Serum Lipid Levels, Body Mass Index, and Their Role in Coronary Artery Calcification

A Polygenic Analysis

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Background—Coronary artery calcification (CAC) is widely regarded as a cumulative lifetime measure of atherosclerosis, but it remains unclear what is the relationship between calcification and traditional risk factors for coronary artery disease (CAD) and myocardial infarction (MI). This study characterizes the genetic architecture of CAC by evaluating the overall impact of common alleles associated with CAD/MI and its traditional risk factors.

Methods and Results—On the basis of summary-association results from the CARDIoGRAMplusC4D study of CAD/MI, we calculated polygenic risk scores in 2599 participants of the Dutch and Belgian Lung Cancer Screening (NELSON) trial, in whom quantitative CAC levels (Agatston scores) were determined from chest computerized tomographic imaging data. The most significant polygenic model explained ≈14% of the observed CAC variance ($P=1.6 \times 10^{-15}$), which points to a residual effect because of many as yet unknown loci that overlap between CAD/MI and CAC. In addition, we constructed risk scores based on published single-nucleotide polymorphism associations for traditional cardiovascular risk factors and tested these scores for association with CAC. We found nominally significant associations for genetic risk scores of low-density lipoprotein-cholesterol, total cholesterol, and body mass index, which were successfully replicated in 2182 individuals of the Heinz Nixdorf Recall Study.

Conclusions—Pervasive polygenic sharing between CAC and CAD/MI suggests that a substantial fraction of the heritable risk for CAD/MI is mediated through arterial calcification. We also provide evidence that genetic variants associated with serum lipid levels and body mass index influence CAC levels. (Circ Cardiovasc Genet. 2015;8:327-333. DOI: 10.1161/CIRCGENETICS.114.000496.)

Key Words: coronary artery disease • genome-wide association study • myocardial infarction

Over the past few years, several collaborative genome-wide association studies (GWAS) have identified many loci associated with coronary artery disease (CAD) and myocardial infarction (MI).1,2 The CARDIoGRAMplusC4D Consortium reported 46 loci at genome-wide significance in an association analysis of as many as 64,000 cases and 131,000 controls, where the lead variants at these loci collectively explain 10.6% of CAD heritability.2 In parallel, several studies have also focused on the genetic basis of known CAD risk factors, including circulating lipid levels,3,4 hypertension,5–7 type 2 diabetes mellitus (T2D),8 body mass index (BMI),9,10 and arterial calcification,11–13 altogether pinpointing hundreds of loci across the genome. An important question now is to what extent the identified genetic variants overlap across traits and which biological mechanisms are shared.

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In this study, we focus on coronary artery calcification (CAC), a strong and independent risk factor for cardiovascular events.\textsuperscript{14–18} To date, only 2 loci (CDKN2A/CDKN2B and \textit{PHACTR1}) have been consistently associated with CAC at genome-wide significance in 3 independent studies, and these loci are also linked to CAD and MI risk.\textsuperscript{2,11–13} Going beyond these 2 \textit{bona fide} loci, we and others have demonstrated a significant concordance in direction of effect for 25 single-nucleotide polymorphisms (SNPs) associated with CAD/MI\textsuperscript{11,13} (identified by the initial CARDIoGRAM Study\textsuperscript{1}). It was subsequently suggested that there is a strong causal overlap between vascular calcification and cardiovascular events, even though the associated SNPs discovered to date explain only a modest fraction of the heritability of CAC, CAD, or MI.\textsuperscript{19}

Here, we test the hypothesis that CAD/MI SNPs associated below genome-wide significance influence the degree of calcification in the coronary arteries beyond the overlapping associations already identified. Because power is limited to detect small effects for single SNPs, we evaluated the collective—polygenic—impact of SNPs published by the CARDIoGRAMplusC4D consortium\textsuperscript{11} on CAC. We also investigated whether SNPs associated with serum lipid levels, T1D, T2D, height, BMI, and blood pressure have a measurable influence on CAC levels.

**Materials and Methods**

**Cohort Characteristics**

Details on sample collection, genotyping, and measurement of phenotypes were described elsewhere.\textsuperscript{13} In brief, the Dutch and Belgian Lung Cancer Screening trial (NELSON trial) was designed to study the early detection of lung cancer in an at-risk population. The study was approved by the Ministry of Health of the Netherlands, and written informed consent was obtained from all participants. Low-dose, non-ECG synchronized, non–contrast-enhanced baseline chest computed tomographies were available for all participants. We used a computer-aided detection system for automatic identification and quantification of CAC.\textsuperscript{20} Scores were manually inspected and corrected when needed. CAC burden was expressed in terms of Agatston scores.\textsuperscript{21} All individuals were male smokers or former smokers.

**Genotype Data and SNP Imputation**

Genome-wide SNP genotype data were collected in 3082 participants on the Illumina Human610-Quad BeadChip, and quality control was performed to remove low-quality SNPs and samples.\textsuperscript{13} After extensive quality control of the data (including principal components analysis), we kept 2599 samples for all downstream analyses. We performed imputation of untyped SNPs with Minimac,\textsuperscript{22} splitting all samples into random batches of \( \approx 500 \) individuals. As reference panel, we used the 998 phased haplotypes from the Genome of the Netherlands Project release 4 encompassing 19 763 454 SNPs.\textsuperscript{23}

**Association Testing Framework**

We used a linear regression model to test genetic risk scores for association to the log-transformed Agatston scores (\( \ln(\text{Agatston score}+1) \)), including as covariates the first principal component of the genotype data (which was the only statistically significant component at univariate \( P<0.05 \), age, and smoking history (in pack years). The baseline model included only these 3 covariates. In the 3-SNP model, we included the risk alleles of 3 SNPs (rs4977574 at 9p21, rs3825807 at \textit{ADAMTS7}, and rs12526453 at \textit{PHACTR1}) as independent terms in the regression model. These 3 SNPs reach genome-wide significance in a combined analysis of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium,\textsuperscript{11} the Heinz Nixdorf Recall Study,\textsuperscript{22} and the NELSON trial.\textsuperscript{23} The 45-SNP model includes the risk alleles of all 45 CAD/-MI-associated SNPs identified by the CARDIoGRAMplusC4D Study\textsuperscript{2} as independent terms in the regression model. Of the 46 associations originally reported by CARDIoGRAMplusC4D, one SNP, rs6903956 at \textit{Cobrj0105}, was not included because this SNP was identified in the Han Chinese population\textsuperscript{24} and has not been replicated in Europeans to date. Statistical software R 3.0.2 was used for the analysis. To calculate the explained variance of the 3 CAC SNPs and 45 CAD/MI SNPs, we subtracted the variance explained by the baseline model from that explained by the 3-SNP and 45-SNP models, respectively.

**Polygenic Risk Scores for CAD/MI**

We calculated polygenic risk scores for all individuals in the NELSON trial using publicly available association results for 79 128 SNPs from the CARDIoGRAMplusC4D consortium.\textsuperscript{2} We extracted all SNPs that were present in both the CARDIoGRAMplusC4D data and our NELSON GWAS data. After removing A/T and C/G SNPs we used PLINK\textsuperscript{25} to prune remaining SNPs based on linkage disequilibrium (LD), preferentially keeping SNPs with lower \( P \) values in the CARDIoGRAMplusC4D results and leaving no pairs of SNPs with \( r^2 > 0.05 \). We calculated multiple polygenic scores based on SNPs with \( P \) values reported by the CARDIoGRAMplusC4D consortium\textsuperscript{2} that reached a predefined threshold\textsuperscript{1,2} \( (p<5 \times 10^{-5}, <5 \times 10^{-4}, <5 \times 10^{-3}, <5 \times 10^{-2}, <10^{-3}, <0.05, <0.1, <0.2, <0.3, <0.4, <0.5)\).\textsuperscript{2} This resulted in 11 polygenic models containing between 39 and 475 SNPs. Allelic dosages for the risk alleles for all SNPs were calculated from the posterior probabilities provided by the imputation software Minimac,\textsuperscript{22} as follows:

\[
\text{Dosage(A allele)} = 2 \times P \left( \frac{\text{AA genotype}}{\text{AB genotype}} \right) + P \left( \frac{\text{AB genotype}}{\text{AA genotype}} \right),
\]

where \( A \) is the risk allele and \( B \) is the alternative allele. We calculated the genetic risk score using the following formula:

\[
\text{Genetic risk score} = \sum_{i=1}^{n} \beta_i x_i + \ldots + \beta_n x_n\]

where \( \beta_i \) is the natural log of the reported odds ratio for CAD/MI, and \( x_i \) is the estimated allele dosage (between 0 and 2) in a given individual for the \( i \)th SNP. The genetic risk score was then divided by the total number of SNPs in the model to account for missing data, and added as a single term to the regression model. We corrected for the effects of the 45 SNPs associated with CAD/MI by adding them as covariates to the regression model. We subtracted the explained variance of the 45-SNP model to estimate the explained variance because of the polygenic signal.

We partitioned the polygenic scores by chromosome and tested each separately for association to CAC levels using linear regression and including the same covariates as for the 12 polygenic models listed above.\textsuperscript{27} This allowed us to test whether there was a relation between the explained variance and chromosome size for each chromosome. Under a polygenic model, the assumption is that larger chromosomes explain more of the trait than smaller ones. We used a binomial test to assess whether effect directions across a set of SNPs are more concordant than expected by chance.

We evaluated whether the variance explained per chromosome could be explained by particular gene sets from Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology, Biocarta, Reactome, canonical, and chemical pathways, all obtained from the Molecular Signatures Database (MsigDB).\textsuperscript{28} We mapped SNPs to genes using GENCODE annotations,\textsuperscript{29} and selected only pathways with \( \geq 50 \) genes. For each gene set, we tested for an association between the number of genes per chromosome and the explained trait variance.

**Polygenic Risk Scores for Traditional Risk Factors**

We tested 9 polygenic risk score models on the basis of known SNP associations for circulating lipids (high-density lipoprotein [HDL]-cholesterol, low-density lipoprotein [LDL]-cholesterol, triglycerides, and total cholesterol),\textsuperscript{14–18} T2D,\textsuperscript{17} height,\textsuperscript{30} BMI,\textsuperscript{17} and T1D\textsuperscript{31–33} and...
a combined set of blood pressure SNPs as described by Van’t Hof et al.5-7,34 For each individual, we calculated the polygenic risk score by summing up the number of risk alleles of the trait-associated SNPs, weighted by the reported effect size of each SNP and dividing this by the total number of included SNPs (similar to CAD/MI as described above). We used linear regression to test this genetic risk score for association to CAC, using the same covariates as the baseline regression model described above. We subtracted the explained variance of the baseline model to estimate the explained variance because of the polygenic signal. We claimed statistical significance after multiple testing correction (P<0.0056 for 9 independent polygenic risk score models tested to achieve a type 1 error of 5%).

Replication in the Heinz Nixdorf Recall Study
We replicated polygenic models in the Heinz Nixdorf Recall Study. Study rationale, study design, and methods have been described previously.12,35 The study was approved by the local ethics committees, and informed consent was obtained from all participants. CAC levels were assessed by nonenhanced electron-beam computerized tomography (C-150 scanner; GE Imatron, San Francisco, CA). Individuals were genotyped using 3 different genotyping chips: Illumina HumanOmni1-Quad, Illumina HumanOmni15S, and Illumina HumanOmniExpress. After extensive quality control, 2182 samples were included in downstream analyses. SNPs were imputed with IMPUTE version 2.1.12 using the 1000 Genomes Project (release 2012) as a reference panel. Using SNPTEST version 2.1.1,36 scores were tested for association with log-transformed Agatston scores (ln[Agatston score+1]) using a linear regression model correcting for age and sex. We reported 1-sided P-values because these tests are based on hypotheses with a prespecified effect direction. We claimed statistical significance after correcting for the total number of included SNPs (similar to CAD/MI as described above). We tested each for association with CAC, while adjusting for the collective effects of the 45 known CAD/MI risk alleles. All models were significantly associated with CAC with P-values ranging from 10−3 to 10−11 (Table II in the Data Supplement). The most significant model (P=1.6x10−11) was for the 8918 SNPs with P<0.2 in CARDIoGRAMplusC4D, explaining an additional 1.5% of the observed CAC variance (Figure 1).

To test whether these polygenic associations are evenly distributed over the genome, we partitioned all SNPs with P<0.2 in the CARDIoGRAMplusC4D analysis for each chromosome separately, again adjusting for the effects of the 45 CAD/MI SNPs, and estimated the explained variance of CAC for each chromosome. The correlation between the explained variance per chromosome and chromosome number is significant (r²=0.39; P=0.0019; Figure 2). We obtained similar results for the relation between explained variance and SNPs on each chromosome after LD pruning (r²=0.45, P=0.0006; Table III in the Data Supplement), and explained variance and physical chromosome length (r²=0.45, P=0.0006).

Chromosomes 2 and 6 explained more of the variance than expected on the basis of their physical size or number of SNPs. For both chromosomes, we observed an excess of SNPs with concordant direction of effects between CAD/MI and CAC (binomial P=1.5x10−3 for chromosome 2 and P=1.5x10−3 for chromosome 6).

Results
CAC Variance Explained by Known Genetic Risk Factors
First, we assessed the explained variance of CAC by a baseline model containing only age, smoking (pack years), and the first principal component to correct for population structure within the Netherlands as covariates. This baseline model explained 8.3% of the phenotypic variance (Figure 1). Next, we tested the additional variance explained by the collective effect of 3 SNPs (at 9p21, PHACTR1, and ADAMTS7) associated with CAD/MI and with CAC. This 3-SNP model explained an additional 2.4% of the variance of the CAC phenotype (Figure 1).

To test whether other published CAD/MI risk SNPs are also associated with CAC, we included 45 established CAD/MI risk SNPs in the model.6 The total variance explained by this 45-SNP model was 12.3%, of which 4% could be attributed to the 45 SNPs associated with CAD/MI risk (Figure 1). Association results for the 45 SNPs with CAC are shown in Table I in the Data Supplement. To characterize the impact of CAD/MI-associated SNPs that never reached genome-wide significance in the CARDIoGRAMplusC4D study, we created 11 polygenic models and tested each for association with CAC, while adjusting for the collective effects of the 45 known CAD/MI risk alleles. All models were significantly associated with CAC with P-values ranging from 10−3 to 10−11 (Table II in the Data Supplement). The most significant model (P=1.6x10−11) was for the 8918 SNPs with P<0.2 in CARDIoGRAMplusC4D, explaining an additional 1.5% of the observed CAC variance (Figure 1).

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Influence of Traditional Risk Factors on CAC

Finally, we evaluated whether there was a shared genetic basis between CAC and 7 traits involving traditional cardiovascular risk factors that influence risk for CAD and MI. To this end, we tested polygenic risk score models for HDL-cholesterol, LDL-cholesterol, triglycerides, total cholesterol, height, BMI, blood pressure, T1D, and T2D; all based on validated SNP associations from the GWAS literature (Table). The model containing LDL-cholesterol SNPs was significantly associated with CAC (P=0.002) and another 3 models (BMI, T2D, and total cholesterol) were associated with CAC at nominal significance (P<0.05). We attempted to replicate the findings for these 4 genetic risk score models in the Heinz Nixdorf Recall Study assuming consistent effect directions (Table). We observed significant replication evidence for the model for LDL-cholesterol (P=0.0097), total cholesterol (P=0.0063), and BMI (P=0.0018). This suggests that alleles that increase LDL or total cholesterol levels or BMI will collectively tend to increase levels of CAC, even though their overall quantitative contribution is modest (<1% variance explained). Summary statistics for the individual SNPs are shown in Table IV in the Data Supplement. The association signal for the T2D-based model (which was nominally significant in the NELSON trial) seemed to be predominantly driven by a single SNP (rs944801) at the pleiotropic 9p21 locus.

Discussion

The 3 loci that have been associated with CAC thus far (CDKN2A/B at 9p21, PHACTR1, and ADAMTS7) were first discovered for their association with CAD and MI. This observation motivated us to test the hypothesis that other loci associated with CAD/MI might also influence CAC. Because single-variant association testing has limited power to detect modest effects, we adopted a polygenic approach by aggregating the effects of ≤15 000 independent SNPs into a single genetic risk score and then tested each for association with CAC. The polygenic score is based on the assumption that markers act additively; that is, gene–gene interactions (epistasis) are ignored in these models. Our results demonstrate that there is at least a polygenic component with alleles acting additively, and its quantitative contribution may represent a lower bound estimate if we assume a non-negligible contribution because of epistatic effects.

In our data, the 3 known CAC SNP associations found by recent GWAS explained 2.4% of the phenotypic variance, and the 45 CAD/MI risk SNPs identified by the CARDIoGRAMplusC4D study accounted for an additional 4%. Accounting for the effects of these 45 SNPs, the most significant polygenic model was based on 8918 SNPs with P<0.2 in the CARDIoGRAMplusC4D study, and collectively, these SNPs explained another 1.5% of the observed CAC variance, indicating nontrivial genetic overlap between CAC and CAD/MI. This number is likely an underestimate because causal variants are only poorly tagged by the sparse set of 8918 unlinked SNPs throughout the genome. In addition, it should be noted that the Metabochip is biased toward gene regions prioritized by early GWAS of cardiometabolic traits. That is to say, other (unsuspected) gene regions may also play a role. The relative contributions of the 3-SNP, 45-SNP, and polygenic models to the variance explained are consistent with the expectation that variants with stronger effects were the first ones to be identified in discovery GWAS. These common variants will consequently explain a correspondingly larger fraction of the phenotypic variance. The effect size and variance explained for individual variants captured by the polygenic

<table>
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<tr>
<th>Genetic Trait</th>
<th>NELSON Study (n=2599)</th>
<th>Heinz Nixdorf Recall Study (n=2182)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trait</td>
<td>No. of SNPs</td>
<td>Explained Variance, %</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
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</tr>
<tr>
<td>Body mass index</td>
<td>31</td>
<td>8.5×10^{-3} +</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus</td>
<td>55</td>
<td>2.6×10^{-2} +</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>72</td>
<td>4.0×10^{-2} +</td>
</tr>
<tr>
<td>Height</td>
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</tr>
<tr>
<td>Type 1 diabetes mellitus</td>
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</tr>
<tr>
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<td>71</td>
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<tr>
<td>Blood pressure</td>
<td>38</td>
<td>0.928</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>40</td>
<td>0.939</td>
</tr>
</tbody>
</table>

Genetic risk scores are based on bona fide SNP associations for the traits listed. Results are sorted by decreasing significance in the NELSON Study. The explained variance and effect direction are only given for traits that are nominally significant in the NELSON Study. Nominally, significant models were replicated in the Heinz Nixdorf Recall Study. Results of the NELSON Study are corrected for age, smoking history (in pack years), and the first principal component of the genotype data. Results of the Heinz Nixdorf Recall Study are corrected for age and sex. HDL indicates high-density lipoprotein; and LDL, low-density lipoprotein; NELSON Study, the Dutch and Belgian Lung Cancer Screening Study; SNP, single-nucleotide polymorphism.
association signal will be much lower, but they may still give important insights into the underlying biology.

Considering the phenotypic heterogeneity among the many CAD/MI studies that were part of the CARDiOGRAMplusC4D study, it is not straightforward to interpret the magnitude of effect of the SNPs because they may not necessarily reflect the true impact on the CAC phenotype. We, therefore, expect that the estimated variance explained in our analysis is probably not that precise although the qualitative result should be robust. Indeed, ignoring the effect sizes for the CAD/MI-associated SNPs (as weights in the polygenic score) produced essentially identical results (data not shown). These observations are consistent with the notion that the effect sizes of the individual SNPs underlying the polygenic association are small and hard to detect individually at the genome-wide significance level. An important result is that the cumulative effects on CAC seem to be distributed all over the genome, again consistent with a polygenic architecture.

To investigate the role of traditional risk factors on CAC, we tested genetic risk scores based on validated SNPs associated with LDL-cholesterol, HDL-cholesterol, triglycerides, total cholesterol, height, BMI, blood pressure, T1D, and T2D. Of these, we found statistically significant positive associations for LDL-cholesterol, total cholesterol, and BMI. One interpretation of these results is that certain genes underlying these traits have pleiotropic effects on CAC.

Previous observational studies have demonstrated the relation between LDL and total cholesterol levels and CAC levels. Our results confirm that common SNPs associated with LDL and total cholesterol affect calcification levels and are in agreement with findings from a similar genetic risk score analysis in 1987 elderly individuals from the Rotterdam Study. We did not observe a significant association for the HDL-cholesterol genetic risk score, which is consistent with recent evidence that genes influencing HDL-cholesterol may not have a significant impact on MI risk. This finding appears at odds, however, with recent results from the Diabetes Heart Study where HDL-based genetic risk scores were found to be inversely associated with CAC. With respect to the impact of obesity on CAC, several studies have described (positive) associations between CAC and BMI (or obesity), which is consistent with our results.

We did not observe an association for the genetic risk scores for triglycerides and hypertension, which is perhaps somewhat more surprising given their established causal role in cardiovascular disease. On the basis of these observations, we found that increasing blood pressure or triglyceride levels are likely to exert their harmful effects on cardiovascular disease with no direct effect on calcification.

It is interesting to speculate whether LDL and total cholesterol and BMI may have a direct impact on CAC levels in the sense that their effects on CAD/MI risk are (partially) mediated through CAC, but we caution that our study was not designed to make such causal inferences. Even so, the results presented here are consistent with genes having a direct (that is, pleiotropic) effect on calcification or an indirect effect on calcification because of, for example, LDL-cholesterol.

In this study, we characterized the genetic architecture of CAC in relation to CAD and MI and traditional risk factors. First, we identified a polygenic model based on common SNPs associated with CAD/MI that could explain up to 13.9% of the observed CAC variance. This suggests that a substantial fraction of the genetic risk for CAD/MI is mediated through CAC. Second, polygenic models based on published risk alleles for traditional cardiovascular risk factors showed that SNPs associated with LDL-cholesterol, total cholesterol, and BMI may also be involved in the cause of CAC. Further insight into the underlying CAC mechanisms may lead us toward novel treatment opportunities for CAD/MI beyond traditional risk factor management. Collaborative GWAS of larger sample sizes will remain a useful activity as they continue to identify novel loci, especially for imaging-based traits where sample numbers have not been as large as compared with other traits. Such a strategy will likely lead to a better understanding of the underlying processes of arterial calcification and its role in CAD and MI.

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Disclosures

Dr de Koning participated once in the Medical Advisory Board of Roche Diagnostics, for which the department was compensated. Dr Viergever has received grants from Philips Healthcare and from Pie Medical Imaging and Dr Figum has received a grant from Pie Medical Imaging on cardiovascular image analysis, but these do not relate directly to the subject matter of the present article. The other authors report no conflicts.

References


**CLINICAL PERSPECTIVE**

Understanding the root causes for cardiovascular disease and associated risk factors is a key priority for biomedical research. Calcification of the coronary arteries is a strong and independent risk factor for cardiovascular events including myocardial infarction. In this study, we analyzed the genetic architecture of coronary calcification in relation to genetic associations for coronary artery disease and traditional risk factors including low-density lipoprotein-cholesterol, type 2 diabetes mellitus, blood pressure, and obesity. Our analyses underscore a substantial genetic overlap between quantitative calcification levels (measured by computed tomography) and coronary artery disease (including myocardial infarction), reinforcing the notion that calcification plays a causal role in myocardial infarction. In addition, we tested if bona fide single-nucleotide polymorphisms associated with traditional risk factors were also associated with coronary calcification, and found that variants influencing low-density lipoprotein and total cholesterol levels, body mass index, and type 2 diabetes mellitus risk also have an effect on coronary calcification. This finding is consistent with the notion that at least some of the adverse effects of these risk factors may be mediated through calcification.
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**SUPPLEMENTAL MATERIAL**

**Supplementary Table 1:** The CARDioGRAMplusC4D consortium described 45 CAD/MI risk SNPs. This table shows the association results of these SNPs with Agatston score in the NELSON study.

<table>
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<th>SNP</th>
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<th>Position</th>
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<td>A2 Allele Frequency</td>
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**Supplementary Table 2**: Influence of polygenic risk score calculated from CAD/MI associated SNPs on quantitative coronary artery calcification levels in the NELSON Cohort Study. The threshold refers to the $P$-value below which SNPs from the CARDioGRAMplusC4D study are included in the model. The models are adjusted for age, smoking history (in pack years), the first principal component of the genotype data, and 45 known CAD/MI risk SNPs.

<table>
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<th># SNPs</th>
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<th>Explained variance (%)</th>
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Supplementary Table 3: Explained CAC variance per chromosome based on the model with all CAD/MI associated SNPs with \( P < 0.2 \) in the CARDIOGRAMplusC4D study. The explained variance correlates significantly with the number of SNPs that overlap between the CARDIoGRAMplusC4D and NELSON Studies, after removal of A/T and C/G SNPs and after LD pruning (leaving no SNP pairs with \( r^2 > 0.05 \)).

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<th>P-value</th>
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**Supplementary Table 4:** Association results for CAC levels in the NELSON Cohort Study and the Heinz Nixdorf Recall Study for validated SNPs associated with nine traits/diseases.

**Supplementary Table 4a: Total cholesterol**

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### Supplementary Table 4d: LDL-cholesterol

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