

## ORIGINAL ARTICLE

# Recapitulation of two genomewide association studies on blood pressure and essential hypertension in the Korean population

Kyung-Won Hong<sup>1</sup>, Hyun-Seok Jin<sup>1</sup>, Ji-Eun Lim<sup>1</sup>, Sangsoo Kim<sup>2</sup>, Min Jin Go<sup>3</sup> and Bermseok Oh<sup>1</sup>

Essential hypertension causes high rates of morbidity and mortality, primarily due to its complications, and its development is regulated by genetic risk and environmental factors. However, until recent genomewide association studies (GWASs) were reported, the genetic factors were unknown. Two GWASs on systolic blood pressure (SBP), diastolic blood pressure (DBP) and hypertension in Caucasians—Global Blood Pressure Genetics (Global BPgen) and Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE)—reported 51 single-nucleotide polymorphisms (SNPs) in 12 loci at  $P < 4 \times 10^{-7}$ . Because the prevalence, age of onset and severity of complications of hypertension vary between ethnic groups, we wanted to investigate these results in other ethnic groups. We examined the association of 27 of the 51 SNPs in 8512 unrelated individuals from Korean Association REsource (KARE), a GWAS that was based on epidemiological cohorts in Korea. Four loci—ATP2B1 (ATPase, Ca<sup>++</sup> transporting, plasma membrane 1), CSK (c-src tyrosine kinase), CYP17A1 (cytochrome P450 17A1) and PLEKHA7 (pleckstrin homology domain-containing family A member 7)—were associated with blood pressure and hypertension in the Korean population.

*Journal of Human Genetics* (2010) 55, 336–341; doi:10.1038/jhg.2010.31; published online 23 April 2010

**Keywords:** blood pressure; CHARGE; global BPgen; KARE; replication

## INTRODUCTION

Hypertension, which is defined as systolic blood pressure (SBP) of  $\geq 140$  mm Hg and/or diastolic blood pressure (DBP) of  $\geq 90$  mm Hg, is a polygenic disease<sup>1</sup> that is regulated by complex interactions between networked genes and environmental stimuli. Hypertension likely contributes to the development of metabolic syndrome and cardiovascular risk factors.<sup>2,3</sup> To identify the genetic risk factors for hypertension, two genomewide association studies (GWASs) were recently reported by the Global Blood Pressure Genetics (Global BPgen)<sup>4</sup> consortium and the Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) consortium.<sup>5</sup>

The study by Global BPgen performed three-stage analyses of 118 547 subjects of primarily European ancestry,<sup>4</sup> identifying the significant genomewide ( $P < 5 \times 10^{-7}$ ) associations in eight loci that are listed in Supplementary Table S1a. In another GWAS, the CHARGE consortium,<sup>5</sup> examining 29 136 individuals from six population-based cohorts, conducted a meta-analysis of all significant single-nucleotide polymorphisms (SNPs;  $P < 1 \times 10^{-6}$ ). Supplementary Table S1b lists the 43 SNPs that were significantly associated with SBP, DBP or hypertension status.

In a previous study, we reported the GWAS results of Korean Association REsource (KARE) using 8842 unrelated individuals who

were recruited from two community-based epidemiological cohorts.<sup>6</sup> Eight novel loci associated with seven quantitative traits and a plasma membrane calcium pump, ATP2B1 (ATPase, Ca<sup>++</sup> transporting, plasma membrane 1), were significantly associated with blood pressure in Koreans. In this study, we analyzed the association of the SNPs that were reported by two blood pressure consortiums, Global BPgen and CHARGE, to determine whether these SNPs also influenced blood pressure and hypertension risk in the Korean population.

## MATERIALS AND METHODS

### Subjects

Subjects and their genotypes were reported in a previous GWAS.<sup>6</sup> In brief, subjects came from two community-based cohorts, the rural community Ansung and the urban community Ansan, in Kyunggi-Do province, near Seoul, Korea. The initial numbers of individuals, aged 40–69 years, were 5018 and 5020 from Ansung and Ansan, respectively. This study was approved by the international review board committees of the Korea National Institute of Health.

The basic characteristics and blood pressures of the patients are listed in Table 1. For this study, blood pressure measurements were taken three times in the supine position. Before the first measurement, participants rested for 5 min, and three measurements were taken at least 30 s apart. The average of the three measurements was used for this study. Hypertensive status was defined as

<sup>1</sup>Department of Biomedical Engineering, School of Medicine, Kyung Hee University, Seoul, Korea; <sup>2</sup>Department of Bioinformatics, Soongsil University, Seoul, Korea and <sup>3</sup>Center for Genome Science, National Institute of Health, Seoul, Korea  
Correspondence: Dr B Oh, Department of Biomedical Engineering, School of Medicine, Kyung Hee University, 1 Hoeki-dong, Dongdaemun-gu, Seoul 130-702, Korea.  
E-mail: ohbs@khu.ac.kr

Received 15 January 2010; revised 16 March 2010; accepted 18 March 2010; published online 23 April 2010

**Table 1** Basic characteristics of study subjects

	Total	Ansung	Ansan	P-value
<i>Quantitative trait analysis</i>				
Number of individuals	7551	3470	4081	
Gender (men (%) /women (%))	3747 (50)/3804 (50)	1584 (46)/1886 (54)	2163 (53)/1918 (47)	<0.01
Age (mean year $\pm$ s.d.)	51.44 $\pm$ 8.79	55.0 $\pm$ 8.8	48.4 $\pm$ 7.5	<0.01
BMI (mean kg m <sup>-2</sup> $\pm$ s.d.)	24.42 $\pm$ 3.08	24.2 $\pm$ 3.4	24.6 $\pm$ 2.9	<0.01
SBP (mean mm Hg $\pm$ s.d.)	115.65 $\pm$ 17.25	120.5 $\pm$ 17.2	111.6 $\pm$ 16.21	<0.01
DBP (mean mm Hg $\pm$ s.d.)	74.21 $\pm$ 11.27	76.6 $\pm$ 10.0	72.2 $\pm$ 11.9	<0.01
<i>Case-control analysis</i>				
Control (SBP <120 and DBP <80mm Hg)	4451	1701	2751	
Subjects with hypertension therapy	961	579	382	
Cases (subjects of untreated hypertensive patients and of hypertension therapy)	1968	1181	787	

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure. P-values were obtained using the  $\chi^2$  test for sex and by independent Student's *t*-test for other variables.

having SBP of  $\geq 140$  mm Hg and/or DBP of  $\geq 90$  mm Hg, and normal controls were defined as having SBP of <120 mm Hg and DBP of <80 mm Hg, excluding the prehypertensive status.

### Genotyping and quality control

Most DNA samples were isolated from the peripheral blood of participants and genotyped using Affymetrix Genomewide Human SNP array 5.0 (Affymetrix, Inc., Santa Clara, CA, USA). The quality controls are described in a previous report.<sup>6</sup> In brief, the accuracy of the genotyping was determined by Bayesian Robust Linear Modeling using the Mahalanobis distance genotyping algorithm.<sup>7</sup>

Samples that had accuracies of <98% and a high missing genotype call rate ( $\geq 4\%$ ), high heterozygosity (>30%) or inconsistency in sex were excluded from subsequent analyses. Individuals who had any tumor were excluded. Related individuals whose estimated identity-by-state value was high (>0.80)<sup>8</sup> were also excluded.

After the quality control steps, 8842 samples were selected. To analyze blood pressure traits, 330 subjects who had been treated with drugs that likely influenced blood pressure were also excluded. Finally, we used 8512 individuals. The 351 677 genotyped SNPs had a missing gene call rate <0.1, a minor allele frequency >0.01 and no deviation from the Hardy-Weinberg equilibrium ( $P > 1 \times 10^{-6}$ ).

### Genotype imputation

Of the 51 SNPs that were reported by Global BPgen and CHARGE, only 10 occurred in the KARE genotype data. To maximize the association analysis in Koreans, we used imputation methods using the Chinese-Japanese HapMap data set. An additional 17 imputed SNPs (see Table 2, column G/I) were analyzed in this study.

The detailed imputation procedure has been published earlier.<sup>9</sup> In brief, the KARE data set, comprising 351 677 SNPs for 8842 individuals, was merged with that of International HapMap Phase II JPT (Japanese)+HCB (Chinese) panel 2, resulting in 2 180 896 SNPs. The genotypes of the KARE individuals were imputed using PLINK (version 1.07, <http://pngu.mgh.harvard.edu/~purcell/plink/>).<sup>10</sup> A total of 333 418 SNPs were shared between the KARE and HapMap data sets. The concordance rates for these SNPs were calculated, for which the first quartile and median were 0.964 and 0.987, respectively. Profiling the concordance rates at various intervals of the key parameters that were reported by PLINK allowed us to develop a filtering scheme—PHWE >  $10^{-6}$ , IMPUTED  $\geq 0.9$  and INFO  $\geq 0.9$ —which improved the first quartile and median to 0.985 and 0.994, respectively. From 1 026 596 SNPs that passed the filters, we selected an additional 17 SNPs that matched the 51 SNPs that were reported by the Global BPgen and CHARGE studies.

To identify differences between the KARE (Korean) and other populations (CHARGE, Global BPgen, CEU (European), HCB (Chinese), JPT (Japanese)

and YRI (African)), pairwise  $F_{st}$  distances (measures of genetic differentiation<sup>11</sup>) were calculated for each tested SNP in Table 2.  $F_{st}$  measures the proportion of genetic diversity due to differences in allele frequencies between populations relative to the within-population variation.  $F_{st}$  values were estimated using Arlequin ver. 3.1 (<http://cmpg.unibe.ch/software/arlequin3/>).<sup>12</sup>

### Association tests

*Quantitative trait analysis.* Of the 8512 subjects in this study, 961 subjects were undergoing antihypertensive therapy. For quantitative analysis of SBP and DBP, these individuals were excluded, yielding a final sample size of 7551 individuals. Linear regression was used to analyze SBP and DBP as quantitative traits, controlling for the subject's cohort, age, sex and body mass index. Statistical analyses were performed using PLINK.<sup>10</sup> All tests were based on an additive model, and P-values were not adjusted for multiple tests.

*Case-control analysis.* In addition to 1007 subjects who had SBP of  $\geq 140$  mm Hg and/or DBP of  $\geq 90$  mm Hg, 961 subjects who were using antihypertensive medications were included, generating a total of 1968 cases. The 2029 prehypertensive subjects, defined as having  $120 \leq \text{SBP} < 140$  mm Hg and/or  $80 \leq \text{DBP} < 90$  mm Hg, were excluded from the control groups in the hypertension case-control analysis. The normotensive controls were defined as having SBP of <120 mm Hg and DBP of <80 mm Hg and without antihypertensive treatment. In the end, 1968 hypertensive cases and 4451 normotensive controls were used for the case-control study.

Logistic regression analysis was used for the hypertensive cases and normotensive controls, controlling for cohort, age, sex and body mass index as covariates. Statistical analyses were performed using PLINK.<sup>10</sup> All tests were based on an additive model, and P-values were not adjusted for multiple tests. The estimated sample sizes for 80% study power at  $\alpha=0.05$  in Tables 2 and 3 were respectively based on CHARGE and KARE parameters, including minor allele frequency, effect size and mean value of blood pressure.

## RESULTS

We analyzed 8512 individuals in the KARE study for their genotypic association with blood pressure and hypertension. The basic characteristics of the subjects are listed in Table 1. Of the 51 significant SNPs, 10 that were reported by the Global BPgen and CHARGE were genotyped in the KARE study. To examine more SNPs, imputation was performed, and an additional 17 SNPs were analyzed.

The minor allele frequencies of the 27 SNPs in the KARE data were compared with those from the Global BPgen, CHARGE and dbSNP data. As shown in Table 2, the allele frequencies in the KARE data were similar to those in the HCB (Han Chinese in Beijing, average  $F_{st}$  0.005) and JPT (Japanese in Tokyo, average  $F_{st}$  0.006) groups but

**Table 2** Proximal gene symbol and minor allele frequencies of 27 SNPs reported in Global BPgen and CHARGE

ID	CHR	SNP	G	BP	Near gene symbol	Minor Minor allele	Effect size	Minor allele frequency								Sample size for 80% study power ( $\alpha=0.05$ )
								Global		dbSNP						
								BPgen	CHARGE	KARE	CEU	HCB	JPT	YRI		
K1	1	rs17367504	I	11785365	MTHFR	G	-0.50±0.12	0.14	0.16	0.09	0.17	0.10	0.10	0.06	11676	
K2	2	rs11895934	I	190510498	PMS1	C	0.96±0.19		0.18	0.06	0.20	0.07	0.14	0.25	2881	
K3	2	rs7571613	I	190513907	PMS1	G	0.96±0.19		0.18	0.06	0.22	0.07	0.16	0.23	2881	
K4	2	rs7564968	I	190520217	PMS1	C	0.96±0.19		0.18	0.06	0.22	0.07	0.16	0.15	2881	
K5	3	rs7640747	I	37571809	ITGA9	G	0.56±0.16		0.38	0.14	0.39	0.14	0.13	0.34	5308	
K6	3	rs9815354	I	41887655	ULK4	A	0.60±0.12		0.17	0.16	0.23	0.17	0.12	0.17	7722	
K7	10	rs11014166	I	18748804	CACNB2	T	-0.74±0.15		0.34	0.05	0.37	0.09	0.03	0.11	3190	
K8	10	rs11191548	G	104836168	CYP17A1	C	1.17±0.22	0.09	0.08	0.25	0.06	0.24	0.21	0.02	3891	
K9	11	rs381815	I	16858844	PLEKHA7	T	0.84±0.17		0.26	0.19	0.21	0.15	0.19	0.30	2887	
K10	11	rs11024074	G	16873795	PLEKHA7	C	0.79±0.16		0.28	0.23	0.31	0.13	0.31	0.28	3115	
K11	11	rs7926335	I	16874445	PLEKHA7	T	0.85±0.17		0.26	0.19	0.29	0.11	0.28	0.26	2819	
K12	12	rs2681472	I	88533090	ATP2B1	G	-1.29±0.19		0.17	0.37	0.11	0.37	0.40	0.13	1667	
K13	12	rs2681492	I	88537220	ATP2B1	C	-1.26±0.19		0.19	0.37	0.12	0.37	0.40	0.17	1602	
K14	12	rs11105354	I	88550654	ATP2B1	G	-1.3±0.20		0.17	0.37	0.10	0.37	0.39	0.13	1642	
K15	12	rs12579302	I	88574634	ATP2B1	G	-1.29±0.20		0.17	0.37	0.11	0.37	0.43	0.14	1667	
K16	12	rs17249754	G	88584717	ATP2B1	A	-1.30±0.20		0.17	0.37	0.11	0.34	0.35	0.17	1642	
K17	12	rs11105364	I	88593407	ATP2B1	G	-1.30±0.20		0.17	0.37	0.11	0.36	0.39	0.13	1642	
K18	12	rs11105368	I	88598572	ATP2B1	C	-1.30±0.20		0.17	0.37	0.11	0.36	0.39	0.16	1642	
K19	12	rs11105378	I	88614872	ATP2B1	T	-1.31±0.20		0.17	0.37	0.11	0.36	0.39	0.11	1617	
K20	12	rs1991391	G	113837049	TBX3-TBX5	A	-0.71±0.15		0.35	0.12	0.34	0.18	0.10	0.45	3418	
K21	12	rs2384550	G	113837114	TBX3-TBX5	A	-0.71±0.15		0.35	0.12	0.34	0.18	0.10	0.38	3418	
K22	12	rs6489992	G	113837152	TBX3-TBX5	T	-0.71±0.15		0.37	0.13	0.34	0.18	0.12	0.59	3336	
K23	12	rs10744835	G	113838232	TBX3-TBX5	T	-0.68±0.16		0.30	0.12	0.25	0.18	0.10	0.35	4038	
K24	12	rs7977406	G	113843807	TBX3-TBX5	T	-0.69±0.16		0.30	0.12	0.25	0.17	0.10	0.46	3921	
K25	15	rs1378942	G	72864420	CSK	A	0.62±0.13	0.65	0.65	0.17	0.67	0.17	0.19	0.00	4484	
K26	15	rs6495122	I	72912698	CSK	C	0.64±0.15		0.55	0.13	0.58	0.12	0.20	0.23	3867	
K27	17	rs16948048	G	44795465	ZNF652	C	0.41±0.13	0.37	0.37	0.17	0.35	0.37	0.33	0.15	10011	

Abbreviations: CEU, European; CHARGE, Cohorts for Heart and Aging Research in Genome Epidemiology; dbSNP, single-nucleotide polymorphism database; Global BPgen, Global Blood Pressure Genetics; HCB, Han-Chinese; JPT, Japanese; KARE, Korean Association Resource; SNP, single-nucleotide polymorphism; YRI, sub-Saharan African. G/I indicates the experimental genotyped marker (G) or the imputed marker (I); minor alleles were defined from KARE; minor allele frequencies were referred from Global BPgen and CHARGE;<sup>4,5</sup> dbSNP minor allele frequencies were obtained from the NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp&cmd=search&term=>); sample size for 80% power at  $\alpha=0.05$  is based on CHARGE parameters, including minor allele frequency, effect size and mean value of the markers.

differed from those of the Global BPgen, CHARGE and CEU cohorts (Caucasians in Europe, average  $F_{st}$  0.085, 0.057 and 0.075, respectively).

The association results are shown in Table 3 for SBP, DBP and hypertension, as are significant  $P$ -values. In all, 14 SNPs in 4 genes were significantly associated with blood pressure and hypertension with  $P$ -value  $<0.05$ .

The strongest signals were detected in the *ATP2B1* gene region of chromosome 12q21.3 ( $P$ -value  $<5\times 10^{-7}$  for all three blood pressure traits). rs17249754 had the strongest association of the SNPs in the *ATP2B1* gene region, and individuals with minor allele (A) of rs17249754 experienced significantly lower SBP ( $\beta=-1.34\pm 0.26$ ,  $P=3.35\times 10^{-7}$ ), DBP ( $\beta=-0.90\pm 0.18$ ,  $P=3.76\times 10^{-7}$ ) and hypertension risk (odds ratio=0.79, confidence interval 0.72–0.86,  $P=1.60\times 10^{-7}$ ).

The second set of robust signals for all three blood pressure traits were detected in the *CSK* (*c-src tyrosine kinase*) gene region of chromosome 15q23–q25. In KARE, individuals who had minor allele (A) of rs1378942 showed significantly reduced SBP ( $\beta=-1.42\pm 0.34$ ,  $P=2.53\times 10^{-5}$ ), DBP ( $\beta=-0.94\pm 0.23$ ,  $P=4.20\times 10^{-5}$ ) and hypertension risk (odds ratio=0.73, confidence interval 0.65–0.89,  $P=2.09\times 10^{-7}$ ).

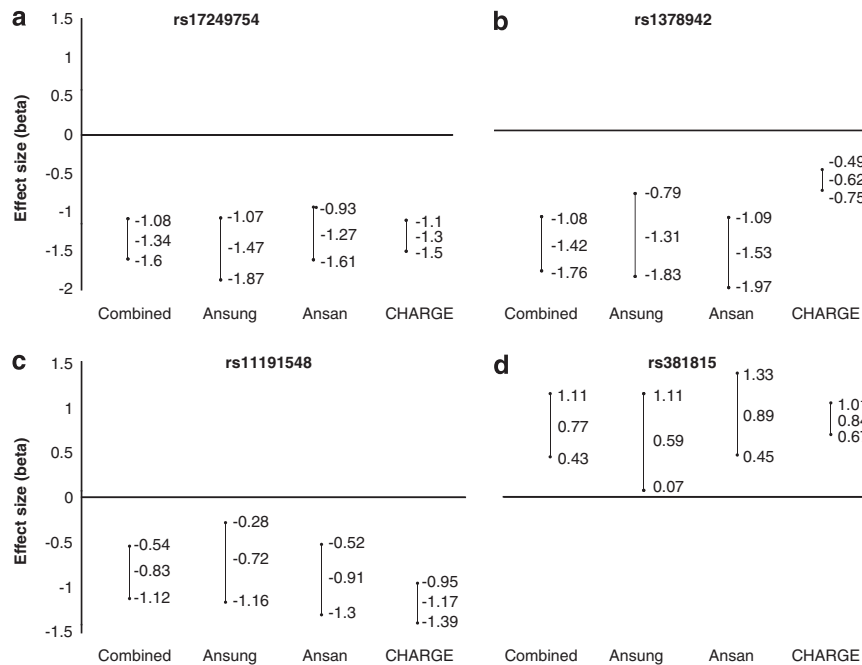
The remaining two signals were found in the *CYP17A1* (*cytochrome P450 17A1*) and *PLEKHA7* (*pleckstrin homology domain-containing, family A member 7*) loci. Individuals who had the minor allele (C) of rs11191548 in *CYP17A1* experienced significantly reduced SBP ( $\beta=-0.83\pm 0.29$ ,  $P=4.76\times 10^{-3}$ ), DBP ( $\beta=-0.51\pm 0.20$ ,  $P=1.01\times 10^{-2}$ ) and hypertension risk (odds ratio=0.84, confidence interval 0.76–0.92,  $P=4.85\times 10^{-4}$ ). With regard to *PLEKHA7*, individuals who had the minor allele (T) of rs381815 showed significantly increased SBP ( $\beta=0.77\pm 0.34$ ,  $P=2.28\times 10^{-2}$ ), DBP ( $\beta=0.35\pm 0.23$ ,  $P>0.05$ ) and hypertension risk (odds ratio=1.19, confidence interval 1.07–0.33,  $P=1.70\times 10^{-3}$ ).

To determine the differences in genetic effects between populations, the effects of the SNPs, expressed as the  $\beta$ -values of the regression analysis for SBP, were compared with those in Caucasians, as shown in Figure 1. All four SNPs had similar effects as those that have been reported, and except for rs1378942 in *CSK*, the SNPs had similar effect sizes as those in Caucasians. The extent of the rs1378942 effect was  $-1.42\pm 0.34$  and  $-0.62\pm 0.13$  in Koreans and Caucasians, respectively, showing a greater influence of genetic variations in *CSK* on Koreans. The results on DBP and hypertension were similar to those for SBP (data not shown).

**Table 3** Linear regression analysis results for SBP, DBP and hypertension case-control study using the 27 SNPs in KARE

ID	SNP	Near gene symbol	Affymetrix allele	SBP		Sample size for 80% study		DBP		Sample size for 80% study power ( $\alpha=0.05$ )		Hypertension case-control		Number of cases for 80% study power ( $\alpha=0.05$ )	
				$\beta$	P-value	power ( $\alpha=0.05$ )	$\beta$	P-allele	power ( $\alpha=0.05$ )	OR	Lower	Upper	P-value		
K1	rs17367504	MTHFR	G	-0.22 ± 0.45			-0.32 ± 0.30			0.95	0.82	1.10			
K2	rs11895934	PMS1	C	-0.27 ± 0.53			-0.09 ± 0.36			1.03	0.87	1.23			
K3	rs7571613	PMS1	G	-0.27 ± 0.53			-0.09 ± 0.36			1.03	0.87	1.23			
K4	rs7564968	PMS1	C	-0.27 ± 0.53			-0.09 ± 0.36			1.03	0.87	1.23			
K5	rs7640747	ITGA9	G	0.01 ± 0.38			-0.34 ± 0.25			0.96	0.85	1.09			
K6	rs9815354	ULK4	A	-0.45 ± 0.35			-0.20 ± 0.24			0.99	0.88	1.12			
K7	rs11014166	CACNB2	T	0.13 ± 0.56			-0.31 ± 0.38			1.00	0.83	1.20			
K8	rs11191548	CYP17A1	C	-0.83 ± 0.29	$4.76 \times 10^{-3}$	9037	-0.51 ± 0.20	$1.01 \times 10^{-2}$		10217	0.84	0.76	0.92	$4.58 \times 10^{-4}$	1408
K9	rs381815	PLEKHA7	T	0.77 ± 0.34	$2.28 \times 10^{-2}$	12794	0.35 ± 0.23			26435	1.19	1.07	1.33	$1.70 \times 10^{-3}$	1658
K10	rs11024074	PLEKHA7	C	0.63 ± 0.30	$3.76 \times 10^{-2}$	16609	0.30 ± 0.21			31269	1.14	1.03	1.26	$1.30 \times 10^{-2}$	2553
K11	rs7926335	PLEKHA7	T	0.77 ± 0.34	$2.28 \times 10^{-2}$	12794	0.35 ± 0.23			26435	1.19	1.07	1.33	$1.70 \times 10^{-3}$	1658
K12	rs2681472	ATP2B1	G	-1.33 ± 0.26	$4.10 \times 10^{-7}$	2828	-0.89 ± 0.18	$4.97 \times 10^{-7}$		2696	0.79	0.72	0.86	$1.59 \times 10^{-7}$	619
K13	rs2681492	ATP2B1	C	-1.33 ± 0.26	$4.10 \times 10^{-7}$	2828	-0.89 ± 0.18	$4.97 \times 10^{-7}$		2696	0.79	0.72	0.86	$1.59 \times 10^{-7}$	619
K14	rs11105354	ATP2B1	G	-1.33 ± 0.26	$4.10 \times 10^{-7}$	2828	-0.89 ± 0.18	$4.97 \times 10^{-7}$		2696	0.79	0.72	0.86	$1.59 \times 10^{-7}$	619
K15	rs12579302	ATP2B1	G	-1.33 ± 0.26	$4.10 \times 10^{-7}$	2828	-0.89 ± 0.18	$4.97 \times 10^{-7}$		2696	0.79	0.72	0.86	$1.59 \times 10^{-7}$	619
K16	rs17249754	ATP2B1	A	-1.34 ± 0.26	$3.35 \times 10^{-7}$	2828	-0.90 ± 0.18	$3.76 \times 10^{-7}$		2696	0.79	0.72	0.86	$1.60 \times 10^{-7}$	619
K17	rs11105364	ATP2B1	G	-1.33 ± 0.26	$4.10 \times 10^{-7}$	2828	-0.89 ± 0.18	$4.97 \times 10^{-7}$		2696	0.79	0.72	0.86	$1.59 \times 10^{-7}$	619
K18	rs11105368	ATP2B1	C	-1.33 ± 0.26	$4.10 \times 10^{-7}$	2828	-0.89 ± 0.18	$4.97 \times 10^{-7}$		2696	0.79	0.72	0.86	$1.59 \times 10^{-7}$	619
K19	rs11105378	ATP2B1	T	-1.33 ± 0.26	$4.10 \times 10^{-7}$	2828	-0.89 ± 0.18	$4.97 \times 10^{-7}$		2696	0.79	0.72	0.86	$1.59 \times 10^{-7}$	619
K20	rs1991391	TBX3-TBX5	A	-0.19 ± 0.39			-0.05 ± 0.27				1.01	0.88	1.15		
K21	rs2384550	TBX3-TBX5	A	-0.21 ± 0.39			-0.05 ± 0.27				1.01	0.88	1.15		
K22	rs6489992	TBX3-TBX5	T	0.43 ± 0.38			0.31 ± 0.26				1.05	0.92	1.19		
K23	rs10744835	TBX3-TBX5	T	-0.22 ± 0.40			-0.05 ± 0.27				1.00	0.88	1.14		
K24	rs7977406	TBX3-TBX5	T	-0.23 ± 0.39			-0.03 ± 0.27				1.01	0.89	1.15		
K25	rs1378942	CSK	A	-1.42 ± 0.34	$2.53 \times 10^{-5}$	4100	-0.94 ± 0.23	$4.20 \times 10^{-5}$		3994	0.73	0.65	0.82	$2.09 \times 10^{-7}$	593
K26	rs6495122	CSK	C	-1.15 ± 0.38	$2.27 \times 10^{-3}$	7803	-0.78 ± 0.26	$2.35 \times 10^{-3}$		7240	0.78	0.68	0.89	$2.08 \times 10^{-4}$	1175
K27	Rs16948048	ZNF652	C	0.02 ± 0.34			0.24 ± 0.23				1.08	0.97	1.21		

Abbreviations: BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; KARE, Korean Association Resource; OR, odds ratio; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism. Age, sex, BMI and cohort were used as covariates; P-values are <0.10; sample size for 80% power at  $\alpha=0.05$  is based on KARE parameters, including minor allele frequency, effect size and mean value of the markers.



**Figure 1** Effects of four significant SNPs, based on systolic blood pressure. (a) rs17249754 in *ATP2B1*; (b) rs1378942 in *CSK*; (c) rs11191548 in *CYP17A1*; and (d) rs381815 in *PLEKHA7*.

## DISCUSSION

We examined 27 SNPs that were associated with blood pressure in Global BPgen and CHARGE. Among them, 14 SNPs in 4 genes, *ATP2B1*, *CSK*, *CYP17A1* and *PLEKHA7*, were recapitulated in the Korean population, implying that the functions of these genes in regulating blood pressure are conserved between ethnic groups. In support of this model, the effect sizes of three of the four SNPs that we examined were similar to those in Caucasians. The estimated sample size for 80% study power in Table 2 showed that our sample size was enough to exclude the false-negative result in most cases. However, we could not exclude the possibility that some negative results in this study might come from the low study power ( $1-\beta < 80\%$ ) because the minor allele frequencies and effect sizes of the SNPs in Koreans were different from those in Caucasians.

*ATP2B1* belongs to the family of P-type primary ion transport ATPases. *ATP2B1* regulates the homeostasis of cellular calcium ion levels, which is important in controlling vascular smooth muscle contraction and dilation.<sup>13,14</sup> However, it has been difficult to speculate the exact role of *ATP2B1* in physiology because of the large number of isoforms and the multiplicity of interacting molecules.<sup>15</sup> Related to its role in blood pressure, it is not clear yet whether *ATP2B1* directly regulates the contraction and dilation of vascular smooth muscle by pumping out intracellular  $Ca^{++}$  or it indirectly regulates the blood pressure by transmitting signals from vascular endothelial cells into the vascular smooth muscle cells.

*CSK* is a member of nonreceptor tyrosine kinases, which are widely expressed in human tissues.<sup>16</sup> To date, at least 14 members have been identified, of which the 60-kDa c-Src is the prototype.<sup>16</sup> Of the many Src kinases, c-Src is highly expressed in vascular smooth muscle cells.<sup>17</sup> It is rapidly activated by angiotensin II and regulates signaling events that are associated with vascular smooth muscle cell contraction, growth and migration.<sup>18–20</sup> The direct involvement of *CSK* in mediating blood pressure was not known until the results of recent blood pressure GWASs. We are interested in validating the underlying

mechanism of *CSK* in blood pressure regulation through angiotensin II and vascular smooth muscle cell contraction.

*CYP17A1* encodes a member of the cytochrome P450 superfamily of enzymes.<sup>21</sup> The cytochrome P450 proteins are monooxygenases that catalyze many reactions in drug metabolism and synthesis of cholesterol, steroids and other lipids. Because *CYP17A1* has both 17 $\alpha$ -hydroxylase and 17,20-lyase activity, a deficiency in *CYP17A1* activity causes congenital disorders that are characterized by sexual infantilism and hypertension.<sup>22,23</sup> A total of 66 cases of mutations in *CYP17A1* have been reported (Human Gene Mutation Database: www.hgmd.cf.ac.uk), in more than 15 cases of which patients suffered from hypertension, strongly suggesting that *CYP17A1* regulates blood pressure. One possible underlying mechanism proposes that overexpression of mineralocorticoids in patients who lack *CYP17A1* causes hypokalemic hypertension.<sup>24</sup> Our study, in addition to the two GWASs on blood pressure, confirmed the involvement of *CYP17A1* in blood pressure, and we sought to analyze the differences in levels of mineralocorticoids—such as deoxycorticosterone and corticosterone—renin and aldosterone in the blood of individuals who bear different *CYP17A1* genotypes.

The function of *PLEKHA7* was unknown until last year, when it was reported to be a component of the zonula adherens, a specialized cadherin-based cell–cell junction.<sup>25</sup> *PLEKHA7* seems to promote the incorporation of cadherin clusters, including E-cadherin and p120-catenin, into the higher-order structure of the zonula adherens. Many functions have been proposed for the zonula adherens beyond basic cadherin adhesion, including cell signaling. However, it is difficult to determine how *PLEKHA7* affects blood pressure. One method of examining *PLEKHA7* function in blood pressure would be to identify patients who have mutations in *PLEKHA7* and show familial hypertension. A pathophysiological study in these patients might provide definitive results. Another means might to develop a mouse that overexpresses or underexpresses *PLEKHA7* and measure the phenotypic changes that occur.

Because the prevalence, age of onset, severity and complications of hypertension vary between ethnic groups, we assume that the contributions of dissimilar gene sets differentially affect the development of hypertension in diverse populations.<sup>3</sup> However, the consistent results in this study, suggest that some genetic architecture that affects the development of essential hypertension is similar between Europeans and Asians. It should also be noted that our conclusion is limited by the other 24 SNPs that were associated in the previous GWASs but not tested in this study. Moreover, the allele frequencies of SNPs replicated in Koreans were quite different from those in Caucasians, drawing the possibility that the effect of susceptibility loci may be different between Caucasians and Koreans at the population level.

#### ACKNOWLEDGEMENTS

This study was supported by a grant of the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Replication of Korea (A090318). The consortium for the large-scale genomewide association study was supported by genotyping data (Genomewide association analysis of community-based cohort study; 2007) from the Korean Genome Analysis Project (4845-301) and the Korea National Institute of Health (Korea Center for Disease Control, Ministry for Health, Welfare and Family Affairs), Republic of Korea.

- 1 Saavedra, J. M. Studies on genes and hypertension: a daunting task. *J. Hypertens.* **23**, 929–932 (2005).
- 2 Himmelman, A., Hedner, T., Hansson, L., O'Donnell, C. J. & Levy, D. Isolated systolic hypertension: an important cardiovascular risk factor. *Blood Press* **7**, 197–207 (1998).
- 3 Noto, D., Cefalu, A. B., Barbagallo, C. M., Sapienza, M., Cavera, G., Nardi, I. *et al.* Hypertension and diabetes mellitus are associated with cardiovascular events in the elderly without cardiovascular disease. Results of a 15-year follow-up in a Mediterranean population. *Nutr. Metab. Cardiovasc. Dis.* **19**, 321–326 (2008).
- 4 Newton-Cheh, C., Johnson, T., Gateva, V., Tobin, M. D., Bochud, M., Coin, L. *et al.* Genome-wide association study identifies eight loci associated with blood pressure. *Nat. Genet.* **41**, 666–676 (2009).
- 5 Levy, D., Ehret, G. B., Rice, K., Verwoert, G. C., Launer, L. J., Dehghan, A. *et al.* Genome-wide association study of blood pressure and hypertension. *Nat. Genet.* **41**, 677–687 (2009).
- 6 Cho, Y. S., Go, M. J., Kim, Y. J., Heo, J. Y., Oh, J. H., Ban, H. J. *et al.* A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat. Genet.* **41**, 527–534 (2009).
- 7 Rabe, N. & Speed, T. P. A genotype calling algorithm for Affymetrix SNP arrays. *Bioinformatics* **22**, 7–12 (2006).
- 8 WTCCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661–678 (2007).
- 9 Lee, K. & Kim, S. A scheme for filtering SNPs imputed in 8,842 Korean individuals based on the International HapMap Project data. *Genomics Inform.* **7**, 136–140 (2009).
- 10 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
- 11 Wright, S. The genetical structure of populations. *Ann. Eugen.* **15**, 323–354 (1951).
- 12 Excoffier, L., Laval, G. & Schneider, S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* **1**, 47–50 (2005).
- 13 Afroz, T., Yang, L. L., Wang, C., Gros, R., Kalair, W., Hoque, A. N. *et al.* Calcineurin-independent regulation of plasma membrane Ca<sup>2+</sup> ATPase-4 in the vascular smooth muscle cell cycle. *Am. J. Physiol. Cell Physiol.* **285**, C88–95 (2003).
- 14 Gros, R., Afroz, T., You, X. M., Kabir, G., Van Wert, R., Kalair, W. *et al.* Plasma membrane calcium ATPase overexpression in arterial smooth muscle increases vaso-motor responsiveness and blood pressure. *Circ. Res.* **93**, 614–621 (2003).
- 15 Di Leva, F., Domi, T., Fedrizzi, L., Lim, D. & Carafoli, E. The plasma membrane Ca<sup>2+</sup> ATPase of animal cells: structure, function and regulation. *Arch. Biochem. Biophys.* **476**, 65–74 (2008).
- 16 Martin, G. S. The hunting of the Src. *Nat. Rev. Mol. Cell Biol.* **2**, 467–475 (2001).
- 17 Oda, Y., Renaux, B., Bjorge, J., Saifeddine, M., Fujita, D. J. & Hollenberg, M. D. cSrc is a major cytosolic tyrosine kinase in vascular tissue. *Can. J. Physiol. Pharmacol.* **77**, 606–617 (1999).
- 18 Mureebe, L., Nelson, P. R., Yamamura, S., Lawitts, J. & Kent, K. C. Activation of pp60c-src is necessary for human vascular smooth muscle cell migration. *Surgery* **122**, 138–144 discussion 144–145 (1997).
- 19 Touyz, R. M., He, G., Wu, X. H., Park, J. B., Mabrouk, M. E. & Schiffrin, E. L. Src is an important mediator of extracellular signal-regulated kinase 1/2-dependent growth signaling by angiotensin II in smooth muscle cells from resistance arteries of hypertensive patients. *Hypertension* **38**, 56–64 (2001).
- 20 Touyz, R. M., Wu, X. H., He, G., Park, J. B., Chen, X., Vacher, J. *et al.* Role of c-Src in the regulation of vascular contraction and Ca<sup>2+</sup> signaling by angiotensin II in human vascular smooth muscle cells. *J. Hypertens.* **19**, 441–449 (2001).
- 21 Chung, B. C., Picado-Leonard, J., Haniu, M., Bienkowski, M., Hall, P. F., Shively, J. E. *et al.* Cytochrome P450c17 (steroid 17 alpha-hydroxylase/17,20 lyase): cloning of human adrenal and testis cDNAs indicates the same gene is expressed in both tissues. *Proc. Natl Acad. Sci. USA* **84**, 407–411 (1987).
- 22 Kagimoto, M., Winter, J. S., Kagimoto, K., Simpson, E. R. & Waterman, M. R. Structural characterization of normal and mutant human steroid 17 alpha-hydroxylase genes: molecular basis of one example of combined 17 alpha-hydroxylase/17,20 lyase deficiency. *Mol. Endocrinol.* **2**, 564–570 (1988).
- 23 Yanase, T., Sanders, D., Shibata, A., Matsui, N., Simpson, E. R. & Waterman, M. R. Combined 17 alpha-hydroxylase/17,20-lyase deficiency due to a 7-basepair duplication in the N-terminal region of the cytochrome P45017 alpha (CYP17) gene. *J. Clin. Endocrinol. Metab.* **70**, 1325–1329 (1990).
- 24 Auchus, R. J. The genetics, pathophysiology, and management of human deficiencies of P450c17. *Endocrinol. Metab. Clin. North Am.* **30**, 101–119 (2001).
- 25 Meng, W., Mushika, Y., Ichii, T. & Takeichi, M. Anchorage of microtubule minus ends to adherens junctions regulates epithelial cell-cell contacts. *Cell* **135**, 948–959 (2008).

Supplementary Information accompanies the paper on Journal of Human Genetics website (<http://www.nature.com/jhg>)