Genome-Wide and Gene-Based Meta-Analyses Identify Novel Loci Influencing Blood Pressure Response to Hydrochlorothiazide

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Abstract—This study aimed to identify novel loci influencing the antihypertensive response to hydrochlorothiazide monotherapy. A genome-wide meta-analysis of blood pressure (BP) response to hydrochlorothiazide was performed in 1739 white hypertensives from 6 clinical trials within the International Consortium for Antihypertensive Pharmacogenomics Studies, making it the largest study to date of its kind. No signals reached genome-wide significance ($P<5\times10^{-8}$), and the suggestive regions ($P<10^{-5}$) were cross-validated in 2 black cohorts treated with hydrochlorothiazide. In addition, a gene-based analysis was performed on candidate genes with previous evidence of involvement in diuretic response, in BP regulation, or in hypertension susceptibility. Using the genome-wide meta-analysis approach, with validation in blacks, we identified 2 suggestive regulatory regions linked to gap junction protein α1 gene (*GJA1*) and forkhead box A1 gene (*FOXA1*), relevant for cardiovascular and kidney function. With the gene-based approach, we identified hydroxy-delta-5-steroid dehydrogenase, 3 β- and steroid δ-isomerase 1 gene (*HSD3B1*) as significantly associated with BP response ($P<2.28\times10^{-4}$). *HSD3B1* encodes the 3β-hydroxysteroid dehydrogenase enzyme and plays a crucial role in the biosynthesis of aldosterone and endogenous ouabain. By amassing all of the available pharmacogenomic studies of BP response to hydrochlorothiazide, and using 2 different analytic approaches, we identified 3 novel loci influencing BP response to hydrochlorothiazide. The gene-based analysis, never before applied to pharmacogenomics of antihypertensive drugs to our knowledge, provided a powerful strategy to identify a locus of interest, which was not identified in the genome-wide meta-analysis because of high allelic heterogeneity. These data pave the way for future investigations on new pathways and drug targets to enhance the current understanding of personalized antihypertensive treatment. (***Hypertension*. 2017;69:00-00. DOI: 10.1161/HYPERTENSIONAHA.116.08267.)** • **Online Data Supplement**

Key Words: blood pressure response ■ diuretics ■ genome-wide association study ■ hydrochlorothiazide ■ hypertension ■ meta-analysis ■ pharmacogenomics

Hypertension is a major global risk factor for stroke, coronary heart disease, renal failure, and heart failure and is the most common chronic disease for which medications are prescribed.[1] The primary goal of hypertension treatment is the reduction of blood pressure (BP), which is strongly associated with the prevention of adverse cardiovascular outcomes. However, only ≈50% of the treated hypertensive patients achieve BP control, despite the availability of many classes of antihypertensive drugs.[2,3]

Thiazide diuretics (TD), inhibitors of the Na^+/Cl^- symporter, are first-line agents to treat uncomplicated hypertension as they are effective, relatively safe, and well tolerated.[4]

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However, as with other antihypertensive drugs, there is substantial interindividual variation in BP response to TD which can be at least partially attributed to genetic differences among individuals.[5] Pharmacogenomics, the study of the influence of genomic variations on drug response, could be a useful tool to select the most effective antihypertensive therapy for an individual, based on genetic profile, once replicated drug–gene pairs have been discovered.[6] Many groups have conducted pharmacogenetic studies on BP response to TD using candidate gene(s)[7–11] or genome-wide association studies (GWAS).[12–15] These studies, recently reviewed,[6,16–18] have advanced the knowledge surrounding hypertension pharmacogenomics and suggest several genetic variants that may be important determinants of response to TD. Nevertheless, only a small percentage of the variability in BP response has been explained to date.

The International Consortium for Antihypertensive Pharmacogenomics Studies (icaps-htn.org) was created to promote the collaboration between independent research groups, share knowledge, and increase the likelihood for genetic discoveries in the field of antihypertensive pharmacogenomics.

Here, we present the largest genome-wide meta-analysis of BP response to hydrochlorothiazide (a TD) monotherapy to date in whites with uncomplicated hypertension, from 6 clinical trials included in the International Consortium for Antihypertensive Pharmacogenomics Studies. Because all the currently available clinical trials for hydrochlorothiazide monotherapy in whites with genome-wide genetic data were included in our meta-analysis, we used cohorts of black individuals for validation. This validation approach among multiple race groups increases the confidence of a functional finding. Moreover, as a useful complement to the GWAS, we conducted a gene-based analysis focused on 219 candidate genes with previous evidence of involvement in diuretic response or BP regulation, selected after a comprehensive literature search.

Methods

Study Participants and Inclusion Criteria

Six study cohorts contributed data to the meta-analysis: the GENRES study (Genetics of Drug Responsiveness in Essential Hypertension)[15,19]; the GERA-1 study (Genetic Epidemiology of Responses to Antihypertensives-1)[20]; the HCTZ-Milan study (Milan Hydrochlorothiazide)[13]; the NORDIL study (Nordic Diltiazem)[9,21]; the PEAR-1 study (Pharmacogenomic Evaluation of Antihypertensive Responses-1)[22]; and the PHSS study (Pharmacogenomics of Hydrochlorothiazide Sardinian Study).[13] Detailed information about each of 6 cohorts is included in the online-only Data Supplement. Across all studies, participants with uncomplicated hypertension were included if they had a baseline untreated BP level (ie, prehydrochlorothiazide treatment) in the hypertensive range (systolic BP [SBP] >140 mm Hg or diastolic BP [DBP] >90 mm Hg). Participants previously taking antihypertensive medications underwent a washout period during which all antihypertensive medications were withdrawn. All participants were treated with hydrochlorothiazide as monotherapy for at least 4 weeks. All participants voluntarily signed ethics committee approved informed consent forms, and all clinical trials were conducted in accordance with regulations set forth by the Declaration of Helsinki and local regulatory agencies.

BP Response Phenotype

We used the most precise measure of BP response available for each study. In the GENRES and the NORDIL studies, BP response was determined as the difference between the averaged BP measurements before and at the end of hydrochlorothiazide treatment. In the other studies, in which BP was measured at least once between the baseline and the end of the treatment period, or by multiple methodologies (eg, office, home, and ambulatory BP), we used a single model to take into account all available BP measurements, including the intermediate time points and multiple methods of measurement. PEAR-1 generated a weighted average of the office, home, and ambulatory daytime and nighttime BP responses calculated on the sums of the inverse of the intermethod covariance matrices.[23] In the GERA-1, the HCTZ-Milan, and the PHSS studies, office BP was measured at 1 intermediate time point between the baseline and the final measurement. These data were fit to a general linear model that included baseline BP, sex, age, the first 10 principal components, and time point. The residuals from this model represent adjusted measurements of treatment response. There were typically 2 such residuals per individual, calculated for the 2 time points, which were combined using a weighted average.

Table 1. Baseline Characteristics of Study Participants

Characteristic*	GENRES (n=192)†	GERA-1 (n=282)	HCTZ-Milan (n=207)	NORDIL (n=381)	PEAR-1 (n=228)	PHSS (n=449)
Men/women	192/0	161/121	177/30	148/233	137/91	293/156
Age, y	50.8±6.2	46.3±8.1	45.7±7.98	61.5±6.7	50.0±9.5	50.9±10.1
BMI, kg/m^2	26.8±2.6	30.9±5.5	26.14±3.1	28.4±4.7	30.3±4.9	27.6±4.0
Pretreatment SBP, mm Hg	152.3±12.2	142.4±12.5	149.76±12.5	172.5±15.6	151.8±12.4	158.1±13.0
Pretreatment DBP, mm Hg	100.2±6.1	95.4±5.4	98.96±7.8	103.0±4.5	98.1±5.8	100.4±9.9
Treatment dose	25 mg/d	25 mg/d	12.5 mg/d 25 mg/d	at physician discretion	12.5 mg/d 25 mg/d	25 mg/d
Period of treatment	4 wk	4 wk	8 wk (time point at 4 wk)	6 mo	8 wk (time point at 2 wk)	8 wk (time point at 4 wk)
Run-in period	4 wk placebo	4 wk	Never treated	2 wk	≈ 31 d	Never treated

BMI indicates body mass index; DBP, diastolic blood pressure; GENRES, Genetics of Drug Responsiveness in Essential Hypertension Study; GERA-1, Genetic Epidemiology of Responses to Antihypertensives-1 study; HCTZ-Milan, Milan Hydrochlorothiazide study; NORDIL, Nordic Diltiazem study; PEAR-1, Pharmacogenomic Evaluation of Antihypertensive Responses-1 study; PHSS, Pharmacogenomics of Hydrochlorothiazide Sardinian Study; and SBP, systolic blood pressure.

*Numeric characteristics were presented as mean±SD; categorical variables were presented as number.

†The mean blood pressure level of 4 placebo treatment periods was used as the baseline (pretreatment) level.

Genotyping and Imputation

Genome-wide genotyping was done on commercially available platforms from Illumina or Affymetrix. All genotype data were imputed to HapMap CEU II (Utah residents of northern and western European ancestry; build 36, version 22), and standard quality control procedures were applied. Details of the genotyping and quality control procedures are included in the Data Supplement.

Statistical Analysis

Continuous variables are presented as mean and SD, and categorical variables as numbers and percentages. Between-group comparison of continuous variables was performed using 1-way analysis of variance and Tukey Honestly Significant Difference post hoc test. Categorical data were compared between groups using the χ^2 test.

A total of 1739 white individuals from the 6 study cohorts are included in the GWAS for BP response. Using mach2qtl[24] or SNPtest[25] software, we performed linear regressions of the BP response phenotype on single nucleotide polymorphism (SNP) dosages adjusting for sex, age, prediuretic treatment BP, and principal components. All GWAS results underwent quality control using the EasyQC package.[26] Genome-wide SNP meta-analysis was performed using the software METAL.[27] Details of these analysis procedures are included in the Data Supplement.

SNPs with $P<5\times10^{-8}$ were considered genome-wide significant, and those with $P<10^{-5}$ were considered suggestive.

We then performed a transethnic validation in black hypertensives treated with hydrochlorothiazide from the GERA-1 and PEAR-1 studies (Table S1) for the suggestive SNPs with $P<10^{-5}$. We tested the genetic regions harboring the suggestive signals as well because we did not necessarily expect to observe the same SNPs, because of the differences in linkage disequilibrium (LD) across the genome between white and black populations. Neighboring SNPs were not required to have effects in the same direction; because of differences in LD and allele frequency, neighboring SNPs could tag the same unknown causal variant.[28]

We conducted a candidate gene-based association analysis in each cohort separately using the VEGAS program (Versatile Gene-based Association Study)[29] and then performed a meta-analysis of the results applying the Fisher method[30] using the sumlog R function.[31] We selected the candidate genes beginning with the catalogue recently reviewed by Padmanabhan et al[32] with the inclusion of additional genes found in PubMed using the key search terms diuretic response, hypertension, and blood pressure regulation. Following this comprehensive literature search, 219 autosomal genes (Table S2) were identified based on evidence from candidate studies or GWAS of involvement in diuretic response, in BP regulation, or in hypertension susceptibility and included in the gene-based analysis.

Results

Demographic and baseline characteristics of the 6 cohorts included in the GWAS meta-analysis are summarized in Table 1. All participants were white and from the United States or Europe. With the exception of GENRES, all cohorts included a majority or exclusively males. Overall, mean age and body mass index were significantly different among the cohorts. GENRES, HCTZ-Milan, and PEAR-1 had similar pretreatment SBP (P=0.09), whereas pretreatment DBP levels were similar between the Milan Hydrochlorothiazide study and PEAR-1 (P=0.19) and between GENRES and PHSS studies (P=0.80).

GWAS Meta-Analysis

Manhattan and q–q plots for the meta-analysis of SBP and DBP response to hydrochlorothiazide are shown in Figure 1. Although no SNP achieved Bonferroni-corrected

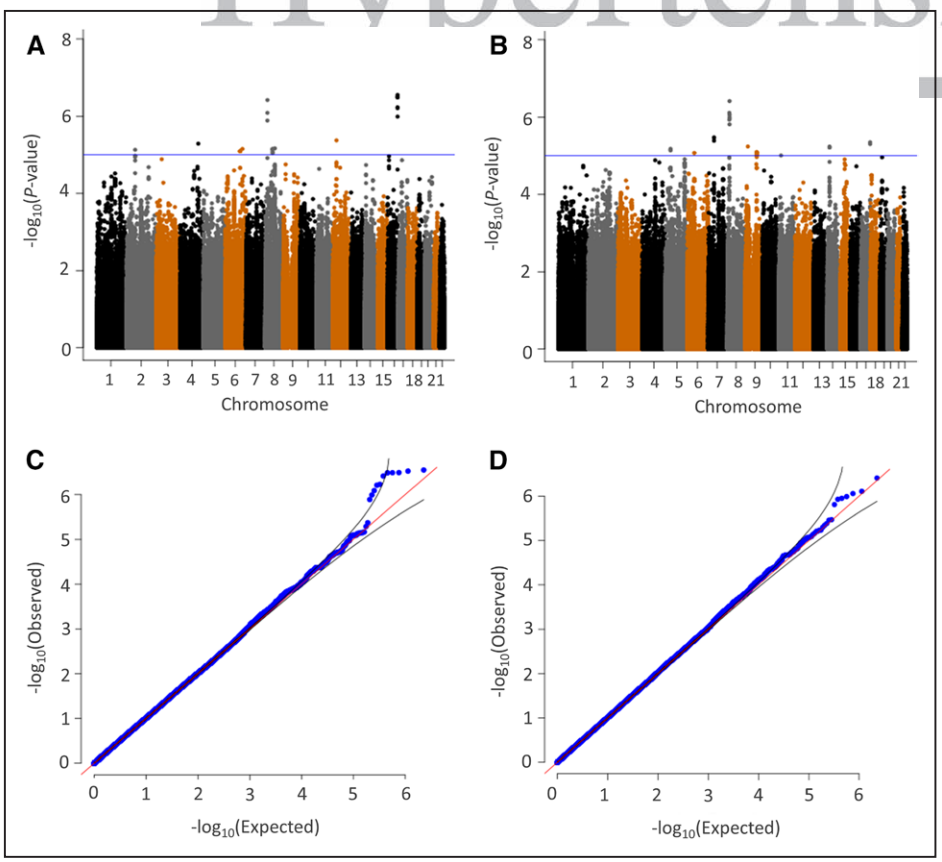

Figure 1. Manhattan (**A** and **B**) and quantile–quantile (**C** and **D**) plots from meta-analysis of genome-wide association for systolic (**A** and **C**) and diastolic (**B** and **D**) blood pressure response to hydrochlorothiazide. Each dot signifies a SNP. In the Manhattan plots, genomic coordinates are displayed along the *x* axis with the negative logarithm of the association *P* value displayed on the *y* axis. The blue line refers to $-\log_{10}(P)=5$. The quantile–quantile plots compare the observed results ($-\log_{10}(P)$, *x* axis) versus the theoretically expected values (*y* axis).

Table 2. Meta-Analysis Results for Blood Pressure Response to Hydrochlorothiazide With $P<10^{-5}$

Trait	Marker	Chromosome	Position (bp)	Gene/Region	Function	Coded/Other Alleles	Coded Allele Frequency	β	*P* Value	Tagged SNPs ($r^2>0.80$)
SBP	*rs7565329*	2	67959340	*ETAA1/C1D*	Intergenic	T/C	0.09	−1.7	7.42×10^{-6}	
	rs12642634	4	147205077	*ZNF827/ LSM6*	Intergenic	A/G	0.78	1.1	5.15×10^{-6}	
	rs11750990	6	121977301	*GJA1/HSF2*	Intergenic	A/G	0.96	2.44	8.11×10^{-6}	rs11755230, rs11755743, rs11752212, rs11754717
	rs2876449	6	141099634	*CITED2/ NMBR*	Intergenic	T/G	0.03	−2.8	7.06×10^{-6}	
	rs11784910	8	17119626	*ZDHHC2*	Intronic	A/T	0.95	2.31	3.85×10^{-7}	rs11775427, rs11786857
	rs2925663	8	59424423	*FAM110B/ UBXN2B*	Intergenic	T/C	0.12	−1.4	7.11×10^{-6}	rs747401, rs733665, rs2970756, rs2925666
	rs13253998	8	78697117	*PEX2/PKIA*	Intergenic	T/G	0.51	−0.9	6.80×10^{-6}	
	rs7960884	12	31251907	*FAM60A*	Intergenic	A/G	0.27	1.09	4.23×10^{-6}	
	rs16962897	16	82766581	*DNAAF1*	Intronic	A/G	0.12	−1.7	3.25×10^{-7}	rs1056616, rs2288025, rs1056612, rs3743642, rs4150162
	rs4150161	16	82771737	*TAF1C*	Intronic	T/C	0.86	1.53	1.02×10^{-6}	
DBP	*rs822127*	5	41582716	*PLCXD3/ OXTC1*	Intergenic	A/G	0.37	−0.6	6.64×10^{-6}	rs1495757, rs4957159
	rs9370524	6	56327712	*COL21A1*	Intronic	A/G	0.36	0.58	8.51×10^{-6}	
	rs10951933	7	47873739	*PKD1L1*	Intronic	T/G	0.2	0.75	3.37×10^{-6}	rs10951934, rs17131904
	rs2198596	8	15045137	*SGCZ*	Intronic	C/G	0.91	−1.3	7.76×10^{-7}	rs17575278
	rs13256445	8	17106584	*ZDHHC2*	Intronic	T/C	0.85	0.96	1.02×10^{-6}	rs13278086
	rs11784910	8	17119626	*ZDHHC2*	Intronic	A/T	0.95	1.48	3.89×10^{-7}	rs11775427, rs11786857
	rs17755650	9	21770037	*MTAP*	Intergenic	T/C	0.9	1.17	5.78×10^{-6}	
	rs12685849	9	93570641	*ROR2*	Intronic	A/G	0.04	−1.7	9.98×10^{-6}	rs12685213, rs3935544, rs10992090, rs3802377, rs3802378, rs16907776, rs10992095
	rs4757718	11	18761510	*PTPN5*	Intronic	A/G	0.67	−0.6	9.87×10^{-6}	
	rs177848	14	37173757	*TTC6/FOXA1*	Intronic/5′-flanking	A/C	0.59	−0.6	5.81×10^{-6}	rs1998125, rs177829
	rs11657217	17	75323934	*ENPP7*	Coding	C/G	0.69	−0.7	4.50×10^{-6}	rs8081537

SNPs are ranked by chromosome and position based on hg18 (NCBI 36) assembly. Tagged SNPs are in high linkage disequilibrium ($r^2>0.80$) with markers. *P* refers to the meta-analysis results of genome-wide association studies from the 6 International Consortium for Antihypertensive Pharmacogenomics Studies cohorts.

genome-wide significance ($P<5\times10^{-8}$), the q–q plots indicated that the SNPs with $P<10^{-5}$ deviated above the straight line, indicating that there is a suggestion of relationships between SNPs and BP response. Thus, we considered SNPs with $P<10^{-5}$ as suggestive. Accordingly, there were 10 SNPs with suggestive associations with SBP response, and there were 11 SNPs with suggestive associations with DBP response (Table 2). These SNPs were in high LD ($r^2>0.80$) with 34 additional SNPs according to the HapMap reference (Tagged SNPs, Table 2).

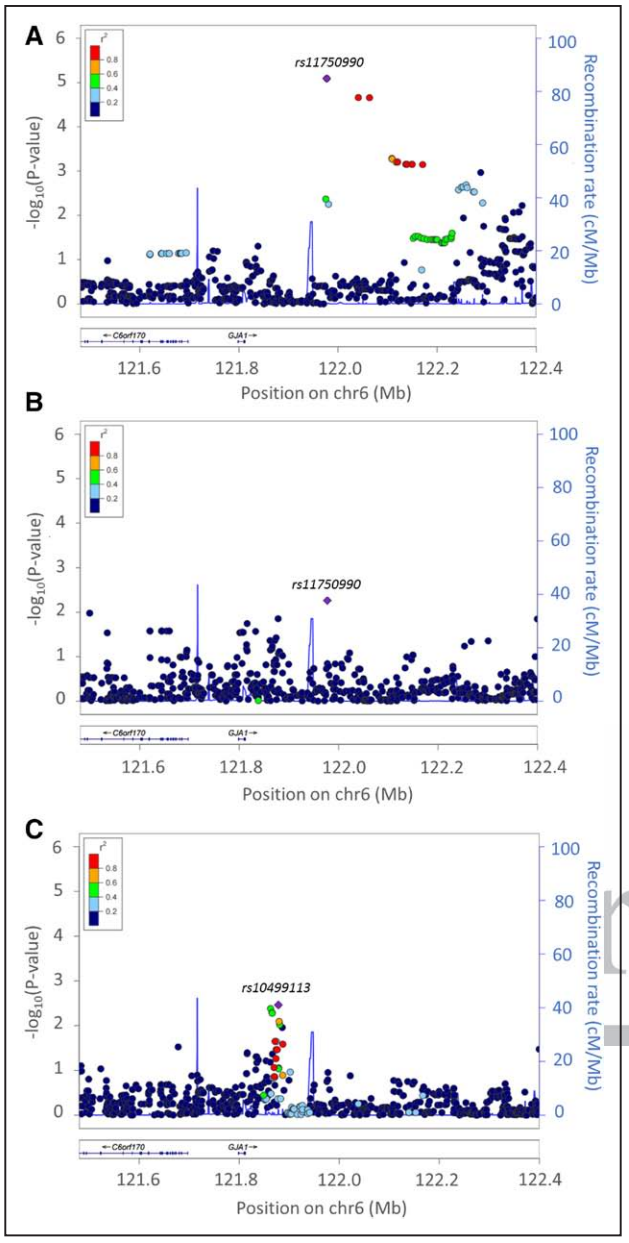

Figure 2. Local regional plot for chromosome 6q22.31. **A**, Meta-analysis results for white samples; (**B**) replication results in meta-analysis of PEAR-1 (Pharmacogenomic Evaluation of Antihypertensive Responses-1) and GERA-1 (Genetic Epidemiology of Responses to Antihypertensives-1) black samples; and (**C**) replication results in GERA-1 black sample. Each dot signifies a SNP. Genomic coordinates are displayed along the *x* axis with the negative logarithm of the association *P* value displayed on the *y* axis. SNPs are colored based on their r^2 with the top-signal SNP that has the lowest *P* value in the region.

Transethnic Replication

We then assessed the association of suggestive meta-analysis loci ($P<10^{-5}$) and neighboring regions in 2 black ancestry samples from GERA-1 and PEAR-1. Sample characteristics are detailed in the Data Supplement and in Table S1. GERA-1 and PEAR-1 black participants had similar mean age and body mass index. Pretreatment DBP was higher in GERA-1 group than in PEAR-1 (P=0.004), whereas pretreatment SBP was not significantly different between the cohorts (P=0.069).

In blacks, we identified 2 regions of interest on chromosome 6 and 14, neighboring the identified suggestive SNPs in whites, for SBP and DBP response to hydrochlorothiazide.

With regard to SBP associations, we identified a signal of interest located in the 3′-flanking region of gap junction protein α1 gene (*GJA1*) on chromosome 6q22.31, where *rs11750990* was associated with SBP in the meta-analysis of white participants ($P=8.11\times10^{-6}$ and β=2.44 mm Hg per A allele; Figure 2A; Table 2). This variant was nominally associated in the meta-analysis of PEAR-1 and GERA-1 black individuals ($P=5.46\times10^{-3}$) with opposite β coefficient (β=−6.17) per A allele (Figure 2B). In the same region, a peak of suggestive significance was observed in the GERA-1 sample with a different SNP, *rs10499113*, associated with SBP response ($P=3.46\times10^{-3}$ and β=−3.14 per C allele; Figure 2C).

With regard to DBP associations, we identified a signal of interest in the 5′-flanking region of forkhead box A1 gene (*FOXA1*) on chromosome 14q21.1 (Figure 3). In the meta-analysis of whites, *rs177848* was associated with DBP response ($P=5.8\times10^{-6}$ and β=−0.60 per A allele; Table 2; Figure 3A). *Rs177852*, 7.9 kb upstream of *rs177848*, was associated with DBP response in the PEAR-1 black cohort ($P=1.43\times10^{-3}$, β=−2.95 per C allele; Figure 3B).

Gene-Based Meta-Analysis

Results of the gene-based analysis are shown in Table S2. Applying a Bonferroni-corrected significance threshold for gene-based analysis ($P<2.28\times10^{-4}$), we identified the hydroxy-delta-5-steroid dehydrogenase, 3 β- and steroid δ-isomerase 1 gene (*HSD3B1*) on chromosome 1p12 as significantly associated with DBP response ($P=2.80\times10^{-5}$) and SBP response ($P=7.5\times10^{-5}$) to hydrochlorothiazide (Table S2).

In the GWAS meta-analysis results for *HSD3B1*, we observed a cluster of SNPs with $P\leq10^{-4}$ associated with BP response to hydrochlorothiazide (Figure 4; Table S3). For SBP response, we identified the 3′-flanking SNP *rs7553527* (β=−0.86, $P=3.67\times10^{-5}$ per C allele) in high LD with the coding SNP *rs6203* and the 3′-flanking SNP *rs10754403* (β=−0.83, $P=8.07\times10^{-5}$ per G allele), which is in LD with 58 other SNPs within the 3′- to 5′-flanking region (Table S3; Figure 4A). The coding SNP *rs6203* had a $P=2.32\times10^{-3}$ for DBP response (Table S3; Figure 4B).

Although gene-based analysis for *HSD3B1* in blacks was not significant, it is important to consider that (1) the LD pattern of the *HSD3B1* locus is different comparing CEU and YRI (Yoruba in Ibadan, Nigeria) HapMap populations (Figure S1) and (2) *rs7553527* and *rs6203* are not present in HapMap data for the YRI population.

Discussion

This article describes the largest genome-wide meta-analysis of loci influencing the antihypertensive response to hydrochlorothiazide monotherapy and includes a total of 1739 white individuals from 6 independent cohorts from the International Consortium for Antihypertensive Pharmacogenomics Studies. Using the single-SNP GWAS meta-analysis, we identified 2 suggestive regulatory regions on chromosomes 6 and 14, potentially linked to genes relevant for cardiovascular and kidney function. These signals

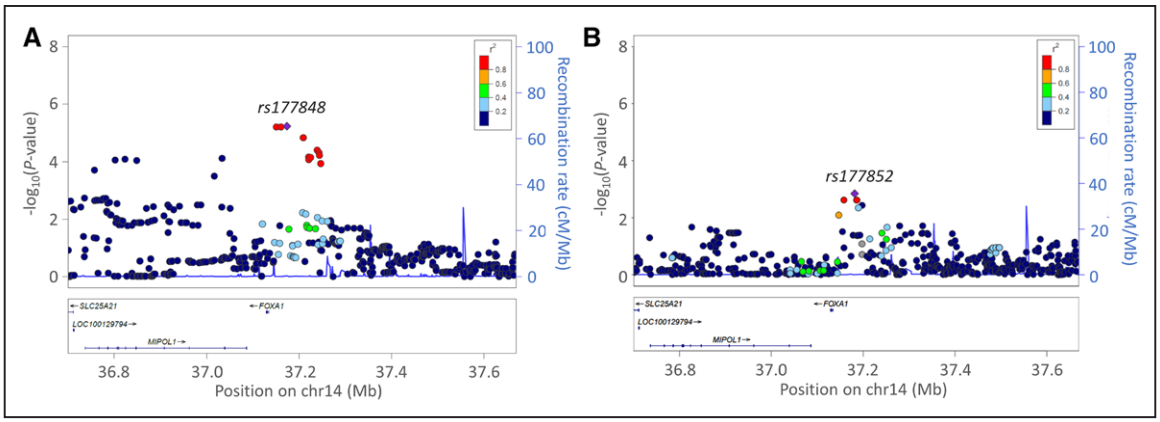

Figure 3. Local regional plot for chromosome 14q21. **A**, Meta-analysis results for white samples and (**B**) replication results in PEAR-1 black sample. Each dot signifies a SNP. Genomic coordinates are displayed along the *x* axis with the negative logarithm of the association *P* value displayed on the *y* axis. SNPs are colored based on their r^2 with the top-signal SNP that has the lowest *P* value in the region.

were nominally validated in 2 independent black cohorts. Through a complementary candidate gene-based approach, we identified *HSD3B1*, at a significance level taking Bonferroni correction into account, which was not detected using the single-SNP GWAS meta-analysis.

In the genome-wide meta-analysis, no SNP reached the genome-wide significance level, which is not surprising given the total sample size included in the meta-analysis. In fact, despite being the largest hydrochlorothiazide monotherapy meta-analysis in whites (1739 individuals), we calculated that a sample size ranging from 2860 to 5571 samples would be required to achieve 80% power at $P=5\times10^{-8}$, assuming an expected effect of at least 2 mm Hg and an allele frequency ranging from 0.15 to 0.45.[33] The sample size of this study was powered to find variants with effects ≥3 mm Hg and frequencies ≥0.2, whereas, for the suggestive SNPs identified, the average effect observed was ≈1.3 mm Hg.

The suggestive SNPs, as well as SNPs in neighboring regions, that were identified in the meta-analysis were tested in 2 samples of different ancestries.

The first interesting region for SBP response is located in the 3′-flanking region of *GJA1*. *GJA1* encodes the Connexin43 (Cx43), the predominant gap junction protein in myocardial and aortic smooth muscle cells with the involvement in the regulation of cell-to-cell communication and elasticity and contractility of the vascular wall.[34] The expression of Cx43 was observed to be increased in aortic wall[34] and muscular artery[35] of hypertensive rats and was decreased after the exposure to the combination of hydralazine–hydrochlorothiazide and candesartan.[35] According to the HaploReg database,[36] the suggestive 3′-flanking region of *GJA1* contains expression quantitative trait loci, transcription factor binding sites and histone marks. SNP *rs10499113* is reported to be an expression quantitative trait locus for *GJA1* in sun-exposed skin tissue with *C* allele carriers exhibiting increased gene expression compared with the *GG* carriers. In the GERA-1 black cohort, the *C* allele was associated with greater SBP response (effect size=−3.1 mm Hg and $P=3.5\times10^{-3}$). In addition, *rs10499113* is in high LD with another expression quantitative trait locus for *GJA1*, *rs2104334* (not present in the HapMap reference). According to the ChiP-Seq experiments from ENCODE Project Consortium 2011, in HUVEC cells, rs2104334 maps within a probable peak of binding of GATA2 transcription factor, 35 base pairs upstream the GATA2 consensus binding motif. Moreover, in aorta, *rs2104334* colocalizes with H3K4me3 histone modifications that mark active promoters in chromatin regions and with H3K4me1 and H3K27ac histone marks associated with enhancers. SNP *rs11750990*, which we identified to be associated with SBP response in the GWAS meta-analysis of whites, colocalizes with H3K4me1 and H3K27ac.[37]

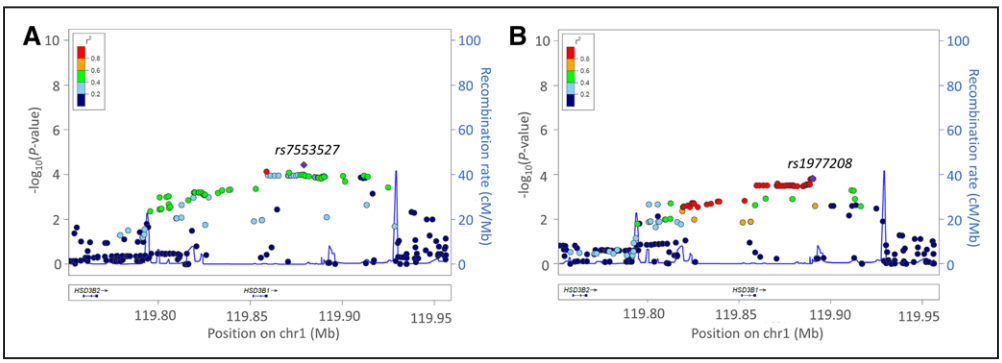

Figure 4. Local regional plot for HSD3B1 gene. **A**, systolic blood pressure and (**B**) diastolic blood pressure meta-analysis results for white samples. Each dot signifies a SNP. Genomic coordinates are displayed along the *x* axis with the negative logarithm of the association *P* value displayed on the *y* axis. SNPs are colored based on their r^2 with the top-signal SNP that has the lowest *P* value in the region.

The 5′-flanking region of *FOXA1* was associated with DBP response to hydrochlorothiazide in the GWAS meta-analysis and nominally validated with different SNPs in the PEAR-1 black cohort. According to the HaploReg annotation,[36] *rs177852*, identified to be associated with hydrochlorothiazide response in PEAR-1 black cohorts, is related to *FOXA1* expression in the brain cortex.[38] *FOXA1* is also expressed in the collecting duct of the kidney.[39,40] Its putative binding sites were found in the promoters of several genes expressed in the urothelium of the renal pelvis, including genes encoding the vasopressin receptor, several subunits of the Na/K ATPase and E-cadherin.[39–41] *Foxa1* has also been identified as a vasopressin-induced gene in a differentiated mouse clonal cortical collecting duct cell line.[42] Furthermore, *Foxa1*-deficient mice develop nephrogenic diabetes insipidus with a defect in renal water reabsorption.[40]

For all of the above-mentioned variants, we observed a greater effect size in blacks compared with whites. This could be related to the greater antihypertensive efficacy of hydrochlorothiazide in blacks because of their greater volume expansion, salt sensitivity, and lower renin activity, compared with white hypertensives.[43]

Our gene-based meta-analysis provided an interesting, biologically plausible, and statistically significant signal that would have remained indistinguishable from random noise with the traditional single-SNP GWAS approach. Gene-based tests can highlight regions that display substantial allelic heterogeneity, defined as the presence of multiple alleles that act through 1 gene to influence a trait. Furthermore, gene-based tests can increase statistical power by combining single variants from GWAS into a gene-based score, which substantially reduces the burden of multiple testing.[29]

With this approach, we identified *HSD3B1*. *HSD3B1* is expressed as 3β-hydroxysteroid dehydrogenase with a crucial role in the biosynthesis of hormonal steroids, including aldosterone.[44] *HSD3B1*, markedly overexpressed in the hypothalamus of Milan hypertensive rats, is involved in endogenous ouabain synthesis in an adrenal medullary–derived cell line (PC12).[45] Hypertensive patients have elevated circulating endogenous ouabain levels, which are positively correlated with higher BP, higher plasma Na concentrations, and increased proximal tubular reabsorption.[46,47] Endogenous ouabain is also higher in patients with kidney failure,[48] myocardial infarction,[49] and congestive heart failure.[50] Multiple studies have described the association of genetic variants in *HSD3B1* with hypertension or BP variation. The *CC* genotype at *rs6203* was associated with hypertension[51] and higher BP.[51–54] This association was reported as stronger in males as also confirmed by our single-GWAS data (Table S4). In addition, *rs3765945* and *rs1047303* have been significantly associated with SBP.[53] The *T-C* haplotype, established by *rs3088283–rs1047303*, correlated with significantly higher level of aldosterone and BP,[54] and the *G-C-C* haplotype of *rs2236780–rs3765945–rs6203* was related to left ventricular diastolic function.[55]

In conclusion, the following can be gathered from our study: (1) this is the largest genome-wide meta-analysis of BP response to hydrochlorothiazide conducted to date, (2) although ours is the largest hydrochlorothiazide meta-analysis conducted to date, our sample size still lacked sufficient power, (3) using the single-SNP approach with validation in blacks, we identified 2 suggestive regions linked to the regulation of *GJA1* and *FOXA1*, and (4) the gene-based approach, never applied before to pharmacogenomics of antihypertensive drugs to our knowledge, highlights *HSD3B1* as a susceptibility gene of BP response to hydrochlorothiazide. This gene was not identified in the single-SNP analysis because of high allelic heterogeneity.

These data pave the way for future research on new pathways and drug targets in hypertension toward better-personalized therapeutic approaches.

Perspectives

Hypertension is a major risk factor for global disease burden and is also the most common chronic disease for which medications are prescribed. Pharmacogenomics may represent a useful tool in the future to select antihypertensive therapy with the greatest efficacy, based on individual's genetic profile. This study performed the largest pharmacogenomic genome-wide meta-analysis of BP response to hydrochlorothiazide in hypertensive cohorts from the International Consortium for Antihypertensive Pharmacogenomics Studies applying both SNP-based and gene-based approaches. Three new biologically plausible loci linked to hypertension and BP regulation were identified as markers of BP response to hydrochlorothiazide. Further investigations of the associated regions may enhance the current understanding of personalized antihypertensive treatment.

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Disclosures

None.

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Novelty and Significance

What Is New?

- This is the largest pharmacogenomics study of blood pressure (BP) response to hydrochlorothiazide.
- The genome-wide association studies SNP-based approach identified 2 novel loci of BP response to hydrochlorothiazide linked to the regulation of *GJA1* and *FOXA1*.
- The gene-based approach, never before applied to pharmacogenomics of antihypertensive drugs, highlighted *HSD3B1* as new marker of BP response to hydrochlorothiazide.

What Is Relevant?

- The identified variants can be considered new biologically plausible loci associated with hypertension and BP regulation.

Summary

By amassing all the available pharmacogenomic studies of BP response to hydrochlorothiazide, and using 2 different analysis approaches, we identified 3 novel loci influencing BP response to hydrochlorothiazide. These data open the way for future research on new pathways and drug targets in hypertension toward better-personalized therapeutic approaches.