Original Article

Associations between Genetic Variants and Angiographic Characteristics in Patients with Coronary Artery Disease

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***Aim*: In this study, we investigated the genetic determinants of lesion characteristics and the severity of coronary artery disease (CAD) using a genome-wide association study (GWAS) and replication genotyping.**
***Methods*: The discovery set for GWAS consisted of 667 patients exhibiting angiographically diagnosed CAD with symptoms. For replication genotyping, 837 age- and sex-matched CAD patients were selected. Genetic determinants of lesion characteristics (diffuse vs. non-diffuse lesions), the number of diseased vessels (multi-vessel vs. single vessel disease) and the modified Duke score (high vs. low), which indicates the severity of CAD, were analyzed after adjusting for confounding factors.**
***Results*: Single nucleotide polymorphisms (SNPs) rs12917449, rs10152898 and rs231150 were associated with diffuse lesions, while rs1225006 and rs6745588 were associated with multi-vessel disease. However, on replication genotyping, no significant associations were found between any of these five SNPs and the lesion characteristics or CAD severity. In contrast, in the combined population of both the discovery and replication sets, genotypes rs125006 of *CPNE4* and rs231150 of *TRPS1* were found to be significantly associated with the modified Duke score. The addition of rs1225006 to conventional risk factors had significant incremental value in the model of the score.**
***Conclusions*: The associations between five SNPs identified using GWAS and angiographic characteristics were not significant in the current replication study. However, two variants, particularly rs1225006, were found to be associated with the severity of CAD in the combined set. These results indicate the potential clinical implication of these variants with respect to the risk of CAD.**

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Key words: Coronary artery disease, Angiography, Genome-wide association study

Introduction

It is well known that the etiology of atherosclerotic cardiovascular disease is multi-factorial[1]. In past decades, numerous efforts have been made to understand the pathophysiology of coronary artery disease (CAD) beyond clinical risk factors. The heritability of CAD is known to vary widely, with most estimates ranging between 30% and 50%[2, 3]. Recently, genome-wide association studies (GWAS) and combined analyses have identified several genetic loci associated with the risk of CAD, characterizing the possible biological functions of these genes[4]. Such studies have proposed some candidate genes as determinants of CAD, and further investigations of variants are under way.

The treatment of patients with complex coronary lesions, such as diffuse lesions, remains a major concern for cardiologists[5]. Because various clinical and

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angiographic characteristics are considered during the process of clinical decision making in CAD management, enhancing knowledge of variants related to specific vascular phenotypes would be beneficial. However, studies exploring the genetic variants associated with detailed phenotypes of atherosclerosis and/or CAD are extremely limited. Therefore, we investigated the genetic determinants of lesion characteristics and the severity of CAD using GWAS and replication genotyping.

Methods

Study Population

We used a two-stage approach: the identification of variants associated with lesion characteristics or the severity of CAD using GWAS, followed by replication genotyping of the identified candidate variants. The sample size calculation is described in **Supplementary Table 1**. The discovery set consisted of 667 CAD patients enrolled in the Genomics Research in Cardiovascular Disease (GenRIC) consortium, which was established for the genomic study of CAD in Korea. Men <55 years of age and women <65 years of age were included. The patients underwent coronary angiography for chest discomfort or pain. The diagnosis of CAD was made based on the detection of angiographic findings showing significant stenosis (≥ 50%) in at least one epicardial coronary artery. In the replication study, 837 age- and sex-matched CAD patients were selected from the patient database of the Cardiovascular Genome Center at Yonsei University Health System, Seoul, Korea. The inclusion criteria were the same for both sets. This study was approved by the local institutional review board, and all subjects or their representatives provided their written informed consent.

Clinical and Angiographic Data Collection

Trained nurses obtained the clinical data, including demographic variables and medical history. The characteristics and severity of CAD were assessed according to lesion characteristics, number of diseased vessels and modified Duke score. The lesion characteristics were classified as diffuse if the coronary arteries contained at least one lesion measuring ≥ 20 mm[6] and non-diffuse if the arteries contained no such lesion. The modified Duke score is the sum of the parameters associated with coronary lesions, including the number of diseased vessels, degree of stenosis and involvement of the proximal left anterior descending or left main coronary arteries[7]. All angiographic findings were confirmed by at least two interventional cardiologists. Blood samples were collected from all study subjects during enrollment.

GWAS and Replication Genotyping

On GWAS, the samples were genotyped using the Affymetrix Genome-Wide Human SNP array 6.0. The BirdSeed genotyping algorithm was used for the genotype analysis. Genotyping was performed as previously described[8]. Briefly, the samples were analyzed on a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) for purity, yield and concentration and separated on 1% agarose gels for integrity. Purity was determined according to the A260/A280 and A260/A230 ratios. Following GWAS, variants with a *p*-value in the additive model of $< 1.0 \times 10^{-5}$ were selected for replication. Genomic DNA was extracted from 5-mL peripheral blood samples using a commercially available kit (QuickGene SNP Kit DNA whole blood; Fujifilm, Tokyo, Japan). Genotyping was performed with 5' exonuclease (Taqman) chemistry on the ABI Prism 7000 device (Applied Biosystems, Foster City, CA, USA).

Statistical Analysis

Baseline demographic and laboratory data are presented as the mean ± standard deviation for continuous variables and frequencies for discrete variables. An additive model was employed to examine the associations between SNPs and the lesion characteristics and severity of CAD, with a logistic regression analysis adjusted for age, sex, hypertension, hyperlipidemia, diabetes mellitus and chronic kidney disease. Common SNPs (N=542,675) that met the quality control criteria were analyzed (Plink v1.07)[9]. Associations of common variants reaching $P \leq 5 \times 10^{-8}$ are considered to be significant on GWAS testing[10]. Combined statistics were calculated from meta-analysis assuming a fixed-effects model using the R program (version 3.0.2). Regional plots were drawn with the LocusZoom standalone software package, version 1.1, based on HapMap phase II JPT+CHB for five SNPs (**Fig. 1**). In order to increase the power, the discovery and replication sets were combined to determine the associations between the SNPs and the modified Duke score. The score was categorized as low (≤ 42) or high (>42) when evaluating the associations among the variants. A multivariate logistic regression analysis was used for the association analyses following adjustment for the confounding variables listed above. The statistical power of the odds ratio (OR) was calculated using the Quanto program, version 1.2.4. We calculated the integrated discrimination improvement (IDI) in order to measure the incremental value of adding SNPs to

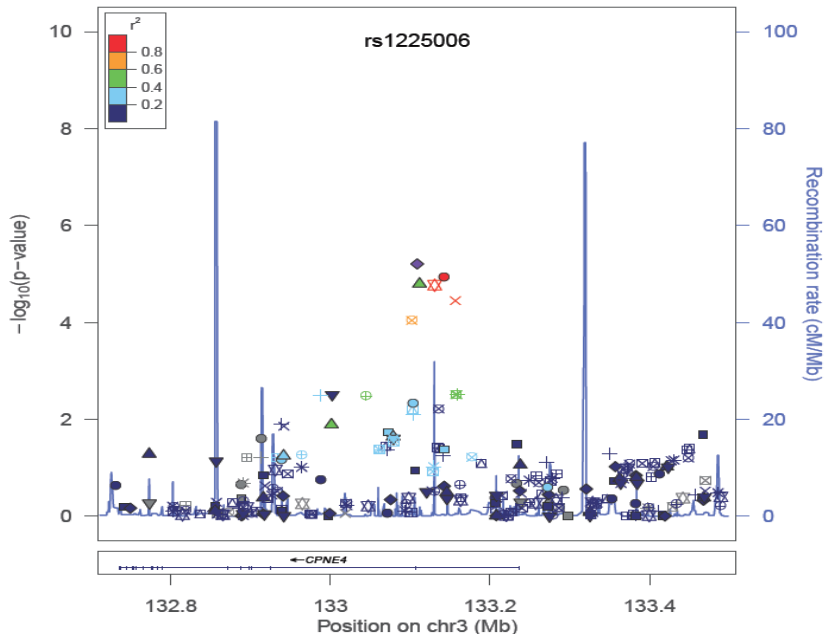

Fig. 1. Regional associations for rs1225006

the conventional prediction model of CAD severity. The R-package PredictABEL software program (version 3.0.1) was used to calculate the IDI.

Results

Clinical Characteristics of the Study Population

The mean age of the discovery set was 49.9 years, and men comprised 60% of the set. Sixty-four percent of the subjects had acute coronary syndrome, 48% had diffuse lesions and 34% had multi-vessel disease. Current smokers were more common in the discovery set, whereas the prevalence of hypertension, diabetes mellitus, hyperlipidemia and multi-vessel disease and the modified Duke scores were higher in the replication set. (**Table 1**)

Associations between the SNPs and the Lesion Characteristics and Severity of CAD

The minor allele of rs12917449 of *PML* at chromosome 15 exhibited a positive association with the presence of diffuse lesions (OR: 2.45, $p=4.17\times10^{-6}$). The direction and strength of the association between the minor allele of rs10152898 and the presence of diffuse characteristics were similar to those for rs12917449, while another variant, rs231150 of *TRPS1* at chromosome 8, showed a negative association with the findings of diffuse lesions (OR: 0.53, $p=1.44\times10^{-5}$). In addition, a variant genotype of rs1225006 of *CPNE4* at chromosome 3 exhibited a negative association with multi-vessel disease (OR: 0.41, $p=7.55\times10^{-6}$), whereas a variant of rs6745588 of *STK39* at chromosome 2 demonstrated a positive association (OR: 2.02, $p=5.84\times10^{-5}$). However, none of these associations reached the GWAS significance level (**Table 2**). Interestingly, a variant of rs1225006 clustered with several other SNPs with high *p*-values (**Fig. 1**). In the replication genotyping analysis, none of the five SNPs mentioned above displayed a significant association with the presence of diffuse lesions or multi-vessel disease.

Combined Analysis

In the combined population of the discovery and replication sets, none of the five SNPs were significantly associated with lesion characteristics or number of diseased vessels (**Table 2**). However, a univariate logistic regression analyzing the associations between the SNPs and the modified Duke score identified the frequencies of genotypes of rs231150, rs1225006 and rs6745588 in the low-score group as being different from those in the high-score group. When the AA genotype was set as a reference, the AT and TT genotypes of rs231150 showed a negative association with the modified Duke score. Compared to the AA genotype, the AG and GG genotypes of rs125006 displayed a negative correlation with the score. Conversely, the subjects with AG or GG genotypes of rs6745588 exhibited a positive association with the score (**Table 3**). In the multivariate logistic regression

Table 1. Clinical characteristics of the study subjects

	Discovery set (*N*=667)	Replication set (*N*=853)	*p*
Age, years	49.9±7.9	49.5±7.6	0.35
Male	399 (59.8)	517 (60.6)	0.80
Hypertension	306 (46.0)	462 (54.2)	0.001
Diabetes mellitus	143 (21.5)	249 (29.2)	0.001
Hyperlipidemia	101 (15.2)	237 (27.8)	<0.001
Current smoker	169 (24.2)	179 (21.0)	0.002
Chronic kidney disease*	40 (6.9)	61 (7.8)	0.53
Body mass index, kg/m^2	25.1±3.2	25.4±3.3	0.79
Clinical presentation of CAD			
Stable angina	222 (33.7)	272 (31.9)	
Unstable angina	197 (29.9)	277 (32.5)	
Myocardial infarction	240 (36.4)	304 (35.6)	0.59
Lesion characteristics			
Non-diffuse	336 (51.1)	417 (48.9)	
Diffuse	321 (48.9)	436 (51.1)	0.21
Number of diseased vessels			
Single	430 (65.7)	331 (38.8)	
Multiple	224 (34.3)	522 (61.2)	<0.001
Modified Duke score	39.0±19.7	44.6±20.6	<0.001

The values are presented as the mean±SD or number (%). CAD: coronary artery disease; Missing covariates were excluded.

*Chronic kidney disease was defined as a decline in GFR to <60 mL/min/1.73 m^2

Table 2. Associations between the top five SNPs and the lesion characteristics and number of diseased vessels

CHR	SNP	Gene	Genomic location[a]	Discovery set			Replication set		Combined set	
				Risk allele	OR (95% CI)[b]	*P*	OR (95% CI)[b]	*P*	OR (95% CI)[b]	*P*
Lesion characteristics (diffuse)										
15	rs12917449	*PML*	72118712	C	2.45 (1.67-3.59)	4.17×10^{-6}	1.08 (0.80-1.47)	6.05×10^{-1}	1.50 (1.19-1.89)	6.66×10^{-4}
8	rs231150	*TRPS1*	116489503	A	0.53 (0.40-0.70)	1.44×10^{-5}	1.09 (0.88-1.34)	4.37×10^{-1}	0.84 (0.71-0.99)	3.49×10^{-2}
15	rs10152898		72042174	T	2.57 (1.73-3.81)	2.80×10^{-6}	1.16 (0.85-1.59)	3.51×10^{-1}	1.59 (1.25-2.03)	1.66×10^{-4}
Number of diseased vessels (multiple)										
3	rs1225006	*CPNE4*	133108849	A	0.41 (0.28-0.60)	7.55×10^{-6}	0.86 (0.66-1.13)	2.80×10^{-1}	0.68 (0.55-0.84)	3.33×10^{-4}
2	rs6745588	*STK39*	168664268	G	2.02 (1.44-2.85)	5.84×10^{-5}	1.15 (0.87-1.52)	3.19×10^{-1}	1.46 (1.18-1.80)	4.59×10^{-4}

[a]location is based on NCBI Build 37. [b]adjusted for age, sex, hypertension, diabetes mellitus, hyperlipidemia, chronic kidney disease and body mass index. CHR: chromosome; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval

analysis, the genotype distributions of rs1225006 and rs231150 were found to be significantly associated with the modified Duke score. Meanwhile, the AG and GG genotypes of rs1225006 showed a negative association with the score (OR: 0.79, $p=0.03$), while the AT and TT genotypes of rs231150 exhibited similar results (OR: 0.85, $p=0.048$). Following adjustment for confounding variables, the relationship between the rs6745588 genotype and the score was no longer significant.

The degree of improvement in sensitivity obtained by adding the variants was examined in a model of the modified Duke score. Notably, only rs1225006 displayed significant incremental value compared to the conventional model (**Supplementary Table 2**).

Table 3. Associations between the five SNPs and the modified Duke score in the combined set

Genotype	Modified Duke score		Univariate analysis		Multivariate analysis	
	Low (*N*=859)	High (*N*=658)	OR (95%CI)	*p*	OR (95%CI)	*p*
rs12917449						
AA	657 (76.7)	487 (74.1)	1.00		1.00	
AC+CC	200 (23.3)	170 (25.9)	1.15 (0.91-1.45)	0.25	1.17 (0.93-1.47)	0.17
rs231150						
AA	338 (40.1)	302 (46.2)	1.00		1.00	
AT+TT	504 (59.9)	352 (53.8)	0.78 (0.64-0.96)	0.01	0.85 (0.72-0.99)	0.048
rs10152898						
GG	668 (78.4)	495 (75.7)	1.00		1.00	
GT+TT	185 (21.6)	159 (24.3)	1.17 (0.92-1.49)	0.21	1.20 (0.96-1.51)	0.13
rs1225006						
AA	550 (64.3)	456 (69.3)	1.00		1.00	
AG+GG	305 (35.7)	202 (30.7)	0.80 (0.64-0.99)	0.04	0.79 (0.64-0.98)	0.03
rs6745588						
AA	601 (70.2)	428 (65.1)	1.00		1.00	
AG+GG	255 (29.8)	230 (34.9)	1.27 (1.02-1.57)	0.03	1.22 (0.99-1.50)	0.057

The score was defined as low (≤ 42) or high (>42). OR: odds ratio; CI: confidence interval. Missing genotypes for each SNP were excluded in the analysis.
The multivariate analysis was adjusted for age, sex, hypertension, diabetes mellitus, hyperlipidemia, chronic kidney disease and body mass index

Discussion

The present study investigated the genetic determinants of lesion characteristics and severity of CAD using GWAS and replication genotyping. Consequently, the associations between five candidate SNPs identified on GWAS and the CAD-related variables were not significant in the replication set. However, the analysis of the combined population of the discovery and replication sets showed that the genotypes rs1225006 of *CPNE4* and rs231150 of *TRPS1* were associated with the modified Duke score. Furthermore, the addition of rs1225006 to conventional risk factors exhibited significant incremental value in the model of the score. These results suggest that these two variants, particularly rs1225006, are associated with the risk of atherosclerosis and CAD.

Several studies have been conducted to identify genetic determinants of the angiographic and/or clinical severity of CAD. Recently, two studies demonstrated that the 9p21 locus is associated with the angiographic severity of CAD[11, 12]. In a subsequent meta-analysis, the association between 9p21 and multi-vessel disease was noted[13]. In the current study, we examined the associations between 9p21 (rs1333049) and lesion characteristics and the number of diseased vessels. However, we found no significant associations between these factors. Additionally, a case of diffuse coronary and carotid atherosclerosis was recently reported in a patient with a mutation in *ABCA1*, which is involved in cellular cholesterol efflux[14]. Subsequently, it was reported that a polymorphism of *hOGG1*, which encodes a key component in nuclear and mitochondrial DNA repair, determines the susceptibility to multi-vessel CAD[15]. The number of known loci relevant to CAD severity, however, remains low. Apart from the previously highlighted loci, two additional variants have been identified, although functional studies are required to determine their clinical relevance.

Our efforts to identify novel variants associated with lesion characteristics did not result in findings with genome-wide significance. The morphological characteristics of CAD are influenced by clinical factors, including age[16], diabetes mellitus and impaired glucose tolerance[17]. Impaired glucose metabolism is reported to affect the onset of CAD via the effects of endothelial dysfunction, a hyperplastic vasculature and prothrombotic state[18, 19]. Negative remodeling and the presence of fibrous and calcified plaque also contribute to the development of diffuse lesions[20]. However, to our knowledge, studies of the genetic determinants of these lesion characteristics are significantly limited, and no well-characterized genetic links have been established. In most previous studies, the lesion characteristics were assessed using quantitative

coronary angiography. Conducting lesion phenotyping with more precise imaging modalities, such as intravascular ultrasound, can be difficult to undertake in large populations, which are usually required for modern genomic studies. This may explain why few well-established genetic variants associated with lesion characteristics have been identified.

SNP rs1225006 is one of the two variants found to be associated with CAD severity in the present study. This SNP is located in an intron of *CPNE4* (copine Ⅳ), which belongs to the highly conserved copine family, and encodes a Ca^{2+}-dependent phospholipid-binding protein that may play a role in membrane trafficking, mitogenesis and development[21, 22]. However, data regarding the functional implications of *CPNE4* variants are limited. One animal study showed that *CPNE4* is one of the genes downregulated most significantly following mild ischemic exposure in cortical neurons and may participate in cell death or survival. In addition, *CPNE4* is upregulated in the myocardium of children with chronic hypoxia. Whether the link between *CPNE4* variants and CAD severity is dependent on the aforementioned cellular adaptation pathway remains to be determined.

The second variant identified in this study, rs231150, is located in *TRPS1*, which encodes a transcription factor bound to a dynein light chain protein. The binding of the encoded protein affects subsequent binding to GATA consensus sequences, thereby suppressing its transcriptional activity[23, 24]. Although the pathophysiological and/or clinical relevance of this gene in the setting of cardiovascular disease is unclear, a few studies have suggested that such variants are associated with the total cholesterol or high-density lipoprotein levels[25, 26]. Furthermore, *TRPS1* has been shown to be involved in smooth muscle cell differentiation via transcriptional regulation[27]. Interestingly, in a transcriptome profiling study, *TRPS1* was found to be highly expressed in the macrophages of large atherosclerotic lesions in apoE-deficient mice[28].

The current study assessed lesion characteristics and the severity of CAD using a comprehensive review of the findings of coronary angiography, which has not always been possible in prior large studies. The relatively small size of our study population is an acknowledged limitation of this study. Furthermore, the modified Duke score can be influenced by multiple risk factors and may not be the optimal parameter for evaluating the significance of specific SNPs. However, we evaluated the associations between the SNPs and the score after adjusting for major risk factors in an attempt to minimize this limitation. Although no significant genetic variants were validated according to the GWAS scale and replication genotyping, this study identified two novel variants associated with the modified Duke score. These data extend our understanding of the effects of genomics on CAD.

In conclusion, the associations between five candidate SNPs identified on GWAS and the lesion characteristics and severity of CAD were not significant in this study. However, the analysis of the combined population of the discovery and replication sets revealed that the genotypes rs1225006 of *CPNE4* and rs231150 of *TRPS1* were associated with the severity of CAD based on the modified Duke score. Furthermore, the addition of rs1225006 to conventional risk factors exhibited incremental value in the model of the severity score. These findings increase our understanding of the genetic influences on atherosclerosis and suggest that additional research is required to determine the genetic risks associated with CAD.

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Conflicts of Interest

None.

References

1) Ellis SG, Vandormael MG, Cowley MJ, DiSciascio G, Deligonul U, Topol EJ, Bulle TM: Coronary morphologic and clinical determinants of procedural outcome with angioplasty for multivessel coronary disease. Implications for patient selection. Multivessel angioplasty prognosis study group. Circulation, 1990; 82: 1193-1202
2) Mayer B, Erdmann J, Schunkert H: Genetics and heritability of coronary artery disease and myocardial infarction. Clin Res Cardiol, 2007; 96: 1-7
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
4) Consortium CAD, Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, Ingelsson E, Saleheen D, Erdmann J, Goldstein BA, Stirrups K, Konig IR, Cazier JB, Johansson A, Hall AS, Lee JY, Willer CJ, Chambers JC, Esko T, Folkersen L, Goel A, Grundberg E, Havulinna AS, Ho WK, Hopewell JC, Eriksson N, Kle-

ber ME, Kristiansson K, Lundmark P, Lyytikainen LP, Rafelt S, Shungin D, Strawbridge RJ, Thorleifsson G, Tikkanen E, Van Zuydam N, Voight BF, Waite LL, Zhang W, Ziegler A, Absher D, Altshuler D, Balmforth AJ, Barroso I, Braund PS, Burgdorf C, Claudi-Boehm S, Cox D, Dimitriou M, Do R, Consortium D, Consortium C, Doney AS, El Mokhtari N, Eriksson P, Fischer K, Fontanillas P, Franco-Cereceda A, Gigante B, Groop L, Gustafsson S, Hager J, Hallmans G, Han BG, Hunt SE, Kang HM, Illig T, Kessler T, Knowles JW, Kolovou G, Kuusisto J, Langenberg C, Langford C, Leander K, Lokki ML, Lundmark A, McCarthy MI, Meisinger C, Melander O, Mihailov E, Maouche S, Morris AD, Muller-Nurasyid M, Mu TC, Nikus K, Peden JF, Rayner NW, Rasheed A, Rosinger S, Rubin D, Rumpf MP, Schafer A, Sivananthan M, Song C, Stewart AF, Tan ST, Thorgeirsson G, van der Schoot CE, Wagner PJ, Wellcome Trust Case Control C, Wells GA, Wild PS, Yang TP, Amouyel P, Arveiler D, Basart H, Boehnke M, Boerwinkle E, Brambilla P, Cambien F, Cupples AL, de Faire U, Dehghan A, Diemert P, Epstein SE, Evans A, Ferrario MM, Ferrieres J, Gauguier D, Go AS, Goodall AH, Gudnason V, Hazen SL, Holm H, Iribarren C, Jang Y, Kahonen M, Kee F, Kim HS, Klopp N, Koenig W, Kratzer W, Kuulasmaa K, Laakso M, Laaksonen R, Lee JY, Lind L, Ouwehand WH, Parish S, Park JE, Pedersen NL, Peters A, Quertermous T, Rader DJ, Salomaa V, Schadt E, Shah SH, Sinisalo J, Stark K, Stefansson K, Tregouet DA, Virtamo J, Wallentin L, Wareham N, Zimmermann ME, Nieminen MS, Hengstenberg C, Sandhu MS, Pastinen T, Syvanen AC, Hovingh GK, Dedoussis G, Franks PW, Lehtimaki T, Metspalu A, Zalloua PA, Siegbahn A, Schreiber S, Ripatti S, Blankenberg SS, Perola M, Clarke R, Boehm BO, O'Donnell C, Reilly MP, Marz W, Collins R, Kathiresan S, Hamsten A, Kooner JS, Thorsteinsdottir U, Danesh J, Palmer CN, Roberts R, Watkins H, Schunkert H, Samani NJ: Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet, 2013; 45: 25-33

5) Rodes-Cabau J, Gutierrez M, Courtis J, Larose E, Dery JP, Cote M, Nguyen CM, Gleeton O, Proulx G, Roy L, Noel B, Barbeau G, De Larochelliere R, Rinfret S, Bertrand OF: Importance of diffuse atherosclerosis in the functional evaluation of coronary stenosis in the proximal-mid segment of a coronary artery by myocardial fractional flow reserve measurements. Am J Cardiol, 2011; 108: 483-490
6) Krone RJ, Laskey WK, Johnson C, Kimmel SE, Klein LW, Weiner BH, Cosentino JJ, Johnson SA, Babb JD: A simplified lesion classification for predicting success and complications of coronary angioplasty. Registry committee of the society for cardiac angiography and intervention. Am J Cardiol, 2000; 85: 1179-1184
7) Miller JM, Rochitte CE, Dewey M, Arbab-Zadeh A, Niinuma H, Gottlieb I, Paul N, Clouse ME, Shapiro EP, Hoe J, Lardo AC, Bush DE, de Roos A, Cox C, Brinker J, Lima JA: Diagnostic performance of coronary angiography by 64-row ct. N Engl J Med, 2008; 359: 2324-2336
8) Lee JY, Lee BS, Shin DJ, Woo Park K, Shin YA, Joong Kim K, Heo L, Young Lee J, Kyoung Kim Y, Jin Kim Y, Bum Hong C, Lee SH, Yoon D, Jung Ku H, Oh IY, Kim BJ, Lee J, Park SJ, Kim J, Kawk HK, Lee JE, Park HK, Lee JE, Nam HY, Park HY, Shin C, Yokota M, Asano H, Nakatochi M, Matsubara T, Kitajima H, Yamamoto K, Kim HL, Han BG, Cho MC, Jang Y, Kim HS, Euy Park J, Lee JY: A genome-wide association study of a coronary artery disease risk variant. J Hum Genet, 2013; 58: 120-126
9) Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC: Plink: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet, 2007; 81: 559-575
10) Panagiotou OA, Ioannidis JP, Genome-wide significance project: What should the genome-wide significance threshold be? Empirical replication of borderline genetic associations. Int J Epidemiol, 2012; 273-286
11) Dandona S, Stewart AF, Chen L, Williams K, So D, O'Brien E, Glover C, Lemay M, Assogba O, Vo L, Wang YQ, Labinaz M, Wells GA, McPherson R, Roberts R: Gene dosage of the common variant 9p21 predicts severity of coronary artery disease. J Am Coll Cardiol, 2010; 56: 479-486
12) Patel RS, Su S, Neeland IJ, Ahuja A, Veledar E, Zhao J, Helgadottir A, Holm H, Gulcher JR, Stefansson K, Waddy S, Vaccarino V, Zafari AM, Quyyumi AA: The chromosome 9p21 risk locus is associated with angiographic severity and progression of coronary artery disease. Eur Heart J, 2010; 31: 3017-3023
13) Chan K, Patel RS, Newcombe P, Nelson CP, Qasim A, Epstein SE, Burnett S, Vaccarino VL, Zafari AM, Shah SH, Anderson JL, Carlquist JF, Hartiala J, Allayee H, Hinohara K, Lee BS, Erl A, Ellis KL, Goel A, Schaefer AS, El Mokhtari NE, Goldstein BA, Hlatky MA, Go AS, Shen GQ, Gong Y, Pepine C, Laxton RC, Whittaker JC, Tang WHW, Johnson JA, Wang QK, Assimes TL, Nothlings U, Farrall M, Watkins H, Richards AM, Cameron VA, Muendlein A, Drexel H, Koch W, Park JE, Kimura A, Shen WF, Simpson IA, Hazen SL, Horne BD, Hauser ER, Quyyumi AA, Reilly MP, Samani NJ, Ye S: Association between the chromosome 9p21 locus and angiographic coronary artery disease burden a collaborative meta-analysis. J Am Coll Cardiol, 2013; 61: 957-970
14) Dimitroulis D, Kelm M, Vorpahl M: Single heterozygote splice mutation in the abca1 gene is associated with diffuse atherosclerotic disease in a low high-density lipoprotein syndrome. Eur Heart J, 2012; 33: 2281-2281
15) Wang CL, Lin TH, Lin HY, Sheu SH, Yu ML, Hsiao PJ, Lin KD, Hsu C, Yang YH, Shin SJ: The 8-oxoguanine glycosylase i (hogg1) ser326cys variant affects the susceptibility to multi-vessel disease in taiwan coronary artery disease patients. Thromb Res, 2010; 126: 319-323
16) Bigi R, Cortigiani L, Colombo P, Desideri A, Bax JJ, Parodi O: Prognostic and clinical correlates of angiographically diffuse non-obstructive coronary lesions. Heart, 2003; 89: 1009-1013
17) Kataoka Y, Yasuda S, Morii I, Otsuka Y, Kawamura A, Miyazaki S: Quantitative coronary angiographic studies of patients with angina pectoris and impaired glucose tolerance. Diabetes Care, 2005; 28: 2217-2222
18) Davi G, Catalano I, Averna M, Notarbartolo A, Strano A,

Ciabattoni G, Patrono C: Thromboxane biosynthesis and platelet-function in type-ii diabetes-mellitus. N Engl J Med, 1990; 322: 1769-1774
19) Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA: Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. Circulation, 1993; 88: 2510-2516
20) Fujii K, Mintz GS, Kobayashi Y, Carlier SG, Takebayashi H, Jacoboff D, Yasuda T, Moussa I, Dangas G, Mehran R, Lansky AJ, Reyes A, Kreps E, Collins M, Stone GW, Leon MB, Moses JW: Vascular remodeling and plaque composition between focal and diffuse coronary lesions assessed by intravascular ultrasound. Am J Cardiol, 2004; 94: 1067-1070
21) Creutz CE, Tomsig JL, Snyder SL, Gautier MC, Skouri F, Beisson J, Cohen J: The copines, a novel class of c2 domain-containing, calcium-dependent, phospholipid-binding proteins conserved from paramecium to humans. J Biol Chem, 1998; 273: 1393-1402
22) Tomsig JL, Snyder SL, Creutz CE: Identification of targets for calcium signaling through the copine family of proteins. Characterization of a coiled-coil copine-binding motif. J Biol Chem, 2003; 278: 10048-10054
23) Kaiser FJ, Tavassoli K, Van den Bemd GJ, Chang GT, Horsthemke B, Moroy T, Ludecke HJ: Nuclear interaction of the dynein light chain lc8a with the trps1 transcription factor suppresses the transcriptional repression activity of trps1. Hum Mol Genet, 2003; 12: 1349-1358
24) Malik TH, Shoichet SA, Latham P, Kroll TG, Peters LL, Shivdasani RA: Transcriptional repression and developmental functions of the atypical vertebrate gata protein trps1. EMBO J, 2001; 20: 1715-1725
25) Sayols-Baixeras S, Lluis-Ganella C, Lucas G, Elosua R: Pathogenesis of coronary artery disease: Focus on genetic risk factors and identification of genetic variants. Appl Clin Genet, 2014; 7: 15-32
26) Vimaleswaran KS, Cavadino A, Hypponen E: Apoa5 genotype influences the association between 25-hydroxyvitamin d and high density lipoprotein cholesterol. Atherosclerosis, 2013; 228: 188-192
27) Donati C, Marseglia G, Magi A, Serrati S, Cencetti F, Bernacchioni C, Nannetti G, Benelli M, Brunelli S, Torricelli F, Cossu G, Bruni P: Sphingosine 1-phosphate induces differentiation of mesoangioblasts towards smooth muscle. A role for gata6, PloS one. 2011; 6(5): e20389
28) Smith JD, Peng DQ, Dansky HM, Settle M, Baglione J, Le Goff W, Chakrabarti E, Xu YM, Peng XJ: Transcriptome profile of macrophages from atherosclerosis-sensitive and atherosclerosis-resistant mice. Mamm Genome, 2006; 17: 220-229

Supplementary Methods for GWAS and Replication Genotyping

Preparation and Quality Control

For preparation and quality control of the DNA, stringent criteria were used to assure the quality of the data. Peripheral whole blood samples for genomic DNA were collected at the time of enrollment and visually inspected for an adequate fluid volume in individual tubes.

Genetic variants were excluded from the GWAS analysis if they met the following quality control criteria: (1) a genotype call rate of $<95\%$; (2) a minor allele frequency of $<1\%$; (3) deviation from the Hardy-Weinberg equilibrium with a *p*-value of $<10^{-6}$; (4) a poor cluster plot (5) multiple positioning and the presence of mitochondrial SNPs. Therefore, in total, 542,675 SNPs from the Affymetrix array were used in the association study.

Genotyping Cluster Plots

Genotype calls for the Affymetrix array were determined using two separate case and control batches according to the Birdseed algorithm. In order to create a cluster plot for a given SNP, total signal information was processed to generate an integrated summary file. The summary file was then translated into cluster plot format using an algorithm similar to SST 1.0 (SNP signal tool, Affymetrix). The cluster plots were inspected manually for five SNPs considered for the replication studies.

Replication Genotyping

Variants with a p-value of $<1.0\times10^{-5}$ in the additive model in the GWAS analysis were selected for replication. Peripheral whole blood samples were used to obtain genomic DNA. All samples were monitored according to the same quality control method. We obtained a high call rate of 99%.

Supplementary Table 1. Power calculations for the GWAS study

Numbers	Power			
	OR=1.5	OR=1.75	OR=2.0	OR=2.25
600	0.10	0.67	0.97	0.99
700	0.17	0.81	0.99	0.99
900	0.37	0.96	0.99	0.99
1100	0.58	0.99	0.99	0.99
1300	0.76	0.99	0.99	0.99
1500	0.88	0.99	0.99	0.99

The assumptions are as follows: an additive inheritance model with an effect allele frequency of 20%, alpha threshold of 5×10^{-8} and CAD prevalence of 2%.

Supplementary Table 2. Degree of model improvement according to the integrated discrimination improvement (IDI)

	C- index	IDI (95%CI)	*P*-value
Model for modified Duke score			
Conventional risk factors	53.5		
Conventional risk factors + rs231150	54.7	0.028 (− 0.0001-0.006)	0.061
Conventional risk factors + rs1225006	54.4	0.036 (0.0002-0.007)	0.036

Conventional risk factors included age, sex, hypertension, diabetes mellitus, hyperlipidemia, chronic kidney disease and body mass index.