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## Genome-wide Association Study for Coronary Artery Calcification with Follow-up in Myocardial Infarction

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## Abstract

**Background**—Coronary artery calcification (CAC) detected by computed tomography is a non-invasive measure of coronary atherosclerosis, that underlies most cases of myocardial infarction (MI). We aimed 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 carried out in 9,961 men and women from five independent community-based cohorts, with replication in three additional independent cohorts (n=6,032). 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 with 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.

### Keywords

cardiac computed tomography; coronary artery calcification; coronary atherosclerosis; genome-wide association studies; myocardial infarction

## Introduction

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.<sup>1</sup> Extent of coronary atherosclerosis may be determined with non-invasive, high resolution computed tomography (CT) to measure coronary artery calcification (CAC).<sup>2</sup> CAC quantity is heritable<sup>3</sup>, significantly higher in persons with a parental history of CHD<sup>4</sup>, correlates with increased burden of subclinical coronary plaque<sup>5</sup>, and predicts incident CHD in multiple ethnic populations after adjustment for other traditional CHD risk factors.<sup>2;6;7</sup>

Genome-wide association (GWA) studies identified common genetic variation influencing risk of MI, including a strongly replicated association on chromosome 9p21,<sup>8–13</sup> as well as strong associations with *PHACTR1*<sup>11</sup> and over nine other loci.<sup>8;11</sup> Recently, a large meta-analysis of 14 GWAS 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.<sup>14</sup> 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 9,961 participants of European ancestry from five large cohorts. The study was performed in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium<sup>15</sup> including data from the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik),<sup>16;17</sup> the Framingham Heart Study (FHS),<sup>18–20</sup> the Rotterdam Study I (RS I), and Rotterdam Study II (RS II).<sup>15;21</sup> In addition, participants from the Genetic Epidemiology Network of Arteriopathy Study (GENOA) were included.<sup>22</sup> Each study received institutional review board approval and all participants gave written informed consent. These cohorts are described in the online-only Supplemental Materials.

### Measures

**CAC Measurement**—Different cohorts used different CT scanners to assess CAC as described in the online-only Supplemental Materials. Total calcium score, based on the sum of the individual coronary arteries (left main, left anterior descending, circumflex, and right coronary arteries), was quantified using the Agatston method<sup>23</sup> 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.<sup>24;25</sup> Because the two scanning techniques yield similar results and the available evidence for prediction of CVD risk is similar regardless of use of electron beam CT or multi-detector CT, consensus clinical guidelines recommending clinical use of CT allows for the use of either electron beam CT or multi-detector CT.<sup>26</sup>

**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 non-monomorphic, autosomal single nucleotide polymorphisms (SNPs) using 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 Supplemental Materials). 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 Supplemental Materials.

### Statistical Analyses

We conducted a GWA analyses in each discovery cohort independently. Each study evaluated population substructure in their cohort, primarily using principal components from EIGENSTRAT (<http://genepath.med.harvard.edu/~reich/EIGENSTRAT.htm>). To reduce non-normality, total CAC score was natural log transformed after adding one and adjusted for age and sex variation. Data were analyzed using linear regression in AGES-Reykjavik, RS I and RS II and 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 using the inverse variance weighted approach in METAL (<http://www.sph.umich.edu/csg/abecasis/metal>). Genomic control was applied to each cohort prior to meta-analysis. The inflation of the association test statistic,  $\lambda_{gc}$ , was: 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 using METAL. The analyses were repeated with further adjustment for total cholesterol, HDL-cholesterol, type II diabetes, hypertension status, current smoking and use of statins. The analyses were also repeated excluding 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,<sup>7</sup> was also investigated in each discovery cohort. Details of the analyses are found in the online-only Supplemental Material.

The *a priori* threshold for genome-wide significance was  $5 \times 10^{-8}$ , and a P-value  $> 5 \times 10^{-8}$  but  $< 5 \times 10^{-6}$  was considered moderate evidence for association. With a sample size of 9,961, a minor allele frequency of 0.25, an additive model with a mean of 0 and standard deviation of 2 (mean and standard deviation similar to residuals from  $\log(\text{CAC score} + 1)$  adjusted for age and sex), and alpha at  $5 \times 10^{-8}$ , we had at least 80% power to detect a beta-coefficient of 0.21 or  $-0.21$  in analysis of CAC quantity.

### Replication

Replication cohorts included 6,032 participants from the Family Heart Study, the Multi-Ethnic Study of Atherosclerosis (MESA), and the Amish Family Calcification Study. Details about these cohorts, the CAC measures, the genotyping, and statistical analysis are provided in the online-only Supplemental Material. 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 less than or equal to  $10^{-3}$ , that were non-redundant at a linkage disequilibrium (LD) threshold of  $r^2 \leq 0.8$  among the HapMap CEU using SNAP.<sup>27</sup> The four studies, including participants from three studies independent of the CHARGE consortium, were: Heart and Vascular Health (HVH) Study, the Myocardial Infarction Genetics Consortium (MIGen),<sup>11</sup> the Gutenberg Heart Study/Atherogene Study (CADomics),<sup>28</sup> and the CHARGE Consortium.<sup>15</sup> These studies, including 34,508 participants (6,811 with MI), are described in Supplemental Tables S2a and S2b. A Bonferroni adjusted P-value of  $4.3 \times 10^{-5}$  was used as the threshold for significance.

## Results

### CAC Discovery

Characteristics of the five 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 one 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 reports.

The strong association on 6p24 in the *PHACTR1* gene represents a new finding for CAC. The two 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. Supplemental Figures 1a and 1b provide 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 five other loci— *COL4A1/COL4A2*, *SERPINI*, *HAL*, *CDC7* and *IRS2*—moderately associated with CAC quantity. Supplemental Table S3 shows the results for the most strongly associated SNPs in each individual cohort while Supplemental Table S4 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 Tables 2 or S4 have a  $p < 0.01$  for the test for SNP heterogeneity. The results for SNP associations were also similar after adjustment for additional CHD risk factors in addition to age and sex (data not shown).

The results 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 (Supplemental Table S5).

### CAC Replication and Association with MI

Table 3 presents the results in the replication cohorts for the eight 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,<sup>8;11</sup> 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  $4.3 \times 10^{-5}$  (Table 4). When we examined the association of the same SNPs in the GWAS results from the CARDIoGRAM Consortium,<sup>14</sup> a total of ten out of 16 SNPs were associated with coronary disease, after Bonferroni adjustment, including the five SNPs above as well as rs380946 (in *COL4A2*), rs599839 (near *PSRC1*), and rs1199337 (in *MRAS*); for the ten SNPs, the direction of effect was the same for CAC and coronary disease (Supplementary Table 6).

Twelve of 14 SNPs strongly associated with MI in recent GWAS were available in the discovery cohorts (Table 5). In addition to the known associations with SNPs in 9p21 and 6p24, four 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 the our discovery GWAS for CAC, a total of seven out of 25 SNPs were associated with CAC after Bonferroni adjustment, with the same direction of effect for CAC and coronary disease for the seven SNPs (Supplementary Table 7).

## Discussion

Our replicated CAC association of rs1333049 on chromosome 9p21 and our novel replicated associations of rs93493979 on 6p24 (the *PHACTR1* locus), as well as the strong evidence for association in the combined discovery and replication cohorts for rs2026458, provides evidence 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 over 100,000 bp away from, and are poorly correlated (ie., in low LD) with the nearest protein-coding genes, the cell cyclin-dependent kinase inhibitors *CDKN2B* 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).<sup>29</sup> 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 this region may affect atherosclerosis progression via vascular cell proliferation.<sup>30</sup> Of note, SNPs in this region are also strongly associated with abdominal aortic aneurysms<sup>31;32</sup> and vascular stiffness,<sup>33</sup> but not with carotid intimal medial wall thickness determined by ultrasonography.<sup>33;34</sup>

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 of protein phosphatase 1, a ubiquitous serine/threonine phosphatase known to regulate multiple cellular processes through dephosphorylation of different substrates.<sup>35</sup> 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.<sup>8;11</sup> The M-ras protein encoded by *MRAS* (3q22) belongs to the ras superfamily of GTP-binding proteins and is expressed in the heart and aorta,<sup>8</sup> and may be involved in adhesion signaling, which is important in the atherosclerotic process.<sup>36</sup> 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 study, as in prior smaller GWA studies, SNPs in this locus were shown to be associated with MI and with circulating LDL-cholesterol levels.<sup>37</sup> In previous work, rs646776, which is highly correlated with rs6657811 ( $D' = 0.93$ ), was strongly associated with human liver transcript concentrations of the three nearby genes, *SORT1*, *CELSR2*, and *PSRC1*.<sup>38</sup> 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,  $D'=1.0$ ) serves as a proxy for rs646776 directly implicates hepatic SORT1 gene expression in the causal mechanism of altered plasma LDL and risk for MI.<sup>39</sup> These findings are consistent with a genetic component to the association of LDL-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 13q34. While 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.<sup>14</sup> Type IV collagen is a basement-membrane collagen that does not form ordered fibrillar structures but instead forms 4 molecules crosslinked 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 brain;<sup>40</sup> small arterial vessel disease and cerebral hemorrhage;<sup>41</sup> and a syndrome including hereditary angiopathy, nephropathy, and aneurysms.<sup>42</sup> A recent GWA study for vascular stiffness measures reported a strong replicated association of SNPs in *COL4A1* with arterial stiffness.<sup>43</sup> 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 significance ( $P<0.05$ ).<sup>44</sup> While 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.<sup>2;6;7</sup> In comparing SNPs for MI with the CAC GWA, we note there is evidence for association of one other SNP reported to be associated with MI, rs1746048<sup>11</sup> 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 *HFN1A* (Table 5). rs17465637 on 1q41 near *MIA3* was not assessed in our meta-analysis as it is not localized in HapMap, although there are non-significant associations with CAC quantity of SNPs within a 25,000 bp interval (e.g., 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 GWAS for CAC, a total of seven out 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 (Supplementary Table 7). 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 GWAS for coronary atherosclerosis detected by angiography in selected cases of diseased patients reported novel GWA associations with *ADAMTS7* and *ABO* and also reported concordance of association with several other loci including 9p21, *PHACTR1*, *MRAS* and *SORT1*.<sup>45</sup>

Strengths of the present study include data from large community-based studies, similarity in CAC assessment and quality control measures across cohorts, as well as 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 impacted 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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

See Separate Supplemental Materials.

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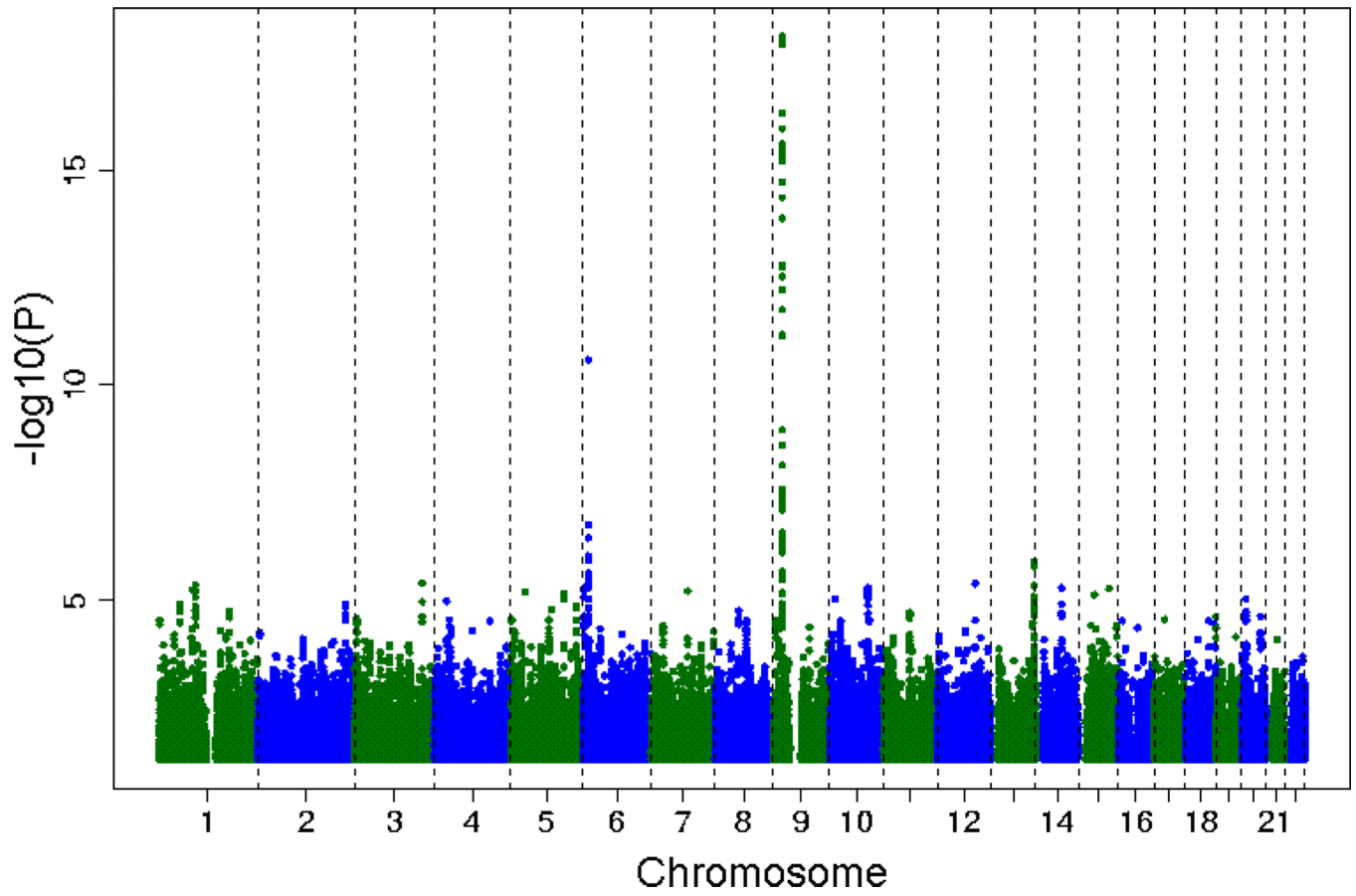
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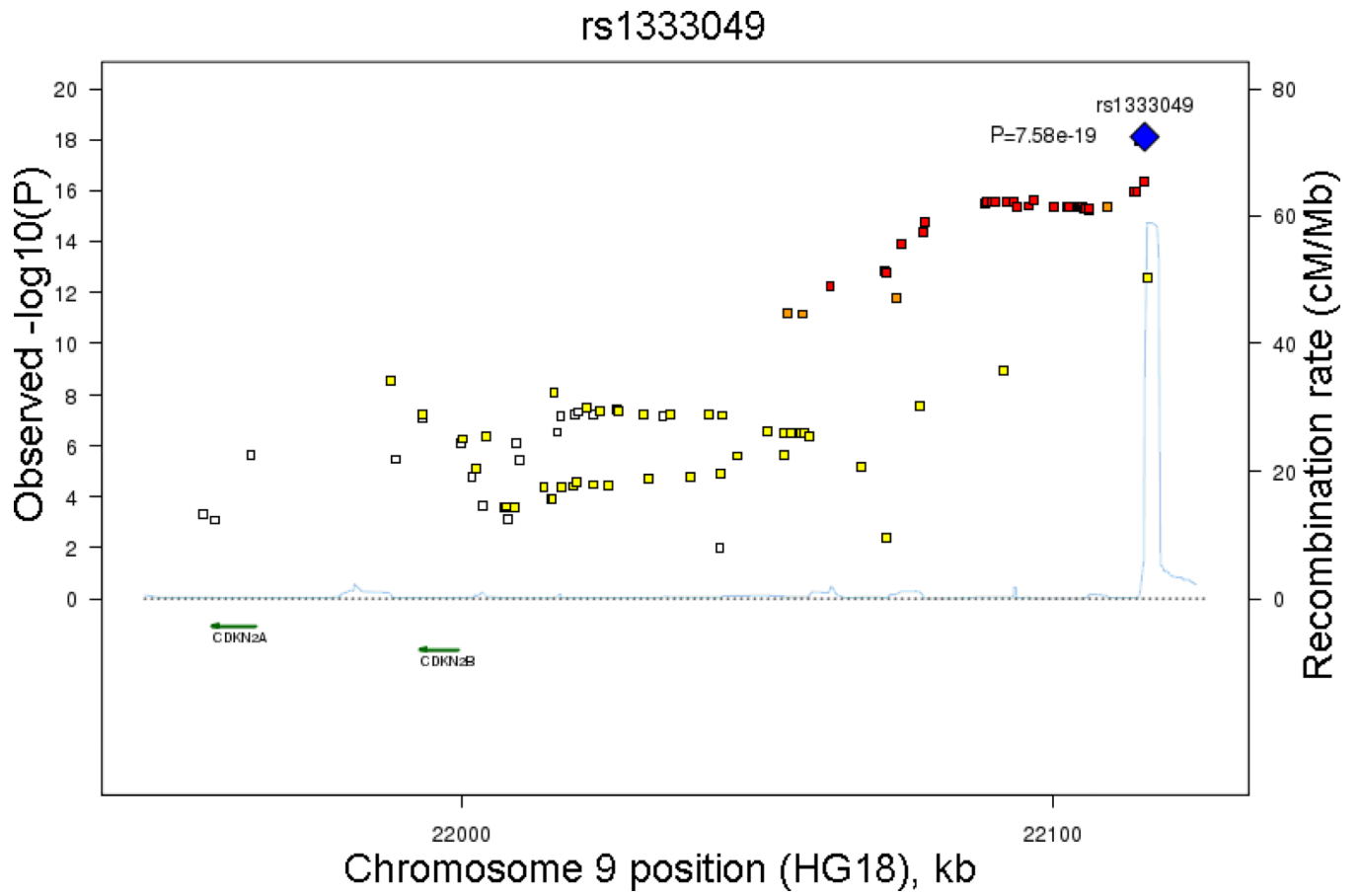
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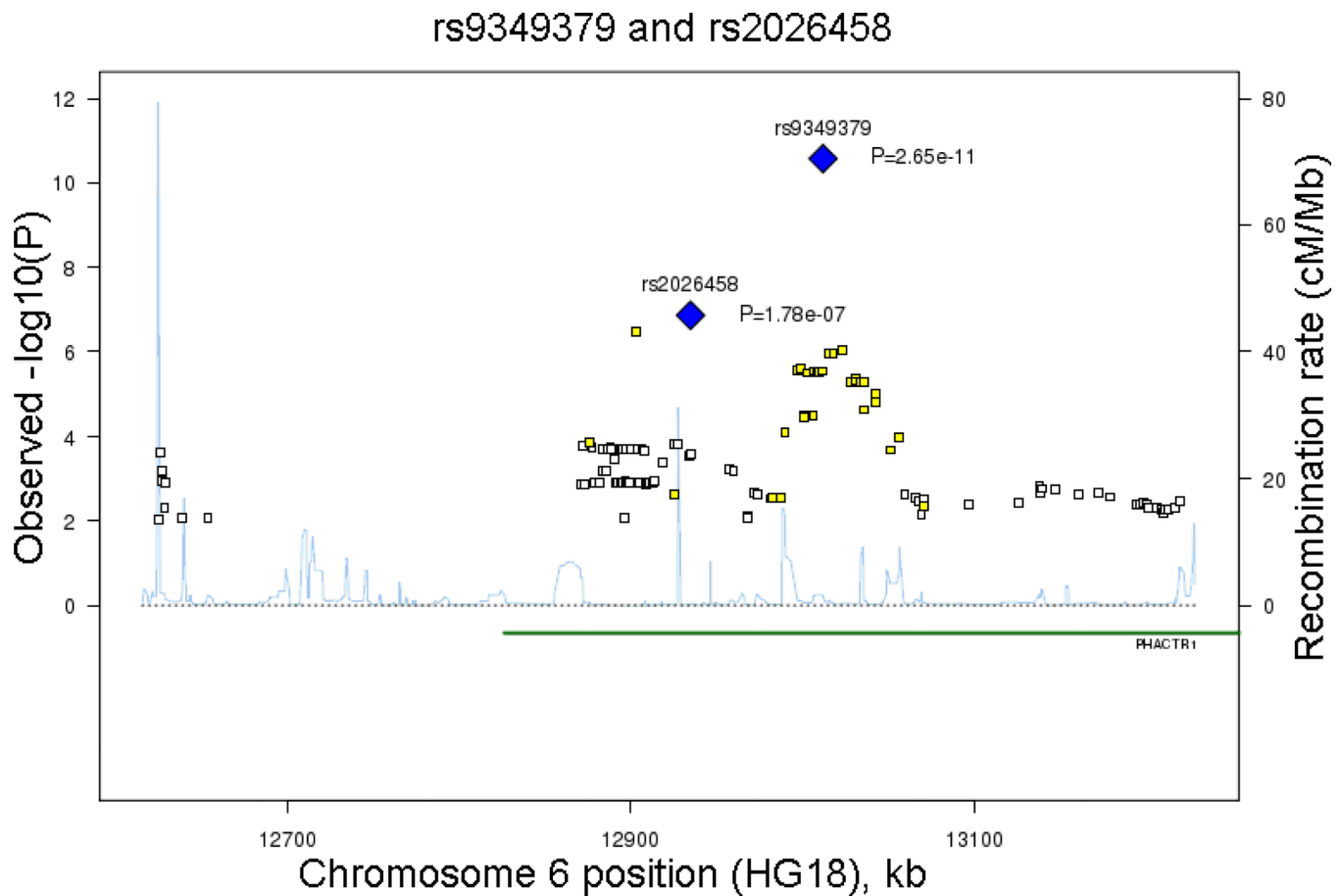
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**Figure 1.** Plot of  $-\log_{10}(P)$  for association of SNPs and chromosomal position for all autosomal SNPs analyzed in the age and sex-adjusted model of CAC quantity in the meta-analysis of five independent discovery cohorts.





**Figure 2.**

Observed  $-\log(P)$  and recombination rates by chromosomal position for all associated SNPs nearby (a) rs1333049 near CDKN2B on 9p21.3 and (b) rs9349379 near PHACTR on 6p24. Results from the genome-wide association analysis of SNPs versus age and sex-adjusted CAC quantity in the meta-analysis of five independent discovery cohorts. Association plots were conducted using SNAP.[27] Top SNPs of interest and P values in each region are indicated (blue diamonds). Color coding indicates the strength of LD of each SNP with the top SNP in each region: red ( $r^2 < 0.08$ ), orange ( $r^2 0.08-0.2$ ), yellow ( $r^2 0.2-0.5$ ), white with no color ( $r^2 < 0.2$ ).



Baseline participant characteristics of the five discovery cohorts in the meta-analysis of genome-wide association studies of coronary artery calcification

**Table 1**

Characteristic Mean (SD) or N (Percent)	AGES-Reykjavik N=3,177	FHS N=3,207	GENOA N=629	RS I N=1,720	RS II N=1,228
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, years	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	8,673	5,016	4,867	12,611	8,636
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, %	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)
Triglyceride, mmol/L	1.22 (0.67)	1.44 (1.01)	1.80 (1.19)	1.54 (0.79)	NA
BMI, kg/m <sup>2</sup>	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

Abbreviations: CAC=coronary artery calcification; AGES-Reykjavik=Age, Gene/Environment Susceptibility Study; BMI=body mass index; FHS=Framingham Heart Study; HDL=high density lipoprotein; GENOA=Genetic Epidemiology Network of Arteriopathy; RS=The Rotterdam Study; MDCT=multidetector computed tomography; EBCT=electron beam computed tomography; MI=myocardial infarction; NA=not available.

Table 2

Top SNP association results for coronary artery calcification quantity in the meta-analysis of five discovery cohorts

SNP	Chr	Position	Closest Reference Gene <sup>†</sup>	Distance from Nearest Transcript <sup>††</sup>	Beta Coefficient	SE	P	Coded Allele Frequency	Coded allele	Non-coded allele
<b>Associations with <math>P &lt; 5.0 \times 10^{-8}</math></b>										
rs1333049 <sup>*</sup>	9	22,115,503	( <i>CDKN2B</i> )	116,191	0.269	0.030	$7.58 \times 10^{-19}$	0.47	C	G
rs9349379 <sup>**</sup>	6	13,011,943	<i>PHACTR1</i>	186,125	-0.211	0.032	$2.65 \times 10^{-11}$	0.59	A	G
<b>Associations with <math>5.0 \times 10^{-8} &lt; P &lt; 5.0 \times 10^{-6}</math></b>										
rs2026458 <sup>**</sup>	6	12,933,860	<i>PHACTR1</i>	108,042	0.162	0.031	$1.78 \times 10^{-7}$	0.46	T	C
rs3809346	13	109,758,944	<i>COL4A2</i>	1,313	0.154	0.032	$1.25 \times 10^{-6}$	0.43	A	G
rs6783981	3	169,010,823	<i>SERPINI1</i>	15,225	-0.140	0.030	$3.94 \times 10^{-6}$	0.51	T	C
rs17676451	12	94,899,916	<i>HAL</i>	8,644	-0.170	0.037	$4.08 \times 10^{-6}$	0.22	A	G
rs6604023	1	91,717,485	( <i>CDC7</i> )	21,546	0.184	0.040	$4.29 \times 10^{-6}$	0.18	C	G
rs8001186	13	109,174,856	( <i>IRS2</i> )	29,328	-0.148	0.032	$4.51 \times 10^{-6}$	0.67	T	G

<sup>\*</sup> An additional 66 SNP's with  $P < 5.0E-07$  were located near rs1333049 in a 128,041 bp region of chromosome 9 between position 21,987,872 and 22,115,913. See Supplemental Table S3.

<sup>\*\*</sup> SNP rs9349379 and rs2026458 reside 78,083 bp apart and are only moderately correlated ( $r^2$  0.374, D: 0.715).

<sup>†</sup> Genes for SNPs that are outside the transcript boundary of the protein-coding gene are shown in parentheses [e.g., (*CDKN2B*)].

<sup>††</sup> Distance in base pairs from nearest start or stop site for transcription.

Table 3

Association of top coronary artery calcification quantity SNPs in replication panel of three cohorts and combined with the discovery cohorts

SNP	Chr	Position	Closest Reference Gene <sup>†</sup>	Distance from Nearest Transcript <sup>††</sup>	Coded allele	Replication		Discovery + Replication <sup>*</sup>	
						Beta (SE)	P	Beta (SE)	P
rs1333049	9	22,115,503	( <i>CDKN2B</i> )	116,191	C	0.147 (0.026)	$1.41 \times 10^{-8}$	0.199 (0.020)	$3.33 \times 10^{-24}$
rs9349379	6	13,011,943	<i>PHACTR1</i>	186,125	A	-0.187 (0.026)	$1.22 \times 10^{-12}$	-0.196 (0.020)	$3.90 \times 10^{-22}$
rs2026458	6	12,933,860	<i>PHACTR1</i>	108,042	T	0.082 (0.026)	$1.73 \times 10^{-3}$	0.115 (0.020)	$8.10 \times 10^{-9}$
rs3809346	13	109,758,944	<i>COL4A2</i>	1,313	A	0.064 (0.027)	$1.79 \times 10^{-2}$	0.102 (0.021)	$8.64 \times 10^{-7}$
rs6783981	3	169,010,823	<i>SERPINI1</i>	15,225	T	0.009 (0.026)	$7.26 \times 10^{-1}$	-0.054 (0.019)	$5.85 \times 10^{-3}$
rs17676451	12	94,899,916	<i>HAL</i>	8,644	A	-0.003 (0.036)	$9.24 \times 10^{-1}$	-0.084 (0.026)	$1.12 \times 10^{-3}$
rs6604023	1	91,717,485	( <i>CDC7</i> )	21,546	C	0.051 (0.036)	$1.59 \times 10^{-1}$	0.111 (0.027)	$3.66 \times 10^{-5}$
rs8001186	13	109,174,856	( <i>IRS2</i> )	29,328	T	-0.005 (0.028)	$8.59 \times 10^{-1}$	-0.068 (0.021)	$1.37 \times 10^{-3}$

<sup>†</sup> Genes for SNPs that are outside the transcript boundary of the protein-coding gene are shown in parentheses [e.g., (*CDKN2B*)].

<sup>††</sup> Distance in base pairs from nearest start or stop site for transcription.

<sup>\*</sup> Combined p-values in **bold** are below the genome-wide threshold; other combined p-values are not significant at a genome-wide level.

**Table 4**

Association of top coronary artery calcification quantity SNPs with myocardial infarction in a meta-analysis of four studies

SNP	Chr	Position	Closest Reference Gene <sup>†</sup>	Distance from Nearest Transcript <sup>††</sup>	Beta Coefficient	SE	P	Coded Allele Frequency	Coded Allele	Non-coded allele
<b>Associations with <math>P &lt; 4.3 \times 10^{-5}</math></b>										
rs10811647	9	22,055,002	( <i>CDKN2B</i> )	55,690	-0.133	0.026	$2.81 \times 10^{-7}$	0.56	C	G
rs9349379*	6	13,011,943	<i>PHACTR1</i>	186,125	-0.122	0.029	$2.06 \times 10^{-5}$	0.61	A	G
<b>Associations with <math>4.3 \times 10^{-5} &lt; P &lt; 1 \times 10^{-2}</math></b>										
rs874203	13	109,625,575	<i>COL4A1</i>	26,265	0.123	0.032	$8.88 \times 10^{-5}$	0.37	A	T
rs6657811	1	109,608,806	<i>CELSR2</i>	11,095	0.148	0.043	$5.92 \times 10^{-4}$	0.88	A	T
rs1720819**	3	139,583,229	<i>MRAS</i>	23,838	-0.125	0.040	$1.91 \times 10^{-3}$	0.89	T	G
rs3809346	13	109,758,944	<i>COL4A2</i>	1,313	0.084	0.027	$1.99 \times 10^{-3}$	0.44	A	G
rs11984041	7	18,998,460	<i>HDAC9</i>	5,049	0.136	0.045	$2.67 \times 10^{-3}$	0.09	T	C
rs13170330	5	7,498,712	<i>ADCY2</i>	49,370	-0.098	0.033	$2.96 \times 10^{-3}$	0.24	A	C
rs599839	1	109,623,689	( <i>PSRC1</i> )	12	0.089	0.031	$3.86 \times 10^{-3}$	0.77	A	G
rs2330341 <sup>‡</sup>	7	53,173,719	( <i>DKFZp564N2472</i> )	101,608	-0.089	0.032	$5.05 \times 10^{-3}$	0.75	A	G
rs1199337**	3	139,570,754	<i>MRAS</i>	20,557	0.096	0.035	$5.37 \times 10^{-3}$	0.16	C	G
rs12146493 <sup>++</sup>	11	65,303,909	<i>DKFZp761E198</i>	489	-0.074	0.027	$7.38 \times 10^{-3}$	0.33	A	G
rs163189	5	122,470,526	( <i>PPIC</i> )	70,202	-0.090	0.034	$7.53 \times 10^{-3}$	0.83	T	C
rs1021505	5	95,377,193	( <i>ELL2</i> )	53,662	0.080	0.031	$8.59 \times 10^{-3}$	0.66	A	T
rs2026458*	6	12,933,860	<i>PHACTR1</i>	108,042	0.071	0.027	$8.67 \times 10^{-3}$	0.44	T	C
rs12772023	10	108,115,181	( <i>SORCS1</i> )	208,230	0.307	0.119	$9.82 \times 10^{-3}$	0.02	T	C

\* SNP rs9349379 and rs2026458 reside 78,083 bp apart, ( $r^2$  0.374, D' 0.715).

\*\* SNP rs1720819 and rs1199337 reside 12,475 bp apart, ( $r^2$  0.483, D' 1).

<sup>†</sup> Genes for SNPs that are outside the transcript boundary of the protein-coding gene are shown in parentheses [e.g., (*CDKN2B*)].

<sup>††</sup> Distance in base pairs from nearest start or stop site for transcription.

<sup>‡</sup> Hypothetical protein LOC285877

<sup>†\*</sup>Hypothetical protein LOC91056

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Table 5

Association results for coronary artery calcification quantity for SNPs with strong association with premature onset myocardial infarction.

SNPs associated with early MI in past GWAS				SNPs associated with CAC in the meta-analysis of five discovery cohorts				
SNP	Chr	Position	Gene(s)	Risk Allele	MI GWAS P	Beta	SE	P
rs4977574	9	220,885,74	<i>CDKN2A, CDKN2B</i>	G	$2.7 \times 10^{-44}$	0.244	0.030	$3.2 \times 10^{-16}$
rs12526453	6	13,035,530	<i>PHACTR1</i>	C	$1.3 \times 10^{-9}$	0.137	0.033	$2.2 \times 10^{-5}$
rs1746048	10	44,096,080	<i>CXCL12</i>	C	$7.4 \times 10^{-9}$	0.141	0.044	$1.5 \times 10^{-3}$
rs9818870	3	139,604,812	<i>MRAS</i>	T	$7.4 \times 10^{-13}$	0.131	0.042	$1.8 \times 10^{-3}$
rs10455872	6	160,930,108	<i>LPA</i>	G	$3.4 \times 10^{-15}$	0.225	0.075	$2.8 \times 10^{-3}$
rs646776	1	109,620,303	<i>CELSR2, PSRC1, SORT1</i>	T	$7.9 \times 10^{-12}$	0.127	0.037	$5.3 \times 10^{-4}$
rs6725887	2	203,454,380	<i>WDR12</i>	C	$1.3 \times 10^{-8}$	0.077	0.046	0.09
rs1122608	19	11,024,851	<i>LDLR</i>	G	$1.9 \times 10^{-9}$	0.058	0.035	0.10
rs9982601	21	34,521,248	<i>SLC5A3, MRP56, KCNE2</i>	T	$6.4 \times 10^{-11}$	0.055	0.044	0.21
rs11206510	1	55,268,877	<i>PCSK9</i>	T	$9.6 \times 10^{-9}$	0.020	0.041	0.62
rs3184504	12	110,368,991	<i>SH2B3</i>	T	$8.6 \times 10^{-8}$	-0.010	0.031	0.75
rs2259816	12	119,919,970	<i>HNF1A</i>	T	$4.8 \times 10^{-7}$	-0.007	0.031	0.81

Abbreviations: MI=myocardial infarction; GWAS=genome-wide association study; Beta=beta coefficient; SE=standard error; CAC=coronary artery calcification; NA=not available.