CE, Chen XJ, Henkelman RM, Bruneau BG. Tbx5-dependent rheostatic control of cardiac gene expression and morphogenesis. Dev Biol 2006;297:566–586.
37. Hsu J, Hanna P, Van Wagoner DR, Barnard J, Serre D, Chung MK, Smith JD. Whole genome expression differences in human left and right atria ascertained by RNA sequencing. Circ Cardiovasc Genet 2012;5:327–335.
38. Xiao R, Boehnke M. Quantifying and correcting for the winner's curse in genetic association studies. Genet Epidemiol 2009;33:453–462.