ORIGINAL RESEARCH

Genetic Variants Associated With Uncontrolled Blood Pressure on Thiazide Diuretic/β-Blocker Combination Therapy in the PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses) and INVEST (International Verapamil-SR Trandolapril Study) Trials

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***Background*—**The majority of hypertensive individuals require combination antihypertensive therapy to achieve adequate blood pressure (BP) control. This study aimed to identify genetic variants associated with uncontrolled BP on combination therapy with a thiazide diuretic and a β-blocker.

***Methods and Results*—**A genome-wide association study of uncontrolled BP on combination therapy was conducted among 314 white participants of the PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses) trial. Multivariable logistic regression analysis was used. Genetic variants meeting a suggestive level of significance ($P<1.0E-05$) were tested for replication in an external cohort, INVEST (International Verapamil-SR Trandolapril study). We also examined genome-wide variant associations with systolic and diastolic BP response on combination therapy and tested for replication. We discovered a single nucleotide polymorphism, the rs261316 major allele, at chromosome 15 in the gene *ALDH1A2* associated with an increased odds of having uncontrolled BP on combination therapy (odds ratio: 2.56, 95% confidence interval, 1.69–3.88, $P=8.64E-06$). This single nucleotide polymorphism replicated (odds ratio: 1.86, 95% confidence interval, 1.35–2.57, $P=0.001$) and approached genome-wide significance in the meta-analysis between discovery and replication cohorts (odds ratio: 2.16, 95% confidence interval, 1.63–2.86, $P=8.60E-08$). Other genes in the region surrounding rs261316 (*ALDH1A2)* include *AQP9* and *LIPC*.

***Conclusions*—**A single nucleotide polymorphism in the gene *ALDH1A2* may be associated with uncontrolled BP following treatment with a thiazide diuretic/β-blocker combination.

***Clinical Trial Registration*—**URL: https://www.clinicaltrials.gov. Unique identifier: NCT00246519. **(*J Am Heart Assoc*. 2017;6: e006522. DOI: 10.1161/JAHA.117.006522.)**

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Hypertension is a leading risk factor for cardiovascular morbidity and mortality, affecting 1.4 billion people worldwide[1] and 1 of every 3 adults in the United States.[2] While many effective antihypertensive medications are available, 75% of hypertensive patients require at least 2 drugs to achieve blood pressure (BP) control, 25% require at least 3

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Accompanying Tables S1 through S4 and Figure S1 are available at http://jaha.ahajournals.org/content/6/11/e006522/DC1/embed/inline-supplementary-material-1.pdf

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Clinical Perspective

What Is New?

- We discovered a variant in the gene *ALDH1A2* associated with uncontrolled blood pressure on combination therapy with a thiazide diuretic/β-blocker in the PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses) trial and replicated this variant in an independent cohort, INVEST (International Verapamil-SR Trandolapril Study).
- We discovered a potential biomarker for identifying patients at risk of uncontrolled blood pressure on thiazide diuretic/β-blocker combination therapy.

What Are the Clinical Implications?

- Clinicians may use the information to prescribe more appropriate alternative antihypertensive medications for patients carrying the risk allele; however, further replication of the identified variant in other external cohorts is needed to confirm the finding.

drugs, and a subset, defined as resistant hypertensives, requires at least 4 agents of different drug classes to achieve BP control.[3] Given these data, the current US hypertension recommendations for adult BP management include suggestions for initiation of combination therapy in those with stage II hypertension or systolic BP (SBP) ≥20 mm Hg or diastolic BP (DBP) ≥10 mm Hg above goal.[4] However, whether all patients with high baseline BP would benefit from starting on combination therapy and on which drug combinations is unclear. Among patients on dual therapy, combination of an angiotensin-converting enzyme (ACE) inhibitor and calcium-channel blocker (CCB) was associated with better cardiovascular outcomes than the ACE inhibitor/hydrochlorothiazide or β-blocker/hydrochlorothiazide combinations in the ACCOMPLISH (Avoiding Cardiovascular Events in Combination Therapy in Patients Living with Systolic Hypertension) and the ASCOT-BPLA (Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm) trials, respectively.[5,6] However, in the INVEST study (International Verapamil-SR Trandolapril Study), both ACE inhibitor/CCB and β-blocker/hydrochlorothiazide combinations showed comparable reductions in BP and adverse cardiovascular outcomes.[7] Because most hypertensive patients need at least 2-drug combinations, gaining a better understanding of which patients would most benefit from initiation on different combination therapies would not only be clinically useful but may also lead to the discovery of novel BP regulatory mechanisms and drugs that would work in different subsets of hypertensive patients.

The widespread use of combination therapy also underscores the need for more effective targeted therapies. While adding optimized doses of additional drugs from different drug classes increases the likelihood of BP control in most patients, poor adherence to antihypertensive therapy is an already prevalent issue that increases as drug regimens become more complex.[8] Indeed, developing targeted therapies that achieve the desired effect while optimizing as well as minimizing the number of drugs needed will be essential to the successful delivery of precision medicine.

There has been a long-standing interest in understanding the genetic underpinnings of differential response to common antihypertensive agents.[9] A few candidate gene studies have examined the associations of genes in the renin–angiotensin–aldosterone system or sodium/volume regulation pathways with BP response to dual combination therapy[10–12]; however, to our knowledge, no study has taken a non-hypothesis-driven genome-wide association study (GWAS) approach to examine BP response to 2-drug antihypertensive combination therapy. A few studies have examined the genetics of a more serious form of drug nonresponse, resistant hypertension, including recent GWAS and candidate gene studies.[13–15] Additionally, several BP or hypertension GWAS and large candidate gene studies have been conducted and have included participants treated with antihypertensive therapy, typically with an adjustment of the BP values to account for the effects of medication[16,17]; however, these studies were not specifically designed to evaluate the genetics of antihypertensive drug response.

In this study, using data from the PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses) trial, we sought to identify genetic variants associated with uncontrolled BP in participants taking a thiazide diuretic and a β-blocker combination. Findings from this study may contribute to the discovery of novel BP regulatory mechanisms and drugs that would work for different subsets of hypertensive patients with different genetic backgrounds.

Methods

Study Design and Study Population

PEAR Study

PEAR was a prospective, randomized controlled clinical trial with a parallel-group design that aimed to identify the genetic associations of the effects of the thiazide diuretic hydrochlorothiazide, the β-blocker atenolol, and their combination, on BP and adverse metabolic responses (clinicaltrials.gov: NCT00246519).[18] Institutional Review Boards at the University of Florida, the Mayo Clinic, and Emory University, where the participants were enrolled, approved the study, and voluntary written informed consent was obtained from all participants before enrollment. Additional details on the PEAR study have been published previously.[18] Briefly, male and female participants aged 17 to 65 years with mild-to-moderate essential hypertension (DBP 90–110 mm Hg) and no major

comorbidities were enrolled and randomized, following a washout period of ≈4 weeks duration, as necessary, to hydrochlorothiazide 12.5 mg or atenolol 50 mg once-daily monotherapy. After 3 weeks on monotherapy, participants with BP >120/70 mm Hg underwent dose titration to hydrochlorothiazide 25 mg or atenolol 100 mg once daily for an additional 6 weeks of treatment. If BP remained >120/70 mm Hg at the end of monotherapy treatment, the alternate agent was added, followed by a similar dose-titration step for another 6 to 9 weeks. BP and laboratory measurements were obtained at baseline and after monotherapy and combination therapy. Participants with severe or secondary hypertension, diabetes mellitus, cardiovascular disease, or renal disease were excluded. A nested case–control study was created within PEAR that comprised participants with uncontrolled office BP following 6 to 9 weeks of combination therapy (cases) and controlled office BP following 6 to 9 weeks of monotherapy or combination therapy (controls). Uncontrolled office BP was defined as having SBP ≥140 mm Hg and/or DBP ≥90 mm Hg, and controlled office BP was defined as having SBP <130 mm Hg and DBP <85 mm Hg to account for elevated daytime BP and "white coat" or isolated office hypertension. A total of 314 genetically defined white participants or individuals of European ancestry (123 cases, 191 controls) met these criteria and were included in the study. Black participants were not included because of insufficient numbers of cases and controls for discovery and replication studies.

INVEST Study

The top genetic variants discovered in PEAR were tested for replication in INVEST (clinicaltrials.gov: NCT00133692).[19] Details of the INVEST study have been published elsewhere.[19] Briefly, INVEST was an international, multicenter, outcomes-based randomized controlled clinical trial in which male and female participants of multiple race groups aged ≥50 years with a history of hypertension and coronary artery disease were randomized to treatment with a CCB (verapamil) or a β-blocker (atenolol), followed by add-on therapy with an ACE inhibitor (trandolapril) in the CCB arm or a thiazide diuretic (hydrochlorothiazide) in the β-blocker arm. Only participants of the INVEST-GENES (INVEST-Genetic Substudy) were studied,[20] and only participants who were randomized to the β-blocker arm were included in order to create a replication cohort for the PEAR discovery analysis. Additionally, only BP measurements taken at the end of β-blocker and thiazide diuretic combination therapy, before a third drug add-on, were used to assess BP control status. The replication cohort included 414 participants, including genetically defined 221 whites (85 cases, 136 controls) and 193 Hispanics (81 cases, 112 controls). We considered INVEST Hispanics as a valid replication cohort for PEAR whites because INVEST Hispanics were recruited from Puerto Rico where Hispanics have more European genetic ancestry.[21]

Genotyping, Imputation, and Quality Control

Genome-wide single nucleotide polymorphism (SNP) genotype data on PEAR participants were obtained through genotyping on the Illumina Human 1M-duo beadchip (Illumina, San Diego, CA). All genome-wide genotypes, including autosomal and sex chromosome variants, underwent quality control steps in PLINK.[22] To confirm participants' self-identified race, Principal Components Analysis for genetic ancestry was performed on a linkage disequilibrium pruned data set using the EIGENSTRAT method.[23] Genotypes of variants that passed quality control underwent haplotype phasing in Markov Chain Haplotyping (MaCH) software[24] followed by imputation to 1000 Genomes phase III reference panels using minimac/minimac2.[25,26] Variants with imputation quality r^2<0.30 and minor allele frequency <3% were excluded.

INVEST DNA samples were genotyped on the Illumina OmniExpressExome Beadchip (Illumina, San Diego, CA). The 1000 Genomes phase III imputed genome-wide variants data were used for replication analysis. Imputation was performed using a similar strategy as in PEAR. Principal Components Analysis for genetic ancestry was performed on a data set of linkage disequilibrium–pruned high-quality SNPs using EIGENSTRAT.[23] Race/ethnicity was genetically classified as white or Hispanic based on Principal Components Analysis.

Statistical Analysis

Discovery analysis in the PEAR study

Descriptive statistics were obtained using the Student *t* test for continuous variables and the χ^2 or Fisher exact test for categorical variables. Continuous variables are shown as means±SDs and categorical variables as numbers and percentages. Multivariable logistic regression modeling was used for the GWAS analyses. The dependent variable or phenotype was a dichotomous variable: uncontrolled versus controlled BP on combination therapy, as described previously. In all models, covariates that met P<0.20 in the univariate analyses were selected for inclusion in a stepwise logistic regression model building procedure, with P<0.05 used as the criterion for a variable to stay in the model. The list of tested covariates is shown in Table. The final model included the covariates age, sex, ancestry-specific principal components (PCs) 1 and 2, randomization assignment, baseline SBP, whether a higher dose of the add-on drug was used, and smoking status. An additive genetic model was assumed, and variants with minor allele frequency <3% were

excluded. All regression analyses were conducted using 1000 Genomes imputed expected genotype dosage files implemented in ProbABEL.[27] Genetic race was defined using Principal Components Analysis as described in the Methods section. In all analyses, variants meeting $P<5.0E-08$ were considered as genome-wide significant markers of uncontrolled BP on combination therapy, and variants with $P<1.0E-05$ were considered as having a suggestive level of evidence. Additionally, in all analyses, the effect of the allele associated with uncontrolled BP was reported. Regional plots of the top variants were visualized in LocusZoom[28] and variant function was assessed using HaploReg and GTEx browsers.[29,30]

We also conducted additional genome-wide association analyses on the whole sample with the phenotypes SBP or DBP response on combination therapy, defined as the difference in BP between baseline and end of combination therapy. Multivariable linear regression modeling was used, with a similar strategy for covariate selection as previously described.

Replication analysis in the INVEST study

Phenotypes were defined similarly to PEAR: uncontrolled versus controlled BP on combination therapy (dichotomous), SBP or DBP response on combination therapy (continuous). In order to ensure that participants were on the maximal needed or tolerated dose of atenolol and hydrochlorothiazide, we used BP measurements taken at the last visit at which INVEST participants were on atenolol and hydrochlorothiazide combination therapy before a third drug add-on. Participants with controlled BP on atenolol or hydrochlorothiazide monotherapy were included among controls based on the assumption that if a participant is already controlled on 1 drug, they would be controlled on 2 drugs; and only BP measurements taken before a cardiovascular event were used for participants with an event. Further, BP values used to define "controlled BP" were the same as in PEAR and were also the same as those used to define "controlled BP" among diabetic participants in INVEST, in accordance with Sixth Report of the Joint National Commission (JNC VI) guidelines at the time of the trial.

Multivariable logistic or linear regression modeling was used depending on the phenotype. Covariates were selected using a similar strategy as in PEAR and included age, sex, ancestry-specific PCs, atenolol dose, and hydrochlorothiazide dose. Atenolol and hydrochlorothiazide dose categories were collapsed into 3 groups based on clinically meaningful dosing cut points (hydrochlorothiazide $\geq$25 mg once daily or atenolol $\geq$100 mg once daily; hydrochlorothiazide $<$25 mg once daily or atenolol $<$100 mg once daily; none or "no dose" for the alternate drug, if controlled on 1 drug). Models with the dichotomous phenotype were further adjusted for history of left ventricular hypertrophy, and models with the continuous phenotypes were further adjusted for history of stroke and peripheral vascular disease as indicated by the stepwise covariate selection process.

The top variant in each gene region with lowest P value was selected for replication analysis in INVEST. Variants with consistent direction of effect between PEAR whites and INVEST whites, or between PEAR whites and INVEST white-Hispanic meta-analysis (but not INVEST Hispanics only) that met $P<0.05/\#$ of variants tested were considered as validated genetic markers of uncontrolled BP on combination therapy. A 1-sided P value threshold was used, consistent with a 1-sided hypothesis. Further, in all analyses, the effect of the allele associated with uncontrolled BP was reported.

Sensitivity analyses

We tested the robustness of the primary genome-wide association analyses in PEAR with further model adjustment for baseline lipid levels. We also conducted sensitivity replication analysis in INVEST excluding individuals with uncontrolled BP on a low dose of both combination therapy drugs despite having a normal heart rate. Further, we performed sensitivity analysis in INVEST with additional adjustment for history of diabetes mellitus, heart failure, and renal insufficiency to account for the fact that INVEST included participants with these comorbidities while PEAR did not. Finally, we tested whether the top findings from PEAR validate among INVEST participants treated with CCB/ACE inhibitor combination therapy.

Results

Study Population Demographic and Clinical Characteristics

Table shows characteristics of PEAR study participants by case–control status (uncontrolled or controlled BP on combination therapy). In general, participant characteristics were similar between cases and controls, with the exception of sex, baseline SBP and DBP, use of higher dose of add-on drug, and smoking status.

Characteristics of INVEST participants by case–control status within each race/ethnicity group are summarized in Table S1. In total, there were 221 white and 193 Hispanic participants. Participant characteristics did not differ between cases and controls in each race/ethnicity group, with the exception of baseline SBP and DBP, hydrochlorothiazide and atenolol dose, and history of left ventricular hypertrophy (Hispanics).

Results of Discovery GWAS and Replication Analysis

No signals met the threshold of genome-wide significance in PEAR; however, 24 variants were associated with uncontrolled BP on combination therapy at a suggestive level of significance (Table S2); all 24 variants imputed with good quality ($r^2 \geq 0.80$). These 24 variants arose from 5 distinct gene regions, and the top SNP in each region was tested for replication in INVEST (Table S2). Figure 1 summarizes the results for the top SNP. As shown, rs261316 in *ALDH1A2* (aldehyde dehydrogenase 1 family member A2) was associated with uncontrolled BP on combination therapy with suggestive significance in PEAR whites, with the major allele (T) showing increased odds of having uncontrolled BP (odds ratio [OR]: 2.56, 95% confidence interval [CI], 1.69–3.88, *P*=8.64E-06). This SNP showed consistent direction of association in INVEST whites (OR: 1.94, 95% CI, 1.23–3.06, *P*=0.008) and Hispanics (OR: 1.79, 95% CI, 1.13–2.82, *P*=0.018), replicated in white–Hispanic meta-analysis (OR: 1.86, 95% CI, 1.35–2.57, *P*=0.001), and approached genome-wide significance in the meta-analysis between PEAR whites and INVEST whites and Hispanics (OR: 2.16, 95% CI, 1.63–2.86, *P*=8.60E-08). The major allele frequency of rs261316 was 0.59–0.63, consistent with frequencies reported in 1000 genomes for individuals of European and Hispanic (Puerto Rico) ancestry.[21] A plot of the genomic region surrounding the top variant rs261316 is shown in Figure 2. Other genes in the region include *AQP9* and *LIPC*.

We also identified a variant rs35123024 near *OR5H14* that was associated with the phenotype at a suggestive level of significance in PEAR, with the minor allele (C) associated with an increased odds of having uncontrolled BP on combination therapy (OR: 3.71, 95% CI, 2.13–6.45, *P*=3.46E-06). rs35123024 replicated with nominal significance in the INVEST white-Hispanic meta-analysis (OR: 1.72, 95% CI, 1.06–2.79, *P*=0.033) and showed consistent direction of associations across all cohorts. This SNP did not reach genome-wide significance in the meta-analysis between PEAR whites and INVEST whites and Hispanics (OR: 2.56, 95% CI, 1.72–3.82, *P*=3.77E-06). Minor allele frequencies of rs35123024 ranged from 0.12 to 0.18, consistent with those reported in 1000 genomes for individuals of European and Hispanic ancestry.[21] A regional plot is provided in Figure S1. Results on the top variants from the additional GWAS of SBP and DBP response on combination therapy are summarized in Tables S3 and S4, respectively. In these additional analyses, none of the variants that met a suggestive level of significance in PEAR replicated in INVEST.

Table. Characteristics of PEAR White Study Participants

Characteristics	Cases (N=123)	Controls (N=191)	*P* Value
Age, y	50.8±9.8	49.5±9.3	0.288
Male	89 (72)	93 (49)	<0.001
BMI, kg/m^2	29.3±4.0	30.3±5.4	0.063
Systolic BP, mm Hg (baseline)	157.0±12.8	147.7±10.6	<0.001
Diastolic BP, mm Hg (baseline)	100.1±6.3	96.4±5.1	<0.001
Randomized to hydrochlorothiazide (vs atenolol)	65 (53)	94 (49)	0.530
Current smoker	22 (18)	19 (10)	0.042
Duration of hypertension, y	6.7±6.8	6.2±6.5	0.552
Family history of hypertension*	88 (71)	139 (73)	0.812
Add-on drug dose increased	105 (85)	144 (75)	0.033

Means±SD or numbers with percentages in parentheses are shown, *P* values are based on Student *t* test and χ^2 or Fisher exact test. Cases: participants with uncontrolled BP on combination therapy. Controls: participants with controlled BP on combination therapy. BMI indicates body mass index; BP, blood pressure; HCTZ, hydrochlorothiazide; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses.
*Family history of hypertension defined as hypertension in a parent or sibling.

Results of Sensitivity Analysis

The top replicated variant, rs261316, in the primary analysis identified a region containing the gene *LIPC*, which has well-known associations with lipid levels. We therefore conducted sensitivity GWAS analyses with additional models separately

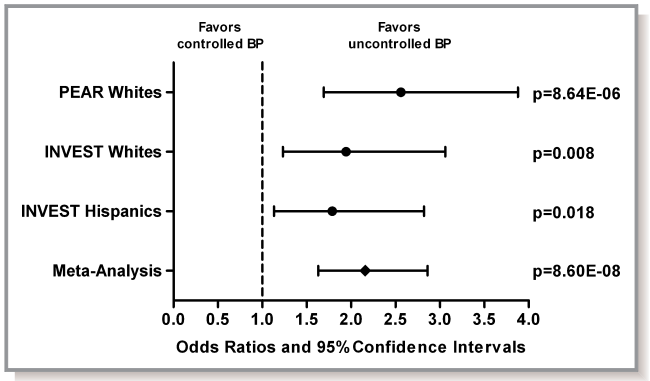

Figure 1. Association of rs261316 allele (T) in *ALDH1A2* with uncontrolled BP on combination therapy in PEAR with replication analysis in INVEST. *P* values and 95% confidence intervals for INVEST cohorts are 1-sided, consistent with a 1-sided hypothesis for replication; sample sizes: PEAR whites (n=314), INVEST whites (n=221), and INVEST Hispanics (n=193). BP indicates blood pressure; INVEST, International Verapamil-SR Trandolapril Study; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses.

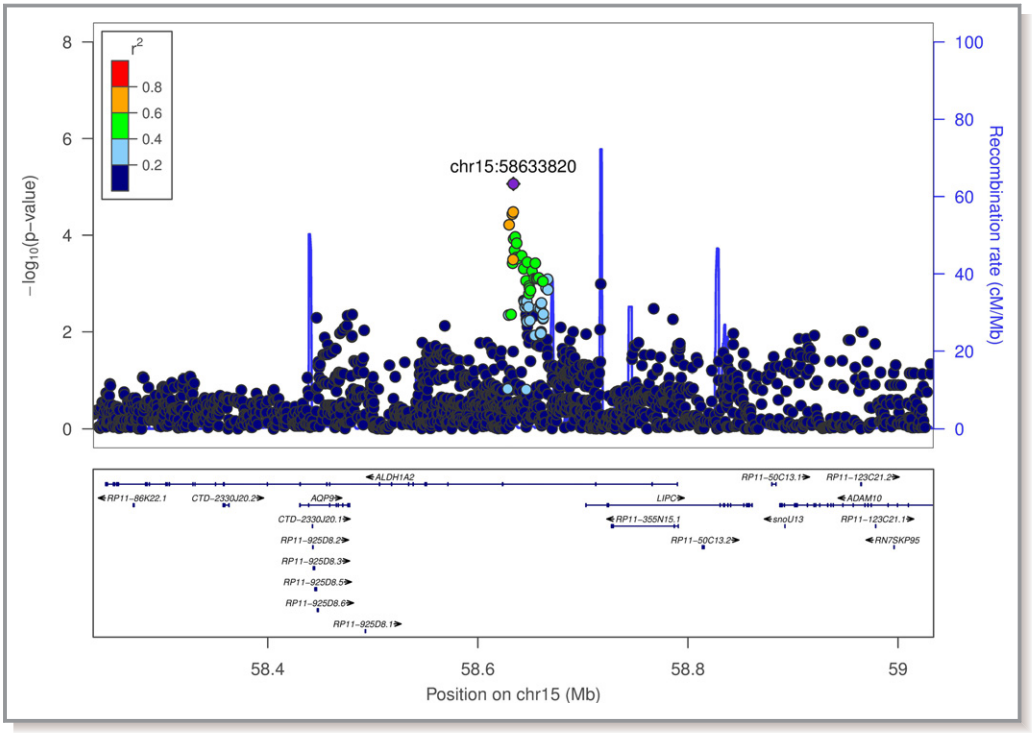

Figure 2. Regional plot of top genetic variant associated with uncontrolled BP on combination therapy in PEAR with replication in INVEST. rs261316 associated with uncontrolled BP on combination therapy (PEAR whites: *P*=8.64E-06; INVEST white–Hispanic meta-analysis: 1-sided *P*=0.001; PEAR and INVEST meta-analysis: *P*=8.60E-08); BP indicates blood pressure; INVEST, International Verapamil-SR Trandolapril Study; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses.

adjusted for baseline level of serum low-density lipoprotein, high-density lipoprotein, triglycerides, or total cholesterol in PEAR. rs261316 remained associated with uncontrolled BP on combination therapy in a consistent direction (adjusted for low-density lipoprotein: *P*=1.79E-06; high-density lipoprotein: *P*=2.42E-06; triglycerides: *P*=1.88E-06; total cholesterol: *P*=1.95E-06). Second, to ensure that most INVEST participants were on the maximum needed or tolerated doses of combination therapy, we identified and excluded the few individuals in INVEST (n=6) who were on a low dose of both combination therapy drugs despite having a normal heart rate. The top signal remained associated with the primary phenotype in the replication cohort (INVEST white–Hispanic meta-analysis *P*=0.005). This issue did not arise in PEAR because the protocol required dose optimization on the first drug before addition of the second drug. Furthermore, because participants with diabetes mellitus were included in INVEST but not in PEAR, we performed sensitivity replication analysis in INVEST, with further model adjustment for history of diabetes mellitus. The top signal remained associated with the primary phenotype (INVEST white–Hispanic meta-analysis *P*=0.002). The association also held after concurrently adjusting for history of diabetes mellitus, heart failure, and renal insufficiency (INVEST white–Hispanic meta-analysis *P*=0.005). Lastly, we tested whether the top variant, rs261316, was associated with uncontrolled BP on combination therapy with a CCB and an ACE inhibitor in INVEST. rs261316 showed a trend toward significance in the INVEST white–Hispanic meta-analysis (OR: 1.22, 95% CI, 0.94–1.59, *P*=0.104), with the association driven by INVEST Hispanics only (OR: 1.56, 95% CI, 1.05–2.32, *P*=0.030). The direction of association was consistent with PEAR discovery results.

Discussion

To our knowledge, this is the first pharmacogenomic GWAS to investigate the genome-wide associations of uncontrolled BP in people taking a combination of a thiazide diuretic and a β-blocker. We identified a region on chromosome 15 containing the genes *ALDH1A2*, *AQP9,* and *LIPC* associated with uncontrolled BP on combination therapy, and replicated this finding in an external cohort. *ALDH1A2,* also known as *RALDH2*, is a highly conserved gene located at chromosome 15q21.3 that encodes the protein aldehyde dehydrogenase 1 family member A2. This enzyme catalyzes the irreversible oxidation of retinaldehyde from vitamin A to produce retinoic acid, which is essential for normal mammalian development of the kidneys and heart.[31,32] SNPs in *ALDH1A2* have previously

been associated with kidney size.[33] Smaller kidneys may indicate having fewer nephrons, which has been associated with hypertension in animal and human studies.[34] A GWAS among black participants identified an intergenic SNP near *ALDH1A2* that was associated with hypertension (rs1550576, OR: 0.52, *P*=1.03E-05).[35] It is also notable that a recent meta-analysis of pharmacogenomics GWAS by Hiltunen et al identified an intronic SNP in *ALDH1A3*, also important for retinoic acid synthesis, associated with BP response to hydrochlorothiazide (rs3825926, β: 6.7 mm Hg, *P*=5.6E-06).[36]

The primary function of ALDH1A2 is to synthesize retinoic acid from vitamin A. Retinoic acid has been extensively studied in relation to kidney disease and has been considered a potential therapeutic agent because of its anti-inflammatory, antifibrotic, and cell differentiation properties.[31] In regard to BP regulation, retinoic acid administration has been shown to modify the expression of renin–angiotensin–aldosterone system components[37,38] and leads to decreased BP in a rat model.[39,40] Studies have also shown that the retinoic acid receptor RXR dimerizes with peroxisome-proliferator-activated receptor-γ to upregulate renin gene expression.[41] This may be of significance given that peroxisome-proliferator-activated receptor-γ has well-established roles in many cardiovascular and metabolic syndrome phenotypes, including hypertension.[42] Other mechanisms through which retinoic acid may modulate BP include epigenetic alterations[43] and endothelium-dependent NO-cGMP signaling.[44] Retinoic acid may also inhibit the development of atherosclerosis[45,46] and reduce the risk of cardiovascular mortality.[47]

The region surrounding the top variant also includes the genes *LIPC* and *AQP9. LIPC* encodes hepatic lipase, which has well-known associations with lipids[48] and coronary artery disease.[49,50] *LIPC* has also been linked to BP and hypertension in some studies.[51,52] Of note, the top variant was in moderate linkage disequilibrium (r^2: 0.51) with rs4775032, an expression quantitative trait loci (eQTL) in *ALDH1A2* for *LIPC* expression.[29] The third gene in the region, *AQP9*, encodes a member of a subset of aquaporins involved in transferring water, glycerol, and other small noncharged solutes across membranes. Association of *AQP9* with BP regulation is unclear; however, 1 study found that it was upregulated in preeclamptic placenta.[53] AQP9, in coordination with AQP7, may regulate adiposity and glucose homeostasis.[54] Notably, other members of the aquaporin family (AQP1, AQP2, and AQP4) have been associated with BP in animal studies.[55,56]

Retinoic acid–induced signaling, and the enzymes involved in its biosynthesis, may regulate BP through a variety of molecular mechanisms and pathophysiological processes. Further, retinoic acid may have BP-lowering effects. Because the top variant in *ALDH1A2* is intronic, it may be an eQTL, though this is not yet known, or it may be in linkage disequilibrium with the causal or functional variant. How that functional variant may affect retinoic acid is unclear, but it is possible that the T allele alters ALDH1A2 function such that levels of retinoic acid are decreased, leading to downstream effects on the renin–angiotensin–aldosterone system and peroxisome-proliferator-activated receptor-γ pathways that may ultimately result in higher BP.

We also identified a top variant near *OR5H14* that replicated with nominal significance and showed consistent direction of effect across discovery and replication cohorts. With additional replication studies, it is possible that this signal may be validated. *OR5H14* has not previously been linked to BP; however, there is a growing body of literature on the role of sensory receptors, including olfactory receptors, in modulating BP.[57,58] Further, in additional analysis with SBP and DBP response phenotypes, a few variants showed nominal significance in the replication analysis, including a variant in the genes *SETD7*, *MIR720*, and *RP11-461F11.3* associated with DBP response. *SETD7* may play a role in mediating high glucose-induced vascular dysfunction.[59] Less is known about the other genes, which are micro- and long non-coding RNAs, in regard to the cardiovascular system.

The strengths of the study include the use of data from a randomized controlled clinical trial with a robust design for evaluating the pharmacogenomics of antihypertensive drug response. We used a non-hypothesis-driven GWAS approach to identify the genetic correlates of uncontrolled BP on combination therapy with the 2 well-known antihypertensive drug classes, thiazide diuretics and β-blockers. Through this approach, we identified genetic variants corresponding to genes with plausible connections to the cardiovascular system and BP regulation. The top variant replicated in an external cohort of complicated hypertensives, indicating that the finding may have broader validity beyond uncomplicated hypertensives. More research is needed to understand the role of the identified genes in the cardiovascular system.

Our study also adds to the growing literature on the pharmacogenomics of antihypertensive drug nonresponse to combination therapy. A few GWAS and candidate gene studies have studied a more extreme form of drug nonresponse, namely, resistant hypertension,[13–15] defined as the use of at least 4 drugs to achieve BP control. Further, many BP or hypertension GWAS and large candidate gene studies have been conducted that have included treated individuals,[16,17] including 1 study that identified BP associations with the gene *ALDH2*, which encodes aldehyde dehydrogenase.[60] It must be noted, however, that these studies were not specifically designed to investigate the genetics of drug response, and therefore, the genes identified may not always overlap with those of hypertension pharmacogenomic studies.

Lastly, another strength of the study is that both PEAR and INVEST had well-documented drug and dose optimization protocols, with good adherence in PEAR (>85%),[18] and while INVEST did not directly assess adherence, study drugs were delivered to participants' homes (with receipt confirmations), and we observed expected reductions in BP and heart rate in both treatment arms, consistent with drug utilization.[61,62]

The limitations of the study are acknowledged. We focused on the discovery and replication of variants associated with uncontrolled BP on thiazide diuretic and β-blocker combination, and did not examine other drug class combinations. While β-blocker combinations are necessary in many patients, particularly those with ischemic heart disease or other indications for a β-blocker, we acknowledge that other combinations, particularly CCB/ACE inhibitor combinations, are now more widely used for uncomplicated hypertensives in light of the results of landmark studies.[5,6] As such, we also tested whether the top variant, rs261316, was associated with uncontrolled BP on CCB/ACE inhibitor combination therapy in INVEST, which showed that rs261316 trended toward association in INVEST white–Hispanic meta-analysis, with consistent direction across INVEST cohorts and with PEAR. Although this would need to be studied in another, larger cohort, these results suggest that rs261316 might also be a marker of uncontrolled BP among patients treated with CCB/ACE inhibitor combination therapy.

Other study limitations are noted. We did not identify variants that reached Bonferroni-corrected level of genome-wide significance in the discovery analyses; however, we selected variants that met the suggestive level of significance and focused on those that replicated or trended toward replication in a secondary cohort. Further, with the exception of the top variant in *ALDH1A2*, other top variants from the discovery analysis did not meet the multiple-comparisons corrected *P* value threshold for replication and did not approach genome-wide significance in the meta-analysis across discovery and replication cohorts. The relatively small sample size of the study is likely a contributing factor. Despite the reduced power, we had moderate-to-large effect sizes, which is consistent with findings from pharmacogenomics GWAS.[63] Differences in participants' baseline characteristics as well as differences in treatment protocol between the discovery and replication cohorts may also have precluded identifying additional significant signals. We tried to overcome these differences by adjusting for participant and study-related differences. Given that we were able to identify a replicated signal in or close to genes with plausible connections to various cardiovascular phenotypes suggests that our findings may not be spurious. Lastly, our study focused on white and Hispanic participants only. Further replication of the findings in other race/ethnic groups is warranted.

In summary, our study identified a SNP in the gene *ALDH1A2* where the major allele T was associated with an increased odds of uncontrolled BP on a thiazide diuretic and a β-blocker combination therapy. *ALDH1A2* and its byproduct, retinoic acid, may influence BP through a variety of alternative pathways. Additionally, we found suggestive evidence that the top SNP may also be associated with better BP control among individuals treated with a CCB/ACE inhibitor combination. More research on the genes corresponding to the top SNP, particularly *ALDH1A2*, and their pathways, are needed, which may illuminate novel or understudied mechanisms of BP regulation. Additionally, replication of the study's findings among participants treated with the same or different drug combinations is needed to determine their clinical utility for identifying patients who would benefit from initiation of combination therapy.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Table S1. Characteristics of INVEST-GENES study participants

Characteristics	All	Whites, N=221			Hispanics, N=193		
	N=414	Cases N=85	Controls N=136	P-value	Cases N=81	Controls N=112	P-value
Age, years	67.4 ± 10.0	71.5 ± 9.2	69.8 ± 9.5	0.186	63.2 ± 9.3	65.6 ± 10.6	0.139
Male	194 (47)	39 (46)	72 (53)	0.307	31 (38)	52 (46)	0.259
BMI, m/kg^2	28.7 ± 5.1	28.4 ± 5.6	28.2 ± 5.0	0.736	30.1 ± 5.1	29.1 ± 4.9	0.230
Baseline SBP, mmHg	149.6 ± 17.3	155.7 ± 15.0	146.4 ± 16.9	<0.001	152.7 ± 16.0	146.5 ± 20.7	0.031
Baseline DBP, mmHg	86.4 ± 10.5	86.5 ± 9.9	81.6 ± 9.8	<0.001	91.4 ± 10.0	88.0 ± 10.3	0.033
Current smoker	160 (39)	35 (41)	61 (45)	0.592	25 (31)	39 (35)	0.564
Disease history							
DM	53 (13)	12 (14)	22 (16)	0.680	12 (15)	7 (6)	0.055
Stroke/TIA	25 (6)	6 (7)	12 (9)	0.802	2 (2)	5 (4)	0.701
MI	109 (26)	31 (36)	55 (40)	0.556	7 (8)	16 (14)	0.267
LVH	60 (14)	12 (14)	19 (14)	0.976	18 (22)	11 (10)	0.017
CHF	10 (2)	1 (1)	6 (4)	0.254	2 (2)	1 (1)	0.573
RI	5 (1)	2 (2)	1 (1)	0.560	2 (2)	0 (0)	0.175
PVD	45 (11)	11 (13)	13 (9)	0.506	13 (16)	8 (7)	0.062
HCTZ dose*							
≥25 mg	259 (63)	79 (93)	42 (31)	<0.001	79 (97)	59 (53)	<0.001
12.5-25 mg	30 (7)	6 (7)	16 (12)		2 (2)	6 (5)	
None	125 (30)	0 (0)	78 (57)		0 (0)	47 (42)	
Atenolol dose							
≥100 mg	140 (34)	36 (42)	26 (19)	<0.001	50 (62)	28 (25)	<0.001
50-100 mg	253 (61)	49 (58)	95 (70)		31 (38)	78 (70)	
None	21 (5)	0 (0)	15 (11)		0 (0)	6 (5)	

*Doses of HCTZ and atenolol are once daily

Means ± standard deviation or numbers with percentages in parentheses; p-values are based on Student's t-test and χ^2 or Fisher's exact test; Cases: participants with uncontrolled BP on combination therapy; Controls: participants with controlled BP on combination therapy;

Abbreviations: INVEST-GENES, INternational VErapamil SR Trandolapril STudy Genetic Substudy; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; TIA, transient ischemic attack; MI, myocardial infarction; LVH, left ventricular hypertrophy; CHF, congestive heart failure; RI, renal insufficiency; PVD, peripheral vascular disease; HCTZ, hydrochlorothiazide

Table S2. Association of top genetic variants with uncontrolled BP on combination therapy in PEAR with replication analysis of top variant per region in INVEST

Variants associated with uncontrolled BP on combination therapy					Discovery			Replication			Meta-Analysis			
Locus					PEAR Whites			INVEST Whites-Hispanics*			PEAR Whites-INVEST Whites-Hispanics			
Variant	Chr:Position	Nearest gene	Function	A1	Freq A1	OR	P-value	Freq A1	OR	P-value	Freq A1	OR	P-value	Direction
rs798071	1:34939605	*MIR552*	Intergenic	G	0.48	3.27	3.88E-07	0.43	1.09	0.328	0.45	1.71	3.26E-04	+-+
rs35123024	3:97859694	*RP11-343D2.11*	Intronic	C	0.15	3.71	3.46E-06	0.15	1.72	0.033	0.15	2.57	3.77E-06	+++
rs59124417	3:97863708	*RP11-343D2.11*	Intronic	A	0.15	3.70	3.57E-06	-	-	-	-	-	-	-
rs4857072	3:97866177	*RP11-343D2.11*	Intronic	A	0.15	3.69	3.71E-06	-	-	-	-	-	-	-
rs7630491	3:97871082	*OR5H14*	Intergenic	T	0.15	3.69	3.71E-06	-	-	-	-	-	-	-
rs13098247	3:97880870	*OR5H15*	Intergenic	G	0.15	3.69	3.72E-06	-	-	-	-	-	-	-
rs4305454	3:97877963	*OR5H14*	Intergenic	T	0.15	3.69	3.72E-06	-	-	-	-	-	-	-
rs141791233	3:97877320	*OR5H14*	Intergenic	I	0.15	3.69	3.76E-06	-	-	-	-	-	-	-
rs6783261	3:97883239	*OR5H15*	Intergenic	A	0.15	3.68	3.90E-06	-	-	-	-	-	-	-
rs12489147	3:97883334	*OR5H15*	Intergenic	T	0.15	3.68	3.93E-06	-	-	-	-	-	-	-
rs150161264	6:12669218	*PHACTR1*	Intergenic	G	0.11	4.75	4.00E-06	0.14	1.14	0.324	0.13	2.04	9.49E-04	+-+
rs143212921	6:12669257	*PHACTR1*	Intergenic	C	0.11	4.75	4.00E-06	-	-	-	-	-	-	-
rs149142795	6:12669244	*PHACTR1*	Intergenic	C	0.11	4.75	4.00E-06	-	-	-	-	-	-	-
rs138629443	6:12669230	*PHACTR1*	Intergenic	T	0.11	4.75	4.00E-06	-	-	-	-	-	-	-
rs35009841	18:71053441†	*CTD-2354A18.1*	Intergenic	G	0.64	3.13	4.13E-06	-	-	-	-	-	-	-
rs144122711	6:12669115	*PHACTR1*	Intergenic	C	0.11	4.42	4.61E-06	-	-	-	-	-	-	-
rs141150884	6:12669158	*PHACTR1*	Intergenic	C	0.11	4.44	4.63E-06	-	-	-	-	-	-	-
rs11876414	18:71062198	*CTD-2354A18.1*	Intergenic	T	0.59	2.64	6.01E-06	0.57	0.79	0.378	0.58	1.38	0.027	+--
rs140264450	6:12669037	*PHACTR1*	Intergenic	C	0.12	4.17	6.11E-06	-	-	-	-	-	-	-
rs10753308	1:34907148	*C1orf94*	Intergenic	A	0.33	2.67	6.58E-06	-	-	-	-	-	-	-
rs10753309	1:34908206	*C1orf94*	Intergenic	A	0.34	2.67	6.63E-06	-	-	-	-	-	-	-
rs1954981	18:71055969	*CTD-2354A18.1*	Intergenic	G	0.61	2.64	8.56E-06	-	-	-	-	-	-	-
rs261316	15:58633820	*ALDH1A2*	Intronic	T	0.59	2.56	8.64E-06	0.60	1.86	0.001	0.60	2.16	8.60E-08	+++
rs112779628	6:12644689	*RP11-125M16.1*	Intergenic	G	0.12	3.80	9.96E-06	-	-	-	-	-	-	-

*Meta-analysis of INVEST Whites and Hispanics; P-values shown for INVEST replication cohort are one-sided, consistent with a one-sided hypothesis for replication;

† The top signal in the chromosome 18 region was unavailable in the replication dataset;

(-) indicates that the variant was not moved forward for replication analysis because only the top variant per distinct gene region was tested for replication;

+++ or --- indicates consistent direction of association across PEAR Whites, INVEST Whites, and INVEST Hispanics;

++- or --+ indicates consistent direction of association across PEAR Whites and INVEST Whites;

Sample sizes: PEAR Whites (N=314), INVEST Whites (N=221), INVEST Hispanics (N=193);

Base pair positions are based on build 37;

Abbreviations: BP, blood pressure; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; INVEST, INternational VErapamil SR Trandolapril STudy; A1, allele associated with uncontrolled BP; Freq A1, frequency of A1

Table S3. Association of top genetic variants with SBP response on combination therapy in PEAR with replication analysis in INVEST

Variants associated with SBP response on combination therapy					Discovery			Replication			Meta-Analysis			
Locus					PEAR Whites			INVEST Whites-Hispanics*			PEAR Whites-INVEST Whites-Hispanics			
Variant	Chr:Position	Nearest gene	Function	A1	Freq A1	β	P-value	Freq A1	β	P-value	Freq A1	β	P-value	Direction
rs7562028	2:105051172	*AC013402.2*	Intronic	G	0.08	-7.48	8.06E-06	0.06	-3.38	0.069	0.07	-6.05	7.64E-06	---
rs163434	5:156626607	*ITK*	Intronic	C	0.04	10.25	4.12E-06	0.05	2.92	0.123	0.04	7.04	2.46E-05	++-
rs150598951	3:73518178	*PDZRN3*	Intronic	D	0.04	10.11	5.49E-06	0.03	1.70	0.314	0.04	7.70	4.14E-05	+++
rs10214709	6:166066893	*PDE10A*	Intronic	T	0.25	4.50	5.31E-06	0.24	0.88	0.263	0.24	3.28	4.64E-05	+++
rs2114713	15:80528373	*RP11-2E17.1*	Intronic, eQTL	G	0.43	-4.07	3.86E-06	0.42	-0.78	0.233	0.43	-2.74	5.56E-05	--+
rs77106793	20:49866387†	*AL035457.1*	Intergenic	A	0.05	14.05	5.52E-06	0.03	2.78	0.219	0.04	9.25	7.90E-05	+++
rs73006036	19:9239010†	*OR7G3*	Intergenic	C	0.05	11.60	8.85E-06	0.02	0.92	0.416	0.04	8.74	9.16E-05	++-
rs798071	1:34939605	*MIR552*	Intergenic	G	0.46	4.13	3.04E-06	0.44	-0.07	0.524	0.45	2.49	3.10E-04	++-
rs10802909	1:240985581	*RGS7*	Intronic	G	0.24	4.35	9.89E-06	0.27	-1.67	0.915	0.25	1.97	1.02E-02	++-

*Meta-analysis of INVEST Whites and Hispanics; P-values shown for INVEST replication cohort are one-sided, consistent with a one-sided hypothesis for replication;

+++ or --- indicates consistent direction of association across PEAR Whites, INVEST Whites, and INVEST Hispanics;

++- or --+ indicates consistent direction of association across PEAR Whites and INVEST Whites;

†Variants with imputation quality $0.30 < r^2 < 0.70$

Sample sizes: PEAR Whites (N=401), INVEST Whites (N=221), INVEST Hispanics (N=193);

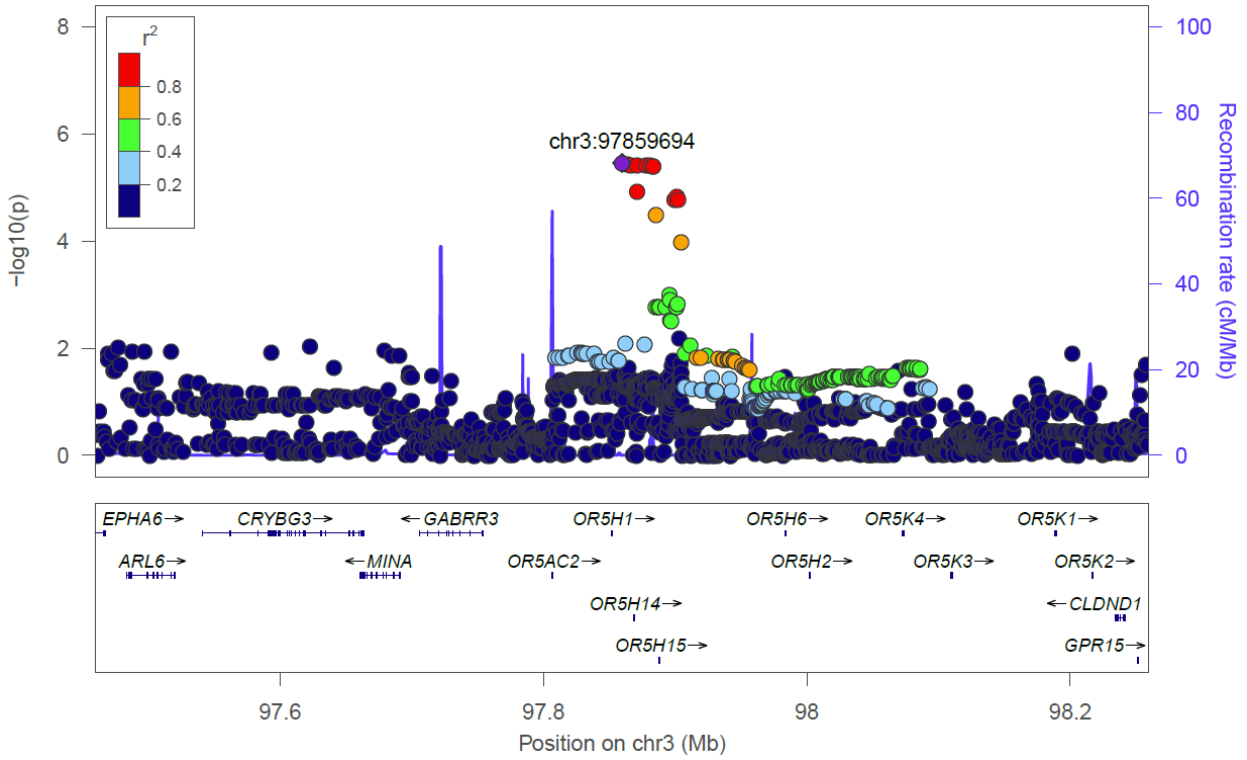
Base pair positions are based on build 37; Abbreviations: SBP, systolic blood pressure; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; INVEST, INternational VErapamil SR Trandolapril STudy; A1, allele associated with uncontrolled BP; Freq A1, frequency of A1; eQTL, expression quantitative trait loci

Table S4. Association of top genetic variants with DBP response on combination therapy in PEAR with replication analysis in INVEST

Variants associated with DBP response on combination therapy					Discovery			Replication			Meta-Analysis			
Locus					PEAR Whites			INVEST Whites-Hispanics*			PEAR Whites-INVEST Whites-Hispanics			
Variant	Chr:Position	Nearest gene	Function	A1	Freq A1	β	P-value	Freq A1	β	P-value	Freq A1	β	P-value	Direction
rs148514273	4:140465078†	*SETD7*	Intronic	A	0.04	-9.32	2.01E-06	0.02	-5.59	0.011	0.03	-7.85	2.69E-07	---
rs142521164	3:164060970	*MIR720*	Intergenic	T	0.42	2.63	5.99E-06	0.38	1.19	0.037	0.40	2.01	4.56E-06	+++
rs192336334	14:40391173†	*RP11-111A21.1*	Intergenic	T	0.07	7.54	3.03E-06	0.03	2.61	0.074	0.05	5.35	8.78E-06	+++
rs62123267	19:8194515	*FBN3*	Intronic	A	0.03	8.50	8.10E-06	0.02	2.81	0.177	0.03	6.89	1.94E-05	+++
rs143166465	15:98153795†	*RP11-461F11.3*	Intergenic	D	0.27	5.28	4.50E-06	0.13	1.73	0.032	0.19	3.14	1.52E-05	+++
rs3745429	19:54937593	*TTYH1*	Intronic, eQTL	C	0.08	4.87	6.91E-06	0.30	0.79	0.136	0.23	2.05	6.46E-04	+++
rs9406603	9:16270602	*C9orf92*	Intronic	C	0.17	3.35	7.32E-06	0.17	0.03	0.484	0.17	1.87	7.60E-04	++-
rs72654162	13:110883496†	*COL4A1*	Intronic	T	0.06	7.70	8.86E-06	0.05	0.75	0.317	0.05	3.89	8.43E-04	++-
rs75278792	11:104161452	*RN5S348*	Intergenic	T	0.04	7.43	4.74E-06	0.03	-0.89	0.672	0.04	4.13	1.03E-03	++-
rs117289276	13:107219470†	*ARGLU1*	Intronic	T	0.05	9.84	9.87E-06	0.03	0.48	0.409	0.04	4.81	1.49E-03	++-

*Meta-analysis of INVEST Whites and Hispanics; P-values shown for INVEST replication cohort are one-sided, consistent with a one-sided hypothesis for replication;
+++ or --- indicates consistent direction of association across PEAR Whites, INVEST Whites, and INVEST Hispanics;
++- or --+ indicates consistent direction of association across PEAR Whites and INVEST Whites;
†Variants with imputation quality $0.30 < r^2 < 0.70$
Sample sizes: PEAR (N=401), INVEST Whites (N=221), INVEST Hispanics (N=193);
Base pair positions are based on build 37;
Abbreviations: DBP, diastolic blood pressure; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; INVEST, INternational VErapamil SR Trandolapril STudy; A1, allele associated with uncontrolled BP; Freq A1, frequency of A1; eQTL, expression quantitative trait loci

Figure S1. Regional plot of top genetic variant associated with uncontrolled BP on combination therapy in PEAR



rs35123024 associated with uncontrolled BP on combination therapy (PEAR Whites: p=3.46E-06; INVEST White-Hispanic meta-analysis: one-sided p=0.033; PEAR and INVEST meta-analysis p=3.77E-06);

Abbreviations: BP, blood pressure; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; INVEST, INternational VErapamil SR Trandolapril STudy