Genome-Wide Association Approach Identified Novel Genetic Predictors of Heart Rate Response to β-Blockers

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Background—For many indications, the negative chronotropic effect of β-blockers is important to their efficacy, yet the heart rate (HR) response to β-blockers varies. Herein, we sought to use a genome-wide association approach to identify novel single nucleotide polymorphisms (SNPs) associated with HR response to β-blockers.

Methods and Results—We first performed 4 genome-wide association analyses for HR response to atenolol (a β1-adrenergic receptor blocker) as: (1) monotherapy or (2) add-on therapy, in 426 whites and 273 blacks separately from the PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses) study. A meta-analysis was then performed between the genome-wide association analysis performed in PEAR atenolol monotherapy and add-on therapy, in each race separately, using the inverse variance method assuming fixed effects. From this analysis, SNPs associated with HR response to atenolol at a $P<$1E-05 were tested for replication in whites (n=200) and blacks (n=168) treated with metoprolol (a β1-adrenergic receptor blocker). From the genome-wide association meta-analyses, SNP rs17117817 near olfactory receptor family10 subfamily-p-member1 (*OR10P1*), and SNP rs2364349 in sorting nexin-9 (*SNX9*) replicated in blacks. The combined studies meta-analysis *P* values for the rs17117817 and rs2364349 reached genome-wide significance (rs17117817G-allele; Meta-β=5.53 beats per minute, Meta-*P*=2E-09 and rs2364349 A-allele; Meta-β=3.5 beats per minute, Meta-*P*=1E-08). Additionally, SNPs in the *OR10P1* and *SNX9* gene regions were also associated with HR response in whites.

Conclusions—This study highlights *OR10P1* and *SNX9* as novel genes associated with changes in HR in response to β-blockers.

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Over the past 5 decades, β-adrenergic receptor blockers (β-blockers) have been a cornerstone therapy for heart failure, post–myocardial infarction, symptomatic angina, and other cardiovascular diseases.[1,2] Additionally, they remain one of the most commonly prescribed classes of drugs in the United States.[3] β-Blockers work by inhibiting the β-adrenergic receptors in the heart, which prevent the binding of

epinephrine and norepinephrine to these receptors and eventually reduce cardiac contractility and heart rate (HR).[4] HR lowering in response to β-blockers has been associated with lower risk of incident heart failure and cardiovascular diseases.[5,6] Additionally, results from clinical trials and meta-analyses have shown a significant association between β-blockers' HR-lowering effect and improvement in clinical

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outcomes.[7–10] Altogether, these data highlight that the HR-modulating effects of β-blockers are a critical efficacy component for this class of drugs and contributes to their beneficial therapeutic effects.

Despite the widespread use of β-blockers, interindividual differences in HR-lowering response to β-blockers have been observed.[11,12] These differences might be attributed to several factors, including genetics.[13] Over the past 2 decades, considerable resources have been allocated to elucidate the genetic contributors of variability in drug response to β-blockers. Most of this research has focused on explaining differences in their effects on blood pressure, heart failure phenotypes, and effects on cardiovascular outcomes.[14–18] However, only a few candidate gene pharmacogenetic studies have focused on evaluating the role of genetics in explaining the differences in β-blockers' negative chronotropic response. Not surprisingly, *ADRB1*, the gene encoding the β_1 receptor, has been the focus of most studies evaluating variability in negative chronotropic response to β-blockers.[12,19–24] However, there is a paucity of information about other potential genes that may be associated with changes in β-blockers' negative chronotropic response.

Since autonomic regulation of HR is dependent on a vast array of proteins, change in HR in response to a β-blocker may be controlled via multiple genes. Therefore, a thorough understanding of genetic determinants associated with changes in HR in response to a β-blocker is needed. Accordingly, in this study, we sought to conduct a genome-wide association analysis to identify novel genetic predictors associated with changes in HR in response to β-blockers therapy in European-Americans (whites) and African-Americans (blacks).

Methods

Study Design and Participants

PEAR study

A description of the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study (clinicaltrials.gov #NCT00246519), including the study rationale, design, protocol, and safety procedures, has been previously published.[25] PEAR genotype and phenotype data used in this study have been made publicly available at the database of Genotypes and Phenotypes (dbGaP; https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000649.v1.p1; dbgap study accession: phs000649.v1.p1). Briefly, PEAR was a randomized, open-label, multicenter, prospective study designed to evaluate the role of genetic variation on the blood pressure response to the β-blocker atenolol and the thiazide diuretic hydrochlorothiazide (HCTZ). In total, 768 individuals with mild-to-moderate essential hypertension, of any race or ethnicity, and between the ages of 17 and 65 years completed the PEAR study. Following a washout period and collection of baseline data, study participants were randomized to either atenolol 50 mg or HCTZ 12.5 mg, both administered once daily, with the dose doubled (ie, atenolol 100 mg or HCTZ 25 mg) in patients with a systolic blood pressure >120 mm Hg and/or diastolic blood pressure >70 mm Hg. Responses to treatment were assessed after 6 to 9 weeks of monotherapy (response assessment #1; Figure 1), after which those subjects who were still not at goal had the alternate drug added with a similar dose titration scheme and a response assessment following 6 to 9 weeks on combination therapy (response assessment #2). In PEAR, >85% of the participants treated with atenolol as a mono- or add-on therapy had atenolol dose titrated to 100 mg per day. Data collected included home, office, and 24-hour ambulatory systolic blood pressure, diastolic blood pressure, and HR. Both HR measurement and blood pressure response were used to guide dosage adjustments, as those individuals with HR <55 beats per minute were precluded from receiving a higher dose of atenolol. Institutional Review Boards at each clinical research center approved the study protocol
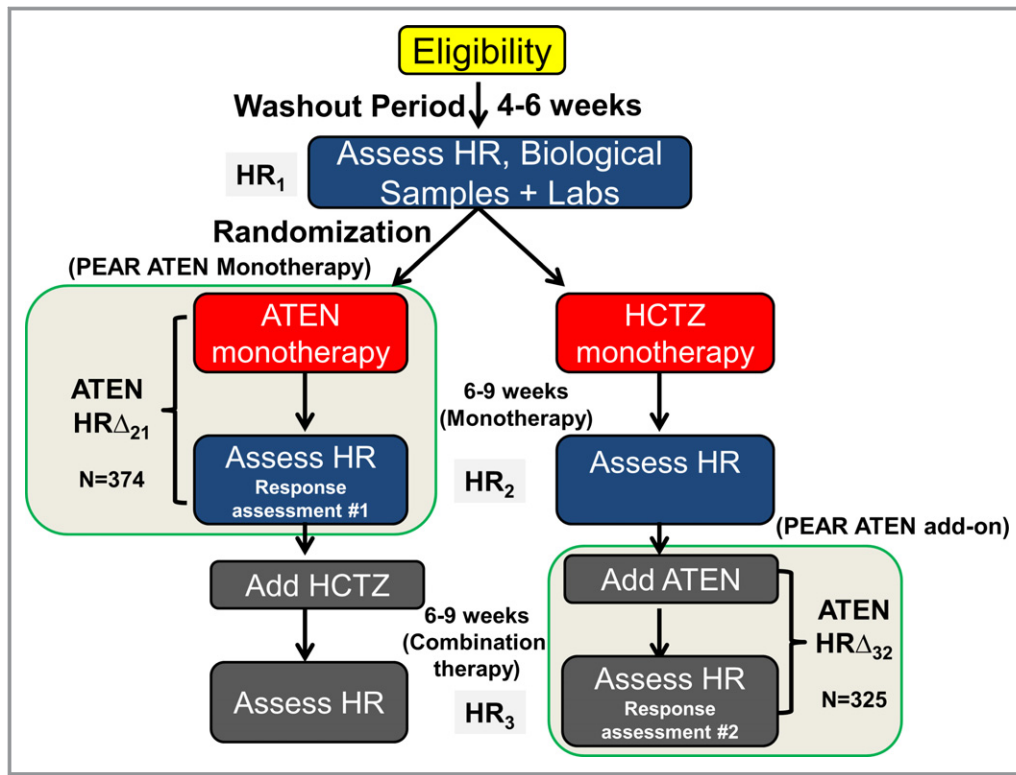
Figure 1. PEAR study design. ATEN indicates atenolol; HCTZ, hydrochlorothiazide; HR, heart rate; PEAR, Pharmacogenomics Evaluation of Antihypertensive Response.

(University of Florida, Gainesville, FL; Emory University, Atlanta, GA; Mayo Clinic, Rochester, MN) and written informed consent was obtained from all participants.

PEAR-2 study

PEAR-2 was a multicenter, prospective, open-label, sequential monotherapy clinical trial (clinicaltrials.gov #NCT01203852).[26] The PEAR-2 genotype and phenotype data used in this study are currently in the process of being uploaded to dbGaP and will soon be available to other researchers under dbGaP accession phs000649.v2.p1. The Institutional Review Boards of each enrolling institution (University of Florida, Gainesville, FL; Emory University, Atlanta, GA; Mayo Clinic, Rochester, MN) approved the study and all subjects provided written informed consent. Eligible study participants received metoprolol tartrate 50 mg twice daily, and after 2 weeks if an inadequate response was observed (>120/70 mm Hg and HR >55 bpm), the dose was increased to 100 mg twice daily for an additional 6 weeks. Of note, >95% of the participants treated with metoprolol had their dose titrated to 100 mg twice day. Metoprolol and atenolol are both β1-adrenergic receptor blockers, and they have the same mechanism of reducing HR via their inhibitory effect on the β1-adrenergic receptor. Thus, we used data from PEAR-2 metoprolol-treated participants for replicating the results discovered from PEAR atenolol-treated participants.

Heart Rate Assessment

In both the PEAR and PEAR-2 studies, HR data were generated from measurements collected using a home blood pressure monitor, the Microlife model 3AC1-PC home blood pressure monitor (Minneapolis, MN), which has been validated for accuracy.[27] Blood pressure and HR were measured in triplicate mode in the morning and evening, averaged by the blood pressure, and recorded with a date and time stamp. At each assessment period, study participants were required to have 5 morning and evening recordings in the previous 7 days. Thus, HR values used in the analysis described herein were based on a minimum of 30 HR recordings. Baseline HR was measured at the end of an average 4-week washout of antihypertensive drugs (HR_1). HR response following monotherapy with atenolol or HCTZ was measured after 6 to 9 weeks (HR_2), and HR response following addition of atenolol to HCTZ was measured after 6 to 9 weeks of combination therapy (HR_3). In PEAR-2, the HR response to metoprolol was noted as HR_2 and the baseline HR was HR_1.

Genetic Analyses and Genotyping

Genome-wide association study approach

Details of the genome-wide genotyping, quality control, and imputation performed on PEAR samples were previously
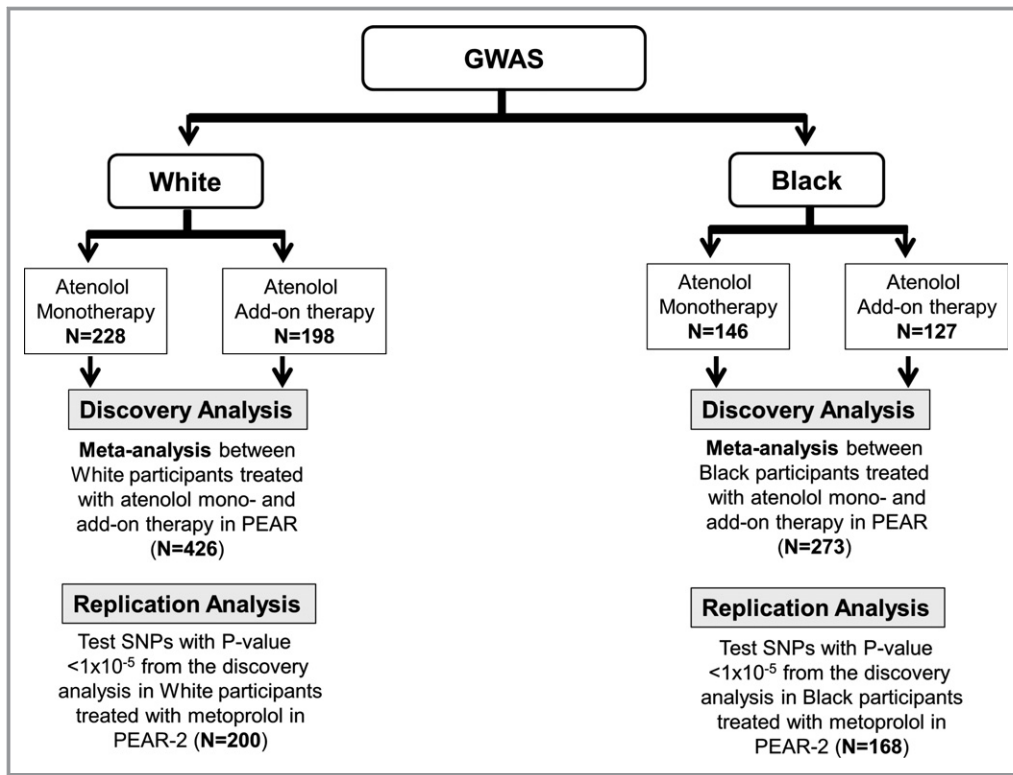
Figure 2. The overall analysis framework of the study. GWAS indicates genome-wide association study; PEAR, Pharmacogenomics Evaluation of Antihypertensive Response; SNP, single nucleotide polymorphism.

described.[28] In brief, PEAR DNA samples underwent genotyping using the Illumina Human Omni-1Million Quad BeadChip (Illumina, San Diego CA). Genotypes were called using GenTrain2 Illumina clustering algorithm in the software package GenomeStudio (Illumina, San Diego, CA). MaCH software (version 1.0.16) was used to impute single nucleotide polymorphisms (SNPs) based on HapMapIII haplotypes. SNPs with minor allele frequency <5% or imputation r^2 <0.3 were excluded from the analysis. After quality control, we had ≈1.1 million SNPs that were included in the genome-wide association discovery analyses in white participants in PEAR monotherapy and PEAR add-on therapy and ≈1.2 million SNPs in blacks. For PEAR-2, genotyping was conducted using Human Omni2.5 S BeadChip (Illumina), and imputation based on 1000 Genomes Phase I reference panels was performed using Minimac.[29] Participants from PEAR or PEAR-2 were excluded if sample genotype call rates were below 95%. Sample contamination was tested by checking sex mismatches using X chromosome genotype data, and those who were discordant were excluded. Additionally, cryptic relatedness was estimated by pairwise identity-by-descent analysis implemented using PLINK.[30] We also ran principal component analysis on all samples, using the EIGENSTRAT method,[30] to assess the ancestral background. Heterozygosity was also assessed using PLINK, by estimating the inbreeding coefficient. After imputing PEAR2 genotyping data

and running quality control procedures, as explained above, we had ≈6.5 million SNPs in whites and ≈8.9 million SNPs in blacks treated with metoprolol therapy, which were used for the replication efforts in this study.

The analysis approach used in this study is summarized in Figure 2. First, we aimed to identify new genetic variants that contribute to the observed interindividual variability in HR-lowering effect in response to atenolol by conducting a genome-wide association study (GWAS). A meta-analysis was then performed, in each race separately, between PEAR atenolol monotherapy and PEAR atenolol add-on therapy. To replicate our findings, SNPs with $P<1E-05$ from the PEAR meta-analysis were evaluated for association with changes in HR in response to metoprolol in PEAR-2. Given the fact that patterns of linkage disequilibrium often differ across different ancestral groups, further validation of SNPs of interest was performed by looking up regions of the SNPs of interest in the alternate ancestral group.

Statistical Analysis

In PEAR, the change in HR in response to atenolol was determined as follows (Figure 1). For the monotherapy, HR_1 was subtracted from HR_2 ($HR\Delta_{21}$); for the add-on therapy, HR_2 was subtracted from HR_3 ($HR\Delta_{32}$). Hence, HR_1 and HR_2 values were considered baseline HR for monotherapy and

add-on therapy, respectively. In PEAR-2, the change in HR in response to metoprolol was obtained from subtracting HR_2 from HR_1 ($HR\Delta_{21}$).

For the GWAS discovery analyses, 4 separate genome-wide association analyses were conducted for association with β-blockers' negative chronotropic response; analyses of response to atenolol as: (1) monotherapy, and (2) add-on therapy, in whites and blacks. Association analyses between SNPs and HR responses were performed using a linear regression analysis in PLINK.[30] All analyses were conducted under an additive model of inheritance and adjusted for age, sex, baseline HR, and principal components 1 and 2. Although >85% of the atenolol-treated participants in PEAR monotherapy and add-on therapy were taking the same dose of therapy (atenolol 100 mg per day), we adjusted for differences in dose in the analyses to prevent any confounding results. A meta-analysis was then performed between PEAR atenolol monotherapy and PEAR atenolol add-on therapy, in each race separately, assuming fixed effects and using inverse-variance weighting as implemented in the METAL software.[31] For the GWAS analysis, a typical genome-wide *P* value of <5E-08 was used to judge the significance of SNPs in both black and white participants. SNPs were considered suggestive with meta-analysis *P*<1E-05. Power analysis was performed using Quanto (http://biostats.usc.edu/software) to identify the power and effect sizes that can be detected using our study sample size. We found that our GWAS discovery analysis in whites (n=426) has >80% power to detect a 5 bpm difference in β-blocker HR response by genotype, assuming a SD of 10 bpm and SNPs of minor allele frequency ≥15%, with 2-sided α level=1E-05 (suggestive α level used in the study) (Table S1). This shows that this study is only powered to detect common SNPs with large effect size. Similarly, the power calculation in blacks shows that we only have power to detect common SNPs with large effect sizes (Table S1). This reveals that the sample size used in this study is not powered to detect SNPs with small effect sizes.

PLINK[30] software was used to explore the linkage disequilibrium between SNPs with *P*<1E-05 from PEAR atenolol mono- and add-on therapy GWAS meta-analysis. SNPs were then pruned based on linkage disequilibrium by removing any SNP with an r^2 >0.5 with any other SNP in a 50 SNP window. SNPs that remained after pruning were considered independent SNPs and were moved forward for replication in PEAR-2 participants treated with metoprolol (Figure 2). A Bonferroni correction was used to define a significant threshold for this analysis (0.05/number of independent SNPs tested). Deviation from Hardy–Weinberg equilibrium was tested by χ^2 or Fisher exact test in each race separately, and SNPs with Hardy-Weinberg *P*<1E-06 were excluded from the analysis.

To evaluate the effect of multiple response alleles on the HR response to β-blockers and to investigate the relative contribution of our genetic findings toward the phenotype, we constructed a genetic response score based on replicated SNPs. Points were given for the genotypes of the replicated SNPs in which the homozygous genotype of each SNP with the greatest HR-lowering effect had 2 points, while heterozygous genotype had 1 point, and homozygous genotype associated with the worst HR-lowering effect had 0. Alleles with HR-lowering effect were then summed up for inclusion in a linear regression model to test the association between the response score and changes in HR in response to β-blockers in PEAR and PEAR-2 participants. The latter analyses were adjusted for age, sex, baseline HR, differences in dose, and PC1 and 2.

Network and In Silico Analyses

To further investigate the potential physiological roles of the replicated SNPs identified in this study and the possible interactions between them, we used STRING database (version 10.5) (https://string-db.org/).[32] Replicated SNPs, from the GWAS meta-analysis, were used to build networks showing the potential interactions between the identified SNPs/genes and other potential interacting genes that might be involved in the mechanism underlying β-blocker HR-lowering effects.

Results

Baseline demographic characteristics for the PEAR and PEAR-2 study participants treated with β-blockers are summarized in Table 1. Age, sex, body mass index, baseline HR, and HR response were similar between white atenolol mono- and add-on therapy and metoprolol monotherapy–treated participants. Similarly, age, sex, body mass index, baseline HR, and HR response were similar between black atenolol mono- and add-on therapy and metoprolol monotherapy-treated participants. The mean and 95% confidence interval for atenolol HR-lowering response following mono- and add-on therapy were similar, as shown in Table S2, suggesting minimal chronotropic response following HCTZ treatment, which provides the justification for combining these in the meta-analysis. We did not conduct any comparisons between whites and blacks because analyses in these groups were all done separately and the purpose of this study is not to evaluate differences between whites and blacks.

Genome-Wide Association Analyses

Four separate genome-wide association analyses were conducted for association with β-blockers' negative chronotropic response; these included analyses of response to atenolol as monotherapy and add-on therapy in whites and blacks,

Table 1. Characteristics of β-Blocker-Treated Participants in PEAR and PEAR-2

Characteristics	PEAR Atenolol Monotherapy (N=374)		PEAR Atenolol Add-on (N=325)		PEAR-2 Metoprolol Monotherapy (N=368)	
	White (n=228)	Black (n=146)	White (n=198)	Black (n=127)	White (n=200)	Black (n=168)
Age, mean (SD) y	49.5±9.5	47.2±8.5	49.9±9.5	47.4±8.8	51.0±9.0	50.0±9.2
Women, N (%)	109 (47.3)	107 (73.2)	91 (45.9)	92 (77.3)	110 (55.0)	89 (53.0)
BMI, mean (SD) kg×m^{-2}	30.3±5.6	31.6±6.3	30.3±4.9	31.5±5.4	30.8±5.1	30.8±5.2
Pretreatment HR, mean (SD) bpm	76.5±9.2	79.9±9.2	77.8±9.7	81.2±9.6	77.7±9.6	79.8±9.5
Change in HR, mean (SD) bpm	−12.8±5.5	−11.0±6.8	−13.4±6.0	−11.1±7.4	−12.3±7.2	−11.2±7.0

BMI indicates body mass index; bpm, beats per minute; HR, heart rate; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses.

followed by meta-analysis of the 2 data sets within race. SNPs with a $P<$1E-05 for atenolol monotherapy associations in whites and blacks are reported in Tables S3 and S4, respectively, and those for associations with response to atenolol add-on therapy are in Tables S5 and S6, respectively. Manhattan plots and q-q plots for each GWAS analysis are presented in Figures S1 through S4.

In blacks, the meta-analysis of atenolol monotherapy and add-on therapy revealed 35 SNPs achieving the suggestive P value level, representing 20 independent genetic signals (Table 2); these were tested for replication in PEAR-2 blacks treated with metoprolol. Replicated SNPs were defined as those with a Bonferroni corrected $P<$0.0025 (0.05/20 independent genetic signals), and with HR-lowering effects in the same direction observed in the discovery analysis. From this analysis, we found 2 SNPs—SNP rs17117817 located 7 kb away from 5′ of olfactory receptor family10 subfamily p-member1 (*OR10P1*) gene, and SNP rs2364349 within the sorting nexin-9 gene (*SNX9*)—to be significantly associated with metoprolol's HR-lowering effect in black participants (Table 2). Using an additive genetic model, blacks carrying the rs17117817 G-allele or rs2364349 A-allele had a smaller HR-lowering response to β-blockers (Figure 3). The combined 3-cohort meta-analysis P value for the rs17117817 and rs2364349 reached genome-wide significance (rs17117817G-allele; β=5.53 bpm, $P=$2E-09 and rs2364349 A-allele; β=3.5 bpm, $P=$1E-08, respectively). Additionally, we validated the association between the *OR10P1* and *SNX9* genetic regions and changes in HR in response to β-blockers in whites where we found significant SNPs in the *OR10P1* and *SNX9* genetic regions associated with HR changes in response to β-blockers (rs79860936 in *OR10P1* gene region; $P=$7.6E-04, rs7772983 in the *SNX9* gene region; $P=$1.7E-03, Figure 4).

To make sure that the 2 replicated signals were not previously associated with HR and that they are pharmacogenomics independent signals, we searched NHGRI GWAS Catalog, (https://www.ebi.ac.uk/gwas/search?query=SNX9)

and the dbGap phenotype-genotype integrator (https://www.ncbi.nlm.nih.gov/gap/phegeni) for any GWAS associations that have been previously reported between *OR10P1* or *SNX9* and HR. The results from this search showed no previously reported association between *SNX9* or *OR10P1* and HR. Although we adjusted for baseline HR in our analysis, we also tested the association between SNP rs2364349 and SNP rs17117817 and baseline HR in black participants treated with β-blockers in PEAR and PEAR-2 participants included in this study. We found no association between SNP rs2364349 and baseline HR in PEAR monotherapy ($P=$0.23), PEAR add-on therapy ($P=$0.37), and PEAR-2 ($P=$0.70). Similarly, we found no association between SNP rs17117817 and baseline HR in PEAR monotherapy ($P=$0.18), PEAR add-on therapy ($P=$0.79), and PEAR-2 ($P=$0.30).

To assess the relative contribution of the replicated SNPs toward our changes in HR in response to β-blockers, we created a response score as previously discussed in the Methods section. As expected, in black participants treated with atenolol mono- and add-on therapy in PEAR and metoprolol in PEAR2, individuals with a higher score had a greater HR reduction in response to β-blockers compared with individuals with lower score (Figure 5). The meta-analysis of the 3 data sets reveal that, on average, for each HR-lowering allele carried by an individual, there is ≈3.4 bpm reduction in HR in response to β-blockers ($P=$2.4E-12). Such an approach requires validation in an independent cohort but highlights the potential approach to clinical use of such data.

In whites, the meta-analysis between PEAR atenolol mono- and add-on therapy revealed 13 SNPs with a $P<$1E-05, which represent 5 independent genetic signals (Table 2). However, none of these SNPs were replicated when tested in white participants treated with metoprolol in PEAR-2 (Table 2).

Network Analysis

Lastly, we conducted a network analysis using the genes discovered in this study (*OR10P1* and *SNX9*) and the

Table 2. Replication of the Linkage-Disequilibrium Pruned SNPs With *P* Value <0.00001 From the GWAS Meta-Analysis of β-Blockers HR Response in PEAR-2 Participants Treated With Metoprolol

Race	SNP	A1	FRQ	Rsq*	Meta Effect	Meta SE	Meta *P* Value	Dir	PEAR-2 Effect	PEAR-2 SE	PEAR-2 *P* Value	Rsq†
Black‡	rs17117817‡	T‡	0.93‡	1.00 to 0.82‡	−5.73‡	1.11‡	2E-07‡	−−‡	−5.05‡	1.65‡	2.2E-03‡	1.00‡
Black	rs10809249	T	0.82	0.95 to 0.99	−3.52	0.74	2E-06	−−	−0.57	0.98	0.56	0.99
Black	rs12890215	T	0.82	1.01 to 1.03	−3.4	0.72	2E-06	−−	−0.48	0.92	0.602	1.00
Black	rs1045580	T	0.85	0.95 to 1.02	3.56	0.76	2E-06	++	0.62	1.02	0.53	1.00
Black	rs2806495	A	0.10	0.96 to 0.82	4.67	0.99	2E-06	++	−0.53	1.29	0.67	1.00
Black	rs7738600	A	0.51	1.05 to 1.03	−2.6	0.56	4E-06	−−	−0.24	0.71	0.73	1.00
Black	rs11757000	T	0.77	0.99 to 0.91	−3.05	0.67	5E-06	−−	0.35	0.91	0.69	1.00
Black	rs7042878	C	0.09	1.05 to 1.10	4.31	0.95	5E-06	++	1.65	1.35	0.22	1.00
Black‡	rs2364349‡	A‡	0.18‡	0.87 to 0.92‡	3.43‡	0.76‡	7E-06‡	++‡	3.64‡	1.03‡	4E-04‡	0.98‡
Black	rs4733278	T	0.46	0.82 to 0.99	−2.62	0.58	7E-06	−−	0.01	0.75	0.98	0.99
Black	rs11069252	T	0.92	0.92 to 1.00	−4.76	1.06	7E-06	−−	1.21	1.37	0.37	1.00
Black	rs12417208	A	0.84	0.89 to 0.82	−3.76	0.84	8E-06	−−	−1.43	1.09	0.19	0.93
Black	rs3759422	T	0.46	0.91 to 1.06	2.5	0.56	8E-06	++	0.77	0.69	0.26	1.00
Black	rs1018353	T	0.9	0.95 to 0.85	−4.29	0.96	8E-06	−−	0.94	1.37	0.49	1.00
Black	rs4554901	T	0.07	0.93 to 1.03	4.85	1.09	8E-06	++	−0.09	1.41	0.94	0.99
Black	rs10145648	T	0.74	0.98 to 1.00	−2.85	0.64	8E-06	−−	−0.34	0.77	0.65	1.00
Black	rs10809367	A	0.14	1.18 to 1.18	3.41	0.77	9E-06	++	0.95	1.18	0.42	1.00
Black	rs10499	A	0.95	0.94 to 1.10	−5.67	1.28	9E-06	−−	−2.01	2.00	0.31	1.00
Black‡	rs6455914‡	T‡	0.22‡	0.97 to 0.93‡	3.08‡	0.7‡	1E-05‡	++‡	1.73‡	0.83‡	0.03‡	0.99‡
Black	rs6470259	A	0.80	0.94 to 0.88	−3.2	0.72	1E-05	−−	−0.76	1.01	0.44	0.94
White	rs955395	A	0.36	0.99 to 0.96	−1.7	0.35	9E-07	−−	0.21	0.59	0.69	0.99
White	rs11727192	T	0.21	0.94 to 0.97	−1.92	0.42	5E-06	−−	−0.80	0.77	0.29	1.00
White	rs10516175	A	0.12	1.09 to 1.02	2.17	0.48	6E-06	++	0.46	0.79	0.64	0.99
White	rs13160161	A	0.37	1.07 to 0.97	−1.5	0.33	7E-06	−−	−0.34	0.67	0.71	1.00
White	rs11641210	T	0.89	0.99 to 1.00	2.32	0.52	8E-06	++	−0.50	1.00	0.61	0.99

A1 indicates coded allele; Dir, the direction of effect; FRQ, coded allele frequency; GWAS, genome-wide association study; HR, heart rate; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; POS, position; SNP, single nucleotide polymorphism.

*Rsq, *r*-squared imputation quality metric in the discovery analysis in which the first number represents the imputation quality for the presented SNP in PEAR monotherapy and the second number represents the imputation quality for the presented SNP in PEAR add-on therapy.

†Rsq, *r*-squared imputation quality metric in the replication analysis (PEAR2).

‡Represent polymorphisms with a *P* value <0.05 in the replication cohort and the effect of the polymorphism on HR in response to β-blockers is in the same direction in the discovery (Meta Effect) and replication (PEAR-2 Effect) analysis. Meta represents meta-analysis between PEAR β-blocker mono- and add-on therapy.

β–adrenergic receptor genes (*ADRB1* and *ADRB2*), which encode the protein targets for β-blockers, to identify potential interactions between the replicated genetic signals that might be involved in β-blockers' HR-lowering effects mechanism. From this analysis, using STRING database, we found a direct interaction between SNX9 and ADRB2, and an indirect interaction between OR10P1 and ADRB1 and ADRB2 via the effect of OR10P1 on either ADRBK1 or GNB1 (Figure 6). These results further support the association we found between *OR10P1* and *SNX9* and changes in HR in response to β-blockers and suggest that OR10P1 and SNX9 may be playing a potential role in the HR-lowering mechanism underlying β-blocker therapy.

Discussion

β–Blockers are one of the most commonly prescribed classes of drugs and have been used for decades to treat various cardiovascular conditions, including myocardial infarction, heart failure, hypertension, and angina.[1,3] The HR-lowering effect of β-blockers is a critical efficacy component for this class of drugs and contributes substantially to their beneficial effects in acute- and post–myocardial infarction, heart failure, angina, and arrhythmias.[5,6,33] However, considerable interpatient variability in HR response to β-blockers has been observed,[11,12] indicating that a substantial proportion of β-blocker-treated patients fail to achieve cardioprotection
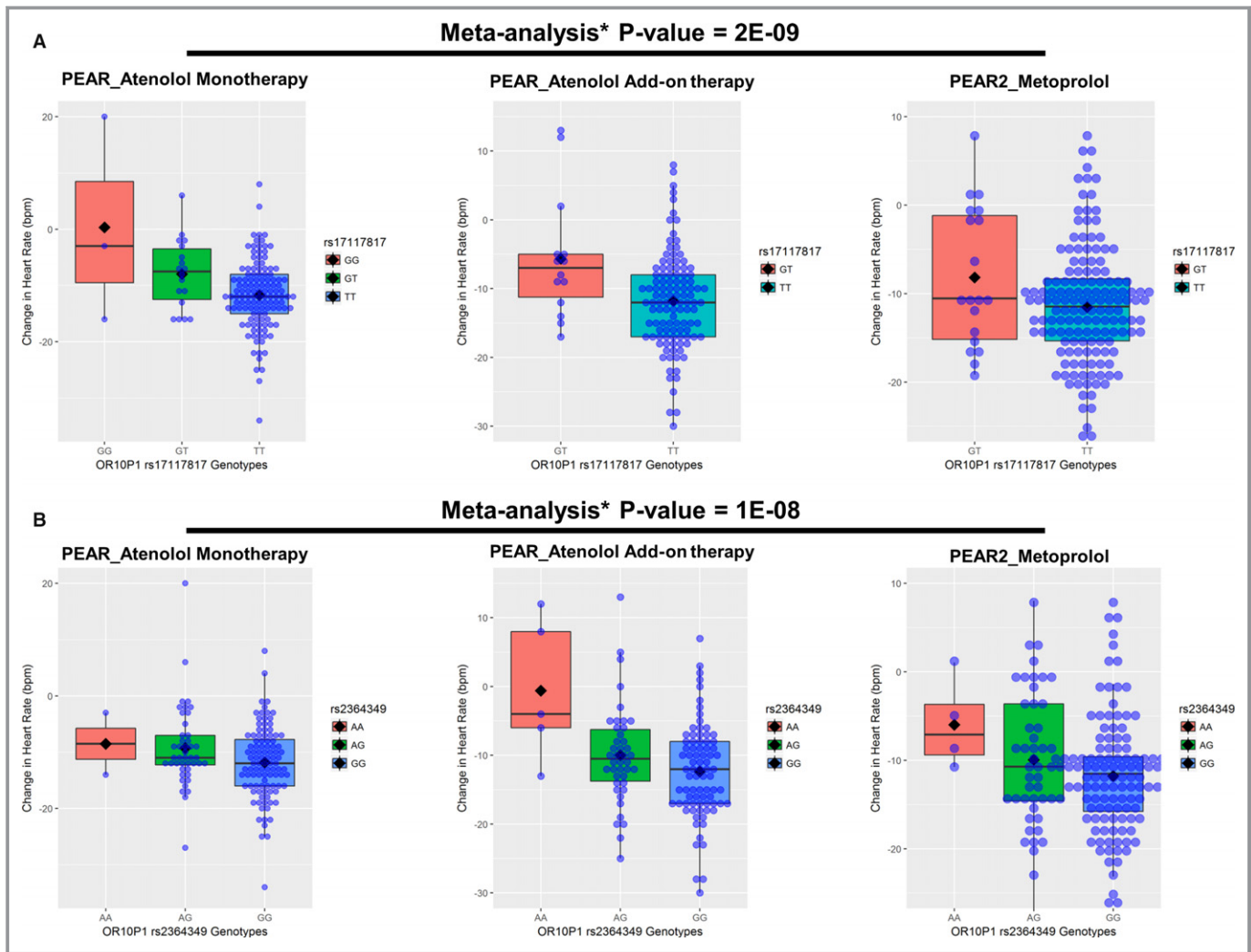
Figure 3. Effect of rs17117817 and rs2364349 polymorphisms on the β-blocker HR response in whites within PEAR and PEAR-2 studies. A, rs17117817. B, rs2364349. The box represents the values from the 25% to 75% percentile. The horizontal line represents the median. The black diamond represents the mean. The vertical line extends from the minimum to the maximum value. Each blue dot represents an individual. HR response was adjusted for age, sex, baseline HR, differences in dose, and principal components 1 and 2. Two-sided *P* values represented are for the contrast of adjusted means between different genotype groups. *Meta-analysis was performed assuming fixed effects and using inverse-variance weighting. bpm indicates beats per minute; HR, heart rate; OR10P1, olfactory receptor family 10 subfamily p member 1; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; SNX9, sorting nexin 9.

with β-blockers. This reveals that the current approach for β-blockers selection and achieving target HR is suboptimal. Thus, identifying factors that are associated with the observed changes in HR in response to β-blockers is important and holds the promise to optimize the use of this class of drugs. Given the growing evidence in the literature highlighting the significant influence of genetics on HR,[34–36] we sought herein to identify genetic factors associated with the observed interindividual variability in the negative chronotropic response to atenolol and metoprolol using a genome-wide association approach. To our knowledge, the present study is the first GWAS to investigate genetic variations associated with changes in HR in response to β-blocker therapy in whites and blacks.

The results from the GWAS analyses have revealed 2 novel genetic signals, SNP rs17117817 in the *OR10P1* genetic region, and SNP rs2364349 in the *SNX9* gene, with clinically relevant effects on HR in response to β-blocker therapy used in blacks. We also validated these findings in whites, by identifying significant signals, in the *OR10P1* and *SNX9* genetic regions, associated with changes in HR of whites treated with β-blockers. According to the meta-analysis results, rs17117817 T/T and G/T genotype carriers have ≈11 and 5.5 bpm reductions in their HR in response to β-blockers, respectively, compared with noncarriers. Similarly, rs2364349 G/G and A/G genotype carriers have ≈7 and 3.5 bpm reductions in their HR in response to β-blockers, respectively, compared with noncarriers. Moreover, we created
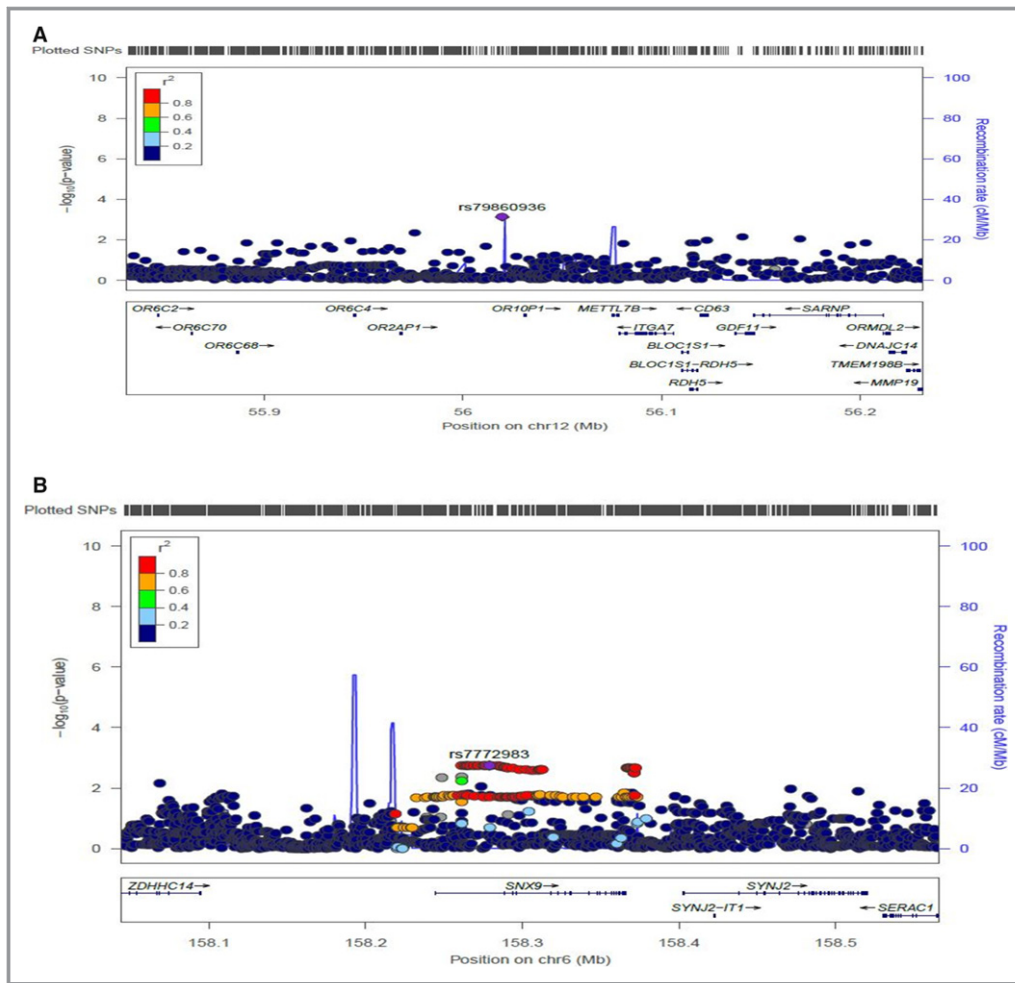
Figure 4. Regional plot showing the significance of the associations of SNPs with changes in HR in response to β-blockers used in whites. A, In olfactory receptor family 10 subfamily p member 1 (*OR10P1*) genetic region. B, In sorting nexin-9 (*SNX9*) genetic region. HR indicates heart rate; SNP, single nucleotide polymorphism.

a response score to assess the relative contribution of the replicated SNPs and their effect on changes in HR in response to β-blockers. From this analysis, we found that for each HR-lowering allele carried by an individual, there is ≈3.4 bpm reduction in HR in response to β-blockers (*P*=2.4E-12). These observed reductions in HR in response to β-blockers represent clinically significant differences that have been previously reported to be significantly associated with cardiovascular morbidity and mortality in the general population and in patients with heart disease.[37–40] Several studies have reported the pivotal role of β-blockers HR reduction effect in reducing cardiovascular mortality in heart failure.[41,42] For instance, a meta-analysis of 35 studies has shown that β-blocker HR reduction of 5 bpm could provide 14% risk reduction in mortality.[10] These data reveal the clinical significance of our findings on HR response to β-blockers and their clinical implications on cardiovascular mortality in patients treated with β-blockers. However, further validation of the response

score and the genetic variants, identified in this study, in large well-designed independent cohorts are still needed.

OR10P1 is a member of the olfactory receptor gene family, which represents a large family of genes that encode G-protein coupled receptors. OR10P1 has been shown to be highly expressed in the heart,[43] yet little is known about the role of OR10P1 in the regulation of HR. Herein, using network analyses, we have seen an indirect interaction between OR10P1 and ADRB2. Consistent with this finding, previous studies have demonstrated the interaction between protein members of the olfactory receptor family and β₂-adrenergic receptors.[44] Additionally, several studies have shown that inactivation of β-adrenergic receptor kinase-2 and β-arrestin both have a well-known regulatory role on β-adrenergic receptors, results in increased olfactory receptor stimulation of cAMP formation,[45,46]; the latter mediates the catecholaminergic control on HR and contractility.[47] Moreover, a recent study by Jovancevic et al[48] has shown that the
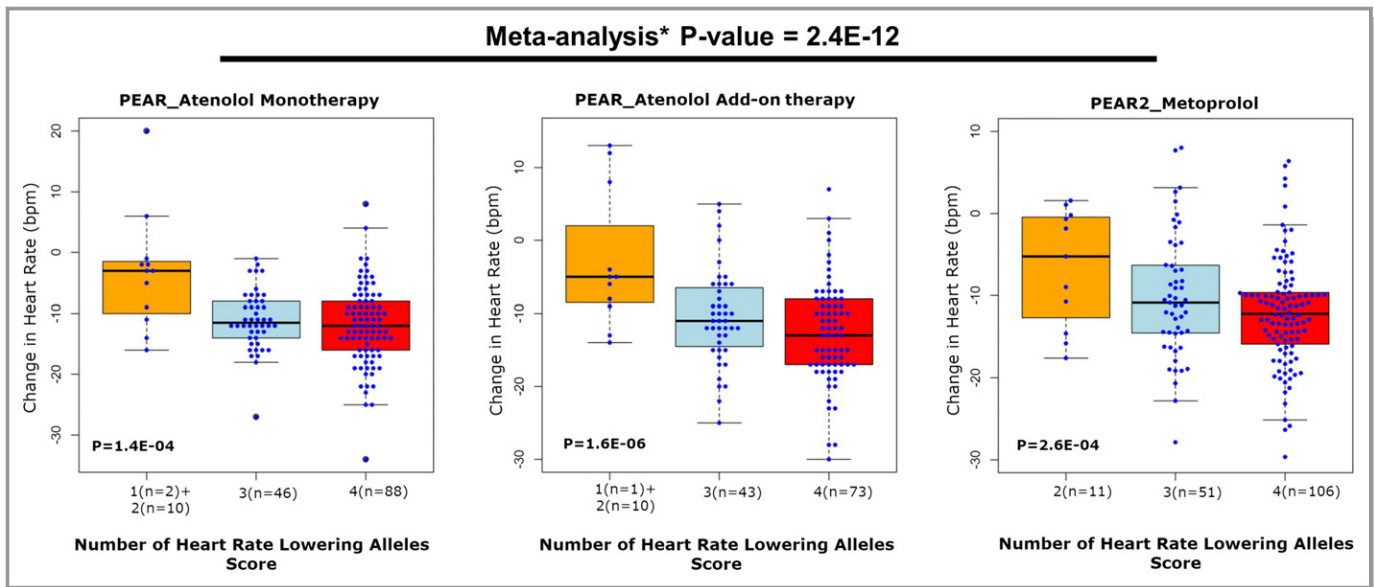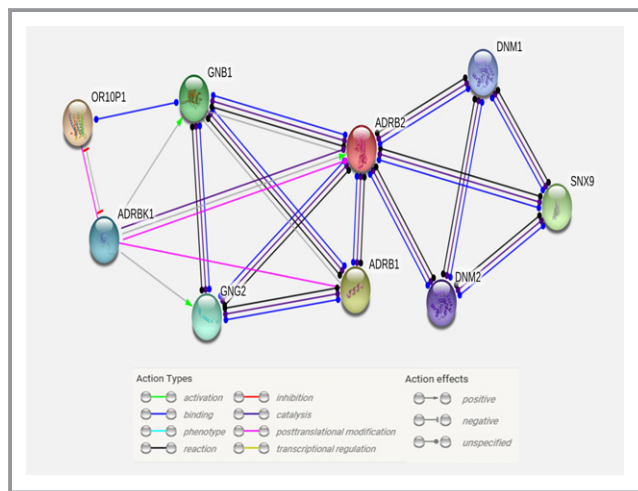
Figure 5. β-Blocker's HR response score in PEAR and PEAR-2. HR responses were adjusted for age, sex, baseline HR, differences in dose, and principal components 1 and 2. Genetic variants were coded as follows: (A) rs17117817 (OR10P1 genetic region) T/T=2, G/T=1, G/G=zero, and (B) rs2364349 (SNX9 genetic region) G/G=2, A/G=1, A/A=zero. The box represents the values from the 25% to 75% percentile. The horizontal line represents the median. The vertical line extends from the minimum to the maximum value. Each blue dot represents an individual. bpm indicates beats per minute HR, heart rate; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses. *Meta-analysis was performed between PEAR atenolol monotherapy, PEAR atenolol add-on therapy, and PEAR-2 metoprolol groups, assuming fixed effects and using inverse-variance weighting.

activation of olfactory receptors (ie, OR51E1) induces a negative chronotropic effect in human stem cell–derived cardiomyocytes in a dose-dependent manner. This literature evidence, along with the results of this study, suggests that olfactory receptors, including OR10P1, may be important regulators of HR in response to β-blockers.

SNX9, a member of the sorting nexin gene family, has been shown to be highly expressed in the heart and placenta.[49] It

Figure 6. Interaction network of replicated genetic signals and protein targets for β-blockers. Network was created using the STRING database (https://string-db.org/).

was first described in 1999 as a Src homology 3–domain and phox homology-domain protein that regulates metallopro-teinases ADAM9 and ADAM15.[49] SNX9 has also been shown to play a substantial role in endocytosis via clathrin-coated pits, which is well identified in the downregulation of transmembrane signal transduction but can also promote sustained signal transduction.[50] Interestingly, studies have reported that SNX9 interacts and stimulates the basal GTPase activity of dynamin, a GTPase that plays a central role in clathrin-induced endocytosis and agonist-mediated seques-tration of ADRB2.[51–53] Studies have also shown that the rapid and efficient recycling of G-protein coupled receptors, like ADRB2, to the plasma membrane after ligand-induced endo-cytosis is essential for the functional resensitization of receptors-mediated signaling.[54,55] This highlights the impor-tance of clathrin-mediated endocytosis, which is regulated by SNX9, in controlling the activity of G-protein coupled recep-tors like ADRB2.

In addition, studies have also revealed an interaction between SNX9 and adaptor protein 2,[56,57] an important multimeric protein that binds to cargo proteins and regulates the assembly of the clathrin-coated vesicle and their endocytosis.[58] Furthermore, adaptor protein 2 has been shown to interact with β-arrestin,[59] an interaction that is required for the recycling of the ADRB2. Since β-arrestin is known to block the activation of adenylyl cyclase, it eventually reduces ADRB responsiveness to catecholamines.[60] Thus, it

may be possible that SNX9 mediates its effect on HR response to β-blockers via β-arrestin. Moreover, the results of the network analysis that we performed revealed that SNX9 might be interacting with ADRB2 in an indirect way via its effect on dynamin 1 (DNM1) or dynamin 2 (DNM2) proteins. Although all these possible hypotheses are intriguing, follow-up studies are needed to test these hypotheses.

The present study has several strengths. To our knowledge, this is the first GWAS conducted to identify genetic variants associated with changes in HR in response to β-blockers in whites and blacks. Using this genome-wide approach, we are the first to uncover the novel association between *OR10P1* and *SNX9* and changes in HR in response to β-blockers. Additionally, the replication and the validation of the *OR10P1* and *SNX9* signals in blacks and whites, respectively, highlight the novelty of these findings. Moreover, the highly consistent effect of the *OR10P1* and *SNX9* genetic signals on changes in HR, across 3 independent cohorts, with 2 β-blockers, emphasizes the importance of these findings and suggests the involvement of the *OR10P1* and *SNX9* in the mechanism underlying β-blockers' HR-lowering effect.

The present study also has a few noteworthy limitations. First, the small sample sizes used for the genetic analyses may have limited our power to identify additional novel markers and to replicate some of our genetic signals. Second, we have used resting instead of exercise HR as an end point. However, in clinical practice, dose titration of β-blockers is performed based on resting HR and blood pressure response. Thus, this is the more clinically relevant phenotype.

In summary, the present study sheds light on *OR10P1* and *SNX9* and their association with changes in HR in response to β-blockers. Future studies are still needed to investigate the role of *OR10P1* and *SNX9* in the mechanism underlying HR response to β-blockers. Such studies may provide new insights into the HR-lowering mechanism, and help in the identification of novel drug targets based on a deeper understanding of the determinants of HR response to β-blockers. Perhaps as we move forward towards a personalized medicine approach, future use of the genetic signals identified in this study might help in guiding the selection of β-blockers and optimize their use.

Acknowledgments

Disclosures

None.

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SUPPLEMENTAL MATERIAL

Table S1. Power calculation for whites and blacks involved in the discovery analysis.

White participants in PEAR monotherapy and add-on therapy (n=426)			Black participants in PEAR monotherapy and add-on therapy (n=273)		
MAF	Difference in HR (bpm)	Power α=1E-05	MAF	Difference in BP (mmHg)	Power α=1E-05
0.05	5	11.18%	0.05	5	3.21%
	7	77.40%		7	20.90%
0.1	5	50.47%	0.1	5	19.17%
	7	99.75%		7	72.69%
0.15	5	81.06%	0.15	5	42.99%
	7	>99.9%		7	94.73%
0.2	5	93.86%	0.2	5	63.83%
	7	>99.9%		7	99.19%
0.25	5	98.02%	0.25	5	77.84%
	7	>99.9%		7	99.88%
0.3	5	99.30%	0.3	5	86.12%
	7	>99.9%		7	99.98%

Power was calculated using Quanto (http://biostats.usc.edu/software). Bpm, beats-per-minute; MAF, minor allele frequency

Table S2. Heart rate response to atenolol and hydrochlorothiazide in the PEAR study participants.

Change in Heart Rate (HRΔ) Mean (95% Confidence Interval)	White	Black
Atenolol as Monotherapy (HRΔ_{21})	-12.8 (-13.5, -12.1)	-11.0 (-12.1, -9.9)
Atenolol as Add-on Therapy (HRΔ_{32})	-13.4 (-14.3, -12.6)	-11.1 (-12.4, -9.8)

Table S3. Single nucleotide polymorphisms with p-value less than 0.00001 from the genome-wide association analysis of β-blockers heart rate response in Whites treated with atenolol monotherapy in the PEAR study.

CHR	SNP	POS*	A1	A2	FRQ	BETA	SE	P
3	rs11928048	1.69E+08	C	T	0.86	-2.91	0.64	9.67E-06
8	rs7461108	1.36E+08	G	A	0.7	2.68	0.51	3.87E-07
8	rs4132831	1.36E+08	G	A	0.66	2.44	0.52	4.81E-06
15	rs11072312	31269236	A	G	0.86	-3.37	0.69	2.36E-06
15	rs4780091	31271629	C	G	0.86	-3.32	0.7	4.72E-06
19	rs7255998	61348825	A	C	0.88	-3.25	0.7	7.51E-06

Single nucleotide polymorphisms were sorted in ascending order according to chromosome number. *SNPs positions were based on build 36 (NCBI36/hg18). CHR, chromosome; SNP, single nucleotide polymorphism; POS, position; A1, coded allele; A2, alternative allele; FRQ, coded allele frequency; SE, standard error.

Table S4. Single nucleotide polymorphisms with p-value less than 0.00001 from the genome-wide association analysis of β-blockers heart rate response in Blacks treated with atenolol monotherapy in the PEAR study.

CHR	SNP	POS*	A1	A2	FRQ	BETA	SE	P
1	rs547441	2.43E+08	A	C	0.54	3.69	0.75	2.15E-06
1	rs3002655	1.82E+08	T	C	0.76	-4.21	0.86	2.81E-06
1	rs804136	15006255	C	A	0.22	4.06	0.88	8.23E-06
1	rs6685658	97762924	C	A	0.9	-5.57	1.2	8.44E-06
2	rs12619068	72922807	C	T	0.47	-3.46	0.74	7.66E-06
2	rs7593084	72867344	C	T	0.47	-3.46	0.75	8.13E-06
2	rs16829119	1.49E+08	G	A	0.3	3.78	0.82	8.40E-06
2	rs6713372	1.52E+08	A	G	0.93	-6.44	1.4	9.37E-06
4	rs4315759	1.55E+08	C	T	0.94	-8.53	1.82	6.86E-06
5	rs4274995	1.62E+08	C	T	0.13	5.95	1.16	1.05E-06
8	rs6470258	1.26E+08	C	G	0.8	-5.63	1.08	6.34E-07
8	rs6470259	1.26E+08	A	G	0.78	-4.21	0.9	7.03E-06
9	rs10115550	1.27E+08	C	T	0.84	-4.48	0.93	3.38E-06
10	rs2804864	33835919	A	G	0.93	-6.17	1.3	5.41E-06
11	rs11034161	37454092	A	G	0.88	-5.08	1.01	1.42E-06
11	rs16930242	37397243	G	A	0.93	-6.49	1.39	6.92E-06
18	rs3730662	50049273	C	T	0.92	-6.4	1.15	1.46E-07
18	rs3730810	50072499	T	C	0.92	-6.4	1.15	1.46E-07
18	rs17662631	34979891	T	C	0.95	-7.52	1.53	2.45E-06
18	rs1006609	20368055	A	G	0.92	-6.6	1.35	2.89E-06
18	rs11082886	47528563	A	G	0.77	-4.41	0.91	3.33E-06
21	rs2830261	26795766	T	C	0.13	5.53	1.14	3.07E-06
21	rs2823008	15309774	T	C	0.89	-5.24	1.13	7.94E-06
21	rs2823005	15302300	A	G	0.89	-5.23	1.13	8.05E-06

Single nucleotide polymorphisms were sorted in ascending order according to chromosome number. *SNPs positions were based on build 36 (NCBI36/hg18). CHR, chromosome; SNP, single nucleotide polymorphism; POS, position; A1, coded allele; A2, alternative allele; FRQ, coded allele frequency; SE, standard error.

Table S5. Single nucleotide polymorphisms with p-value less than 0.00001 from the genome-wide association analysis of β-blockers heart rate response in Whites treated with atenolol add-on therapy in the PEAR study.

CHR	SNP	POS*	A1	A2	FRQ	BETA	SE	P
4	rs6822628	1.65E+08	G	A	0.93	-5.29	0.92	3.69E-08
4	rs17044565	1.65E+08	T	C	0.93	-5.29	0.92	3.69E-08
4	rs723730	1.65E+08	A	T	0.93	-5.29	0.92	3.69E-08
4	rs6835078	1.65E+08	C	T	0.93	-5.29	0.92	3.76E-08
4	rs6840738	1.65E+08	A	C	0.93	-5.29	0.92	3.94E-08
4	rs985676	1.65E+08	T	C	0.93	-5.29	0.92	4.05E-08
4	rs17044630	1.65E+08	T	A	0.93	-5.28	0.93	4.64E-08
4	rs11930019	1.65E+08	T	C	0.93	-5.28	0.93	4.83E-08
4	rs11943491	1.65E+08	G	A	0.93	-5.27	0.93	5.02E-08
4	rs9991538	1.65E+08	T	G	0.93	-5.27	0.93	5.12E-08
4	rs10002082	1.65E+08	A	G	0.93	-5.27	0.93	5.23E-08
4	rs11931264	1.65E+08	T	C	0.93	-5.27	0.93	5.38E-08
4	rs1431001	1.65E+08	A	T	0.93	-5.26	0.93	5.72E-08
4	rs1430999	1.65E+08	G	A	0.93	-5.26	0.93	5.96E-08
4	rs1120384	1.65E+08	G	A	0.93	-5.26	0.93	6.02E-08
4	rs1430998	1.65E+08	A	G	0.93	-5.17	0.95	1.74E-07
4	rs10517800	1.65E+08	C	T	0.93	-5.04	0.96	3.95E-07
4	rs17044711	1.65E+08	C	T	0.93	-5.04	0.96	3.95E-07
4	rs13435288	1.65E+08	A	G	0.93	-5.03	0.96	3.96E-07
4	rs7673540	1.65E+08	G	A	0.93	-5.03	0.96	3.96E-07
4	rs7659131	1.65E+08	G	A	0.93	-5.03	0.96	3.96E-07
4	rs1835507	1.65E+08	A	G	0.93	-5.03	0.96	3.96E-07
17	rs12952770	73964590	A	G	0.87	-3.54	0.72	1.96E-06
17	rs2289754	73966813	G	A	0.85	-3.02	0.66	9.58E-06

Single nucleotide polymorphisms were sorted in ascending order according to chromosome number. *SNPs positions were based on build 36 (NCBI36/hg18). CHR, chromosome; SNP, single nucleotide polymorphism; POS, position; A1, coded allele; A2, alternative allele; FRQ, coded allele frequency; SE, standard error.

Table S6. Single nucleotide polymorphisms with p-value less than 0.00001 from the genome-wide association analysis of β-blockers heart rate response in Blacks treated with atenolol add-on therapy in the PEAR study.

| CHR	SNP	POS*	A1	A2	FRQ	BETA	SE	P
1	rs12130603	14617376	C	G	0.9	-7.11	1.44	2.52E-06
4	rs13118928	1.46E+08	A	G	0.87	-5.44	1.17	8.94E-06
6	rs9385619	1.33E+08	C	T	0.72	4.35	0.93	8.23E-06
8	rs16917667	96521004	G	A	0.93	-8.83	1.56	1.10E-07
8	rs1392797	96522028	C	G	0.91	-7.17	1.47	3.61E-06
8	rs1017660	15747621	C	T	0.43	4	0.86	7.89E-06
8	rs2736012	15746643	G	A	0.28	4.38	0.95	9.64E-06
8	rs1017659	15747493	A	T	0.42	3.91	0.85	9.78E-06
11	rs2658796	92850464	A	G	0.31	4.27	0.92	9.14E-06
14	rs10142635	33962358	A	G	0.88	-6.61	1.25	5.80E-07
14	rs7144173	33969051	T	C	0.81	-4.95	1.07	9.84E-06
14	rs10150887	33969816	A	G	0.81	-4.95	1.07	9.84E-06
14	rs28448660	33971784	T	C	0.81	-4.95	1.07	9.84E-06
15	rs8035503	24544752	A	G	0.91	-6.7	1.39	4.23E-06
18	rs2000887	55370384	A	G	0.08	7.01	1.43	2.83E-06

Single nucleotide polymorphisms were sorted in ascending order according to chromosome number. *SNPs positions were based on build 36 (NCBI36/hg18). CHR, chromosome; SNP, single nucleotide polymorphism; POS, position; A1, coded allele; A2, alternative allele; FRQ, coded allele frequency; SE, standard error.
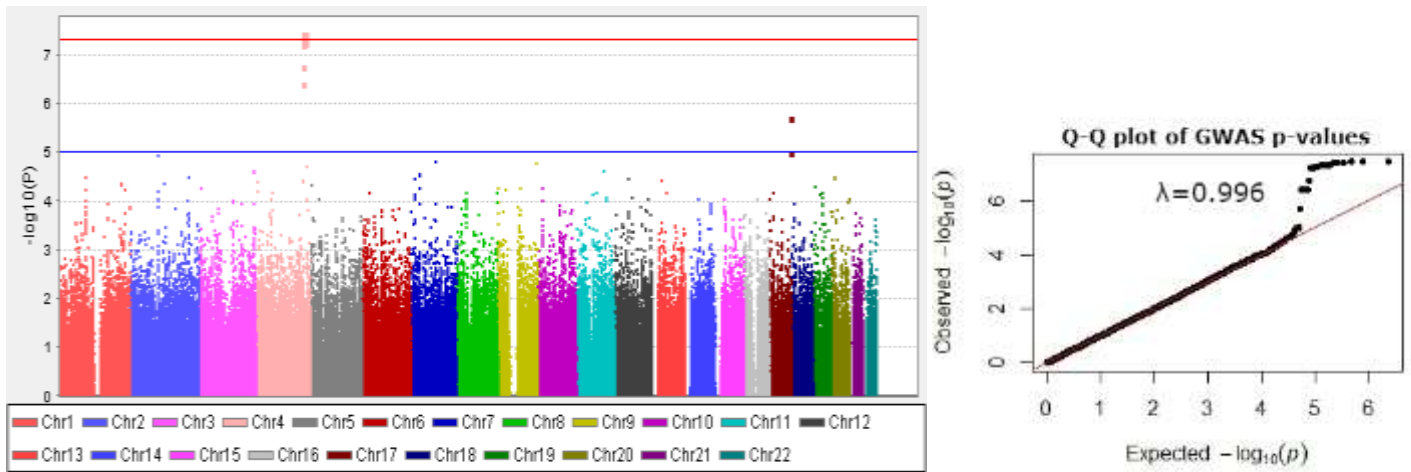
Figure S1. Manhattan plot and q-q plot obtained from the genome-wide association analysis conducted in PEAR White participants treated with atenolol monotherapy using $HR\Delta_{21}$ as the phenotype.
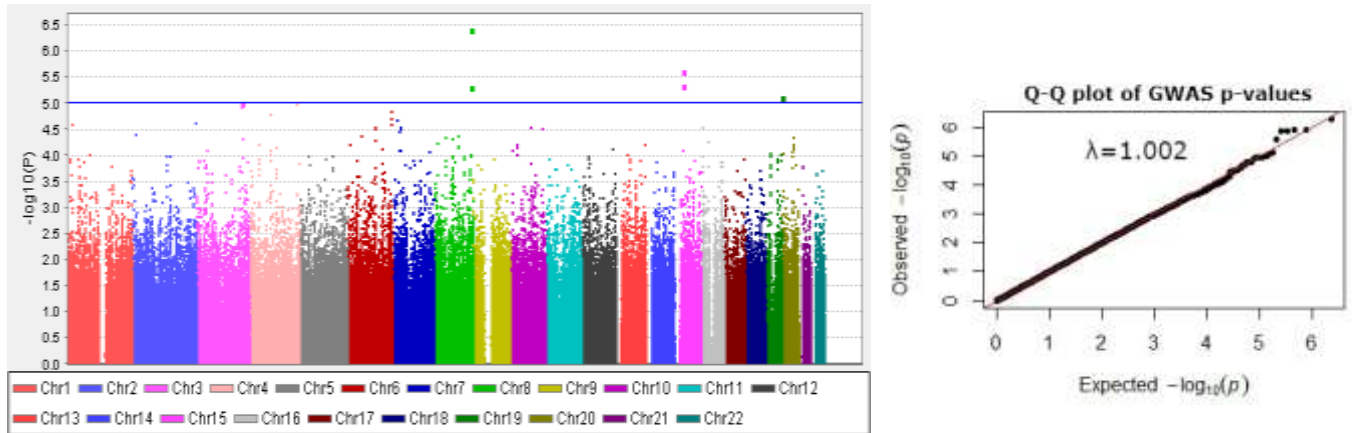
Figure S2. Manhattan plot and q-q plot obtained from the genome-wide association analysis conducted in PEAR Black participants treated with atenolol monotherapy using $HR\Delta_{21}$ as the phenotype.
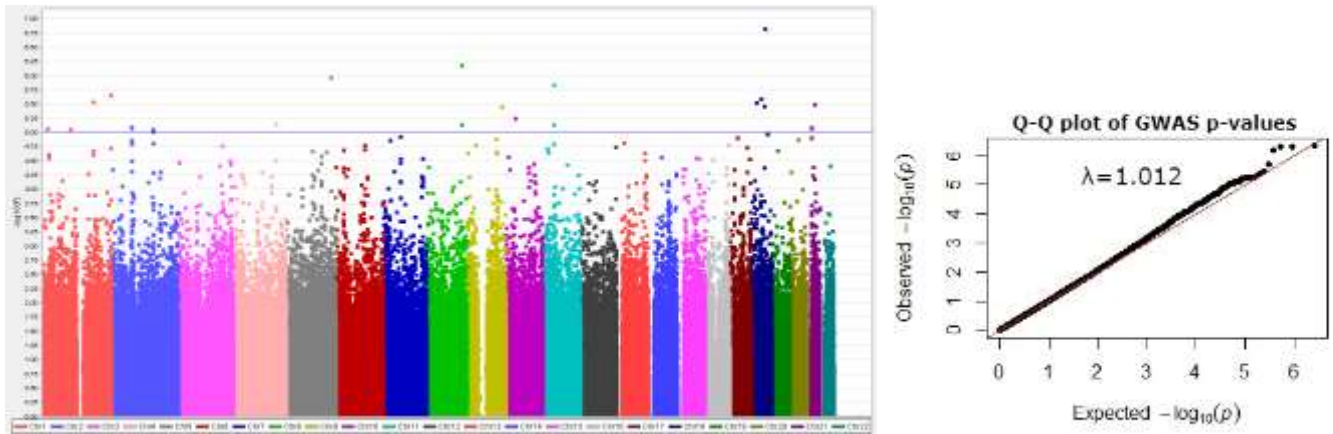
Figure S3. Manhattan plot and q-q plot obtained from the genome-wide association analysis conducted in PEAR White participants treated with atenolol add-on therapy using HR$_{\Delta32}$ as the phenotype.

Figure S4. Manhattan plot and q-q plot obtained from the genome-wide association analysis conducted in PEAR Black participants treated with atenolol add-on therapy using HRΔ_{32} as the phenotype.