ORIGINAL RESEARCH

Pharmacogenomics of Hypertension: A Genome-Wide, Placebo-Controlled Cross-Over Study, Using Four Classes of Antihypertensive Drugs

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***Background*—**Identification of genetic markers of antihypertensive drug responses could assist in individualization of hypertension treatment.

***Methods and Results*—**We conducted a genome-wide association study to identify gene loci influencing the responsiveness of 228 male patients to 4 classes of antihypertensive drugs. The Genetics of Drug Responsiveness in Essential Hypertension (GENRES) study is a double-blind, placebo-controlled cross-over study where each subject received amlodipine, bisoprolol, hydrochlorothiazide, and losartan, each as a monotherapy, in a randomized order. Replication analyses were performed in 4 studies with patients of European ancestry (PEAR Study, N=386; GERA I and II Studies, N=196 and N=198; SOPHIA Study, N=372). We identified 3 single-nucleotide polymorphisms within the *ACY3* gene that showed associations with bisoprolol response reaching genome-wide significance ($P<5\times10^{-8}$); however, this could not be replicated in the PEAR Study using atenolol. In addition, 39 single-nucleotide polymorphisms showed *P* values of 10^{-5} to 10^{-7}. The 20 top-associated single-nucleotide polymorphisms were different for each antihypertensive drug. None of these top single-nucleotide polymorphisms co-localized with the panel of >40 genes identified in genome-wide association studies of hypertension. Replication analyses of GENRES results provided suggestive evidence for a missense variant (rs3814995) in the *NPHS1* (nephrin) gene influencing losartan response, and for 2 variants influencing hydrochlorothiazide response, located within or close to the *ALDH1A3* (rs3825926) and *CLIC5* (rs321329) genes.

***Conclusions*—**These data provide some evidence for a link between biology of the glomerular protein nephrin and antihypertensive action of angiotensin receptor antagonists and encourage additional studies on aldehyde dehydrogenase–mediated reactions in antihypertensive drug action. **(*J Am Heart Assoc*. 2015;4:e001521 doi: 10.1161/JAHA.114.001521)**

Key Words: antihypertensive drug • association study • drug response • genome-wide • hypertension

By 2010, elevated blood pressure (BP) became the leading risk factor of disease burden on a global basis.[1] The insidious nature of hypertension is substantiated by its increasing prevalence, its mostly asymptomatic nature, and its poor drug control. Indeed, globally more than 1 billion people suffer from hypertension, but only 40% to 50% of those on therapy reach the targets of treatment.[2,3] This is most unfortunate since even a small

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Accompanying Tables S1 through S3 are available at http://jaha.ahajournals.org/content/4/1/e001521/suppl/DC1

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lowering of elevated BP results in significant reduction of cardiovascular events.

Family, twin, and adoption studies have suggested that heritability accounts for 30% to 50% of interindividual variation of BP.[4–6] Recent extensive genome-wide association studies have revealed >40 genetic loci associated with essential hypertension,[4–6] but even combined they account for only 2% of variability of BP, and no variant is of proven clinical value in guiding antihypertensive drug treatment. Until 2009, pharmacogenomic studies of hypertension suffered from narrow selection of candidate genes, small sample sizes, and weaknesses in study design.[7] In subsequent studies, suggestive but inconsistent associations were reported when panels of gene variants selected by data from the hypertension genome-wide association studies were screened in the Genetics of Drug Responsiveness in Essential Hypertension (GENRES)[8] and Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR)[9] cohorts. Recently, the first applications of genome-wide association studies principles in pharmacogenomics of hypertension have taken place,[10–12] but there is still a need for controlled randomized studies with adequate replication of data using results from other laboratories.

The GENRES study is a randomized, double-blind, crossover, placebo-controlled study of 228 hypertensive men who received 4 different classes of antihypertensive drugs (a diuretic, β-blocker, calcium-channel blocker, and angiotensin receptor antagonist), each as a monotherapy, in a rotational manner.[13] Both office (OBP) and 24-hour ambulatory (ABP) blood pressure responses were measured. We here report genome-wide analysis of association of 0.7M single-nucleotide polymorphisms (SNPs) to the different antihypertensive drug responses, and also provide results from attempts to replicate findings using meta-analysis data from the PEAR,[14] Genetic Epidemiology of Responses to Antihypertensives (GERA) I,[15] GERA II,[10] and Study of the Pharmacogenomics in Italian hypertensive patients treated with the Angiotensin receptor blocker losartan (SOPHIA)[16] studies.

Methods

Study Participants

The design of the GENRES Study with initial clinical and biochemical data has been described previously.[13,17] In brief, a total of 313 moderately hypertensive Finnish men (aged 35 to 60 years) were initially screened.[13] Inclusion criteria were diastolic BP ≥95 mm Hg in repeated measurements or use of antihypertensive medication. Exclusion criteria were use of 3 or more antihypertensive drugs, secondary hypertension, or significant comorbidity. There was no evident heart, cerebrovascular, liver, pulmonary, or kidney disease, and no patient had drug-treated diabetes. No participant had signs of abuse of alcohol or drugs. Each study participant received losartan 50 mg, bisoprolol 5 mg, hydrochlorothiazide 25 mg, and amlodipine 5 mg daily, each as a monotherapy in randomized order for 4 weeks. The study started with a 4-week run-in placebo period, and all 4 drug treatment periods were separated by 4-week placebo periods. Twenty-four-hour ABP readings were recorded at the end of each treatment period with a device equipped with a QRS complex detector and a position sensor (Diasys Integra; Novacor, Rueil-Malmaison, France); in addition, OBP measurements were carried out with repeated measurements after a 30-minute rest in the sitting position using a semiautomatic oscillometric device. In this study, ABP responses to the 4 monotherapies were analyzed. The 228 subjects who were successfully genotyped and had ABP response data from at least 1 drug treatment period are included in this study. Of these subjects, 212, 204, and 177 had ABP response data from 2, 3, and 4 drug treatments, respectively.

The clinical part of the GENRES study was conducted in accordance with the Declaration of Helsinki and Guidelines for Good Clinical Practice (1996) at Helsinki University Central Hospital between years 1999 and 2004. The study was approved by the Ethical Committee of Helsinki University Central Hospital and the National Agency for Medicines of Finland. All subjects gave signed informed consent before any study-related activities.

PEAR is a study of mild–to–moderate hypertensives, with diastolic OBP >90 mm Hg (and ≤110 mm Hg) and diastolic home BP >85 mm Hg. The details of the study design have been described previously.[14] In brief, the patients had no history of cardiovascular disease or diabetes and were between the ages of 17 and 65 years. After an average washout period of 28 days, they had baseline data collected, which included measurement of home, office, and 24-hour ABP, along with collection of biological samples. They were then randomized to atenolol 50 mg daily or hydrochlorothiazide 12.5 mg daily. Following 3 weeks on this dose, those with BP >120/70 mm Hg had the dose doubled, followed by an additional 6 weeks of treatment, after which BP data were collected along with biological samples. The 24-hour ABP data were used for this analysis. Only subjects of European ancestry were included in the present study.

For each of the GERA I and II studies, 300 whites (in Rochester, MN) and 300 African Americans (in Atlanta, GA) were enrolled.[18,19] The participants had uncomplicated primary hypertension, stage 1 to 2, and were 30 to 59.9 years of age. They were instructed to discontinue previous antihypertensive medications for ≥4 weeks. Once stable elevation of the BP was achieved (diastolic OBP ≥90 mm Hg), the study drug was administered orally: hydrochlorothiazide 25 mg daily for 4 weeks or candesartan 16 mg daily for 2 weeks followed by 32 mg daily for an 4 additional weeks. At the end of the

drug-free and drug-treatment periods, 3 readings of BP were made by a trained assistant after the participant had been seated quietly for at least 5 minutes. The difference between averages of the second and third diastolic BP readings taken before and at the end of drug treatment was calculated as the BP response. Only subjects of European ancestry were included in the present study.

The SOPHIA Study is a study of mild-to-moderate, asymptomatic, never-treated hypertensive patients (85% of participants), or patients out of treatment for at least 6 months (15% of participants).[16] At the screening visit (week -8), participants ranging 18 to 59 years of age had to display systolic OBP from 140 to 179 mm Hg and diastolic OBP from 90 to 109 mm Hg. At each visit, OBP was measured 3 times, using a certified electronic device, with the subject in the sitting position after 5 minutes' rest. During a run-in period of 8 weeks, the participants followed a diet program that provided 100 to 140 mEq of sodium and 50 to 70 mEq of potassium daily to minimize the lifestyle differences. At the end of this period, 50 mg/day of losartan as open-label was prescribed for 4 weeks.

Genotyping Methods

The DNA samples of 228 GENRES study subjects were successfully genotyped (success rates >99%) at the Institute for Molecular Medicine Technology Centre, University of Helsinki using the Illumina HumanOmniExpress-12 BeadChip (Illumina, Inc, San Diego, CA). The genotypes (NCBI build 37, hg19) were called and quality controlled using GenomeStudio v. 2011.1 software (Illumina, Inc) and in-house-developed database tools. Further quality steps, including identity-by-state clustering and gender check, were performed using Plink software and PLINK v1.07 toolset.[20] Of the total of 709 357 genotyped autosomal SNPs, 707 658 passed these quality-control steps. After this, SNPs with Hardy-Weinberg equilibrium P value $<1\times10^{-5}$ (393 SNPs) and minor allele frequency <0.01 (75 421 SNPs) were excluded, which resulted in 631 844 autosomal SNPs that were used for analysis.

DNA samples from PEAR were genotyped using Illumina Human Omni1-Quad BeadChip (Illumina) as previously described.[11] DNA samples from GERA I and II study participants were genotyped using the Affymetrix GeneChip Human Mapping 6.0 Array Sets.[10,11] SOPHIA samples were genotyped using the Illumina Human1M-Duo array (Illumina) and imputed using MACH software and the HapMap CEU haplotypes (release 22) as reference.

Statistical Analyses

As the first step of the present study, systolic (ASBP) and diastolic (ADBP) 24-hour ambulatory blood pressure responses to each of the 4 monotherapies were analyzed separately in the GENRES Study (discovery sample). The BP response was calculated as BP after 4 weeks' drug treatment minus mean BP after placebo periods. The mean BP level of all (up to 4) placebo periods, as opposed to the 1 preceding placebo period, was used as the baseline level to reduce BP variation. The approach was supported by several analyses. First, the BP responses to study drugs showed clearly lower variation when mean of all placebo periods was used as the baseline level (Table S1). Second, compared with the other drugs, amlodipine seemed to have a small carry-over effect ($\approx-1.5/-0.5$ mm Hg) based on placebo BP levels 4 weeks after amlodipine treatment (Table S2). The randomized cross-over design and the use of all placebo periods as the baseline level eliminates any systematic effect of this finding on the results. In addition, the effect was probably even smaller after an additional 4 weeks when BP response to the next study drug was assessed. Third, the higher variation of placebo BP levels when only 1 placebo period was used can be seen in Table S2 as higher SDs. Finally, the preceding study treatment and the order of the drug treatment periods had no effect on BP response to any of the study drug when they were tested with regression analysis (GLM Univariate procedure of IBM SPSS Statistics program, version 19).

For the genome-wide analyses, ASBP and ADBP response residuals were generated using IBM SPSS Statistics program and stepwise linear regression. The following covariates were tested with $P<0.10$ as an inclusion condition: corresponding

Table 1. Characteristics of the Subjects From the Genetics of Drug Responsiveness in Essential Hypertension (GENRES) Study

Number of subjects	228
Age, y	50.6 (6.4)
Body mass index, kg/m^2	26.7 (2.7)
ABP during placebo periods (systolic/diastolic, mm Hg)	135 (10)/93 (6)
OBP during placebo periods (systolic/diastolic, mm Hg)	152 (13)/100 (7)
ABP responses (systolic/diastolic, mm Hg)	
Amlodipine (N=205)	−7.4 (7.2)/−4.9 (4.0)
Bisoprolol (N=207)	−11.1 (6.2)/−8.3 (4.2)
Hydrochlorothiazide (N=206)	−4.8 (6.3)/−1.7 (4.1)
Losartan (N=203)	−9.1 (6.7)/−6.1 (4.7)
dU-sodium, mmol/24 h	173 (70)
Fasting serum glucose, mmol/L	5.4 (0.6)
Serum creatinine, mmol/L	86 (13)

Subjects with genome-wide genetic data and ambulatory blood pressure recordings after at least 1 antihypertensive monotherapy are included. All subjects were males. Data are presented as mean (SD). ABP indicates 24-h ambulatory blood pressure; dU, daily urinary excretion of; OBP, office blood pressure.

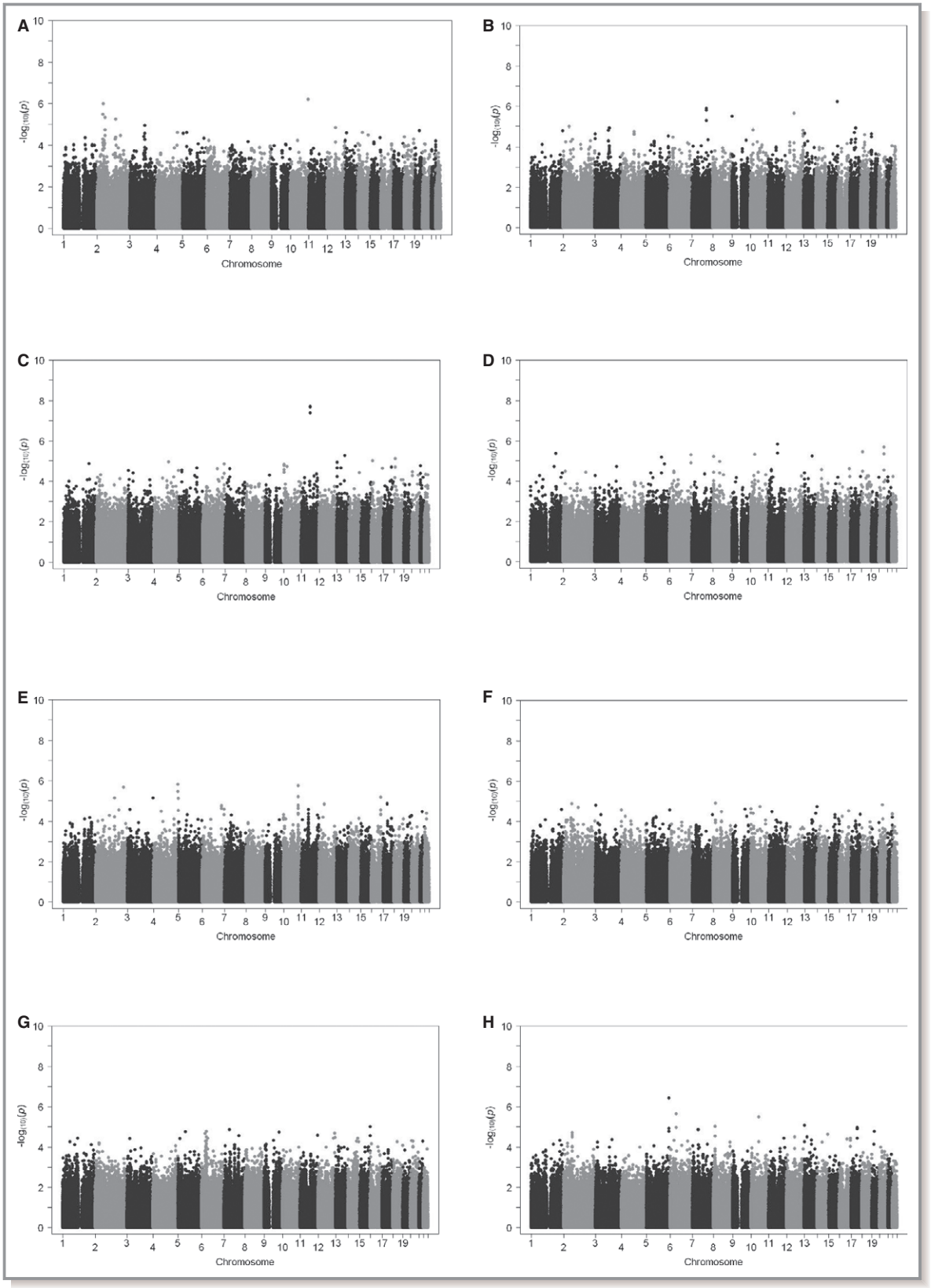

Figure 1. Manhattan plots from the genome-wide association analysis of the ambulatory blood pressure responses using an additive model. (A) Losartan, systolic; (B) losartan, diastolic; (C) bisoprolol, systolic; (D) bisoprolol, diastolic; (E) amlodipine, systolic; (F) amlodipine, diastolic; (G) hydrochlorothiazide, systolic; (H) hydrochlorothiazide, diastolic.

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Table 2. Single-Nucleotide Polymorphisms Associated With 24-H Ambulatory Blood Pressure Response to Bisoprolol in the Genetics of Drug Responsiveness in Essential Hypertension (GENRES) Study

P Value Rank	SNP	Chr	Position (Build 37)	N	CA/NCA	CAF	SBP/DBP	BP Response			Other SNPs From the Same Locus With Low *P* Values (r^2 Value With the Listed SNP)
								β	SE	*P* Value	
1	rs2514036	11	67 415 054	207	G/A	0.11	SBP	−5.4	±0.9	2.0E-8	rs948445 (r^2=1.00), rs2514037 (r^2=0.61)
							DBP	−3.1	±0.6	1.4E-6	
2	rs7268800	20	38 580 738	207	G/A	0.42	SBP	−2.1	±0.6	5.2E-4	rs6071822 (r^2=0.52), rs211841 (r^2=0.37), rs211840 (r^2=0.37), rs7261610 (r^2=0.61), rs11699371 (r^2=0.58), rs16988591 (r^2=0.68), rs11699530 (r^2=1.00), rs958523 (r^2=0.94), rs6124201 (r^2=0.68)
							DBP	−1.9	±0.4	2.1E-6*	
3	rs12967284	18	12 532 098	207	A/G	0.25	SBP	2.9	±0.6	7.8E-6	rs11663391 (r^2=0.95)
							DBP	2.0	±0.4	3.6E-6*	
4	rs4357510	1	192 612 394	207	A/C	0.06	SBP	−5.4	±1.2	1.4E-5	
							DBP	−3.8	±0.8	4.2E-6*	
5	rs2506143	10	33 468 169	207	G/A	0.09	SBP	3.4	±1.0	4.7E-4	rs2506140 (r^2=1.0), rs2506144 (r^2=1.0)
							DBP	3.0	±0.6	4.7E-6*	
6	rs2029870	6	164 888 366	204	A/G	0.14	SBP	−3.4	±0.8	4.3E-5	rs10945994 (r^2=0.83)
							DBP	−2.6	±0.5	5.0E-6*	
7	rs7984003	13	82 866 573	206	A/G	0.25	SBP	3.1	±0.7	5.4E-6*	rs7984202 (r^2=0.42), rs9531328 (r^2=0.34), rs7993722 (r^2=0.34)
							DBP	2.1	±0.5	5.8E-6	
8	rs11777699	8	6 714 699	206	G/A	0.50	SBP	−1.8	±0.6	1.4E-3	
							DBP	−1.7	±0.4	5.9E-6*	
9	rs10519585	5	118 676 529	207	G/A	0.31	SBP	−2.5	±0.6	5.5E-5	
							DBP	−1.9	±0.4	6.6E-6*	
10	rs6501061	16	8 092 171	207	G/A	0.09	SBP	4.7	±1.0	9.9E-6*	
							DBP	2.3	±0.7	1.5E-3	
11	rs16918900	8	54 147 242	207	A/G	0.01	SBP	−8.4	±2.4	6.9E-4	
							DBP	−7.3	±1.6	1.1E-5*	
12	rs2194860	4	109 948 511	207	A/G	0.19	SBP	3.2	±0.7	1.1E-5*	
							DBP	1.9	±0.5	1.7E-4	
13	rs2765115	13	24 529 399	207	G/A	0.16	SBP	−3.7	±0.8	1.2E-5*	rs6490847 (r^2=0.89), rs2147990 (r^2=0.49), rs2765089 (r^2=0.33),
							DBP	−2.3	±0.6	8.1E-5	rs2492084 (r^2=0.47), rs9510968 (r^2=0.32), rs2765114 (r^2=0.59), rs2765119 (r^2=0.37)
14	rs17642669	5	140 628 277	207	G/A	0.06	SBP	−3.9	±1.3	2.1E-3	rs17629216 (r^2=1.0)
							DBP	−3.7	±0.8	1.4E-5*	

Continued

Table 2. Continued

P Value Rank	SNP	Chr	Position (Build 37)	N	CA/NCA	CAF	SBP/DBP	BP Response			Other SNPs From the Same Locus With Low *P* Values (r^2 Value With the Listed SNP)
								β	SE	*P* Value	
15	rs7895038	10	2 432 266	207	G/A	0.41	SBP	−2.7	±0.6	1.5E-5*	rs826474 (r^2=0.47), rs826489 (r^2=0.52)
							DBP	−1.4	±0.4	1.1E-3	
16	rs150210	21	19 362 312	207	G/A	0.28	SBP	−2.7	±0.6	1.8E-5*	
							DBP	−1.5	±0.4	7.2E-4	
17	rs10910862	1	181 045 421	207	A/G	0.06	SBP	−5.1	±1.3	9.0E-5	
							DBP	−3.7	±0.8	1.9E-5*	
18	rs2148117	10	27 213 946	207	G/A	0.02	SBP	−9.1	±2.1	1.9E-5*	
							DBP	−5.8	±1.4	5.6E-5	
19	rs1150433	3	163 653 210	207	A/G	0.13	SBP	−3.6	±0.9	8.9E-5	
							DBP	−2.6	±0.6	2.0E-5*	
20	rs969981	17	63 818 989	206	A/G	0.16	SBP	−3.3	±0.7	2.0E-5*	
							DBP	−1.6	±0.5	2.2E-3	

Twenty loci with the lowest *P* values based on either systolic or diastolic response are listed. BP indicates blood pressure; CA, coded allele; CAF, coded allele frequency; Chr, chromosome; DBP, diastolic blood pressure; NCA, noncoded allele; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism.

*Lowest *P* value of each locus.

placebo ABP (mean of all periods), age, earlier use of antihypertensive medication (coded as 0/1), current smoking (coded as 0/1), body mass index, daily urinary sodium excretion after the first placebo period, and serum creatinine level after the first placebo period. For non-normally distributed covariates, normalized values were used. The covariates selected for calculation of BP response residuals (in mm Hg) for each study drug are listed in Table S3.

The genome-wide association analysis was done using covariate-adjusted BP responses and linear regression under an additive genetic model with program PLINK.[20] For each of the 4 study drugs, we report here the 20 genetic loci with the lowest *P* values based on either ASBP or ADBP responses. *P* values $<5\times10^{-8}$ were considered as significant at the genome-wide level.

In the second step, replication analyses of the 20 best loci associated with losartan, bisoprolol, and hydrochlorothiazide responses were carried out using the data from PEAR, GERA I, GERA II, and SOPHIA studies. In each case, the analyses were confined to study participants of European ancestry only, and age, gender, baseline BP, and principal components to account for ancestry were used as covariates. Accordingly, we replicated losartan/GENRES data using losartan/SOPHIA and candesartan/GERA II data, bisoprolol/GENRES data using atenolol/PEAR data, and hydrochlorothiazide/GENRES data using both hydrochlorothiazide/PEAR and hydrochlorothiazide/GERA I data. Successful replication was defined as *P* values below the Bonferroni-corrected level of 2.5×10^{-4} (number of individual tests: 99 SNPs in 5 replication study analyses×SBP+DBP responses=198) and the same direction of effect. Suggestive replication was defined as *P* values <0.05 and the same direction of effect.

As the third step, meta-analyses of the top 20 GENRES 24-hour ambulatory blood pressure response SNPs in all available studies were performed using inverse-variance model with fixed effects in METAL.[21] We defined significant results as *P* values $<5\times10^{-8}$. In addition, *P* values $<1\times10^{-5}$ were considered to represent a suggestive association.

Results

Study Subjects

Principal demographic and clinical data from the subjects of the GENRES Study are summarized in Table 1. The patients were all males, had a slightly elevated body mass index (none had body mass index >32 kg/m^2) and a moderate hypertension (mean OBP 152/100 mm Hg). No patient had drug-treated diabetes or reduced kidney glomerular filtration rate. The BP reductions, as assessed by the ABP measurements, ranged from 4.8/1.7 mm Hg (hydrochlorothiazide) to 11.1/8.3 mm Hg (bisoprolol). Note that the study design used fixed

Table 3. Single-Nucleotide Polymorphisms Associated With 24-H Ambulatory Blood Pressure Response to Losartan in the GENRES Study

P Value Rank	SNP	Chr	Position (Build 37)	N	CA/NCA	CAF	SBP/DBP	BP Response			Other SNPs From the Same Locus With Low *P* Values (r^2 Value With the Listed SNP)
								β	SE	*P* Value	
1	rs1370555	15	97 049 985	203	G/A	0.40	SBP	−2.6	±0.6	4.4E-5	
							DBP	−2.1	±0.4	6.2E-7*	
2	rs7086428	10	130 778 452	203	A/G	0.37	SBP	3.3	±0.6	6.5E-7*	
							DBP	1.7	±0.5	2.8E-4	
3	rs4953045	2	44 268 800	203	G/A	0.25	SBP	−3.5	±0.7	1.1E-6*	rs17424646 (r^2=0.28), rs4131366 (r^2=0.32), rs17496908(r^2=0.59)
							DBP	−2.2	±0.5	1.0E-5	
4	rs711513	7	109 368 769	200	G/A	0.36	SBP	−2.4	±0.7	5.6E-4	rs2841921 (r^2=0.95), rs10953645 (r^2=0.39)
							DBP	−2.3	±0.5	1.3E-6*	
5	rs12814605	12	63 438 145	203	A/G	0.15	SBP	−4.0	±0.9	1.4E-5	rs1249935 (r^2=0.33), rs12813083 (r^2=0.30), rs699615 (r^2=0.36)
							DBP	−3.0	±0.6	2.3E-6*	rs699618 (r^2=0.37), rs12823849 (r^2=0.44), rs12815621 (r^2=1.00)
6	rs2279989	9	1 041 524	203	A/G	0.10	SBP	−4.0	±1.0	1.6E-4	
							DBP	−3.3	±0.7	3.3E-6*	
7	rs7597606	2	41 836 313	202	A/G	0.40	SBP	2.9	±0.6	3.3E-6*	
							DBP	1.4	±0.4	8.5E-4	
8	rs1559557	2	57 332 673	203	G/A	0.32	SBP	3.3	±0.7	4.8E-6*	rs1424642 (r^2=0.70), rs17048681 (r^2=0.74)
							DBP	1.9	±0.5	2.1E-4	
9	rs1432232	2	137 799 664	203	A/C	0.18	SBP	3.8	±0.8	5.8E-6*	rs1347033 (r^2=0.79)
							DBP	2.2	±0.6	1.2E-4	
10	rs1993802	3	110 595 402	203	G/A	0.17	SBP	−3.9	±0.9	1.1E-5*	rs7637068 (r^2=0.94), rs1462795 (r^2=0.94), rs4450855 (r^2=0.94)
							DBP	−2.6	±0.6	1.2E-5	rs1477841 (r^2=0.94)
11	rs12602832	17	42 396 896	202	A/G	0.14	SBP	−2.8	±0.9	1.6E-3	
							DBP	−2.7	±0.6	1.2E-5*	
12	rs2038912	10	20 207 349	203	A/G	0.49	SBP	2.0	±0.6	1.7E-3	
							DBP	1.8	±0.4	1.4E-5*	
13	rs4759885	12	131 736 813	203	G/A	0.46	SBP	−2.7	±0.7	7.3E-5	rs4387437 (r^2=0.99), rs7488647 (r^2=0.99), rs12578896 (r^2=0.50)
							DBP	−2.0	±0.5	1.5E-5*	
14	rs771574	3	101 620 682	203	G/A	0.24	SBP	−3.2	±0.8	3.9E-5	
							DBP	−2.3	±0.5	1.6E-5*	
15	rs10754459	1	245 785 508	203	G/A	0.30	SBP	−2.3	±0.7	1.3E-3	
							DBP	−2.1	±0.5	1.6E-5*	

Continued

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Table 3. Continued

P Value Rank	SNP	Chr	Position (Build 37)	N	CA/NCA	CAF	SBP/DBP	BP Response			Other SNPs From the Same Locus With Low *P* Values (r^2 Value With the Listed SNP)
								β	SE	*P* Value	
16	rs1392874	4	101 466 820	203	A/C	0.45	SBP	−1.8	±0.6	4.3E-3	rs11725047 (r^2=0.09), rs4699824 (r^2=0.17)
							DBP	−1.9	±0.4	1.7E-5*	
17	rs1357365	17	34 436 532	203	A/G	0.22	SBP	2.6	±0.7	5.2E-4	
							DBP	2.2	±0.5	2.0E-5*	
18	rs3814995	19	36 342 212	202	A/G	0.35	SBP	−2.8	±0.7	2.0E-5*	
							DBP	−1.6	±0.5	5.1E-4	
19	rs11841583	13	31 495 179	203	A/G	0.10	SBP	−4.5	±1.0	2.6E-5	
							DBP	−3.1	±0.7	2.1E-5*	
20	rs17271855	19	5 524 721	203	A/G	0.18	SBP	−2.9	±0.8	5.0E-4	
							DBP	−2.4	±0.6	2.2E-5*	

Twenty loci with the lowest *P* values based on either systolic or diastolic response are listed. BP indicates blood pressure; CA, coded allele; CAF, coded allele frequency; Chr, chromosome; DBP, diastolic blood pressure; NCA, noncoded allele; SBP, systolic blood pressure; SE, standard error of β; SNP, single-nucleotide polymorphism.
*Lowest *P* value of each locus.

doses of antihypertensive drugs, and no attempt was made to use equipotent doses.

First Step: Genome-Wide Association Analyses in GENRES

Manhattan plots providing genome-wide associations of ≈630 000 SNPs with the antihypertensive responses to losartan, bisoprolol, amlodipine, and hydrochlorothiazide are illustrated in Figure 1. Three SNPs on chromosome 11 (rs2514036, rs948445, and rs2514037) provided evidence for association reaching genome-wide significance for ASBP response to bisoprolol (Figure 1C and Table 2). These 3 SNPs map within the coding and regulatory regions of *ACY3*, coding for aminoacylase III. Altogether, 42 SNPs in 31 distinct regions were identified having at least 1 SNP associated with the treatment response at $P \leq 1 \times 10^{-5}$ (Figure 1 and Tables 2 through 5).

The quantile-quantile plots (Figure 2) show little evidence for genomic inflation and provide some support for the existence of significant associations with genomic loci influencing responsiveness to losartan and bisoprolol.

The strongest associations for the 24-hour ABP responses to the 4 different drug responses are listed in Tables 2 through 5. In each case, 20 loci with the lowest *P* values for either ASBP or ADBP responses are indicated. For each locus listed, there was a remarkable congruence between the direction and relative extent of the systolic and diastolic BP lowering. None of the top 20 loci were shared between 2 or more drugs.

Second Step: Replication Analyses Using Individual Pharmacogenomics Studies

We carried out replication analyses of the top 20 significant associations with each antihypertensive response noted in the GENRES study, using data from 4 available studies: SOPHIA (losartan responses compared to those in GENRES), GERA II (candesartan responses, compared to losartan responses in GENRES), PEAR (atenolol responses, compared to bisoprolol responses in GENRES), and GERA I+PEAR (hydrochlorothiazide responses in all 3 studies). The participants of the replication studies are described in Table 6. Note that while the data from the GENRES and PEAR studies were based on ABP recordings, OBP measurements were used in SOPHIA, GERA I, and II. Composite results from these replication analyses are listed in Tables 7 through 9. Two SNPs analyzed in GENRES were not available in the replication material GERA II (losartan/candesartan responses, Table 7) and 3 SNPs were not available in GERA I (hydrochlorothiazide responses, Table 9). Two flanking SNPs were included in the replication analysis of the GERA I data (Table 9). Unfortunately, data on

Table 4. Single-Nucleotide Polymorphisms Associated With 24-H Ambulatory Blood Pressure Response to Amlodipine in the GENRES Study

P Value Rank	SNP	Chr	Position (Build 37)	N	CA/NCA	CAF	SBP/DBP	BP Response			Other SNPs From the Same Locus with Low *P* Values (r^2 Value With the Listed SNP)
								β	SE	*P* Value	
1	rs7687961	4	187 122 009	205	C/A	0.21	SBP	3.6	±0.7	1.6E-6*	rs3736456 (r^2=0.36)
							DBP	1.6	±0.4	1.5E-4	
2	rs602618	10	112 843 085	205	C/A	0.24	SBP	3.6	±0.7	1.8E-6*	rs4917589 (r^2=0.36), rs10787287 (r^2=0.55), rs10787292
							DBP	1.4	±0.4	1.5E-3	(r^2=0.66), rs521674 (r^2=0.92), rs7923122 (r^2=0.25)
3	rs17406681	2	212 403 387	205	A/G	0.20	SBP	3.4	±0.7	2.2E-6*	
							DBP	1.5	±0.4	2.4E-4	
4	rs12921986	16	72 312 727	205	G/A	0.03	SBP	7.4	±1.6	6.8E-6*	rs12933482 (r^2=0.64), rs17604662 (r^2=0.64)
							DBP	3.9	±0.9	3.0E-5	rs17606532 (r^2=0.78), rs34522712 (r^2=0.78)
5	rs354706	2	143 886 216	205	G/A	0.40	SBP	2.7	±0.6	7.3E-6*	
							DBP	1.2	±0.3	3.9E-4	
6	rs9814527	3	193 533 703	205	G/A	0.36	SBP	2.6	±0.6	7.4E-6*	
							DBP	0.9	±0.3	7.4E-3	
7	rs10021692	4	188 080 985	205	G/A	0.17	SBP	3.5	±0.8	7.6E-6*	rs882227 (r^2=0.97)
							DBP	1.6	±0.4	3.4E-4	
8	rs352796	8	15 613 270	205	G/A	0.02	SBP	−8.9	±2.3	1.2E-4	
							DBP	−5.6	±1.3	1.3E-5*	
9	rs11125895	2	62 069 358	205	G/A	0.19	SBP	2.5	±0.8	1.7E-3	
							DBP	1.9	±0.4	1.3E-5*	
10	rs220457	17	30 102 635	205	G/A	0.25	SBP	2.9	±0.6	1.4E-5*	
							DBP	1.4	±0.4	1.8E-4	
11	rs1947234	12	39 450 491	205	A/C	0.23	SBP	−3.0	±0.7	1.4E-5*	
							DBP	−1.3	±0.4	1.1E-3	
12	rs6083017	20	23 119 766	205	A/G	0.51	SBP	−1.7	±0.6	4.6E-3	
							DBP	−1.4	±0.3	1.5E-5*	
13	rs1504058	3	1 090 602	204	G/A	0.43	SBP	1.7	±0.6	4.2E-3	
							DBP	1.4	±0.3	1.6E-5*	
14	rs526847	6	148 236 385	204	G/A	0.39	SBP	−2.7	±0.6	1.8E-5*	rs498291 (r^2=0.99)
							DBP	−1.0	±0.4	3.8E-3	
15	rs9577581	13	114 252 002	205	A/G	0.38	SBP	2.2	±0.6	8.0E-4	
							DBP	1.6	±0.4	1.8E-5*	

Continued

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Table 4. Continued

P Value Rank	SNP	Chr	Position (Build 37)	N	CA/NCA	CAF	SBP/DBP	BP Response			Other SNPs From the Same Locus with Low *P* Values (r^2 Value With the Listed SNP)
								β	SE	*P* Value	
16	rs17238902	10	67 649 378	205	A/G	0.13	SBP	−3.2	±0.9	3.0E-4	
							DBP	−2.1	±0.5	1.9E-5*	
17	rs6706577	2	111 796 833	205	A/G	0.48	SBP	1.7	±0.6	4.1E-3	
							DBP	1.4	±0.3	2.0E-5*	
18	rs2018975	10	317 501	205	A/G	0.40	SBP	2.5	±0.6	4.7E-5	rs2379078 (r^2=0.81)
							DBP	1.5	±0.3	2.4E-5*	
19	rs10821312	9	96 944 581	205	G/A	0.20	SBP	2.2	±0.7	2.4E-3	rs7038156 (r^2=1.00)
							DBP	1.7	±0.4	2.4E-5*	
20	rs9364385	6	168 415 372	205	A/G	0.50	SBP	2.4	±0.6	2.4E-5*	rs1858675 (r^2=1.00), rs3807061 (r^2=0.96)
							DBP	0.9	±0.3	4.2E-3	

Twenty loci with the lowest *P* values based on either systolic or diastolic response are listed. BP indicates blood pressure; CA, coded allele; CAF, coded allele frequency; Chr, chromosome; DBP, diastolic blood pressure; NCA, noncoded allele; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism.
*Lowest *P* value of each locus.

responses to amlodipine could not be replicated in this collaborative study.

Of the 60 SNPs with the strongest associations to losartan, bisoprolol, or hydrochlorothiazide responses in GENRES (Tables 2, 3 and 5), no SNP reached the Bonferroni-corrected level of significance (2.5×10^{-4}). Only 1 SNP (rs3814995 on chromosome 19) emerged that gave a 2-sided *P* value <0.05 (suggestive significance level), with the same direction of BP effect, for both systolic and diastolic blood pressure responses to a particular drug in 2 other studies (Table 7). Accordingly, rs3814995 was associated with systolic ($P=2.0\times10^{-5}$) and diastolic ($P=5.1\times10^{-4}$) BP responses to losartan in GENRES, with systolic ($P=0.03$) and diastolic ($P=0.02$) BP responses in GERA II, and diastolic BP responses ($P=0.03$) in SOPHIA (Table 7); there was a trend toward association for systolic BP response in SOPHIA ($P=0.19$). The SNP rs3814995 corresponds to a Glu117Lys missense mutation in the *NPHS1* gene coding for the protein nephrin.

Third Step: Meta-Analyses Based on GENRES, PEAR, GERA I, GERA II, and SOPHIA Data

A meta-analysis employing inverse-variance model with fixed effects was carried out using SNP data from GENRES, GERA I, GERA II, PEAR, and SOPHIA studies (Table 10). *P* values $<1\times10^{-5}$ were considered to indicate a suggestive association; no SNP reached the genome-wide level of significance (5×10^{-8}).

Of the top 20 SNPs associated with losartan responses in the GENRES Study, rs4953045 on chromosome 2 was associated with BP response ($P=5.1\times10^{-7}$) and rs12814605 on chromosome 12 with diastolic BP response ($P=6.4\times10^{-6}$) in the meta-analysis utilizing responses to losartan in SOPHIA and candesartan in GERA II (Tables 10 and 11). The closest gene to the intergenic SNP rs4953045 is *LRPPRC*, which is located 46 kbp apart from it and codes for mitochondrial leucine-rich PPR motif-containing protein. rs12814605 is likewise an intergenic variant, located approximately 100 kb apart from the closest protein-coding genes *AVPR1A* (arginine vasopressin receptor 1A) and *PPM1H* (Mg^{2+}/Mn^{2+} dependent protein phosphatase 1H). However, the slightly higher *P* value for this SNP in the meta-analysis than in the discovery study (Table 10) renders the significance of this finding uncertain.

Next, a meta-analysis of the 20 SNPs with the highest association scores with bisoprolol responses in GENRES was performed in conjunction with the PEAR Study, using 24-hour ABP responses to atenolol for comparison (Tables 10 and 12). Two SNPs on chromosome 13, rs7984003 ($P=7.8\times10^{-7}$) and rs2765115 ($P=3.6\times10^{-6}$) showed suggestive evidence of association when systolic ABP responses of both

Table 5. Single-Nucleotide Polymorphisms Associated With 24-H Ambulatory Blood Pressure Response to Hydrochlorothiazide in the GENRES Study

P Value Rank	SNP	Chr	Position (Build 37)	N	CA/NCA	CAF	SBP/DBP	BP Response			Other SNPs From the Same Locus With Low *P* values (r^2 value With the Listed SNP)
								β	SE	*P* Value	
1	rs4867623	5	170 071 480	206	A/G	0.20	SBP	−2.2	±0.8	5.6E-3	rs9968589 (r^2=0.64), rs9968699 (r^2=0.78)
							DBP	−2.6	±0.5	3.7E-7*	rs4868010 (r^2=0.78), rs11747249 (r^2=0.78)
1	rs4868010	5	170 092 760	206	A/C	0.16	SBP	−2.1	±0.8	1.1E-2	(listed because of replication analysis in PEAR and
							DBP	−2.4	±0.5	1.7E-5	GERA)
2	rs321320	6	45 702 779	205	G/A	0.14	SBP	−3.3	±0.8	3.7E-5	rs321329 (r^2=0.24)
							DBP	−2.5	±0.5	2.3E-6*	
2	rs321329	6	45 722 988	206	A/G	0.41	SBP	−2.2	±0.6	1.2E-4	(Listed because of replication analysis in PEAR and
							DBP	−1.7	±0.4	1.1E-5	GERA), rs3846898 (r^2=0.57)
3	rs11006074	10	59 941 452	206	G/A	0.13	SBP	3.3	±0.9	1.5E-4	
							DBP	2.7	±0.6	3.2E-6*	
4	rs3117915	13	22 605 580	206	G/A	0.21	SBP	−2.0	±0.7	3.7E-3	
							DBP	−2.1	±0.5	8.6E-6*	
5	rs7821547	8	13 541 779	206	A/G	0.22	SBP	2.6	±0.7	1.9E-4	rs4831455 (r^2=0.91)
							DBP	2.0	±0.4	9.5E-6*	
6	rs3825926	15	101 445 441	206	A/G	0.03	SBP	7.3	±1.6	1.0E-5*	rs4646660 (r^2=0.62), rs4646672 (r^2=0.87)
							DBP	3.8	±1.1	6.6E-4	rs4246323 (r^2=0.87), rs4246326 (r^2=0.87) rs3803430 (r^2=0.87), rs4646683 (r^2=0.82) rs3803426 (r^2=1.00), rs1802603 (r^2=0.66)
7	rs2056531	17	47 283 058	206	A/G	0.11	SBP	3.1	±0.9	7.1E-4	rs4438351 (r^2=0.77), rs8077444 (r^2=0.96), rs11868965
							DBP	2.7	±0.6	1.1E-5*	(r^2=0.96), rs2293215 (r^2=1.00)
8	rs6977301	7	42 497 247	206	A/G	0.08	SBP	4.4	±1.0	1.4E-5*	
							DBP	2.9	±0.7	1.4E-5	
9	rs1553009	7	45 908 994	206	A/G	0.10	SBP	3.5	±0.9	1.7E-4	
							DBP	2.7	±0.6	1.4E-5*	
10	rs2776906	6	37 431 757	206	A/G	0.36	SBP	2.6	±0.6	1.7E-5*	rs10692 (r^2=1.00)
							DBP	1.3	±0.4	9.6E-4	
11	rs964132	19	19 243 175	206	A/G	0.04	SBP	5.3	±1.5	6.5E-4	
							DBP	4.4	±1.0	1.7E-5*	
12	rs158210	5	55 737 637	206	A/G	0.10	SBP	−4.1	±0.9	1.8E-5*	
							DBP	−2.5	±0.6	8.8E-5	
13	rs1324210	9	114 791 150	206	A/G	0.25	SBP	2.8	±0.6	1.9E-5*	
							DBP	1.6	±0.4	1.6E-4	
14	rs6546025	2	63 923 561	206	G/A	0.24	SBP	−1.8	±0.6	5.0E-3	rs10201844 (r^2=0.90), rs10187013 (r^2=0.89)
							DBP	−1.8	±0.4	2.0E-5*	rs6546038 (r^2=0.84)

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Table 5. Continued

P Value Rank	SNP	Chr	Position (Build 37)	N	CA/NCA	CAF	SBP/DBP	BP Response			Other SNPs From the Same Locus With Low *P* values (r^2 value With the Listed SNP)
								β	SE	*P* Value	
15	rs11059985	12	129 347 891	206	A/G	0.29	SBP	2.8	±0.6	2.1E-5*	
							DBP	1.0	±0.4	2.6E-2	
16	rs3799369	6	25 912 634	206	G/A	0.12	SBP	3.7	±0.8	2.1E-5*	
							DBP	1.7	±0.6	4.0E-3	
17	rs17099050	14	100 255 923	206	C/A	0.03	SBP	7.2	±1.7	5.4E-5	
							DBP	5.0	±1.2	2.3E-5*	
18	rs329668	11	133 795 219	205	A/G	0.13	SBP	3.5	±0.8	2.6E-5*	
							DBP	2.0	±0.6	5.8E-4	
19	rs776472	7	114 352 862	206	G/A	0.43	SBP	−2.3	±0.5	2.8E-5*	
							DBP	−1.5	±0.4	7.4E-5	
20	rs1922117	12	63 789 836	206	C/A	0.37	SBP	−2.1	±0.6	6.4E-4	
							DBP	−1.7	±0.4	3.1E-5*	

Twenty loci with the lowest *P* values based on either systolic or diastolic response are listed. BP indicates blood pressure; CA, coded allele; CAF, coded allele frequency; Chr, chromosome; DBP, diastolic blood pressure; NCA, noncoded allele; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism.
*Lowest *P* value of each locus.

studies were analyzed. Two pseudogenes (*PTMAP* and *GYG1P2)* but no obvious protein-coding gene candidates are located in the vicinity of rs7984003. Of the protein-coding genes, *SPATA13* coding for spermatogenesis-associated protein 13 lies closest (24 kbp) to rs2765115. A corresponding meta-analysis of DBP responses to bisoprolol revealed an association to rs7268800 ($P=8.6\times10^{-7}$), which is an intergenic polymorphism, with 2 long intergenic non-protein coding RNA species but no apparent candidate genes in its vicinity.

Finally, we carried out a similar meta-analysis with the hydrochlorothiazide response data, using 24-hour ABP responses in PEAR and OBP responses in GERA I for comparison (Tables 10 and 13). rs3825926 on chromosome 15 was found to associate with systolic BP responses ($P=5.6\times10^{-6}$; GERA I data lacking). This SNP is located in the intron of the *ALDH1A3* gene coding for aldehyde dehydrogenase 1 family member A3, and is 14 kbp apart from the *LRRK1* gene coding for leucine-rich repeat kinase 1. A corresponding meta-analysis of diastolic BP responses revealed 3 suggestive associations to 3 SNPs (Table 10). rs4867623 is an intronic variant of *KCNIP1* coding for potassium (Kv) channel interacting protein 1; however, a higher *P* value was obtained in the meta-analysis than in the discovery sample (Table 10). rs321329 and rs321320 are 2 adjacent intergenic variants on chromosome 6, lying ≈90 kbp of *RUNX2* encoding the runt-related transcription factor 2 and ≈145 kb from *CLIC5* encoding the chloride intracellular channel 5.

Discussion

The majority of the pharmacogenomic studies on essential hypertension carried out until now suffer from weaknesses in their design.[7] The GENRES Study represents a careful attempt to avoid some of the most important problems. Accordingly, this study is prospective, placebo-controlled, and rotational in nature, implying that every test subject received 4 different antihypertensive drugs as a 4 weeks′ monotherapy in a randomized order, with 4 intervening 4 weeks′ placebo periods. The use of the mean of 4 placebo periods increases the accuracy of the estimation of baseline BP levels. The performance of the GENRES study has been validated by a number of observations. For example, the within-subject resemblance of BP responses, as analyzed by pairwise correlation matrixes, was found to be highest for responses to bisoprolol and losartan (r=0.32 to 0.39), followed by responses to amlodipine and hydrochlorothiazide (r=0.20 to 0.35), as would be expected.[13] In addition, plasma renin activity was positively correlated with BP responses to losartan (*P* values 0.001 to 0.005) and bisoprolol (*P* values

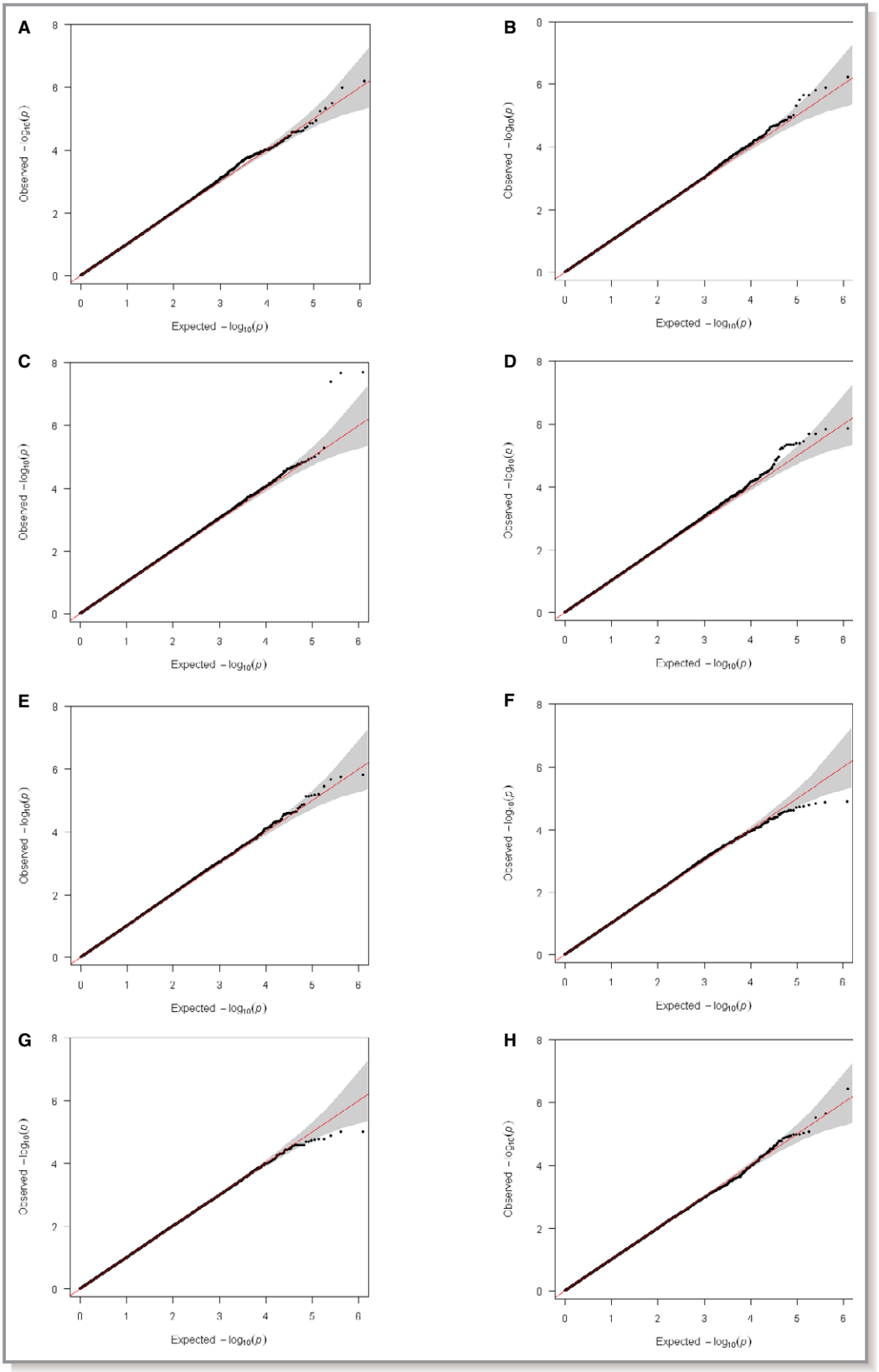

Figure 2. Quantile-quantile plots from the genome-wide association analysis of the ambulatory blood pressure responses using an additive model. Single-nucleotide polymorphisms with minor allele frequency <0.01 are excluded. (A) Losartan, systolic; (B) losartan, diastolic; (C) bisoprolol, systolic; (D) bisoprolol, diastolic; (E) amlodipine, systolic; (F) amlodipine, diastolic; (G) hydrochlorothiazide, systolic; (H) hydrochlorothiazide, diastolic.

Table 6. Description of Subjects in Replication Studies

	PEAR	PEAR	GERA I	GERA II	SOPHIA
Treatment	Atenolol	HCTZ	HCTZ	Candesartan	Losartan
Blood pressure measurement method	Ambulatory	Ambulatory	Office	Office	Office
Number of subjects in replication analyses	193	193	196	198	372
Women, N (%)	85 (44%)	80 (42%)	84 (43%)	98 (49%)	92 (25%)
Age, y	50.0±9.4	50.6±9.1	48.6±7.3	49.1±6.8	45.7±7.4
Body mass index, kg/m^2	30.1±5.4	30.2±4.9	31.3±5.6	29.9±3.9	26.9±2.9
Baseline systolic blood pressure, mm Hg	138±10	139±11	143±13	147±13	149±7
Baseline diastolic blood pressure, mm Hg	87±8	87±9	96±6	95±5	97±4
Blood pressure responses					
Atenolol, systolic response, mm Hg	−14.2 ±10.6				
Atenolol, diastolic response, mm Hg	−10.6±7.9				
HCTZ, systolic response, mm Hg		−8.4±10.1	−10.9±13.0		
HCTZ, diastolic response, mm Hg		−4.5±7.2	−6.3±8.8		
Candesartan, systolic response, mm Hg				−18.4±14.7	
Candesartan, diastolic response, mm Hg				−13.4±10.2	
Losartan, systolic response, mm Hg					−11.8±9.1
Losartan, systolic response, mm Hg					−8.8±6.2

Only participants of European ancestry are included. Data are presented as mean±SD. HCTZ, hydrochlorothiazide.

0.03 to 0.17), and negatively with BP responses to hydrochlorothiazide (*P* values 0.01 to 0.07).[17]

There are several important limitations in the present study. First, an obvious methodological limitation of the GENRES study is the sample size of 228 individuals, resulting in insufficient power to detect effect sizes of 0.5 to 1 mm Hg, characteristic of gene loci revealed in genome-wide association studies of complex diseases. For example, we calculated that in order to reach a power of 80% to detect an antihypertensive response in GENRES, an effect size of 4 mm Hg is needed for a SNP with a minor allele frequency of 0.30. It should be noted, however, that in carefully controlled pharmacogenetic studies, common polymorphisms with small effects on BP levels may well have larger effects on BP responsiveness. Second, the GENRES study included only males with mild-to-moderate hypertension. Third, since equipotent drug effects were not designed to be reached, less variability in responses to certain drugs may have affected our data. Fourth, it is to be emphasized that while ABP measurements were used in GENRES and PEAR, OBP measurements were carried out in SOPHIA, GERA I, and GERA II, which may have affected the meta-analysis data.

Even using the strictly controlled experimental conditions in GENRES, we failed to identify pharmacogenomic associations of genome-wide significance ($P<5\times10^{-8}$), with the exception of 3 SNPs (rs2514036, rs948445, and rs2514037) reaching this value for bisoprolol responses. In fact, we consider this lack of stronger associations as the most significant finding of our study, because it emphasizes the importance of even larger samples of patients in studies with similar strict design. Furthermore, it is of note that upon listing of 80 different SNPs (20 for each drug, Tables 2 through 5) showing the strongest associations to drug responses, we failed to identify any SNP common to more than a single drug class, supporting the notion that the genomic pathways regulating the BP-lowering mechanisms of different classes of antihypertensive drugs are specific to each class of drugs.

A meta-analysis using losartan response data from the GENRES and SOPHIA studies and candesartan data from the GERA II study revealed 2 gene loci of potential interest. rs4953035 showing association with systolic BP responses is located ≈46 kbp from *LRPPRC* coding for mitochondrial leucine-rich PPR motif-containing protein that is expressed in a variety of tissues, including the heart and kidney. There were also other SNPs within or close to *LRPPRC* that showed a significant association (Table 3). Another gene of note, *PPM1B* coding for Mg^{2+}/Mn^{2+} dependent protein phosphatase, lies 126 kb from rs4953035. This gene may be involved in the cell cycle and is richly expressed (eg, in the heart). Recent data indicate that the phosphatase coded by *PPB1B* selectively modulates PPAR activity.[22]

In addition, based on ranking of strengths of associations of the various drug responses in GENRES and available

Table 7. Replication Analysis of the Best GENRES Losartan 24-H Ambulatory Blood Pressure Response SNPs With Office Blood Pressure Responses to Candesartan in GERA II and to Losartan in SOPHIA

				Replication Results From GERA II (N=198)							Replication Results From SOPHIA (N=372)						
Rank in GENRES	SNP	Chr	Position (Build 37)	CA/NCA	CAF	r^2 imp.	SBP/DBP	β	*P* Value	Direction of Replication	CA/NCA	CAF	r^2 imp.	SBP/DBP	β	*P* Value	Direction of Replication
1	rs1370555	15	97 049 985	C/T	0.51	1.00	SBP	−0.3	0.84	Same	T/C	0.42	—	SBP	0.2	0.76	Same
							DBP	0.2	0.82	Opposite				DBP	0.2	0.60	Same
2	rs7086428	10	130 778 452	A/G	0.35	—	SBP	−1.6	0.22	Opposite	A/G	0.36	—	SBP	0.7	0.26	Same
							DBP	−0.6	0.55	Opposite				DBP	0.1	0.74	Same
3	rs4953045	2	44 268 800	C/T	0.24	0.90	SBP	−1.9	0.25	Same	C/T	0.28	—	SBP	−1.3	0.06	Same
							DBP	−2.1	0.10	Same				DBP	0.2	0.77	Opposite
4	rs711513	7	109 368 769	A/G	0.70	0.98	SBP	−0.2	0.91	Opposite	G/A	0.39	—	SBP	−0.7	0.29	Same
							DBP	−0.7	0.51	Opposite				DBP	−0.3	0.49	Same
5	rs12814605	12	63 438 145	C/T	0.87	0.99	SBP	−0.8	0.69	Opposite	T/C	0.13	—	SBP	−1.0	0.29	Same
							DBP	0.7	0.62	Same				DBP	−1.0	0.14	Same
6	rs2279989	9	1 041 524	A/G	0.06	0.98	SBP	3.0	0.32	Opposite	A/G	0.06	—	SBP	1.3	0.34	Opposite
							DBP	1.6	0.47	Opposite				DBP	0.1	0.93	Opposite
7	rs7597606	2	41 836 313	C/T	0.58	0.96	SBP	−0.6	0.65	Same	T/C	0.42	—	SBP	0.1	0.86	Same
							DBP	−0.8	0.43	Same				DBP	0.2	0.72	Same
8	rs1559557	2	57 332 673	C/T	0.38	0.99	SBP	0.1	0.92	Same	C/T	0.37	—	SBP	−0.6	0.34	Opposite
							DBP	−0.3	0.77	Opposite				DBP	−0.7	0.15	Opposite
9	rs1432232	2	137 799 664	G/T	0.71	—	SBP	2.8	0.06	Opposite	T/G	0.26	—	SBP	−0.3	0.64	Opposite
							DBP	1.8	0.11	Opposite				DBP	−0.6	0.27	Opposite
10	rs1993802	3	110 595 402	C/T	0.08	—	SBP	1.4	0.60	Opposite	C/T	0.10	—	SBP	0.3	0.75	Opposite
							DBP	1.4	0.48	Opposite				DBP	−0.5	0.46	Same
11	rs12602832	17	42 396 896	NA							T/C	0.06	—	SBP	−0.6	0.67	Same
														DBP	−0.5	0.57	Same
12	rs2038912	10	20 207 349	C/T	0.42	1.00	SBP	1.5	0.26	Opposite	C/T	0.42	—	SBP	−0.8	0.22	Same
							DBP	0.8	0.41	Opposite				DBP	−0.4	0.45	Same
13	rs4759885	12	131 736 813	C/T	0.58	0.99	SBP	1.6	0.20	Opposite	T/C	0.34	—	SBP	−0.4	0.48	Opposite
							DBP	1.0	0.31	Opposite				DBP	−0.6	0.21	Opposite
14	rs771574	3	101 620 682	A/G	0.63	1.00	SBP	−0.3	0.84	Opposite	G/A	0.27	—	SBP	0.5	0.51	Opposite
							DBP	−0.0	0.99	Opposite				DBP	0.4	0.40	Opposite
15	rs10754459	1	245 785 508	NA							C/T	0.22	—	SBP	1.2	0.14	Opposite
														DBP	0.9	0.12	Opposite

Continued

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Table 7. Continued

Rank in GENRES	SNP	Chr	Position (Build 37)	Replication Results From GERA II (N=198)							Replication Results From SOPHIA (N=372)							
				CA/NCA	CAF	r^2 imp.	SBP/DBP	β	*P* Value	Direction of Replication	CA/NCA	CAF	r^2 imp.	SBP/DBP	β	*P* Value	Direction of Replication
16	rs1392874	4	101 466 820	A/C	0.43	0.99	SBP	−1.1	0.43	Same	A/C	0.39	—	SBP	0.6	0.38	Opposite
							DBP	−0.4	0.71	Same				DBP	0.0	0.97	Opposite
17	rs1357365	17	34 436 532	A/G	0.30	1.00	SBP	−0.3	0.84	Opposite	A/G	0.35	—	SBP	0.2	0.81	Same
							DBP	0.1	0.95	Same				DBP	0.4	0.44	Same
18	rs3814995	19	36 342 212	C/T	0.68	0.19	SBP	7.1	0.03	Same	T/C	0.28	—	SBP	−0.9	0.19	Same
							DBP	5.9	0.02	Same				DBP	−1.1	0.03	Same
19	rs11841583	13	31 495 179	A/G	0.13	0.83	SBP	0.6	0.78	Opposite	A/G	0.05	—	SBP	2.6	0.07	Opposite
							DBP	0.7	0.67	Opposite				DBP	0.8	0.43	Opposite
20	rs17271855	19	5 524 721	C/T	0.83	0.88	SBP	−2.2	0.23	Opposite	T/C	0.20	—	SBP	−0.0	0.99	Same
							DBP	−0.5	0.72	Opposite				DBP	−0.1	0.81	Same

The loci are listed in the order of their rank in GENRES. CA indicates coded allele; CAF, coded allele frequency; Chr, chromosome; DBP, diastolic blood pressure; NA, not analyzed; NCA, noncoded allele; r^2 imp, r^2 values for imputed SNPs; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism.

replication studies, we found that 1 particular SNP, rs3814995, was significantly associated with responses to angiotensin receptor antagonists in the same direction in 3 separate studies. It is of note that even meta-analysis of the rs3814995 data indicated *P* values close to the 1.0×10^{-5} (Table 11). This missense variant (NM_004646.3:c.349G>A) maps within the coding region of the *NPHS1* gene and causes an amino acid substitution of glutamic acid to lysine (p.Glu117Lys) in the nephrin protein. The amino acid is conserved and the change is predicted to be probably damaging by PolyPhen2 (score: 0.999). The variant is relatively common, with a higher minor allele frequency in populations of European origin (0.30) compared to African Americans (0.09) (Exome Variant Server, http://evs.gs.washington.edu/EVS/). Nephrin is the principal structural protein of the glomerular podocytes, and mutations of *NPHS1* result in the congenital nephrotic syndrome of the Finnish type.[23–25] Increased angiotensin II levels have been shown to result in decrease of renal nephrin expression in a hypertensive rat model.[26] Interestingly, angiotensin blockers irbesartan[27] and valsartan[28] have been shown to attenuate the decrease of nephrin levels and to retard the development of albuminuria in diabetic spontaneously hypertensive rats. The p.Glu117Lys variant does not seem to associate with diabetic proteinuria or end-stage renal disease in type 1 diabetic patients, although carriers of the minor allele had a later onset of diabetes than those with the wild-type allele.[29] Collectively, the present and previous findings should justify additional studies using samples and data from large long-term clinical trials in which nephrin Glu117Lys genotypes are related to blood pressure responses to angiotensin receptor antagonists and to cardiovascular events.

When bisoprolol data were analyzed in the GENRES material only, we obtained the highest *P* values with genome-wide significance (2.0×10^{-8}, 2.1×10^{-8}, and 4.1×10^{-8}) for the tightly linked nearby SNPs rs2514036, rs948445, and rs2514037 present on chromosome 11. rs2514036 is an upstream regulatory region variant of *ACY3*, encoding aminoacylase III, while rs2514037 is an intronic variant of *ACY3*. rs948445, a missense variant mapping within the coding region of the *ACY3* gene, causes an amino acid substitution p.Arg8Gln, which is predicted by PolyPhen2 to be benign. There appears to be no data solidly linking *ACY3* to regulation of blood pressure. It is known to be abundantly expressed in kidney proximal tubules, where it may have role in deacetylating mercapturic acids, and to lesser extent in other tissues including brain and heart.[30] Although β-adrenergic receptors may be more abundant in epithelial cells of distal than proximal parts of the nephron (for review, see Ref. [31]), cultured proximal tubular cells obtained from animal models have been reported to contain β-1- and β-2-adrenergic receptors.[32,33] Other genes next to rs2514036

Table 8. Replication Analysis of the Best GENRES Bisoprolol 24-H Ambulatory Blood Pressure Response SNPs With Ambulatory 24-H Blood Pressure Responses to Atenolol in PEAR

				Replication Results From PEAR (N=192 to 193)					
Rank in GENRES	SNP	Chr	Position (Build 37)	CA/NCA	CAF	SBP/DBP	β	*P* Value	Direction of Replication
1	rs2514036	11	67 415 054	C/T	0.18	SBP	0.7	0.58	Opposite
						DBP	0.5	0.56	Opposite
2	rs7268800	20	38 580 738	G/A	0.37	SBP	−0.5	0.61	Same
						DBP	−0.9	0.14	Same
3	rs12967284	18	12 532 098	T/C	0.34	SBP	0.1	0.90	Same
						DBP	0.2	0.75	Same
4	rs4357510	1	192 612 394	A/C	0.06	SBP	1.5	0.37	Opposite
						DBP	1.0	0.35	Opposite
5	rs2506143	10	33 468 169	G/A	0.17	SBP	1.3	0.28	Same
						DBP	0.9	0.26	Same
6	rs2029870	6	164 888 366	A/G	0.21	SBP	−0.6	0.61	Same
						DBP	0.2	0.84	Opposite
7	rs7984003	13	82 866 573	T/C	0.18	SBP	2.1	0.07	Same
						DBP	0.5	0.52	Same
8	rs11777699	8	6 714 699	G/A	0.45	SBP	−0.0	0.99	Same
						DBP	0.2	0.75	Opposite
9	rs10519585	5	118 676 529	C/T	0.35	SBP	−0.2	0.79	Same
						DBP	0.3	0.63	Opposite
10	rs6501061	16	8 092 171	C/T	0.04	SBP	1.4	0.55	Same
						DBP	0.7	0.65	Same
11	rs16918900	8	54 147 242	T/C	0.04	SBP	0.1	0.97	Opposite
						DBP	0.6	0.67	Opposite
12	rs2194860	4	109 948 511	A/G	0.16	SBP	0.2	0.87	Same
						DBP	−0.3	0.72	Opposite
13	rs2765115	13	24 529 399	G/A	0.13	SBP	−2.2	0.14	Same
						DBP	−0.4	0.71	Same
14	rs17642669	5	140 628 277	G/A	0.05	SBP	−1.5	0.48	Same
						DBP	0.4	0.80	Opposite
15	rs7895038	10	2 432 266	T/C	0.47	SBP	0.0	0.98	Opposite
						DBP	0.3	0.60	Same
16	rs150210	21	19 362 312	C/T	0.25	SBP	1.1	0.30	Opposite
						DBP	0.6	0.43	Opposite
17	rs10910862	1	181 045 421	T/C	0.06	SBP	0.2	0.93	Opposite
						DBP	−0.1	0.96	Same
18	rs2148117	10	27 213 946	C/T	0.05	SBP	−0.6	0.80	Same
						DBP	0.2	0.88	Opposite
19	rs1150433	3	163 653 210	T/C	0.21	SBP	0.4	0.71	Opposite
						DBP	0.0	0.98	Same
20	rs969981	17	63 818 989	T/C	0.23	SBP	0.2	0.85	Opposite
						DBP	0.1	0.86	Opposite

The loci are listed in the order of their rank in GENRES. All SNPs in this table were genotyped. CA indicates coded allele; CAF, coded allele frequency; Chr, chromosome; DBP, diastolic blood pressure; NCA, non-coded allele; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism.

Table 9. Replication Analysis of the Best GENRES Hydrochlorothiazide 24-H Ambulatory Blood Pressure Response SNPs With Office Blood Pressure Responses to Hydrochlorothiazide in GERA I and With 24-H Ambulatory Blood Pressure Responses in PEAR

				Replication Results From GERA I (N=196)							Replication Results From PEAR (N=190 to 193)						
Rank in GENRES	SNP	Chr	Position (Build 37)	CA/NCA	CAF	r^2 imp.	SBP/DBP	β	*P* Value	Direction of Replication	CA/NCA	CAF	r^2 imp.	SBP/DBP	β	*P* Value	Direction of Replication
1	rs4867623	5	170 071 480	NA							T/C	0.14	—	SBP	−0.8	0.49	Same
														DBP	−0.4	0.68	Same
1	rs4868010	5	170 092 760	T/G	0.13	0.95	SBP	−1.8	0.28	Same	T/G	0.11	—	SBP	−1.6	0.23	Same
							DBP	−0.5	0.67	Same				DBP	−1.1	0.29	Same
2	rs321320	6	45 702 779	C/T	0.22	0.82	SBP	1.1	0.49	Opposite	C/T	0.22	—	SBP	0.4	0.71	Opposite
							DBP	−0.8	0.48	Same				DBP	−0.5	0.52	Same
2	rs321329	6	45 722 988	T/C	0.46	0.72	SBP	−2.0	0.16	Same	T/C	0.44	—	SBP	−0.7	0.47	Same
							DBP	−2.3	0.03	Same				DBP	−0.4	0.54	Same
3	rs11006074	10	59 941 452	G/A	0.13	0.94	SBP	4.3	0.01	Same	G/A	0.17	—	SBP	−1.6	0.16	Opposite
							DBP	1.9	0.14	Same				DBP	−0.8	0.37	Opposite
4	rs3117915	13	22 605 580	G/A	0.23	0.66	SBP	−0.4	0.80	Same	G/A	0.29	—	SBP	−0.3	0.73	Same
							DBP	0.4	0.74	Opposite				DBP	0.1	0.86	Opposite
5	rs7821547	8	13 541 779	T/C	0.18	0.94	SBP	−1.7	0.30	Opposite	T/C	0.26	—	SBP	−0.9	0.41	Opposite
							DBP	−0.9	0.47	Opposite				DBP	−0.1	0.94	Opposite
6	rs3825926	15	101 445 441	NA							A/G	0.01	—	SBP	3.6	0.36	Same
														DBP	5.1	0.08	Same
7	rs2056531	17	47 283 058	T/C	0.16	—	SBP	−0.7	0.69	Opposite	T/C	0.20	—	SBP	1.8	0.09	Same
							DBP	−1.1	0.42	Opposite				DBP	1.6	0.05	Same
8	rs6977301	7	42 497 247	A/G	0.07	0.88	SBP	−1.2	0.65	Opposite	A/G	0.07	—	SBP	−1.2	0.51	Opposite
							DBP	−1.3	0.48	Opposite				DBP	−0.5	0.68	Opposite
9	rs1553009	7	45 908 994	A/G	0.21	1.00	SBP	−0.9	0.54	Opposite	A/G	0.22	—	SBP	0.0	0.97	Opposite
							DBP	−2.1	0.05	Opposite				DBP	−0.5	0.55	Opposite
10	rs2776906	6	37 431 757	T/C	0.26	0.99	SBP	0.1	0.94	Same	T/C	0.33	—	SBP	0.6	0.52	Same
							DBP	−1.1	0.26	Opposite				DBP	0.7	0.32	Same
11	rs964132	19	19 243 175	T/C	0.03	0.55	SBP	−5.6	0.28	Opposite	T/C	0.02	—	SBP	1.7	0.62	Same
							DBP	−1.8	0.63	Opposite				DBP	1.1	0.66	Same
12	rs158210	5	55 737 637	T/C	0.11	0.80	SBP	0.5	0.83	Opposite	A/G	0.10	—	SBP	3.5	0.02	Opposite
							DBP	0.4	0.79	Opposite				DBP	3.0	0.01	Opposite
13	rs1324210	9	114 791 150	A/G	0.16	0.63	SBP	−3.2	0.13	Opposite	A/G	0.18	—	SBP	−1.8	0.10	Opposite
							DBP	−2.2	0.16	Opposite				DBP	−1.2	0.16	Opposite

Continued

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Table 9. Continued

Rank in GENRES	SNP	Chr	Position (Build 37)	Replication Results From GERA I (N=196)							Replication Results From PEAR (N=190 to 193)						
				CA/NCA	CAF	r^2 imp.	SBP/DBP	β	*P* Value	Direction of Replication	CA/NCA	CAF	r^2 imp.	SBP/DBP	β	*P* Value	Direction of Replication
14	rs6546025	2	63 923 561	G/A	0.22	0.97	SBP	0.3	0.84	Opposite	G/A	0.22	—	SBP	1.9	0.06	Opposite
							DBP	−0.1	0.94	Same				DBP	1.7	0.03	Opposite
15	rs11059985	12	129 347 891	A/G	0.25	0.46	SBP	−0.6	0.77	Opposite	A/G	0.26	—	SBP	0.8	0.40	Same
							DBP	−0.1	0.94	Opposite				DBP	0.7	0.36	Same
16	rs3799369	6	25 912 634	C/T	0.11	0.95	SBP	0.9	0.65	Same	G/A	0.10	—	SBP	1.0	0.51	Same
							DBP	−0.0	1.00	Opposite				DBP	0.9	0.41	Same
17	rs17099050	14	100 255 923	G/T	0.04	0.87	SBP	−2.6	0.41	Opposite	G/T	0.04	—	SBP	1.5	0.48	Same
							DBP	−2.8	0.23	Opposite				DBP	1.6	0.29	Same
18	rs329668	11	133 795 219	NA							T/C	0.13	—	SBP	0.1	0.94	Same
														DBP	0.0	1.00	Same
19	rs776472	7	114 352 862	C/T	0.44	0.92	SBP	−0.6	0.61	Same	G/A	0.42	—	SBP	1.1	0.21	Opposite
							DBP	−1.7	0.05	Same				DBP	1.1	0.10	Opposite
20	rs1922117	12	63 789 836	G/T	0.29	0.99	SBP	0.0	0.98	Opposite	G/T	0.34	—	SBP	1.0	0.29	Opposite
							DBP	0.5	0.62	Opposite				DBP	0.5	0.49	Opposite

The loci are listed in the order of their rank in GENRES. CA indicates coded allele; CAF, coded allele frequency; Chr, chromosome; DBP, diastolic blood pressure; NA, not analyzed; NCA, non-coded allele; r^2 imp, r^2 values for imputed SNPs; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism.

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Table 10. Meta-Analysis of Blood Pressure Responses to Angiotensin Receptor Antagonists, β-Receptor Blockers, and Hydrochlorothiazide

SNP	Chr	Position	Nearest Gene	CA/NCA	Meta-Analysis				Discovery Study		Replication Study			
					N	CAF	B	*P* Value	β	*P* Value	β	*P* Value	β	*P* Value
Angiotensin receptor antagonists									GENRES		GERA II		SOPHIA	
SBP response														
rs4953045	2	44268800	*LRPPRC*	G/A	775	0.26	−2.4	5.1E-07	−3.5	1.1E-06	−1.9	0.25	−1.3	0.06
DBP response														
rs12814605	12	63438145	*AVPR1A*	A/G	775	0.14	−1.9	6.4E-06	−3.0	2.3E-06	−0.7	0.62	−1.0	0.14
β-Receptor blockers									GENRES		PEAR			
SBP response														
rs7984003	13	82866573	Intergenic	A/G	438	0.23	2.9	7.8E-07	3.1	5.4E-06	2.1	0.07		
rs2765115	13	24529399	*SPATA13*	G/A	440	0.15	−3.3	3.6E-06	−3.7	1.2E-05	−2.2	0.14		
DBP response														
rs7268800	20	38580738	lincRNA	G/A	440	0.40	−1.6	8.6E-07	−1.9	2.1E-06	−0.9	0.14		
Hydrochlorothiazide									GENRES		GERA I		PEAR	
SBP response														
rs3825926	15	101445441	*ALDH1A3*	A/G	399	0.03	6.7	5.6E-06	7.3	1.0E-05	NA	NA	3.6	0.36
DBP response														
rs4867623	5	170071480	*KCNIP1*	A/G	398	0.18	−2.1	1.5E-06	−2.6	3.7E-07	NA	NA	−0.4	0.68
rs321329	6	45722988	*CLIC5, RUNX2*	A/G	595	0.42	−1.5	2.3E-06	−1.7	1.1E-05	−2.3	0.03	−0.4	0.54
rs321320	6	45702779	*CLIC5, RUNX2*	G/A	594	0.17	−1.9	8.5E-06	−2.5	2.3E-06	−0.8	0.48	−0.5	0.52

The top 20 GENRES single-nucleotide polymorphisms of each drug were analyzed using GENRES, GERA1, GERA II, PEAR, and SOPHIA data. Single-nucleotide polymorphisms with significance level $<1\times10^{-5}$ are shown. Physical positions are given in build 37 coordinates and β values in mm Hg. CA indicates coded allele; CAF, coded allele frequency; Chr, chromosome; DBP, diastolic blood pressure; lincRNA, long intergenic noncoding RNA; N, total number of subjects in meta-analysis; NA, data not available; NCA, noncoded allele; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism.

Table 11. Meta-Analysis of Blood Pressure Responses to Angiotensin Receptor Antagonists

SNP	Chr	CA	NCA	N	CAF	β	SE	*P* Value	Direction of β (GENRES/GERA II/SOPHIA)
Systolic blood pressure response									
rs4953045	2	G	A	775	0.26	−2.4	0.5	5.1E-07	− − −
rs3814995	19	A	G	774	0.32	−2.0	0.5	1.5E-05	− − −
rs7086428	10	A	G	775	0.37	1.6	0.4	2.1E-04	+ − +
rs7597606	2	A	G	774	0.41	1.5	0.4	3.2E-04	+ + +
rs12814605	12	A	G	775	0.14	−2.2	0.6	3.5E-04	− + −
rs1370555	15	G	A	775	0.49	−1.3	0.4	2.1E-03	− − −
rs1993802	3	G	A	775	0.14	−1.9	0.6	2.7E-03	− + +
rs711513	7	G	A	772	0.37	−1.3	0.4	3.1E-03	− + −
rs12602832	17	A	G	574	0.11	−2.1	0.7	4.0E-03	− ? −
rs2038912	10	A	G	775	0.54	1.1	0.4	9.0E-03	+ − +
rs1357365	17	A	G	775	0.29	1.1	0.5	1.7E-02	+ − +
rs1559557	2	G	A	775	0.35	1.1	0.5	1.8E-02	+ + −
rs771574	3	G	A	775	0.27	−1.1	0.5	2.9E-02	− + +
rs11841583	13	A	G	775	0.09	−1.7	0.8	2.9E-02	− + +
rs2279989	9	A	G	775	0.08	−1.6	0.8	3.7E-02	− + +
rs17271855	19	A	G	775	0.19	−1.1	0.5	4.8E-02	− + −
rs1432232	2	A	C	775	0.23	1.0	0.5	5.0E-02	+ −
rs4759885	12	G	A	775	0.57	−0.7	0.4	9.2E-02	− + +
rs1392874	4	A	C	775	0.42	−0.7	0.4	9.8E-02	− +
rs10754459	1	G	A	575	0.26	−0.7	0.5	1.6E-01	− ? +
Diastolic blood pressure response									
rs12814605	12	A	G	775	0.14	−1.9	0.4	6.4E-06	− − −
rs3814995	19	A	G	774	0.32	−1.4	0.3	1.1E-05	− − −
rs12602832	17	A	G	574	0.11	−2.1	0.5	4.2E-05	− ? −
rs1370555	15	G	A	775	0.49	−1.1	0.3	1.0E-04	− + −
rs1993802	3	G	A	775	0.14	−1.7	0.4	2.1E-04	− + −
rs711513	7	G	A	772	0.37	−1.1	0.3	2.6E-04	− + −
rs2279989	9	A	G	775	0.08	−1.9	0.5	4.0E-04	− + +
rs1357365	17	A	G	775	0.29	1.2	0.3	4.5E-04	+ + +
rs4953045	2	G	A	775	0.26	−1.2	0.3	6.0E-04	− +
rs2038912	10	A	G	775	0.54	1.0	0.3	6.2E-04	+ − +
rs1392874	4	A	C	775	0.42	−1.0	0.3	1.2E-03	− +
rs17271855	19	A	G	775	0.19	−1.2	0.4	2.3E-03	− + −
rs7597606	2	A	G	774	0.41	0.9	0.3	4.0E-03	+ + +
rs11841583	13	A	G	775	0.09	−1.6	0.6	4.5E-03	− + +
rs7086428	10	A	G	775	0.37	0.8	0.3	1.3E-02	+ − +
rs771574	3	G	A	775	0.27	−0.8	0.4	1.7E-02	− + +
rs10754459	1	G	A	575	0.26	−0.9	0.4	1.7E-02	− ? +
rs4759885	12	G	A	775	0.57	−0.6	0.3	7.0E-02	− + +
rs1559557	2	G	A	775	0.35	0.5	0.3	1.6E-01	+ −
rs1432232	2	A	C	775	0.23	0.5	0.4	2.1E-01	+ −

The best GENRES losartan 24-h ambulatory blood pressure response SNPs were analyzed using GENRES, GERA II, and SOPHIA data. ? indicates data not available; CA, coded allele; CAF, weighed coded allele frequency; Chr, chromosome; N, total number of subjects in meta-analysis; NCA, noncoded allele; SNP, single-nucleotide polymorphism.

Table 12. Meta-Analysis of Blood Pressure Responses to β-Receptor Blockers

SNP	Chr	CA	NCA	N	CAF	β	SE	*P* Value	Direction of β (GENRES/PEAR)
Systolic blood pressure response									
rs7984003	13	A	G	438	0.23	2.9	0.6	7.8E-07	+ +
rs2765115	13	G	A	440	0.15	−3.3	0.7	3.6E-06	− −
rs6501061	16	G	A	440	0.08	4.1	0.9	1.2E-05	+ +
rs2514036	11	G	A	440	0.14	−3.1	0.7	2.1E-05	− +
rs2194860	4	A	G	440	0.18	2.5	0.6	5.5E-05	+ +
rs12967284	18	A	G	440	0.28	2.0	0.5	1.1E-04	+ +
rs2029870	6	A	G	437	0.16	−2.5	0.7	2.0E-04	− −
rs7895038	10	G	A	440	0.45	−1.8	0.5	2.6E-04	− +
rs10519585	5	G	A	440	0.32	−1.8	0.5	3.7E-04	− −
rs969981	17	A	G	439	0.18	−2.2	0.6	3.9E-04	− +
rs2506143	10	G	A	440	0.12	2.6	0.8	5.5E-04	+ +
rs2148117	10	G	A	440	0.03	−5.1	1.5	8.3E-04	− −
rs10910862	1	A	G	440	0.06	−3.5	1.1	1.1E-03	− +
rs150210	21	G	A	440	0.27	−1.7	0.5	1.2E-03	− +
rs7268800	20	G	A	440	0.40	−1.6	0.5	1.2E-03	− −
rs17642669	5	G	A	440	0.06	−3.3	1.1	2.4E-03	− −
rs4357510	1	A	C	440	0.06	−2.9	1.0	2.6E-03	− +
rs11777699	8	G	A	439	0.49	−1.4	0.5	5.1E-03	− −
rs1150433	3	A	G	440	0.16	−1.9	0.7	5.2E-03	− +
rs16918900	8	A	G	440	0.03	−3.7	1.6	2.3E-02	− +
Diastolic blood pressure response									
rs7268800	20	G	A	440	0.40	−1.6	0.3	8.6E-07	− −
rs2506143	10	G	A	440	0.12	2.2	0.5	1.0E-05	+ +
rs7984003	13	A	G	438	0.23	1.7	0.4	1.4E-05	+ +
rs12967284	18	A	G	440	0.28	1.5	0.4	3.6E-05	+ +
rs11777699	8	G	A	439	0.49	−1.2	0.3	1.2E04	− +
rs2029870	6	A	G	437	0.16	−1.7	0.4	1.6E-04	− +
rs17642669	5	G	A	440	0.06	−2.7	0.7	2.0E-04	− +
rs10910862	1	A	G	440	0.06	−2.6	0.7	2.3E-04	− −
rs2765115	13	G	A	440	0.15	−1.8	0.5	2.4E-04	− −
rs10519585	5	G	A	440	0.32	−1.2	0.3	3.4E-04	− +
rs2514036	11	G	A	440	0.14	−1.7	0.5	4.4E-04	− +
rs1150433	3	A	G	440	0.16	−1.5	0.5	8.0E-04	− −
rs4357510	1	A	C	440	0.06	−2.1	0.7	1.2E-03	− +
rs2194860	4	A	G	440	0.18	1.4	0.4	1.4E-03	+ −
rs6501061	16	G	A	440	0.08	2.0	0.6	1.8E-03	+ +
rs7895038	10	G	A	440	0.45	−1.0	0.3	2.5E-03	− −
rs2148117	10	G	A	440	0.03	−3.0	1.0	4.0E-03	− +
rs16918900	8	A	G	440	0.03	−3.0	1.1	5.9E-03	− +
rs150210	21	G	A	440	0.27	−0.9	0.4	1.1E-02	− +
rs969981	17	A	G	439	0.18	−1.1	0.4	1.3E-02	− +

The best GENRES bisoprolol 24-h ambulatory blood pressure response SNPs were analyzed using GENRES and PEAR data. CA indicates coded allele; CAF, weighed coded allele frequency; Chr, chromosome; N, total number of subjects in meta-analysis; NCA, non-coded allele; SNP, single-nucleotide polymorphism.

Table 13. Meta-Analysis of Blood Pressure Responses to Hydrochlorothiazide

SNP	Chr	CA	NCA	N	CAF	β	SE	*P* Value	Direction of β (GENRES/ GERA I/PEAR)
Systolic blood pressure response									
rs3825926	15	A	G	399	0.03	6.7	1.5	5.6E-06	+ ? +
rs3799369	6	G	A	595	0.11	2.7	0.7	6.6E-05	+ + +
rs321329	6	A	G	595	0.42	−1.8	0.5	7.3E-05	− − −
rs11059985	12	A	G	594	0.28	2.0	0.5	1.0E-04	+ − +
rs2776906	6	A	G	595	0.34	1.8	0.5	1.1E-04	+ + +
rs329668	11	A	G	398	0.13	2.5	0.7	3.1E-04	+ ? +
rs2056531	17	A	G	595	0.15	2.2	0.6	8.3E-04	+ − +
rs6977301	7	A	G	595	0.08	2.7	0.8	1.1E-03	+ −
rs4868010	5	A	C	594	0.14	−2.0	0.7	2.4E-03	− − −
rs776472	7	G	A	595	0.43	−1.3	0.4	2.6E-03	− +
rs17099050	14	C	A	595	0.04	3.7	1.2	2.6E-03	+ − +
rs964132	19	A	G	595	0.04	4.0	1.3	3.0E-03	+ − +
rs11006074	10	G	A	595	0.14	1.9	0.6	3.2E-03	+ + −
rs321320	6	G	A	594	0.17	−1.7	0.6	5.6E-03	− + +
rs4867623	5	A	G	398	0.18	−1.8	0.7	6.3E-03	− ? −
rs3117915	13	G	A	595	0.23	−1.4	0.5	1.1E-02	− − −
rs1324210	9	A	G	595	0.23	1.4	0.5	1.1E-02	+ −
rs1553009	7	A	G	595	0.16	1.5	0.6	1.7E-02	+ −
rs158210	5	A	G	595	0.10	−1.8	0.7	1.9E-02	− + +
rs7821547	8	A	G	595	0.23	1.2	0.5	2.4E-02	+ −
rs1922117	12	C	A	592	0.35	−1.0	0.5	2.7E-02	− + +
rs6546025	2	G	A	595	0.23	−0.6	0.5	2.1E-01	− + +
Diastolic blood pressure response									
rs4867623	5	A	G	398	0.18	−2.1	0.4	1.5E-06	− ? −
rs321329	6	A	G	595	0.42	−1.5	0.3	2.3E-06	− − −
rs321320	6	G	A	594	0.17	−1.9	0.4	8.5E-06	− − −
rs4868010	5	A	C	594	0.14	−1.9	0.4	2.0E-05	− − −
rs2056531	17	A	G	595	0.15	1.9	0.5	2.2E-05	+ − +
rs964132	19	A	G	595	0.04	3.6	0.9	5.8E-05	+ − +
rs3825926	15	A	G	399	0.03	3.9	1.0	1.2E-04	+ ? +
rs11006074	10	G	A	595	0.14	1.7	0.4	1.6E-04	+ + −
rs3117915	13	G	A	595	0.23	−1.3	0.4	4.0E-04	− + +
rs7821547	8	A	G	595	0.23	1.3	0.4	4.5E-04	+ −
rs6977301	7	A	G	595	0.08	1.9	0.6	6.2E-04	+ −
rs17099050	14	C	A	595	0.04	2.9	0.9	7.9E-04	+ − +
rs776472	7	G	A	595	0.43	−1.0	0.3	1.1E-03	− +
rs1922117	12	C	A	592	0.35	−1.0	0.3	2.4E-03	− + +
rs329668	11	A	G	398	0.13	1.4	0.5	2.8E-03	+ ? +
rs2776906	6	A	G	595	0.34	0.9	0.3	4.1E-03	+ − +
rs3799369	6	G	A	595	0.11	1.3	0.5	5.5E-03	+ − +
rs6546025	2	G	A	595	0.23	−0.9	0.3	7.4E-03	− +

Continued

Table 13. Continued

SNP	Chr	CA	NCA	N	CAF	β	SE	*P* Value	Direction of β (GENRES/ GERA I/PEAR)
rs1324210	9	A	G	595	0.23	0.9	0.4	1.7E-02	+ –
rs11059985	12	A	G	594	0.28	0.8	0.4	2.1E-02	+ – +
rs1553009	7	A	G	595	0.16	0.9	0.4	3.3E-02	+ –
rs158210	5	A	G	595	0.10	−1.1	0.5	4.0E-02	– + +

The best GENRES hydrochlorothiazide 24-h ambulatory blood pressure response SNPs were analyzed using GENRES, GERA I, and PEAR data. ? indicates data not available; CA, coded allele; CAF, weighed coded allele frequency; Chr, chromosome; N, total number of subjects in meta-analysis; NCA, noncoded allele; SNP, single-nucleotide polymorphism.

include ALDH3B2 (15 kbp apart) coding for aldehyde dehydrogenase 3 family member B2 expressed mainly in salivary gland and placenta,[34] and *TBX10* (8 kbp apart) coding for a member of the T-box family of transcription factors involved in organogenesis and embryonic development. However, rs2514036 data were not at all replicated in the PEAR study using atenolol as the β-blocker (Table 8). In the meta-analysis combining atenolol data of PEAR, 3 SNPs provided suggestive associations. rs7984003 and rs7268800 appear to be intergenic variants, with no obvious candidate genes for BP regulation in their vicinity, but rs7984003 maps within an ENCODE transcription factor binding site (ERα_a). rs2765115 is likewise an intergenic variant located 24 kbp from *SPATA13* coding for spermatogenesis-associated protein 13, also known as APC-stimulated guanine nucleotide exchange factor 2 (ASEF2). ASEF2 appears to specifically activate Rho-family GTPases and may thus influence a wide range of cellular functions, including smooth-muscle contraction. It is also of interest that a genetic association study has suggested that *SPATA13-AS1* (gene coding for an antisense RNA that overlaps *SPATA13*) may serve as a pharmacogenomic predictor of effectiveness of inhaled β-agonists.[35]

In the meta-analysis of association with hydrochlorothiazide responsiveness, 3 different gene loci were identified that showed *P* values $<1.0\times10^{-5}$ when replication data from GERA I and/or PEAR studies were used (Table 10). rs3825926 is of interest because the genotype-related β values for thiazide responses were the highest in both GENRES and PEAR (Tables 5 and 9); unfortunately, it could not be analyzed in GERA I. This SNP represents an intronic variant of *ALDH1A3*, coding for aldehyde dehydrogenase 1 family member A3. This gene is expressed in a variety of tissues including retina and kidney, and mutations of *ALDH1A3* are known to result in anophthalmia or microophthalmia.[36] Another SNP, rs321329, deserves note since responses to hydrochlorothiazide followed a logical pattern (although *P* value remained nonsignificant in PEAR; Table 9) in all 3 studies. It should, however, be pointed out that the SNP rs321329 was not the top SNP of that locus in GENRES and the top SNP rs321320 showed no evidence of replication in GERA I or in PEAR (Table 9). The closest candidate genes, located 90 to 145 kbp from this SNP, include *RUNX2*, encoding a transcription factor involved in skeletal morphogenesis, and *CLIC5*, encoding the chloride intracellular channel protein 5. *CLIC5* is expressed (except in placenta and cochlea) in renal glomerular podocytes and endothelial cells, and is postulated to function in the maintenance of glomerular and podocyte architecture.[37] It is not known, however, whether the *CLIC5* channel plays any role in thiazide action.

Certain general delineations of our findings should be recapitulated. First, none of the 80 SNPs (20 for each drug) listed in Tables 2 through 5 proved to give a hit when compared to the list of >40 hypertension candidate loci[6] derived from genome-wide association studies of essential hypertension. Second, the association of the *NPHS1* (nephrin) gene Glu117Lys variant with losartan responsiveness is of interest in view of the experimental findings linking together angiotensin II levels, angiotensin II receptor blockers, nephrin expression, and development of albuminuria. Third, 2 different members of the aldehyde dehydrogenase (ALDH) gene family got some support as candidate genes, 1 for bisoprolol (*ALDH3B2*) and the other for hydrochlorothiazide (*ALDH1A3*) responsiveness. The human ALDH gene family contains 19 members.[34] It is intriguing that 2 other members of this gene family (*ALDH1A2* and *ALDH7*) were found to be associated with the presence of hypertension in African Americans,[38] and yet another member (*ALDH2*) associated with BP variation in East Asians.[39] ALDHs constitute an important family of enzymes that are able to oxidize a variety of endogenous and exogenous aldehydes that play a role in cell proliferation, differentiation, and responsiveness to environmental stress.[34] The links, if any, of ALDHs to regulation of BP and/or antihypertensive drug action remain unknown at present.

Perspectives

Genomic loci influencing responsiveness to antihypertensive drugs are proving difficult to identify, reflecting similar difficulties to identify genetic variants underlying elevated

BP per se. We have carried out a genome-wide analysis of responses of 4 different antihypertensive drugs using very carefully designed experimental conditions. Using GENRES data alone, we could only identify 1 candidate gene locus for hypertension pharmacogenomics: *ACY3* associating with bisoprolol responsiveness. However, use of replication data from 3 other trials provided several additional gene candidates, including those coding for nephrin and members of the aldehyde dehydrogenase family, all of which require additional studies. We did not find evidence for gene loci associating with responsiveness to more than 1 particular class of antihypertensive drugs, suggesting that genetic control of pathways influencing antihypertensive drug responsiveness are drug class–specific. However, since many hypertension genes may show pleiotropic effects on blood pressure pathways, it is possible that the power of our study may simply have been insufficient to detect gene loci interacting with more than 1 class of drugs. In future, even larger carefully controlled prospective clinical studies are needed in which several different antihypertensive drugs are tested. It should be emphasized that although pharmacogenomic prediction of antihypertensive response augmentation on the order of 2 mm Hg may appear minor, in the long term it is translated into a 7% to 10% lower risk of mortality from ischemic heart disease and stroke.[40] It is realistic to expect that this level of predictive accuracy in individualized antihypertensive drug therapy could be reached by pharmacogenomic approaches.

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Disclosures

None.

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