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A genome-wide association study of the association between single nucleotide polymorphisms and brachial-ankle pulse wave velocity in healthy Koreans

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Purpose: Pulse wave velocity (PWV) is an indicator of arterial stiffness, and is considered a marker of vascular damage. However, a genome-wide association study analyzing single nucleotide polymorphisms (SNPs) associated with brachial-ankle PWV (baPWV) has not been conducted in healthy populations. We performed this study to identify SNPs associated with baPWV in healthy populations in Korea.

Materials and Methods: Genomic SNPs data for 2,407 individuals from three sites were analyzed as part of the Korean Genomic Epidemiologic Study. Without replication samples, we performed multivariable analysis as a post hoc analysis to verify the findings in site adjusted analysis. Healthy subjects aged between 40 and 70 years without self-reported history or diagnosis of hypertension, diabetes, hyperlipidemia, heart disease, cerebrovascular disease and cancer were included. We excluded subjects with a creatinine level >1.4 mg/dL (men) and 1.2 mg/dL (women).

Results: In the site-adjusted association analysis, significant associations ($P<5\times10^{-8}$) with baPWV were detected for only 5 SNPs with low minor allele frequency. In multivariable analysis adjusted by age, sex, height, body mass index, mean arterial pressure, site, smoking, alcohol, and exercise, 11 SNPs were found to be associated ($P<5\times10^{-8}$) with baPWV. The 5 SNPs ($P<5\times10^{-8}$) linked to three genes (*OPCML*, *PRR35* and *RAB40C*) were common between site-adjusted analysis and multivariable analysis. However, meta-analysis of the result from three sites for the 11 SNPs showed no significant associations.

Conclusion: Using the recent standard for genome-wide association study, we did not find any evidence of significant association signals with baPWV.

Key words: Genome-wide association study, Association, Nucleotides, Pulse.

Introduction

Cardiovascular disease is a substantial cause of morbidity and mortality worldwide and aging is an independent risk factor for cardiovascular disease. Aging of the arterial system is accompanied by structural changes including fragmentation and degeneration of elastin, an increase in collagen, thickening of the arterial walls, and progressive dilatation of arteries. These changes result in a gradual stiffening of the vasculature.

Arterial stiffness is a general term that refers to the elasticity of arteries, reflecting the state of vessel wall diastolic function and buffering capacity. Arterial stiffness occurs as a consequence of age and arteriosclerosis. Age-related stiffness occurs when elastic fibers within the arterial wall (elastin) begin to fray due to mechanical stress. The two leading causes of death in the developed world, myocardial infarction and stroke, are both a direct consequence of atherosclerosis. Atherosclerosis progression can be clinically evaluated by examinations that are able to identify its different aspects [1].

Increased arterial stiffness is associated with an increased risk of cardiovascular events. Pulse wave velocity (PWV) is an indicator of arterial stiffness [2,3], and a marker of vascular damage [4–6]. Thus, in the review of 2013 Korean Society of Hypertension guidelines for the management of hypertension, PWV was also advanced as a method of detecting subclinical organ damage [7]. PWV is the velocity of propagation of forward and backward pressure waves between two points on the arterial tree [8], generally between the carotid and femoral arteries, and represents the mean rigidity between the two points. Carotid-femoral PWV (cfPWV) measurement is one of the most popular techniques for estimating the level of arterial stiffness; however, accurate measurement of cfPWV levels requires specialized training, and the procedure can be cumbersome.

Brachial-ankle PWV (baPWV) measurement, which is easy to perform, has recently become available in clinical practice [9]. For example, the well-known study by Kawai et al. [10,11] used baPWV as a measure of arterial stiffness. Its validity and utility have been proven in various clinical settings [12–17] and in meta-analyses [18,19], and many studies have investigated its clinical significance [9,20]. baPWV can easily be calculated using pressure cuffs on the brachial and tibial arteries. It reflects the stiffness of central to middle-sized arteries, and can be applied as a first line tool. Additionally, baPWV is closely correlated with cfPWV.

Recent genetic studies have identified specific genes that contribute to arterial stiffening, and genome-wide linkage analyses have revealed genetic polymorphisms associated with arterial stiffness. However, PWV is affected by various factors other than genetic background including blood pressure, diabetes, hypercholesterolemia, and renal function. Genetic association studies with statistical adjustment for these covariates have not been reported. We performed this study to identify the single nucleotide polymorphisms (SNPs) associated with baPWV in three rural areas in Korea.

Materials and Methods

1. Study population

The study subjects were selected from the cohort of an ongoing population-based study designated as the Korean Genome and Epidemiology Study (KoGES). Participants were recruited from among residents aged 38-89 years from three rural areas: Yangpyeong in Gyeonggi-do province, Goryeong in Gyeongsangbuk-do province, and Namwon in Jeollabuk-do province. In total, 8,702 males and females were recruited for the baseline study between 2004 and 2008. The study was approved by the Institutional Review Board (IRB) of the Korea National Institute of Health and all study subjects gave written informed consent before participation (IRB No. HYUH IRB 2005-15, HYUH IRB 2006-32 and HYUH IRB 2007-04).

2. General characteristics and anthropometric measurements

All participants were interviewed by trained interviewers, and general information (such as age, sex, smoking status, alcohol consumption, past medical history) was collected using a structured questionnaire. The criterion for regular exercise was ≥3 times per week and ≥30 minutes per session. Smoking status was classified as non-smoker, past smoker, or current smoker. Alcohol consumption was classified as non-drinker, past drinker, or current drinker.

In addition, 10 mL samples of fasting venous blood were collected in plain tubes. Sera were isolated by centrifugation at 3,000 rpm for 10 minutes at 4℃ and were stored in liquid nitrogen. Biochemical tests including fasting serum glucose, fasting serum insulin, total cholesterol, triglyceride (TG), high density lipoprotein-cholesterol (HDL-cholesterol), and others were conducted.

The anthropometric characteristics (such as height, weight, waist circumference, blood pressure) were measured according to standardized protocols. Waist circumference was measured half way between the lowest rib margin and the iliac crest. For measurement of blood pressure, subjects had been sitting for at

least 5 minutes. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a standard mercury sphygmomanometer using the first and fifth Korotkoff sounds, to the nearest 2 mmHg. If two systolic or DBP readings were more than 5 mmHg apart, an additional measurement was performed, and the mean of the last two measurements was used for subsequent analyses.

From the total of 8,702 subjects that were identified, we excluded subjects that had self-reported disease histories and were receiving drug-treatment for hypertension, type 2 diabetes mellitus, hyperlipidemia, heart disease, cerebrovascular diseases, and cancer. We also excluded subjects who were diagnosed with hypertension by supine position blood pressure measurements (SBP ≥140 mmHg and/or DBP ≥90 mmHg), and type 2 diabetes by fasting plasma glucose level (Glu ≥126 mg/dL). Finally, 4,034 healthy subjects that did not have a history or characteristics of any of the six diseases were selected for SNP genotyping, and 3,667 of these subjects fulfilled the quality control specifications included in the study.

The following participants were excluded: participants who were ≤40 or ≥70 years of age; those with missing height or weight values; with total cholesterol ≥250 mg/dL or creatinine >1.4 mg/dL for men and creatinine >1.2 mg/dL for women; subjects with missing alcohol consumption or smoking histories, or unmeasured right brachial SBP or pulse rate >100 beats/min during arterial stiffness measurement; and those with missing exercise variable values in the structured questionnaire. Based on these exclusion criteria, 2,407 of the 3,667 individuals were included in the study.

3. Genotyping and quality control

In total, 4,034 KoGES Cardiovascular Disease Association study samples were genotyped from the three rural areas using HumanOmni1 Quad v1.0 (Illumina, San Diego, CA, USA). Genotypes were called using Bead Studio (Illumina, San Diego, CA, USA). Samples that exhibited the following properties were excluded: low genotyping calls <98% (n=185), excessive heterozygosity (n=61), sex inconsistency (n=18), and cryptic relatedness (n=4). Markers with high missing gene call rates (>5%), rare minor allele frequency (MAF; <0.01), or significant deviation from the Hardy-Weinberg equilibrium ($P<1\times10^{-6}$) were also excluded. To detect the population substructure, we performed multidimensional scaling and principal component analyses using PLINK software (PLINK v1.07; Purcell Lab, New York, NY, USA) and R programming language (R Foundation for Statistical Computing, Vienna, Austria). Dimensions were calculated on the basis of

identity-by-descent pair-wise distances between all individuals. The degree of relation was determined in individuals (n=99) whose estimated identity-by-state values were high (>0.80). After these quality control steps, 3,667 samples were selected. Markers with high missing gene call rates (>5%), rare MAF (<0.01), and significant deviations from the Hardy-Weinberg equilibrium ($P<1\times10^{-6}$) were excluded, leaving a total of 723,056 markers for examination in 3,667 individuals.

4. Measurement of brachial–ankle pulse wave velocity and right brachial systolic pressure

Arterial tonometry was conducted to assess arterial stiffness in all the cohort participants during their examinations. Two primary tonometry phenotypes were analyzed: left and right PWV. PWV was measured using the VP-2000. Tonometry was performed in a supine position after 5 minutes of rest. Arterial tonometry was obtained with simultaneous ECG recording.

5. Statistical analysis

General characteristics were analyzed using SAS 9.2. (SAS Institute, Cary, NC, USA) Measurement data were expressed as means±standard deviations, and count data as percentages. Genetic analysis was conducted using PLINK software (PLINK v1.07; Purcell Lab, New York, NY, USA). In the genetic analysis, standardized multivariable-adjusted PWV and tonometry residuals were generated as described above. Mean arterial pressure (MAP) was calculated from the systolic and DBP. The dependent variable, mean baPWV, was transformed to a normal distribution using reciprocal transformation methods, and the covariates adjusted were age, sex, height, body mass index (BMI), MAP, site information, smoking status, alcohol consumption, and exercise. The numerical covariates were also transformed to form as normal a distribution as possible. A P-value $<5\times10^{-8}$ was regarded as statistically significant.

Results

1. General characteristics

The mean age of the 2,407 individuals was 56.6 years; 36.1% of the participants were male and the mean BMI was 24.0±3.0 kg/m^2. Subjects that smoked accounted for 16.2% of the total study population. Alcohol consumption was noted in 47.3% of the study subjects. Of the total study population, 30.2% exercised regularly. Casual SBP was 115.1±11.1 mmHg, casual MAP was 88.6±7.9 mmHg and casual DBP was 75.4±7.5 mmHg. Blood sample analysis indicated that the mean uric acid level was

4.7±1.3 mg/dL, total cholesterol was 190.6±29.4 mg/dL, HDL-cholesterol was 45.0±10.7 mg/dL, TGs were 133.7±79.1 mg/dL, glucose was 93.8±9.6 mg/dL, and creatinine was 0.9±0.1 mg/dL. Right brachial pulse rates were 64.8±9.3 beats/min. In the baPWV, the right baPWV was 1,390.8±228.5 cm/s, the left was 1,403.0±227.2 cm/s, and the mean baPWV was 1,396.9±224.4 cm/s. Table 1 also shows the characteristics of these variables at each study site, including the observation that mean baPWV differed across the study sites (Yangpyeong: 1,387.0±220.5 cm/s, Namwon: 1,421.2±232.3 cm/s, and Goryeong: 1,388.8±221.2

cm/s; P=0.0049).

2. Site information adjusted association

The arterial stiffness phenotype, mean baPWV, was used as an indicator for the site information adjusted association analysis. After applying the exclusion criteria, 723,056 SNPs were included in the analysis. The separate analysis adjusted by site information is shown in Table 2 and Fig 1. Nine SNPs had P-values $<10^{-6}$ for the association with baPWV. Of these, five SNPs (rs476840, rs11607110, rs1010761, rs11643412, and rs11648607)

Table 1. Characteristics of the general study population

	Yangpyeong (n=957)	Namwon (n=654)	Goryeong (n=796)	Total (n=2407)
Age (yr)	55.0±8.7	56.3±8.1	57.9±7.6	56.6±8.3
Male/female (n)	347/610 (36.3/63.7)	245/409 (37.5/62.5)	276/520 (34.7/65.3)	868/1,539 (36.1/63.9)
BMI (kg/m^2)	24.3±2.9	23.7±2.8	24.0±3.0	24.0±3.0
Smoking (n)	143 (14.9)	127 (19.4)	121 (15.2)	391 (16.2)
Alcohol (n)	485 (50.7)	290 (44.3)	364 (45.7)	1139 (47.3)
Exercise (n)	314 (32.8)	196 (30.0)	217 (27.3)	727 (30.2)
SBP (mmHg)	114.4±10.6	115.7±11.1	115.4±11.7	115.1±11.1
MAP (mmHg)	88.8±7.6	87.8±8.2	89.0±8.0	88.6±7.9
DBP (mmHg)	76.0±7.2	73.8±7.9	75.9±7.3	75.4±7.5
Uric acid (mg/dL)	4.7±1.2	4.9±1.3	4.6±1.3	4.7±1.3
T-cholesterol (mg/dL)	189.4±29.8	194.4±28.4	188.9±29.3	190.6±29.4
HDL-cholesterol (mg/dL)	45.2±10.8	43.8±10.1	45.7±10.8	45.0±10.7
TG (mg/dL)	134.5±78.9	130.3±77.5	135.5±80.7	133.7±79.1
FBG (mg/dL)	95.4±9.3	91.2±9.2	93.9±9.7	93.8±9.6
Creatinine (mg/dL)	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1
Right brachial pulse rate (beats/min)	64.4±9.5	66.3±9.5	64.1±8.7	64.8±9.3
Right baPWV (cm/sec)	1,381.0±225.7	1,416.3±234.5	1,381.6±225.6	1,390.8±228.5
Left baPWV (cm/sec)	1,393.0±221.0	1,426.2±235.9	1,396.0±226.1	1,403.0±227.2
Mean baPWV (cm/sec)	1,387.0±220.5	1,421.2±232.3	1,388.8±221.2	1,396.9±224.4

Values are presented as mean±standard deviation or number (%).
BMI, body mass index; SBP, systolic blood pressure; MAP, mean arterial pressure; DBP, diastolic blood pressure; T-cholesterol, total-cholesterol; HDL-cholesterol, high-density lipoprotein-cholesterol; TG, Triglyceride; FBG, fasting blood glucose; baPWV, brachial ankle pulse wave velocity.

Table 2. Description of SNPs in the association study with adjustment for site information ($P<10^{-6}$)

SNP	Chromosome	Position	Locus	Gene	Location	Minor allele	MAF	RAF	β	SE	P^a
rs2645290	1	119273431	1p11.1	TBX15	Intron	A	0.1586	0.159	−0.1714	0.03462	7.82E-07
rs1766787	1	119254281	1p11.1	TBX15	Intron	C	0.1588	0.159	−0.1714	0.03463	7.9E-07
rs2148150	1	119373580	-	-	-	A	0.1521	0.152	−0.167	0.03381	8.45E-07
rs476840	11	132568537	11q25	OPCML	Intron	C	0.02168	0.0217	0.6169	0.1011	1.21E-09
rs11607110	11	132576733	-	-	-	A	0.02154	0.0215	0.614	0.1006	1.22E-09
rs2073350	14	23614088	14q11.2	CPNE6	Intron	A	0.4138	0.414	0.1066	0.02089	3.57E-07
rs1010761	16	554822	16p13.3	PRR35	Intron	G	0.0251	0.0251	0.7008	0.1148	1.19E-09
rs11643412	16	605324	16p13.3	RAB40C	Intron	G	0.03218	0.0322	0.5031	0.09122	3.86E-08
rs11648607	16	611246	16p13.3	RAB40C	Intron	A	0.03178	0.0318	0.4892	0.08881	4.02E-08

[a]Site information was adjusted.
SNP, single nucleotide polymorphism; MAF, minor allele frequency; RAF, Risk allele frequency; β, regression coefficient; SE, standard error.

had P-values $<5\times10^{-8}$ and were linked to three genes (*OPCML*, *PRR35*, *RAB40C*). However, the MAFs were all low, i.e., between

Fig. 1. Q-Q plot showing the genome-wide P-values for site information adjusted associations. Lambda GC=0.996. The plot shows P-values for all the single nucleotide polymorphisms that were analyzed. Note that the circles in the right half of the plot are significantly above the line. A QQ-plot of these P-values was used to see if the very low P-values differed from the expected distribution (a uniform distribution). GC, genomic control; Q-Q, quantile-quantile.

0.01 and 0.05.

3. Multivariable adjusted association

The variations in age, sex, height, BMI, MAP, site information, smoking status, alcohol consumption, and exercise variables were adjusted for the arterial stiffness phenotype analysis. Covariate analysis included adjustment factors such as age, sex, and multiple variables. After adjusting for multiple confounding factors, it appeared that 18 SNPs had P-values $<10^{-6}$ for the association with baPWV. Of these, 11 SNPs (rs11993165, rs7121368, rs476840, rs11607110, rs10152599, rs2279496, rs7172171, rs16955052, rs1010761, rs11643412, and rs11648607) had P-values $<5\times10^{-8}$ and were linked to five genes (*PDGFRL, OPCML, APBA2, PRR35* and *RAB40C*) as shown in Table 3 and Figs. 2 and 3.

From the above results, five SNPs were common between site information adjusted association and multivariable adjusted association, with a P-value $<5\times10^{-8}$, and were linked to three genes (*OPCML, PRR35*, and *RAB40C*).

OPCML, the opioid-binding protein/cell adhesion molecule, is a protein that is encoded by the *OPCML* gene in humans [21,22]. This gene encodes a member of the IgLON subfamily in the immunoglobulin superfamily of proteins. The encoded pre-protein is proteolytically processed to generate the mature protein.
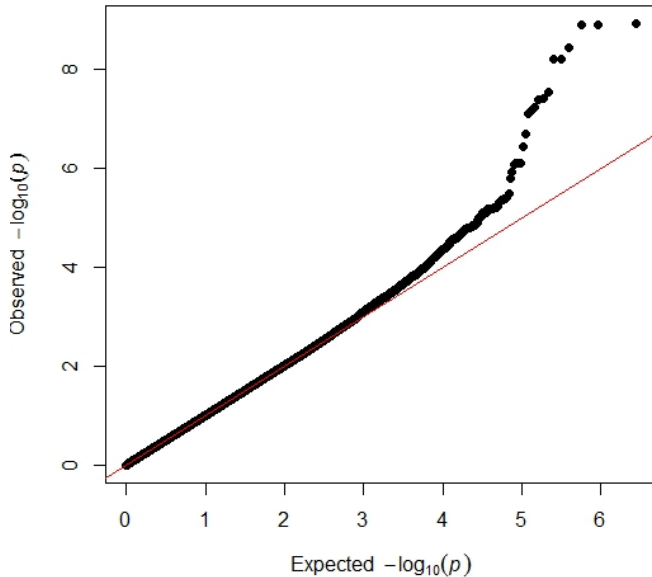
Table 3. Description of SNPs in the multivariable adjusted association study ($P<10^{-6}$)

SNP	Chromosome	Position	Locus	Gene	Location	Minor allele	MAF	RAF	β	SE	P^a
rs2046590	2	186604548	-	-	-	A	0.0629	0.0629	0.2366	0.0461	3.06E-07
rs1038587	2	186614035	2q32.1	*LINC01473*	Intron	A	0.0621	0.0621	0.2727	0.0554	9.26E-07
rs11993165	8	17527398	8p22-p21.3	*PDGFRL*	Intron	A	0.0405	0.0405	0.3015	0.0538	2.26E-08
rs7844026	8	93622913	-	-	-	A	0.0409	0.0409	0.4011	0.0777	2.67E-07
rs510960	11	132565481	11q25	*OPCML*	Intron	G	0.0307	0.0307	0.3524	0.0681	2.51E-07
rs7121368	11	132567618	11q25	*OPCML*	Intron	A	0.042	0.042	0.4712	0.0784	2.12E-09
rs476840	11	132568537	11q25	*OPCML*	Intron	C	0.0217	0.0217	0.6678	0.0797	9.31E-17
rs11607110	11	132576733	-	-	-	A	0.0215	0.0215	0.6646	0.0794	9.37E-17
rs2900264	12	12357203	-	-	-	A	0.0942	0.0942	0.1749	0.0346	4.61E-07
rs10152599	15	27149506	15q11-q12	*APBA2*	Intron	A	0.0296	0.0296	0.3864	0.0681	1.54E-08
rs2279496	15	27155608	15q11-q12	*APBA2*	Intron	G	0.0296	0.0296	0.3866	0.0681	1.50E-08
rs7172171	15	27157470	15q11-q12	*APBA2*	Intron	A	0.0296	0.0296	0.3865	0.068	1.51E-08
rs16955052	15	27167415	15q11-q12	*APBA2*	Intron	G	0.03	0.03	0.3888	0.0685	1.52E-08
rs1010761	16	554822	16p13.3	*PRR35*	Intron	G	0.0251	0.0251	0.7583	0.0906	9.30E-17
rs11643412	16	605324	16p13.3	*RAB40C*	Intron	G	0.0322	0.0322	0.5298	0.0723	3.11E-13
rs11648607	16	611246	16p13.3	*RAB40C*	Intron	A	0.0318	0.0318	0.5153	0.0703	3.22E-13
rs1672228	18	47182761	18q21.2	*LOC100287225*	Intron	A	0.1763	0.176	0.1297	0.0253	3.06E-07
rs821195	18	47190580	18q21.2	*LOC100287225*	Intron	A	0.1668	0.167	0.1314	0.026	4.46E-07

[a]Age, sex, height, body mass index, mean arterial pressure, site information, smoking status, alcohol consumption and exercise variables were adjusted.
SNP, single nucleotide polymorphism; MAF, minor allele frequency; RAF, Risk allele frequency; β, regression coefficient; SE, standard error.
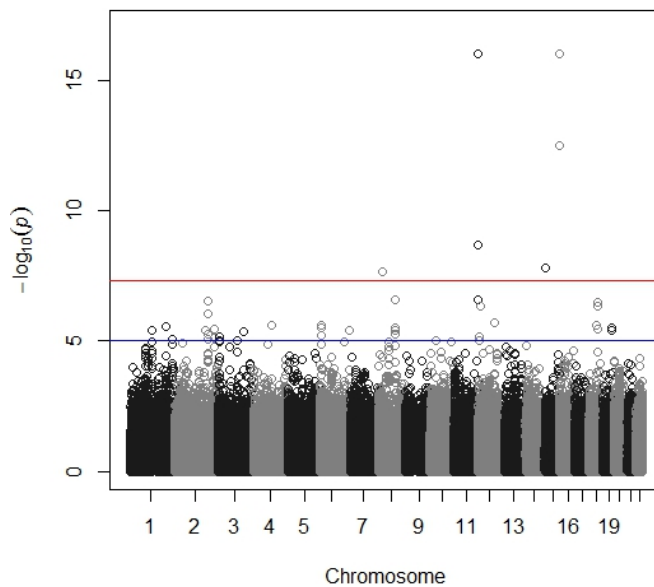
Fig. 2. Manhattan plot showing genome-wide *P*-values of multiple associations. The minimum *P*-values under the genetic models were obtained by linear regression analysis with adjustment for age, sex, height, body mass index, mean arterial pressure, site information, smoking status, alcohol consumption, and exercise. The y axis shows the $-\log_{10}$ *P*-values of 723,056 single nucleotide polymorphisms, and the x axis shows their chromosomal positions. The horizontal red and blue lines represent the thresholds of $P=3.47\times10^{-8}$ for Bonferroni's significance and $P=1\times10^{-5}$ for selecting the SNPs for replication, respectively.

Fig. 3. Q-Q plot showing genome-wide *P*-values in the multiple associations. Lambda GC=0.995. The plot shows *P*-values for all the single nucleotide polymorphisms that were analyzed. Note that the circles in the right half of the plot are significantly above the line. A QQ-plot of these *P*-values was used to see if the very low *P*-values differed from the expected distribution (a uniform distribution).

This protein is localized in the plasma membrane and may have an accessory role in opioid receptor function. This gene has an ortholog in rats and bovines. The opioid binding-cell adhesion molecule encoded by the rat gene binds opioid alkaloids in the presence of acidic lipids, exhibits selectivity for mu ligands, and acts as a glucose-6-phosphate isomerase-anchored protein. Since the encoded protein is highly conserved across species during evolution, it may have a fundamental role in mammalian systems. Two transcript variants encoding different isoforms have been found for this gene [22]. Alternative splicing results in multiple transcript variants, at least one of which encodes an isoform that is proteolytically processed (provided by RefSeq, Jan 2016). *PRR35* or proline rich 35, is a protein that is encoded by the *PRR35* gene in humans [23]. *RAB40C* is a member of the renin–angiotensin system oncogene family, but its specific role and mechanism has not been elucidated in the current study.

4. Multivariable adjusted association analyses for three study sites

For the 11 SNPs, multivariable analyses were preformed separately for three study sites as shown in Table 4, and the effect sizes for all 11 SNPs were extremely large only in Yangpyeong.
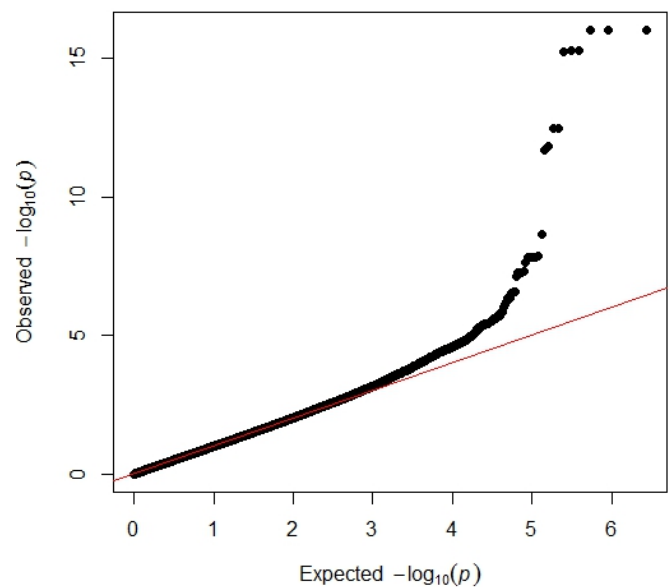
5. Meta–analysis

For the 11 SNPs, the results from the three study sites were meta-analyzed. Among the 11 SNPs, none was associated with baPWV with *P*-values $<5\times10^{-6}$ as shown in Table 5.

Discussion

1. Main findings

The main finding of the present study, which was performed in a healthy and normal general population, was that 5 SNPs were common between site information adjusted association and the multivariable adjusted association analyses with regard to baPWV. The multiple adjusted model was provided as a post hoc analysis to verify the findings of the site information adjusted association analysis. However, such associations with low MAF seemed to be noise signals because the multivariable analyses performed for the three individual study sites and the corresponding meta-analyses showed no significant SNPs associated with baPWV. From the statistical viewpoint, these were considered as negative findings.

2. Previous genome–wide association studies on arterial stiffness

Previous investigations of the association between arterial stiffness and SNPs using genome-wide association studies

Table 4. Description of SNPs in the multivariable adjusted association at each study site

Chromosome	SNP	Position	A1	β	SE	L95	U95	STAT	P^a
In Yangpyeong									
8	rs11993165	17527398	A	0.608	0.07685	0.4574	0.7586	7.912	7.06E-15
11	rs7121368	132567618	A	0.4816	0.07932	0.3261	0.6371	6.072	1.83E-09
11	rs476840	132568537	C	0.7149	0.08424	0.5498	0.88	8.487	8.13E-17
11	rs11607110	132576733	A	0.7149	0.08424	0.5498	0.88	8.487	8.13E-17
15	rs10152599	27149506	A	0.3873	0.06787	0.2543	0.5203	5.707	1.54E-08
15	rs2279496	27155608	G	0.3873	0.0679	0.2542	0.5204	5.704	1.57E-08
15	rs7172171	27157470	A	0.3873	0.06787	0.2543	0.5203	5.707	1.54E-08
15	rs16955052	27167415	G	0.3873	0.06787	0.2543	0.5203	5.707	1.54E-08
16	rs1010761	554822	G	0.7414	0.08737	0.5701	0.9126	8.486	8.21E-17
16	rs11643412	605324	G	0.5093	0.06738	0.3772	0.6414	7.559	9.59E-14
16	rs11648607	611246	A	0.502	0.06636	0.3719	0.632	7.565	9.21E-14
In Namwon									
8	rs11993165	17527398	A	−0.01274	0.0324	−0.07624	0.05077	−0.3931	6.94E-01
11	rs7121368	132567618	A	0.009672	0.03205	−0.05315	0.07249	0.3018	7.63E-01
11	rs476840	132568537	C	−0.004828	0.03207	−0.06768	0.05802	−0.1506	8.80E-01
11	rs11607110	132576733	A	−0.004828	0.03207	−0.06768	0.05802	−0.1506	8.80E-01
15	rs10152599	27149506	A	−0.003536	0.03217	−0.06658	0.05951	−0.1099	9.13E-01
15	rs2279496	27155608	G	−0.003491	0.03215	−0.06651	0.05952	−0.1086	9.14E-01
15	rs7172171	27157470	A	−0.003491	0.03215	−0.06651	0.05952	−0.1086	9.14E-01
15	rs16955052	27167415	G	−0.003491	0.03215	−0.06651	0.05952	−0.1086	9.14E-01
16	rs1010761	554822	G	0.03157	0.03205	−0.03125	0.09439	0.9849	3.25E-01
16	rs11643412	605324	G	0.02053	0.0323	−0.04277	0.08384	0.6357	5.25E-01
16	rs11648607	611246	A	−0.01115	0.03223	−0.07433	0.05202	−0.346	7.30E-01
In Goryeong									
8	rs11993165	17527398	A	−0.05761	0.02744	−0.1114	-0.00382	−2.099	3.61E-02
11	rs7121368	132567618	A	−0.0159	0.02746	−0.06971	0.03791	−0.5792	5.63E-01
11	rs476840	132568537	C	0.004207	0.02744	−0.04958	0.058	0.1533	8.78E-01
11	rs11607110	132576733	A	−0.0009364	0.02745	−0.05474	0.05287	−0.03411	9.73E-01
15	rs10152599	27149506	A	−0.01507	0.02743	−0.06883	0.0387	−0.5492	5.83E-01
15	rs2279496	27155608	G	−0.01488	0.02741	−0.0686	0.03883	−0.543	5.87E-01
15	rs7172171	27157470	A	−0.01523	0.02742	−0.06896	0.03851	−0.5553	5.79E-01
15	rs16955052	27167415	G	−0.02166	0.0274	−0.07536	0.03204	−0.7904	4.30E-01
16	rs1010761	554822	G	−0.009659	0.02751	−0.06359	0.04427	−0.351	7.26E-01
16	rs11643412	605324	G	0.005239	0.02761	−0.04888	0.05935	0.1897	8.50E-01
16	rs11648607	611246	A	−0.002022	0.02765	−0.05622	0.05217	−0.07313	9.42E-01

[a]Age, sex, height, body mass index, mean arterial pressure, smoking status, alcohol consumption and exercise variables were adjusted.
SNP, single nucleotide polymorphism; A1, first allele code; β, regression coefficient; SE, standard error; L95, lower bound of 95% confidence interval; U95, upper bound of 95% confidence interval; STAT, coefficient t-statistic.

(GWAS) involved pulse pressure and PWV. The genes identified in the various studies using pulse pressure, which included the Framingham Heart Study, did not yield consistent findings [24–26]. PWV is currently considered an optimal measure, as recently described in the consensus statement on arterial stiffness [27]. cfPWV is the gold-standard index of arterial stiffness; however, the complexity of measuring cfPWV [28] prevents its use in routine clinical practice. Measurement of baPWV is simple, noninvasive, and more practical for population surveys. However, in the Framingham Offspring Study, as in studies using carotid-brachial PWV, rs10514688, rs770189, and rs7042864 had significant associations after adjustment for age, sex, age[2], height, and weight ($P < 10^{-5}$) [29]. However, these SNPs were not associated with baPWV in our study. These results of our study

Table 5. Meta-analysis results of three study sites

Chromosome	Position	SNP	A1	A2	N	P-value[a]	P-value[b]	β	β(R)	Q	I²
8	17527398	rs11993165	A	G	3	0.7727	0.203	0.0058	0.1655	0.0000	97.02
11	132567618	rs7121368	A	G	3	0.1907	0.1468	0.0264	0.1386	0.0000	94.38
11	132568537	rs476840	C	A	3	0.03969	0.09631	0.0416	0.2201	0.0000	97.05
11	132576733	rs11607110	A	C	3	0.05497	0.1002	0.0388	0.2184	0.0000	97.07
15	27149506	rs10152599	A	G	3	0.2264	0.2093	0.0241	0.1093	0.0000	93.63
15	27155608	rs2279496	G	A	3	0.2252	0.2087	0.0242	0.1093	0.0000	93.62
15	27157470	rs7172171	A	G	3	0.228	0.2094	0.024	0.1092	0.0000	93.64
15	27167415	rs16955052	G	A	3	0.3013	0.2228	0.0206	0.1072	0.0000	93.78
16	554822	rs1010761	G	A	3	0.01945	0.07779	0.0474	0.2347	0.0000	97.04
16	605324	rs11643412	G	A	3	0.005439	0.1258	0.0557	0.1676	0.0000	95.99
16	611246	rs11648607	A	G	3	0.04409	0.1749	0.0403	0.1529	0.0000	96.25

[a]Fixed-effects meta-analysis, [b]random-effects meta-analysis.
SNP, single nucleotide polymorphism; A1, first allele code; A2, second allele code; N, number of valid studies for this SNP; P, fixed-effects meta-analysis P-value; β, fixed-effects β estimate; β(R), random-effects β estimate; Q, P-value for Cochrane's Q statistic; I, I² heterogeneity index (0-100).

may be related to differences in ethnicity, study subjects, and other factors. In addition, recent studies also showed that common genetic variants associated with baPWV could not be identified in cardiovascular patients [30]. Compared with our study, this study was based on cardiovascular patients as subjects and factors such as age, sex, SBP, and DBP were adjusted in the model.

3. Study population

Previously arterial stiffness and GWAS studies did not exclude subjects with chronic diseases, but the present study was undertaken in a healthy population to exclude the effect of acquired or environmental influences. McEniery et al. [31] and Giannattasio et al. [32] showed that arterial stiffening is associated with aging, hypertension, diabetes mellitus, and chronic kidney disease. We performed a GWAS for arterial stiffness in Korean individuals aged 40 to 70 years. The study subjects included three rural populations, and the analysis focused on distinct SNPs that regulate a specific, pathophysiologically relevant pathway that can alter downstream arterial properties. Moreover, there is increasing evidence that arterial stiffness and cardiovascular events are closely parallel. To the best of our knowledge, this study is the first GWAS to investigate the association between SNPs and baPWV levels in a healthy and normal general population.

4. Biological consideration: explanation for the genes and SNPs

Recently, GWAS have shown associations between measures of arterial stiffness and the loci on different chromosomes (chromosomes 2, 7, 13, and 15). However, the pathophysiologi-

cal links between the genetic and physiological components remain largely unknown [26,33,34]. In addition, some studies have reported that SNPs can affect arterial stiffness [8]; examples include SNPs in genes such as those encoding the renin-angiotensin-aldosterone system, matrix protein/metalloproteinases, the nitric oxide pathway, β-adrenergic/endothelin receptors, the inflammatory cascade, and others [35–39]. Compared with the current research, this study did not identify specific and relevant SNPs after accounting for various factors. The specific role and mechanism of the 5 SNPs and 3 genes has not been elucidated in the current research, but may be related to arterial stiffness and needs to be studied further.

5. Limitations of the present study

The present study has several limitations. First, with regard to age, the study subjects were a middle-aged cohort from three rural populations; therefore, a study needs to be conducted using a young population cohort. Secondly, the study involved a relatively small range of geographical areas and future studies should be conducted in the broader context of national and international populations. Third, in our study, we found 5 SNPs for 3 genes with a P-value less than 5×10^{-8} and MAFs about 0.02. With the current knowledge, the implication of an association by these low or rare MAFs cannot be interpreted properly. Because MAFs below 0.05 are usually excluded in standard GWAS, such SNPs with low MAFs shown in our study should not be regarded as an evidence of significant association and further studies for these low or rare MAFs are needed to properly interpret our results [40].

In conclusion, employing the recent standard applied to

GWAS and due to the lack of replication analysis in the present study, we found no evidence of a significant association signal for baPWV.

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