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Genome-Wide Association Analyses on Blood Pressure Using Three Different Phenotype Definitions

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Abstract

Hypertension is the most prevalent disease worldwide and is itself a risk factor for cerebral, cardiac, and renal diseases. The inconsistency of candidate genes suggested by previous genomewide association studies (GWASs) may be due to not only differences in study design and genetic or environmental background but also the difference in the power of analysis between continuous traits and discrete traits. We analyzed 352,228 single nucleotide polymorphisms (SNPs) in 8842 unrelated Koreans obtained from Ansan and Ansung cohorts. We performed a series of GWA analyses using three different phenotype models; young hypertensive cases (278 subjects) versus elderly normotensive controls (680 subjects); the upper 25% (2211 hypertensive cases) versus the lower 25% of the SBP distribution (2211 hypotensive controls); and finally SBP and DBP as continuous traits (8842 subjects). The numbers of young hypertensive cases and elderly normotensive controls were not large enough to achieve genomewide significance. The model comparing the upper 25% subjects to the lower 25% of subjects showed a power that was approximate to that of QTL analysis. Two neighboring SNPs of the *ATP2B1* gene, rs17249754 (SBP, $p=2.53^{-10}$; DBP, $p=1.28\times10^{-8}$) and rs7136259 (SBP, $p=1.30\times10^{-9}$; DBP, $p=6.41\times10^{-8}$), were associated with both SBP and DBP. Interestingly, a SNP of the *RPL6* gene, rs11066280, revealed a significant genomewide association with SBP in men only ($p=3.85\times10^{-8}$), and four SNPs located near the *MAN2A1* gene showed a strong association with DBP only in elderly men aged 60-70 years (e.g., rs6421827, $p=4.86\times10^{-8}$). However, we did not observe any gene variant attaining genomewide significance consistently in the three phenotype models except for the *ATP2B1* gene variants. In general, the association signal with blood pressure was stronger in women than in men. Genes identified in GWASs are expected to open the way for prevention, early diagnosis, and personalized treatment of hypertension.

Keywords: genomewide association study, blood pressure, case-control study, continuous trait locus analysis, single nucleotide polymorphism

Introduction

Nearly one billion people (−26%) of the adult population worldwide have hypertension (Kearney *et al.* 2005). According to the Korean National Health and Nutrition Examination Survey (KNHANES 2008, http://knhanes.cdc.go.kr/), the prevalence of hypertension is 26.9% among Korean adults aged above 30 years. Clinically, the diagnosis of hypertension is given when the systolic blood pressure (SBP) and diastolic blood pressure (DB) are above 140/90 mm Hg in the resting position. About 90% to 95% of cases are primary (essential) hypertension, which refers to high blood pressure that is not caused by any other disorders (Carretero and Oparil, 2000).

Hypertension is one of risk factors for cerebral, cardiac, and renal diseases (Pierdomenico *et al.*, 2009). On the other hand, essential hypertension usually occurs with cardiovascular risk factors, such as aging, obesity, type 2 diabetes (T2DM), and hormone disorders. Lifestyle factors that are known to cause hypertension include sedentary lifestyle, high stress, high salt intake, and alcohol consumption (Kyrou *et al.*, 2006). Both blood pressure and hypertension are traditional examples of complex traits controlled by the complex interplay of genes and environmental factors (Pickering *et al.*, 1959). The heritability of blood pressure ranges from 31% to 68%, depending on studies based on different measurements of SBP and DBP (Pilia *et al.*, 2006; Tobin *et al.*, 2005).

Ehret (2010) summarized the 12 genomewide association studies (GWASs) on blood pressure and hyper-

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tension that were recently published. While 10-20 loci were identified in previous GWASs, only two (i.e. *CYP17A1, MTHFR*) genes were reproducibly associated with blood pressure or essential hypertension in large cohorts. Ehret (2010) pointed out the possibility that the differences in significant SNPs that were identified among these 12 GWASs may be due to the difference in power of analysis between continuous traits and discrete traits. A continuous trait provides more variation than a discrete trait; thus, continuous trait locus (QTL) analysis is considered to be more powerful than discrete trait analysis (e.g., case-control analysis) (Potkin *et al*., 2009). However, means of SBPs and DBPs measured in a general population (i.e., a cohort) may not be representative of the true means in the population, since most hypertensive patients have taken antihypertensive drugs to lower their blood pressure. On the other hand, comparing extreme discordant phenotypes, such as individuals at the lowest end versus individuals at the highest end of the blood pressure spectrum, may be a powerful approach in GWASs (Nebert, 2000).

In this study, we have compared the gene variants associated with quantitative blood pressures (SBP and DBP) with those identified in GWASs using different definitions of discrete phenotypes to present an alternative strategy that has increased statistical power over a case-control study in the context of a GWAS for hypertension.

Methods

Study samples and genotype data

The study subjects were obtained from the two community cohorts, Ansung and Ansan, which were established in a rural area and an urban, respectively, in South Korea, as part of the Korean Genome Epidemiology Study (KoGES), launched in 2001. The Korea Association Resource (KARE) project was initiated in 2007, and a total of 10,038 unrelated Koreans were genotyped with the Affymetrix Genomewide Human SNP array 5.0 (Affymetrix Inc., Santa Clara, CA, USA). After exclusion of inadequate samples with high genotype missing rate, high heterozygosity, gender inconsistencies, cancer, and high identity-by-state (>0.8), a total of 8842 individuals aged 40 to 69 years were included in the subsequent analyses. We analyzed 352,228 single nucleotide polymorphism (SNP) markers after excluding 29.6% of the 500K SNPs with low genotype quality (i.e., missing call rate $>5\%$, minor allele frequency (MAF) $<$ 1%, and Hardy-Weinberg Equilibrium (HWE) $p<1\times10^{-6}$). Further details can be found elsewhere (Choi *et al*. 2009), and the characteristics of the 8842 individuals are summarized in Table 1.

Statistical analysis

We calculated the mean and standard deviation (SD) for the continuous variables and counted the number and frequencies for discrete variables by gender to describe the baseline characteristics of study subjects using the STATA software package, v11 (Stata Corp, College station, Texas, USA). We drew box plots of systolic (SBP) and diastolic blood pressure (DBP) by gender and three age groups (39-49, 50-59, and 60-70) using R program, v2.9.1 (http://www.r-project.org).

Three phenotype models were used: first, we compared the younger age group of 39-49 years with high SBP ($\geq$140 mm Hg) and the elderly age group of 60-70 years with low SBP ($<$120 mm Hg); second, we performed a GWAS by comparing the upper 25% and lower 25% of the SBP distribution; and finally, we performed QTL analyses on SBP and DBP. We used PLINK v1.06 to estimate MAF, to test for deviations from HWE, and to conduct single-marker genomewide association tests (Purcell *et al*., 2007). While we tested discrete phe-

Table 1. Baseline characteristics of the study subjects

Variables	Men	Women	Total
Sex, N (%)	4,183 (47.31)	4,659 (52.69)	8,842 (100)
Age, years$\pm$SD[a]	51.78$\pm$8.79	52.61$\pm$9.02	52.22$\pm$8.92
Living in Ansan	2,374 (56.75)	2,263 (48.57)	4,636 (52.44)
Ansung,N (%)	1,809 (43.25)	2,396 (51.43)	4,205 (47.56)
Body mass index, kg/m^2	24.25$\pm$2.93	24.91$\pm$3.26	24.60$\pm$3.12
Systolic blood pressure, mm Hg	122.25$\pm$17.36	121.12$\pm$19.64	121.65$\pm$18.61
Diastolic blood pressure, mm Hg	81.83$\pm$10.94	78.86$\pm$11.74	80.26$\pm$11.46
Hypertension medication[b], no	3,541 (89.67)	3,746 (85.25)	7,287 (87.34)
Yes, N (%)	408 (10.33)	648 (14.75)	1,056 (12.66)

[a]SD represents the standard deviation of the mean of each trait, [b]The numbers did not include 499 subjects with missing data regarding hypertension medication use.

notypes in multiple logistic regression analyses, we carried out multiple linear regression analyses between 352,228 SNP markers with SBP and DBP using models with adjustments for age, sex, place of residence, and BMI under an additive genetic model. Further analyses were performed after stratification of gender with adjustments for age, place of residence, and BMI. We summarized the GWAS results with the integration of PLINK output and annotation data using Python v2.6.2 (http://python.org). We present the Manhattan plots of the GWAS results, which were drawn using R v.2.9.1.

Results

As shown in Table 1, the study subjects were composed of 4183 men (47.3%) and 4659 women (52.7%) with a mean age of 52 and 53 years, respectively. Among 7287 individuals who answered the question on medication use, 10.3% of men and 14.8% of women answered that they had taken antihypertensive drugs. The mean SBP and mean DBP among men (122/82 mm Hg) were slightly higher than those of women (121/79 mm Hg).

The box plots present the distributions of SBP and DBP by gender and age (Fig. 1). Blood pressure tended to increase with age, especially in the case of SBP. The upper 25% and lower 25% of the SBP distribution in men, women, and all subjects were 110/132, 107/133, and 109/132 mm Hg, respectively. The corresponding values of DBP were 74/89, 70/87, and 72/88 mm Hg, respectively. While the distribution of DBP was approximately normal, the distribution of SBP was slightly

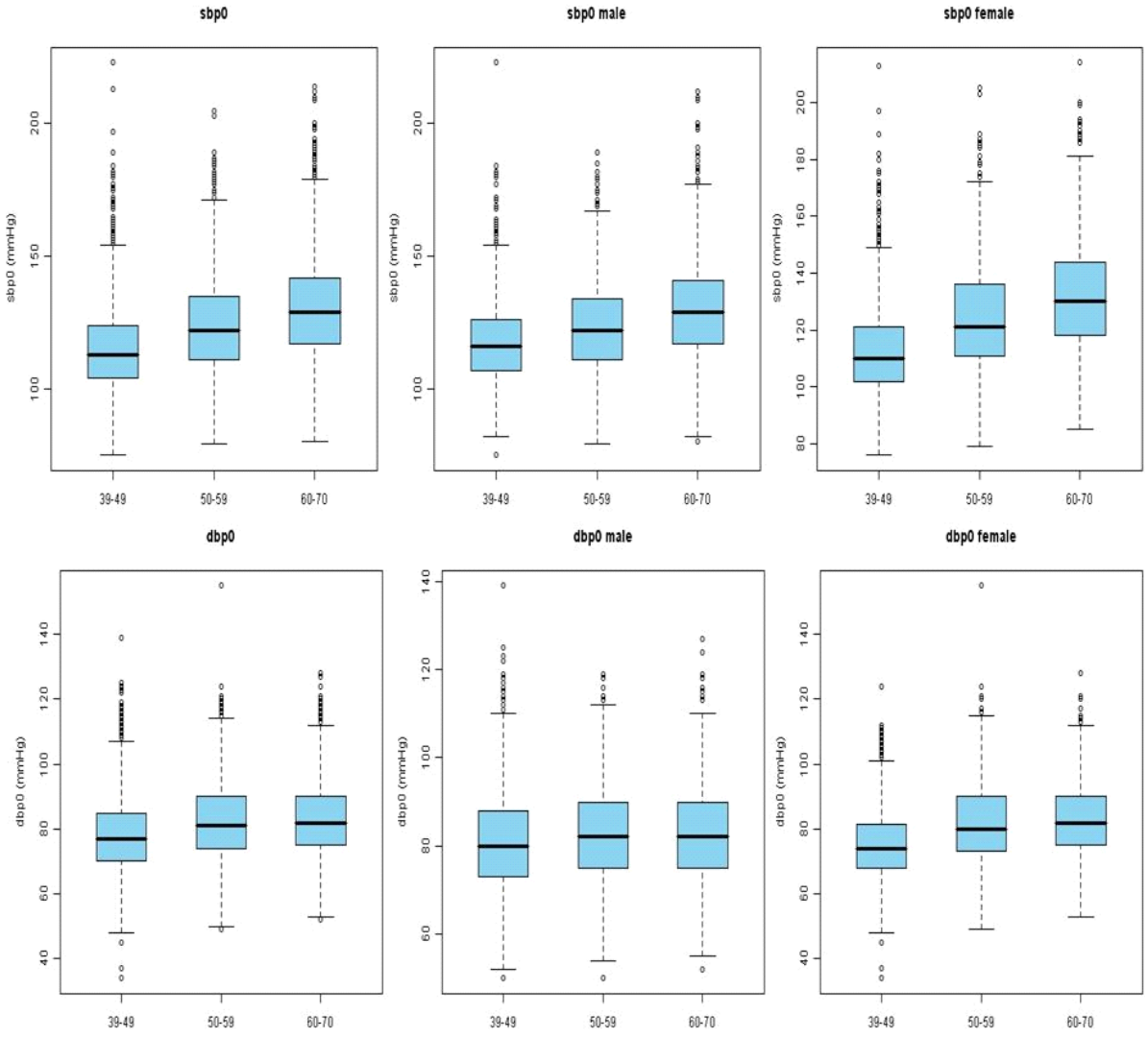

Fig. 1. Box plots of systolic (SBP) and diastolic blood pressure (DBP) by sex and age.

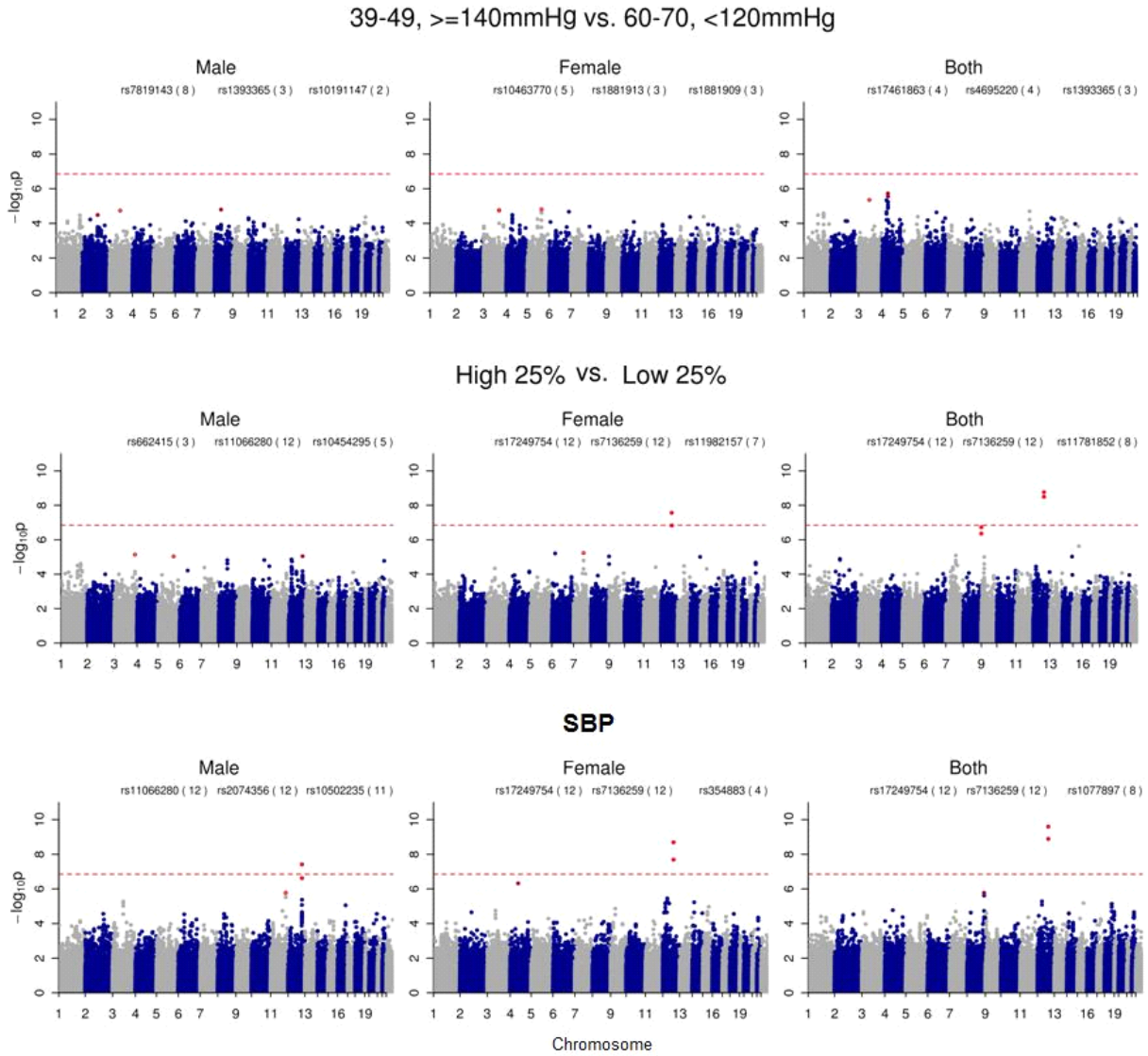

Fig. 2. Manhattan plots of GWASs using three different phenotype definitions of systolic blood pressure (SBP).

skewed to the right. Since most previous GWASs on SBP were conducted without any transformation, we performed a GWAS using the untransformed SBP response variable for comparability with the previous reports.

Initially, we compared the GWAS results obtained by using three different phenotype definitions for SBP (Table 2). Although we compared comparably young hypertensive cases (SBP≥140 mm Hg) with elderly normotensive controls (SBP<120 mm Hg) to increase the statistical power, the numbers of cases and controls were not sufficiently large enough to achieve a genome-wide level of significance. Fig. 2 shows two SNPs that exceeded the genomewide threshold in the QTL analysis as well as in the discrete trait analysis, comparing the upper 25% of individuals with hypertension with the lower 25% of individuals with hypotension. As expected, the strongest evidence for association was observed in the QTL analysis, due to not only the higher power of the QTL analysis over discrete analysis but also the largest pool of subjects being analyzed in this QTL analysis. While the estimate of the effect of this SNP was in the same direction for both genders, the association signal was stronger in women than in men.

In Table 3, we show the candidate genes corresponding to the top 9 SNPs identified from the three GWAS models using different definitions for SBP. Two SNPs, rs17249754 and rs7136259, located approximately 10 kb and 31 kb from the 5' end of the ATPase, Ca++ transporting, plasma membrane 1 (*ATP2B1*) gene, passed

Table 2. Comparison of numbers of SNPs showing significant evidence for association with SBP in GWASs using three different phenotype definitions

Phenotype[a]	Case Control			25% High vs. 25% Low			SBP		
Sex[b]	M	F	T	M	F	T	M	F	T
Cases	157	121	278	1,046	1,165	2,211	4,183	4,659	8,842
Controls	303	377	680	1,046	1,165	2,211			
$p<0.05$	18412	17462	18177	18641	18344	19147	18651	18897	19549
$p<10^{-3}$	322	277	352	364	348	423	453	416	504
$p<10^{-5}$	0	0	7	3	6	7	12	9	9
$p<10^{-7}$	0	0	0	0	1	2	1	2	2
FDR $p<0.05$[c]	0	0	0	0	2	4	2	2	2
Bonf $p<0.05$[c]	0	0	0	0	1	2	1	2	2

[a]Cases were defined as the younger age group (39-49 years) with high SBP (≥140 mm Hg), and controls were the elderly age group (60-70 years) with low SBP (<120 mm Hg); the GWAS results,comparing the upper 25% to the lower 25% of the SBP distributions; QTL analyses on SBP, [b]M, men; F, women; T, both genders combined, [c]FDR, false discovery rate; Bonf $p<0.05$, Bonferroni-corrected significance level of $p<0.05$.

Table 3. Comparison of genetic variants associated with SBP in the genomewide association analyses using three different phenotype definitions

Case control				25% High vs. 25% Low				SBP			
SNP	Gene	Chr.[a]	p	SNP	Gene	Chr.	p	SNP	Gene	Chr.	p
rs17461863*[b]	*GABRB1*	4p12	1.8910^{-6}	rs17249754	*ATP2B1*	12q21.33	1.7410^{-9}	rs17249754	*ATP2B1*	12q21.33	2.5310^{-10}
rs4695220*	*GABRB1*	4p12	2.8110^{-6}	rs7136259	*ATP2B1*	12q21.33	3.1910^{-9}	rs7136259	*ATP2B1*	12q21.33	1.3010^{-9}
rs1393365	*ROBO1*	3p12.3	4.4610^{-6}	rs11781852	*GML*	8q24.3	1.8810^{-7}	rs1077897	*KHDRBS3*	8q24.23	1.6710^{-6}
rs16990689	*ARAP1*	4p15.1	4.7110^{-6}	rs3764795**	*GML*	8q24.3	4.3810^{-7}	rs6578241	*KHDRBS3*	8q24.23	2.4110^{-6}
rs11733050*	*GABRB1*	4p12	5.8610^{-6}	rs1378942*	*SRC*	15q24.1	2.3910^{-6}	rs16918920	*H3F3C*	12p11.21	5.2510^{-6}
rs7677890*	*GABRB1*	4p12	6.0810^{-6}	rs11982157	*RPL7P30*	7q21.11	8.1510^{-6}	rs1378942*	*SRC*	15q24.1	6.6510^{-6}
rs10517184*	*GABRB1*	4p12	8.8210^{-6}	rs8007031	*C14orf64*	14q32.2	9.5810^{-6}	rs1030582*	*PIGA*	18q21.33	7.2810^{-6}
rs4694846*	*GABRB1*	4p12	1.1810^{-5}	rs11125815	*BCL11A*	2p16.1	1.3010^{-5}	rs12458329*	*PIGA*	18q21.33	7.5410^{-6}
rs7928126	*LOC10028*	11q14.1	2.0110^{-5}	rs11125814	*BCL11A*	2p16.1	1.4810^{-5}	rs11051588	*H3F3C*	12p11.21	8.2810^{-6}

[a]Chr, chromosome; [b]*intronic SNP, **nonsynonymous SNP.

the Bonferroni correction in the model for SBP as a QT and the model-namely, 25% High versus 25% Low SBP. However, we did not observe any gene identified consistently in the three GWA analyses, except these two SNPs, which achieved the Bonferroni level of significance. Among the top-ranking SNPs observed in the analysis of young hypertensive cases and elderly normotensive controls, six SNPs were located in the introns of the gamma-aminobutyric acid (GABA) A receptor, beta 1 (*GABRB1*) gene. In the 25% High versus 25% Low SBP GWAS, rs3764795 was located in an exon of glycosylphosphatidylinositol-anchored molecule-like protein (*GML*, 8q24.3); this SNP is a missense mutation that causes an Arg-to-Cys substitution.

We have provided more detailed information on the three SNPs that passed the Bonferroni correction in the GWAS for SBP and DBP in Table 4. The mean differences and 95% confidence intervals (95% CI) that were estimated in men, women, and both genders, respectively, are shown in this table. While the two SNPs of the *ATP2B1* gene showed a stronger effect in women, rs11066280, which is 25.2 kb downstream of the ribosomal protein L6 (*RPL6*) gene, showed a significant genomewide association in men only. The association of *ATP2B1* with SBP was stronger than that with DBP. Interestingly, four SNPs that are 92.4 kb upstream of the mannosidase, alpha, class 2A, member 1 (*MAN2A1*) gene showed strong evidence for an association with DBP in elderly men only (e.g. rs6421827, $p=4.86\times10^{-8}$) (data not shown).

Conclusion

Quantitative phenotype is inherently different from a case-control study. The use of QTs as phenotypes increase the statistical power over categorical association

Table 4. Genetic variants significantly associated with SBP and DBP (Bonferroni $p < 0.05$)

Gene chr.	SNP location	M/m	Sex	MM/Mm/mm	HWE p	MAF	SBP		DBP	
							β (95% CI)	p	β (95% CI)	p
ATP2B1	rs17249754	G/A	Men	1553/1885/571	1	0.38	-1.07 (-1.79-0.35)	3.50×10^{-3}	-0.64 (-1.11–-0.17)	7.80×10^{-3}
12q21.33	10.7kb~5'		Women	1821/2027/648	0.03	0.37	-2.16 (-2.86-1.45)	2.03×10^{-9}	-1.23 (-1.67–-0.79)	3.92×10^{-8}
			Both	3374/3912/1219	0.12	0.37	-1.63 (-2.13-1.13)	2.53×10^{-10}	-0.94 (-1.26–-0.61)	1.28×10^{-8}
	rs7136259	G/A	Men	1548/1909/602	0.74	0.38	-1.10 (-1.81-0.40)	2.28×10^{-3}	-0.63 (-1.10–-0.17)	7.55×10^{-3}
	31.3kb~5'		Women	1761/2132/667	0.59	0.38	-2.02 (-2.72-1.31)	2.04×10^{-8}	-1.16 (-1.60–-0.72)	2.01×10^{-7}
			Both	3309/4041/1269	0.54	0.38	-1.55 (-2.05-1.05)	1.30×10^{-9}	-0.88 (-1.20–-0.56)	6.41×10^{-8}
RPL6	rs11066280	A/T	Men	2785/1142/131	0.30	0.17	-2.54 (-3.44-1.63)	3.85×10^{-8}	-1.321 (-1.91–-0.73)	1.23×10^{-5}
12q24.13	25.2kb~3'		Women	3124/1320/118	0.13	0.17	0.10 (-0.82-1.03)	8.27×10^{-1}	-0.197 (-0.77–0.38)	5.00×10^{-1}
			Both	5909/2462/249	0.73	0.17	-1.18 (-1.83-0.53)	3.62×10^{-4}	-0.749 (-1.16–-0.33)	4.00×10^{-4}

approaches in the context of a GWAS of hypertension. In contrast to a case-control analysis, a QT analysis has considerably greater power. A QT analysis can identify the same risk genes found in a typical case-control study or an entirely different set of genes because of the specificity of the phenotype definition.

In this study, we compared the gene variants associated with quantitative blood pressure (SBP and DBP) with those identified in GWASs using different definitions of discrete phenotype. The numbers of young hypertensive cases and elderly normotensive controls were not large enough to achieve genomewide significance. The model comparing the upper 25% to the lower 25% of subjects showed a power that was approximate to that of the QTL analysis. Two neighboring SNPs, rs17249754 and rs7136259 of the *ATP2B1* gene, were associated with both SBP and DBP. Interestingly, an SNP of the *RPL6* gene, rs11066280, revealed a significant genomewide association with SBP in men only, and four SNPs, located near the *MAN2A1* gene, showed a strong association with DBP in elderly men aged 60-70 years only. However, we did not observe any gene variant that attained genomewide significance consistently in the three phenotypic models, except for the *ATP2B1* gene variants.

So far, 10-20 loci have been proposed as candidate genes for hypertension. However, only two genes reported in previous studies were replicated in the 12 recently published GWASs (Ehret, 2010). Previously, we reported the association of *ATP2B1* with SBP and DBP using the Korea Association Resource (KARE) data obtained from two Korean community-based populations (Cho *et al*., 2009). Among the genes reported in previous studies, two genes, *SRC* (also called *CSK*, rs1378942, 15q24.1) and *ATP2B1* (12q21.33), were also identified in the current study. The association between the *SRC* gene and DBP was shown in the Global BP Gene Study (Newton-Cheh *et al*., 2009), and the *ATP2B1* gene was associated with SBP and DBP, as well as hypertension, in the CHARGE study (Levy *et al*., 2009). *ATP2B1* plays a critical role in intracellular calcium homeostasis (Ehret, 2010); thus, it is biologically plausible that the gene variants are involved in blood pressure control.

Previous studies analyzed the KARE data to replicate the association of six SNPs with essential hypertension, reported by the Wellcome Trust GWAS, and to identify nonsynonymous SNPs associated with BP and hypertension through the analysis of 1180 nsSNPs that are included in the Affymerix SNP Array 5.0. A total of 7551 individuals were analyzed after excluding antihypertensive therapy from the SBP and DBP GWAS. Such variables as age, sex, area, and BMI were controlled in their QTL analyses. However, none of the SNPs was replicated in the Korean population at the genomewide significance level in these analyses (Hong *et al*., 2009; 2010a). Recently, Hong *et al*. identified 10 SNPs associated with blood pressure and hypertension risk using the KARE data, composed of 8842 individuals, and replicated three SNPs-17249754 of the *ATP2B1* gene, rs1378942 in the *CSK* gene, and rs12945290 in the ARSG gene-in the Health2 data on 7861 subjects (Hong *et al*., 2010b).

In the current study, we did not exclude 1056 individuals who had taken antihypertensive drugs. The patients who were currently taking antihypertensive drugs might reduce their BP, and therefore, the strength of association might be diluted and the statistical power might decrease when these individuals are included in the QTL analysis. This means that including individuals who are taking antihypertensive drugs increases the false negative rate instead of increasing the false positive association. It may be a reason for the inconsistency of the results of the current study and those of Hong *et al*. The GWASs on blood pressure have provided valuable insights into the underlying genetic architecture of essential hypertension. However, much work remains to be done in understanding the functional and pathophysiological properties of the gene variants that were identified in the previous GWASs. Future studies

on rare variants with intermediate or large effect on BP might facilitate the investigation of heritability of BP. The genes that have been identified in GWASs are expected to open the way for the prevention, early diagnosis, and personalized treatment of hypertension.

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