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## ASSOCIATION OF HYPERTENSION DRUG TARGET GENES WITH BLOOD PRESSURE AND HYPERTENSION IN 86,588 INDIVIDUALS

Andrew D. Johnson, Christopher Newton-Cheh, Daniel I. Chasman, Georg B. Ehret, Toby Johnson, Lynda Rose, Kenneth Rice, Germaine C. Verwoert, Lenore J. Launer, Vilmundur Gudnason, Martin G. Larson, Aravinda Chakravarti, Bruce M. Psaty, Mark Caulfield, Cornelia M. van Duijn, Paul Ridker, Patricia B. Munroe, and Daniel Levy on Behalf of the CHARGE Consortium, Global BPgen Consortium and Women's Genome Health Study

### Abstract

We previously conducted genome-wide association meta-analysis (GWA) of systolic blood pressure (SBP), diastolic blood pressure (DBP) and hypertension in 29,136 people from six cohort studies in the CHARGE Consortium. Here we examine associations of these traits with 30 gene regions encoding known anti-hypertensive drug targets. We find nominal evidence of association of *ADRB1*, *ADRB2*, *AGT*, *CACNA1A*, *CACNA1C*, and *SLC12A3* polymorphisms with one or more BP traits in the CHARGE GWA meta-analysis. We attempted replication of the top meta-analysis SNPs for these genes in the Global BPgen Consortium (GBPG, n=34,433) and the Women's Genome Health Study (WGHS, n=23,019), and found significant results for rs1801253 in *ADRB1* (Arg389Gly), with the Gly allele associated with a lower mean SBP (beta -0.57 (mmHg), se 0.09, meta-analysis  $P=4.7\times 10^{-10}$ ), DBP (beta -0.36, se 0.06, meta-analysis  $P=9.5\times 10^{-10}$ ) and prevalence of hypertension (beta -0.06, se 0.02, meta-analysis  $P=3.3\times 10^{-4}$ ). Variation in *AGT* (rs2004776) was associated with SBP (beta 0.42, se 0.09, meta-analysis  $P=3.8\times 10^{-6}$ ), as well as DBP ( $P=5.0\times 10^{-8}$ ) and hypertension ( $P=3.7\times 10^{-7}$ ). A polymorphism in *ACE* (rs4305) showed modest replication of association with increased hypertension (beta 0.06, se 0.01, meta-analysis  $P=3.0\times 10^{-5}$ ). Two loci, *ADRB1* and *AGT*, contain SNPs that reached a genome-wide significance threshold in meta-analysis for the first time. Our findings suggest that these genes warrant further studies of their genetic effects on BP, including pharmacogenetic interactions.

### Keywords

drug target; genome-wide; SNP; hypertension; blood pressure

### Introduction

Elevated blood pressure (BP) is a critical risk factor for cardiovascular diseases (1), and BP control in hypertensive individuals is an effective intervention for reducing cardiovascular disease risk. Hundreds of compounds representing multiple drug classes have been approved

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**Conflict of Interest/Disclosure** - none

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for treatment of hypertension. Achieving BP control in patients often requires multiple medications, and trial-and-error switching of drug classes to achieve control. This suggests that inter-individual differences in BP and in response to treatment may be influenced by genetic variation, or environmental or other non-genetic factors.

We recently completed a genome-wide association study (GWAS) and meta-analyses of systolic blood pressure (SBP), diastolic blood pressure (DBP), and hypertension (HTN) in 29,136 individuals from six population-based cohorts of European ancestry in the CHARGE Consortium, identifying and replicating novel BP loci at genome-wide significance levels (2). While GWAS have been successful in identifying new genes with common variants that exhibit small effects on BP, standard methods of analysis ignore all *a priori* information about specific genes. The strict requirements for controlling the occurrence of false positives in such an ‘unbiased’ approach leads to severe multiple testing corrections, whereby true positive associations will be missed, particularly when replication resources are limited.

Examining subsets of GWAS associations based on *a priori* hypotheses is one way to identify genes of interest for further investigation (3) while paying a smaller penalty for multiple testing. Evidence from lipid GWAS and candidate gene studies indicates some polymorphisms in drug target genes (e.g. *HMGCR*, *APOE*) are associated with main effects on lipids as well as effects on drug response (4,5). We hypothesized that GWAS approaches have missed some true BP association signals in antihypertensive drug target genes. We identified 30 drug target genes, including the targets of alpha blockers, ACE inhibitors, beta-blockers, angiotensin-receptor blockers, calcium-channel blockers, diuretics, and vasodilators, and analyzed single nucleotide polymorphisms (SNPs) in these gene regions for association with BP and hypertension.

## Methods

### Description of cohorts, participants, genotypes and phenotypes

The CHARGE consortium cohorts, their genotyping, SNP imputation (6) and BP and hypertension GWAS have been previously described (2). Participants underwent standardized resting seated BP readings (means of two repeated measures used in analysis) and had GWAS results available (n=29,136). BP readings from the first examination attended were used. Hypertension was defined as SBP $\geq$ 140 or DBP $\geq$ 90 mmHg or drug treatment for hypertension at BP assessment.

The Global BPgen consortium (GBPG) included 17 cohorts of European ancestry with either population-based designs or controls drawn from case-control designs (7). In most participants BP analysis was based on the mean of two resting sitting measurements (7).

The WGHS population sample with BP and hypertension data consisted of 23,019 female health professionals of European descent  $\geq$ 45 years of age at enrollment, free of cardiovascular disease or other major chronic illnesses, with GWAS and genotyping previously described (8). BP was determined by self-report in ranges (see the online Supplement, available at <http://hyper.ahajournals.org>), with the midpoint of these ranges used in analyses, and hypertension defined as above.

For individuals in CHARGE who were taking antihypertensive medication, we added 10/5 mmHg to the observed SBP and DBP; for those in GBPG we added 15/10 mmHg. Association results for different treatment adjustments were highly correlated in CHARGE (Table S1, available at <http://hyper.ahajournals.org>). Individual studies obtained approval from their IRBs for consent procedures, examination, data security, and DNA collection and

use in genetic research. All cohorts in the current study conducted imputation using a HapMap CEU reference panel.

### Discovery in CHARGE and replication in GBPG/WGHS

Within each cohort, regression models for BP phenotypes were fit adjusting for sex, age, age squared, and BMI. Genomic control ( $\lambda$ ) parameter values (9) were calculated and applied, to account for within study heterogeneity. Meta-analyses of the SNP-trait association estimates were inverse-variance weighted and reflect the combination of additive model analyses from the cohorts (2).

We identified *a priori* 30 candidate genes that code for proteins that are direct targets of anti-hypertensive drugs based on general knowledge and DrugBank ([www.drugbank.ca](http://www.drugbank.ca)), a database of human drug target genes (10). We analyzed all CHARGE BP/hypertension associations within 60 kb of each target gene, and applied a resampling based test ([11], see Figure S1, available at <http://hyper.ahajournals.org>). To augment SNPs for replication, we additionally selected SNPs at a  $P < 1/(\text{the number of SNPs tested})$ . SNPs selected for replication are in bold in Table S2 (available at <http://hyper.ahajournals.org>).

For selected gene regions, we examined the most significant CHARGE SNP-trait association for the same trait in GBPG and WGHS. Replication was defined *a priori* as allelic association in the same direction as in CHARGE (thresholds: SBP,  $P < 8.3 \times 10^{-3}$ , DBP,  $P < 7.1 \times 10^{-3}$ , hypertension,  $P < 8.3 \times 10^{-3}$ ). We also conducted meta-analysis to provide estimates comparable to GWAS thresholds. We used SNAP (12) to identify SNPs creating a protein change, or (based on HapMap populations) in linkage disequilibrium (LD) with protein-changing variants or SNPs with prior associations with SBP, DBP or hypertension.

## Results

For 30 regions that encode anti-hypertensive drug targets, the single strongest SNP associations for SBP, DBP, and hypertension in/near each drug target gene for the initial CHARGE analysis are in Table S2 (available at <http://hyper.ahajournals.org>), along with the number of SNPs tested within each gene region. The most significant SNP association among the drug target genes tested in CHARGE was rs1985579 in *CACNA1A* with SBP ( $P = 2.6 \times 10^{-5}$ ). Using resampling to account for multiple SNPs per locus, 2 significant SNP associations were identified for SBP (in *ADRB1*, *CACNA1A*), 4 for DBP (in *ADRB1*, *AGT*, *CACNA1A*, *SLC12A3*) and 4 for hypertension (in *ADRB2*, *AGT*, *CACNA1C*, *CACNA1H*). At a less restrictive cutoff of  $P < 1/(\text{the number of SNPs tested in/near a gene})$ , 11 additional SNPs in 9 genes were selected (*ACE*, *ADRB2*, *AGT*, *CA1*, *CACNA1C*, *MME*, *REN*, *SCN11A*, *SLC9A1*), for a total of 19 SNPs in 13 genes selected for replication. Two genes (*CACNA1H*, *MME*) were dropped from replication because their most associated SNPs had poor imputation in  $\geq 2$  groups, and attempts to find satisfactory surrogate SNPs in LD were unsuccessful.

For SBP we replicated associations for variants in *ADRB1* and *AGT* (Table 1). In *ADRB1* the minor allele of rs1801253 (nonsynonymous Arg389Gly) was associated with decreased SBP (replication:  $P = 7.3 \times 10^{-7}$ , meta-analysis: beta (units as mmHg)  $-0.57$ , se 0.09,  $P = 4.7 \times 10^{-10}$ ) and in *AGT* the minor allele of rs2004776 was associated with increased SBP (replication:  $P = 2.8 \times 10^{-5}$ ).

We also replicated associations for *ADRB1* and *AGT* with DBP (Table 2). The minor allele of rs1801253 in *ADRB1* was associated with decreased DBP (replication:  $P = 2.5 \times 10^{-7}$ , meta-analysis: beta  $-0.36$ , se 0.06,  $P = 9.5 \times 10^{-10}$ ) and in *AGT* the minor allele of rs11122587 was associated with increased DBP (replication:  $P = 2.4 \times 10^{-5}$ ).

We sought replication for 6 SNPs where hypertension was the primary trait (Table 3). The minor allele of an intron 5 SNP (rs4305) in *ACE* replicated with all cohort associations with increased odds of hypertension (replication:  $P=7.5\times 10^{-3}$ , meta-analysis: beta 0.06, se 0.01,  $P=3.0\times 10^{-5}$ ). In secondary analysis, this SNP also showed association with increased levels of SBP ( $P=4.6\times 10^{-4}$ ) and DBP ( $P=6.0\times 10^{-5}$ ).

In secondary analyses, the *AGT* SNP selected for SBP, rs2004776, reached a low p-value for DBP (meta-analysis:  $P=5.0\times 10^{-8}$ ). *ADRB1* and *AGT* SNPs were also associated with hypertension, in the same direction as expected based on their BP associations (*ADRB1*: rs1801253  $P=3.3\times 10^{-4}$ , *AGT*: rs2004776  $P=3.7\times 10^{-7}$ ).

Heterogeneity analyses for *ADRB1*, *AGT*, or *ACE* in the multi-study cohorts (CHARGE, GBP) or within the full meta-analysis found no evidence for heterogeneity (all  $I^2<0.50$ ). In additional analyses for *ADRB1* and *AGT*, conditioning on the top variant for SBP or DBP, we found no additional SNPs that contributed to these phenotypes after multiple test correction (see Supplement, available at <http://hyper.ahajournals.org>). Summary results for the three replicated genes and four promising, non-replicated genes (*ADRB2*, *CACNA1C*, *CACNA1A*, *SLC12A3*) compared with prior results and meta-analyses from the literature are presented in Tables S3 and S4, respectively (available at <http://hyper.ahajournals.org>).

## Discussion

Within one of the largest genetic studies of BP traits to date, we examined evidence for associations in gene regions encoding protein targets of anti-hypertensive medications. We conducted a discovery scan in >29,000 individuals from the CHARGE consortium, with validation of significant results in >57,000 individuals from GBP and WGHs. Of note, in previously published GWAS meta-analysis reports from CHARGE and GBP (2,7), none of the SNPs we tested reached genome-wide significance ( $P<5.0\times 10^{-8}$ ). Associations at three loci in our study (*ADRB1*, *AGT*, *ACE*) successfully replicated in independent populations.

The beta-adrenergic receptors (*ADRB1*, *ADRB2*) are targets of a variety of endogenous and pharmacological agonists and antagonists including epinephrine, norepinephrine and beta-blocker drugs, and they mediate important cardiovascular responses including cardiac contractility and heart rate. A nonsynonymous variant of *ADRB1* (rs1801253, Arg389Gly) was reported to alter BP response to beta blocker therapy in multiple studies (e.g., 13,14) and was also reported to affect outcomes following treatment (15-17). However, tests of this variant with baseline BP have generally been conducted in modestly-sized samples not drawn from general population cohorts, with conflicting reports about association of the Arg389 allele with increased BP ([14,16,18-23], Table S3, available at <http://hyper.ahajournals.org>). In our survey of 142 SNPs in/near *ADRB1*, we found the strongest association at rs1801253 with the Gly389 allele being associated with decreased SBP ( $P=4.7\times 10^{-10}$ ) and DBP ( $P=9.5\times 10^{-10}$ ). Our study is consistent with several studies (Table S3, available at <http://hyper.ahajournals.org>) indicating that there is a small reduction in BP associated with Gly389 (16,19,21-23). This result is also consistent with experimental observations that Gly389 acts functionally to reduce basal and agonist-stimulated receptor responses (24-26).

The renin-angiotensin system plays critical roles in BP regulation, is targeted by multiple drug classes, and has been the subject of prior genetic studies for candidate genes (e.g., *AGT*, *ACE*). Among 3 *AGT* SNPs (rs2004776, rs12046196, rs11122587) associated with BP in CHARGE, rs2004776, in intron 1 (between the *AGT* 5'UTR and exon 1), showed the strongest validation in an independent European ancestry cohort (meta-analysis  $P=5.0\times 10^{-8}$ ). Of note, rs2004776 is in partial LD ( $r^2=0.49$ , CEU) with a Met235Thr (rs699)

that has been widely studied for association with hypertension (27). Prior analyses suggest a role for Met235Thr (27,28) or other variants affecting AGT function (29,30) in hypertension, with multiple variants being supported in meta-analyses (27,29, Table S3, available at <http://hyper.ahajournals.org>). In CHARGE, the minor allele of Met235Thr showed nominal association with hypertension ( $P=0.0011$ ), SBP ( $P=0.012$ ), and DBP ( $P=0.0042$ ). These results suggest that Met235Thr may not be the most relevant AGT variant and that there may be other functional alleles to be discovered and characterized.

Another important gene within the renin-angiotensin system, ACE, showed significant positive association with hypertension (rs4305,  $P=3.0\times 10^{-5}$ ), and with SBP ( $P=4.6\times 10^{-4}$ ) and DBP ( $P=6.0\times 10^{-5}$ ). ACE genotypes have been studied for association with various traits, including BP and hypertension where results from a literature-based meta-analysis indicate no significant effect (31). A large study of anti-hypertensive drug response also indicates no genotype-treatment effect (32). However; most previous studies focused only on the well-known intronic I/D polymorphism (Table S3, available at <http://hyper.ahajournals.org>) and lacked detailed information on the genetic architecture of ACE. The importance of this point is emphasized by a recent resequencing study of ACE in African-Americans that found novel functional variation associated with myocardial infarction in hypertensives (33). Furthermore, a recent GWAS for ACE enzyme activity found strong association of the minor allele of rs4343 with increased activity in Han Chinese ( $P=3.0\times 10^{-25}$ ) (34). The nonsynonymous SNP rs4343 is in moderate-high LD in Asian and European populations with our replicated BP SNP, rs4305 (HapMap CEU  $r^2=0.48$ , JPT/CHB  $r^2=0.80$ ), suggesting a potential common link between the studies. We examined a sample of 944 unrelated individuals in Framingham and found rs4350-I/D are also in modest LD ( $r^2=0.55$ ).

The effect of variation in *ADRB1*, *AGT*, and *ACE* on BP variation and treatment response were the subject of prior research (Table S3, available at <http://hyper.ahajournals.org>). Our large study validates their role in BP genetics. The effect sizes of the *ADRB1* and *AGT* variants are on par with variants identified in GWAS for BP, lipids and similar traits, accounting for only ~0.25-0.50% of the variation in BP (2,7,35). These results demonstrate that surveying prior biological candidates in large genetic studies may be a useful approach to identify and replicate additional loci. This observation is consistent with two recent surveys of published GWAS, that indicate some *a priori* candidates show true associations (36,37). Candidate genes for variation in lipid levels have also been validated in GWAS and shown to have treatment effects (4,5,35).

The loci and variants identified here may also influence treatment response, a hypothesis that we did not assess. Our study is limited in that treated and untreated individuals are included, with variable ascertainment of treatment across cohorts. We applied differing treatment adjustments in different cohorts to impute expected baseline effects. Further analysis in CHARGE indicates +15/10 and +10/5 adjustments generate similar results for BP associations so this is unlikely to have greatly affected replication (Table S1, Figures S2 and S3, available at <http://hyper.ahajournals.org>). Since treatment of participants in our GWAS was non-randomized, it is difficult to assess gene-by-treatment interactions without confounding. Prospective studies, or more sophisticated cross-sectional analyses will be required to determine whether gene variants identified from large GWAS influence treatment outcomes; our results indicate these could be worthwhile pursuits. Another potential limitation of the study is reliance in WGHS on self-reported BP values. However, past studies indicate that self-report is reasonably reliable in assessment of BP and hypertension (e.g.,38,39). Furthermore, we independently found high replication rates for BP GWAS loci in the WGHS (40).

## Perspectives

Our results indicate that candidate genes, with clinical and physiological relevance by virtue of their role as antihypertensive drug targets, harbor true BP associated variants. Such loci, not identified in prior large GWAS meta-analyses but detected in our drug target gene approach, account for a portion of the unexplained proportion of BP variance. These results suggest that re-visiting GWAS scans from the perspective of biological and clinical knowledge may be useful for discovery and validation of new genetic associations.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Results for SNPs associated with SBP in CHARGE.

Gene	SNP	Minor allele	CHARGE (5 cohorts, n=29,136)				GBPG (17 cohorts, n=34,433)				WGHS (1 cohort, n=23,019)				Meta-analysis (n=86,588)			
			Beta	S.E.	p*	MAF	Beta	S.E.	p*	MAF	Beta	S.E.	p*	MAF	Trait	Beta	S.E.	p
ADRB1	rs1801253	G	-0.60	0.17	7.3×10 <sup>-4</sup>	27.3%	-0.31	0.15	0.048	31.7%	-0.82	0.16	7.3×10 <sup>-7</sup>	27.0%	SBP	-0.57	0.09	4.7×10 <sup>-10</sup>
					I <sup>2</sup> =	0%			I <sup>2</sup> =	13%					DBP	-0.36	0.06	9.5×10 <sup>-10</sup>
AGT	rs2004776	T	0.58	0.17	9.4×10 <sup>-4</sup>	23.8%	0.09	0.15	0.58	22.8%	0.69	0.16	2.8×10 <sup>-5</sup>	24.1%	HTN	-0.06	0.02	3.3×10 <sup>-4</sup>
					I <sup>2</sup> =	27%			I <sup>2</sup> =	6%					SBP	0.42	0.09	3.8×10 <sup>-6</sup>
CACNA1A	rs1985579	A	-0.70	0.16	2.6×10 <sup>-5</sup>	36.4%	-0.09	0.14	0.53	39.8%	-0.21	0.15	0.16	34.5%	SBP	-0.29	0.08	4.4×10 <sup>-4</sup>
					I <sup>2</sup> =	0%			I <sup>2</sup> =	0%					DBP	-0.14	0.05	8.7×10 <sup>-3</sup>
ADRB2	rs6580586	C	0.76	0.23	1.6×10 <sup>-3</sup>	11.0%	0.09	0.20	0.66	8.3%	0.03	0.23	0.89	10.1%	HTN	-0.04	0.01	2.5×10 <sup>-3</sup>
					I <sup>2</sup> =	27%			I <sup>2</sup> =	21%					SBP	0.27	0.13	0.033
CACNA1C	rs2239101	C	-0.92	0.27	9.6×10 <sup>-4</sup>	12.8%	0.09	0.22	0.70	12.5%	0.15	0.25	0.55	11.5%	SBP	-0.16	0.14	0.24
					I <sup>2</sup> =	52%			I <sup>2</sup> =	55%					DBP	-0.14	0.09	0.13
SCNN1A	rs11064160	C	-0.90	0.33	7.8×10 <sup>-3</sup>	12.0%	0.55	0.29	0.07	12.5%	-0.39	0.38	0.32	10.8%	HTN	-0.01	0.02	0.65
					I <sup>2</sup> =	0%			I <sup>2</sup> =	98%					SBP	-0.15	0.19	0.41
														DBP	-0.12	0.12	0.30	
														HTN	-0.01	0.03	0.85	

\* p-values have been taken from GWAS results corrected by genomic control<sup>9</sup>. λ values for SBP: CHARGE (λ=1.06), GBPG (λ=1.08), WGHS (λ=1.06). Beta reflects the unit change in mmHG in SBP/DBP or in log odds of HTN per allele dose. S.E. indicates standard error of the mean. Replicated SNPs are in bold.



**Table 3**

Results for SNPs associated with HTN in CHARGE.

Gene	SNP	Minor allele	CHARGE (5 cohorts, n=29,136)			GBPG (17 cohorts, n=34,433)			WGHS (1 cohort, n=23,019)			Meta-analysis					
			Beta	S.E.	p*	MAF	Beta	S.E.	p*	MAF	Beta	S.E.	p*	Trait	Beta	S.E.	p
ACE	rs4305	A	0.06	0.02	9.3×10 <sup>-3</sup>	44.7%	0.07	0.03	7.5×10 <sup>-3</sup>	39.7%	0.05	0.03	0.07	44.4%	0.06	0.01	3.0×10 <sup>-5</sup>
					I <sup>2</sup> =	47%			I <sup>2</sup> =	0%				HTN	0.29	0.08	4.6×10 <sup>-4</sup>
AGT	rs12046196	A	0.14	0.04	5.6×10 <sup>-4</sup>	9.1%	-0.01	0.05	0.80	7.5%	0.07	0.04	0.11	9.3%	0.21	0.05	6.0×10 <sup>-5</sup>
					I <sup>2</sup> =	91%			I <sup>2</sup> =	15%				HTN	0.25	0.15	0.09
CACNA1A	rs1985579	A	-0.07	0.02	2.6×10 <sup>-5</sup>	36.3%	-0.003	0.03	0.90	39.8%	-0.05	0.03	0.08	34.5%	-0.04	0.01	2.5×10 <sup>-3</sup>
					I <sup>2</sup> =	86%			I <sup>2</sup> =	11%				HTN	0.21	0.09	0.03
ADRB2	rs2082382	G	-0.07	0.02	7.0×10 <sup>-4</sup>	41.1%	0.04	0.03	0.19	32.4%	-0.05	0.02	0.04	43.9%	-0.04	0.01	5.0×10 <sup>-3</sup>
					I <sup>2</sup> =	89%			I <sup>2</sup> =	0%				HTN	-0.02	0.08	0.84
SLC9A1	rs484677	T	0.07	0.02	3.3×10 <sup>-3</sup>	41.5%	0.04	0.03	0.24	38.3%	0.01	0.03	0.77	42.0%	0.04	0.01	6.5×10 <sup>-3</sup>
					I <sup>2</sup> =	0%			I <sup>2</sup> =	0%				HTN	0.05	0.08	0.53
CACNA1C	rs16929470	T	-0.19	0.05	1.3×10 <sup>-4</sup>	6.9%	0.05	0.07	0.46	8.3%	0.03	0.06	0.63	4.5%	-0.07	0.03	0.04
					I <sup>2</sup> =	94%			I <sup>2</sup> =	0%				HTN	-0.14	0.19	0.48
														DBP	0.02	0.12	0.85

\* p-values have been taken from GWAS results corrected by the genomic control approach<sup>9</sup>. λ values for HTN: CHARGE (λ=1.04), GBPG (λ=1.01), WGHS (λ=1.07). Beta reflects the unit change in mmHG in SBP/DBP or in log odds of HTN per allele dose. S.E. indicates standard error of the mean. Replicated SNP is in bold.