Heterogeneous Effects of Association Between Blood Pressure Loci and Coronary Artery Disease in East Asian Individuals

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Background: A coronary artery disease (CAD) association study of genetic loci previously identified as being associated with blood pressure (BP) was performed in east Asian populations.

Methods and Results: Nine single nucleotide polymorphisms (SNPs) from 9 candidate loci robustly confirmed to be associated with BP in east Asian people, were genotyped. Genotyping was done in up to 17,785 CAD case-control samples (6,522 cases and 11,263 controls). We then tested the associations with other metabolic traits ($n \leq 17,900$) and with type 2 diabetes (931 cases and 1,404 controls), and looked up the datasets in silico in other populations. Significant (adjusted $P < 0.05$) CAD associations were found for 5 BP loci: 3 new CAD associations at *FIGN*, *FGF5* and *NPR3*, and 2 previously reported ones at *ATP2B1* and *CNNM2*. The strongest CAD association was detected at *ATP2B1* rs2681472 ($P = 1.7 \times 10^{-8}$), in the direction inverted to what is generally recognized for BP in the epidemiological studies. *CNNM2* rs12413409 showed significant association with CAD ($P = 8.7 \times 10^{-7}$) and BMI ($P = 3.5 \times 10^{-8}$, when meta-analyzed with 75,807 east Asian people). The genetic risk score combining BP-raising alleles at each of the SNPs was positively associated with CAD ($P = 0.011$).

Conclusions: A substantial proportion of genetic variants associated with BP were also associated with the risk of CAD in east Asian people, and there was some counter-evidence for causal inference. (*Circ J* 2015; **79**: 830–838)

Key Words: Blood pressure; Coronary artery disease; Genetic susceptibility

Genetic, environmental and demographic factors and their interaction determine an individual's risk for hypertension, one of the most common disorders; essential hypertension may consist of disparate mechanisms that lead to elevations in systemic blood pressure (BP). Until recently, the inherently complex nature of hypertension had hampered progress in the elucidation of the genes involved, despite considerable efforts made in the study of molecular genetics of hypertension.¹ With the remarkable progress in molecular techniques, genome-wide association (GWA) stud-

ies involving tens of thousands of individuals have succeeded in the identification of more than 40 BP loci, most of which are located near genes previously unsuspected in the BP regulation or in the intergenic regions.^{2,3}

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Elevated BP is related to an increased risk of cardiovascular conditions such as coronary artery disease (CAD) in the population at large.^{4,5} Given that there is substantial evidence for

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clinical benefit of lowering BP in the prevention or treatment of cardiovascular disease,⁶ it is generally assumed that single nucleotide polymorphism (SNP) alleles that increase BP are associated with an increased risk of CAD, in proportion to the genetic effects on BP. A large-scale study has recently reported that this assumption holds true for the overall effect of BP-associated polymorphisms in people of European descent (from the CARDIoGRAM⁷ data set), where 23 of 26 (88%) tested SNPs were associated with odds ratio (OR) >1 for CAD in a direction consistent with their effect on BP.⁸ We have found, however, that this is not always pertinent to a BP locus in east Asian people; at 12q24.13 near *ALDH2*, the SNP alleles associated with elevated BP have turned out to be associated with a reduced risk of CAD.⁹ While these appear to be counterintuitive based on what is known about the relationship between BP and CAD, such observations are due, in part, to pleiotropic effects: that is, the deleterious effects of the variant (at 12q24.13) on BP were balanced by protective effects on high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C).

In the present study, to further address this issue, we performed a CAD case-control study for candidate SNPs that had significant evidence for BP association in east Asian people, in a total of 6,522 cases and 11,263 controls from Japanese and Korean populations. We also examined the relevance of 9 SNPs to other cardiovascular risk traits in Japanese individuals in order to test the potential presence of pleiotropy.

Methods

Case-Control Association Study for CAD

A case-control association study for CAD was performed in a multi-tier design. Detailed characteristics of the individuals analyzed in each tier are described in [Table 1](#). All participants were of east Asian ancestry; tier 1 and tier 2 panels were Japanese and the tier 3 panel was Korean. In the Japanese panels (tier 1 and tier 2), case subjects were enrolled from clinical practices or annual medical checkups at medical institutions and university hospitals in accordance with the uniformly defined criteria.¹⁰ These criteria included (1) a validated history of either myocardial infarction (MI) or coronary revascularization (coronary artery bypass grafting or percutaneous coronary intervention); or (2) subjective symptoms of angina pectoris with 1 or more major coronary vessels having $\geq 75\%$ stenosis on coronary angiography. In the Korean panel (tier 3), case subjects were enrolled from teaching hospitals according to the GenRIC working group criteria.¹¹ In all panels, controls were individuals randomly selected from a cross-sectional study of cardiovascular risk factors in the recruitment areas and were deemed free of MI on history, physical examination, and electrocardiogram.

Following the current standard of testing SNP-trait association for common disease such as CAD, we performed a meta-analysis of effect sizes in multiple groups: Japanese GWA-scanned samples and Japanese validation samples,¹⁰ and Korean GWA-scanned samples,¹¹ for the 9 target SNP, which were first chosen, based on the results for east Asian GWA meta-analysis of BP,⁹ as listed in the following section. Because the major purpose was to test association between a selected list of SNP with the strongest association signal with a primary target trait (ie, BP) at individual loci, and a secondary target trait (ie, CAD), the present study was designed as such, in accordance with the previous study in European-descent people.⁸

Metabolic Trait Association Study

We also performed an association study of the BP-associated variants with cardiovascular risk (or metabolic) traits – body mass index (BMI), waist-to-hip ratio (WHR), blood lipid concentration, fasting plasma glucose (FPG) and HbA1c – in the general Japanese population ([Table 1](#)). Specifically, 5,331 Japanese participants (referred to hereafter as the Amagasaki Study panel) were consecutively enrolled in the population-based setting as described previously;¹² and 12,569 other Japanese participants (referred to hereafter as the Fukuoka Cohort Study panel) were randomly selected from residents aged 50–74 years in the general population.¹³ We further examined the genetic associations in silico with type 2 diabetes (T2D), using GWA-scanned data sets that we previously published: 931 cases and 1,404 controls.¹⁴ For the replication study of BMI association (rs12413409 at *CNNM2*), the results for 75,807 east Asian people from the Asian Genome Epidemiology Network (AGEN)¹⁵ were included in meta-analysis.

In the Amagasaki Study panel, blood samples that were collected after ≥ 6 -h fast and for which there were measurement data on the corresponding phenotypes, were used for tests of association with FPG ($n=4,813$) and blood lipid concentration ($n=4,990$). In the Fukuoka Cohort Study panel, in contrast, because blood was drawn not strictly on the condition of overnight fast, it was not used for tests of association with FPG, LDL-C or triglyceride (TG) concentration. Here, LDL-C was calculated using the Friedewald formula, with missing values assigned to individuals with TG >400 mg/dl.

All participants from the different studies provided written informed consent, and the local ethics committees approved the protocols.

SNP Genotyping and Quality Control

Apart from 806 cases and 1,337 controls (part of the tier 1 panel, genotyping done using the Infinium HumanHap550 or Human610-Quad BeadArray [Illumina, San Diego, CA, USA] as previously reported),¹⁰ Japanese samples were genotyped using the TaqMan assay (Life Technologies, Carlsbad, CA, USA) for 9 SNPs from 9 BP loci robustly confirmed ($P<5\times 10^{-8}$) in populations of east Asian descent.⁹ These SNPs included rs880315 (*CASZ1*), rs17030613 (*ST7L*), rs16849225 (*FIGN*), rs16998073 (*FGF5*), rs6825911 (*ENPEP*), rs1173766 (*NPR3*), rs12413409 (*CNNM2*), rs2681472 (*ATP2B1*), and rs35444 (*TBX3*). The genotype distribution of all tested SNPs was in Hardy-Weinberg equilibrium ($P>10^{-3}$). We obtained successful genotyping call rates of >99% for the whole characterized sample (across 9 SNPs) with the TaqMan assay.

Korean samples (the tier 3 panel of CAD case-control study) were genotyped as part of the Korean GWA study for CAD with the Affymetrix Genome-Wide Human SNP array 6.0. Data cleaning and analysis were performed as described elsewhere.¹¹

Statistical Analysis

Individual SNP Association The SNPs were tested for association with dichotomous traits (CAD and T2D) and quantitative traits (BMI, WHR, blood lipid concentration, FPG, and HbA1c) using the Cochran-Armitage trend test and linear regression analysis, respectively. In the linear regression models, we adjusted BMI and WHR for sex and age classes; lipid traits for age classes separately by sex and BMI; and FPG and HbA1c for sex, age, and BMI. Age classes were defined according to age distribution in the individual panels, and included ≤ 40 , 41–50, 51–60, and >60 years for the Amagasaki Study panel, and ≤ 55 , 56–60, 61–65, 66–70, and >70 years for

Table 1. Baseline Participant Characteristics

	Amagasaki Study panel	Fukuoka Cohort Study panel	CAD case-control study panel					
			Japanese tier 1		Japanese tier 2		Korean	
			Cases	Controls	Cases	Controls	Cases	Controls
n	5,331	12,569	1,347	1,337	3,052	6,335	2,123	3,591
% female	39.8	54.9	22.4	44.7	22.3	41.1	38.6	55.7
BMI (kg/m ²)	23.0±3.2	23.1±3.0	23.8±3.2	23.4±3.2	23.7±3.0	23.0±2.9	25.3±3.0	24.0±2.9
Age at recruitment (years)	47.8±12.3	62.6±6.8	66.3	65.6	62.7	62.4	51.6±7.5	53.1±8.3
Age at first event (years)	–	–	63.3	–	62.7	–	–	–
Former or current smoker	45.4	40.1	65.5	41.3	65.7	44.1	50.7	32.7
Hypertension [†]	21.5	56.9	65.2	44.8	53.7	50.1	–	–
SBP (mmHg)	124.3±17.3	138.8±21.2	–	–	–	–	125.0±20.5	122.5±14.3
DBP (mmHg)	75.9±11.0	83.9±11.7	–	–	–	–	77.3±12.1	77.7±9.9
Diabetes mellitus [‡]	5.2	7.6	47.9	25.5	37.9	16.7	–	–
FPG (mmol/L)	5.37±0.98	–	–	–	–	–	–	–
HbA1c (%) [¶]	5.26±0.53	5.23±0.77	–	–	–	–	–	–
Dyslipidemia ^{§,††}	41.3	–	56.7	54.8	51.9	49.9	68.5	46.9
Total cholesterol (mg/dl)	206.9±35.2	215.0±35.0	–	–	–	–	180.1±44.0	196±35.0
LDL-C (mg/dl) ^{††}	123.2±31.2	–	107.1±29.7	124.1±30.9	–	–	110.4±41.1	119.2±31.7
HDL-C (mg/dl)	62.8±17.7	62.5±16.8	51.2±14.1	61.0±16.6	–	–	42.5±10.6	54.7±13.3
TG (mg/dl)	110.1±87.5	146.6±99.3	155.1±83.9	118.7±68.9	–	–	161.6±109.4	123.4±92.1
Alcohol drinking			–	–	–	–	–	–
None or abstainer	24.1	53.6	–	–	–	–	–	–
Drinker	75.9	46.4	–	–	–	–	–	–

Data given as mean±SD or %. All clinical assessments were performed using uniform standards in each population. Blood samples were taken after ≥6-h fast in the Amagasaki Study panel; without setting strict fasting condition in the Fukuoka Cohort Study panel. [†]SBP ≥140 mmHg and/or DBP ≥90 mmHg, or taking anti-hypertensive medication. [‡]Fasting plasma glucose ≥7.0 mmol/L and/or HbA1c ≥6.5%, or taking blood glucose-lowering medication. [§]Defined according to the Japan Atherosclerosis Society Guidelines.³⁰ [¶]HbA1c was measured for 596 subjects in the Amagasaki Study panel, whereas for all participants in the Fukuoka Cohort Study panel. ^{††}LDL-C was calculated in the Amagasaki Study panel using the Friedewald formula, with missing values assigned to individuals with TG >400 mg/dl. Given that blood samples were taken without setting strict fasting condition, LDL-C and the prevalence of dyslipidemia are not shown for the Fukuoka Cohort Study panel according to the Japan Atherosclerosis Society Guidelines.³⁰ BMI, body mass index; CAD, coronary artery disease; DBP, diastolic blood pressure (BP); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; SBP, systolic BP; TG, triglycerides.

the Fukuoka Cohort Study panel. HDL-C, TG, and HbA1c were log-transformed before linear regression analysis. Significance was set at $P < 0.05$ after adjustment for multiple testing with Bonferroni correction. For a CAD association study, we combined association results for Japanese and Korean samples using the inverse variance method to meta-analyze effect sizes in multiple populations; we pooled association results from 2 tiers of Japanese samples, because we found no significant regional differences in the allele frequencies at 9 tested loci in Japanese.¹⁰ We used PLINK,¹⁶ R (version 2.10.0; <http://cran.r-project.org/>), and the rmeta and meta packages to test for the associations.

Estimation of Predicted Effect of BP-Associated SNPs on CAD We calculated the predicted effect size for each BP-associated SNP on CAD risk, based on the association between BP level and the incident risk of CAD (or MI) estimated on meta-analysis of Japanese cohort studies¹⁷ and the reported effects of the selected SNPs on BP level.⁹ In this calculation, we corrected for measurement error or within-person variability (i.e. regression dilution bias), as described previously.¹⁸ For each BP-associated SNP, the type of lead BP traits (either systolic BP [SBP] or diastolic BP [DBP]) was determined following the previous report.⁹

Genetic Risk Score To assess association of the variants

in aggregate with prevalent CAD, we created a genetic risk score. The risk score was weighted using the average of SBP and DBP effects $[(\text{SBP effect} + \text{DBP effect})/2]$ ³ for the 9 SNPs previously reported in east Asian people.⁹

Results

Association With CAD at BP Loci

In this study, we tested CAD associations at 9 SNP loci that had attained genome-wide significance ($P < 5 \times 10^{-8}$) in previous GWA meta-analysis for BP in east Asian people.⁹ In the combined sample (6,522 cases and 11,263 controls; **Table 2**), we found a significant CAD association for 5 SNPs ($P < 0.0056 = 0.05/9$): they were rs16849225 (*FGN*), rs16998073 (*FGF5*), rs1173766 (*NPR3*), rs12413409 (*CNNM2*), and rs2681472 (*ATP2B1*). All of these had nominally significant association in both Japanese and Korean populations (**Table 2**; $P < 0.05$). The effect sizes of association with CAD were almost identical across the study panels (tier 1, tier 2, and tier 3) (**Tables 2, S1**). Regional plots were done for 3 loci with prominent CAD association: *FGN*, *CNNM2* and *ATP2B1* (**Figure S1**).

To assess heterogeneity in effect sizes across 3 populations (Japanese tier 1, including GWA-scanned samples, and tier 2

Table 2. Effects of BP-Associated SNPs on CAD

SNP	Chr	Position (Build 36)	Nearby gene(s)	Alleles (coded/other)*	Japanese				
					Coded allele frequency		OR (95% CI)	P-value	n total
					Case	Control			
rs880315	1	10,719,453	<i>CASZ1</i>	C/T	0.677	0.685	0.96 (0.91–1.02)	0.214	12,048
rs17030613	1	112,992,330	<i>ST7L</i>	C/A	0.490	0.497	0.97 (0.92–1.03)	0.345	12,032
rs16849225	2	164,615,066	<i>FIGN</i>	C/T	0.643	0.618	1.12 (1.06–1.18)	6.6E-05	12,057
rs16998073	4	81,403,365	<i>FGF5</i>	T/A	0.315	0.300	1.07 (1.01–1.14)	0.015	12,049
rs6825911†	4	111,601,087	<i>ENPEP</i>	C/T	0.553	0.541	1.05 (1.00–1.11)	0.057	12,049
rs1173766†	5	32,840,285	<i>NPR3</i>	C/T	0.592	0.578	1.06 (1.01–1.12)	0.030	12,020
rs12413409	10	104,709,086	<i>CNNM2</i>	G/A	0.754	0.732	1.12 (1.06–1.19)	1.8E-04	12,059
rs2681472	12	88,533,090	<i>ATP2B1</i>	T/C	0.594	0.628	0.87 (0.82–0.91)	2.5E-07	12,007
rs35444†	12	114,036,820	<i>TBX3</i>	A/G	0.753	0.739	1.07 (1.01–1.14)	0.020	12,006

SNP	Korean					Combined		
	Coded allele frequency		OR (95% CI)	P-value	n total	OR (95% CI)	P-value	N
	Case	Control						
rs880315	N/A					0.96 (0.91–1.02)	0.214	12,048
rs17030613	0.500	0.510	0.930 (0.86–1.00)	0.060	5,685	0.96 (0.92–1.00)	0.066	17,717
rs16849225	0.610	0.600	1.140 (1.05–1.23)	0.002	5,619	1.12 (1.08–1.18)	2.2E-07	17,676
rs16998073	0.36	0.330	1.12 (1.03–1.22)	0.008	5,315	1.09 (1.04–1.14)	6.3E-04	17,364
rs6825911†	0.493	0.483	1.040 (0.96–1.13)	0.334	5,417	1.05 (1.00–1.10)	0.033	17,466
rs1173766†	0.619	0.597	1.10 (1.01–1.19)	0.018	5,594	1.07 (1.03–1.12)	0.002	17,614
rs12413409	0.776	0.751	1.15 (1.05–1.26)	0.004	5,690	1.13 (1.08–1.19)	8.7E-07	17,749
rs2681472	0.590	0.610	0.92 (0.85–1.00)	0.037	5,458	0.88 (0.84–0.92)	1.7E-08	17,465
rs35444†	0.760	0.750	1.04 (0.95–1.14)	0.318	5,690	1.06 (1.01–1.12)	0.015	17,696

A CAD association study comprises 2 sample populations: the Japanese sample (4,399 cases and 7,672 controls) and the Korean sample (2,123 cases and 3,591 controls). In the Japanese, association results from the 2 tiers were combined by pooling the genotype counts (Table S1), given that we found no significant regional differences in the allele frequencies at 9 tested loci in the Japanese sample.¹⁰ Association results for the Japanese and Korean subjects were combined using inverse variance weighting with the *rmeta* package of R. †In part of the tier 1 panel (806 cases and 1,337 controls), proxy SNPs were genotyped at *ENPEP* (rs6825911 is replaced by rs4358460, $r^2=1.000$ in HapMap JPT+CHB), *NPR3* (rs1173766 is replaced by rs1147225, $r^2=0.952$ in JPT+CHB), and *TBX3* (rs35444 is replaced by rs35441, $r^2=0.883$ in JPT+CHB). *Alleles associated with elevated BP are defined as coded. Alleles are nominated as those in dbSNP Build 130 mapped on the strand of Human Genome Build 36.3. SNP, single nucleotide polymorphism. Other abbreviations as in Table 1.

(validation samples) panels, and Korean GWA-scanned samples), we prepared forest plots and analyzed Cochran's Q-test for the 9 SNPs; we found a lack of significant evidence for heterogeneity at 5 significantly associated SNPs (Figure S2).

Among the 5 significant SNP loci, the T-allele of rs2681472 at *ATP2B1*, associated with elevated BP, was found to be associated with a reduced risk of CAD (OR, 0.88; 95% CI: 0.84–0.92, $P=1.7\times 10^{-8}$ in the combined sample; Table 2). To

evaluate the inter-trait correlation, we then produced scatter plots (Figure 1) and observed that, for 9 BP-associated SNP loci, there was no apparent correlation of effect sizes between SBP and CAD; and there was a fair correlation of effect sizes between SBP and DBP.

To examine the possibility that CAD association of BP loci reflects a causal relationship of increasing BP with CAD, we displayed the predicted effect and the observed effect (both

Figure 1. Correlation of effect sizes for coronary artery disease (CAD) risk and blood pressure (BP) traits at 9 loci tested in the current study: (A) CAD vs. systolic BP (SBP); and (B) SBP vs. diastolic BP (DBP). Genetic impacts on BP (β) and CAD risk (odds ratio, OR) are compared for the 9 SNP loci that were previously reported to be associated with BP in east Asian people (Table 2).⁹ Whiskers represent 95% CI. Effect sizes for SBP and DBP were derived from meta-analysis in samples from Amagasaki and Fukuoka Cohort Study panels, and those for CAD were from meta-analysis in samples from the tier 1 and tier 2 Japanese panels.

Figure 2. Genetic risk scores (OR, ♦) for coronary artery disease (CAD) for (Left) systolic blood pressure (SBP) and (Right) diastolic blood pressure (DBP). Whiskers, ± 1 SE. Blue bars, sample size for BP risk score groups. The P-values for slope across BP risk score groups were significant: $P=0.009$ and $P=0.017$ for SBP and DBP risk scores, respectively, and $P=0.011$ for the average of SBP and DBP effects (for all BP effects: OR, 1.05; 95% CI: 1.01–1.09; per 1-SD effect of risk score).

quantified as OR) of each individual SNP on CAD (Figure S3). The predicted effects of 9 SNPs were in the range OR=1.01–1.03 and showed substantial disagreement with their observed effects, suggesting that some of the association between BP loci and CAD is likely through non-BP-mediated pathways genetically determined.

The genetic risk score was positively associated with CAD ($P=0.011$; OR, 1.05; 95% CI: 1.01–1.09 per 1 SD increase in the average of SBP and DBP effects) in the Japanese CAD case-control panels (Figure 2).

Genetic Associations With Other Cardiovascular Risk Traits

Assuming the presence of pleiotropy, we examined the asso-

ciation of the 9 SNPs with other cardiovascular risk traits in Japanese individuals (Table 3). After adjustment for multiple testing, there were 3 significant association signals: at rs12413409 (*CNNM2*) for BMI, and at rs880315 (*CASZ1*) and rs1173766 (*NPR3*) for HbA1c ($P<0.0056\sim 0.05/9$). The association between rs12413409 and BMI was replicated in an independent large sample of east Asian people ($n=75,807$; β , -0.0365 ; SE, 0.008; $P=2.5\times 10^{-6}$), reaching genome-wide significance when meta-analyzed ($n=93,707$; β , -0.0358 ; SE, 0.006; $P=3.5\times 10^{-8}$). Notably, the direction of this BMI association appeared to be counterintuitive in terms of CAD risk. That is, the G-allele of rs12413409 associated with elevated BP was associated with reduction of BMI, whereas it was associated with an increased

Table 3. Effects of BP-Associated SNPs on Metabolic Traits

SNP ID	Chr	Position (Build 36)	Coded/ Other allele	CAF	Obesity measure				Lipid			
					BMI (kg/m ²)		WHR		Total cholesterol (mg/dl)		LDL-C (mg/dl)	
					β (SE)	P-value	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value
rs880315	1	10,719,453	C/T	0.66	-0.007 (0.011)	0.520	-0.002 (0.013)	0.857	-0.132 (0.394)	0.739	-0.120 (0.620)	0.847
rs17030613	1	112,971,190	C/A	0.49	0.003 (0.010)	0.810	-0.006 (0.012)	0.599	0.129 (0.371)	0.728	0.435 (0.592)	0.463
rs16849225	2	164,615,066	C/T	0.61	-0.022 (0.011)	0.036	-0.027 (0.013)	0.033	0.058 (0.380)	0.879	0.104 (0.595)	0.861
rs16998073	4	81,541,520	T/A	0.31	-0.011 (0.011)	0.333	0.0001 (0.013)	0.992	-0.392 (0.404)	0.332	0.341 (0.642)	0.595
rs6825911	4	111,601,087	C/T	0.53	-0.005 (0.010)	0.658	-0.010 (0.012)	0.402	0.240 (0.373)	0.520	0.764 (0.590)	0.196
rs1173766	5	32,840,285	C/T	0.58	0.014 (0.011)	0.191	0.011 (0.012)	0.394	-0.179 (0.380)	0.637	-0.227 (0.601)	0.706
rs12413409	10	104,709,086	G/A	0.73	-0.035 (0.012)	0.003	-0.015 (0.014)	0.262	0.314 (0.417)	0.452	-0.239 (0.666)	0.719
rs2681472	12	88,533,090	T/C	0.64	0.012 (0.011)	0.268	0.007 (0.012)	0.262	-0.270 (0.383)	0.481	-0.879 (0.615)	0.153
rs35444	12	114,036,820	A/G	0.74	-0.010 (0.012)	0.412	-0.002 (0.014)	0.908	-0.277 (0.432)	0.521	0.095 (0.685)	0.889

SNP ID	Lipid				T2D-related trait				T2D†	
	HDL-C (mg/dl)		TG (mg/dl)		FPG (mmol/L)		HbA1c (%)			
	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value	OR (95% CI)	P-value
rs880315	0.002 (0.001)	0.083	−0.002 (0.004)	0.709	0.009 (0.011)	0.417	−0.0022 (0.0007)	0.002	0.97 (0.85–1.10)	0.583
rs17030613	0.002 (0.001)	0.062	−0.005 (0.004)	0.210	0.020 (0.010)	0.060	0.0001 (0.0007)	0.902	0.95 (0.85–1.07)	0.404
rs16849225	−0.001 (0.001)	0.503	0.001 (0.004)	0.865	−0.001 (0.010)	0.887	0.0002 (0.0007)	0.814	0.99 (0.88–1.12)	0.881
rs16998073	0.0001 (0.001)	0.923	−0.001 (0.005)	0.893	0.005 (0.011)	0.630	0.0001 (0.0007)	0.902	1.04 (0.92–1.17)	0.563
rs6825911	0.002 (0.001)	0.207	−0.004 (0.004)	0.381	−0.0001 (0.010)	0.990	−0.0002 (0.0007)	0.714	0.92 (0.82–1.04)	0.195
rs1173766	−0.001 (0.001)	0.389	0.001 (0.004)	0.854	0.008 (0.011)	0.431	−0.0019 (0.0007)	0.004	1.03 (0.92–1.17)	0.572
rs12413409	0.003 (0.001)	0.017	−0.008 (0.005)	0.075	−0.015 (0.012)	0.209	0.0009 (0.0008)	0.249	1.02 (0.89–1.16)	0.819
rs2681472	0.0002 (0.001)	0.860	−0.0001 (0.004)	0.988	−0.005 (0.011)	0.661	0.0003 (0.0007)	0.662	0.97 (0.86–1.09)	0.579
rs35444	0.002 (0.001)	0.191	−0.008 (0.005)	0.116	0.004 (0.012)	0.767	−0.0004 (0.0008)	0.650	1.08 (0.95–1.24)	0.247

Variables adjusted for in the regression models: sex and age-class in testing association with BMI and WHR; sex, age-class, and BMI in testing association with total cholesterol, LDL-C, HDL-C (log-transformed), and TG (log-transformed); and sex, age, and BMI in testing association with FPG and HbA1c (log-transformed). LDL-C was calculated in the Amagasaki Study panel (n=5,331) using the Friedewald formula, with missing values assigned to individuals with TG >400 mg/dl. Given that blood samples were taken without setting strict fasting condition, LDL-C was not calculated in the Fukuoka Cohort Study panel (n=12,569) according to the Japan Atherosclerosis Society Guidelines.³⁰ Trait-SNP association is meta-analyzed in samples from the Amagasaki Study (AMA; n=5,331) and Fukuoka Cohort Study (FUK; n=12,569) except for FPG, LDL-C, and TG (AMA samples alone) and WHR and HbA1c (FUK samples alone). Variables adjusted in the regression models are: age classes by sex for BMI (normalized) and WHR (normalized); sex, age, age-squared and BMI for total cholesterol, LDL-C, HDL-C (log₁₀-transformed), and TG (log₁₀-transformed); and sex, age and BMI for FPG and HbA1c (log₁₀-transformed). [†]Association with T2D is tested in the CAGE Network samples (931 cases and 1,404 controls).¹⁴ CAF, coded allele frequency; T2D, type 2 diabetes; WHR, waist-to-hip ratio. Other abbreviations as in Tables 1,2.

risk of CAD (OR, 1.13; 95% CI: 1.08–1.19, $P=8.7 \times 10^{-7}$) in the combined sample (Table 2). No significant association with HbA1c was replicated for rs880315 (*CASZ1*) and rs1173766 (*NPR3*) in a large GWA-scanned sample (n=20,160) of east Asian people (data not shown).

Discussion

The present study shows that 5 common variant loci influencing BP are significantly associated with CAD in a total of 17,785 east Asian samples, with the presence of some counter-

Figure 3. Meta-analysis of coronary artery disease (CAD) association at 2 principal loci. At *CNNM2*, rs12413409 was used in both current and previously reported studies.²⁰ At *ATP2B1*, in contrast, rs2681472 and rs7136259 were used in the current and previous studies,¹⁹ respectively; the 2 SNPs are in fair linkage disequilibrium ($r^2=0.738$, $D'=0.859$) in east Asian people.

evidence for causal inferences. Despite the small number of SNP loci tested here (9 SNPs), we found a BP locus, rs2681472 at *ATP2B1*, to demonstrate a counterintuitive relationship between BP and CAD, similar to the locus at 12q24.13 (near *ALDH2*) previously reported in east Asian people.⁹ Although the BP-associated SNPs at 12q24.13 showed substantial pleiotropic effects on risk factors for cardiovascular disease, it did not appear to be the case with rs2681472 at *ATP2B1* for a series of risk factors tested in the present study (Table 3). Also, rs12413409 at *CNNM2* had significant association with CAD and BMI, whereas the alleles associated with elevated BP and risk of CAD were associated with reduced BMI, suggesting the complexity in causal relationship.

For more than half of the tested BP loci (5 out of 9 SNPs), the CAD association reached significance after adjustment for multiple testing ($P<0.0056$) in the combined sample of east Asian people and also had nominal significance ($P<0.05$) concordantly in the Japanese and Korean populations (Table 2). In the previous GWA studies of CAD,^{19,20} a genome-wide significant association has been identified at 2 of 9 loci: *ATP2B1* and *CNNM2* (Figure 3). At *ATP2B1*, rs7136259 was shown to be associated with CAD in a Chinese GWA study;¹⁹ rs7136259 and rs2681472 were in fair linkage disequilibrium (LD; $r^2=0.738$, $D'=0.859$) in east Asian people (the HapMap CHB+JPT panel). Although the counterintuitive relationship had not been recognized in the Chinese GWA study,¹⁹ the T allele of rs7136259, which is in LD with a BP-lowering allele (C-allele) of rs2681472 tested in the present study, has turned out to be associated with elevated risk of CAD. At *CNNM2*, in contrast, rs12413409 was shown to be associated with CAD in a meta-analysis of European GWA studies;²⁰ the G allele of rs12413409 associated with higher BP was also associated with elevated risk of CAD and with BMI in European people,²¹ both of these findings were confirmed in east Asian people. Such counterintuitive associations can be explained by the potential presence of pleiotropic effects of a single allele and/or multiple genes, and alleles that affect multiple independent traits. In this line, it has been reported that the variants at the *CDKAL1* and *KCNQ1* loci are associated with an increased risk of T2D as well as with decreased BMI.²² Despite the strong link between the 2 CAD-associated traits, the directions of genetic association are inverted in terms of susceptibility to CAD risk. One clinical study has suggested that suppression of insulin secretion, which can increase a risk for T2D, is associated with loss of body weight and fat mass.²³ To proceed with investigation of the cause-and-effect relationships between the CAD-associated traits, the findings of the counteractive

effects in the present study are also of note. For the other 7 BP loci, little corresponding data have been provided to date with regard to CAD association, apart from the Lieb et al study,⁸ in which CAD association was also indicated for *FGF5* ($P=0.038$ at rs1458038) and *NPR3* ($P=0.02$ at rs1173771) but not for *FIGN* ($P=0.65$ at rs1446468) in European people.

Among the 5 loci thus identified as having significant CAD association in east Asian people, *ATP2B1* is of particular note. Several lines of evidence have supported *ATP2B1* as a candidate causative gene for the BP association at 12q21.3. Besides physical proximity of lead SNP to the gene, first, *ATP2B1* mRNA expression was found to be associated with genotypes of a lead SNP, rs11105738, in umbilical artery smooth muscle cells; and second, significant BP elevation was demonstrated in mice with vascular smooth muscle cell-specific knockout of *ATP2B1*, which was assumed through alteration of calcium handling and vasoconstriction.²⁴ Moreover, third, an SNP (rs17249754), which is in strong LD with rs2681472 and rs11105738, was found to be associated with arterial stiffness, measured using carotid-femoral pulse wave velocities (cf-PWV);²⁵ the alleles associated with faster cf-PWV were also associated with higher BP, in accordance with their functional relationship. Although we could not find evidence for pleiotropic effects that directly or indirectly influence the outcome (ie, CAD) other than through its risk factor (ie, BP elevation) in the present study, it is possible that *ATP2B1* and/or other causative genes at 12q21.3 exert some unnoticed effects on the coronary artery, thereby producing a counterintuitive action based on what is known about the relationship between BP and CAD. Without eventual identification of the causal variant(s) underlying the relevant genetic associations, we cannot clearly explain the molecular mechanisms as to how BP-elevating alleles exert CAD-protective effects at 12q21.3 near *ATP2B1*. Largely, however, 2 possibilities seem to be possible in such a case. One is that at a given locus there are multiple genes and alleles that participate in the regulation of multiple independent traits through diverse mechanisms. These multiple variants might have arisen at different times in the historical context, with each affecting CAD risk phenotypes (as-yet unnoticed, non-BP traits) independently, while the separate alleles with balancing effects on BP have been fixed on a haplotype. In the BP-associated region at 12q21.3, for example, there exists a potential candidate gene for CAD, *GALNT4*, adjacent to *ATP2B1*; the *GALNT4* gene, encoding the polypeptide N-acetylgalactosaminyltransferase 4, plays a role in modifying glycoproteins, which have critical function in both platelet and endothelial cells.²⁶ The other possibility is that a

single causal variant accounts for the observed associations with multiple CAD risk phenotypes in a pleiotropic manner, as mentioned. One such example is the reported association of P446L variant in *GCKR* with raised TG and lower glucose levels, both of which are likely to be mediated by indirect increase in glucokinase activity.²⁷

A number of prospective epidemiological studies have established the relation between BP elevation and the risk of incident CAD.^{6,28} This may largely support causal inferences about the effect of BP elevation on CAD. Nevertheless, given the complex nature of BP regulation, it is of interest to assess its causal relevance to CAD by using naturally occurring genetic variants as instruments. Using these 9 genetic variants, which are among those most significantly associated with BP in east Asian people, we tested the hypothesis that genetically raised BP might increase the risk for CAD, in a manner similar to a previous study.³ We investigated the association of genetic risk score for BP with CAD in 4,399 cases and 7,672 controls of Japanese descent. In the previous meta-analysis involving European and south Asian ancestry (a total of 30,657 cases and 71,911 controls),³ which included the CARDIoGRAM7 data set and 2 additional samples, the genetic risk score for BP (using 29 SNPs from 28 unique loci) was positively associated with CAD ($P=8.1 \times 10^{-29}$). Discrepancies in the estimated, unit effect sizes between the 2 studies (0.100, previous GWA meta-analysis³ vs. 0.049, present study; $\ln(\text{odds})$ per SD of genetic risk score) may be due, in part, to the counteractive effects of the alleles at *ATP2B1* on CAD association, which seem to be prominent in east Asian people, although CAD association itself was similarly detectable in European people at *ATP2B1* rs17249754 ($P=0.013$), in a direction inconsistent with its effect on BP.⁸ Further investigation is warranted to evaluate such inter-population discrepancies in the estimated effect sizes for genetic risk score, using the same set of BP SNPs among different ethnic groups, although care has to be taken, given the possible presence of ethnic differences in LD structure and allele frequency. Also, it needs to be determined whether the genetic mechanism at the *ATP2B1* locus involves disrupted regulation of a single gene (eg, *ATP2B1*) with pleiotropic effects or more than 1 genes with mutually discrete effects on BP and CAD, by pinpointing the true functional variant (or variants) in the target region of association and by performing a combination of gene function experiments in vivo and in vitro.

Whether individual BP loci exert genetic effects on CAD through non-BP-mediated pathways genetically determined or not, SNP-related BP effects cannot be neglected, although average SNP effects on BP are relatively modest, that is, approximately 1 mmHg for SBP. A meta-analysis of pharmacological studies has shown that a mean BP decrease of 1.04 mmHg may reduce CAD risk by only 2.3%,²⁹ but it is possible that SNP-related BP effects capture a lifetime exposure to a difference in BP, whereas clinical studies reflect medium-term effects.⁸ This will lead to potential underestimation of the true risk mediated by genetic effects on BP and suggests that the small sizes of SNP-related BP effect may have clinical relevance.

Conclusions

A substantial proportion of genetic variants associated with BP are also associated with the risk of CAD in east Asian subjects, whereas the effect sizes are not necessarily correlated between the 2 traits. Polymorphisms at the *ATP2B1* locus are found to be associated with CAD in the direction opposite to

what is generally recognized for BP in the epidemiological studies. These findings provide evidence for the inherently complex nature of hypertension and cardiovascular complications at the level of individual susceptibility genes.

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Disclosures

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Supplementary Files

Supplementary File 1

Figure S1. Regional association plots of 3 loci (A–C).

Figure S2. Forest plots showing the results for the 9 single nucleotide polymorphisms (SNPs) examined in the validation samples (Japanese tier 2) as well as genome-wide association (GWA)-scanned samples.

Figure S3. Predicted and observed effect sizes for the association of each blood pressure (BP)-associated single nucleotide polymorphism (SNP) with coronary artery disease (CAD).

Table S1. Effects of BP-associated SNPs on CAD in 2-tiered Japanese sample

Appendix S1. AGEN Consortium

Please find supplementary file(s);
<http://dx.doi.org/10.1253/circj.CJ-14-0841>