

Genome-Wide Association Study for Coronary Artery Calcification With Follow-Up in Myocardial Infarction

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Background—Coronary artery calcification (CAC) detected by computed tomography is a noninvasive measure of coronary atherosclerosis, which underlies most cases of myocardial infarction (MI). We sought to identify common genetic variants associated with CAC and further investigate their associations with MI.

Methods and Results—Computed tomography was used to assess quantity of CAC. A meta-analysis of genome-wide association studies for CAC was performed in 9961 men and women from 5 independent community-based cohorts, with replication in 3 additional independent cohorts (n=6032). We examined the top single-nucleotide polymorphisms (SNPs) associated with CAC quantity for association with MI in multiple large genome-wide association studies of MI. Genome-wide significant associations with CAC for SNPs on chromosome 9p21 near *CDKN2A* and *CDKN2B* (top SNP: rs1333049; $P=7.58 \times 10^{-19}$) and 6p24 (top SNP: rs9349379, within the *PHACTR1* gene; $P=2.65 \times 10^{-11}$) replicated for CAC and for MI. Additionally, there is evidence for concordance of SNP associations with both CAC and MI at a number of other loci, including 3q22 (*MRAS* gene), 13q34 (*COL4A1/COL4A2* genes), and 1p13 (*SORT1* gene).

Conclusions—SNPs in the 9p21 and *PHACTR1* gene loci were strongly associated with CAC and MI, and there are suggestive associations with both CAC and MI of SNPs in additional loci. Multiple genetic loci are associated with development of both underlying coronary atherosclerosis and clinical events. (*Circulation*. 2011;124:2855-2864.)

Key Words: cardiac computed tomography ■ coronary artery calcification ■ coronary atherosclerosis
■ genome-wide association studies ■ myocardial infarction

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Atherosclerosis in coronary arteries underlies most cases of myocardial infarction (MI) and other clinical coronary heart diseases (CHD). CHD comprises the leading cause of death in Western countries.¹ Extent of coronary atherosclerosis may be determined with noninvasive, high-resolution computed tomography (CT) to measure coronary artery calcification (CAC).² CAC quantity is heritable,³ significantly higher in people with a parental history of CHD,⁴ correlates with increased burden of subclinical coronary plaque,⁵ and predicts incident CHD in multiple ethnic populations after adjustment for other traditional CHD risk factors.^{2,6,7}

Clinical Perspective on p 2864

Genome-wide association (GWA) studies identified common genetic variations influencing risk of MI, including a strongly replicated association on chromosome 9p21,^{8–13} as well as strong associations with *PHACTR1*¹¹ and >9 other loci.^{8,11} Recently, a large meta-analysis of 14 GWA studies for coronary disease phenotypes in the Coronary Artery Disease Genome Wide Replication and Meta-Analysis (CARDIoGRAM) Consortium reported a total of 24 loci including the previously known loci for MI.¹⁴ Neither chromosome 9p21 nor any other locus associated with CHD has been shown to be associated with the CAC quantity at a genome-wide significance level. We conducted a meta-analysis to identify loci underlying variation in extent of CAC. We further assessed whether single-nucleotide polymorphisms (SNPs) associated with CAC quantity were also associated with MI and whether SNPs previously shown to be associated with MI are associated with CAC quantity.

Methods

Setting

We conducted a meta-analysis of GWA data in 9961 participants of European ancestry from 5 large cohorts. The study was performed in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium¹⁵ including data from the Age, Gene/Environment Susceptibility–Reykjavik Study (AGES-Reykjavik),^{16,17} the Framingham Heart Study (FHS),^{18–20} the Rotterdam Study I (RS I), and the Rotterdam Study II (RS II).^{15,21} In addition, participants from the Genetic Epidemiology Network of Arteriopathy Study (GENOA) were included.²² Each study received institutional review board approval, and all participants gave written informed consent. These cohorts are described in the online-only Data Supplement.

Measures

CAC Measurement

Different cohorts used different CT scanners to assess CAC, as described in the online-only Data Supplement. Total calcium score, based on the sum of the individual coronary arteries (left main, left anterior descending, circumflex, and right coronary arteries), was quantified by the Agatston method²³ and used as CAC quantity in analyses. Prior studies by others confirm the highly significant association between CAC scores obtained by the different scanners used in the present study.^{24,25} Because the 2 scanning techniques yield similar results and the available evidence for prediction of CVD risk is similar regardless of use of electron beam CT or multidetector CT, consensus clinical guidelines recommending clinical use of CT allow for the use of either electron beam CT or multidetector CT.²⁶

Genotyping and Imputation

Different discovery cohorts used different genotyping platforms: Illumina 370CNV for AGES-Reykjavik, AffymetrixHuman 500K and gene-centric 50K for FHS, Illumina 550K version 3 for RS I and II, and Affymetrix 6.0 for GENOA. Each study imputed genotype data to 2.5 million nonmonomorphic, autosomal SNPs with the use of HapMap haplotypes (CEU population, release 22, build 36) with the imputation software MACH (<http://www.sph.umich.edu/csg/abecasis/MACH/>) and SNPs that passed quality control criteria (described in the online-only Data Supplement). All studies imputed genotype dosage, from 0 to 2, which is the expected number of alleles. Extensive quality control analyses were performed as described in the online-only Data Supplement.

Statistical Analyses

We conducted GWA analyses in each discovery cohort independently. Each study evaluated population substructure in their cohort, primarily with the use of principal components from EIGENSTRAT. (<http://gene.path.med.harvard.edu/~reich/EIGENSTRAT.htm>). To reduce nonnormality, total CAC score was natural log transformed after adding 1 and was adjusted for age and sex variation. Data were analyzed with the use of linear regression in AGES-Reykjavik and RS I and RS II and with linear mixed-effects models to account for family covariance structure in FHS and GENOA with an additive genetic model. Fixed-effects meta-analysis was conducted with the inverse variance weighted approach in METAL (<http://www.sph.umich.edu/csg/abecasis/metal>). Genomic control was applied to each cohort before meta-analysis. The inflation of the association test statistic, λ_{gc} , was as follows: 1.10 for AGES-Reykjavik, 1.00 for FHS, 1.01 for GENOA, 1.04 for RS I, and 1.01 for RS II. Tests for homogeneity of observed effect sizes across cohorts were conducted with the use of METAL. The analyses were repeated with further adjustment for total cholesterol, high-density lipoprotein cholesterol, type 2 diabetes mellitus, hypertension status, current smoking, and use of statins. The analyses were also repeated with exclusion of individuals with a prior MI.

The association of each SNP with CAC score >100 versus <100, a threshold that is an independent predictor of clinical events,⁷ was also investigated in each discovery cohort. Details of the analyses are found in the online-only Data Supplement.

The a priori threshold for genome-wide significance was 5×10^{-8} , and a P value $>5 \times 10^{-8}$ but $<5 \times 10^{-6}$ was considered moderate evidence for association. With a sample size of 9961, a minor allele frequency of 0.25, an additive model with a mean of 0 and SD of 2 [mean and SD similar to residuals from $\log(\text{CAC score} + 1)$ adjusted for age and sex], and α at 5×10^{-8} , we had at least 80% power to detect a β -coefficient of ≥ 0.21 or ≤ -0.21 in analysis of CAC quantity.

Replication

Replication cohorts included 6032 participants from the Family Heart Study, the Multi-Ethnic Study of Atherosclerosis (MESA), and the Amish Family Calcification Study. Details about these cohorts, CAC measures, genotyping, and statistical analysis are provided in the online-only Data Supplement. Meta-analysis was repeated for the replication cohorts alone and then for the discovery and replication cohorts combined.

Association With MI

To test for association with MI, we chose a set of 1150 SNPs associated with CAC quantity, at a P value of $\leq 10^{-3}$, that were nonredundant at a linkage disequilibrium threshold of $r^2 \geq 0.8$ among the HapMap CEU using SNAP.²⁷ The 4 studies, including participants from 3 studies independent of the CHARGE consortium, were the Heart and Vascular Health (HVH) Study, Myocardial Infarction Genetics Consortium (MIGen),¹¹ Gutenberg Heart Study/Atherogene Study (CADomics),²⁸ and CHARGE Consortium.¹⁵ These studies, including 34 508 participants (6811 with MI), are described in Tables IIa and IIb in the online-only Data Supplement. A Bonferroni-adjusted P value of 4.3×10^{-5} was used as the threshold for significance.

Table 1. Baseline Participant Characteristics of the 5 Discovery Cohorts in the Meta-Analysis of Genome-Wide Association Studies of Coronary Artery Calcification

Characteristic	AGES-Reykjavik (n=3177)	FHS (n=3207)	GENOA (n=629)	RS I (n=1720)	RS II (n=1228)
Years of CAC measurements	2002–2006	2002–2005	2000–2004	1997–2000	2003–2006
Scanner type	MDCT, 4 detector	MDCT, 8 detector	EBCT (C-150)	EBCT (C-150)	MDCT, 16 or 64 detector
Age, y	76.4 (5.5)	52.2 (11.6)	58.0 (9.8)	70.7 (5.5)	67.2 (6.7)
Women, %	58	49	58	54	53
Mean CAC score	686 (1011)	131 (432)	191 (487)	505 (969)	312 (712)
Maximum CAC score	8673	5016	4867	12 611	8636
Detectable CAC, %	88.2	41.6	68.2	91.0	80.0
CAC score >100, %	66.7	19.0	28.3	54.4	40.5
CAC score >300, %	48.8	10.3	14.3	36.3	25.7
Hypertension, %	80.1	28.0	71.2	61.9	63.8
Diabetes mellitus, %	11.5	5.2	13.4	13.7	10.2
Current cigarette smoker, %	12.7	12.9	9.5	17.3	14.9
Former cigarette smoker, %	45.3	34.5	34.2	54.7	54.1
Total cholesterol, mmol/L	5.70 (1.17)	5.08 (0.91)	5.18 (0.88)	5.83 (0.96)	5.70 (0.98)
HDL cholesterol, mmol/L	1.61 (0.47)	1.40 (0.44)	1.35 (0.41)	1.40 (0.39)	1.45 (0.38)
Triglycerides, mmol/L	1.22 (0.67)	1.44 (1.01)	1.80 (1.19)	1.54 (0.79)	NA
Body mass index, kg/m ²	27.1 (4.4)	27.7 (5.3)	30.7 (6.3)	27.0 (3.9)	27.8 (4.9)
Waist circumference, cm	101 (12)	97 (16)	101 (16)	94 (11)	94 (12)
Prevalent MI, %	7.5	1.2	0	7.7	4.2

Values are mean (SD) or No. (%). AGES-Reykjavik indicates Age, Gene/Environment Susceptibility Study–Reykjavik; FHS, Framingham Heart Study; GENOA, Genetic Epidemiology Network of Arteriopathy; RS, Rotterdam Study; CAC, coronary artery calcification; HDL, high-density lipoprotein; MI, myocardial infarction; MDCT, multidetector computed tomography; EBCT, electron beam computed tomography; and NA, not available.

Results

CAC Discovery

Characteristics of the 5 discovery cohorts are presented in Table 1. The cohort differences in the distribution of CAC reflect the cohort differences in the age and sex distributions. Figure 1 provides a plot of the meta-analysis *P* values by chromosome position. Forty-eight SNPs located on chromosome 9p21 near *CDKN2B* and *CDKN2A* and 1 SNP on chromosome 6p24 attained genome-wide significance. Table

2 lists the genome-wide significant SNPs as well as those considered moderately associated with CAC quantity. Figure 2A provides a plot of meta-analysis *P* values for SNPs near rs1333049, the variant most strongly associated with CAC quantity in our meta-analysis as well as with CHD in previously reported GWA study results.

The strong association on 6p24 in the *PHACTR1* gene represents a new finding for CAC. The 2 most strongly associated SNPs, rs9349379 and rs2026458, are modestly correlated ($r^2=0.37$) and reside 78 083 bp apart (Table 2). Figure 2B provides a plot of meta-analysis *P* values for SNPs near rs9349379. Figure 1a and 1b in the online-only Data Supplement provides plots of observed $-\log_{10}(P)$ versus expected $-\log_{10}(P)$ with and without the genome-wide significantly associated SNPs in the analysis.

Table 2 presents results for SNPs in 5 other loci—*COL4A1/ COL4A2*, *SERPINI*, *HAL*, *CDC7*, and *IRS2*—moderately associated with CAC quantity. Table III in the online-only Data Supplement shows the results for the most strongly associated SNPs in each individual cohort, and Table IV in the online-only Data Supplement presents results of all SNP associations for CAC quantity with $P < 5 \times 10^{-6}$ from the meta-analysis, the associations when those with prior MI are excluded, and the results of tests for homogeneity. The results are similar when those with prior MI are excluded, and there is no evidence for significant heterogeneity. None of the SNPs in Table 2 or Table IV in the online-only Data Supplement have a $P < 0.01$ for the test for SNP heterogeneity.

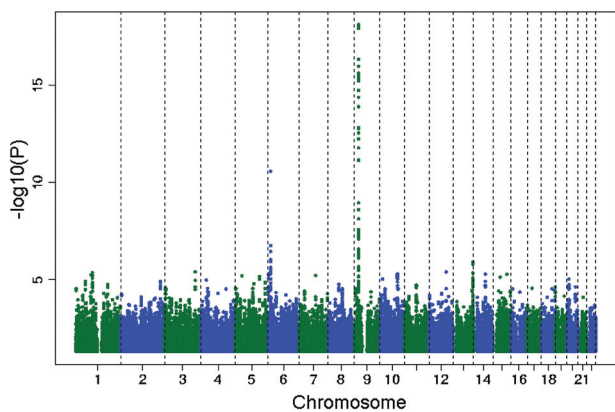


Figure 1. Plot of $-\log_{10}(P)$ for association of single-nucleotide polymorphisms and chromosomal position for all autosomal single-nucleotide polymorphisms analyzed in the age- and sex-adjusted model of coronary artery calcification quantity in the meta-analysis of 5 independent discovery cohorts.

Table 2. Top SNP Association Results for Coronary Artery Calcification Quantity in the Meta-Analysis of 5 Discovery Cohorts

SNP	Chromosome	Position	Closest Reference Gene*	Distance From Nearest Transcript†	β -Coefficient	SE	<i>P</i>	Coded Allele Frequency	Coded Allele	Noncoded Allele
rs1333049‡	9	22 115 503	(<i>CDKN2B</i>)	116 191	0.269	0.030	7.58×10^{-19}	0.47	C	G
rs9349379§	6	13 011 943	<i>PHACTR1</i>	186 125	-0.211	0.032	2.65×10^{-11}	0.59	A	G
rs2026458§	6	12 933 860	<i>PHACTR1</i>	108 042	0.162	0.031	1.78×10^{-7}	0.46	T	C
rs3809346	13	109 758 944	<i>COL4A2</i>	1313	0.154	0.032	1.25×10^{-6}	0.43	A	G
rs6783981	3	169 010 823	<i>SERPINI1</i>	15 225	-0.140	0.030	3.94×10^{-6}	0.51	T	C
rs17676451	12	94 899 916	<i>HAL</i>	8644	-0.170	0.037	4.08×10^{-6}	0.22	A	G
rs6604023	1	91 717 485	(<i>CDC7</i>)	21 546	0.184	0.040	4.29×10^{-6}	0.18	C	G
rs8001186	13	109 174 856	(<i>IRS2</i>)	29 328	-0.148	0.032	4.51×10^{-6}	0.67	T	G

SNP indicates single-nucleotide polymorphism.

*Genes for SNPs that are outside the transcript boundary of the protein-coding gene are shown in parentheses [eg, (*CDKN2B*)].

†Distance in base pairs from nearest start or stop site for transcription.

‡An additional 66 SNPs with $P < 5.0 \times 10^{-7}$ were located near rs1333049 in a 128 041-bp region of chromosome 9 between position 21 987 872 and 22 115 913. See Table III in the online-only Data Supplement.

§SNPs rs9349379 and rs2026458 reside 78 083 bp apart and are only moderately correlated ($r^2 = 0.374$, $D' = 0.715$).

The results for SNP associations were also similar after adjustment for additional CHD risk factors in addition to age and sex (data not shown). In addition, inferences for the CAC threshold of 100 were similar to those for CAC quantity (Table V in the online-only Data Supplement).

CAC Replication and Association With MI

Table 3 presents the results in the replication cohorts for the 8 SNPs with the strongest association in the discovery cohorts. The meta-analysis *P* value for the combined discovery and replication cohorts remained genome-wide significant and lower than the discovery *P* value for rs1333049 and rs9349379 and moderately associated ($P < 1 \times 10^{-6}$) for rs2026458 and rs3809346 (*COL4A1/COL4A2*).

Table 4 presents association of selected SNPs with MI. In addition to known associations near *CDKN2B* and *PHACTR1* with MI, several loci recently reported to be associated with MI,^{8,11} including rs874203 (intronic SNP in *COL4A2*), rs6657811 (nearest *CELSR2* and also near *SORT1*), and rs1720819 (near *MRAS*), had concordant associations (same direction of effect for same allele) with both CAC and MI in our meta-analysis, although the associations were not below a Bonferroni-adjusted *P* value of 4.3×10^{-5} (Table 4). When we examined the association of the same SNPs in the GWA study results from the CARDIoGRAM Consortium,¹⁴ a total of 10 of 16 SNPs were associated with coronary disease after Bonferroni adjustment, including the aforementioned 5 SNPs as well as rs380946 (in *COL4A2*), rs599839 (near *PSRC1*), and rs1199337 (in *MRAS*); for the 10 SNPs, the direction of effect was the same for CAC and coronary disease (Table VI in the online-only Data Supplement).

Twelve of 14 SNPs strongly associated with MI in recent GWA studies were available in the discovery cohorts (Table 5). In addition to the known associations with SNPs in 9p21 and 6p24, 4 other loci—*CXCL12*, *MRAS*, *LPA*, and *CELSR2/PSRC1/SORT1*—were associated with CAC quantity (after Bonferroni adjustment for 12 tests). When we examined the association of the 25 SNPs in the CARDIoGRAM Consortium that are significantly associated with coronary disease

and available in our discovery GWA studies for CAC, a total of 7 of 25 SNPs were associated with CAC after Bonferroni adjustment, with the same direction of effect for CAC and coronary disease for the 7 SNPs (Table VII in the online-only Data Supplement).

Discussion

Our replicated CAC association of rs1333049 on chromosome 9p21 and our novel replicated associations of rs9349379 on 6p24 (the *PHACTR1* locus), as well as the strong evidence for association in the combined discovery and replication cohorts for rs2026458, provide evidence that these loci contribute to variation in coronary atherosclerotic calcified plaque. Although both loci have been associated with MI and now with CAC, the specific causal variants have not been elucidated for either locus, and our GWA study is not able to provide evidence for causal association with nearby genes. SNPs in 9p21 reside >100 000 bp away from and are poorly correlated (ie, in low linkage disequilibrium) with the nearest protein-coding genes, the cell cyclin-dependent kinase inhibitors *CKDN2B* and *CDKN2A*. SNPs associated with both CAC and MI overlap with the upstream portion of a newly annotated antisense noncoding RNA (also known as *ANRIL* or DQ485453).²⁹ The mechanism of association of *ANRIL* is not known; however, the strength and consistency of SNP associations with CAC and MI strongly suggest involvement in formation of calcified atherosclerotic lesions in coronary arteries.

A recent report implicates the 9p21 risk interval in regulation of cardiac *CDKN2A/B* expression, suggesting that this region may affect atherosclerosis progression via vascular cell proliferation.³⁰ Of note, SNPs in this region are also strongly associated with abdominal aortic aneurysms^{31,32} and vascular stiffness³³ but not with carotid intimal-medial wall thickness determined by ultrasonography.^{33,34}

Our newly identified association between CAC and *PHACTR1* SNPs, similar to the association between MI and these SNPs, may represent a novel pathway for development of clinical atherosclerosis. *PHACTR1* in 6p24 is an inhibitor

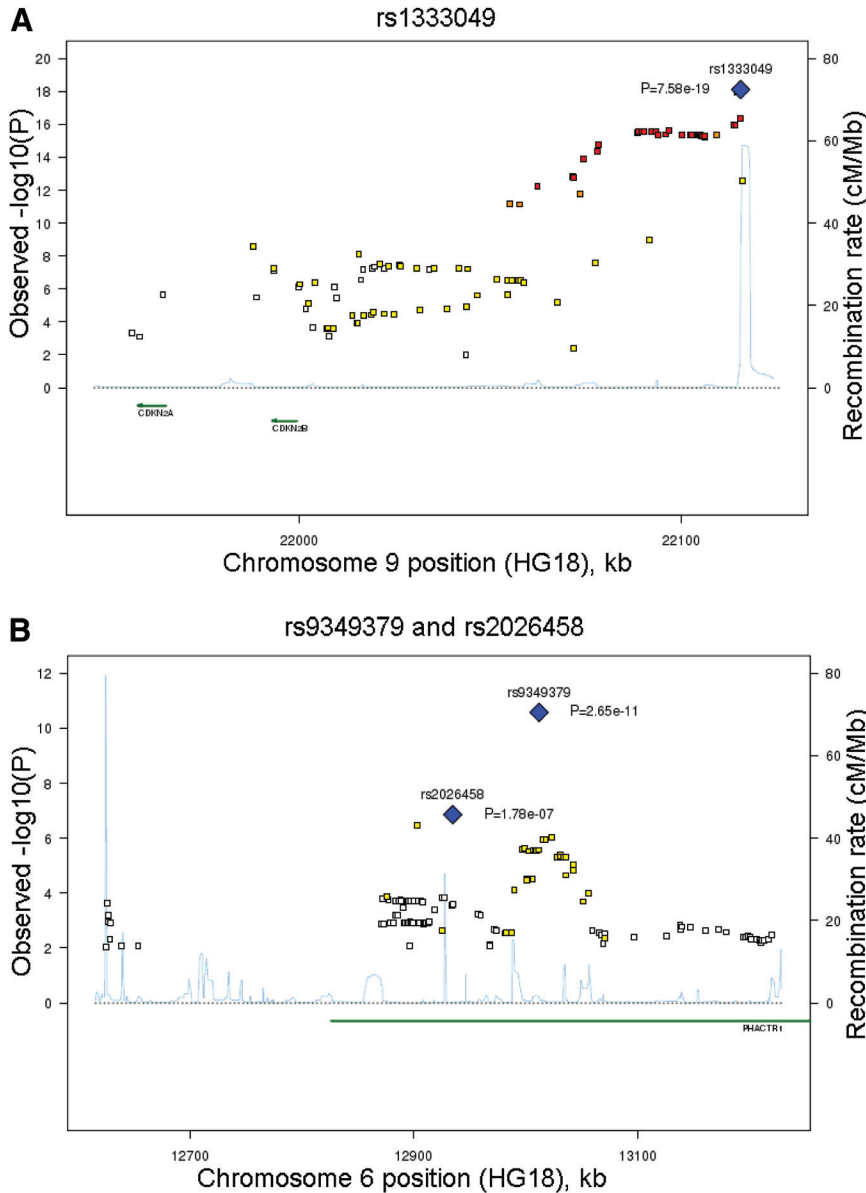


Figure 2. Observed $-\log(P)$ and recombination rates by chromosomal position for all associated single-nucleotide polymorphisms near rs1333049 near *CDKN2B* on 9p21.3 (A) and rs9349379 near *PHACTR1* on 6p24 (B). Results from the genome-wide association analysis of single-nucleotide polymorphisms vs age- and sex-adjusted coronary artery calcification quantity in the meta-analysis of 5 independent discovery cohorts are shown. Association plots were conducted with the use of SNAP.²⁷ Top single-nucleotide polymorphisms of interest and *P* values in each region are indicated (blue diamonds). Color coding indicates the strength of linkage disequilibrium of each single-nucleotide polymorphism with the top single-nucleotide polymorphism in each region: red ($r^2 \geq 0.08$), orange ($r^2 \geq 0.5$), yellow ($r^2 \geq 0.2$), and white with no color ($r^2 < 0.2$).

of protein phosphatase 1, a ubiquitous serine/threonine phosphatase known to regulate multiple cellular processes through dephosphorylation of different substrates.³⁵ The exact mechanism of association of this locus or pathway with CAC and MI is unknown.

In addition, SNPs on 3q22.3 near *MRAS*, on 1p13 near *CELSR2/PSRC1/SORT1*, and in *CXCL12* and *LPA* showed concordant association with CAC and MI, although the CAC associations did not reach genome-wide significance.^{8,11} The M-ras protein encoded by *MRAS* (3q22) belongs to the ras superfamily of GTP-binding proteins and is expressed in the heart and aorta⁸ and may be involved in adhesion signaling, which is important in the atherosclerotic process.³⁶ The locus containing the *CELSR2* (cadherin EGF LAG seven-pass G-type receptor 2) gene also harbors *PSRC1* and *SORT1*. In recent very large GWA studies, as in prior smaller GWA studies, SNPs in this locus were shown to be associated with MI and with

circulating low-density lipoprotein cholesterol levels.³⁷ In previous work, rs646776, which is highly correlated with rs6657811 ($D' = 0.93$), was strongly associated with human liver transcript concentrations of the 3 nearby genes *SORT1*, *CELSR2*, and *PSRC1*.³⁸ In our CAC meta-analysis, rs646776 had the same direction of effect with continuous CAC ($P = 5.3 \times 10^{-4}$) and with CAC > 100 ($P = 1.6 \times 10^{-3}$) as reported for MI (data not shown). A recent investigation, incorporating both mouse and human models, demonstrated that a nearby common noncoding polymorphism at the 1p13 locus (rs12740374, $D' = 1.0$) that serves as a proxy for rs646776 directly implicates hepatic *SORT1* gene expression in the causal mechanism of altered plasma low-density lipoprotein and risk for MI.³⁹ These findings are consistent with a genetic component to the association of low-density lipoprotein cholesterol levels with both CAC and MI.

A new and potentially interesting finding is the concordant association with SNPs in the *COL4A1/COL4A2* locus at

Table 3. Association of Top Coronary Artery Calcification Quantity SNPs in the Replication Panel of 3 Cohorts and Combined With the Discovery Cohorts

SNP	Chromosome	Position	Closest Reference Gene*	Distance From Nearest Transcript†	Coded Allele	Replication		Discovery + Replication‡	
						β -Coefficient (SE)	P	β -Coefficient (SE)	P
rs1333049	9	22 115 503	(<i>CDKN2B</i>)	116 191	C	0.147 (0.026)	1.41×10^{-8}	0.199 (0.020)	3.33×10^{-24} §
rs9349379	6	13 011 943	<i>PHACTR1</i>	186 125	A	-0.187 (0.026)	1.22×10^{-12}	-0.196 (0.020)	3.90×10^{-22} §
rs2026458	6	12 933 860	<i>PHACTR1</i>	108 042	T	0.082 (0.026)	1.73×10^{-3}	0.115 (0.020)	8.10×10^{-9} §
rs3809346	13	109 758 944	<i>COL4A2</i>	1313	A	0.064 (0.027)	1.79×10^{-2}	0.102 (0.021)	8.64×10^{-7}
rs6783981	3	169 010 823	<i>SERPINI1</i>	15 225	T	0.009 (0.026)	7.26×10^{-1}	-0.054 (0.019)	5.85×10^{-3}
rs17676451	12	94 899 916	<i>HAL</i>	8644	A	-0.003 (0.036)	9.24×10^{-1}	-0.084 (0.026)	1.12×10^{-3}
rs6604023	1	91 717 485	(<i>CDC7</i>)	21 546	C	0.051 (0.036)	1.59×10^{-1}	0.111 (0.027)	3.66×10^{-5}
rs8001186	13	109 174 856	(<i>IRS2</i>)	29 328	T	-0.005 (0.028)	8.59×10^{-1}	-0.068 (0.021)	1.37×10^{-3}

*Genes for single-nucleotide polymorphisms (SNPs) that are outside the transcript boundary of the protein-coding gene are shown in parentheses [eg, (*CDKN2B*)].

†Distance in base pairs from nearest start or stop site for transcription.

‡§Combined P values marked with § are below the genome-wide threshold; other combined P values are not significant at a genome-wide level.

13q34. Although not reaching genome-wide significance, the region was moderately associated with both CAC and clinically apparent MI. Importantly, *COL4A1/COL4A2* is a new gene identified in the CARDIoGRAM Consortium.¹⁴ Type IV collagen is a basement-membrane collagen that does not form ordered fibrillar structures but instead forms 4 molecules cross-linked together at the ends. The *COL4A1* and *COL4A2* genes are transcribed divergently by the same promoter. Mutations in *COL4A1* have been implicated in

several rare familial conditions including porencephaly, manifested by cystic cavities of the brain⁴⁰; small arterial vessel disease and cerebral hemorrhage⁴¹; and a syndrome including hereditary angiopathy, nephropathy, and aneurysms.⁴² A recent GWA study for vascular stiffness measures reported a strong replicated association of SNPs in *COL4A1* with arterial stiffness.⁴³ A prior candidate gene case-control study in Japan provided the first unreplicated report of an association of SNPs in *COL4A1* with MI at a modest level of

Table 4. Association of Top Coronary Artery Calcification Quantity SNPs With Myocardial Infarction in a Meta-Analysis of 4 Studies

SNP	Chromosome	Position	Closest Reference Gene*	Distance From Nearest Transcript†	β -Coefficient	SE	P	Coded Allele Frequency	Coded Allele	Noncoded Allele
Associations with $P < 4.3 \times 10^{-5}$										
rs10811647	9	22 055 002	(<i>CDKN2B</i>)	55 690	-0.133	0.026	2.81×10^{-7}	0.56	C	G
rs9349379‡	6	13 011 943	<i>PHACTR1</i>	186 125	-0.122	0.029	2.06×10^{-5}	0.61	A	G
Associations with $4.3 \times 10^{-5} < P < 1 \times 10^{-2}$										
rs874203	13	109 625 575	<i>COL4A1</i>	26 265	0.123	0.032	8.88×10^{-5}	0.37	A	T
rs6657811	1	109 608 806	<i>CELSR2</i>	11 095	0.148	0.043	5.92×10^{-4}	0.88	A	T
rs1720819§	3	139 583 229	<i>MRAS</i>	23 838	-0.125	0.040	1.91×10^{-3}	0.89	T	G
rs3809346	13	109 758 944	<i>COL4A2</i>	1313	0.084	0.027	1.99×10^{-3}	0.44	A	G
rs11984041	7	18 998 460	<i>HDAC9</i>	5049	0.136	0.045	2.67×10^{-3}	0.09	T	C
rs13170330	5	7 498 712	<i>ADCY2</i>	49 370	-0.098	0.033	2.96×10^{-3}	0.24	A	C
rs599839	1	109 623 689	(<i>PSRC1</i>)	12	0.089	0.031	3.86×10^{-3}	0.77	A	G
rs2330341	7	53 173 719	(<i>DKFZp564N2472</i>)	101 608	-0.089	0.032	5.05×10^{-3}	0.75	A	G
rs1199337§	3	139 570 754	<i>MRAS</i>	20 557	0.096	0.035	5.37×10^{-3}	0.16	C	G
rs12146493¶	11	65 303 909	<i>DKFZp761E198</i>	489	-0.074	0.027	7.38×10^{-3}	0.33	A	G
rs163189	5	122 470 526	(<i>PPIQ</i>)	70 202	-0.090	0.034	7.53×10^{-3}	0.83	T	C
rs1021505	5	95 377 193	(<i>ELL2</i>)	53 662	0.080	0.031	8.59×10^{-3}	0.66	A	T
rs2026458‡	6	12 933 860	<i>PHACTR1</i>	108 042	0.071	0.027	8.67×10^{-3}	0.44	T	C
rs12772023	10	108 115 181	(<i>SORCS1</i>)	208 230	0.307	0.119	9.82×10^{-3}	0.02	T	C

*Genes for single-nucleotide polymorphisms (SNPs) that are outside the transcript boundary of the protein-coding gene are shown in parentheses [eg, (*CDKN2B*)].

†Distance in base pairs from nearest start or stop site for transcription.

‡SNPs rs9349379 and rs2026458 reside 78 083 bp apart ($r^2=0.374$, $D'=0.715$).

§SNPs rs1720819 and rs1199337 reside 12 475 bp apart ($r^2=0.483$, $D'=1$).

||Hypothetical protein LOC285877.

¶Hypothetical protein LOC91056.

Table 5. Association Results for Coronary Artery Calcification Quantity for SNPs With Strong Association With Premature-Onset MI

SNPs Associated With Early MI in Past GWA Studies						SNPs Associated With CAC in the Meta-Analysis of 5 Discovery Cohorts CAC (Continuous)			
SNP	Chromosome	Position	Gene(s)	Risk Allele	MI GWA Study <i>P</i>	β -Coefficient	SE	<i>P</i>	
rs4977574	9	220 885 74	<i>CDKN2A, CDKN2B</i>	G	2.7×10^{-44}	0.244	0.030	3.2×10^{-16}	
rs12526453	6	13 035 530	<i>PHACTR1</i>	C	1.3×10^{-9}	0.137	0.033	2.2×10^{-5}	
rs1746048	10	44 096 080	<i>CXCL12</i>	C	7.4×10^{-9}	0.141	0.044	1.5×10^{-3}	
rs9818870	3	139 604 812	<i>MRAS</i>	T	7.4×10^{-13}	0.131	0.042	1.8×10^{-3}	
rs10455872	6	160 930 108	<i>LPA</i>	G	3.4×10^{-15}	0.225	0.075	2.8×10^{-3}	
rs646776	1	109 620 303	<i>CELSR2, PSRC1, SORT1</i>	T	7.9×10^{-12}	0.127	0.037	5.3×10^{-4}	
rs6725887	2	203 454 380	<i>WDR12</i>	C	1.3×10^{-8}	0.077	0.046	0.09	
rs1122608	19	11 024 851	<i>LDLR</i>	G	1.9×10^{-9}	0.058	0.035	0.10	
rs9982601	21	34 521 248	<i>SLC5A3, MRPS6, KCNE2</i>	T	6.4×10^{-11}	0.055	0.044	0.21	
rs11206510	1	55 268 877	<i>PCSK9</i>	T	9.6×10^{-9}	0.020	0.041	0.62	
rs3184504	12	110 368 991	<i>SH2B3</i>	T	8.6×10^{-8}	-0.010	0.031	0.75	
rs2259816	12	119 919 970	<i>HNF1A</i>	T	4.8×10^{-7}	-0.007	0.031	0.81	

SNP indicates single-nucleotide polymorphism; MI, myocardial infarction; GWA, genome-wide association; and CAC, coronary artery calcification.

significance ($P < 0.05$).⁴⁴ Although to date there have been no reports of common variants in the *COL4A1/COL4A2* locus having genome-wide significant associations with CAC, our findings for CAC taken together with findings from the CARDIoGRAM Consortium provide evidence in support of a role for variants in this locus in both coronary atherosclerosis and clinically apparent MI.

Not all individuals with high CAC quantity develop MI, and some cases of MI occur in the absence of high levels of CAC.^{2,6,7} In comparing SNPs for MI with the CAC GWA, we note that there is evidence for association of 1 other SNP reported to be associated with MI, rs1746048¹¹ near *CXCL12* on 10q11.21 ($P = 1.5 \times 10^{-3}$ in our CAC GWA meta-analysis). In contrast, there was no evidence for association with CAC quantity of several other SNPs associated with MI, including SNPs on 2q33.1 near *WDR12*, 19p13.2 near *LDLR*, 21q22.1 near *KCNE2*, 1p32.3 near *PCSK9*, 12q24.1 near *SH2B3*, and 12q24.3 near *HFNIA* (Table 5). rs17465637 on 1q41 near *MIA3* was not assessed in our meta-analysis because it is not localized in HapMap, although there are nonsignificant associations with CAC quantity of SNPs within a 25 000-bp interval (eg, rs17011666; $P = 0.0098$). When we examined the association of the 25 SNPs in the CARDIoGRAM Consortium that are significantly associated with coronary disease and available in our discovery GWA studies for CAC, a total of 7 of 25 SNPs were associated with CAC after Bonferroni adjustment, including SNPs in 9p21 and 6p24 as well as SNPs near *CXCL12*, *SORT1*, *MRAS*, *COL4A1/COL4A2*, and *ADAMTS7*; the remainder of the 25 SNPs were not significantly associated with CAC quantity (Table VII in the online-only Data Supplement). The absence of a genetic association may be due to limited power or confounding factors. However, the fact that some loci are risk factors for both CAC and CAD and others are

not raises the possibility that some but not all genetic mechanisms for CAD are strongly related to the presence and burden of coronary atherosclerosis. It is notable that a recent report of a GWA study for coronary atherosclerosis detected by angiography in selected cases of diseased patients reported novel genome-wide associations with *ADAMTS7* and *ABO* and also reported concordance of association with several other loci including 9p21, *PHACTR1*, *MRAS*, and *SORT1*.⁴⁵

Strengths of the present study include data from large community-based studies, similarity in CAC assessment and quality control measures across cohorts, and similarity in imputation strategies and analytical methods. Limitations include heterogeneity in the cohorts and differences in actual CT scanners. Moreover, we have modest statistical power to detect associations with low-frequency SNPs or poorly imputed SNPs. This limitation in power may have affected our ability to identify additional SNPs associated with both CAC and MI. Examination of larger cohorts with CAC may detect more genome-wide significant associations. In addition, identified associations with genomic regions require further follow-up studies to establish the actual functional variants and elucidate actual mechanisms of association. Finally, we cannot generalize our findings to individuals of non-European ancestry.

The associations of SNPs in 9p21 and 6p24 in the discovery and replication studies for CAC and in the MI studies, as well as the association with SNPs in or near other genes including *MRAS*, *COL4A1/COL4A2*, and *SORT1*, suggest that the common mechanism of some genetic loci underlying MI is development of early, underlying coronary atherosclerosis. Investigations to understand mechanisms underlying the genetic associations with coronary atherosclerosis may ultimately suggest new strategies for prediction, prevention, and treatment of CHD.

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See the online-only Data Supplement.

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CLINICAL PERSPECTIVE

Coronary artery calcification, detected by computed tomography, is a noninvasive measure of coronary atherosclerosis and an independent predictor of myocardial infarction. We conducted a genome-wide association study with a discovery sample of nearly 10 000 participants of European origin and a replication sample of >6000 participants of European origin. We report strong evidence for genetic variants in 9p21 near the *CDKN2B* and *CDKN2A* genes and in 6p24 within the *PHACTR1* gene. Additionally, we found evidence for concordance of single-nucleotide polymorphism associations with both coronary artery calcification and some but not all other loci known to be associated with myocardial infarction, including 3q22 (*MRAS* gene), 13q34 (*COL4A1/COL4A2* genes), and 1p13 (*SORT1* gene). These findings reinforce the important role of variation at multiple genes in the pathogenesis of coronary atherosclerosis. Although the functional mechanism of many of these genetic associations remains to be determined, our comprehensive study lays the groundwork for future studies to determine the role of several genes in treatment, prediction, and prevention of coronary atherosclerosis and resulting myocardial infarction and other clinically apparent forms of coronary heart disease.