Genome-Wide Meta-Analysis of Blood Pressure Response to β₁-Blockers: Results From ICAPS (International Consortium of Antihypertensive Pharmacogenomics Studies)

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Background—There exists a wide interindividual variability in blood pressure (BP) response to β₁-blockers. To identify the genetic determinants of this variability, we performed a pharmacogenomic genome-wide meta-analysis of genetic variants influencing β₁-blocker BP response.

Methods and Results—Genome-wide association analysis for systolic BP and diastolic BP response to β₁-blockers from 5 randomized clinical trials consisting of 1254 patients with hypertension of European ancestry were combined in meta-analysis and single nucleotide polymorphisms (SNPs) with $P<10^{-4}$ were tested for replication in 2 independent randomized clinical trials of β₁-blocker–treated patients of European ancestry (n=1552). Regions harboring the replicated SNPs were validated in a β₁-blocker–treated black cohort from 2 randomized clinical trials (n=315). A missense SNP rs28404156 in *BST1* was associated with systolic BP response to β₁-blockers in the discovery meta-analysis ($P=9.33\times10^{-5}$, β=−3.21 mm Hg) and replicated at Bonferroni significance ($P=1.85\times10^{-4}$, β=−4.86 mm Hg) in the replication meta-analysis with combined meta-analysis approaching genome-wide significance ($P=2.18\times10^{-7}$). This SNP in *BST1* is in linkage disequilibrium with several SNPs with putative regulatory functions in nearby genes, including *CD38*, *FBXL5*, and *FGFBP1*, all of which have been implicated in BP regulation. SNPs in this genetic region were also associated with BP response in the black cohort.

Conclusions—Data from randomized clinical trials of 8 European ancestry and 2 black cohorts support the assumption that *BST1* containing locus on chromosome 4 is associated with β₁-blocker BP response. Given the previous associations of this region with BP, this is a strong candidate region for future functional studies and potential use in precision medicine approaches for BP management and risk prediction. (***J Am Heart Assoc***. 2019;8:e013115. DOI: 10.1161/JAHA.119.013115.)

Key Words: blood pressure • hypertension • meta-analysis • pharmacogenomics • β₁-blocker • β–blocker

Hypertension is a preventable risk factor for stroke, chronic heart failure, coronary artery disease, and kidney disease resulting in premature death and disability.[1] Hypertension remains a global burden with an estimated direct cost of ≈$200 billion by 2030.[2] More than 1.3 billion people worldwide are estimated to have hypertension and this estimate is expected to increase to 1.56 billion people globally by 2025.[1,3] Moreover, ≈4 in 10 adults older than

Clinical Perspective

What Is New?

- Although multiple genome-wide association studies have attempted to investigate the genetic determinants of the large interindividual variability observed in the blood pressure (BP) response to β_1-blockers, most of these studies have been plagued by small sample sizes and lack of replication.
- Using genome-wide association study data from 8 distinct cohorts of β_1-blocker–treated participants, we performed the largest genome-wide meta-analysis to identify the genetic determinants of BP response to β_1-blockers and identified a locus near *BST1* on chromosome 4p15.32 that was significantly associated with BP response with multiple levels of evidence.

What Are the Clinical Implications?

- These findings indicate that the *BST1* locus is involved in the BP response to β_1-blockers with multiple genes in this region having been previously implicated in pathways related to BP regulation and hypertension.
- Additional investigations to understand the exact mechanism of this locus and the genes involved in mediating BP response can aid in the advancement of personalized β_1-blocker therapy for improved BP control and management.

25 years have hypertension, and 9 of 10 adults living up to 80 years will develop hypertension.[4]

β-Blockers, one of the many available pharmacotherapeutic interventions, have been used for the past 45 years and previously were recommended as first-line agents for treating uncomplicated hypertension. However, over recent years, β-blockers have fallen out of favor as first-line therapy because of results of large meta-analyses documenting poorer outcomes when compared with other drug classes such as diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or calcium channel blockers, for treating hypertension.[5] Based on findings from these large studies, recent guidelines including American College of Cardiology/American Heart Association and National Institute for Health and Care Excellence no longer recommend β-blockers as a first line of treatment in all or in a subset of patients.[6,7] While β-blockers are not recommended as first-line agents, they are still a preferred choice for treating patients with hypertension who have comorbid cardiovascular conditions such as angina, chronic heart failure, and arrhythmias, and as part of combination therapy for resistant hypertension.[8,9] As a result of this continued use of β-blockers, there remains a need for individualizing β-blocker–based antihypertensive therapy for

better hypertension management, blood pressure (BP) control, and improved outcomes of patients who require β-blocker therapy.

Studies have documented that patients of white ancestry usually respond favorably to β-blocker therapy compared with other antihypertensive medications such as diuretics that result in better BP control in patients of black ancestry.[10] However, there exists a wide interindividual variability in BP response to β-blockers among patients of white ancestry, suggesting a need to understand related factors (genetic and nongenetic) that contribute to variability in BP response to β-blockers, with an ultimate goal of optimizing BP management.[11] Furthermore, β_1-selective blockers are the β-blocker of choice for patients with hypertension who require β-blocker therapy.[16,17] Identification of genetic factors of β_1-selective blocker therapy response through pharmacogenomics analyses could have the potential to achieve some of the goals for individualizing therapy among individuals with hypertension. To this end, several genome-wide analyses conducted through individual studies have identified a few replicated signals of β_1-selective blocker therapy response.[12–15] To the best of our knowledge, there are no genome-wide analyses for nonselective β-blockers, which are not currently recommended as first line for treatment of hypertension. In this article, we aimed to perform the largest 2-stage genome-wide meta-analysis using data from randomized clinical trials with genome-wide data, as part of the ICAPS (International Consortium for Antihypertensive Pharmacogenomics Studies). However, as mentioned before, ICAPS only had pharmacogenomics trials with whole genome data for β_1-selective blocker–centered studies. Based on the phenotype of interest, our meta-analysis study reported in this article focused on BP response to β_1-selective blockers, which comprised a total of 8 cohorts of hypertensive patients of white ancestry treated with β_1-blockers to identify pharmacogenetic markers associated with BP response.

Methods

Study Participants and Inclusion Criteria

The discovery genome-wide association study (GWAS) meta-analysis consisted of 5 study cohorts: PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses),[18] PEAR-2 (Pharmacogenomic Evaluation of Antihypertensive Responses 2),[19] LIFE-Fin (Finnish arm of the Losartan Intervention For Endpoint Reduction in Hypertension Study),[20] GENRES (Genetics of Drug Responsiveness in Essential Hypertension),[13] and BB-SS (Pharmacogenomics of Beta-Blockers Sardinian Study). Detailed information of each of these

cohorts is described in Data S1. For all participating cohorts, patients with uncomplicated hypertension and untreated systolic BP (SBP) >140 mm Hg or diastolic BP (DBP) >90 mm Hg were included in the study. Patients from all participating studies in the discovery cohort underwent an antihypertensive medication washout before starting the study, after which they were treated with β1-blocker monotherapy for varying lengths of time according to each study-specific protocol.

For replication, a meta-analysis consisting of 3 study cohorts (INVEST [International Verapamil SR-Trandolapril Study],[21] ASCOT-UK [Anglo-Scandinavian Cardiac Outcomes Trial-United Kingdom],[22] and ASCOT-SC [Anglo-Scandinavian Cardiac Outcomes Trial-Scandinavian][22]) was used. ASCOT-UK, ASCOT-SC, and INVEST did not have a hypertensive medication washout period before starting the β1-blocker therapy. Black participants from PEAR and PEAR-2 were used for ethnic validation.

All of the studies were approved by institutional review committee, all of the participants signed informed consent, and studies were conducted in accordance with the regulations set forth by the Declaration of Helsinki. The data for PEAR have been made publicly available in the database of Genotypes and Phenotypes (dbGaP)[23] (Accession: phs000649.v1.p1). The PEAR-2 and INVEST data are currently in the process of being uploaded and will soon be available to other researchers in dbGaP. Clinical Trials included in this meta-analysis can be found at: URL: https://www.clinicaltrials.gov. Unique identifiers: PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses): NCT00246519; PEAR-2 (Pharmacogenomic Evaluation of Antihypertensive Responses 2): NCT01203852; INVEST (International Verapamil SR Trandolapril Study): NCT00133692; GENRES (A Study on Molecular Genetics of Drug Responsiveness in Essential Hypertension): NCT03276598; and LIFE (Losartan Intervention For Endpoint Reduction in Hypertension): NCT00338260.

BP Response Phenotype

Multiple measures of BP response were available for each study and the most accurate BP measurement available for each study was used to determine the phenotype. PEAR-2[19] used home BP measurements, whereas GENRES,[24] BB-SS, LIFE-Fin,[20] INVEST,[21] ASCOT-UK, and ASCOT-SC used office BP measurements to define response to β1-blockers. PEAR used a composite weighted average of BP response from office, home, and ambulatory day and nighttime values.[25]

For all studies, using the above-mentioned BP measurements, β1-blocker BP responses (diastolic and systolic BP response) were calculated as the difference between the start to the end of the β1-blocker treatment.

Genotyping and Imputation

For each study, participants were genotyped on either an Illumina or an Affymetrix panel. Each study underwent standard quality control (QC) protocols for their genotype data to obtain high-quality single nucleotide polymorphisms (SNPs) for their study, which were then imputed to the 1000G phase 3 reference panel for a total number of ≈47 million SNPs.

Statistical Analysis

Continuous variables are presented as mean±SD and categorical variables are presented as numbers and percentages.

We performed a 2-stage genome-wide meta-analysis. The first stage comprised of a discovery GWAS meta-analysis, which was followed by testing the top signals in a meta-analysis replication cohort. The second stage was a combined meta-analysis of the findings from both discovery and replication meta-analysis. The detailed flowchart outlining the steps in the study is presented in Figure 1.

GWAS Per Study

A total of 1254 individuals from 5 cohorts (PEAR, PEAR-2, LIFE-Fin, GENRES, and BB-SS) were included as part of the discovery GWAS meta-analysis for BP response to β1-blocker treatment. First, a GWAS for imputed SNPs was performed separately in each study for association with SBP and DBP response using linear regression adjusting for age, sex, pretreatment BP, and principal components for ancestry. Regression analysis was conducted using either EPACTS or SNPTEST. Each study filtered the tested SNPs at a minor allele frequency cutoff of >1% and imputation QC (Rsq) of >0.30.

QC Per Study

Results from each study further underwent stringent standard QC procedures using the EasyQC package as per protocol described by Winkler et al,[26] to account for heterozygosity, missingness, and allele mismatches. Quantile-quantile plots, P-Z test, and allele frequency mismatch plots were generated using EasyQC.

Discovery GWAS Meta-Analysis

After QC, the GWAS results from each cohort were combined in a meta-analysis using the inverse variance method with fixed effect model performed using Meta-Analysis Helper (METAL).[27] A genomic inflation (λ) correction was applied if genomic inflation was deemed to exist (λ >1) for any of the studies. As mentioned previously, each of the studies in the discovery meta-analysis GWAS were imputed to the same reference panel. However, each of the GWAS results from the study was subjected to stringent QC and a different number of SNPs were removed from each study based on the QC criteria. However, for the purpose of discovery meta-analysis
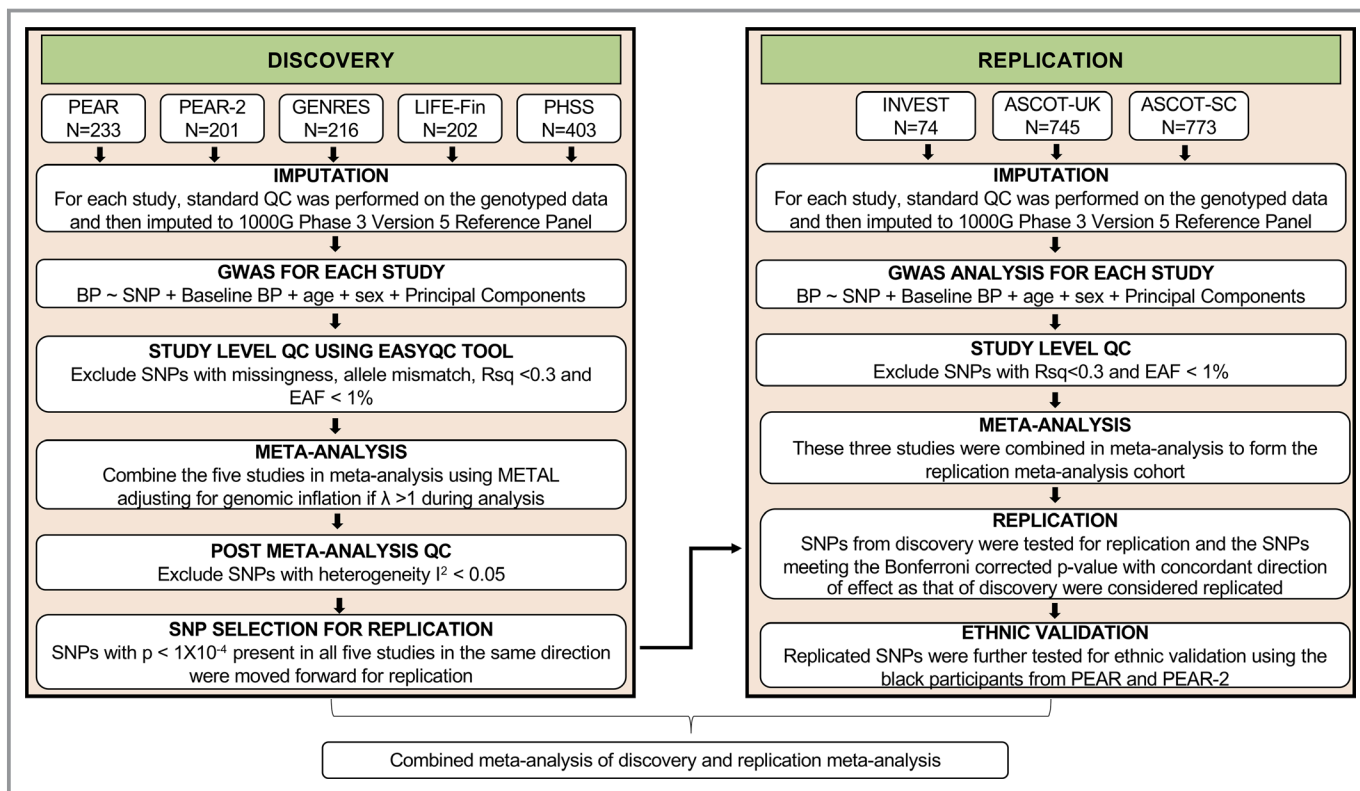
Figure 1. Overall design framework for the discovery and replication meta-analysis. ASCOT-SC indicates Anglo-Scandinavian Cardiac Outcomes Trial-Scandinavian; ASCOT-UK, Anglo-Scandinavian Cardiac Outcomes Trial-United Kingdom; BB-SS, Pharmacogenomics of Beta-Blockers Sardinian Study; BP, blood pressure; EAF, effect allele frequency; GENRES, Genetics of Drug Responsiveness in Essential Hypertension; GWAS, genome-wide association study; INVEST, International Verapamil SR-Trandolapril Study; LIFE-Fin, Finnish arm of the Losartan Intervention For Endpoint Reduction in Hypertension Study; METAL, Meta-Analysis Helper; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; QC, quality control; Rsq, imputation quality; SNPs, single nucleotide polymorphisms.

we focused only on the 6596214 SNPs that were present in all 5 studies. After meta-analysis, we applied a stringent prioritization criterion to select SNPs from the discovery meta-analysis and focused only on SNPs with the same direction of association across all 5 studies in the discovery meta-analysis and deemed SNPs that met the conventionally acceptable $P<5\times10^{-8}$ as genome-wide significant. The suggestive level of association was set at a threshold of $P=1\times10^{-4}$. SNPs that met the suggestive level of association with a concordant direction of association across all 5 studies were further linkage disequilibrium (LD) pruned at $r^2>0.2$ using LD Link,[28] to obtain single independent SNPs representative of each associated locus that were tested for replication. The number of independent signals were used to calculate a Bonferroni-corrected α level (0.05/# of independent signals), to determine whether SNPs were replicated for SBP and DBP responses in the replication cohort.

Replication GWAS Meta-Analysis

A total of 1592 individuals from 3 study cohorts (INVEST, ASCOT-UK, and ASCOT-SC) were included in the replication

meta-analysis. Since replication cohorts did not have a hypertensive medication washout before starting the β₁-Blocker therapy, the analysis was adjusted for a dummy variable indicative of patient treatment status (yes=1, no=0), if patients were or were not treated, respectively, with any antihypertensive medication at the start of their β₁-blocker therapy. Summary statistics for SNPs meeting the suggestive level of significance from the discovery GWAS meta-analysis were looked up in each study of the defined replication cohorts, which were then combined in meta-analysis to form the replication meta-analysis cohort. SNPs were deemed replicated if they met the Bonferroni-corrected P value for association with SBP (3.4×10^{-4}) and DBP (4.3×10^{-4}) response, with concordant direction of association as in the discovery meta-analysis.

Combined Discovery and Replication Meta-Analysis

Summary statistics of discovery and replication GWAS and meta-analyses were further combined in a meta-analysis. Genome-wide significance for this combined meta-analysis was set at $P=5\times10^{-8}$.

Secondary Validation

The SBP and DBP association results for black participants with hypertension from PEAR-2 and PEAR were combined in a meta-analysis and used for ethnic validation of SNPs that were successfully replicated in an INVEST/ASCOT-UK/ASCOT-SC replication cohort. Given the differences in LD between the genome of people with white and African ancestry, we did not expect the same SNPs to be necessarily validated. Hence, we scanned the genetic region surrounding the index SNP (±500 kb) for any SNPs that were associated with the SBP or DBP response in the same direction as that of the index SNP at a Bonferroni-corrected *P* value.

In Silico Analysis

In an attempt to identify the putative functional SNP, all SNPs in high LD (r^2>0.8) with the replicated SNP (rs28404156 of *BST1* locus) based on the 1000G phase 3 reference panel were extracted using the rAggar.[29] RegulomeDB[30] was used to annotate and score these SNPs to identify potentially regulatory SNPs in LD with the index SNP using a stringent score of ≤4.[30] The expression Quantitative Trait Locus (eQTL) of all of these SNPs in LD with the replicated SNPs was evaluated using HaploReg v4.1 databases.[31]

Results

Clinical characteristics of all 8 participating cohorts in the β₁-blocker discovery and replication meta-analysis are summarized in Table 1. All participants were of European ancestry. Men and women were similarly represented in all of the studies, with the exceptions of GENRES, which was composed of men only, and ASCOT, which was composed of predominantly men. The clinical characteristics of the black patients from PEAR-2 and PEAR treated with a β₁-blocker, and included in the secondary validation, are summarized in Table S1.

Discovery GWAS Meta-Analysis

GWAS discovery meta-analysis for SBP and DBP responses to β₁-blockers was performed using 5 cohorts (PEAR, PEAR-2, LIFE-Fin, GENRES, and BB-SS). Manhattan and QQ plots for association are shown in Figure 2. The QQ plots for both SBP and DBP do not exhibit any inflation indicative of population substructure beyond that expected by chance alone. None of the SNPs reached genome-wide significance for either SBP or DBP response. However, 1207 SNPs for SBP and 842 SNPs for DBP met the suggestive level of significance ($P<1\times10^{-4}$). With LD pruning (r^2>0.2), a total of 280 and 218 independent

signals associated with SBP and DBP, respectively, were identified and tested for replication.

Replication GWAS Meta-Analysis

For association with SBP response, the SNP rs28404156 was successfully replicated with a Bonferroni-corrected *P* value and was associated in the same direction as in the discovery meta-analysis. (Discovery: $P=9.33\times10^{-5}$ and β=−3.21±0.82; replication: $P=1.85\times10^{-4}$ and β=−4.86±1.3) (Table 2). rs28404156 (G->A) is a missense variant that causes an arginine to histidine (Arg153His) amino acid change in BST1 and is an eQTL[31] for *FAM200B*(Artery_Tibial). The regional plot for rs28404516 for the white population is presented in Figure 3A.

The least square adjusted means for SBP response by genotype for rs28404156 was plotted for the studies that were part of the discovery meta-analysis (PEAR, PEAR-2, LIFE-Fin, GENRES, and BB-SS) as these were the only studies for which this analysis was available. Given the low minor allele frequency of rs28404156 (0.08), only heterozygotes but no homozygotes for the minor allele were available in all of the discovery studies except BB-SS. Among the 5 studies, the A allele carriers had better SBP response compared with the noncarriers (Figure S1).

In contrast, for association with DBP responses, none of the SNPs that were tested met the Bonferroni-corrected *P* value for replication.

Combined Discovery and Replication Meta-Analysis

None of the SNPs met genome-wide significance in the combined discovery-replication meta-analysis and the association of the replicated SNP rs28404156 with SBP response reached a meta-analysis *P* value of 1.15×10^{-7}.

Ethnic Validation

The black cohort from PEAR and PEAR-2 were used for ethnic validation of the replicated SNP rs28404156. rs28404156 was not significantly associated in the black cohort. However, considering the region (±500 kb) surrounding the SNP rs28404156, another SNP, rs193131744, was found to be significantly associated with reduction in SBP in response to β₁-blocker treatment with a concordant direction as that of discovery meta-analysis (discovery: $P=2.64\times10^{-6}$ and β=−16.87) (Figure 3B).

In Silico Analysis

A total of 12 SNPs in high LD (r^2>0.8) with the replicated SNP (rs28404156) were extracted using rAggar. Of these, the

Table 1. Clinical Characteristics of all White Participants for the 8 Studies in the Discovery and Replication Meta-Analysis

	Discovery Meta-Analysis					Replication Meta-Analysis		
	PEAR	PEAR-2	GENRES	LIFE-Fin	BB-SS	ASCOT-UK	ASCOT-SC	INVEST
No.	233	201	216	202	403	745	773	74
β-Blocker	Atenolol	Metoprolol	Bisoprolol	Atenolol	Atenolol	Atenolol	Atenolol	Atenolol
Treatment duration	8 wk	8 wk	4 wk	61 d (average)	4 wk	6 wk	6 wk	6 wk
Age, y	49±9.52	51±8.99	50.5±6.3	64.0±6.5	51.7±11.4	63.1±8.3	60.8±8.9	68.35±9.21
Women, No. (%)	109 (46.78)	65 (48.14)	0	97 (48.0)	214 (53.1)	114 (15.2)	177 (22.9)	41 (54.66)
Body mass index, kg/m²	30.30±4.90	30.66±4.95	26.7±2.7	27.1±3.6	26.9±4.08	28.4±4.6	28.4±4.2	29±6
Baseline SBP, mm Hg	145.46±9.68	147.49±10.83	151.9±13.1	166.4±13.2	159.3±15.3	159.7±14.9	164.8±16.5	146.41±15.43
Baseline DBP, mm Hg	93.20±5.54	93.94±5.63	100.0±7.2	97.6±6.4	102.5±10.7	92.6±9.1	96.3±9.8	84.01±10.21
Post-treatment SBP, mm Hg	136.11±11.46	137.32±12.82	138.9±15.2	145.1±13.1	136.3±16.5	148.8±20.7	156.4±22.1	136.82±15.48
Post-treatment DBP, mm Hg	86.05±7.75	84.90±7.45	90.2±8.7	84.8±6.4	84.9±10.9	83.9±10.1	89.6±10.5	77.53±8.63
ΔSBP, mm Hg	−12.67±8.61	−10.19±9.20	−13.0±10.5	−21.3±12.8	−23.0±17.7	−10.8±19.7	−8.4±20.7	−9.59±16.96
ΔDBP, mm Hg	−10.50±5.77	−9.05±6.07	−9.8±6.9	−12.8±6.6	−17.7±11.2	−8.5±10.6	−6.7±10.4	−6.47±10.84

Values are presented as mean±SD unless otherwise noted. ASCOT-SC indicates Anglo-Scandinavian Cardiac Outcomes Trial-Scandinavian; ASCOT-UK, Anglo-Scandinavian Cardiac Outcomes Trial-United Kingdom; BB-SS, Pharmacogenomics of Beta-Blockers Sardinian Study; DBP, diastolic blood pressure; GENRES, Genetics of Drug Responsiveness in Essential Hypertension; INVEST, International Verapamil SR-Trandolapril Study; LIFE-Fin, Finnish arm of the Losartan Intervention For Endpoint Reduction in Hypertension Study; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; SBP, systolic blood pressure.

functional prediction performed using regulome DB identified 3 variants that met the stringent cutoff threshold of <4. We also investigated the eQTL traits of these 12 SNPs using HaploReg v4.1, and 10 of these variants were also eQTLs for either *BST1* or other nearby genes of this locus such as *CD38*, *FBXL5*, and *FAM200B*. The details of the LD (r^2 and D'), eQTLs and the correlated genes and tissues, the RegulomeDB scores, and the potential regulatory functions have been outlined in Table S2. There were 3 variants that were positively predicted by both tools used (RegulomeDB and Haplroreg v4.1), further strengthening their putative roles as functional/regulatory variants (Table S2).

Discussion

To our knowledge, this is the largest genome-wide meta-analysis for antihypertensive response to β₁-blocker treatment, consisting of 5 randomized controlled trials for discovery and 3 randomized clinical trials for replication, followed by ethnic validation of the replicated SNPs among a cohort of black patients from 2 randomized clinical trials. We successfully identified and replicated a missense variant,

rs28404156 in the *BST1* locus, which was significantly associated with SBP response to β₁-blockers. The carriers of the A allele of this SNP had a significantly better SBP response in both discovery and the replication meta-analysis. Given LD differences across race groups, even though the exact SNP did not replicate in the black cohort, we were able to validate the genomic region by identifying other SNPs that were significantly associated with BP response to β₁-blockers. This further points towards the potential importance of this locus in β₁-blocker BP responsiveness.

The SNP rs28404156 is present in the *BST1* gene and represents a missense SNP along with being an eQTL for *FAM200B*. This SNP results in an arginine to histidine substitution. Arginines are usually involved in formation of salt bridges needed for protein stability and, accordingly, its replacement could have implication on the BST1 protein function. *BST1*, also known as CD157, encodes for ADP-ribosyl cyclase 2, which is a cell surface receptor expressed in bone marrow stromal cells and is primarily involved in regulation of pre−B-cell growth. It is now known that *BST1* is also expressed in multiple other cell types including vascular endothelial stem cells, monocytes, and mast cells.[32] Although
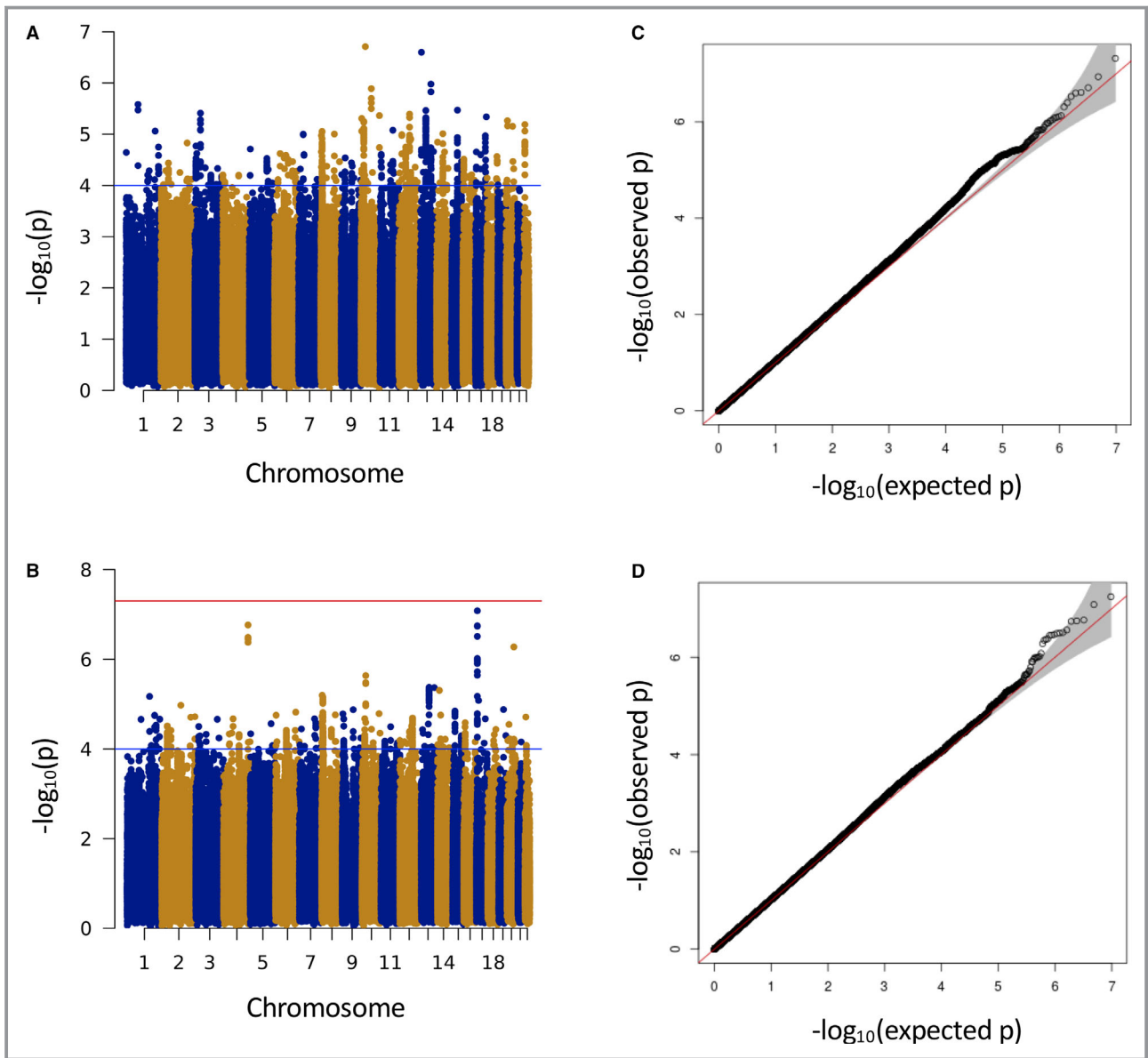
Figure 2. Manhattan (**A** and **B**) and quantile–quantile (**C** and **D**) plots from meta-analysis of genome-wide association for systolic (**A** and **C**) and diastolic (**B** and **D**) blood pressure responses to β$_1$-blocker treatment. The blue line refers to the suggestive level of significance ($P<1\times10^{-4}$). The red line refers to the genome-wide significance ($P=5\times10^{-8}$).

no direct association between *BST1* and BP regulation has been reported, there are various pathways and signaling components that *BST1* may interact with to influence the pathophysiology of hypertension. For example, *BST1/CD157* influences the phosphorylation of focal adhesion kinase[33] and calcium homeostasis,[34] which are known to be involved in the hypertension-vascular signaling cascade. *BST1/CD157* is also involved in various immune and inflammation responses, including regulation of motility and transendothelial migration of monocytes and their adhesion to extracellular matrix proteins.[32] The fact that *BST1* is expressed in a larger number of tissue subtypes was only recently discovered, implying that

the interaction of *BST1* with these signaling cascades with respect to hypertension may warrant further study.

This replicated SNP is located on chromosome 4p15.32 on the distal part of the small segment of chromosome 4. Multiple previous studies have identified and reported the distal region of chromosome 4p to be involved with BP regulation, which further highlights the importance of this locus in BP response.[35,36] There are several genes present in this locus, with variants in high LD with our successfully replicated index SNP (±500 kb) such as *CD38, FAM200B, FBXL5, FGFBP1*, and *FGFBP2*. Many of these genes have been implicated in various physiological and pathophysiological

Table 2. Discovery Meta-Analysis Association Result and Replication Meta-Analysis and Combined Meta-Analysis of Discovery and Replication Meta-Analysis

SNP	CHR	BP	EA	OA	Gene/Region	Discovery Meta-Analysis				Replication Meta-Analysis				Combined Meta-Analysis		
						EAF	Effect	SE	P Value	EAF	Effect	SE	P Value	Effect	SE	P Value
rs28404156	4	1573732	A	G	BST1	0.080	−3.21	0.82	9.33×10^{-05}	0.072	−4.86	1.30	1.8×10^{-4}	−3.60	0.69	2.18×10^{-07}

β indicates regression coefficient for effect allele; BP, base pair position (hg19); CHR, chromosome; EA, effect allele; EAF, effect allele frequency; OA: other allele; SE, standard error of the beta coefficient; SNP, single nucleotide polymorphism.

components of hypertension signaling. To further investigate the plausible involvement of this locus in BP response, we used in silico approaches and identified several SNPs in high LD with the replicated SNP in *BST1* as well as in other genes in this genomic locus (±500 kb). Further investigation using in silico analysis revealed that several of these SNPs were predicted to have regulatory function and are eQTLs for the genes in the same locus.

Some of these SNPs with potential regulatory function were found to be in high LD with our index replicated SNP and were present in *CD38*. Interestingly, *CD38* is a transmembrane protein that shares 33% homology with *BST1* and has both ADP-ribosyl cyclase and cyclic adenosine 5′-diphosphate ribose hydrolase activities.[37] *CD38* has been shown to be involved in multiple key processes such as vascular contraction, apoptosis, and neural signaling via its involvement in calcium regulation, which plays a key role in hypertension as well as cardiovascular diseases, renal regulation, and energy metabolism.[38] Studies have also shown that elimination of *CD38* prevents mice from gaining weight and becoming obese while on a high-fat diet via a SIRT-dependent mechanism.[39] Some recent human studies have found a similar association between *CD38* and metabolic syndrome in which high BP is an important factor.[40] Of note, one study has shown that *CD38*-deficient mice have increased plasma renin activity.[41] This could have implications for β₁-blocker BP response given that higher plasma renin activity has been reported to be associated with better BP responses to β₁-blocker monotherapy as well as to various add-on therapies.[42] Several studies have reported the direct activation of *CD38* by angiotensin II (Ang II), endothelin-1, and norepinephrine, as well as indirect activation via the generation of reactive oxygen species.[43,44] Upon activation, *CD38* generates the endogenous calcium secondary messengers, ie, cyclic adenosine 5′-diphosphate ribose and nicotinic acid adenosine dinucleotide phosphate, which modulate the intracellular calcium transients resulting in vasoconstriction.[38,45] These studies indicate that *CD38* plays an important role in the modulation of agonist-induced vascular smooth muscle cell responses. These responses might have an important implication in the regulation of BP by β₁-blockers and need further elucidation.

FBXL5 is another gene harboring SNPs in LD with the index SNPs. *FBXL5* belongs to the F-box family of proteins and encodes for F-box and leucine-rich repeat protein 5. It acts as an iron sensor and has been reported to be a tumor suppressor.[46] One of the meta-analyses of data from 6 global whole blood gene expression studies for BP and hypertension found *FBXL5* to be one of the differentially expressed genes and to be significantly associated with DBP.[47] The index SNP as well as several other SNPs in LD with the index SNP were also eQTLs for *FAM200B*. However, not much information is
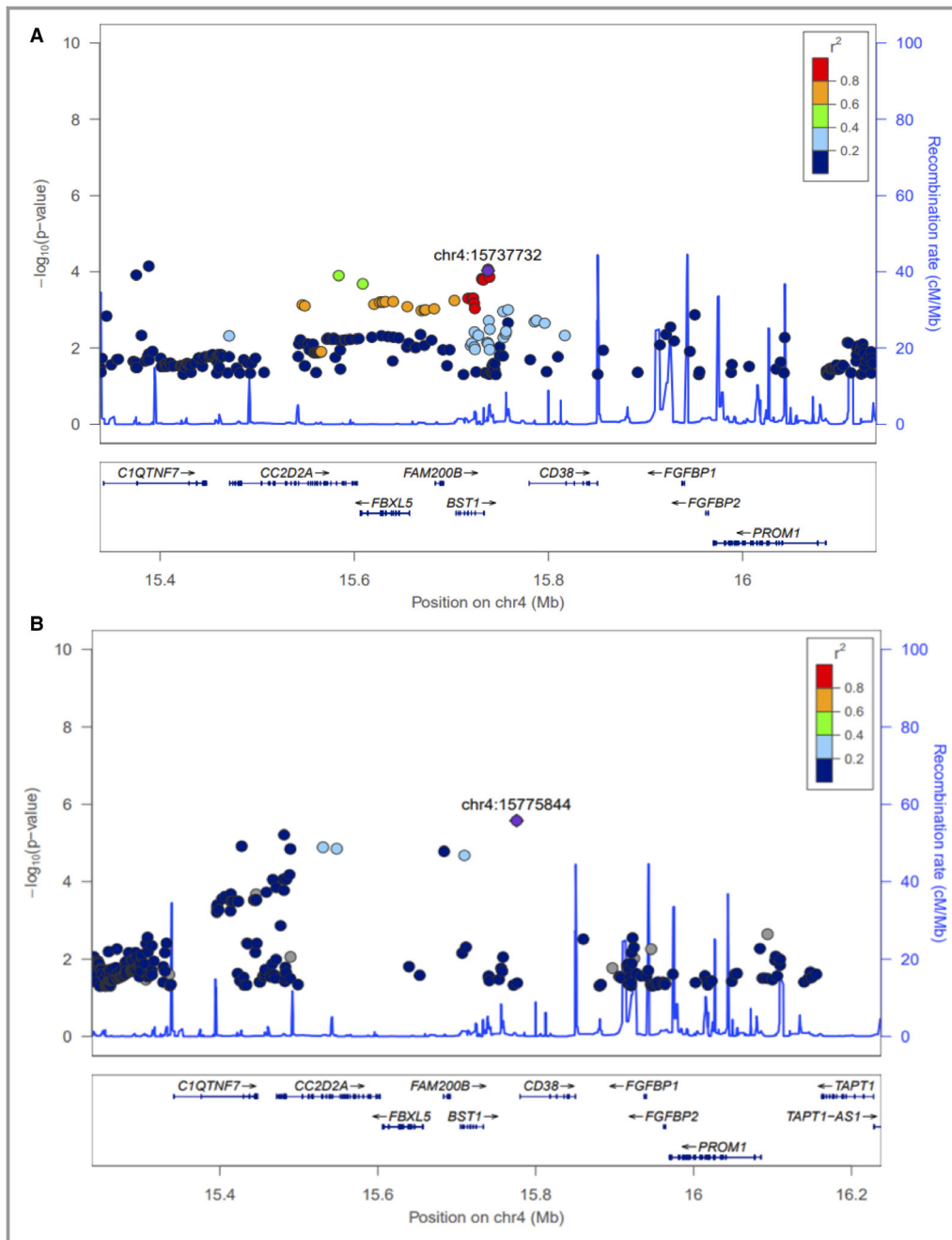
Figure 3. Regional plot of the *BST1* locus in white (**A**) and blacks (**B**).

available in the literature regarding *FAM200B* in relation to the BP phenotype or related pathways.

FGFBP1 is among other genes in this associated locus. Even though we could not find SNPs in *FGFBP1* that were in LD with the rs28404156 SNP, the importance of this gene in hypertension and BP response cannot be overlooked. *FGFBP1* encodes for fibroblast growth factor (FGF)–binding protein 1 (FGFBP1) and is involved in the modulation of the FGF signaling. FGFs are a family of proteins, many of which have been implicated in pathways related to hypertension.[48,49]

Specifically, *FGFBP1* is a precursor protein that releases FGF1 from the extracellular matrix and has been investigated for its role in modulating BP. Cuevas et al[50,51] reported a decrease in the endothelial FGF in the blood vessels of spontaneously hypertensive rats and further showed that systemic administration of FGF improves the impaired endothelial cells and nitric oxide synthase resulting in improvement in hypertension. Similarly, studies in hypertensive rats have deemed the *FGFBP1* locus to be important in the development of glomerular damage and hypertension.[52] Further, a recent

family-based study from Poland reported a novel association between variants in the *FGFBP1* and familial hypertension. This report also showed an increased expression of the FGFBP1 in the kidneys of these patients with hypertension.[51,53] More recently, studies in transgenic mice have shown that increased expression of FGFBP1 resulted in an increase of >30 mm Hg in arterial pressure by increasing the sensitivity of the blood vessels to Ang II.[54] The investigators of this study postulated that targeted inhibition of the FGF signaling using FGF inhibitors, which are conventionally used for cancer treatment, might help in controlling hypertension as well. They tested this theory by using an FGF inhibitor in transgenic mice overexpressing FGFBP1 and found that Ang II sensitivity of the vasculature was reversed, resulting in decreased BP.[54] Given the fact that β₁-blockers are known to suppress the renin system and Ang II levels in the plasma,[55] it is important to consider the cross-talk between β₁-blockers, Ang II, and *FGFBP1*. Additional investigations to better understand this cross-talk is needed to further elucidate the possible contribution of FGFBP1 to β₁-blocker BP response.

Study Strengths and Limitations

Overall, this study has certain noteworthy strengths. To our knowledge, this is the largest pharmacogenomic GWAS assessing the underlying genetic variants contributing to variable BP response. Through our discovery, replication, and ethnic validation efforts we were able to identify and thoroughly validate the association of the *BST1*-containing locus with β₁-blocker BP response. Although various genes in this locus have been previously implicated in BP-related signaling, this is the first time a direct association with response to β₁-blocker treatment has been identified. Furthermore, our criteria for selecting markers from the discovery meta-analysis for testing in replication were stringent, which ensured the consistency of direction across the data sets for the identified association.

We acknowledge the limitations of our study as well. While this is considered the largest pharmacogenomics analysis of β₁-blockers response including 8 cohorts (5 discovery and 3 replication) and consisted of >2000 participants, our results did not identify genome-wide significance, which may be partly attributed to reduced power driven by variants with low-modest effect size. Additionally, subtle heterogeneity in BP response phenotype across the different studies may have led to failure to identify genome-wide significant signals. Nevertheless, the association of SNPs in the replication cohort at a Bonferroni-corrected *P* value, as well as ethnic validation of the region, suggest the importance of the genomic locus. Another limitation is the use of different β₁-blockers across studies included in this meta-analysis. Although the majority of the studies used

atenolol in their design, GENRES and PEAR-2 used bisoprolol and metoprolol, respectively. Bisoprolol was used at 5 mg/d, which is equivalent to 50 mg/d as that of atenolol[56] and is the dose that was used in the majority of the atenolol studies in this article, except for PEAR, in which the target atenolol dose was 100 mg/d upon titration. Likewise, for metoprolol, the patients were started on 50 mg/d with uptitration to 100 mg twice per day in PEAR-2; in this case, the equivalent atenolol doses are ≈100 mg once per day.[57] Hormones are known to play a role in modulating BP, and hormone replacement therapy can interfere with BP levels. Another limitation of this study is that exclusion of women taking any kind of hormone replacement therapy was not consistently across all of the studies that are part of this meta-analysis. While some studies excluded women taking oral contraceptives or hormone replacement therapy, others did not. This precludes the ability to include hormone replacement therapy, which may influence BP, as a covariate in our association analysis. However, using our stringent thresholds and multistage GWAS efforts, we were able to identify a strong association with multiple levels of evidence. This article leverages the power of collaborative efforts within ICAPS to investigate genetic determinants of BP response, and showcases potential opportunities to investigate other important related phenotypes such as heart rate reduction response to β-blockers in future

Conclusions

We performed the largest genome-wide meta-analysis of BP response to β₁-blockers and successfully identified and replicated an SNP in the BST1 locus that was significantly associated with BP response to β₁-blockers. We were further able to gather additional evidence in support of the region containing this SNP using in silico approaches, and further identified SNPs in high LD with the index SNP with putative regulatory and functional effects. These data collectively suggest the importance of this region in mediating BP response to β₁-blockers, warranting further studies to understand the exact underlying mechanism(s). Deeper insights about such pharmacogenomic markers and loci can ultimately pave the way for personalized β₁-blocker use with optimum BP control and outcomes.

Disclosures

None.

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Supplemental Material

Data S1.

Supplemental Methods:

Study samples

All the study protocols for each of the studies was approved by the local institutional review boards or equivalent committees. Informed consent forms were voluntary signed by all the participants. The clinical trials at each of the institutions included in this meta-analysis were conducted in accordance with regulations set forth by the Declaration of Helsinki and local regulatory agencies.

PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses)

The details of the PEAR study have been described previously[1]. PEAR was a prospective randomized, crossover clinical trial, which included hypertensive patients with mild to moderate uncomplicated hypertension. In brief, patients of both genders and of any race and ethnicity between ages of 17-65 years were included in the study. Secondary HTN, known history of cardiovascular disease (CVD) or diabetes were exclusions from PEAR. Before the initiation of HTN treatment, patients went on a washout for at least three-four weeks to revert to their hypertensive BP state. Patients were then randomized to an eight-week, monotherapy treatment with either atenolol (ATN) 50mg daily or hydrochlorothiazide (HCTZ) 12.5mg daily, followed by the addition of the other drug (i.e. ATN added to HCTZ, and HCTZ added to ATN). For BP phenotype, a composite BP response of home, office and ambulatory BP measurement was used. For the analysis included herein, only participants with European American ancestry (EA) who were treated with β-blocker monotherapy and had available genome-

wide genotype data were included. Genotyping was performed using Illumina Human Omni1M Quad Beadchip. (Illumina, San Diego, CA, USA)

PEAR-2

The details of PEAR-2 study have been published previously[2, 3]. PEAR-2 was a prospective, randomized trial that assessed the genetic factors contributing to variable BP response following sequential monotherapy with metoprolol (β-blocker) and chlorthalidone (thiazide-like diuretic). Like PEAR, adult participants between 18 and 65 years of age, from both sex, and of any race/ethnicity who had mild to moderate, uncomplicated, HTN, were recruited into the study and underwent an anti-hypertensive treatment washout period for three-four weeks. Exclusion criteria was like that of PEAR. Upon confirmation of HTN criteria and BP readings, participants were started on metoprolol tartrate (50mg twice daily), and treatment was up titrated to a maximum dose of 100mg twice daily, followed by a second hypertensive washout, and treatment with chlorthalidone. While office and home BP readings were collected in PEAR-2, the average home BP measurements were used to create the BP response phenotype to metoprolol monotherapy. For this analysis, participants with EA ancestry who were treated with metoprolol monotherapy and had available genome-wide data were included. Genome-wide data were generated using Illumina Human Omni2.5S Beadchip (Illumina, San Diego, CA).

LIFE-Fin (Finnish Arm of the Losartan Intervention For Endpoint Reduction in Hypertension Study)

LIFE was a prospective, randomized, multicenter (Scandinavia, UK, USA), double-blind, double-dummy, active-controlled study that aimed at evaluating the long-term treatment effects of losartan compared with atenolol among 9193 hypertensive patients with signs of left ventricle hypertrophy (LVH) indicated by their ECG. The details of the study have been published elsewhere[4]. Adult patients with 55-80 years of age, who had office systolic BP (SBP) of 160-200 mmHg or diastolic BP (DBP) of 95–115 mmHg, were included in the study. They underwent a two-week antihypertensive treatment-washout period during which placebo treatment was used. The baseline BP was derived from BP at the end of the washout period. Patients were then randomized to either losartan or atenolol, with a subsequent addition of HCTZ, to achieve a target BP of <140/90mmHg. Patients were followed at two, four and six months intervals after starting the medication, then every six-months for a total follow-up period of 4.8 years. Office BP readings were recorded at each visit and used to create BP response phenotype.

For the current study, DNA samples from Finnish participants of the LIFE pharmacogenetic sample were available. Herein, we selected 202 participants on monotherapy treatment with atenolol 50 mg daily at two months of the study and used office BP data at 2 months after start of the treatment to calculate the BP responses. The DNA samples were genotyped using Illumina Human OmniExpress-12 BreadChip (Illumina, Inc., San Diego, CA, USA).

GENRES (Genetics of Drug Responsiveness in Essential Hypertension)

GENRES is a randomized, placebo-controlled, double-blind, cross-over, single-center study that investigated the molecular genetics of drug response in essential HTN. The details of the study have been described previously[5, 6]. Moderately hypertensive Finnish men with 35–60 years of age who had repeated measurements of DBP >=95mmHg or used antihypertensive medication(s) were included in the study. Exclusion criteria included the use of three or more antihypertensive drugs, secondary hypertension or significant additional co-morbidity. Patients' antihypertensive treatment was stopped for at least 4 weeks prior to starting the study drugs. The study included a four-week, run-in placebo period, followed by four-week drug monotherapy periods that were separated by four-week placebo treatment periods. During the drug periods, the patients received (in a randomized order, in a rotational fashion) losartan 50mg, bisoprolol 5mg, HCTZ 25mg, or amlodipine 5mg daily. Office BP measurements after a 30-minute rest in the sitting position were recorded at the end of each treatment or placebo period. For the present study, we selected 216 participants with office BP response data to bisoprolol treatment available. Means of all (up to four) placebo treatment periods were used as the baseline levels for the calculation of BP responses. These patients had genome-wide genotype data on Illumina Human OmniExpress-12 BreadChip (Illumina, Inc., San Diego, CA, USA).

Pharmacogenomics of beta blocker Sardinian Study (BBSS)

PHSS was a Pharmacogenomic study conducted at Hypertension and Related Disease Centre of the AOU-University of Sassari, Italy. The aim of the study was to find genetic markers of response to the antihypertensive drug atenolol in Essential Hypertension

(EH). Four hundred and three never treated Caucasian essential hypertensive patients, whose blood pressure was found SBP ≥ 140 or DBP≥ 90 mmHg at the first visit (week-8), were screened. Only patients with asymptomatic, mild-to-moderate essential hypertension were screened. A complete absence of major systemic disease and of any kind of treatment was also required. The absence of whatever kind of concurrent disease was a prerequisite for patients' eligibility to the study.

After a run-in period of 8 weeks under controlled dietary conditions (sodium 100-140 mEq/day and potassium 50-70 mEq/day), during which a complete diagnostic workout was performed to define the presence of high BP and to exclude secondary forms of hypertension (week 0), all the patients started Atenolol 50 mg b.i.d for 4 weeks. To avoid the interference of the effect of previous treatments which may last up to six months after drug withdrawal, only never treated EH were enrolled in the study. The run-in period to confirm the presence of BP≥140/90 was decided according to International Guidelines on High Blood Pressure and to the Ethics Committee of the University of Sassari,

During this period, BP and side effects were monitored every 2 weeks. BP was measured by automated electronic devices (OMRON 7051 IT) between 8:00 and 10:00 AM, in the sitting position, on the dominant arm: three readings were obtained in each occasion by the same nurse in a quiet room and in the absence of the physician: the average of the three readings was used as reference value.Genomic DNA, collected at week 0 from the peripheral blood, was extracted with Macherey-Nagel kit: NucleoSpin Blood XL, Düren, Germany. All samples were genotyped at "Genomic and Bioinformatics laboratory" of University of Milan, using the Illumina Human1M-Duo array

within the HYPERGENES project[7] and the Illumina HumanOmniExpress array (Illumina Inc, San Diego, CA, USA), within the InterOmics project (http://www.interomics.eu/).

INVEST (INternational VErapamil SR-Trandolapril)

INVEST was an international, prospective, multicenter, randomized trial. The details of the study including the inclusion/exclusion criteria have been published elsewhere[8, 9]. Patients older than 50 years of age, with essential hypertension and documented coronary artery disease (CAD) were randomized to receive either verapamil SR (calcium antagonist strategy; CAS) or atenolol (β-B strategy). Trandolapril or HCTZ were added respectively, to the treatment arms if target BP control was not achieved (<140/90 mm Hg or <130/85 mm Hg if patients were diabetic or have renal impairment). Patients were initially followed up every six weeks for the first six months, then every 6 months thereafter for recording of BP, assessing of patients' overall well-being, adverse events, and drug compliance, for an average follow-up period of 2.7 years. INVEST-GENES is the genetic sub-study of INVEST which included 5979 INVEST participants with DNA samples of whom, 1529 had genome-wide data generated on Illumina OmniExpress Exome chip. Herein, a total of 74 participants with EA ancestry who had BP response data to atenolol monotherapy treatment were analyzed.

ASCOT UK/ASCOT SC

ASCOT study is an investigator-led, multi-center trial, which included over 19,000 hypertensive patients, aged 40-79 years at baseline, with an average SBP of 140/90 mmHg on-treatment and 160/100 mmHg off-treatment. The details of this study have

been published previously[10]. Patients with no history of coronary heart disease who had at least three risk factors for CVD such as LVH, type II diabetes mellitus, peripheral artery disease, previous stroke/TIA, smoking, and male sex older than 55 years. The study investigated the outcome of amlodipine, a CAS compared with atenolol (βB-based strategy) among hypertensive patients at a moderate risk for CVD. BP was measured at randomization clinic visit and subsequently, at follow-up visits scheduled initially at six weeks, three months, six months, and every six months interval thereafter. BP measurement post-atenolol monotherapy treatment taken at each person's first follow-up visit was used to define BP response phenotype. 745 Europeans from UK and Ireland were genotyped using Illumina HumanCNV 370 chip and 773 Europeans from Scandinavia were genotyped using the Illumina Human Omni Exome Express v8.1 and comprised the ASCOT UK and ASCOT-Sc cohorts, respectively.

Table S1. Clinical Characteristics of African American participants from PEAR and PEAR-2.

	Ethnic Validation Cohorts	
	PEAR	**PEAR-2**
N	150	168
Age, years	47.2 ± 8.5	50.0 ± 9.2
Female, N (%)	107 (71.3%)	89 (53.0%)
Body Mass Index, kg/m^2	31.6 ± 6.3	30.8 ± 5.2
Baseline SBP (mm/Hg)	151.3 ± 12.1	147.5 ± 10.6
Baseline DBP(mm/Hg)	99.0 ± 5.7	95.6 ± 6.1
Post treatment SBP (mm/Hg)	137.3±12.8	142.7±13.1
Post treatment DBP (mm/Hg)	84.9±7.4	89.8±7.9
Delta SBP (ΔSBP) (mm/Hg)	-5.1±9.2	-4.7±10.5
Delta DBP (ΔSBP) (mm/Hg)	-5.1±5.9	-5.7±6.7

Values are presented as mean ± standard deviation unless otherwise noted. SBP:

Systolic Blood Pressure, DBP: Diastolic Blood Pressure

Table S2. *In silico* analysis of the SNPs in LD with the replicated SNP rs28404156 using regulome DB and Haploreg v4.1.

| SNP | BP | LD with the index SNP (rs28404156) | | Functional prediction using Regulome DB | | eQTL annotation (Haploreg v4.1) | |
		R-squared	D'	Score	Potential Regulatory Function	Correlated gene	Tissue
rs28404156	15737732	1	1	No data	-	FAM200B	
rs4301112	15717226	0.82	0.96	No data	-	CD38	Heart_Atrial_Appendage
rs28532698	15721619	0.82	0.96	6	other	CD38	Heart_Atrial_Appendage
rs10001565	**15722573**	**0.82**	**0.96**	**1f**	**eQTL + TF binding / DNase peak**	**CD38 BST1 FBXL5**	**Heart_Atrial_Appendage Whole Blood Whole Blood**
rs12643475	15723514	0.86	0.97	5	TF binding or DNase peak	BST1 FBXL5	Heart_Atrial_Appendage Whole Blood
rs9942212	15724150	0.88	0.98	No data	-	-	-
rs7667512	**15731560**	**0.93**	**0.97**	**3a**	**TF binding + any motif + DNase peak**	**BST1 FBXL5**	**Whole Blood Whole Blood**
rs7672311	**15731690**	**0.95**	**0.98**	**1f**	**eQTL + TF binding / DNase peak**	**BST1 FBXL5**	**Whole Blood Whole Blood**
rs12649015	15733020	0.95	0.98	No data	-	-	-
rs33936701	15737559	0.97	0.98	6	other	FAM200B	Arterial_Tibial
rs28641514	15737722	0.98	1	6	other	FAM200B	Arterial_Tibial
rs12646913	15739276	0.91	0.96	5	TF binding or DNase peak	BST1	Whole Blood

The SNPs that have evidence of potential regulatory function from both the databases are in bold.

Figure S1. Change in systolic blood pressure post β1-blocker treatment by rs28404156 genotype.
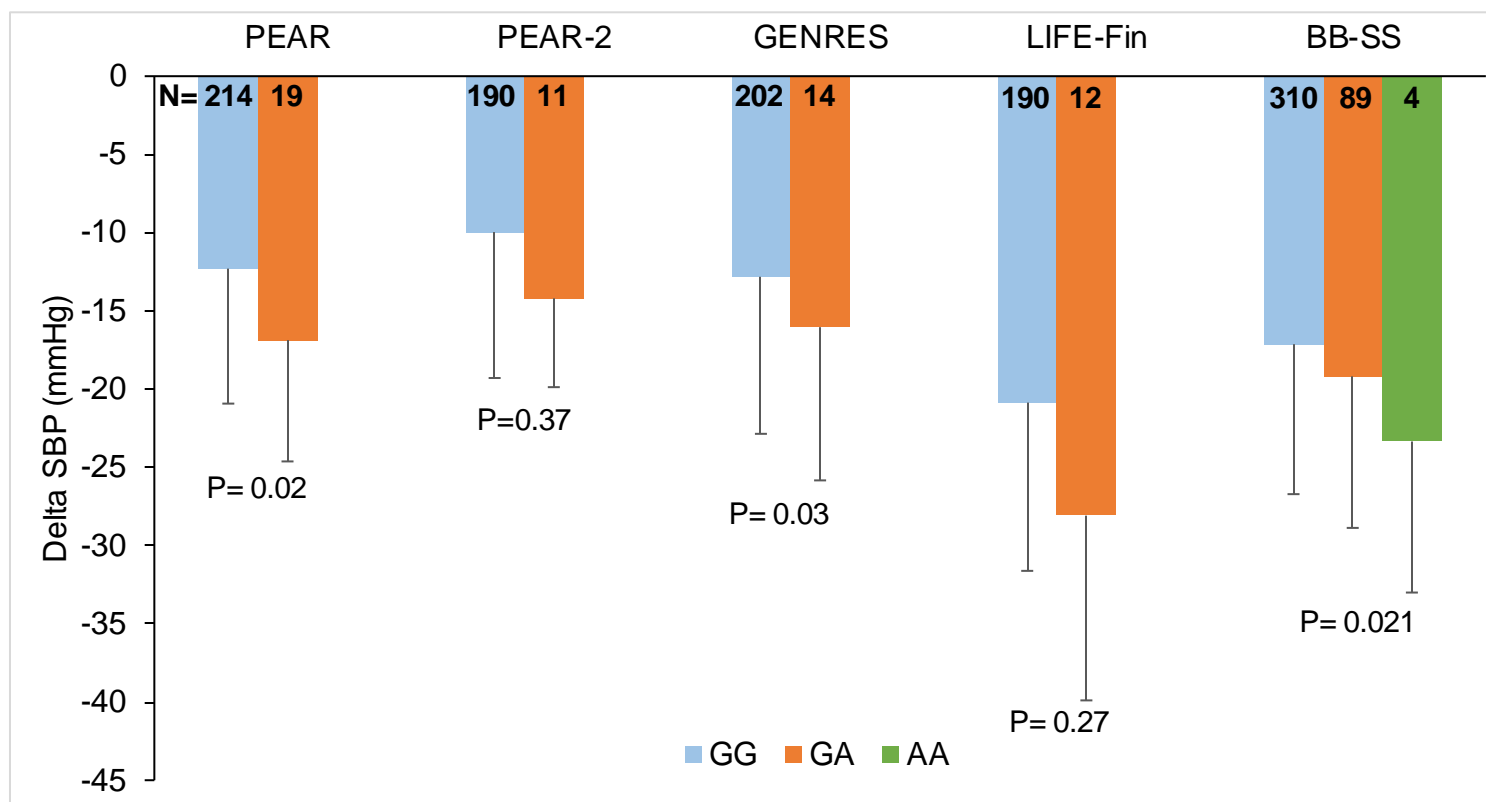
Systolic Blood Pressure change post to β1-blocker treatment among PEAR, PEAR-2, GENRES, LIFE-Fin and BB-SS by BST1 rs28404156 genotype. Systolic blood pressure change for all studies is adjusted for pretreatment systolic blood pressure levels, age, sex, and principal components. P values are for contrast of least square adjusted means between genotype groups. PEAR: Pharmacogenomic Evaluation of Antihypertensive Response, GENRES: Genetics of Drug Responsiveness in Essential Hypertension, LIFE-Fin: Finnish Arm of the Losartan Intervention For Endpoint Reduction in Hypertension Study and BB-SS: Pharmacogenomics of beta blocker Sardinian Study

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