ORIGINAL ARTICLE

Reevaluation of the association of seven candidate genes with blood pressure and hypertension: a replication study and meta-analysis with a larger sample size

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To obtain evidence for blood pressure (BP) trait association, we conducted an association study of selected candidate gene variants. In Japan, a total of 19 426 individuals underwent testing for genetic associations with systolic BP (SBP)/diastolic BP (DBP) and 9271 individuals (3460 cases and 5811 controls) underwent testing for genetic associations with dichotomous hypertension. Association with seven notable candidate genes was tested, namely, *ACE*, *ADD1*, *ADRB2*, *AGT*, *CYP11B2*, *GNB3* and *NOS3*, followed by a joint meta-analysis involving previously reported multi-study populations, including >20 000 individuals (for SBP/DBP) and >17 000 individuals (for hypertension). BP trait associations at two loci (*AGT* rs699 and *CYP11B2* rs1799998) were consistently replicated in the Japanese association study and joint meta-analysis involving the populations described above. Hypertension association reached genome-wide significance for the two variants, specifically, $P=7.3 \times 10^{-10}$ for *AGT* rs699 and $P=3.9 \times 10^{-8}$ for *CYP11B2* rs1799998. In our study panels, the most significant association was found for *CYP11B2* rs1799998 with all three BP traits: $P=1.5 \times 10^{-5}$ for SBP, $P=1.8 \times 10^{-5}$ for DBP and $P=2.3 \times 10^{-5}$ for hypertension. A suggestive association with SBP ($P=0.042$), DBP ($P=0.01$) and hypertension ($P=1.4 \times 10^{-5}$) was also detected for *ACE* rs4340 (a proxy for *ACE* D/I polymorphism) in the joint meta-analysis. Our data provide evidence for true BP trait associations with two candidate gene variants. These variants were not identified in the previous genome-wide association studies, presumably because they did not reach a given threshold in the discovery stage. Thus, certain variants in genes with clinical and physiological relevance are likely to account for a portion of BP variance in the general population and are worth following up via a target gene approach.

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INTRODUCTION

Despite the high heritability estimate (30–50%) of blood pressure (BP) and hypertension,^{1–3} to date, associations among candidate genes and these traits have rarely been convincingly demonstrated. With recent technological advances, genome-wide association (GWA) studies, which interrogate a large proportion of the common genetic variants (that is, by genotyping hundreds of thousands of common single-nucleotide polymorphisms (SNPs)) throughout the genome, have allowed the investigation of genetic susceptibility to these traits without *a priori* hypotheses regarding the underlying pathophysiology.³ In the first wave of GWA studies,^{4–9} few studies

appeared to identify loci associated with BP traits at a genome-wide significance level ($P<5 \times 10^{-8}$) when adjusted for multiple testing. In 2009, two large-scale meta-analyses of GWA studies from the Global BP Genetics (Global BPgen) and Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) consortia identified a total of 13 independent loci significantly associated with BP.^{10,11} Subsequently, meta-analyses of GWA studies in East Asians¹² and in a combined population of Global BPgen and CHARGE¹³ further identified a total of 21 additional loci. Most of these associations are in or near genes that had not been suspected of involvement in BP regulation. The sample size of genome-wide exploration in these

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meta-analyses ($n = 19608\text{--}69395$)^{10–13} was much larger than that of the previous GWA studies of BP traits,^{4–9} indicating that very large samples are required to identify molecular variants with modest genetic effects on BP variation in the general population. In addition, continuous BP traits are implied to have greater power than dichotomous hypertension traits, at least for the genetic loci commonly affecting both types of traits; that is, the chances of obtaining significant results are higher with a continuous trait, either systolic (SBP) or diastolic (DBP) BP.³

Therefore, we conducted a large-scale association study of eight variants from seven notable candidate genes, namely, *ACE* (angiotensin-converting enzyme), *ADD1* (adducin 1), *ADRB2* (beta-2 adrenergic receptor), *AGT* (angiotensinogen), *CYP11B2* (aldosterone synthase), *GNB3* (G protein, beta-3 subunit), and *NOS3* (nitric oxide synthase 3), in the Japanese ($n = 21851$ in total). The individual contribution of each of these genes to BP variation and/or hypertension in the general population has not been thoroughly elucidated, although a number of studies have repeatedly investigated them as candidate genes.^{14–20} Moreover, we performed meta-analyses of the current data on the Japanese and the data from previous studies to verify consistency and to increase the statistical power of SNP trait associations.

METHODS

Study population

Detailed characteristics of the subjects analyzed in each study panel are described in the Supplementary Information and Table 1. Our genetic studies for BP and hypertension were originally organized as part of an ongoing GWA study for cardiometabolic disorders among Japanese subjects in the Cardiometabolic Genome Epidemiology (CAGE) network.²¹ Three CAGE study panels were used for the association analysis with continuous BP traits, SBP and DBP, and dichotomous hypertension. The CAGE study panel 1 comprised 1526 Japanese subjects who were genotyped for a GWA scan and included 403

cases and 452 controls. The CAGE study panel 2 involved 5331 Japanese subjects (hereafter known as the Amagasaki cohort) consecutively enrolled in a population-based setting as described elsewhere²² and 2425 subjects primarily recruited for the case-control study; in total, 1750 cases and 1776 controls were selected for case-control comparison from panel 2 (see the footnote to Table 1). The CAGE study panel 3 also comprised 12 569 subjects (hereafter called the Fukuoka cohort) who were randomly selected from the general Japanese population.²³ From panel 3, 1307 cases and 3583 control subjects were further chosen for case-control comparison. All participants in these studies provided written informed consent, and the local ethics committees approved the protocols.

BP levels were classified according to the Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH2009) as described elsewhere.²⁴ Cases (except participants in the Fukuoka cohort) were enrolled from clinical practices or during annual medical checkups at medical institutions and university hospitals in accordance with uniformly defined criteria. These criteria included (i) SBP ≥ 160 mm Hg and/or DBP ≥ 100 mm Hg for untreated subjects (Grade II and III hypertension in JSH2009); (ii) currently receiving chronic antihypertensive treatment(s); (iii) no secondary form of hypertension as evaluated by an extensive workup, including serum creatinine and electrolytes, chest radiography, electrocardiogram, urinalysis, and other hematological screening tests; and (iv) age of hypertension onset ≤ 60 years. Normotensive controls were defined as follows: (i) SBP < 130 mm Hg and DBP < 85 mm Hg without antihypertensive treatments (normal BP in JSH2009) and (ii) age ≥ 50 years.

SNP genotyping

In panel 1, genotyping was performed with the Infinium HumanHap550 BeadArray system (Illumina, San Diego, CA, USA), interrogating 555352 SNPs as previously described.²¹ The lambda value for the genomic control was 1.00 to 1.02, indicating the absence of systematic confounding, such as population stratification, in our GWA study samples (panel 1). With the exception of four variants (which were on the HumanHap550 BeadArray) in panel 1, samples were genotyped using the TaqMan assay for eight SNPs from seven unique candidate genes (Table 2).^{14–20} These genes included *AGT* (rs699, M235T),

Table 1 Clinical characteristics of study sample

Study panel	N	Women (%)	Age, years (s.d.)	SBP mm Hg (s.d.)	DBP mm Hg (s.d.)	BMI kg m ⁻² (s.d.)	HTN (%)	Antihypertensive therapy (%)
CAGE study panel 1								
GWA study sample								
Total subjects examined	1526	43.1	66.1 (8.0)	134.1 (20.3)	76.8 (12.0)	23.5 (3.3)	52.7	38.2
Cases	403	37.0	63.3 (7.5)	149.0 (20.3)	85.3 (13.0)	24.8 (3.5)	100	74.7
Controls	452	51.0	64.4 (7.1)	115.4 (10.5)	69.0 (8.2)	22.2 (2.7)	0	0
CAGE study panel 2								
Population cohort								
Amagasaki Study	5331	39.8	47.8 (12.3)	124.3 (17.3)	75.9 (11.0)	23.0 (3.2)	21.5	9.0
Case-control study sample*								
Cases	1750	40.0	58.9 (11.2)	157.1 (19.6)	96.3 (11.3)	24.5 (3.4)	100	73.5
Controls	1776	44.1	60.8 (8.6)	117.9 (12.1)	73.5 (8.6)	22.4 (2.8)	0	0
CAGE study panel 3								
Population cohort								
Fukuoka Cohort Study	12 569	54.9	62.6 (6.8)	138.9 (21.2)	83.9 (11.7)	23.1 (3.0)	57.9	23.9
Case-control study sample								
Cases	1307	46.4	56.1 (2.7)	162.7 (17.1)	101.7 (10.2)	24.2 (3.4)	100	53.6
Controls	3583	68.1	60.4 (6.4)	116.5 (8.8)	73.5 (6.8)	21.9 (2.7)	0	0

Abbreviations: BMI, body mass index; CAGE, Cardiometabolic Genome Epidemiology Network; DBP, diastolic blood pressure; SBP, systolic blood pressure. Hypertension (HTN) is defined as SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg or taking antihypertensive medication.

*In the case-control study samples of panel 2, 204 cases and 897 controls were derived from the Amagasaki Study panel, whereas the remaining subjects were enrolled independently.

ADD1 (rs4961, G460W), *ADRB2* (rs1042713, R16G; rs1042714, E27Q), *NOS3* (rs1799983, D298E), *CYP11B2* (rs1799998, -344C/T), *GNB3* (rs5443, C825T) and *ACE* (rs4341,²⁵ a proxy SNP for D/I polymorphism). The genotypic distribution of all tested SNPs was in Hardy–Weinberg equilibrium ($P > 10^{-3}$). We obtained successful genotyping call rates of $>99\%$ for the entire characterized sample.

Statistical Analyses

SNP association analysis. The SNPs were tested for associations with BP and hypertension using linear regression analysis and the Cochran–Armitage trend test, respectively. In the linear regression models, we adjusted continuous SBP and DBP for age, age squared, and body mass index stratified by sex (all panels) and sample enrollment site (panel 1 only). For individuals receiving antihypertensive therapies, BP was imputed by adding 15 mm Hg and 10 mm Hg to SBP and DBP, respectively.^{10,21,26} One-tailed $P < 0.006 \approx 0.05/8$ (two-tailed $P < 0.012$) was considered significant with Bonferroni's correction for multiple testing; for an association to be considered significant, it had to involve the same risk allele as that previously reported and was assessed accordingly with the one-tailed test. For both the quantitative trait analysis and case–control study, the genetic effects estimated in each of the Japanese panels were combined with the inverse variance method (Supplementary Information). We used PLINK, the R software, and the *rmeta* and *meta* packages to test for the associations.

Meta-analysis. First, the previous multi-ethnic meta-analysis studies of each candidate gene variant were identified in the PubMed database and included in the current meta-analysis. When >1 meta-analysis studies were identified per variant, only the latest and most comprehensive one was used. Next, we identified additional studies published after the chosen meta-analysis studies by extended computer-based searches of PubMed. In terms of BP association, meta-analyses had not been published, except for the *AGT* and *NOS3* variants.^{17,20} In these searches, we used the following search criteria: ('gene name' or 'gene symbol (official and alias)') and 'polymorphism' and ('hypertension' or 'blood pressure'). The retrieved studies were read in their entirety to assess their appropriateness for inclusion in the meta-analysis; eligible studies were included if they fulfilled the following criteria: (i) evaluated one of the eight variants using either a case–control or cross-sectional design; (ii) contained non-overlapping data; (iii) showed the relevant data to calculate the effect size and its variance; and (iv) involved a sample size >1000 (for either case–control or quantitative trait analysis). We included the fourth criterion to reduce possible fluctuation in genetic effects due to small sample sizes, that is, publication bias, which seemed to be particularly pertinent to studies that were published after the meta-analysis report(s) of a given variant.

The main endpoints were the influence of candidate gene variants on BP as continuous traits (SBP and DBP) and the risk of hypertension as a categorical variable. The strength of association with hypertension was measured using an odds ratio (OR) and its 95% confidence interval (CI). Heterogeneity among the study results was formally assessed using Cochran's Q-statistic or Woolf's test, with $P < 0.05$ being considered to be significant.

RESULTS

Replication of eight variants from seven candidate genes in Japanese

From the three CAGE study panels, a total of 19 426 individuals and 9271 individuals (3460 cases and 5811 controls) were subjected to quantitative trait analysis and a case–control study, respectively (Table 1). Among the eight variants tested in the Japanese, rs1799998 in *CYP11B2* had the most significant association with all three BP traits: $\beta = 0.91$ mm Hg, $P = 1.5 \times 10^{-5}$ for SBP; $\beta = 0.53$ mm Hg, $P = 1.8 \times 10^{-5}$ for DBP; and OR = 1.15 (95% CI, 1.08–1.23), $P = 2.3 \times 10^{-5}$ for hypertension (Table 2). A significant association (two-tailed $P < 0.012$) was also detected for rs699 in *AGT* with SBP ($\beta = 0.78$ mm Hg, $P = 0.002$) and DBP ($\beta = 0.49$ mm Hg, $P = 0.001$), whereas a borderline association was replicated for

Table 2 Blood pressure and hypertension association at seven candidate genes in the Japanese population

Chr	Gene	Common variant name	SNP ID	Risk/ non-risk allele	Risk allele freq.*	Systolic blood pressure (N = 19 426)			Diastolic blood pressure (N = 19 426)			Hypertension (3460 case/5811 control)					
						Beta (mm Hg)	s.e.	P-value	Phet	Beta (mm Hg)	s.e.	P-value	Phet	OR	(95% CI)	P-value	Phet
1	AGT	T235M	rs699	C/T	0.81	0.78	0.26	0.002	0.385	0.49	0.15	0.001	0.253	1.09	(1.01, 1.19)	0.033	0.768
4	ADD1	W460G	rs4961	T/G	0.55	0.05	0.20	0.789	0.559	0.13	0.12	0.258	0.412	0.99	(0.93, 1.05)	0.805	0.656
5	ADRB2	R16G	rs1042713	A/G	0.48	0.07	0.20	0.708	0.009	0.14	0.12	0.235	0.012	1.06	(1.00, 1.13)	0.071	0.159
5	ADRB2	Q27E	rs1042714	C/G	0.93	-0.35	0.39	0.368	0.229	-0.24	0.23	0.289	0.106	1.06	(0.93, 1.20)	0.391	0.582
7	NOS3	D298E	rs1799983	T/G	0.08	0.67	0.37	0.071	0.901	0.32	0.22	0.144	0.499	1.03	(0.91, 1.17)	0.615	0.558
8	CYP11B2	-344T/C	rs1799998	T/C	0.65	0.91	0.21	1.5E-05	0.794	0.53	0.12	1.8E-05	0.912	1.15	(1.08, 1.23)	2.3E-05	0.659
12	GNB3	T825C	rs5443	T/C	0.50	-0.32	0.20	0.107	0.938	-0.19	0.12	0.096	0.906	0.97	(0.91, 1.03)	0.340	0.539
17	ACE	D/I	rs4341	G/C	0.35	0.22	0.21	0.288	0.004	0.16	0.12	0.199	0.048	1.05	(0.98, 1.12)	0.145	0.018

Abbreviations: CAGE, Cardiometabolic Genome Epidemiology Network; freq., frequency; SNP, single-nucleotide polymorphism.

In the genome-wide association study sample (CAGE study panel 1), proxy SNPs were genotyped at two loci: rs10087214 at *CYP11B2* ($r^2 = 1.0$ to rs1799998 in HapMap JPT/CHB) and rs2285084 at *ADD1* ($r^2 = 0.951$ to rs4961 in HapMap JPT/CHB); rs4961 is a proxy to rs4341, see Nanri *et al.*⁽²⁵⁾.

Inter-panel heterogeneity of per-allele effect (Phet) among CAGE study panels 1–3 is assessed by the Cochran's Q-test for blood pressure and the Woolf's test of heterogeneity for a dichotomous trait of hypertension. *Risk allele frequency is estimated from the data for 5,811 control samples in the case-control study.

this variant with hypertension (OR=1.09 (95% CI, 1.01–1.19), $P=0.033$). For the two SNPs mentioned above, the major alleles—T of rs1799998 and C of rs699—were associated with elevated levels of SBP and DBP and increased risks of hypertension in accordance with the previous meta-analysis findings.^{17,18}

Upon examining the association results separately by panel, we found that the direction and strength of the associations with BP traits were almost concordant for the two SNPs; that is, *CYP11B2* rs1799998 and *AGT* rs699 (Supplementary Table 1). In addition, we found significant associations of *ADRB2* rs1042713 and *ACE* rs4341 with SBP ($P=0.004$ for rs4341), DBP ($P=0.005$ for rs1042713 and $P=0.0118$ for rs4341), and/or hypertension ($P=0.008$ for rs1042713 and $P=0.002$ for rs4341) in the CAGE study panel 3, in directions that were consistent with those of previous studies,^{16,27,28} however, these associations were not replicable in panel 1 and panel 2 (Supplementary Table 1).

Joint meta-analysis for seven candidate genes

In the PubMed database, we identified multi-ethnic meta-analyses of BP association for two (of eight tested) SNPs and those of hypertension association for eight SNPs (Supplementary Tables 2 and 3).^{14–20} Compared with Japanese samples genotyped in this study, the relative increase in joint meta-analysis samples appeared to be modest (that is, 5–40%) at all loci except *AGT* rs699 for SBP/DBP association, whereas this increase was prominent (that is, 105–586%) for hypertension association, thereby providing the joint meta-analysis, in particular, for case-control study (5757–17399 cases vs. 11240–23338 controls) with reasonable power. For SBP and DBP, there were significant associations (two-tailed $P<0.012$, putative risk-allele homozygote vs. non-risk-allele homozygote) with *AGT* rs699 ($P=0.001$ for SBP), *CYP11B2* rs1799998 ($P=2.0 \times 10^{-6}$ for SBP; $P=3.1 \times 10^{-6}$ for

DBP), and *ACE* rs4340 ($P=0.01$ for DBP) (Supplementary Table 2). For hypertension, conversely, reproducible association was detected at *AGT* rs699 ($P=7.3 \times 10^{-10}$), *CYP11B2* rs1799998 ($P=3.9 \times 10^{-8}$), and *ACE* rs4340 ($P=1.4 \times 10^{-5}$) (Figures 1 and 2 and Supplementary Table 3). In this study, heterogeneity analyses in multi-study populations identified some heterogeneity at *ACE* rs4340 ($P_{\text{hetero}}=0.019$, DD vs. II).

DISCUSSION

We examined evidence for the association of three BP traits (SBP, DBP and hypertension) with seven notable candidate genes assumed to have etiological roles in BP regulation. We conducted a large-scale replication study by testing eight previously reported variants in a total of >21 000 Japanese individuals from the CAGE network followed by joint meta-analyses involving an additional sample of 876–17492 individuals for BP association and 10 133–54764 individuals (cases plus controls) for hypertension association. Notably, BP trait associations at two loci (*AGT* and *CYP11B2*) were consistently replicated in both a Japanese association study in the CAGE network and the joint meta-analysis of multi-study populations. In addition, a suggestive association with DBP and hypertension was detected for *ACE* rs4340 in the joint meta-analysis. For the first time, our data demonstrate that an association with hypertension of two candidate variants, namely, *AGT* rs699 and *CYP11B2* rs1799998, reached genome-wide significance ($P<5.0 \times 10^{-8}$), thereby confirming the validity of the target gene approach, which has been an issue of debate, given the complex nature of hypertension genetics.

The necessity of large study samples (roughly >10 000 individuals) has been recognized in identifying genes associated with BP traits following the great success of consortium-based GWA studies.^{10–12} Until those studies were conducted, people had raised general

Figure 1 Forest plots showing the association with BP traits (SBP, DBP and hypertension) in the studies included in the present meta-analysis (individually and in meta-analysis) for the two SNPs robustly associated ($P<5 \times 10^{-8}$) with hypertension. See also Supplementary Tables 2 and 3. For dichotomous hypertension, the effect size (OR and 95% CI) is depicted for risk/risk vs. non-risk/non-risk homozygous subjects

Figure 2 Forest plots showing the association with hypertension in the studies included in the present meta-analysis (individually and in meta-analysis) for the five SNPs tested for hypertension association, except *AGT* M235T, *CYP11B2* -344T/C and *GNB3* C825T. The corresponding data (the effect size for risk/risk vs. non-risk/non-risk homozygous subjects) are not available for *GNB3* C825T.

concerns about the utility of genetic association studies for BP traits. Effect sizes of each risk allele have turned out to be modest for the variants previously identified in the GWA studies using common SNPs;^{10–12,29} 1 mm Hg for SBP and 0.5 mm Hg for DBP, on average, and 5–20% increase in dichotomous disease risk. On the basis of these findings, it appears that solid evidence of genetic association with BP traits is attainable when conducting large-scale association studies. The present study did enable us accordingly to determine association signals at several candidate SNP loci.

Components of the renin–angiotensin system (RAS) were most repeatedly investigated as candidate genes for essential hypertension. In particular, suggestive or significant association was detected in certain studies for the angiotensinogen M235T (*AGT* rs699),^{17,30} angiotensin I-converting enzyme D/I (a proxy for *ACE* rs4340),^{27,28} and aldosterone synthase C-344T (*CYP11B2* rs1799998)^{18,31} polymorphisms, although the results remained largely inconclusive.^{32–35} Besides reproducible associations in the present study (Figure 1), a recent, large-scale association study of hypertension drug target genes in populations of European descent (involving a total of 86 588 individuals)³⁶ confirmed BP trait associations with two candidate genes—*AGT* and *ACE*—in the RAS. Using the GWA scan data without focusing on particular variants selected *a priori*, the authors reported that rs2004776 in *AGT* ($P=3.8 \times 10^{-6}$ for SBP, $P=5.0 \times 10^{-8}$ for DBP, and $P=3.7 \times 10^{-7}$ for hypertension) and rs4305 in *ACE* ($P=3.0 \times 10^{-5}$ for SBP, $P=4.6 \times 10^{-4}$ for DBP, and $P=6.0 \times 10^{-5}$ for hypertension) showed prominent association signals in each gene.³⁶ Two pairs of SNPs—rs2004776 and rs699 in *AGT* ($r^2=0.362$ in HapMap JPT/CHB; $r^2=0.49$ in HapMap CEU) and rs4305 and rs4341 in *ACE* ($r^2=0.80$ in HapMap JPT/CHB; $r^2=0.48$ in HapMap CEU)—exhibit moderate to high levels of

linkage disequilibrium in East Asian and European populations, suggesting a potential common link between the studies. Another locus in the RAS demonstrating significant BP trait association in the present study is *CYP11B2* rs1799998. This SNP (T to C substitution in the 5'-distal promoter region of the gene at position -344) disrupts a putative recognition site for the transcriptional factor, steroidogenic factor 1, and is considered to have functional relevance to gene expression,³⁷ despite conflicting reports about its association with hypertension. One previous meta-analysis involving 5343 cases and 5882 controls reported a significant association with hypertension, OR = 0.834 (95% CI, 0.76–0.914) for CC vs. TT, $P<0.0001$.¹⁸ By further increasing the sample size, hypertension association of *CYP11B2* rs1799998 reached genome-wide significance with an almost equivalent effect size (OR = 0.81 for non-risk alleles, $P=3.9 \times 10^{-8}$), and BP association was also found to be highly significant ($P=2.0 \times 10^{-6}$ for SBP and $P=3.1 \times 10^{-6}$ for DBP) in our meta-analysis (Supplementary Tables 2 and 3).

The strength of the current study lies in its extensive, quantitative trait analysis conducted in two general Japanese populations, specifically, the Amagasaki ($n=5331$, CAGE panel 2) and Fukuoka ($n=12569$, CAGE panel 3) cohorts, in addition to the GWA study samples ($n=1526$, CAGE panel 1), which have been validated for the absence of systematic confounding such as population stratification (see Methods) with genome-wide SNP genotyping data.²¹ These cohorts are among the largest reported to date for quantitative trait analysis of BP (Supplementary Table 2) and hypertension case-control studies (Supplementary Table 3). Nevertheless, given the relatively modest effect sizes for BP/hypertension susceptibility loci, statistical power may not be sufficient to robustly confirm or refute the BP trait associations in the present study (Figure 2 and

Supplementary Table 4). Moreover, several limitations of the use of continuous BP traits in the association study must be noted. For instance, we applied treatment adjustments to impute expected baseline effects; that is, the individuals receiving antihypertensive therapies were imputed to have 15 mm Hg higher SBP and 10 mm Hg higher DBP than the observed measurements. Although this imputation has been shown to improve statistical power as a whole,²⁶ it could lead to over- or underadjustment at the individual level, depending on the treatment intensity. The confounding effects of non-genetic factors (for example, obesity, sex, age and lifestyle factors such as diet) may also result in inter-individual variation in BP measurements and hamper the identification of true association signals to a substantial extent,²¹ although determining the effects of these factors is beyond the scope of the present study.

Apart from a targeted, candidate gene approach,³⁶ neither of the two loci (*AGT* and *CYP11B2*) has been reported to show genome-wide significant association with BP traits via GWA scans. Because *AGT* rs699 and *CYP11B2* rs1799998 themselves and/or their proxies are included in the list of SNPs that are assayed by most of the GWA scan platforms, it is unlikely that these SNPs have failed to be tested for BP trait associations in the previous GWA studies.^{4–13} Rather, it is probable that the associations were not followed up in the multi-staged screening approach, principally because their statistical significance in the discovery stage did not attain a given threshold (for example, $P < 1 \times 10^{-5}$). This value may not indicate the lack of modest association at these loci, as we reported in the present study (Figure 3). During the preparation of our manuscript, a multi-staged

GWA study of hypertension reported a new susceptibility locus (rs3918226) in the promoter region of *NOS3* in populations of European descent;³⁸ this SNP does not appear to be polymorphic in East Asians (according to HapMap JPT/CHB data), although further examination is warranted.

In the present study, we chose seven gene variants based on the existing biological knowledge of the genetics of hypertension. In addition to studying such 'biologically plausible candidate genes', the target gene approach should be used to test suggestive associations with 'positional candidate genes', such as those genes (for example, *ADD2* and *M6PR*) that we previously reported in a genome-wide exploratory approach,⁵ which was, by current standards, modest with regard to sample size.

In summary, our data provide evidence for true BP trait associations with two candidate gene variants that were not identified in previous GWA studies.^{4–13} Although the effect sizes could be rather modest, certain variants in genes with clinical and physiological relevance are likely to account for a proportion of the BP variance in the general population and are worth further investigation via the target gene approach to complement GWA scans.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Figure 3 Relationship of two genome-wide significant loci (two loci on the left hand) and those previously reported²¹ (seven loci on the right hand, which are presented here for the purpose of comparability of effect sizes) for SBP, DBP and hypertension. Bars at each locus show the effects of each variant on continuous SBP and DBP and on the OR for dichotomous hypertension compared with normotension. For comparability, SBP and DBP effects are shown on the s.d. scale (SBP s.d. = 22.2 mm Hg, DBP s.d. = 12.7 mm Hg). In addition, for comparability, the OR for hypertension is scaled to have the same average level as BP over the nine loci. Whiskers indicate standard error. Alleles are coded as shown in Table 2 and in the previous report.²¹ See Supplementary Tables 2 and 3 for the association with BP traits at *AGT* rs699 (M235T) and *CYP11B2* rs1799998 (−344T/C). A full color version of this figure is available at the *Hypertension Research* journal online.

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