RESEARCH ARTICLE

Genome-wide association study of coronary artery calcification in asymptomatic Korean populations

Su-Yeon Choi1,2*, Eunsoon Shin3*, Eun Kyung Choe4, Boram Park5, Heesun Lee1,2, Hyo Eun Park1,2*, Jong-Eun Lee3, Seung Ho Choi1,2*

1 Department of Internal Medicine, Seoul National University Hospital Healthcare System Gangnam Center, Seoul, Korea, 2 Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea, 3 DNA Link, Inc., Seoul, Korea, 4 Department of Surgery, Seoul National University Hospital Healthcare System Gangnam Center, Seoul, Korea, 5 Department of Public Health Science, Seoul National University, Seoul, Korea

* These authors contributed equally to this work.

© sychoi9@gmail.com (SYC); cshmed@snuh.org (SHC)

Abstract

Epidemiologic evidence indicates that the prevalence and severity of coronary artery disease vary depending on ethnicity. In this study, a genome-wide association study for coronary artery calcification (CAC) was performed in a Korean population-based sample of 400 subjects without prior coronary artery disease and replicated in another of 1,288 subjects. CAC score, as assessed by multi-detector computed tomography, was evaluated in volunteers for screening purposes as part of a routine health examination. CAC score greater than the 90th percentile across the age in each sex group was considered severe CAC. Single nucleotide polymorphisms (SNPs) associated with severe CAC after adjusting for age, sex, hypertension, and diabetes were investigated using the additive model of logistic regression. One SNP (rs10757272 in the intronic region of the CDKN2B-AS1 gene in chromosome 9p21.3) met Bonferroni correction in the discovery set (p = 7.55E-08) and was also significant in the validation set by TaqMan assay (p = 0.036). Subjects with rs10757272 were found to have an increased odds ratio (OR) of having severe CAC in multivariate logistic regression analysis after adjusting for age, sex, hypertension, and diabetes (adjusted OR 3.24 and 95% CI 2.11–4.97). In conclusion, SNP rs10757272 in chromosome 9p21.3 was associated with severe CAC based on age and sex in an asymptomatic community-based Korean population. Therefore, it was associated with promotion of coronary artery calcification in subclinical state.

Introduction

Coronary artery calcium score (CACS) represents a measure of overall coronary atheromatous plaque burden and is a strong predictor of coronary artery disease (CAD). Epidemiological studies have identified that the development of coronary atherosclerosis is associated with
many risk factors [1,2]. These factors include plasma lipid concentrations, blood pressure, smoking, diabetes, inflammation markers, and lifestyle. However, the role of traditional risk factors has been proven only for some. A twin and family study in the Swedish Twin Registry has estimated the total heritability of susceptibility to CAD to be a significant proportion (40–50%) [3]. Therefore, genetic analysis has the potential to identify pathways of and therapeutic targets against CAD. A recent study has provided insights into the genetic basis of CAD and identified key biological pathways [4]. Genetic factors are defined as an important risk contributor for the pathogenesis of CAD and myocardial infarction (MI) [5–10].

Epidemiologic evidence indicates that the prevalence and severity of CAD vary depending on ethnicity [11]. Moreover, ethnic disparity with regard to coronary artery calcification (CAC) has been reported. In asymptomatic individuals without known CAD, Korean adults have a lower prevalence and severity of CAC compared with American adults [12]. Meanwhile, African Americans are less likely to develop CAC and have markedly lower levels of CACS than European Americans despite exposure to more severe conventional CVD risk factors [13]. The precise mechanisms that might influence these ethnic disparities are not well understood, but genetic factors may explain this variability by providing the basis for differences in individual susceptibility to CAD [14–16].

Most genome-wide association studies (GWASs) have been performed mainly in populations of European descent. The exact genetic markers and mechanisms to influence the risk of CAD in Koreans remain largely unknown.

In this study, we aimed to identify single nucleotide polymorphisms (SNPs) that could be associated with severe CAC in asymptomatic Korean populations using a GWAS.

Materials and methods
Study design and population

After obtaining informed consent for blood storage, blood samples were collected from subjects during a routine health check-up program at the Seoul National University Hospital Healthcare System Gangnam Center from January 2014 to March 2016. Blood samples were then stored at a biorepository. Among the subjects, asymptomatic subjects (n = 2,188) who had no history of CAD and underwent CACS by multi-detector computed tomography (CT) were retrospectively enrolled. All subjects opted to have a CACS evaluation for screening purposes as part of a routine health examination.

We defined CACS percentiles using the KOICA registry as the Korean reference score, which is a large multicenter registry of a single-ethnicity (Korean) sample of 85,945 asymptomatic individuals who underwent a health examination at 5 health-care centers including the Seoul National University Hospital Healthcare System Gangnam Center between 2002 and 2014 [17]. We defined severe CAC as a CACS greater than the 90th percentile for their age and sex (5-year interval, from 20–24 to ≥75 years of age (S1 Table). Subjects with a CACS less than the 50th percentile for their age and sex were used as the control group. Individuals with CACS between the observed 50th and 90th percentile for age and sex were excluded. Of the original 2,188 subjects evaluated, 1,688 subjects were included (n = 327 in severe CAC group and n = 1,361 in control group). Genotype data were produced using the Korean Chip (K-CHIP) in 400 samples (n = 100 in severe CAC group and n = 300 in control group) based on the time of enrollment, and GWAS was conducted with this sample as the discovery set.

The genome-wide significant SNP associated with severe CAC in the discovery set was screened using the TaqMan fluorogenic 5’ nuclease assay (ABI, Foster City, CA, USA) in the replication set (n = 227 in severe CAC group and n = 1,061 in control group).
Ethics statement
The Institutional Review Board of the Seoul National University Hospital approved the storage of bio-specimens with informed consent (IRB no. 1103-127-357). We used the bio-specimens retrospectively. The board approved this study protocol (IRB no. 1504-017-662), and informed consent for research use of bio-specimens was waived by the board. The study was conducted in accordance with the Declaration of Helsinki.

Hybridization on Affymetrix Axiom KORV1.0–96 array
Axiom2.0 Reagent Kit (Affymetrix, Santa Clara, CA, USA) was used according to the manufacturer's protocol. About 200 ng of genomic DNA was amplified and randomly fragmented into 25 to 125 base pair (bp) fragments. Genomic DNA initial amplification was performed in 40 μl reaction volume, containing 20 μl volume of genomic DNA at a concentration of 10 ng/μl and 20 μl of Denaturation Master Mix. The reaction of initial amplification was performed for 10 min at room temperature. Subsequently, the incubated products were amplified with 130 μl of Axiom 2.0 Neutral Soln, 225 μl of Axiom 2.0 Amp Soln, and 5 μl of Axiom 2.0 Amp Enzyme. The amplification reactions were carried out for 23 ± 1 h at 37˚C. The amplification of products was performed under optimized reaction to amplify fragments between 200 and 1,100 bp. A fragmentation step then reduced the amplified products to segments of approximately 25–50 bp, which were then end-labeled using biotinylated nucleotides. Following hybridization, the bound target was washed under stringent conditions to remove non-specific background to minimize background noise caused by random ligation events. Each polymorphic nucleotide was queried via a multi-color ligation event carried out on the array surface. After ligation, the arrays were stained and imaged on the GeneTitan MC Instrument (Affymetrix, Santa Clara, CA, USA). The image was analyzed using Genotyping Console Software (Affymetrix, Santa Clara, CA, USA). Genotype data were produced using K-CHIP available through the K-CHIP consortium. K-CHIP was designed by the Center for Genome Science, Korea National Institute of Health, Korea (4845–301, 3000–3031).

Genotyping and quality control
We performed systematic quality control steps on the raw genotype data and obtained 550,779 SNPs. SNPs with a minor allele frequency of case and control <1%, a call rate of case or control <95%. A significant deviation from Hardy-Weinberg equilibrium in controls (p <0.0001) were excluded. We also excluded SNPs likely to be false-positive associations due to wrong clustering via visual inspection of the cluster plots.

TaqMan assay
The genotyping of SNP which met Bonferroni correction for genome-wide significance of severe CAC in the discovery set was screened using the TaqMan fluorogenic 5’ nuclease assay (ABI, Foster City, CA, USA) in the replication set. The final volume of polymerase chain reaction (PCR) was 5 ul, containing 10 ng of genomic DNA and 2.5 ul of TaqMan Universal PCR Master Mix, with 0.13 ul of 20X Assay Mix (Assay ID C___2684958_10). Thermal cycle conditions were as follows: 50˚C for 2 min to activate the uracil N-glycosylase and to prevent carry-over contamination, 95˚C for 10 min to activate the DNA polymerase, followed by 45 cycles of 95˚C for 15 s and 60˚C for 1 min. All PCR were performed using 384-well plates by a Dual 384-Well GeneAmp PCR System 9700 (ABI, Foster City, CA, USA), and the endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (ABI, Foster City, CA, USA).
Foster City, CA, USA). Duplicate samples and negative controls were included to ensure accuracy of genotyping.

**Conventional risk factors**

Baseline demographic characteristics, including age, sex, body weight, and height, were recorded. Body mass index (BMI) was calculated as weight divided by height squared in kg/m². Information on risk factors and past medical history of smoking, hypertension, and diabetes mellitus was obtained using self-administered questionnaires. Presence of hypertension was defined as systolic/diastolic blood pressure >140/90 mmHg or a history of being diagnosed with hypertension or current treatment with antihypertensive medication.

Diabetes mellitus was defined by fasting blood glucose ≥7.0 mmol/l (≥126 mg/dl), hemoglobin A1c ≥6.5%, history of diabetes diagnosis, or taking medication for diabetes. Laboratory tests included lipid profiles, fasting glucose, and hemoglobin A1c.

**Cardiac CT and analysis for CACS**

Coronary CT was performed using either a 256-slice multidetector CT scanner (Brilliance iCT 256; Philips Medical Systems, Cleveland, Ohio) or a 16-slice scanner (Somatom Sensation 16; Siemens Medical Solutions, Forchheim, Germany). A calcium scan was performed using standard prospective or retrospective methods with a 330- to 370-ms gantry rotation time. Image data were reconstructed using a 2.5–3 mm slice thickness. All scans were performed with electrocardiogram-gated dose modulation. The estimated radiation doses for CACS ranged between 0.7 and 1.5 mSv. The CACS was calculated quantitatively according to the method described by Agatston et al. [18] and a software program was used (Rapidia 2.8; INFINITT, Seoul, Republic of Korea).

**Statistics**

We performed GWAS in the discovery set (n = 400) with a case–control study between the subjects with severe CAC (>90th percentile for age and sex) and control (≤50th percentile for age and sex). The results were verified using the validation test. The SNP determined to have genome-wide significance after Bonferroni correction in the discovery set was screened using the TaqMan fluorogenic 5’ nuclease assay (ABI, Foster City, CA, USA) in the replication set (n = 1288). A p-value less than 0.05 was considered significant in the replication set.

Logistical regression analyses were used to calculate the odds ratios (OR), 95% confidence intervals (CI), and corresponding p-values of each SNP of severe coronary calcification, controlling for age, sex, hypertension, and diabetes as covariates with additive models. All analyses were two-tailed, and p-values <0.05 were considered statistically significant. Statistical tests were performed using PLINK version 1.9 (https://www.cog-genomics.org/plink2; Free Software Foundation Inc., Boston, MA, USA). R statistical software package, version 3.1.1 (R development Core Team; R Foundation for Statistical Computing, Vienna, Austria) was used to analyze statistical data and to draw the Manhattan plot of -log10. In the regional plot, hg19/1000 Genomes Nov 2014 ASN was used as the reference panel for linkage disequilibrium [19].

**Results**

**Study population characteristics**

The characteristics of the study population including 400 subjects in the discovery set and 1,288 subjects in the replication set are described in Table 1. Between the two sets, the basal characteristics were not significantly different (p>0.05 in all parameters).
Table 1. Baseline characteristics of study population.

|                      | Discovery set | Validation set |
|----------------------|---------------|----------------|
|                      | Total’         | Severe CAC     | p-value | Total’         | Severe CAC | p-value |
| N (%)                | 400           | 100 (25.0)     |         | 1,288         | 227 (17.6) |         |
| Male, %              | 78.0          | 75.2           | 0.676   | 72.5          | 88.1       | <0.001  |
| Age, mean±SD, years  | 52.9±7.1      | 52.9±7.3       | 0.046   | 52.9±7.3      | 52.8±7.4   | 0.403   |
| CACS, median [IQ range] | 93.7±276.3   | 373.5±499.6   | <0.001  | 64.2±265.9    | 362.2±542.5 | <0.001  |
| CACS, %              | 0 [0, 15.4]   | 0 [0, 0]       |         | 0 [0, 0]      | 213 [93.4, 409.9] |         |
| BMI                  | 24.3±2.6      | 24.8±2.6       | 0.031   | 24.2±2.7      | 24.0±2.6   | 0.611   |
| Systolic blood pressure, mmHg | 117.3±13.4 | 119.6±13.1     | 0.054   | 117.5±13.6    | 119.5±12.8 | 0.017   |
| Diastolic blood pressure, mmHg | 77.8±9.9    | 79.8±10.2     | 0.027   | 78.0±10.4     | 76.1±10.7  | 0.83±8.8 | <0.001  |
| Fasting blood glucose, mg/dl | 103±21.1    | 111.3±27.6    | <0.001  | 101±18.2      | 106.9±22.8 | <0.001  |
| Hemoglobin A1c, %    | 5.8±0.6       | 5.8±0.6       | 0.001   | 5.8±0.6       | 5.7±0.5   | 0.001   |
| Low-density lipoprotein cholesterol, mg/dl | 123±29.3 | 121±30.4       | 0.484   | 123±30.9      | 120±30.7  | 0.217   |
| High-density lipoprotein cholesterol, mg/dl | 52.0±12.6 | 52.7±12.1     | 0.090   | 52.2±12.4     | 49.8±13.9 | 0.002   |
| Triglycerides, mg/dl | 133.9±101.7  | 137.7±84.6    | 0.709   | 124.8±73.2    | 138.2±66.5 | 0.003   |
| C-reactive protein, mg/dl | 0.13±0.38  | 0.13±0.18     | 0.998   | 0.12±0.25     | 0.12±0.26 | 0.847   |
| Hypertension, %      | 29.3          | 46.0           | <0.001  | 28.6          | 40.5       | <0.001  |
| Diabetes, %          | 14.0          | 26.0           | <0.001  | 10.7          | 17.6       | <0.001  |
| Current smoker, %    | 19.0          | 21.8           | 0.431   | 17.8          | 21.5       | 0.127   |
| Ex-smoker, %         | 37.7          | 43.7           | 0.200   | 38.0          | 34.8       | 0.531   | <0.001  |

CACS, coronary artery calcium score; IQ, interquartile; BMI, body mass index

*all p-values > 0.05 in discovery set vs. validation set

https://doi.org/10.1371/journal.pone.0214370.t001

Among the 400 subjects in the discovery set, 312 (78.0%) were men. The mean age was 52.9 ±7.1 years, and the median CACS was zero [interquartile range 0, 15.4]. Study participants in the severe CAC group were older and had slightly higher BMI, diastolic blood pressure, fasting glucose, and hemoglobin A1c and higher proportion of hypertension and diabetes. In the control group, 98% of subjects had zero calcium. As shown in S1 Table, CACS was classified as severe with respect to age and sex, and men <40 years old and women <55 years old with any CACS greater than 0 were classified as having severe CAC because individuals below the 90th percentile had a CACS of 0. Therefore, in these age groups, severe CAC indicates earlier CAC presence.

**GWAS of severe coronary calcification**

In the discovery set, an association analysis was conducted for severe CAC using 550,779 SNPs that passed the quality control. Quantile-quantile plots showed generally little evidence for statistical inflation and good agreement, except in the tail where deviations are expected due to genuine associations (Fig 1). Fig 1 also shows the Manhattan plot of the GWAS for cases with severe CAC.

We performed the GWAS in the discovery set after adjusting for age, sex, hypertension, and diabetes using the additive genetic model of logistic regression. One SNP (rs10757272) in
the intronic region of the cyclin-dependent kinase inhibitor 2B anti-sense RNA gene (CDKN2B-AS1) in chromosome 9p21.3 passed Bonferroni correction (at a threshold of \( p = 9.08 \times 10^{-8} \)) for genome-wide significance in the discovery set (\( p = 7.55 \times 10^{-8} \)). Rs10757272 was also significant in the validation set by TaqMan assay (\( p = 0.036 \)).

The regional plot in chromosome 9 is provided in Fig 2, which shows rs10757272 as the top SNP and nearby SNPs with the level of linkage disequilibrium with rs10757272.

The subjects with rs10757272 were found to have an increased OR of having severe CAC in multivariate logistic regression analysis after adjusting for age, sex, hypertension, and diabetes (adjusted OR 3.24 and 95% CI 2.11–4.97) (Table 2).

**Discussion**

In this community-based population without CAD, we demonstrated that one SNP (rs10757272) in the chromosome 9p21.3 locus reached significance for severe CAC for age and sex in Koreans. We also replicated the association of this SNP in subjects in the validation set. Therefore, 9p21.3 variant promoted coronary artery calcification in subclinical state among asymptomatic Koreans. It has mainly been previously reported in the Western population, implying trans-ancestry risk for CAC.

Recently, the National Human Genome Research Institute GWAS catalog shows that >50 SNPs for coronary artery susceptibility variants were identified during the GWAS era, pooling >100,000 subjects of European descent [4]. In multiple cohorts of European descent, other SNPs on chromosome 9p21.3 have been consistently associated with CAD (rs10757274 and rs2383206), and MI (rs2383207 and rs10757278) [5–9]. In a study with Korean patients who had >70% luminal narrowing in at least one vessel by coronary angiography, percutaneous coronary angioplasty, coronary artery bypass graft, or MI, four SNPs (rs10757274, rs2383206, rs2383207, and rs10757278) on chromosome 9p21.3 was reported as an important susceptibility locus [20]. A collaborative meta-analysis reported an association between chromosome
9p21.3 risk locus and a greater burden of angiographically defined CAD in many races including Korean, Chinese, and Japanese [21]. However, it had no association with MI in the presence of background CAD. They hypothesized that the 9p21.3 risk locus primarily precipitates the development of atherosclerotic lesion and significant coronary stenosis rather than promoting plaque rupture or thrombosis. A recent study has reported that variation in 9p21.3 may not only be an independent genetic risk factor for CAD, but may also modify the association between diastolic blood pressure levels and the extent of subclinical coronary atherosclerosis [22].

Table 2. SNP associated with severe coronary calcification in Koreans and the odds ratios.

| SNP   | Chr | Position* | Risk allele | Nearest gene | Allele frequency | HWE   | OR† | 95% CI | p-value |
|-------|-----|-----------|-------------|--------------|------------------|-------|-----|--------|---------|
| rs10757272 | 9   | 22088260  | T           | CDKN2B-AS1   | 0.785            | 0.582 | 0.95 | 2.11–4.97 | 3.34E-08 |

SNP, single-nucleotide polymorphism; Chr, chromosome number; HWE, Hardy–Weinberg equilibrium; OR, odds ratio; CI, confidence interval.
*The SNP positions are indexed to the National Center for Biotechnology Information build 37.
†Association results generated from additive model of logistic regression analyses adjusted with age, sex, hypertension, and diabetes.

https://doi.org/10.1371/journal.pone.0214370.t002

Fig 2. Regional association plot in chromosomes 9. The purple diamonds indicate the associated SNP according to joint analyses. Nearby SNPs are color-coded according to the level of linkage disequilibrium with the top SNP. The left y-axis shows the significance of the association on a -log10 p-value (logistic regression), and the right y-axis shows the recombination rate across the region. Estimated recombination rates from the 1000 Genomes Project Asian base data and hg19 database [19] are plotted with the blue line to reflect the local linkage disequilibrium structure.

https://doi.org/10.1371/journal.pone.0214370.g002
SNPs in this region on chromosome 9p21.3 also have been associated with the quantity of CAC. A meta-analysis reported genome-wide significant associations with CACS >100 versus <100 for SNP rs1333049 on chromosome 9p21.3 mainly in White populations in Northern Europe and North America [10]. Gong et al. reported that the rs1333049 allele in 9p21.3 was associated with the extent or severity of CAC in CAD patients and increased the risk of CV events in the presence of CAC. Therefore, they suggested that the 9p21.3 locus related calcification may affect the susceptibility to CAD [23].

We used reference values for CACS defined according to age and sex in Koreans. In men <40 years old and women <55 years old, the presence of CAC (CAC> 0) was defined as severe CAC, in contrast to the control individuals, who had zero calcium. Therefore, in these age groups, we suggested that SNP rs10757272 promotes the presence of CAC in the population of asymptomatic Koreans without prior history of CAD. CAC is a prognostic factor in asymptomatic subjects as well as CAD patients. Prior asymptomatic population-based studies have documented the relationship between higher CAC and worsened cardiovascular prognosis [24, 25]. A sub-group study of CONFIRM in patients without known CAD demonstrated that patients without luminal narrowing but with CAC experienced greater risk of 5-year mortality than those with zero calcium [26].

However, in GWAS, most of the loci associated with CAD are not related to the risk factors for atherosclerosis. The mechanisms of gene-disease association remain undefined [4]. Even if the suggestive loci in our study have no direct association with known traditional risk factor for CAD, some biological studies have suggested an atherosclerotic mechanism of chromosome 9p21.3 loci. Chromosome 9p21.3 resides in a region distant from known coding regions, with tumor suppressor genes of cyclin-dependent kinase inhibitors (CDKN2A and CDKN2B) and methylthioadenosine phosphorylase. The nearest genes to the 9p21.3 region are CDKN2A and CDKN2B, which are associated with cell cycling and death [27] and relevant to the atherosclerotic process with excessive vascular smooth muscle cell proliferation [28] and proliferation of neointimal content of macrophages [29]. In addition, rs10757272 in the intronic region of CDKN2B-AS1 was reported to be associated with platelet reactivity [30]. These are key pathological mechanisms promoting early development and progression of atherosclerosis. Further studies are necessary to investigate the pathogenesis of atherosclerosis and vascular calcification and clinical utility of genes in 9p21.3 including CDKN2A and CDKN2B.

This study has limitations. First, its sample size was small. The discovery and validation sets were different sets, but drawn from the same population in a single center. A validation study with a different Korean population should be performed in the future. Second, the study population was composed of subjects who underwent regular health check-ups. Thus, this study should be performed using a larger set of clinical CAD patients with MI or coronary revascularization. This study has some strong points. This is the first GWAS study showing the association of CAC quantity in an asymptomatic community-based Korean population. Because Koreans are ideal for case-control association studies as they are ethnically and genetically homogeneous populations, all study subjects were of the same ethnicity (Korean). Moreover, we used a more precise definition of severe CAC. Age is the strongest independent factor for CAC and men and women show a different pattern of increment in CACS. CACS increases with age, and it increases rapidly after age 50 in Korean men and after age 60 in Korean women, with a “time-lag” of about 10 years in women [31]. Therefore, in spite of the absolute CACS, we used the CACS above the 90th percentile of the healthy reference sample in each age and sex in Koreans as a relative cut-off value of severe CAC.
Conclusions
In asymptomatic community-based Korean populations, SNP rs10757272 on chromosome 9p21.3 were associated with severe CAC based on each age and sex. Therefore, it was associated with promoting coronary calcification in subclinical state. These data could support the role of genetic variation as contributors to CAC in Koreans. Subsequent independent replication in large sample size and functional studies including patients with subclinical and clinical CAD are required to elucidate its genetic basis and the molecular pathways underlying the pathogenesis of CAC and CAD.

Supporting information
S1 Table. CACS in control group and severe CAC group in each group as stratified by age and sex.
(DOCX)

Author Contributions
Conceptualization: Su-Yeon Choi, Eunsoon Shin, Eun Kyung Choe, Seung Ho Choi.
Data curation: Su-Yeon Choi, Boram Park, Heesun Lee, Hyo Eun Park.
Formal analysis: Su-Yeon Choi, Eunsoon Shin, Jong-Eun Lee.
Funding acquisition: Eun Kyung Choe, Seung Ho Choi.
Investigation: Su-Yeon Choi, Eunsoon Shin, Jong-Eun Lee.
Methodology: Su-Yeon Choi, Eunsoon Shin, Jong-Eun Lee.
Project administration: Su-Yeon Choi, Eun Kyung Choe, Seung Ho Choi.
Resources: Su-Yeon Choi, Eun Kyung Choe, Heesun Lee, Hyo Eun Park, Seung Ho Choi.
Software: Su-Yeon Choi, Eunsoon Shin, Jong-Eun Lee.
Supervision: Su-Yeon Choi, Eunsoon Shin, Eun Kyung Choe, Seung Ho Choi.
Validation: Eunsoon Shin, Boram Park.
Visualization: Eunsoon Shin.
Writing – original draft: Su-Yeon Choi, Eunsoon Shin.
Writing – review & editing: Su-Yeon Choi.

References
1. Greenland P, Knoll MD, Stamler J, Neaton JD, Dyer AR, Garside DB, et al. Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. JAMA 2003; 290: 891–897. https://doi.org/10.1001/jama.290.7.891 PMID: 12928465
2. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004; 364: 937–952. https://doi.org/10.1016/S0140-6736(04)17018-9 PMID: 15364185
3. Peden JF, Farrall M. Thirty-five common variants for coronary artery disease: the fruits of much collaborative labour. Hum Mol Genet. 2011; 20: R198–R205. https://doi.org/10.1038/hmg/ddi/384 PMID: 21875899
4. CARDioGRAMplusC4D Consortium, Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet. 2013; 45: 25–33. https://doi.org/10.1038/ng.2480 PMID: 23202125
5. Schunkert H, Koenig IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet. 2011; 43: 333–338. https://doi.org/10.1038/ng.784 PMID: 21378990

6. Coronary Artery Disease (CAD) Genetics Consortium. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. Nat Genet. 2011; 43: 339–344. https://doi.org/10.1038/ng.782 PMID: 21378988

7. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al. A common allele on chromosome 9 associated with coronary heart disease. Science. 2007; 316: 1488–1491. https://doi.org/10.1126/science.1142447 PMID: 17478681

8. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, et al. Genomewide association analysis of coronary artery disease. N Engl J Med. 2007; 357: 443–453. https://doi.org/10.1056/NEJMoA072366 PMID: 17634449

9. Helgadottir A, Thorleifsson G, Magnusson KP, Grétarsdottir S, Steinthorsdottir V, Manolescu A, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet. 2008; 40: 217–224. https://doi.org/10.1038/ng.72 PMID: 18176561

10. O’Donnell CJ, Kavousi M, Smith AV, Kardia SL, Feitosa MF, Hwang SJ, et al. Genome-wide association study for coronary artery calcification with follow-up in myocardial infarction. Circulation. 2011; 124: 2855–2864. https://doi.org/10.1161/CIRCULATIONAHA.110.974899 PMID: 22144573

11. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. Circulation. 2001; 104: 2746–2753. PMID: 11723030

12. Han D, O’Harttaih B, Gransar H, Lee JH, Choi SY, Chun EJ, et al. Prevalence and distribution of coronary artery calcification in asymptomatic United States and Korean adults—cross-sectional propensity-matched analysis. Circ J. 2016; 80: 2349–2355. https://doi.org/10.1253/circj.CJ-16-0762 PMID: 27666599

13. Lee TC, O’Malley PG, Feuerstein I, Taylor AJ. The prevalence and severity of coronary artery calcification on coronary artery computed tomography in black and white subjects. J Am Coll Cardiol. 2003; 41: 39–44. PMID: 12570942

14. Saab KR, Kendrick J, Yracheta JM, Lanaspa MA, Pollard M, Johnson RJ. New Insights on the Risk for Cardiovascular Disease in African Americans: The Role of Added Sugars. J Am Soc Nephrol. 2015; 26: 247–57. https://doi.org/10.1681/ASN.2014040393 PMID: 25090991

15. Divers J, Palmer ND, Lu L, Register TC, Carr JJ, Hicks PJ, et al. Admixture mapping of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990; 15: 827–832. PMID: 20981092

16. Wassel CL, Pankow JS, Peralta CA, Choudhry S, Seldin MF, Arnett DK. Genetic ancestry is associated with subclinical cardiovascular disease in African-Americans and Hispanics from the multi-ethnic study of atherosclerosis. Circ Cardiovasc Genet. 2009; 2: 629–636. https://doi.org/10.1161/CIRCGENETICS.109.876243 PMID: 20031644

17. Lee JH, O’Harttaih B, Han D, Park HE, Choi SY, Sung J, et al. Reassessing the usefulness of coronary artery calcium score among varying racial and ethnic groups by geographic locations: relevance of the Korea Initiatives on Coronary Artery Calcium registry. J Cardiovasc Ultrasound. 2015; 23: 195–203. https://doi.org/10.4250/jcu.2015.23.4.195 PMID: 26755926

18. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990; 15: 280–287. PMID: 24077622

19. 1000 Genomes Project Consortium, Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, et al. A map of human genome variation from population-scale sequencing. Nature. 2010; 467: 1061–1073. https://doi.org/10.1038/nature09534 PMID: 20981092

20. Shen GQ, Li L, Rao S, Abdullah KG, Ban JM, Lee BS, et al. Four SNPs on chromosome 9p21 in a South Korean population implicate a genetic locus that confers high cross-race risk for development of coronary artery disease. Arterioscler Thromb Vasc Biol. 2008; 28: 360–365. https://doi.org/10.1161/ATVBAHA.107.157248 PMID: 18048766

21. Chan K, Patel RX, Newcombe P, Nelson CP, Qasim A, Epstein S, et al. Association between the chromosome 9p21 locus and angiographic coronary artery disease burden: a collaborative meta-analysis. J Am Coll Cardiol. 2013; 61: 957–70. https://doi.org/10.1016/j.jacc.2012.10.051 PMID: 23352782

22. Kim DS, Smith JA, Bielak LF, Wu CY, Sun YV, Sheedy PF 2nd, et al. The relationship between diastolic blood pressure and coronary artery calcification is dependent on single nucleotide polymorphisms on chromosome 9p21.3. BMC Med Genet. 2014; 15: 89–96. https://doi.org/10.1186/s12881-014-0089-2 PMID: 25185447
23. Gong L, Chen J, Lu J, Fan L, Zhang Y, Lv B, et al. The 9p21 locus is associated with coronary artery disease and cardiovascular events in the presence (but not in the absence) of coronary calcification. PLoS One. 2014; 9: e9482.

24. Detrano R, Guerci AD, Carr JJ, R Bild DE, Burke G, Folsom AR, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. N Engl J Med. 2008; 358: 1336–1345. https://doi.org/10.1056/NEJMoa072100 PMID: 18367736

25. Cho I, Al’Aref SJ, Berger A, Ó Hartaigh B, Gransar H, Valenti V, et al. Prognostic value of coronary computed tomographic angiography findings in asymptomatic individuals: a 6-year follow-up from the prospective multicentre international CONFIRM study. Eur Heart J. 2018; 39: 934–941. https://doi.org/10.1093/eurheartj/ehx774 PMID: 29365193

26. Cho I, Ó Hartaigh B, Gransar H, Valenti V, Lin FY, Achenbach S, et al. Prognostic implications of coronary artery calcium in the absence of coronary artery luminal narrowing. Atherosclerosis. 2017; 262: 185–190. https://doi.org/10.1016/j.atherosclerosis.2016.12.006 PMID: 28385391

27. Jarinova O, Stewart AF, Roberts R, Wells G, Lau P, Naing T, et al. Functional analysis of the chromosome 9p21.3 coronary artery disease risk locus. Arterioscler Thromb Vasc Biol. 2009; 29: 1671–1677. https://doi.org/10.1161/ATVBAHA.109.189522 PMID: 19592466

28. Visel A, Zhu Y, May D, Atzal V, Gong E, Attanasio C, et al. Targeted deletion of the 9p21 non-coding coronary artery disease risk interval in mice. Nature. 2010; 464: 409–12. https://doi.org/10.1038/nature08801 PMID: 20173736

29. González-Navarro H1, Abu Nabah YN, Vinué A, Andrés-Manzano MJ, Collado M, Serrano M, et al. p19 (ARF) deficiency reduces macrophage and vascular smooth muscle cell apoptosis and aggravates atherosclerosis. J Am Coll Cardiol. 2010; 55: 2258–2268. https://doi.org/10.1016/j.jacc.2010.01.026 PMID: 20381282

30. Musunuru K, Post WS, Herzog W, Shen H, O’Connell JR, McArdle PF, et al. Association of single nucleotide polymorphisms on chromosome 9p21.3 with platelet reactivity: a potential mechanism for increased vascular disease. Circ Cardiovascular Genetics. 2010; 3: 445–453. https://doi.org/10.1161/CIRCGENETICS.109.929508 PMID: 20858905

31. Park HE, Kim MK, Choi SY, Lee W, Shin CS, Cho SH, et al. The prevalence and distribution of coronary artery calcium in asymptomatic Korean population. Int J Cardiovasc Imaging. 2012; 28: 1227–1235. https://doi.org/10.1007/s10554-011-9922-2 PMID: 21789749