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journal or publication title	Biomedical Reports
volume	9
number	5
page range	383-404
year	2018-09
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URL	http://hdl.handle.net/2241/00157110

doi: 10.3892/br.2018.1152

Identification of 26 novel loci that confer susceptibility to early-onset coronary artery disease in a Japanese population

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Received May 31, 2018; Accepted September 5, 2018

DOI: 10.3892/br.2018.1152

Abstract. Early-onset coronary artery disease (CAD) has a strong genetic component. Although genome-wide association studies have identified various genes and loci significantly associated with CAD mainly in European populations, genetic variants that contribute toward susceptibility to this condition in Japanese patients remain to be definitively identified. In the present study, exome-wide association studies (EWASs) were performed to identify genetic variants that confer susceptibility to early-onset CAD in Japanese. A total of 7,256 individuals aged ≤65 years were enrolled in the present study. EWAS were conducted on 1,482 patients with CAD and 5,774 healthy controls. Genotyping of single nucleotide polymorphisms (SNPs) was performed using Illumina Human Exome-12 DNA Analysis BeadChip or Infinium Exome-24 BeadChip arrays. The association between allele frequencies for 31,465 SNPs that passed quality control and CAD was examined using Fisher's exact test. To compensate for multiple comparisons of allele frequencies with CAD, a false discovery rate (FDR) of <0.05 was applied for statistically significant associations. The association between allele frequencies for 31,465 SNPs and CAD, as determined by Fisher's exact test, demonstrated that 170 SNPs were significantly (FDR <0.05) associated with CAD. Multivariable logistic regression analysis with adjustment for age, sex, and the prevalence of hypertension, diabetes mellitus and dyslipidemia revealed that 162 SNPs were significantly (P<0.05) associated with CAD. A stepwise forward selection procedure was performed to examine the effects of genotypes for the 162 SNPs on CAD. The 54 SNPs were significant (P<0.05) and independent [coefficient of determination (R^2), 0.0008 to 0.0297] determinants of CAD. These SNPs together accounted for 15.5% of the cause of CAD. Following examination of results from previous genome-wide association studies and linkage disequilibrium of the identified SNPs, 21 genes (*RNF2*, *YEATS2*, *USP45*, *ITGB8*, *TNS3*, *FAM170B-AS1*, *PRKG1*, *BTRC*, *MKI67*, *STIM1*, *OR52E4*, *KIAA1551*, *MON2*, *PLUT*, *LINC00354*, *TRPM1*, *ADAT1*, *KRT27*, *LIPE*, *GFY* and *EIF3L*) and five chromosomal regions (2p13, 4q31.2, 5q12, 13q34 and 20q13.2) that were significantly associated with CAD were newly identified in the present study. Gene ontology analysis demonstrated that various biological functions were predicted in the 18 genes identified in the present study. The network analysis revealed that the 18 genes had potential direct or indirect interactions with the 30 genes previously revealed to be associated with CAD or with the 228 genes identified in previous genome-wide association studies. The present study newly identified 26 loci that confer susceptibility to CAD. Determination of genotypes for the SNPs at these loci may prove informative for assessment of the genetic risk for CAD in Japanese patients.

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Key words: coronary artery disease, myocardial infarction, ischemic heart disease, genetics, exome-wide association study

Introduction

Coronary atherosclerosis is a chronic inflammatory vascular disease and is initiated as a result of endothelial damage and dysfunction, which lead to the accumulation and oxidation of low density lipoprotein (LDL)-cholesterol in the arterial wall (1,2) Monocytes migrate from the blood into the subendothelial intima and transform into macrophages, which then accumulate lipid particles (foam cells) to form the lipid core

of atherosclerotic plaques (2,3). Inflammatory and thrombotic processes serve central roles in the formation of atherosclerotic lesions and subsequent plaque rupture, which lead toward acute coronary syndrome (2,3).

Coronary artery disease (CAD) and myocardial infarction (MI) are serious clinical conditions that remain the leading cause of mortality in the United States (4). Disease prevention is an important strategy for reducing the overall burden of CAD and MI, with the identification of biomarkers for disease risk being key for risk prediction and for potential intervention, in order to reduce the chance of future adverse coronary events. In addition to conventional risk factors for CAD, including hypertension, diabetes mellitus and dyslipidemia, the importance of genetic factors has been highlighted (5-7). Genes responsible for familial hypercholesterolemia and Tangier disease are prototypical examples of monogenic forms of CAD and MI with Mendelian inheritance (5,8). Familial hypercholesterolemia is an autosomal dominant disorder characterized by marked increases in the circulating concentrations of total cholesterol and LDL-cholesterol caused by mutations of the genes for LDL receptor (*LDLR*), apolipoprotein B (*APOB*), proprotein convertase subtilisin/kexin type 9 (*PCSK9*), cytochrome P450 family 7 subfamily A member 1 (*CYP7A1*) or LDL receptor adaptor protein 1 (*LDLRAP1*) (9,10). Tangier disease is an autosomal recessive disorder characterized by a decrease in the circulating concentration of high density lipoprotein (HDL)-cholesterol as a result of loss-of-function mutations in the ATP-binding cassette subfamily A member 1 gene (*ABCA1*) (11-13). The etiology of common forms of CAD is multifactorial and includes genetic components, as well as environmental and lifestyle factors (5-8). The heritability of common forms of CAD has been estimated to be 40-60% on the basis of family and twin studies (6,7,14).

Genome-wide association studies (GWASs) in European-ancestry (15-21), African American (22) or Han Chinese populations (23,24) have identified various genes and loci that confer susceptibility to CAD or MI. A meta-analysis of GWASs for CAD among European-ancestry populations, including low-frequency variants, identified 202 independent genetic variants at 129 loci with a false discovery rate (FDR) of <5% (25). These genetic variants together accounted for ~28% of the heritability of CAD, demonstrating that genetic susceptibility to this condition is largely determined by common variants with small effect sizes (6,25). A more recent meta-analysis for CAD in European-ancestry populations identified 304 independent genetic variants with an FDR of <5%, and these variants accounted for 21.2% of the heritability of CAD (26). In total, GWASs identified 163 loci associated with CAD at a genome-wide significance level and >300 possible loci for this condition with an FDR of <5% (7). Although several single nucleotide polymorphisms (SNPs) have been revealed to be significantly associated with MI in Japanese patients (27,28), genetic variants that contribute toward susceptibility to CAD and MI in Japanese patients remain to be definitively identified.

A study of monozygotic and dizygotic twins revealed that mortality from CAD at younger ages was significantly influenced by genetic factors in males and females, whereas the genetic effect was smaller at older ages (29,30). A family history of MI is also more apparent in individuals with early-onset MI than in those with late-onset MI, suggestive of a greater heritability in the former (31,32).

The present study included exome-wide association studies (EWASs) for CAD with the use of human exome array-based genotyping methods in order to identify genetic variants that confer susceptibility to this condition in Japanese patients. In order to increase the statistical power of the EWAS, patients with early-onset CAD were examined.

Materials and methods

Study subjects. In our previous EWAS, the median age of subjects with CAD was 69 years (33). Therefore, patients with an age of ≤65 years were defined as individuals with early-onset CAD in the present study. A total of 7,256 Japanese subjects aged ≤65 years [mean age, 51.7 years; age range, 18-65 years; males/females (%), 58.3/41.7; 1,482 with CAD, including 1,152 with MI, and 5,774 controls] were enrolled in the present study. The subjects were individuals who either visited outpatient clinics or were admitted to participating hospitals in Japan (Gifu Prefectural Tajimi Hospital, Tajimi; Gifu Prefectural General Medical Center, Gifu; Japanese Red Cross Nagoya First Hospital, Nagoya; Northern Mie Medical Center Inabe General Hospital, Inabe; and Hirosaki University Hospital and Hirosaki Stroke and Rehabilitation Center, Hirosaki, Japan) due to various symptoms or for an annual health check-up between October 2002 and March 2014, or who were community-dwelling individuals recruited to a population-based cohort study in Inabe between March 2010 and September 2014 (34).

The diagnosis of CAD was based on the detection of stenosis of >50% in any major coronary artery or in the left main trunk by coronary angiography. The diagnosis of MI was based on typical electrocardiographic changes and on increases in the serum activity of creatine kinase (MB isozyme) and in the serum concentration of troponin T. The diagnosis was confirmed by identification of the responsible stenosis in any of the major coronary arteries or in the left main trunk by coronary angiography. The control individuals had no history of MI, CAD, aortic aneurysm or peripheral artery disease; of ischemic or hemorrhagic stroke; or of other atherosclerotic, thrombotic, embolic or hemorrhagic disorders. Although certain control individuals had conventional risk factors for CAD, including hypertension, diabetes mellitus, dyslipidemia and CKD, they did not have any cardiovascular complications.

EWAS. Venous blood (5 or 7 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), peripheral blood leukocytes were isolated, and genomic DNA was extracted from these cells with the use of a DNA extraction kit (Genomix; Talent SRL, Trieste, Italy; or SMITEST EX-R&D; Medical & Biological Laboratories, Co., Ltd., Nagoya, Japan). The EWASs for CAD (1,482 cases and 5,774 controls) was performed with the use of a Human Exome-12 v1.2 DNA Analysis BeadChip or Infinium Exome-24 v1.0 BeadChip (Illumina, Inc., San Diego, CA, USA). These exome arrays include putative functional exonic variants selected from ~12,000 individual exome and whole-genome sequences. The exonic content consists of ~244,000 SNPs from European, African, Chinese and Hispanic individuals (35). SNPs contained in only one of the exome arrays (~2.6% of

all SNPs) were excluded from analysis. Quality control was performed as follows (36): i) Genotyping data with a call rate of <97% were discarded, with the mean call rate for the remaining data being 99.9%; ii) gender specification was checked for each sample, and those for which gender phenotype in the clinical records was inconsistent with genetic sex were discarded; iii) duplicate samples and cryptic relatedness were checked by calculation of identity by descent, and all pairs of DNA samples exhibiting an identity by descent of >0.1875 were inspected and one sample from each pair was excluded; iv) the frequency of heterozygosity for SNPs was calculated for all samples, and those with extremely low or high heterozygosity (>3 standard deviations from the mean) were discarded; v) SNPs in sex chromosomes or mitochondrial DNA were excluded from the analysis, as were nonpolymorphic SNPs or SNPs with a minor allele frequency of <1.0%; vi) SNPs whose genotype distributions deviated significantly (P<0.01) from Hardy-Weinberg equilibrium in control individuals were discarded; and vii) genotype data were examined for population stratification by principal components analysis (37), and population outliers were excluded from the analysis. A total of 31,465 SNPs passed quality control for the EWASs of CAD and these SNPs were subjected to analyses.

Statistical analysis. For analysis of the characteristics of the study subjects, quantitative data were compared between subjects with CAD and controls using the unpaired Student's t-test. Categorical data were compared between the two groups using the Pearson's χ^2 test. Allele frequencies were estimated by the gene counting method, and Fisher's exact test was applied to identify departure from the Hardy-Weinberg equilibrium. In the EWAS, the association between allele frequencies of each SNP and CAD was examined using the Fisher's exact test. The genomic inflation factor (λ) was 0.93. To compensate for multiple comparisons of genotypes with CAD, an FDR was applied for statistical significance of association (38). The significance level was set at an FDR of <0.05 for the EWAS. Multivariable logistic regression analysis was performed with CAD as a dependent variable and independent variables, including age, sex (0, female and 1, male), the prevalence of hypertension, diabetes mellitus, and dyslipidemia (0, no history of these conditions; 1, positive history), as well as the genotype of each SNP. Genotypes of the SNPs were assessed according to dominant [0, AA; 1, AB + BB (A, major allele; B, minor allele)] and recessive (0, AA + AB; 1, BB) genetic models, and the P-value, odds ratio and 95% confidence interval were calculated. A stepwise forward selection procedure was also performed to examine the effects of genotypes on CAD. The P-levels for inclusion in and exclusion from the model were 0.25 and 0.1, respectively. In the stepwise forward selection procedure, each genotype was examined according to a dominant or recessive model on the basis of statistical significance in the multivariable logistic regression analysis. The association between genotypes of SNPs and intermediate phenotypes of CAD was examined using the Pearson's χ^2 test. With the exception of the initial EWAS by the Fisher's exact test (FDR <0.05), P<0.05 was considered to indicate a statistically significant difference. Statistical tests were performed using JMP Genomics version 9.0 software (SAS Institute, Inc., Cary, NC, USA).

Association between genes, chromosomal loci and SNPs identified in the present study and phenotypes previously reported by GWASs. The genes, chromosomal loci, and SNPs identified in the present study were compared with the cardiovascular disease-related phenotypes previously reported by GWASs available in the Genome-Wide Repository of Associations Between SNPs and Phenotypes (GRASP) Search database v. 2.0.0.0 (https://grasp.nhlbi.nih.gov/Search.aspx), developed by the Information Technology and Applications Center at the National Center for Biotechnology Information (National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA) (39,40).

Gene Ontology analysis. Biological functions of the genes were examined by the use of the Gene Ontology and GO Annotations databases (QuickGO version 2018; https://www.ebi.ac.uk/QuickGO/; European Bioinformatics Institute, European Molecular Biology Laboratory, Hinxton, Cambridgeshire, UK) (41,42).

Network analysis of gene-gene interactions. Network analyses were performed to predict functional gene-gene interactions by the use of GeneMANIA Cytoscape plugin (http://apps.cytoscape.org/apps/genemania; Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Canada) (43-45) using Cytoscape v3.4.0 software (http://www.cytoscape.org/; The Cytoscape Consortium, San Diego, CA, USA) (46). To begin with, the 30 genes (*ACE*, *NOS3*, *CCL2*, *PON1*, *CD40LG*, *LOX*, *APOB*, *CRP*, *APOA1*, *LPA*, *ESR1*, *LDLR*, *APOC3*, *VEGFA*, *LTA*, *HMOX1*, *MMP3*, *APOA5*, *PCSK9*, *CDKN2B*, *TLR4*, *GNB3*, *PTGS2*, *NPPB*, *ABCG8*, *ESR2*, *CXCL12*, *MIA3*, *IRS1* and *ABO*) were selected from the DisGeNET database (http://www.disgenet.org/ web/DisGeNET; Integrative Biomedical Informatics Group, Research Programme on Biomedical Informatics, Barcelona Biomedical Research Park, Barcelona, Spain) (47,48), according to the rank order of high scores in association with CAD. Next, the 234 genes previously identified by GWASs (7) were selected, among which six genes were not included in GeneMANIA database and had no interaction with other genes. Therefore, the 228 genes (*SKI, PRDM16, FHL3, PCSK9, PPAP2B, SORT1, NGF, CASQ2, TDRKH, IL6R, ATP1B1, NME7, DDX59, CAMSAP2, LMOD1, HHAT, SERTAD4, DIEXF, MIA3, AGT, APOB, ABCG5, ABCG8, PRKCE, VAMP5, VAMP8, GGCX, ZEB2, FIGN, CALCRL, TFPI, WDR12, NBEAL1, FN1, TNS1, IRS1, KCNJ13, COL6A3, FGD5, ALS2CL, RTP3, CDC25A, SPINK8, MAP4, ZNF589, RHOA, ITGB5, DNAJC13, STAG1, MSL2, NCK1, PPP2R3A, MRAS, ARHGEF26, TIPARP, FNDC3B, RGS12, REST, NOA1, STBD1, PRDM8, FGF5, HNRNPD, UNC5C, MAD2L1, PDE5A, ZNF827, EDNRA, PALLD, SEMA5A, MAP3K1, LOX, SLC22A4, IL5, RAD50, ARHGAP26, FOXC1, PHACTR1, EDN1, HDGFL1, C2, ANKS1A, PI16, KCNK5, VEGFA, RAB23, FAM46A, CENPW, TCF21, PLEKHG1, LPA, PLG, MAD1L1, DAGLB, RAC1, KDELR2, TMEM106B, HDAC9, CCM2, BCAP29, GPR22, CFTR, ZC3HC1, KLHDC10, PARP12, TBXAS1, NOS3, NAT2, LPL, BMP1, ZFPM2, TRIB1, KLF4, SVEP1, DAB2IP, ABO, CDC123, KIAA1462, CXCL12, TSPAN14, FAM213A, LIPA, CYP17A1, CNNM2, NT5C2, SH3PXD2A, HTRA1, TRIM5, TRIM22, TRIM6, SWAP70, CTR9, ARNTL, HSD17B12,*

Table I. Characteristics of control subjects and patients with coronary artery disease.

Characteristic	Control	Coronary artery disease	P-value
No. subjects	5,774	1,482	
Age, years	50.6±10.2	55.9±7.4	<0.0001
Sex, males/females, %	52.1/47.9	82.5/17.5	<0.0001
Smoking, %	42.5	43.0	0.7719
Obesity, %	31.0	43.0	<0.0001
Body mass index, kg/m^2	23.2±3.5	24.5±3.5	<0.0001
Hypertension, %	31.7	70.0	<0.0001
Systolic BP, mmHg	121±18	139±27	<0.0001
Diastolic BP, mmHg	75±13	78±15	<0.0001
Diabetes mellitus, %	12.7	58.7	<0.0001
Fasting plasma glucose, mmol/l	5.66±1.78	7.55±3.39	<0.0001
Blood hemoglobin A_{1c}, %	5.72±0.96	6.89±1.75	<0.0001
Dyslipidemia, %	56.9	84.1	<0.0001
Serum triglycerides, mmol/l	1.32±0.98	1.84±1.34	<0.0001
Serum HDL-cholesterol, mmol/l	1.65±0.45	1.20±0.36	<0.0001
Serum LDL-cholesterol, mmol/l	3.18±0.83	3.18±0.98	0.9770
Chronic kidney disease, %	10.3	29.4	<0.0001
Serum creatinine, μmol/l	69.8±61.0	95.5±119.3	<0.0001
eGFR, ml min^{-1} 1.73 m^{-2}	78.7±17.1	70.7±26.9	<0.0001
Hyperuricemia, %	15.2	25.5	<0.0001
Serum uric acid, μmol/l	321±89	353±102	<0.0001

Quantitative data represent the mean ± standard deviation and were compared between subjects with coronary artery disease and controls with the unpaired Student's t-test. Categorical data were compared between the two groups using Pearson's χ^2 test. P<0.05 was considered to indicate a statistically significant difference. Obesity was defined as a body mass index of ≥25 kg/m2; hypertension as a systolic BP of ≥140 mmHg, diastolic BP of ≥90 mmHg, or the taking of anti-hypertensive medication; diabetes mellitus as a fasting plasma glucose level of ≥6.93 mmol/l, blood hemoglobin A1c content of ≥6.5%, or the taking of anti-diabetes medication; dyslipidemia as a serum triglyceride concentration of ≥1.65 mmol/l, serum HDL-cholesterol concentration of <1.04 mmol/l, serum LDL-cholesterol concentration of ≥3.64 mmol/l or the taking of anti-dyslipidemic medication; chronic kidney disease as an estimated glomerular filtration rate (eGFR) of <60 ml min^{-1} 1.73 m^{-2}; and hyperuricemia as a serum uric acid concentration of >416 μmol/l or the taking of uric acid-lowering medication. BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate.

SIPA1, SERPINH1, ARHGAP42, PDGFD, APOA1, APOC3, APOA4, APOA5, C1S, PRPF31, HOXC4, LRP1, FGD6, SH2B3, KSR2, HNF1A, CCDC92, SCARB1, FLT1, N4BP2L2, PDS5B, COL4A1, COL4A2, MCF2L, CUL4A, ARID4A, PSMA3, TMED10, SERPINA1, HHIPL1, YY1, TRIP4, SMAD3, ADAMTS7, MFGE8, FURIN, FES, CETP, HP, CFDP1, BCAR1, PLCG2, CDH13, SMG6, PEMT, CORO6, BLMH, ANKRD13B, GIT1, SSH2, EFCAB5, COPRS, RAB11FIP4, DHX58, KAT2A, RAB5, NKIRAS2, DNAJC7, KCNH4, HCRT, GHDC, GOSR2, UBE2Z, GIP, BCAS3, PECAM1, DDX5, TEX2, ACAA2, RPL17, PMAIP1, MC4R, LDLR, SMARCA4, FCHO1, COLGALT1, ZNF507, HNRNPUL1, TGFB1, APOE, APOC1, PVRL2, COTL1, SNRPD2, PROCR, EIF6, ZHX3, PLCG1, PLTP, MMP9, ZNF831, BACH1, KCNE2 and *ADORA2A*) were applied to analysis.

Results

Characteristics of subjects. The characteristics of the 7,256 subjects enrolled in the present study are presented in Table I. The age, the frequency of males, and the prevalence of obesity, hypertension, diabetes mellitus (DM), dyslipidemia, chronic kidney disease (CKD) and hyperuricemia, as well as body mass index, systolic and diastolic blood pressure, fasting plasma glucose level, blood glycosylated hemoglobin (hemoglobin A1c) content, and the serum concentrations of triglycerides, creatinine, and uric acid were greater, whereas the serum concentration of HDL-cholesterol and estimated glomerular filtration rate were lower, in patients with CAD than in controls.

EWAS for CAD. The association between allele frequencies for 31,465 SNPs that passed quality control and CAD was examined using the Fisher's exact test, and the 170 SNPs were significantly (FDR <0.05) associated with CAD (Table II).

Multivariable logistic regression analysis of the association between SNPs and CAD. The association between the 170 SNPs identified in the EWAS for CAD and this condition was examined by multivariable logistic regression analysis with adjustment for age, sex and the prevalence of hypertension, diabetes mellitus and dyslipidemia (Table III). The 162 SNPs were significantly (P<0.05 in a dominant or recessive model) associated with CAD.

Table II. 170 SNPs significantly (FDR <0.5) associated with coronary artery disease in the exome-wide association study.

Gene	SNP	Nucleotide substitution[a]	Amino acid substitution	Chromosome	Position	MAF, %	Allele OR	P-value, allele frequency	FDR, allele frequency
PLCB2	rs200787930	C/T	E1106K	15	40289298	1.2	0.03	$1.24x10^{-29}$	$1.56x10^{-26}$
MARCH1	rs61734696	G/T	Q137K	4	164197303	1.2	0.03	$2.09x10^{-29}$	$2.54x10^{-26}$
VPS33B	rs199921354	C/T	R80Q	15	91013841	1.2	0.03	$2.76x10^{-29}$	$3.30x10^{-26}$
CXCL8	rs188378669	G/T	E31*	4	73741568	1.2	0.03	$3.15x10^{-29}$	$3.70x10^{-26}$
TMOD4	rs115287176	G/A	R277W	1	151170961	1.2	0.03	$1.21x10^{-28}$	$1.39x10^{-25}$
COL6A3	rs146092501	C/T	E1386K	2	237371861	1.2	0.04	$2.93x10^{-28}$	$3.27x10^{-25}$
ZNF77	rs146879198	G/A	R340*	19	2934109	1.2	0.04	$2.92x10^{-28}$	$3.27x10^{-25}$
ADGRL3	rs192210727	G/T	R580I	4	61909615	1.3	0.10	$2.92x10^{-23}$	$3.06x10^{-20}$
OR52E4	rs11823828	T/G	F227L	11	5884973	36.6	1.54	$3.40x10^{-21}$	$3.35x10^{-18}$
ALDH2	rs671	G/A	E504K	12	111803962	27.6	1.41	$4.12x10^{-15}$	$3.78x10^{-12}$
ACAD10	rs11066015	G/A		12	111730205	27.5	1.41	$4.92x10^{-15}$	$4.45x10^{-12}$
BRAP	rs3782886	A/G		12	111672685	29.3	1.37	$4.38x10^{-13}$	$3.71x10^{-10}$
HECTD4	rs11066280	T/A		12	112379979	29.0	1.37	$6.94x10^{-13}$	$5.73x10^{-10}$
HECTD4	rs2074356	C/T		12	112207597	25.4	1.36	$1.21x10^{-11}$	$9.78x10^{-9}$
NAA25	rs12231744	C/T	R876K	12	112039251	35.1	0.77	$1.68x10^{-9}$	$1.24x10^{-6}$
GOSR2	rs1052586	T/C		17	46941097	48.7	0.79	$3.94x10^{-8}$	$2.61x10^{-5}$
ATXN2	rs7969300	T/C	N248S	12	111555908	38.8	0.79	$4.41x10^{-8}$	$2.87x10^{-5}$
LILRB2	rs73055442	C/T	R103H	19	54279838	1.6	44.10	$2.00x10^{-7}$	$1.20x10^{-4}$
	rs12229654	T/G		12	110976657	22.5	1.28	$2.09x10^{-7}$	$1.24x10^{-4}$
LOC107987429	rs2844533	T/C		6	31383025	15.3	1.32	$3.49x10^{-7}$	$1.95x10^{-4}$
MTFR2	rs143974258	G/A	R360*	6	136231355	3.3	0.05	$6.66x10^{-7}$	$3.60x10^{-4}$
PSORS1C1	rs3130559	C/T		6	31129524	44.2	0.82	$1.51x10^{-6}$	$7.74x10^{-4}$
	rs2596548	G/T		6	31362769	5.4	1.51	$1.83x10^{-6}$	$9.21x10^{-4}$
EIF3L	rs9466	T/C		22	37877742	21.6	1.28	$1.96x10^{-6}$	$9.77x10^{-4}$
LPGAT1	rs150552771	T/C	K200E	1	211783358	5.0	7.14	$2.26x10^{-6}$	0.0011
LAIR2	rs34429135	T/A	F115Y	19	54508164	2.5	ND	$2.70x10^{-6}$	0.0013
	rs2523644	A/G		6	31374707	8.1	1.40	$2.75x10^{-6}$	0.0013
	rs10757278	A/G		9	22124478	49.5	0.83	$2.92x10^{-6}$	0.0014
CCHCR1	rs130067	T/G	E328D	6	31150734	33.2	0.81	$3.10x10^{-6}$	0.0015
TCHP	rs74416240	G/A		12	109904793	13.3	1.30	$3.25x10^{-6}$	0.0015
	rs1333049	G/C		9	22125504	49.4	1.20	$3.95x10^{-6}$	0.0018
CDKN2B-AS1	rs4977574	A/G		9	22098575	47.1	1.21	$4.18x10^{-6}$	0.0019
CDKN2B-AS1	rs2383207	G/A		9	22115960	33.7	0.81	$4.86x10^{-6}$	0.0022
SLC16A1	rs1049434	T/A	D490E	1	112913924	34.7	0.82	$5.76x10^{-6}$	0.0025
GIT2	rs925368	T/C	N389S	12	109953174	12.5	1.30	$6.02x10^{-6}$	0.0026
	rs1333048	A/C		9	22125348	49.6	1.20	$6.46x10^{-6}$	0.0028
	rs2523578	T/C		6	31360765	8.1	1.39	$6.54x10^{-6}$	0.0028
	rs404890	G/T		6	32231090	30.5	1.22	$8.90x10^{-6}$	0.0037
APOE	rs7412	C/T	R176C	19	44908822	4.3	0.60	$1.06x10^{-5}$	0.0043
CCHCR1	rs130071	G/A		6	31148433	5.1	1.52	$1.06x10^{-5}$	0.0043
	rs602633	C/A		1	109278889	7.6	0.69	$1.15x10^{-5}$	0.0046
CELSR2	rs12740374	G/T		1	109274968	7.7	0.69	$1.15x10^{-5}$	0.0046
MKI67	rs145121731	G/A	S2722L	10	128102595	1.5	2.04	$1.20x10^{-5}$	0.0047
CUBN	rs78201384	C/T	E304K	10	17111024	2.7	0.52	$1.38x10^{-5}$	0.0054
PSORS1C3	rs887466	T/C		6	31175734	41.1	1.20	$1.38x10^{-5}$	0.0054
PSORS1C1	rs3094663	G/A		6	31139310	30.9	1.20	$1.40x10^{-5}$	0.0054
	rs10853110	A/G		17	49241052	39.2	1.20	$1.49x10^{-5}$	0.0057
WDR37	rs10794720	C/T		10	1110225	8.5	0.71	$1.52x10^{-5}$	0.0057
CELSR2	rs629301	A/C		1	109275684	7.8	0.70	$1.52x10^{-5}$	0.0057
SKIV2L	rs592229	G/T		6	31962664	42.4	1.20	$1.57x10^{-5}$	0.0058
	rs12182351	T/C		6	32233930	29.8	1.22	$1.59x10^{-5}$	0.0059

Table II. Continued.

Gene	SNP	Nucleotide substitution[a]	Amino acid substitution	Chromosome	Position	MAF, %	Allele OR	P-value, allele frequency	FDR, allele frequency
POU5F1	rs3130503	G/A		6	31169388	29.5	1.20	$1.64x10^{-5}$	0.0060
PSORS1C3	rs1265155	T/C		6	31175917	41.1	1.19	$1.68x10^{-5}$	0.0061
CELSR2	rs646776	A/G		1	109275908	7.7	0.70	$1.70x10^{-5}$	0.0062
	rs2596503	C/T		6	31353033	19.3	1.24	$1.75x10^{-5}$	0.0063
TRPM1	rs2241493	T/C	N54S	15	31070149	12.6	0.76	$1.81x10^{-5}$	0.0065
CCDC141	rs13419085	T/C	N1170S	2	178837710	1.8	0.46	$1.92x10^{-5}$	0.0068
VARS2	rs9394021	A/G	Q777R	6	30925350	44.9	0.84	$1.98x10^{-5}$	0.0069
SFTA2	rs2286655	T/C		6	30931969	44.9	1.19	$1.99x10^{-5}$	0.0069
	rs3873334	T/C		6	30928370	44.9	1.19	$1.98x10^{-5}$	0.0069
	rs9261800	C/G		6	30408822	2.8	7.21	$2.02x10^{-5}$	0.0069
TCF19	rs3130453	C/T		6	31157072	34.4	0.83	$2.10x10^{-5}$	0.0072
C21orf59	rs76974938	C/T	D67N	21	32609946	2.4	0.00	$2.14x10^{-5}$	0.0073
DDR1	rs2239518	T/C		6	30897948	44.9	1.19	$2.19x10^{-5}$	0.0074
CDSN	rs3130984	C/T	S143N	6	31117187	13.4	1.29	$2.20x10^{-5}$	0.0074
	rs197932	T/C		17	46896981	26.9	0.82	$2.23x10^{-5}$	0.0075
CDSN	rs3130981	C/T	D527N	6	31116036	13.6	1.29	$2.30x10^{-5}$	0.0075
MICB-DT	rs3132469	C/T		6	31488790	5.3	1.46	$2.41x10^{-5}$	0.0078
HLA-DQB1	rs1049056	C/A	A6S	6	32666592	11.9	1.30	$2.51x10^{-5}$	0.0081
DDR1	rs2239517	A/G		6	30897338	44.6	1.19	$2.59x10^{-5}$	0.0083
CCHCR1	rs1265110	G/A		6	31151645	30.2	0.83	$2.69x10^{-5}$	0.0085
CCDC63	rs10774610	T/C		12	110902439	23.7	1.22	$2.76x10^{-5}$	0.0087
GTF2H4	rs2284176	C/T		6	30907845	44.6	1.19	$2.80x10^{-5}$	0.0088
GTF2H4	rs3909130	G/A		6	30906388	44.6	1.19	$2.84x10^{-5}$	0.0089
GTF2H4	rs916920	G/A		6	30909425	44.7	1.19	$2.85x10^{-5}$	0.0089
	rs1264569	A/G		6	30397543	4.6	1.49	$2.98x10^{-5}$	0.0092
CACNA1D	rs35874056	G/A	G460S	3	53702798	2.0	25.00	$3.09x10^{-5}$	0.0094
	rs9468845	A/G		6	30901816	44.7	1.19	$3.12x10^{-5}$	0.0094
DDR1	rs8408	C/T		6	30899889	44.7	1.19	$3.11x10^{-5}$	0.0094
DDR1	rs7756521	C/T		6	30880476	44.7	1.19	$3.10x10^{-5}$	0.0094
CDKN2B-AS1	rs1011970	G/T		9	22062135	5.6	1.41	$3.15x10^{-5}$	0.0095
ADAT1	rs145161932	T/C	R57G	16	75612670	1.4	0.39	$3.29x10^{-5}$	0.0098
POU5F1	rs885950	T/G		6	31172375	34.0	0.83	$3.28x10^{-5}$	0.0098
DDR1	rs4618569	A/G		6	30887474	44.7	1.19	$3.41x10^{-5}$	0.0101
KRT13	rs146918776	A/G	Y281H	17	41502993	1.5	1.94	$3.51x10^{-5}$	0.0103
	rs2523638	G/A		6	31376496	43.1	1.19	$3.53x10^{-5}$	0.0103
PSRC1	rs599839	A/G		1	109279544	7.9	0.71	$3.52x10^{-5}$	0.0103
	rs9275141	G/T		6	32683340	26.4	1.21	$3.62x10^{-5}$	0.0105
CCDC63	rs10849915	T/C		12	110895818	23.6	1.22	$3.63x10^{-5}$	0.0105
HLA-DRA	rs3177928	G/A		6	32444658	5.9	1.41	$3.81x10^{-5}$	0.0108
OAS3	rs2072134	C/T		12	112971371	17.6	1.24	$4.06x10^{-5}$	0.0114
USP45	rs41288947	C/G	T521R	6	99446210	14.9	1.26	$4.11x10^{-5}$	0.0115
CCHCR1	rs1265109	A/C		6	31151812	48.2	1.18	$4.16x10^{-5}$	0.0116
LOC101929163	rs6930777	C/T		6	32383789	5.5	1.43	$4.45x10^{-5}$	0.0122
	rs7333181	G/A		13	111568950	2.5	0.54	$4.45x10^{-5}$	0.0122
DDR1	rs1264323	T/C		6	30888130	38.8	1.19	$4.48x10^{-5}$	0.0122
LINC00243	rs3094111	G/A		6	30820414	14.7	1.25	$4.52x10^{-5}$	0.0123
	rs10484561	T/G		6	32697643	5.9	1.41	$4.55x10^{-5}$	0.0123
PSORS1C1	rs3130558	G/C		6	31129406	13.7	1.27	$4.59x10^{-5}$	0.0124
HLA-DQB1	rs1049060	T/A	S27T	6	32666529	28.8	1.20	$4.92x10^{-5}$	0.0131
	rs2844650	G/A		6	30934756	4.7	1.47	$4.99x10^{-5}$	0.0131
DDR1	rs3132572	T/C		6	30893952	4.7	1.47	$4.99x10^{-5}$	0.0131

Table II. Continued.

Gene	SNP	Nucleotide substitution[a]	Amino acid substitution	Chromosome	Position	MAF, %	Allele OR	P-value, allele frequency	FDR, allele frequency
CCHCR1	rs1265115	T/G		6	31149298	47.7	1.18	$4.96x10^{-5}$	0.0131
CCHCR1	rs3094225	T/C		6	31145275	48.4	1.18	$4.93x10^{-5}$	0.0131
LOC107987453	rs3129987	C/T		6	30798427	14.5	1.25	$5.05x10^{-5}$	0.0132
DPCR1	rs2517451	A/G		6	30946974	4.7	1.47	$5.11x10^{-5}$	0.0133
KIAA1551	rs10771894	A/G	S352G	12	31982009	32.4	1.19	$5.18x10^{-5}$	0.0134
	rs13427905	C/T		2	71846585	18.5	0.80	$5.22x10^{-5}$	0.0134
ABCA1	rs1883025	G/A		9	104902020	28.8	0.83	$5.46x10^{-5}$	0.0139
SFTA2	rs2253705	G/A		6	30932317	18.0	1.23	$5.60x10^{-5}$	0.0141
PLUT	rs954750	G/A		13	27889801	48.3	1.18	$5.86x10^{-5}$	0.0146
TCF19	rs1419881	T/C		6	31162816	48.1	1.18	$6.37x10^{-5}$	0.0156
	rs13209234	G/A		6	32448198	5.9	1.41	$6.47x10^{-5}$	0.0158
PSORS1C1	rs1265100	T/C		6	31137533	32.2	0.83	$6.55x10^{-5}$	0.0159
YEATS2	rs76174573	G/T	C1232F	3	183804099	3.7	0.61	$6.74x10^{-5}$	0.0162
ABO	rs1053878	C/T	P156L	9	133256264	22.8	1.20	$6.78x10^{-5}$	0.0162
	rs4014195	C/G		11	65739351	16.6	1.24	$6.78x10^{-5}$	0.0162
SFTA2	s2253588	C/G		6	30931600	23.6	1.21	$6.93x10^{-5}$	0.0165
CYP4F8	rs201166643	C/A	R488S	19	15629257	1.1	ND	$7.00x10^{-5}$	0.0165
NAXE	rs7516274	C/G	L19V	1	156591859	1.8	0.48	$7.18x10^{-5}$	0.0169
	rs10757283	T/C		9	22134173	33.8	0.84	$7.25x10^{-5}$	0.0170
BTNL2	rs28362680	G/A	A202V	6	32403039	39.7	0.85	$7.40x10^{-5}$	0.0171
BTNL2	rs10947262	C/T		6	32405535	39.7	0.85	$7.40x10^{-5}$	0.0171
KRT27	rs17558532	C/T	A284T	17	40779624	3.6	0.62	$7.71x10^{-5}$	0.0176
GTF2H4	rs3130780	G/T		6	30906531	18.0	1.23	$7.71x10^{-5}$	0.0176
	rs2532934	T/C		6	30926982	24.1	1.20	$7.74x10^{-5}$	0.0176
VARS2	rs753725	G/A		6	30923094	24.1	1.20	$7.68x10^{-5}$	0.0176
PLUT	rs11619319	A/G		13	27913462	48.1	1.18	$7.64x10^{-5}$	0.0176
	rs3095273	C/T		6	29598592	5.5	1.41	$8.16x10^{-5}$	0.0184
TNS1	rs918949	C/T	V1590I	2	217809974	42.8	0.85	$8.39x10^{-5}$	0.0188
LINC00243	rs3130785	C/T		6	30828961	14.6	1.24	$8.37x10^{-5}$	0.0188
VARS2	rs2249464	C/T	R309W	6	30920384	24.1	1.20	$9.39x10^{-5}$	0.0207
	rs3095345	A/G		6	30854636	17.9	1.22	$9.37x10^{-5}$	0.0207
ITGB8	rs80015015	G/A	C481Y	7	20401881	7.1	1.35	$1.01x10^{-4}$	0.0220
VARS2	rs885905	C/T		6	30922654	23.4	1.20	$1.07x10^{-4}$	0.0232
LIPE	rs34052647	G/A	R611C	19	42407617	5.5	1.39	$1.16x10^{-4}$	0.0249
PHACTR1	rs9369640	A/C		6	12901209	9.1	0.74	$1.30x10^{-4}$	0.0275
BTNL2	rs41417449	T/C	M295V	6	32396234	23.0	0.83	$1.35x10^{-4}$	0.0280
BTNL2	rs41441651	C/T	D336N	6	32396111	23.0	0.83	$1.35x10^{-4}$	0.0280
BTNL2	rs28362675	C/A	E454*	6	32394744	23.0	0.83	$1.35x10^{-4}$	0.0280
BTNL2	rs78587369	G/A	T165I	6	32403150	23.0	0.83	$1.35x10^{-4}$	0.0280
BTNL2	rs3763315	G/T		6	32408877	23.0	0.83	$1.35x10^{-4}$	0.0280
BTNL2	rs2076528	T/G		6	32396417	23.0	0.83	$1.35x10^{-4}$	0.0280
PRKG1	rs9414827	G/A		10	51137314	10.1	0.76	$1.37x10^{-4}$	0.0282
	rs6537384	T/G		4	145949613	28.8	1.19	$1.43x10^{-4}$	0.0294
	rs6067640	G/A		20	51092837	38.5	0.85	$1.48x10^{-4}$	0.0302
	rs10514995	A/G		5	66443611	48.7	1.16	$1.51x10^{-4}$	0.0306
BTNL2	rs34423804	T/A	V283D	6	32396269	23.0	0.83	$1.63x10^{-4}$	0.0329
PHACTR1	rs9349379	G/A		6	12903725	34.2	0.85	$1.69x10^{-4}$	0.0341
STIM1	rs116855870	A/G		11	4055527	1.1	1.93	$1.71x10^{-4}$	0.0343
ZNF142	rs3821033	C/T	A1313T	2	218642579	11.2	1.26	$1.78x10^{-4}$	0.0355
LINC00354	rs4907518	G/A		13	111898209	45.6	0.85	$1.82x10^{-4}$	0.0362
TNS3	rs11763932	G/A		7	47567880	42.0	0.85	$1.91x10^{-4}$	0.0378

Table II. Continued.

Gene	SNP	Nucleotide substitution[a]	Amino acid substitution	Chromosome	Position	MAF, %	Allele OR	P-value, allele frequency	FDR, allele frequency
BTRC	rs2270439	C/A	P566H	10	101550817	3.5	0.63	$1.94x10^{-4}$	0.0381
MIA3	rs2936051	A/G	E881G	1	222629862	40.1	0.85	$1.96x10^{-4}$	0.0384
	rs6825911	C/T		4	110460482	45.9	0.86	$2.01x10^{-4}$	0.0391
VNN1	rs2294757	G/A	T26I	6	132713959	37.4	0.85	$2.02x10^{-4}$	0.0393
ZNF860	rs140232911	C/T	S161L	3	31989561	10.4	0.44	$2.09x10^{-4}$	0.0406
	rs838880	C/T		12	124777047	47.5	1.16	$2.23x10^{-4}$	0.0430
MIA3	rs2936052	A/G	K605R	1	222629034	34.4	0.85	$2.26x10^{-4}$	0.0430
DTNBP1	rs2743868	G/A		6	15625577	31.6	1.18	$2.26x10^{-4}$	0.0430
MON2	rs11174549	A/G	I1385V	12	62565357	5.0	1.40	$2.26x10^{-4}$	0.0430
	rs507666	G/A		9	136149399	27.8	1.18	$2.26x10^{-4}$	0.0430
FAM170B	rs73302786	G/T	D252E	10	49131709	3.5	1.47	$2.36x10^{-4}$	0.0445
PSORS1C3	rs3131018	G/T		6	31175805	15.7	1.23	$2.36x10^{-4}$	0.0445
PIEZO2	rs35033671	C/A	C1148F	18	10759842	11.0	1.27	$2.39x10^{-4}$	0.0448
SLC22A3	rs1810126	C/T		6	160451119	49.1	0.86	$2.46x10^{-4}$	0.0460
PANK1	rs11185790	G/A		10	89612776	46.9	1.16	$2.57x10^{-4}$	0.0481
GFY	rs73053944	C/G	T203S	19	49427038	2.9	1.51	$2.58x10^{-4}$	0.0481
RNF2	rs1046592	A/G		1	185100429	33.9	0.85	$2.63x10^{-4}$	0.0488

Allele frequencies were analyzed using Fisher's exact test. [a]Major allele/minor allele. SNP, single nucleotide polymorphisms; MAF, minor allele frequency; OR, odds ratio; FDR, false discovery rate; ND, not determined.

Stepwise forward selection procedure of the effects of SNPs on CAD. A stepwise forward selection procedure was performed to examine effects of genotypes for the 162 SNPs associated with CAD by multivariable logistic regression analysis on this condition (Table IV). The 54 SNPs were significant (P<0.05) and independent [coefficient of determination (R^2), 0.0008 to 0.0297] determinants of CAD. These SNPs together accounted for 15.5% of the cause of CAD.

Association between SNPs associated with CAD and intermediate phenotypes. The association between the 54 SNPs associated with CAD and intermediate phenotypes of this condition, including hypertension, DM, hypertriglyceridemia, hypo-HDL-cholesterolemia, hyper-low density lipoprotein (LDL)-cholesterolemia, CKD, obesity, and hyperuricemia, was examined using Pearson's χ^2 test (Table V).

The SNP rs671 of *ALDH2* was significantly (P<0.05) associated with all the intermediate phenotypes; rs200787930 of *PLCB2* and rs2074356 of *HECTD4* to six of the eight phenotypes; rs9466 of *EIF3L* to five of the eight phenotypes; rs130071 of *CCHCR1*, rs11823828 of *OR52E4* and rs12229654 to four of the eight phenotypes; rs11174549 of *MON2*, rs10514995, rs507666, rs10757283 and rs78201384 of *CUBN* to three of the eight phenotypes; rs1046592 of *RNF2*, rs13427905, rs3094663 of *PSORS1C1*, rs6067640, rs592229 of *SKIV2L*, rs4014195, rs7333181, rs838880, rs1333048, rs10771894 of KIAA1551, rs954750 of *PLUT*, rs10794720 of *WDR37*, rs34052647 of *LIPE*, rs602633, rs145121731 of *MKI67*, rs41288947 of *USP45*, and rs9414827 of *PRKG1* to two of the eight phenotypes; and rs73053944 of *GFY*, rs6825911, rs1011970 of *CDKN2B-AS1*, rs1049434 of *SLC16A1*, rs145161932 of *ADAT1*, rs1052586 of *GOSR2*, rs197932, rs1883025 of *ABCA1*, rs76174573 of *YEATS2*, rs80015015 of *ITGB8*, rs2936051 of *MIA3*, rs7412 of *APOE*, rs4907518 of *LINC00354*, rs6537384, rs17558532 of *KRT27*, rs11185790 of *PANK1*, and rs2523644 to one of the eight phenotypes.

Linkage disequilibrium analyses. Linkage disequilibrium (LD) was examined among SNPs associated with CAD. There was significant LD among rs12229654 at 12q24.1, rs671 of *ALDH2*, and rs2074356 of *HECTD4* [square of the correlation coefficient (r^2), 0.564 to 0.882)].

Association between genes, chromosomal loci and SNPs identified in the present study and phenotypes previously reported by GWASs. The association between genes, chromosomal loci and SNPs identified in the present study and cardiovascular disease-related phenotypes previously reported by GWASs available in the GRASP Search database (Table VI). Chromosomal region 1p13.3, *MIA3*, *PHACTR1*, *SKIV2L*, *CDKN2B-AS1*, *9p21*, *ALDH2* and *HECTD4* were previously revealed to be associated with CAD or MI. *SLC16A1*, *PSORS1C1*, *CCHCR1*, *6p21.3*, *ABCA1*, *9q34.2*, *CUBN*, *PANK1*, *12q24.1*, *12q24.31*, *PLCB2* and *APOE* were previously associated with circulating concentrations of LDL-cholesterol, HDL-cholesterol, triglycerides or insulin, or type 1 diabetes mellitus. Chromosome 4q24, 17q21.3 and *GOSR2* were previously associated with systolic or diastolic blood pressure or pulse pressure. *CCDC141*, *TNS1*, *WDR37* and *11q13*.1 were previously associated with cardiac, pulmo-

Table III. 162 SNPs associated with coronary artery disease as determined by multivariable logistic regression analysis.

			Dominant model			Recessive model		
Gene	SNP		P-value	OR	95% CI	P-value	OR	95% CI
PLCB2	rs200787930	C/T	<0.0001	0.02	0.01-0.09			
MARCH1	rs61734696	G/T	<0.0001	0.02	0.01-0.10			
VPS33B	rs199921354	C/T	<0.0001	0.02	0.01-0.09			
CXCL8	rs188378669	G/T	<0.0001	0.02	0.01-0.09			
TMOD4	rs115287176	G/A	<0.0001	0.02	0.01-0.10			
COL6A3	rs146092501	C/T	<0.0001	0.02	0.01-0.10			
ZNF77	rs146879198	G/A	<0.0001	0.02	0.01-0.10			
ADGRL3	rs192210727	G/T	<0.0001	0.07	0.03-0.16	0.9959		
OR52E4	rs11823828	T/G	<0.0001	1.66	1.41-1.97	<0.0001	2.44	2.01-2.97
ALDH2	rs671	G/A	<0.0001	1.73	1.50-2.01	<0.0001	1.80	1.44-2.26
ACAD10	rs11066015	G/A	<0.0001	1.73	1.49-2.01	<0.0001	1.79	1.42-2.25
BRAP	rs3782886	A/G	<0.0001	1.71	1.48-1.99	<0.0001	1.70	1.36-2.12
HECTD4	rs11066280	T/A	<0.0001	1.73	1.49-2.01	<0.0001	1.73	1.38-2.17
HECTD4	rs2074356	C/T	<0.0001	1.61	1.39-1.87	<0.0001	1.76	1.38-2.26
NAA25	rs12231744	C/T	<0.0001	0.63	0.54-0.73	<0.0001	0.55	0.43-0.70
GOSR2	rs1052586	T/C	0.0003	0.73	0.62-0.87	<0.0001	0.64	0.53-0.77
ATXN2	rs7969300	T/C	<0.0001	0.63	0.55-0.74	<0.0001	0.57	0.45-0.71
	rs12229654	T/G	<0.0001	1.46	1.26-1.69	<0.0001	1.72	1.31-2.25
LOC107987429	rs2844533	T/C	<0.0001	1.36	1.17-1.59	0.8616		
MTFR2	rs143974258	G/A	0.0014	0.04	0.01-0.28			
PSORS1C1	rs3130559	C/T	0.0127	0.82	0.70-0.96	0.0629		
	rs2596548	G/T	<0.0001	1.76	1.41-2.20	0.2047		
EIF3L	rs9466	T/C	0.0053	1.24	1.07-1.44	0.0199	1.47	1.06-2.04
LPGAT1	rs150552771	T/C	0.9970			<0.0001	2.20	1.83-2.64
	rs2523644	A/G	<0.0001	1.59	1.31-1.92	0.9088		
	rs10757278	A/G	<0.0001	0.71	0.60-0.83	0.0023	0.77	0.65-0.91
CCHCR1	rs130067	T/G	0.0010	0.78	0.68-0.91	0.0183	0.73	0.57-0.95
TCHP	rs74416240	G/A	0.0002	1.35	1.15-1.58	0.1725		
	rs1333049	G/C	0.0031	1.29	1.09-1.53	<0.0001	1.41	1.20-1.66
CDKN2B-AS1	rs4977574	A/G	0.0003	1.36	1.15-1.60	<0.0001	1.43	1.21-1.69
CDKN2B-AS1	rs2383207	G/A	<0.0001	0.75	0.65-0.87	0.0171	0.75	0.59-0.95
SLC16A1	rs1049434	T/A	0.0106	0.83	0.71-0.96	<0.0001	0.57	0.45-0.73
GIT2	rs925368	T/C	0.0001	1.37	1.16-1.61	0.3189		
	rs1333048	A/C	0.0036	1.29	1.09-1.53	<0.0001	1.40	1.19-1.64
	rs2523578	T/C	<0.0001	1.55	1.27-1.88	0.8694		
	rs404890	G/T	0.0005	1.29	1.12-1.50	0.0160	1.35	1.06-1.72
APOE	rs7412	C/T	0.0001	0.56	0.42-0.76	0.2259		
CCHCR1	rs130071	G/A	0.0149	1.36	1.06-1.73	0.2668		
	rs602633	C/A	0.0001	0.64	0.51-0.80	0.1782		
CELSR2	rs12740374	G/T	<0.0001	0.63	0.51-0.79	0.1708		
MKI67	rs145121731	G/A	0.0014	1.94	1.29-2.91	0.9957		
CUBN	rs78201384	C/T	0.0003	0.50	0.34-0.73	0.9959		
PSORS1C3	rs887466	T/C	0.0013	1.29	1.11-1.52	0.1666		
PSORS1C1	rs3094663	G/A	<0.0001	1.41	1.21-1.63	0.6404		
	rs10853110	A/G	0.0026	1.26	1.09-1.47	0.0102	1.29	1.06-1.56
WDR37	rs10794720	C/T	0.0003	0.68	0.55-0.84	0.0734		
CELSR2	rs629301	A/C	0.0002	0.65	0.52-0.82	0.1708		
SKIV2L	rs592229	G/T	0.0154	1.22	1.04-1.42	0.0138	1.26	1.05-1.51
	rs12182351	T/C	0.0008	1.28	1.11-1.48	0.0138	1.37	1.07-1.75
POU5F1	rs3130503	G/A	<0.0001	1.41	1.22-1.63	0.6308		
PSORS1C3	rs1265155	T/C	0.0017	1.29	1.10-1.50	0.1666		

Table III. Continued.

Gene	SNP		Dominant model			Recessive model		
			P-value	OR	95% CI	P-value	OR	95% CI
CELSR2	rs646776	A/G	0.0002	0.65	0.52-0.82	0.2660		
	rs2596503	C/T	0.0204	1.19	1.03-1.38	0.1828		
TRPM1	rs2241493	T/C	0.0002	0.71	0.60-0.85	0.0410	0.49	0.25-0.97
CCDC141	rs13419085	T/C	0.0005	0.43	0.27-0.69			
VARS2	rs9394021	A/G	0.0261	0.82	0.69-0.98	0.0117	0.81	0.69-0.95
SFTA2	rs2286655	T/C	0.0097	1.24	1.05-1.45	0.0277	1.22	1.02-1.45
	rs3873334	T/C	0.0117	1.23	1.05-1.44	0.0261	1.22	1.02-1.45
TCF19	rs3130453	C/T	0.0077	0.82	0.71-0.95	0.0024	0.68	0.53-0.87
DDR1	rs2239518	T/C	0.0103	1.23	1.05-1.45	0.0282	1.22	1.02-1.45
CDSN	rs3130984	C/T	<0.0001	1.39	1.18-1.64	0.1220		
	rs197932	T/C	0.0042	0.81	0.70-0.93	0.0348	0.73	0.54-0.98
CDSN	rs3130981	C/T	<0.0001	1.39	1.18-1.64	0.1226		
MICB-DT	rs3132469	C/T	<0.0001	1.63	1.30-2.05	0.3960		
HLA-DQB1	rs1049056	C/A	0.0050	1.28	1.08-1.52	0.1586		
DDR1	rs2239517	A/G	0.0115	1.23	1.05-1.44	0.0326	1.21	1.02-1.44
CCHCR1	rs1265110	G/A	0.0096	0.83	0.71-0.95	0.0948		
CCDC63	rs10774610	T/C	0.0006	1.29	1.12-1.49	0.0214	1.38	1.05-1.82
GTF2H4	rs2284176	C/T	0.0153	1.22	1.04-1.43	0.0306	1.21	1.02-1.45
GTF2H4	rs3909130	G/A	0.0138	1.22	1.04-1.43	0.0326	1.21	1.02-1.44
GTF2H4	rs916920	G/A	0.0139	1.22	1.04-1.43	0.0326	1.21	1.02-1.44
	rs1264569	A/G	0.0004	1.54	1.21-1.97	0.5339		
	rs9468845	A/G	0.0141	1.22	1.04-1.43	0.0332	1.21	1.02-1.44
DDR1	rs8408	C/T	0.0141	1.22	1.04-1.43	0.0326	1.21	1.02-1.44
DDR1	rs7756521	C/T	0.0155	1.22	1.04-1.43	0.0303	1.21	1.02-1.45
CDKN2B-AS1	rs1011970	G/T	0.0047	1.36	1.10-1.69	0.2199		
ADAT1	rs145161932	T/C	0.0104	0.48	0.27-0.84	0.9960		
POU5F1	rs885950	T/G	0.0049	0.81	0.70-0.94	0.0129	0.73	0.57-0.94
DDR1	rs4618569	A/G	0.0141	1.22	1.04-1.43	0.0333	1.21	1.02-1.44
KRT13	rs146918776	A/G	<0.0001	2.21	1.50-3.26			
	rs2523638	G/A	0.0197	1.20	1.03-1.41	0.1032		
PSRC1	rs599839	A/G	0.0004	0.67	0.54-0.83	0.1023		
	rs9275141	G/T	0.0134	1.20	1.04-1.39	0.0065	1.43	1.11-1.85
CCDC63	rs10849915	T/C	0.0005	1.30	1.12-1.50	0.0458	1.33	1.01-1.76
HLA-DRA	rs3177928	G/A	<0.0001	1.58	1.27-1.96	0.7635		
OAS3	rs2072134	C/T	0.0004	1.31	1.13-1.53	0.0268	1.51	1.05-2.16
USP45	rs41288947	C/G	0.0002	1.35	1.15-1.58	0.1360		
CCHCR1	rs1265109	A/C	0.0007	1.35	1.14-1.61	0.0334	1.20	1.01-1.41
LOC101929163	rs6930777	C/T	<0.0001	1.60	1.28-2.00	0.9815		
	rs7333181	G/A	0.0219	0.64	0.44-0.94	0.9969		
DDR1	rs1264323	T/C	0.0240	1.19	1.02-1.39	0.0423	1.22	1.01-1.48
LINC00243	rs3094111	G/A	0.0061	1.24	1.06-1.45	0.5293		
	rs10484561	T/G	<0.0001	1.59	1.28-1.98	0.7637		
PSORS1C1	rs3130558	G/C	<0.0001	1.39	1.18-1.64	0.2712		
HLA-DQB1	rs1049060	T/A	0.0676			0.0077	1.38	1.09-1.75
	rs2844650	G/A	<0.0001	1.66	1.30-2.11	0.4729		
DDR1	rs3132572	T/C	<0.0001	1.66	1.30-2.11	0.4729		
CCHCR1	rs1265115	T/G	0.0004	1.36	1.15-1.62	0.0443	1.19	1.00-1.40
CCHCR1	rs3094225	T/C	<0.0001	1.46	1.23-1.74	0.5822		
LOC107987453	rs3129987	C/T	0.0037	1.26	1.08-1.47	0.5667		
DPCR1	rs2517451	A/G	<0.0001	1.65	1.30-2.11	0.4729		
CCHCR1	rs1265115	T/G	0.0004	1.36	1.15-1.62	0.0443	1.19	1.00-1.40

Table III. Continued.

Gene	SNP		Dominant model			Recessive model		
			P-value	OR	95% CI	P-value	OR	95% CI
CCHCR1	rs3094225	T/C	<0.0001	1.46	1.23-1.74	0.5822		
LOC107987453	rs3129987	C/T	0.0037	1.26	1.08-1.47	0.5667		
DPCR1	rs2517451	A/G	<0.0001	1.65	1.30-2.11	0.4729		
KIAA1551	rs10771894	A/G	0.1122			0.0085	1.35	1.08-1.69
	rs13427905	C/T	0.0024	0.78	0.67-0.92	0.1397		
ABCA1	rs1883025	G/A	0.0051	0.81	0.70-0.94	0.0080	0.68	0.51-0.90
SFTA2	rs2253705	G/A	0.0015	1.28	1.10-1.48	0.6682		
PLUT	rs954750	G/A	0.0409	1.19	1.01-1.41	0.0008	1.33	1.12-1.57
TCF19	rs1419881	T/C	0.0009	1.34	1.13-1.60	0.0487	1.18	1.00-1.39
	rs13209234	G/A	<0.0001	1.55	1.25-1.93	0.7617		
PSORS1C1	rs1265100	T/C	0.0055	0.81	0.70-0.94	0.4135		
YEATS2	rs76174573	G/T	0.0031	0.61	0.44-0.85	0.1756		
ABO	rs1053878	C/T	0.0033	1.25	1.08-1.44	0.1723		
	rs4014195	C/G	0.0083	1.23	1.05-1.43	0.2586		
SFTA2	rs2253588	C/G	0.0021	1.26	1.09-1.45	0.7016		
	rs10757283	T/C	0.0079	0.82	0.71-0.95	0.0181	0.74	0.58-0.95
BTNL2	rs28362680	G/A	0.1143			0.0082	0.76	0.62-0.93
BTNL2	rs10947262	C/T	0.1143			0.0082	0.76	0.62-0.93
KRT27	rs17558532	C/T	0.0004	0.57	0.41-0.78	0.5002		
GTF2H4	rs3130780	G/T	0.0022	1.26	1.09-1.47	0.6706		
	rs2532934	T/C	0.0023	1.25	1.08-1.45	0.5320		
VARS2	rs753725	G/A	0.0021	1.26	1.09-1.45	0.5274		
PLUT	rs11619319	A/G	0.0482	1.18	1.00-1.40	0.0007	1.33	1.13-1.58
	rs3095273	C/T	0.0045	1.38	1.11-1.73	0.1778		
TNS1	rs918949	C/T	0.0028	0.79	0.68-0.92	0.1301		
LINC00243	rs3130785	C/T	0.0060	1.24	1.06-1.45	0.5417		
VARS2	rs2249464	C/T	0.0028	1.25	1.08-1.44	0.5320		
	rs3095345	A/G	0.0021	1.27	1.09-1.47	0.7469		
ITGB8	rs80015015	G/A	<0.0001	1.56	1.28-1.91	0.2511		
VARS2	rs885905	C/T	0.0028	1.25	1.08-1.44	0.7687		
LIPE	rs34052647	G/A	0.0001	1.53	1.23-1.90	0.0074	3.70	1.42-9.65
PHACTR1	rs9369640	A/C	0.0025	0.73	0.60-0.90	0.1527		
BTNL2	rs41417449	T/C	0.1641			0.0017	0.55	0.38-0.80
BTNL2	rs41441651	C/T	0.1586			0.0017	0.55	0.38-0.80
BTNL2	rs28362675	C/A	0.1584			0.0017	0.55	0.38-0.80
BTNL2	rs78587369	G/A	0.1590			0.0018	0.55	0.38-0.80
BTNL2	rs3763315	G/T	0.1637			0.0017	0.55	0.38-0.80
BTNL2	rs2076528	T/G	0.1524			0.0017	0.55	0.38-0.80
PRKG1	rs9414827	G/A	0.0003	0.70	0.58-0.85	0.0395	0.46	0.22-0.96
	rs6537384	T/G	0.0191	1.19	1.03-1.38	0.1787		
	rs6067640	G/A	0.0323	0.85	0.73-0.99	0.0054	0.74	0.60-0.91
	rs10514995	A/G	0.0467	1.19	1.00-1.42	0.0825		
BTNL2	rs34423804	T/A	0.1675			0.0018	0.55	0.38-0.80
PHACTR1	rs9349379	G/A	0.0029	0.80	0.69-0.93	0.0820		
STIM1	rs116855870	A/G	0.0133	1.76	1.13-2.76	0.9967		
ZNF142	rs3821033	C/T	0.0015	1.32	1.11-1.57	0.3469		
LINC00354	rs4907518	G/A	0.0138	0.82	0.70-0.96	0.0050	0.77	0.64-0.92
TNS3	rs11763932	G/A	0.0054	0.81	0.69-0.94	0.0018	0.73	0.60-0.89
BTRC	rs2270439	C/A	0.0063	0.64	0.47-0.88	0.6622		
MIA3	rs2936051	A/G	0.1239			0.0002	0.67	0.55-0.83
	rs6825911	C/T	0.0020	0.78	0.67-0.91	0.0446	0.83	0.70-1.00

Table III. Continued.

Gene	SNP		Dominant model			Recessive model		
			P-value	OR	95% CI	P-value	OR	95% CI
VNN1	rs2294757	G/A	0.2269			0.0334	0.79	0.63-0.98
ZNF860	rs140232911	C/T	0.0013	0.14	0.04-0.46			
	rs838880	C/T	0.0338	1.20	1.01-1.41	0.0308	1.20	1.02-1.43
MIA3	rs2936052	A/G	0.0927			0.0044	0.71	0.56-0.90
DTNBP1	rs2743868	G/A	0.0208	1.19	1.03-1.37	0.0941		
MON2	rs11174549	A/G	0.0427	1.28	1.01-1.61	0.1917		
	rs507666	G/A	0.0098	1.21	1.05-1.40	0.1384		
FAM170B	rs73302786	G/T	0.0003	1.62	1.25-2.11	0.6351		
PSORS1C3	rs3131018	G/T	0.0002	1.35	1.15-1.58	0.9209		
PIEZO2	rs35033671	C/A	0.0035	1.30	1.09-1.54	0.1909		
PANK1	rs11185790	G/A	0.0065	1.26	1.07-1.48	0.0457	1.19	1.00-1.41
GFY	rs73053944	C/G	0.0011	1.60	1.21-2.13	0.0698		
RNF2	rs1046592	A/G	0.0301	0.85	0.74-0.98	0.7186		

Multivariable logistic regression analysis was performed with adjustment for age, sex, and the prevalence of hypertension, diabetes mellitus and dyslipidemia. P<0.05 was considered to indicate a statistically significant difference. SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table IV. 54 SNPs associated with coronary artery disease as determined by a stepwise forward selection procedure.

Gene	SNP	P-value	R^2 (individual)	R^2 (accumulated)
PLCB2	rs200787930	<0.0001	0.0297	0.0297
ALDH2	rs671	<0.0001	0.0061	0.0358
GOSR2	rs1052586	<0.0001	0.0053	0.0411
PSORS1C1	rs3094663	<0.0001	0.0052	0.0463
CCHCR1	rs130071	<0.0001	0.0059	0.0522
	rs13427905	<0.0001	0.0047	0.0569
OR52E4	rs11823828	<0.0001	0.0043	0.0612
EIF3L	rs9466	<0.0001	0.0042	0.0654
KIAA1551	rs10771894	<0.0001	0.0039	0.0693
CCDC141	rs13419085	<0.0001	0.0035	0.0728
MIA3	rs2936051	0.0001	0.0033	0.0761
	rs602633	0.0001	0.0033	0.0794
KRT27	rs17558532	0.0001	0.0032	0.0826
TRPM1	rs2241493	0.0002	0.0030	0.0856
	rs7333181	0.0002	0.0030	0.0886
ADAT1	rs145161932	0.0002	0.0029	0.0915
APOE	rs7412	0.0003	0.0028	0.0943
YEATS2	rs76174573	0.0004	0.0026	0.0969
SLC16A1	rs1049434	0.0005	0.0025	0.0994
RNF2	rs1046592	0.0007	0.0025	0.1019
	rs6825911	0.0006	0.0024	0.1043
ITGB8	rs80015015	0.0007	0.0024	0.1067
USP45	rs41288947	0.0007	0.0024	0.1091
PHACTR1	rs9369640	0.0007	0.0024	0.1115
	rs1333048	0.0008	0.0024	0.1139
	rs838880	0.0011	0.0022	0.1161
STIM1	rs116855870	0.0017	0.0021	0.1182
	rs2523644	0.0016	0.0021	0.1203

Table IV. Continued.

Gene	SNP	P-value	R^2 (individual)	R^2 (accumulated)
MKI67	rs145121731	0.0020	0.0020	0.1223
FAM170B-AS1	rs73302786	0.0019	0.0020	0.1243
	rs6067640	0.0022	0.0020	0.1263
GFY	rs73053944	0.0024	0.0019	0.1282
WDR37	rs10794720	0.0033	0.0018	0.1300
SKIV2L	rs592229	0.0037	0.0018	0.1318
	rs6537384	0.0041	0.0017	0.1335
	rs10757283	0.0058	0.0016	0.1351
CDKN2B-AS1	rs1011970	0.0110	0.0014	0.1365
PRKG1	rs9414827	0.0087	0.0014	0.1379
	rs197932	0.0127	0.0013	0.1392
LINC00354	rs4907518	0.0125	0.0013	0.1405
LIPE	rs34052647	0.0151	0.0013	0.1418
BTRC	rs2270439	0.0143	0.0013	0.1431
TNS3	rs11763932	0.0163	0.0013	0.1444
TNS1	rs918949	0.0158	0.0012	0.1456
	rs12229654	0.0184	0.0011	0.1467
	rs4014195	0.0213	0.0011	0.1478
PANK1	rs11185790	0.0227	0.0011	0.1489
	rs507666	0.0279	0.0010	0.1499
MON2	rs11174549	0.0359	0.0009	0.1508
HECTD4	rs2074356	0.0396	0.0009	0.1517
CUBN	rs78201384	0.0484	0.0009	0.1526
PLUT	rs954750	0.0497	0.0008	0.1534
ABCA1	rs1883025	0.0479	0.0008	0.1542
	rs10514995	0.0493	0.0008	0.1550

SNP, single nucleotide polymorphisms; R^2, coefficient of determination.

Table V. Association between SNPs associated with coronary artery disease and intermediate phenotypes.

Gene	SNP	Hypertension	DM	Hyper-TG	Hypo-HDL	Hyper-LDL	CKD	Obesity	Hyperuricemia
PLCB2	rs200787930	<0.0001[a]	0.0004[a]	0.3432	<0.0001[a]	<0.0001[a]	<0.0001[a]	0.0405[a]	0.9639
ALDH2	rs671	0.0039[a]	0.0074[a]	0.0298[a]	<0.0001[a]	<0.0001[a]	0.0273[a]	0.0350[a]	<0.0001[a]
GOSR2	rs1052586	0.3498	0.0167[a]	0.4457	0.2898	0.2638	0.6185	0.3670	0.4679
PSORS1C1	rs3094663	0.0069[a]	0.0670	0.0947	0.0020[a]	0.3869	0.1080	0.7345	0.5091
CCHCR1	rs130071	0.0865	0.0008[a]	0.2247	0.0143[a]	0.0141[a]	0.5894	0.8651	0.0423[a]
	rs13427905	0.0149[a]	0.0149[a]	0.0524	0.0545	0.6318	0.9487	0.1197	0.0920
OR52E4	rs11823828	0.0024[a]	<0.0001[a]	0.0265[a]	0.1186	0.4445	0.0027[a]	0.1141	0.2815
EIF3L	rs9466	0.0008[a]	0.0204[a]	0.0054[a]	0.2905	0.0114[a]	0.2368	0.2435	0.0312[a]
KIAA1551	rs10771894	0.2439	0.0091[a]	0.9562	0.0343[a]	0.6934	0.3869	0.0974	0.5419
CCDC141	rs13419085	0.3387	0.1255	0.6537	0.1647	0.7447	0.2483	0.8101	0.7938
MIA3	rs2936051	0.4092	0.5246	0.9990	0.0475[a]	0.1222	0.6614	0.8787	0.1949
	rs602633	0.4468	0.2375	0.7350	0.0005[a]	0.0021[a]	0.0842	0.6617	0.9338
KRT27	rs17558532	0.1706	0.2358	0.3643	0.3607	0.7663	0.0133[a]	0.2306	0.5325
TRPM1	rs2241493	0.3861	0.2332	0.7465	0.7106	0.0815	0.1387	0.5698	0.9502
	rs7333181	0.2308	0.0487[a]	0.0379[a]	0.1185	0.2010	0.0795	0.2182	0.6544
ADAT1	rs145161932	0.4468	0.0160[a]	0.3611	0.3357	0.5412	0.7534	0.5836	0.1202
APOE	rs7412	0.3680	0.9184	0.6322	0.1157	<0.0001[a]	0.6367	0.5319	0.2528
YEATS2	rs76174573	0.1305	0.0687	0.0380[a]	0.0606	0.8313	0.6458	0.6338	0.1706

Table V. Continued.

Gene	SNP	Hypertension	DM	Hyper-TG	Hypo-HDL	Hyper-LDL	CKD	Obesity	Hyperuricemia
SLC16A1	rs1049434	0.8319	0.0016[a]	0.1897	0.0686	0.9212	0.0646	0.8850	0.2672
RNF2	rs1046592	0.0007[a]	0.0140[a]	0.5319	0.0544	0.4098	0.7276	0.4643	0.1040
	rs6825911	0.0317[a]	0.4070	0.5755	0.1068	0.1423	0.4050	0.2325	0.6717
ITGB8	rs80015015	0.4001	0.5178	0.4838	0.0075[a]	0.5169	0.3341	0.1339	0.2408
USP45	rs41288947	0.4383	0.1373	0.2641	0.0162[a]	0.6636	0.1341	0.0063[a]	0.0682
PHACTR1	rs9369640	0.1667	0.4673	0.8831	0.5247	0.8191	0.7417	0.6674	0.4133
	rs1333048	0.2947	0.0251[a]	0.5799	0.0156[a]	0.3204	0.6650	0.5825	0.2450
	rs838880	0.9565	0.0108[a]	0.3044	0.0045[a]	0.7818	0.7699	0.8126	0.2552
STIM1	rs116855870	0.1425	0.2455	0.6418	0.8631	0.7116	0.5285	0.7357	0.3365
	rs2523644	0.4105	0.2101	0.6604	0.0773	0.0627	0.0449[a]	0.2968	0.2580
MKI67	rs145121731	0.0903	0.2528	0.2203	0.0138[a]	0.4030	0.0048[a]	0.2459	0.5059
FAM170B-AS1	rs73302786	0.2662	0.6366	0.2511	0.2687	0.8989	0.8203	0.2323	0.5404
	rs6067640	0.0380[a]	0.2144	0.7990	0.0077[a]	0.6995	0.1809	0.6901	0.9347
GFY	rs73053944	0.0145[a]	0.5731	0.5880	0.2471	0.8788	0.6316	0.4534	0.9116
WDR37	rs10794720	0.6272	0.0103[a]	0.9125	0.6954	0.6528	0.1352	0.7804	0.0458[a]
SKIV2L	rs592229	0.0014[a]	0.0754	0.0752	0.0157[a]	0.7557	0.1230	0.3617	0.1727
	rs6537384	0.3890	0.1107	0.2980	0.3818	0.0344[a]	0.0808	0.0620	0.7745
	rs10757283	0.8792	0.0082[a]	0.9667	0.0420[a]	0.4745	0.0342[a]	0.8876	0.4636
CDKN2B-AS1	rs1011970	0.0443[a]	0.0628	0.6524	0.0834	0.5456	0.4293	0.6420	0.3237
PRKG1	rs9414827	0.6287	0.8365	0.6694	0.0424[a]	0.1488	0.6060	0.0291[a]	0.0549
	rs197932	0.1918	0.0272[a]	0.5146	0.5673	0.2395	0.3102	0.7843	0.4625
LINC00354	rs4907518	0.2933	0.8434	0.3915	0.1285	0.0028[a]	0.6454	0.6846	0.7513
LIPE	rs34052647	0.1525	0.7148	0.0040a	0.4199	0.0801	0.0879	0.0940	0.0081[a]
BTRC	rs2270439	0.3191	0.9636	0.1684	0.1393	0.9515	0.2852	0.4689	0.5780
TNS3	rs11763932	0.4812	0.6129	0.9920	0.8857	0.1424	0.9598	0.9307	0.9591
TNS1	rs918949	0.1509	0.0510	0.3218	0.5993	0.7044	0.5484	0.6955	0.9461
	rs12229654	0.0203[a]	0.3290	0.1080	<0.0001[a]	0.0171[a]	0.1167	0.2297	<0.0001[a]
	rs4014195	0.1622	0.0445[a]	0.0270[a]	0.1811	0.7090	0.3732	0.5607	0.2233
PANK1	rs11185790	0.3638	0.4169	0.1750	0.2583	0.3889	0.0149[a]	0.6355	0.3282
	rs507666	0.9872	0.0084[a]	0.7080	0.0370[a]	0.0129[a]	0.4210	0.9126	0.6992
MON2	rs11174549	0.0283[a]	0.0133[a]	0.8790	0.3587	0.0325[a]	0.2617	0.6406	0.9153
HECTD4	rs2074356	0.0285[a]	0.1092	0.0109[a]	<0.0001[a]	0.0002[a]	0.0174	0.1786	<0.0001[a]
CUBN	rs78201384	0.1429	0.9473	0.7525	0.0027[a]	0.0269[a]	0.0327[a]	0.9812	0.3888
PLUT	rs954750	0.9214	0.0212[a]	0.6905	0.8004	0.8585	0.0264[a]	0.2382	0.8865
ABCA1	rs1883025	0.8085	0.2006	0.0134[a]	0.3092	0.0667	0.8891	0.4019	0.3377
	rs10514995	0.0014[a]	0.0353[a]	0.2529	0.3708	0.7542	0.0855	0.5232	0.0365[a]

Data are P-values. The association between genotypes of each SNP and intermediate phenotypes was examined using Pearson's χ^2 test. SNP, single nucleotide polymorphism; DM, diabetes mellitus; hyper-TG, hypertriglyceridemia; hypo-HDL, hypo-HDL-cholesterolemia; hyper-LDL, hyper-LDL-cholesterolemia; CKD, chronic kidney disease. [a]P<0.05 was considered to indicate a statistically significant difference.

nary or renal function. The remaining 21 genes (*RNF2, YEATS2, USP45, ITGB8, TNS3, FAM170B-AS1, PRKG1, BTRC, MKI67, STIM1, OR52E4, KIAA1551, MON2, PLUT, LINC00354, TRPM1, ADAT1, KRT27, LIPE, GFY* and *EIF3L*) and five chromosomal regions (2p13, 4q31.2, 5q12, 13q34 and 20q13.2) identified in the present study have not been revealed to be associated with CAD or cardiovascular disease-related phenotypes in previous GWASs.

Gene Ontology analysis of genes identified in the present study. Biological functions of the 21 genes identified in the present study were estimated using the database of Gene Ontology and GO Annotations (QuickGO; Table VII). Given that *FAM170B-AS1* is the gene for non-coding RNA, *FAM170B* was examined. Various biological functions were predicted in the 18 genes (*RNF2, YEATS2, USP45, ITGB8, TNS3, FAM170B, PRKG1, BTRC, MKI67, STIM1, OR52E4, MON2, TRPM1, ADAT1, KRT27, LIPE, GFY and EIF3L),* although those of *KIAA1551, PLUT* and *LINC00354* were not. Gene ontology analysis revealed that *ITGB8*, *PRKG1*, *STIM1* and *LIPE* may be involved in the development of CAD.

Table VI. Association between genes, chromosomal loci and SNPs associated with coronary artery disease in the present study and previously examined cardiovascular disease-related phenotypes.

Gene/chr. locus	SNP	Chr.	Position	Previously examined phenotypes
1p13.3	rs602633	1	109278889	CAD (23202125, 20032323), LDL-cholesterol (20686565, 23063622, 19060906, 21943158, 18193043, 18262040, 19913121, 21977987, 20339536), HDL-cholesterol (23063622, 20686565), total cholesterol (20686565, 23063622)
SLC16A1	rs1049434	1	112913924	HDL-cholesterol (23063622)
RNF2	rs1046592	1	185100429	None
MIA3	rs2936051	1	222629862	CAD (19198612, 21347282, 23364394, 21378990, 17554300, 22319020, 21966275), MI (19198609)
2p13	rs13427905	2	71846585	None
CCDC141	rs13419085	2	178837710	Heart rate (23583979, 20639392), left ventricular mass (19584346)
TNS1	rs918949	2	217809974	Lung function, forced expiratory volume in 1 second (20010834, 21946350, 23284291)
YEATS2	rs76174573	3	183804099	None
4q24	rs6825911	4	110460482	Systolic BP (21572416), diastolic BP (21572416)
4q31.2	rs6537384	4	145949613	None
5q12	rs10514995	5	66443611	None
PHACTR1	rs9369640	6	12901209	CAD (21378988, 23202125, 22745674, 21347282, 23364394, 21378990, 22751097, 22745674), MI (19198609, 21378990), ischemic stroke (22306652)
PSORS1C1	rs3094663	6	31139310	Type 1 diabetes (17554300, 17632545), triglycerides (20686565), total cholesterol (20686565)
CCHCR1	rs130071	6	31148433	Triglycerides (20686565)
6p21.3	rs2523644	6	31374707	Type 1 diabetes (17554300, 17632545), LDL-cholesterol (23063622, 20686565), triglycerides (23063622, 20686565), total cholesterol (23063622, 20686565)
SKIV2L	rs592229	6	31962664	CAD (21971053), type 1 diabetes (17554300, 17632545), LDL-cholesterol (20686565), triglycerides (20686565), total cholesterol (20686565)
USP45	rs41288947	6	99446210	None
ITGB8	rs80015015	7	20401881	None
TNS3	rs11763932	7	47567880	None
CDKN2B-AS1	rs1011970	9	22062135	CAD (21347282), LDL-cholesterol (23063622), abdominal aortic aneurysm (20622881), type 2 diabetes (17463249)
9p21	rs1333048	9	22125348	CAD (23202125, 21606135, 19198612, 17634449, 20032323, 23364394), MI (17478679), intracranial aneurysm (22961961)
9p21	rs10757283	9	22134173	Type 2 diabetes (20581827)
ABCA1	rs1883025	9	104902020	HDL-cholesterol (20686565, 23505323, 23063622, 21909109, 19060911, 21347282, 19060906, 18193043, 18193044, 18193046, 22629316, 20864672, 21347282, 23726366), LDL-cholesterol (20686565), total cholesterol (20686565, 23063622, 20339536)
9q34.2	rs507666	9	136149399	Venous thrombosis (22675575), VLDL-cholesterol small lipoprotein fraction concentration (19936222), LDL-cholesterol lipoprotein fraction concentration (19936222)
WDR37	rs10794720	10	1110225	Estimated glomerular filtration rate (20383146, 22479191), serum creatinine (20383146)
CUBN	rs78201384	10	17111024	LDL-cholesterol (23063622), HDL-cholesterol (23063622), total cholesterol (23063622)
FAM170B-AS1	rs73302786	10	49131709	None

Table VI. Continued.

Gene/chr. locus	SNP	Chr.	Position	Previously examined phenotypes
PRKG1	rs9414827	10	51137314	None
PANK1	rs11185790	10	89612776	Insulin concentration (19060910)
BTRC	rs2270439	10	101550817	None
MKI67	rs145121731	10	128102595	None
STIM1	rs116855870	11	4055527	None
OR52E4	rs11823828	11	5884973	None
11q13.1	rs4014195	11	65739351	Serum urate (23263486), serum creatinine (20383146), estimated glomerular filtration rate (20383146)
KIAA1551	rs10771894	12	31982009	None
MON2	rs11174549	12	62565357	None
12q24.1	rs12229654	12	110976657	HDL-cholesterol (21909109)
ALDH2	rs671	12	111803962	CAD (21971053, 21572416, 23202125), MI (21971053), LDL-cholesterol (21572416, 20686565), HDL-cholesterol (21572416, 21372407), total cholesterol (20686565), systolic BP (21572416), diastolic BP (21572416, 21909115), serum creatinine (22797727), estimated glomerular filtration rate (22797727), type 1 diabetes (17554300)
HECTD4	rs2074356	12	112207597	CAD (21971053, 21572416, 22751097, 19820697, 23364394, 23202125), MI (19820697), LDL-cholesterol (21572416, 20686565), HDL-cholesterol (21572416, 21909109, 22751097), total cholesterol (20686565), systolic BP (21572416, 21909115), diastolic BP (21572416, 21909115, 19862010, 19430479, 22751097), hypertension (21572416), serum creatinine (22797727), estimated glomerular filtration rate (22797727), type 1 diabetes (18978792)
12q24.31	rs838880	12	124777047	HDL-cholesterol (20686565)
PLUT	rs954750	13	27889801	None
13q34	rs7333181	13	111568950	None
LINC00354	rs4907518	13	111898209	None
TRPM1	rs2241493	15	31070149	None
PLCB2	rs200787930	15	40289298	Triglycerides (23063622)
ADAT1	rs145161932	16	75612670	None
KRT27	rs17558532	17	40779624	None
17q21.3	rs197932	17	46896981	Pulse pressure (21909110), systolic BP (21909110, 21909115)
GOSR2	rs1052586	17	46941097	Pulse pressure (21909110), systolic BP (21909110, 21909115)
LIPE	rs34052647	19	42407617	None
APOE	rs7412	19	44908822	LDL-cholesterol (23100282, 23063622, 20686565, 22629316, 19060911, 23067351, 23696881, 20838585), HDL-cholesterol (21386085), triglycerides (23063622, 20686565, 22629316, 19060911, 21386085), total cholesterol (23063622, 20686565)
GFY	rs73053944	19	49427038	None
20q13.2	rs6067640	20	51092837	None
EIF3L	rs9466	22	37877742	None

Data were obtained from the GRASP Search database (https://grasp.nhlbi.nih.gov/Search.aspx) with a P-value of $<1.0x10^{-6}$. Numbers in parentheses are PubMed IDs. SNP, single nucleotide polymorphisms; Chr., chromosome; HDL, high density lipoprotein; LDL, low density lipoprotein; CAD, coronary artery disease; MI, myocardial infarction; BP, blood pressure.

Network analysis of newly identified genes. Network analysis of the 21 genes identified in the present study was performed using the GeneMANIA Cytoscape plugin with Cytoscape v3.4.0 software (Figs. 1 and 2). FAM170B was applied to the analysis instead of *FAM170B-AS1*. *PLUT* and *LINC00354* were not included in the GeneMANIA database. *GFY* had no interaction with other genes. The network analysis revealed that the 18 genes identified in the present study had potential direct or indirect interactions with the 30 genes previously revealed to be associated with CAD (Fig. 1). Similar analysis

Table VII. Gene ontology analysis of the 21 genes identified in the present study.

Gene	Function	Biological process
RNF2	Ubiquitin-protein transferase activity, chromatin binding, zinc ion binding, transferase activity, metal ion binding, ubiquitin protein ligase activity, RING-like zinc finger domain binding	Histone H2A-K119 monoubiquitination, negative regulation of transcription by RNA polymerase II, regulation of DNA-templated transcription, germ cell development, negative regulation of DNA binding transcription factor activity, negative regulation of G0 to G1 transition
YEATS2	Modification-dependent protein binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding	Negative regulation of transcription by RNA polymerase II, histone H3 acetylation, negative regulation of DNA-templated transcription
USP45	Thiol-dependent ubiquitin-specific protease activity, cysteine-type peptidase activity, zinc ion binding, thiol-dependent ubiquitinyl hydrolase activity	Protein deubiquitination, ubiquitin-dependent protein catabolic process, DNA repair, global genome nucleotide-excision repair
ITGB8	Extracellular matrix protein binding, signaling receptor binding	Ganglioside metabolic process, cell adhesion, integrin-mediated signaling pathway, regulation of gene expression, positive regulation of angiogenesis, cartilage development, extracellular matrix organization, cell-matrix adhesion
TNS3	Protein binding, focal adhesion	Positive regulation of cell proliferation, cell migration, lung alveolus development
FAM170B	Protein binding, outer acrosomal membrane	Positive regulation of acrosome reaction, regulation of fertilization
PRKG1	cGMP-dependent protein kinase activity, calcium channel regulator activity, nucleotide binding, ATP binding, transferase activity, cGMP binding, protein serine/threonine kinase activity, cGMP-dependent protein kinase activity	Negative regulation of vascular smooth muscle cell proliferation and migration, neuron migration, cGMP-mediated signaling, dendrite development, forebrain development, relaxation of vascular smooth muscle, regulation of GTPase activity, negative regulation of platelet aggregation, actin cytoskeleton organization
BTRC	Ubiquitin-protein transferase activity, ubiquitin protein ligase activity, β-catenin binding, protein phosphorylated amino acid binding, protein dimerization activity	Protein polyubiquitination, ubiquitin-dependent protein catabolic process, regulation of circadian rhythm, regulation of canonical Wnt signaling pathway, protein dephosphorylation, mammary gland epithelial cell proliferation, regulation of I-κB kinase/NF-κB signaling, positive regulation of DNA-templated transcription, G2/M transition of mitotic cell cycle, negative regulation of DNA binding transcription factor activity, stress-activated MAPK cascade, interleukin-1-mediated signaling pathway
MKI67	RNA binding, DNA binding, ATP binding, protein binding	Regulation of mitotic nuclear division, regulation of chromosome segregation and organization, cell proliferation
STIM1	Calcium channel regulator activity, calcium ion binding, microtubule plus-end binding, metal ion binding, protein binding	Cellular calcium ion homeostasis, activation of store-operated calcium channel activity, regulation of calcium ion transport, positive regulation of angiogenesis, regulation of cardiac conduction
OR52E4	Olfactory receptor activity, G-protein coupled receptor activity	Signal transduction, G-protein coupled receptor signaling pathway, detection of chemical stimulus involved in sensory perception of smell
KIAA1551	Uncharacterized	
MON2	Protein binding, protein transport	Golgi to endosome transport
PLUT	Uncharacterized	
LINC00354	Uncharacterized	

Table VII. Continued.

Gene	Function	Biological process
TRPM1	Ion channel activity	G-protein coupled glutamate receptor signaling pathway, ion transmembrane transport, protein tetramerization, cellular response to light stimulus
ADAT1	RNA binding, tRNA-specific adenosine deaminase activity, hydrolase activity, metal ion binding	tRNA processing
KRT27	Structural molecule activity, intermediate filament	Hair follicle morphogenesis, keratinization, cornification
LIPE	Triglyceride lipase activity, serine hydrolase activity, protein kinase binding, hormone-sensitive lipase activity	Protein phosphorylation, lipid metabolic process, steroid metabolic process, cholesterol metabolic process, triglyceride catabolic process, long-chain fatty acid catabolic process, diacylglycerol catabolic process
GFY	Protein localization to non-motile cilium, non-motile cilium assembly	Sensory perception of smell, response to stimulus
EIF3L	Translation initiation factor activity, RNA binding, protein binding	Translational initiation, viral translational termination-reinitiation

Data for predicted functions and biological processes of the genes were obtained from database of Gene Ontology and GO Annotations (QuickGO; https://www.ebi.ac.uk/QuickGO/).

revealed that complex networks were observed between the 18 genes identified in the present study and the 228 genes identified in previous GWASs (Fig. 2).

Discussion

Despite recent advances in therapy for acute coronary syndrome, including coronary stent implantation (49), CAD remains the leading cause of mortality and is therefore a key public health problem (4). The identification of genetic variants that confer susceptibility to CAD is therefore clinically important for the prevention and management of this condition.

The EWAS was performed for patients with early-onset CAD, with genetic factors serving a greater role in such patients compared with those with late-onset CAD. The present study identified the 54 SNPs as significant and independent determinants of CAD. These SNPs together accounted for 15.5% of the cause of CAD. Among these loci, 21 genes (*RNF2, YEATS2, USP45, ITGB8, TNS3, FAM170B-AS1, PRKG1, BTRC, MKI67, STIM1, OR52E4, KIAA1551, MON2, PLUT, LINC00354, TRPM1, ADAT1, KRT27, LIPE, GFY* and *EIF3L*) and 5 chromosomal regions (2p13, 4q31.2, 5q12, 13q34 and 20q13.2) that confer susceptibility to CAD have been newly identified.

Among 26 SNPs identified, 14 SNPs were significantly associated with two to five of the eight intermediate phenotypes. The SNP rs9466 of *EIF3L* was associated with hypertension, DM, hypertriglyceridemia, hyper-LDL-cholesterolemia and hyperuricemia; rs11823828 of *OR52E4* with hypertension, DM, hypertriglyceridemia, and CKD; rs11174549 of *MON2* with hypertension, DM, hyper-LDL-cholesterolemia; rs10514995 at 5q12 with hypertension, DM and hyperuricemia; rs1046592 of *RNF2* and rs13427905 at 2p13 with hypertension and DM; rs6067640 at 20q13.2 with hypertension and hypo-HDL-cholesterolemia; rs7333181 at 13q34 with DM and hypertriglyceridemia; rs10771894 of *KIAA1551* with DM and hypo-HDL-cholesterolemia; rs954750 of *PLUT* with DM and CKD; rs34052647 of *LIPE* with hypertriglyceridemia and hyperuricemia; rs145121731 of *MKI67* with hypo-HDL-cholesterolemia and CKD; rs41288947 of *USP45* and rs9414827 of *PRKG1* with hypo-HDL-cholesterolemia and obesity. The seven SNPs were significantly related to one of the eight intermediate phenotypes. The rs73053944 of *GFY* was associated with hypertension; rs145161932 of *ADAT1* with DM; rs76174573 of *YEATS2* with hypertriglyceridemia; rs80015015 of *ITGB8* with hypo-HDL-cholesterolemia; rs4907518 of *LINC00354* and rs6537384 at 4q31.2 with hyper-LDL-cholesterolemia; rs17558532 of *KRT27* with CKD. Given that these intermediate phenotypes are risk factors for CAD (4), the association between these loci and CAD may be attributable, at least in part, to their effects on intermediate phenotypes. By contrast, five SNPs in *TNS3*, *FAM170B-AS1*, *BTRC*, *STIM1* and *TRPM1* were not associated with intermediate phenotypes. The underlying molecular mechanisms of the association between these loci and CAD remain to be elucidated.

Recent GWASs have identified potential biological pathways underlying the association between genetic loci and CAD, including metabolism of LDL-cholesterol, triglycerides and lipoprotein (a); insulin resistance; thrombosis; inflammation, cell adhesion and transendothelial migration; cellular proliferation, vascular remodeling and extracellular matrix metabolism; and vascular tone and nitric oxide signaling (50,51). Network analysis of functional gene-gene interactions may be informative to clarify biological process of CAD and to identify therapeutic targets for this condition (52). Therefore, the present study performed gene ontology

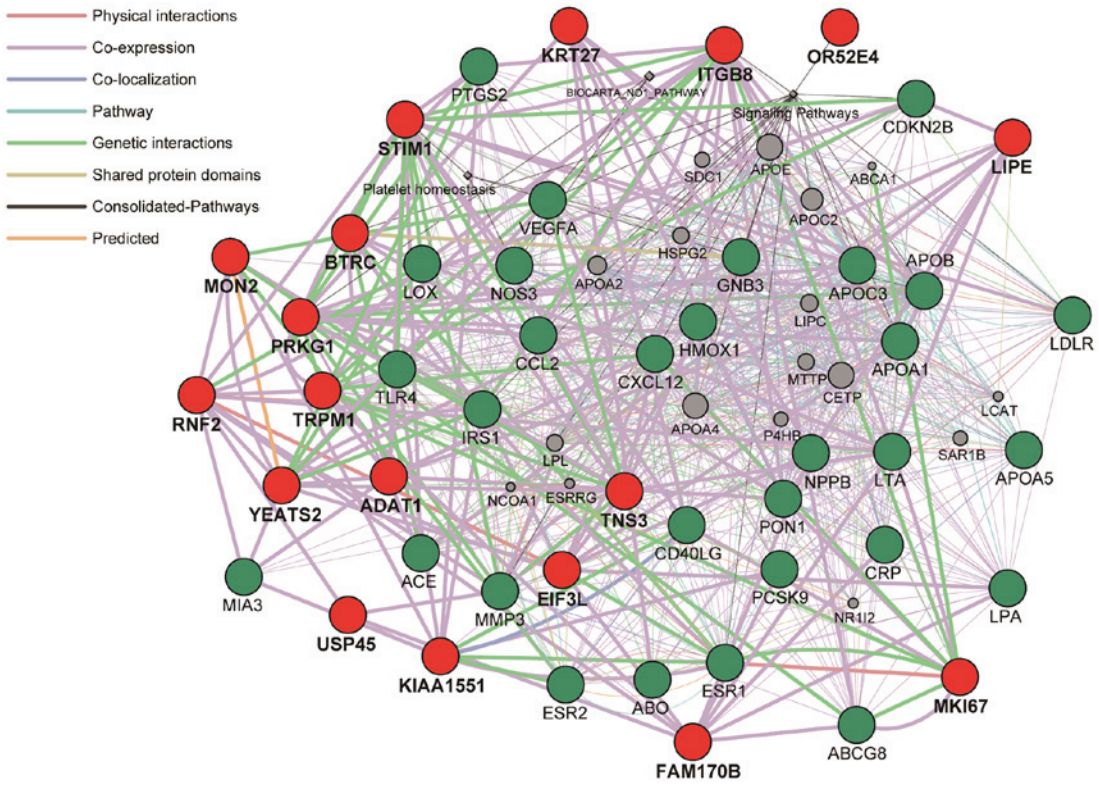

Figure 1. Network analysis of the 18 genes identified in the present study (closed red circle) was performed to predict functional gene-gene interactions by the use of GeneMANIA Cytoscape plugin (http://apps.cytoscape.org/apps/genemania) using Cytoscape v3.4.0 software (http://www.cytoscape.org/). The 30 genes (closed green circle) were selected from the DisGeNET database (http://www.disgenet.org/ web/ DisGeNET) according to the rank order of high scores in association with CAD and applied to analysis.

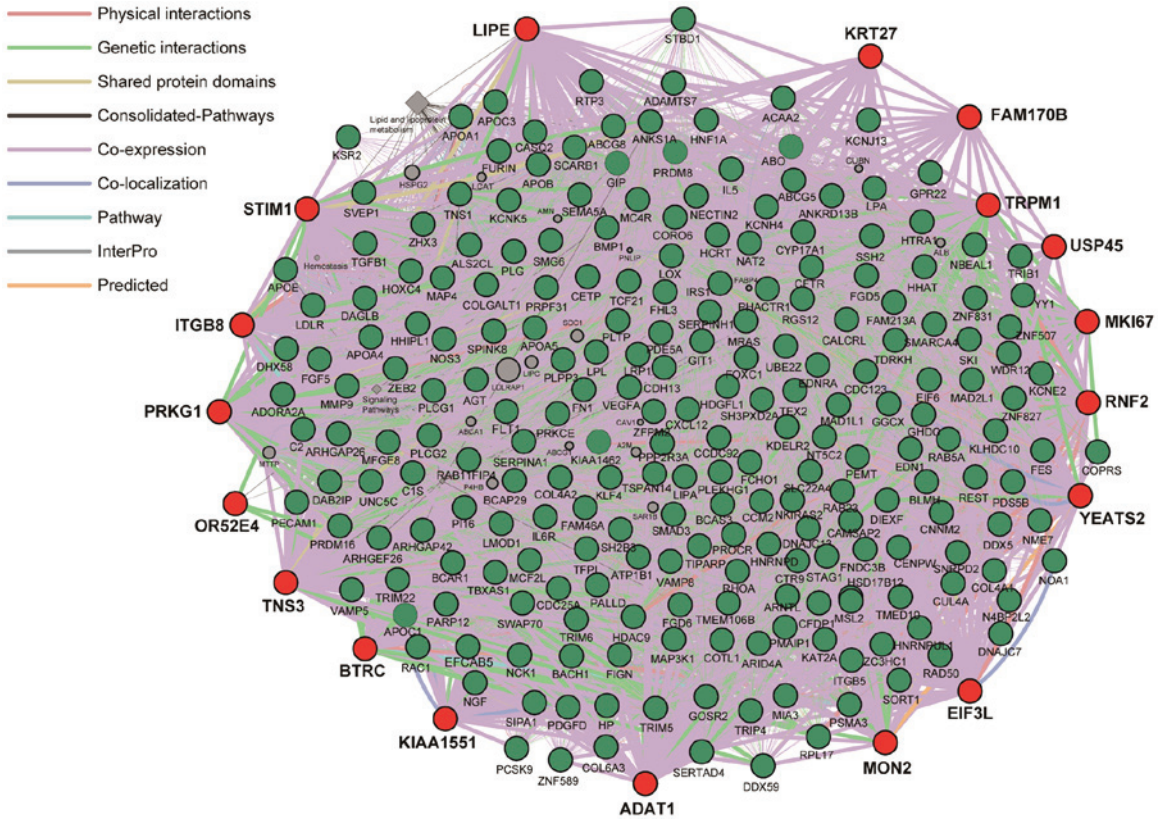

Figure 2. Network analysis of the 18 genes identified in the present study (closed red circle) was performed to predict functional gene-gene interactions by the use of GeneMANIA Cytoscape plugin (http://apps.cytoscape.org/apps/genemania) using Cytoscape v3.4.0 software (http://www.cytoscape.org/). The 228 genes previously identified by GWASs (closed green circle) were applied to analysis. Interactions between closed red circles or between closed red and green circles are shown with bold lines. Molecules shown in closed grey circles represent putative mediators of interactions between the genes.

and network analyses to predict biological processes of the identified genes and interactions between these genes and those previously revealed to be associated with CAD. Gene ontology analysis revealed that biological functions of *ITGB8* (integrin-mediated signaling pathway), *PRKG1* (relaxation of vascular smooth muscle), *STIM1* (activation of store-operated calcium channel activity) and *LIPE* (cholesterol and triglyceride metabolism) may serve roles in the development of CAD. However, the roles of the remaining 17 genes in CAD remain unclear. The network analysis revealed that the 18 genes identified in the present study had direct or indirect interactions with the 30 genes selected from the DisGeNET database (47,48), as well as complex networks with 228 genes previously identified by the GWASs (7). However, the underlying molecular mechanisms of these interactions remain to be elucidated.

It was previously demonstrated that six SNPs were associated with CAD ($P<0.01$), as determined by multivariable logistic regression analysis with adjustment for covariates following an initial EWAS screening of allele frequencies among subjects with early-onset and late-onset forms of this condition (33). The associations between three of the six SNPs [rs202069030 ($P=2.58x10^{-6}$), rs7188 ($P=0.0098$) and rs2271395 ($P=0.0042$)] and CAD were replicated ($P<0.05$) in the present study. These results suggested that genetic variants associated with CAD differ, in part, between early-onset and late-onset patients with this condition. We also examined nine SNPs associated with MI ($P<0.01$) in a previous study (33). Associations between five of the nine SNPs [rs202103723 ($P=0.0033$), rs188212047 ($P=0.0034$), rs1265110 ($P=2.69x10^{-5}$), rs9258102 ($P=0.0374$) and rs439121 ($P=0.0108$)] and CAD ($P<0.05$) were identified in the present study.

There are several limitations to the present study: i) Given that the results were not replicated, their validation will be necessary in independent study populations or in other ethnic groups; ii) it is possible that SNPs identified in the present study are in LD with other genetic variants in the same gene or in other nearby genes that are actually responsible for the development of CAD; and iii) the functional relevance of identified SNPs to the pathogenesis of CAD remains to be elucidated.

In conclusion, the present study identified the 54 SNPs as significant and independent determinants of CAD. Among these loci, 21 genes (*RNF2, YEATS2, USP45, ITGB8, TNS3, FAM170B-AS1, PRKG1, BTRC, MKI67, STIM1, OR52E4, KIAA1551, MON2, PLUT, LINC00354, TRPM1, ADAT1, KRT27, LIPE, GFY* and *EIF3L*) and 5 chromosomal regions (2p13, 4q31.2, 5q12, 13q34 and 20q13.2) that confer susceptibility to CAD were newly identified in the present study. Determination of genotypes for the SNPs at these loci may prove informative for assessment of the genetic risk for CAD in Japanese patients.

Acknowledgements

Not applicable.

Funding

The present study was supported by CREST, Japan Science and Technology Agency, Kawaguchi, Japan (grant no. JPMJCR1302).

Availability of data and materials

All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YY contributed to the conception and design of the study; to acquisition, analysis and interpretation of the data; and to drafting of the manuscript. KK, MO, HH and TF all contributed to the acquisition of the data and to the revision of the manuscript. YY, IT and JS contributed to the analysis and interpretation of the data, as well as to the revision of the manuscript.

Ethics approval and consent to participate

The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University Graduate School of Medicine, Hirosaki University Graduate School of Medicine, and participating hospitals (Gifu Prefectural Tajimi Hospital, Gifu Prefectural General Medical Center, Japanese Red Cross Nagoya First Hospital, Northern Mie Medical Center Inabe General Hospital, and Hirosaki Stroke and Rehabilitation Center). Written informed consent was obtained from all subjects.

Patient consent for publication

All authors approved submission of the final version of the article for publication.

Competing interests

The authors declare that they have no competing interests.

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