Genetic Loci for Coronary Calcification and Serum Lipids Relate to Aortic and Carotid Calcification

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Background—Atherosclerosis in different vessel beds shares lifestyle and environmental risk factors. It is unclear whether this holds for genetic risk factors. Hence, for the current study genetic loci for coronary artery calcification and serum lipid levels, one of the strongest risk factors for atherosclerosis, were used to assess their relation with atherosclerosis in different vessel beds.

Methods and Results—From 1987 persons of the population-based Rotterdam Study, 3 single-nucleotide polymorphisms (SNPs) for coronary artery calcification and 132 SNPs for total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides were used. To quantify atherosclerotic calcification as a marker of atherosclerosis, all participants underwent nonenhanced computed tomography of the aortic arch and carotid arteries. Associations between genetic risk scores of the joint effect of the SNPs and of all calcification were investigated. The joint effect of coronary artery calcification–SNPs was associated with larger calcification volumes in all vessel beds (difference in calcification volume per SD increase in genetic risk score: 0.15 [95% confidence interval, 0.11–0.20] in aorta, 0.14 [95% confidence interval, 0.10–0.18] in extracranial carotids, and 0.11 [95% confidence interval, 0.07–0.16] in intracranial carotids). The joint effect of total cholesterol SNPs, low-density lipoprotein SNPs, and of all lipid SNPs together was associated with larger calcification volumes in both the aortic arch and the carotid arteries but attenuated after adjusting for the lipid fraction and lipid-lowering medication.

Conclusions—The genetic basis for aortic arch and carotid artery calcification overlaps with the most important loci of coronary artery calcification. Furthermore, serum lipids share a genetic predisposition with both calcification in the aortic arch and the carotid arteries, providing novel insights into the cause of atherosclerosis. **(*Circ Cardiovasc Genet.* 2013;6:47-53.)**

Key Words: atherosclerosis ■ coronary artery calcium ■ genetics ■ imaging ■ lipids

Atherosclerosis is a systemic vascular disease[1] with risk factors that overlap across different vessel beds.[2–4] For example, high serum lipid levels are 1 of the strongest risk factors for atherosclerosis in different vessel beds.[1,4–6] However, the question remains whether this shared etiology also extends to genetic risk factors. This is important because it has been shown that the burden of atherosclerosis differs across vessel beds.[2,7–9] Recently, 3 single-nucleotide polymorphisms (SNPs) were identified that were associated with coronary artery calcification (CAC),[10] a marker of atherosclerosis that can be measured in vivo with computed tomography (CT).[11] It is unclear to what extent these CAC–SNPs are also associated with calcification in other major vessel beds.

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Alternatively, the genetic basis of atherosclerosis can be investigated using the genetics of risk factors of atherosclerosis. Serum lipid levels are among the most important risk factors of atherosclerosis,[1,5,12,13] and we hypothesize that SNPs that are associated with lipid levels are also associated with atherosclerosis. Recently, multiple SNPs associated with serum concentrations of total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglycerides were identified.[14] However, the effect of 1 SNP is small. Therefore, genetic risk scores (joint effect of the SNPs) can be constructed to acquire more power.

Hence, the aims of this study were 2-fold. First, we determined whether the CAC–SNPs are also associated with calcification in the aortic arch and carotid arteries. Second, we explored the relation between genetics of serum lipid fractions and calcification in the aortic arch and carotid arteries.

Methods

Study Population

This study was embedded in the Rotterdam Study,[15] a prospective, population-based cohort study that aims to investigate the incidence and determinants of various chronic diseases in the elderly. The original cohort comprised 7983 participants 55 years of age or older and was extended with another 3011 participants in 2000 using the same inclusion criteria. Participants in the Rotterdam Study are virtually all from white origin (96%). From 2003 onward, all participants who

Received May 23, 2012; accepted November 16, 2012.

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The online-only Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.111.963934/-/DC1.

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completed a visit at the research center were invited to undergo CT of the aorta and carotid arteries (as part of a CT-imaging protocol measuring calcification in various vessel beds). In total, 2524 participants were scanned. Due to image artifacts, 2491 participants had a complete and suitable CT examination. Genotype data were present in 1987 of these participants, encompassing the current study population. These genetic data have undergone extensive quality checks, including identity-by-state clustering.[16] Persons >3 SDs away from the population mean were excluded. Therefore, the population used in our analyses is ethnically homogenous with all persons from white origin. This study was approved by the institutional review board, and all participants gave informed consent.

Genotyping

Genotyping was performed as part of a large project on complex diseases.[16] We used the Illumina HumanHap550 Duo BeadChip and the Illumina Infinium II HumanHap 610 Quad Arrays. All genotyping was done at the Human Genotyping Facility, Genetic Laboratory, Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands. As described previously,[16] participant-specific quality controls included filters for call rate, heterozygosity, and number of mendelian errors per individual. SNP-specific quality controls included filters for call rate, minor allele frequency, Hardy-Weinberg equilibrium, and differential missingness by outcome or genotype (mishap test in PLINK, http://pngu.mgh.harvard.edu/purcell/plink/). For imputation, we used the Markov Chain Haplotyping (MaCH) package (http://www.sph.umich.edu/csg/abecasis/MACH, version 1.0.15 or 1.0.16 software). For each imputed SNP, quality of imputation was estimated as the ratio of the empirically observed dosage variance to the expected binomial dosage variance. For this study, we extracted data on 3 recently discovered CAC–SNPs[10] and 132 SNPs,[14] which have been related to serum concentrations of total cholesterol, LDL, HDL, and triglycerides (online-only Data Supplement Table I). Imputation quality (Rsq) was >0.90 (mean, 0.95) for the CAC–SNPs and >0.60 (mean, 0.96) for the lipid SNPs.

CT Acquisition and Processing

CT scans were acquired using a 16-slice (n=591) or 64-slice (n=1396) multidetector CT scanner (Somatom Sensation 16 or 64, Siemens, Forcheim, Germany). No contrast material was administered. To visualize calcification in the aortic arch, the extracranial carotid arteries, and the intracranial carotid arteries, we used a scan that reached from the aortic arch to the intracranial circulation (1 cm above the sella turcica). Detailed information regarding imaging parameters of the scan is described elsewhere.[4]

Calcification in the aortic arch and extracranial carotid artery was quantified with dedicated commercially available software (syngo CalciumScoring, Siemens, Germany) and expressed as calcium volume in cubic millimeters. The aortic arch was measured from the origin to the first centimeter of the common carotid arteries, the vertebral arteries, and the subclavian arteries beyond the origin of the vertebral arteries. The extracranial carotid arteries were measured at both sides within 3 cm proximal and distal of the bifurcation.[4] The intracranial internal carotid artery comprised the horizontal segment of the petrous internal carotid artery to the top of the internal carotid artery. Automatic calcification quantification in this region was not feasible because of the close relation between arterial calcification and the skull. A detailed description of the manual measurement of calcification in this region can be found elsewhere.[8] Briefly, after delineating calcification manually in every consecutive multidetector CT slice, the volume of the intracranial carotid artery calcification was calculated by multiplying the number of pixels above the threshold of 130 Hounsfield units[17] with the pixel size and the slice increment.

Assessment of Serum Lipid Levels and Other Cardiovascular Risk Factors

Serum total cholesterol, HDL, and triglyceride concentrations were determined using an automated enzymatic procedure (Hitachi analyzer, Roche Diagnostics, Washington, DC).[4] The use of lipid-lowering drugs was assessed by interview. These measurements were performed before the CT examination (mean interval, 4.6±0.6 years). LDL was calculated from these 3 parameters using the Friedewald formula.[18] Because this formula does not apply when the triglyceride concentration exceeds 4.51 mmol/L, we could not calculate the LDL concentration in these participants (n=23).

Information on other cardiovascular risk factors was obtained during a home interview and a visit at the research center around the same time as the CT examination.[15] These risk factors included the body mass index, systolic and diastolic blood pressure, the use of blood pressure–lowering medication, diabetes mellitus, and smoking status.[4]

Statistical Analysis

We constructed genetic risk scores[19] for the joint effect of the CAC–SNPs and for the joint effect of the lipid SNPs (52 SNPs for total cholesterol, 47 SNPs for HDL, 37 SNPs for LDL, and 32 SNPs for triglycerides, obtained from Supplementary Table 2 of the GWAS on serum lipids)[14] by summing the number of calcium-increasing or lipid-increasing alleles (lipid-decreasing alleles for HDL) weighted by the reported effect estimate of each CAC–SNP[10] or lipid SNP.[14] Next, for the construction of weighted genetic risk score for the joint effect of all lipid SNPs, we assigned weighting factors to the different components that were derived from the Friedewald equation ([total cholesterol = LDL+HDL+[0.45×triglycerides]).[18] After this, total cholesterol got a weighting factor of 2.45, LDL and HDL of 1, and triglycerides of 0.45. We calculated the score and divided by 4.9 (2.45+1.0+1.0+0.45). Similarly, we calculated a weighted genetic risk score for the joint effect of lipid SNPs, exclusive of total cholesterol (1.0 [LDL]+1.0 [HDL]+0.45 [triglycerides] ÷ 2.45). Additionally, we created quartiles of the weighted compound genetic risk scores. It is important to note that SNPs were allowed to overlap across the risk scores to take into account their pleiotropic effect.

As calcification volumes were positively skewed and non-normally distributed, we used natural log-transformed values and added 1 mm^3 to the nontransformed values (Ln[calcification volume +1.0 mm^3]) to deal with participants with a calcium score of zero. Correlations between calcification volumes across vessel beds were assessed using the Spearman correlation test.

We used linear regression to assess the association between the joint effect of the CAC–SNPs and calcification volume (model 1). In addition, we investigated the associations for the individual CAC–SNPs separately using stepwise linear regression to determine whether all 3 SNPs are relevant for calcification in the different vessel beds. Analyses were additionally adjusted for age, sex, and cardiovascular risk factors (body mass index, systolic and diastolic blood pressure, blood pressure–lowering medication, diabetes mellitus, total cholesterol, lipid-lowering medication, and smoking status; model 2).

Next, we explored associations between the joint effect of the lipid SNPs per lipid fraction and the genetic risk scores for all SNPs with calcification volume using linear regression (model 1). These analyses were additionally adjusted for age, sex, and the following cardiovascular risk factors: body mass index, systolic and diastolic blood pressure, use of blood pressure–lowering medication, diabetes mellitus, and smoking status (model 2). Model 3 was additionally adjusted for the respective serum lipid concentration and use of lipid-lowering medication. Next, linear regression was used to test trends over the calcification quartiles. Finally, we additionally investigated whether adding a quadratic term for age improved the model fit, but this showed no effect and, therefore, is not further reported on in the results.

Associations among serum concentrations of total cholesterol, HDL, LDL, triglycerides, and calcification volume, adjusted for age, sex, and the use of lipid-lowering medication, were also assessed with linear regression. IBM SPSS Statistics version 20 (International Business Machines Corporation, Armonk, NY) was used for statistical analyses.

Table 1. Population Characteristics

Variable	n=1987
Women	991 (49.9)
Age, y	69.8±6.8
BMI, kg/m^2	27.6±3.9
Smoking (ever)	1351 (68.0)
Systolic blood pressure, mm Hg	146.7±20.4
Diastolic blood pressure, mm Hg	80.1±10.9
Use of blood pressure–lowering medication	793 (39.9)
Diabetes mellitus	213 (10.7)
Serum total cholesterol, mmol/L	5.79±0.96
Serum HDL cholesterol, mmol/L	1.38±0.37
Serum LDL cholesterol, mmol/L	3.72±0.88
Serum triglycerides, mmol/L	1.51±0.67
Use of lipid-lowering medication	268 (13.5)
Aortic arch calcification volume, mm^3*	265.5 (44.2–924.7)
Extracranial carotid artery calcification volume, mm^3*	25.9 (0.0–125.9)
Intracranial carotid artery calcification volume, mm^3*	45.9 (8.0–148.1)

Values are mean±SD for continuous variables and number (%) for dichotomous variables. BMI indicates body mass index; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

*Median with interquartile range.

Results

Table 1 shows the characteristics of the study population (online-only Data Supplement Table II also depicts these characteristics for the source population, from which the present study population was derived). The mean age at the time of the CT scan was 69.8±6.8, and 49.9% were women. Of all participants, 7.9% had a zero calcium score for the aortic arch, 26.1% for the extracranial carotid arteries, and 16.9% for the intracranial carotid arteries. Correlations between calcification in the 3 vessel beds are shown in online-only Data Supplement Table III.[8,9]

Associations for the CAC–SNPs, the joint effect of the CAC–SNPs with the calcification volume in the different vessel beds are shown in Table 2. The genetic risk score of the CAC–SNPs was significantly related to calcification in all 3 vessel beds (Table 2) but attenuated after additional adjustment for age, sex, and cardiovascular risk factors (Table 2, model 2). When we investigated the associations for each CAC–SNP separately and calcification volume, rs1333049 was strongly associated with calcification in the aortic arch and the extracranial and intracranial carotid arteries (difference in calcification volume per SD increase in risk allele: 0.05 [95% confidence interval [CI], 0.01–0.09], 0.08 [95% CI, 0.04–0.13], and 0.12 [95% CI, 0.08–0.16], respectively; Table 2). These associations remained after additional adjustments for age, sex, and cardiovascular risk factors (Table 2, model 2).

Table 3 depicts associations between genetic risk scores of the lipid SNPs per fraction and calcification volumes in the different vessel beds. The joint effect of total cholesterol SNPs was significantly associated with larger calcification volume in all 3 vessel beds, and these associations remained after additional adjustment for cardiovascular risk factors (model 2; difference in calcification volume per SD increase in genetic risk score: 0.05 [95% CI, 0.01–0.09] for the aortic arch, 0.06 [95% CI, 0.02–0.10] for the extracranial carotid arteries, and 0.06 [95% CI, 0.02–0.10] for the intracranial carotid arteries). The joint effect of LDL SNPs was associated with larger calcification volume in the aortic arch and the extracranial carotid arteries, whereas the joint effect of HDL SNPs was only associated with calcification in the extracranial carotid arteries (difference in calcification volume per SD increase in genetic risk score: 0.07 [95% CI, 0.03–0.11]). The joint effect of triglyceride SNPs was solely associated with larger calcification volume in the extracranial carotid arteries. We additionally adjusted for the respective serum lipid fraction and the use of lipid-lowering medication

Table 2. Associations of SNPs for Coronary Artery Calcium With Calcification in Other Vessel Beds

	Aortic Arch Calcification	*P*	%EV	Extracranial Carotid Calcification	*P*	%EV	Intracranial Carotid Calcification	*P*	%EV
Model 1									
GRS of CAC–SNPs*	0.15 (0.11 to 0.20)	<0.001	2.3	0.14 (0.10 to 0.18)	<0.001	2.0	0.11 (0.07 to 0.16)	<0.001	1.3
rs1333049†	0.05 (0.01 to 0.09)	0.027	0.2	0.08 (0.04 to 0.13)	<0.001	0.7	0.12 (0.08 to 0.16)	<0.001	1.4
rs9349379‡	0.03 (–0.01 to 0.08)	0.172	0.1	0.03 (–0.02 to 0.07)	0.201	0.1	0.02 (–0.02 to 0.07)	0.331	0.0
rs2026458‡	0.02 (–0.03 to 0.06)	0.397	0.0	0.02 (–0.03 to 0.06)	0.516	0.0	0.01 (–0.03 to 0.06)	0.583	0.0
Model 2									
GRS of CAC–SNPs*	0.01 (–0.03 to 0.06)	0.525	0.0	0.04 (0.00 to 0.08)	0.068	0.1	0.00 (–0.04 to 0.04)	0.968	0.0
rs1333049†	0.04 (0.00 to 0.08)	0.059	0.1	0.07 (0.03 to 0.11)	<0.001	0.5	0.11 (0.07 to 0.15)	<0.001	1.2
rs9349379‡	0.03 (–0.01 to 0.07)	0.114	0.1	0.03 (–0.01 to 0.07)	0.102	0.1	0.03 (–0.01 to 0.07)	0.120	0.1
rs2026458‡	0.02 (–0.02 to 0.06)	0.304	0.0	0.02 (–0.02 to 0.06)	0.319	0.0	0.02 (–0.02 to 0.06)	0.341	0.0

Values represent differences in standardized calcification volumes (Ln[calcification volume +1.0 mm^3]) with 95% confidence intervals per SD increase for the genetic risk score (GRS) of CAC–SNPs and per SD increase in risk allele for each SNP separately. Model 1: unadjusted. Model 2: adjusted for age, sex, and cardiovascular risk factors (body mass index, systolic and diastolic blood pressure, blood pressure–lowering medication, diabetes mellitus, total cholesterol, lipid-lowering medication, and smoking status). %EV indicates percent explained variance; CAC, coronary artery calcification; and SNP, single-nucleotide polymorphism.

*Based on weighted sum of the number of risk alleles of 3 SNPs for coronary artery calcium.

†SNP rs1333049 is located on chromosome 9 near CDKN2B, with coded allele frequency of 0.46 (coded allele: C).

‡SNPs rs9349379 and rs2026458 are both located on chromosome 6 near PHACTR1, with coded allele frequencies of 0.61 and 0.44 (coded alleles: A and T).

Table 3. Genetic Risk Scores for Lipid Fractions and Calcification in Different Vessel Beds

GRS*	Aortic Arch Calcification	*P*	%EV	Extracranial Carotid Calcification	*P*	%EV	Intracranial Carotid Calcification	*P*	%EV
Model 1									
Total cholesterol	0.07 (0.02 to 0.11)	0.003	0.4	0.07 (0.03 to 0.12)	0.002	0.5	0.07 (0.02 to 0.11)	0.004	0.4
LDL cholesterol	0.07 (0.03 to 0.12)	0.002	0.5	0.08 (0.04 to 0.12)	<0.001	0.6	0.05 (0.00 to 0.09)	0.046	0.2
HDL cholesterol	0.04 (–0.01 to 0.08)	0.081	0.2	0.07 (0.03 to 0.11)	0.002	0.5	0.03 (–0.01 to 0.07)	0.183	0.1
Triglycerides	0.04 (–0.01 to 0.08)	0.094	0.1	0.06 (0.01 to 0.10)	0.010	0.3	0.04 (–0.01 to 0.08)	0.121	0.1
Model 2									
Total cholesterol	0.05 (0.01 to 0.09)	0.009	0.3	0.06 (0.02 to 0.10)	0.005	0.3	0.06 (0.02 to 0.10)	0.008	0.3
LDL cholesterol	0.06 (0.02 to 0.10)	0.005	0.3	0.07 (0.03 to 0.11)	0.002	0.4	0.04 (0.00 to 0.08)	0.080	0.1
HDL cholesterol	0.03 (–0.01 to 0.07)	0.109	0.1	0.06 (0.02 to 0.10)	0.003	0.3	0.03 (–0.02 to 0.07)	0.204	0.1
Triglycerides	0.01 (–0.03 to 0.05)	0.531	0.0	0.04 (0.00 to 0.08)	0.053	0.1	0.02 (–0.02 to 0.06)	0.409	0.0
Model 3									
Total cholesterol	0.02 (–0.02 to 0.06)	0.406	0.0	0.01 (–0.04 to 0.05)	0.775	0.0	0.02 (–0.03 to 0.06)	0.404	0.0
LDL cholesterol	0.02 (–0.03 to 0.06)	0.458	0.1	0.02 (–0.02 to 0.06)	0.393	0.1	0.00 (–0.04 to 0.05)	0.907	0.0
HDL cholesterol	0.01 (–0.03 to 0.05)	0.596	0.0	0.05 (0.01 to 0.10)	0.011	0.3	0.02 (–0.02 to 0.06)	0.316	0.1
Triglycerides	–0.01 (–0.05 to 0.04)	0.791	0.0	0.02 (–0.03 to 0.06)	0.426	0.0	0.01 (–0.03 to 0.05)	0.686	0.0

Values represent differences in standardized calcification volumes (Ln[calcification volume +1.0 mm^3]) with 95% confidence intervals per SD increase in the genetic risk score (GRS) of each lipid fraction. Model 1: unadjusted. Model 2: adjusted for age, sex, and cardiovascular risk factors (body mass index, systolic and diastolic blood pressure, blood pressure–lowering medication, diabetes mellitus, and smoking status). Model 3: as model 2, additionally adjusted for the respective serum lipid concentration and use of lipid-lowering medication. %EV indicates percent explained variance; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

*Based on weighted sum of the number of risk alleles of 52 single-nucleotide polymorphisms for total cholesterol, 37 single-nucleotide polymorphisms for LDL cholesterol, 47 single-nucleotide polymorphisms for HDL cholesterol, and 32 single-nucleotide polymorphisms for triglycerides.

to investigate whether serum lipid concentrations (and the use of lipid-lowering medication) are intermediates in the pathway from genetics to atherosclerosis (Table 3, model 3). Now, all associations between the different genetic risk scores and atherosclerotic calcification in the 3 vessel beds attenuated. Only the association between the genetic risk score for the HDL SNPs and extracranial carotid artery calcification remained significant (difference in calcification volume per SD increase in genetic risk score: 0.05 [95% CI, 0.01–0.10]).

The joint effect of LDL, HDL, and triglyceride SNPs together was significantly associated with calcification in the 3 vessel beds (difference in calcification volume per SD increase in genetic risk score: 0.07 [95% CI, 0.03–0.11] for the aortic arch, 0.10 [95% CI, 0.05–0.14] for the extracranial carotid arteries, and 0.05 [95% CI,0.01–0.10] for the intracranial carotid arteries; online-only Data Supplement Table IV). When total cholesterol SNPs were added, these associations became slightly stronger (online-only Data Supplement Table IV). Figure 1A–1C demonstrates the relation between quartiles of the genetic risk score involving all lipid SNPs and calcification volume in all vessel beds.

Associations between the different serum lipid levels and calcification in the various vessel beds are shown in online-only Data Supplement Table V. Furthermore, the relation between the genetic risk scores of the total cholesterol, LDL, HDL, and triglyceride SNPs and the respective serum lipid concentrations are presented in online-only Data Supplement Table VI. The genetic risk scores per lipid fraction were, as expected, associated with the corresponding serum concentrations.

Discussion

In a large sample of community-dwelling older persons, we found genetic risk factors for atherosclerosis across vessel beds. More specifically, we found that previously discovered SNPs for CAC are also jointly associated with calcification in the aortic arch, extracranial, and intracranial carotid arteries. Furthermore, genetic loci for serum lipids are related with calcification in both the aortic arch and the carotid arteries.

We found a relation between the genetic risk score of CAC–SNPs and larger calcification volume in the aortic arch and the extracranial and intracranial carotid arteries. However, the strength of the associations differed across the vessel beds, and, moreover, the percent explained variance of the risk score for calcification also differed. Interestingly, the percent explained variance of the genetic risk score of CAC–SNPs ranged from 1.3% to 2.3% for calcification. When adjusting for cardiovascular risk factors, both the association and the amount of variance explained attenuated. Of the 3 separate CAC–SNPs, only rs1333049 was, significantly and independently of other cardiovascular risk factors, related to atherosclerotic calcification. Again, this association varied in strength across vessel beds. Possibly, differences in pathophysiology of atherosclerosis per vessel bed play a role here.[20] These findings also fit previous findings that correlations for atherosclerotic calcification in different vessel beds are only moderate.[2,8,9] This could indicate that, although atherosclerosis is a systemic disease, various factors play a different role in the development of it across vessel beds. We note, however, that given the relatively small sample size of the current study, some care

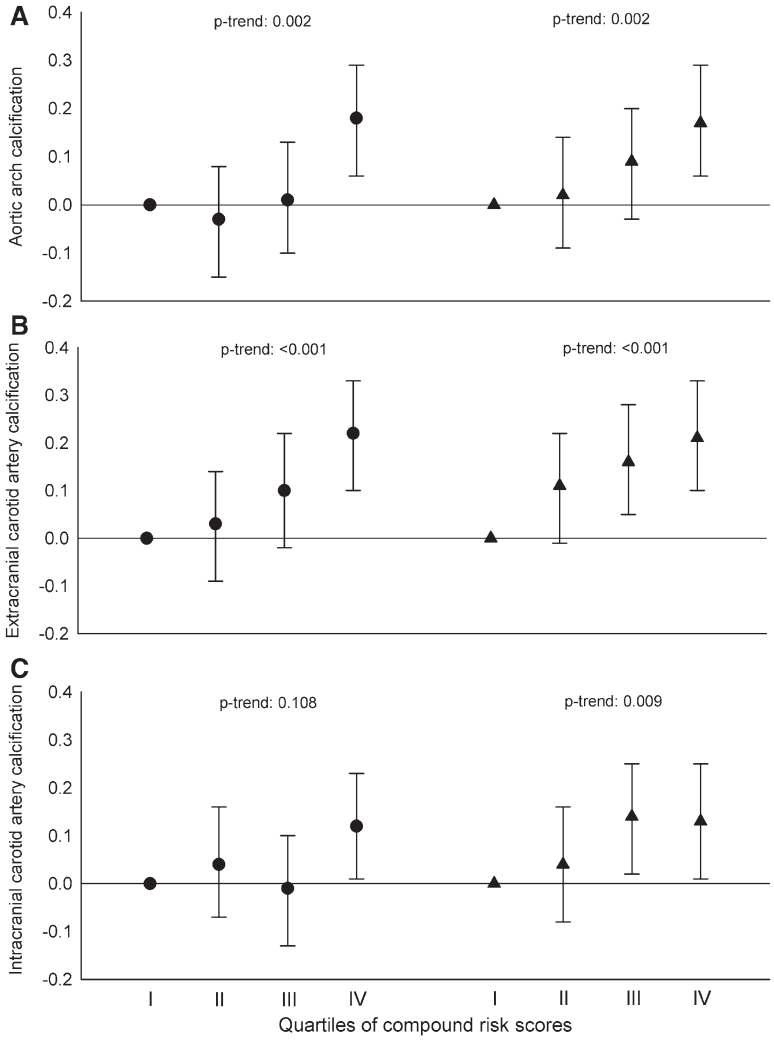

Figure 1. Joint effect of all lipid single-nucleotide polymorphisms (SNPs) and calcification in different vessel beds. In all 3 panels (**A**–**C**), the quartiles of the weighted genetic risk score of the joint effect of all lipid SNPs are displayed on the *x* axis. Values of *y* represent differences in standardized aortic calcification volume, extracranial carotid calcification volume, and intracranial carotid calcification volume. The left column of quartiles (circles) represents the joint effect of high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride SNPs. The right column (triangles) represents the joint effect of HDL, LDL, triglyceride, and total cholesterol SNPs. Adjusted for age and sex. Probability values for linear trends per compound score are displayed.

should be taken in interpreting the variation in associations across vessel beds.

We found that the genetic risk scores of total cholesterol SNPs and LDL SNPs were most prominently associated with larger calcification volumes in all vessel beds. This finding is in line with another study,[14] which described that only several SNPs that coded for LDL were associated with coronary artery disease. Furthermore, we found that the joint effect of all LDL, HDL, and triglyceride SNPs was associated with calcification volume in all vessel beds. These associations became stronger when total cholesterol was included in the genetic risk score. Most likely, part of this can be explained by the pleiotropic effect of the SNPs. However, we acknowledge that it remains difficult to discern with full reliability a pleiotropic effect of a SNP from an effect merely driven by the intercorrelation of fractions. Nevertheless, despite strong correlations across lipid fractions, not all SNPs for a certain fraction also relate with other fractions. This indicates that those SNPs that do relate with >1 fraction have a good likelihood of being pleiotropic.

As with the associations between CAC–SNPs and calcification, we also found differences in the strength of the associations between the genetic risk scores for the lipids and calcification across the vessel beds. This again suggests differences in the cause of atherosclerosis in different vessel beds.

The associations between the genetic risk score of total cholesterol SNPs, the genetic risk score of LDL SNPs, and calcification did not change after adjustment for other cardiovascular risk factors but only after additional adjustment for the serum lipid fraction and the use of lipid-lowering medication. This finding strengthens the hypothesis that serum lipid levels are intermediates in the causal pathway between genes and atherosclerosis.

In contrast, the results for the genetic risk scores of HDL SNPs and for the triglyceride SNPs were less unequivocal. Interestingly, we found a prominent association between the genetic risk score of HDL SNPs (lower serum HDL level) and calcification in the extracranial carotid artery. This association even remained after additional adjustment for the lipid fraction and the use of lipid-lowering medication. Like for this genetic risk score of HDL SNPs, we found an association between the genetic risk score of triglyceride SNPs and extracranial carotid artery calcification only, but this association attenuated after adjustment for age, sex, and serum lipid level. These findings fit the notion that HDL and especially triglycerides are more environmental/lifestyle-dependent (ie, alcohol consumption, obesity, sedentary lifestyle) lipid

fractions.[21,22] Data on the relation among HDL, triglycerides, and carotid atherosclerosis are scarce and inconclusive. A systematic review showed that lower values of HDL were associated with a higher risk of carotid atherosclerosis.[23] Our finding that the HDL genetic risk score is associated with extracranial carotid artery calcification underlines this observation, but further investigation of this relation in larger samples is necessary. However, both the genetic risk scores for triglycerides and HDL were not associated with aortic arch or intracranial carotid artery atherosclerosis, which is in line with a recent finding that a genetic risk score for HDL was not associated with myocardial infarction.[24] Based on our findings, we hypothesize that genetic variants which are associated with higher HDL serum concentrations may not be automatically associated with less atherosclerosis in these vessel beds. This, in turn, suggests that different lipid fractions could play different roles in the cause of atherosclerosis in different vessel beds.

Strengths of our study include the assessment of 3 major vessel beds with the same diagnostic tool (CT) and the fact that we built our genetic risk scores on previous studies. A major advantage of the CT-based calcium scoring is that it provides an accurate measurement of the amount of calcification, and different vessel beds can be measured in 1 session. Several considerations should also be discussed, of which the first is the fact that we used only lipid SNPs, 1 of the strongest determinants for atherosclerosis and calcification,[1,4] and that the effect of other cardiovascular risk factors on calcification is thus not taken into account. Second, we have to note that any negative findings can be due to the relatively small sample size. Another consideration is that calcification is only a part of the atherosclerotic plaque. With CT it is not possible to visualize the complete extent of the noncalcified atherosclerotic plaque. Strong evidence nonetheless suggests that calcification volume is an adequate measure for the total underlying plaque burden.[11,25]

Conclusions

We found that genetic risk factors for atherosclerosis overlap across vessel beds. More specifically, the genetic basis for aortic arch and carotid artery calcification overlaps with the most important loci of CAC. Furthermore, serum lipids share a genetic predisposition with both calcification in the aortic arch and the carotid arteries, providing novel insights into the cause of atherosclerosis.

Sources of Funding

The Rotterdam Study is supported by the Erasmus MC and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research, the Netherlands Organization for Health Research and Development, the Research Institute for Diseases in the Elderly, the Netherlands Genomics Initiative, the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. Drs van der Lugt and Vernooij were supported by an Alzheimer's Association grant (NIRG-08-91391/NIRG-09-13168). Dr Witteman was supported by a grant from the Netherlands Organization for Scientific Research and the Netherlands Organization for Health Research and Development (Vici 918.76.619). Dr Ikram was supported by a grant (2009B102) from the Netherlands Heart Foundation.

Disclosures

None.

References

1. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*. 1995;92:1355–1374.
2. Allison MA, Criqui MH, Wright CM. Patterns and risk factors for systemic calcified atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2004;24:331–336.
3. Newman AB, Naydeck BL, Sutton-Tyrrell K, Feldman A, Edmundowicz D, Kuller LH. Coronary artery calcification in older adults to age 99: prevalence and risk factors. *Circulation*. 2001;104:2679–2684.
4. Odink AE, van der Lugt A, Hofman A, Hunink MG, Breteler MM, Krestin GP, et al. Risk factors for coronary, aortic arch and carotid calcification: the Rotterdam Study. *J Hum Hypertens*. 2010;24:86–92.
5. Goldberg IJ, Eckel RH, McPherson R. Triglycerides and heart disease: still a hypothesis? *Arterioscler Thromb Vasc Biol*. 2011;31:1716–1725.
6. Santos RD, Nasir K. Insights into atherosclerosis from invasive and noninvasive imaging studies: should we treat subclinical atherosclerosis? *Atherosclerosis*. 2009;205:349–356.
7. Allison MA, Hsi S, Wassel CL, Morgan C, Ix JH, Wright CM, et al. Calcified atherosclerosis in different vascular beds and the risk of mortality. *Arterioscler Thromb Vasc Biol*. 2012;32:140–146.
8. Bos D, Ikram MA, Elias-Smale SE, Krestin GP, Hofman A, Witteman JC, et al. Calcification in major vessel beds relates to vascular brain disease. *Arterioscler Thromb Vasc Biol*. 2011;31:2331–2337.
9. Odink AE, van der Lugt A, Hofman A, Hunink MG, Breteler MM, Krestin GP, et al. Association between calcification in the coronary arteries, aortic arch and carotid arteries: the Rotterdam study. *Atherosclerosis*. 2007;193:408–413.
10. O'Donnell CJ, Kavousi M, Smith AV, Kardia SL, Feitosa MF, Hwang SJ, et al. Genome-wide association study for coronary artery calcification with follow-up in myocardial infarction. *Circulation*. 2011;124:2855–2864.
11. Sangiorgi G, Rumberger JA, Severson A, Edwards WD, Gregoire J, Fitzpatrick LA, et al. Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. *J Am Coll Cardiol*. 1998;31:126–133.
12. Lavoie A, Uno K, Bayturan O, Nicholls SJ. Findings of clinical trials that evaluate the impact of medical therapies on progression of atherosclerosis. *Curr Med Res Opin*. 2010;26:745–751.
13. Lee JM, Choudhury RP. Atherosclerosis regression and high-density lipoproteins. *Expert Rev Cardiovasc Ther*. 2010;8:1325–1334.
14. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*. 2010;466:707–713.
15. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, et al. The Rotterdam Study: 2012 objectives and design update. *Eur J Epidemiol*. 2011;26:657–686.
16. Ikram MA, Seshadri S, Bis JC, Fornage M, DeStefano AL, Aulchenko YS, et al. Genomewide association studies of stroke. *N Engl J Med*. 2009;360:1718–1728.
17. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15:827–832.
18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499–502.
19. Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, et al; International Schizophrenia Consortium. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009;460:748–752.
20. Mulvany MJ, Aalkjaer C. Structure and function of small arteries. *Physiol Rev*. 1990;70:921–961.
21. Brinton EA. Effects of ethanol intake on lipoproteins. *Curr Atheroscler Rep*. 2012;14:108–114.
22. Rahilly-Tierney C, Vokonas P, Gaziano JM, Spiro A 3rd. Lifestyle change and high-density lipoprotein change: the US Department of Veterans Affairs Normative Aging Study. *Clin Cardiol*. 2012;35:437–442.
23. Amarenco P, Labreuche J, Touboul PJ. High-density lipoprotein-cholesterol and risk of stroke and carotid atherosclerosis: a systematic review. *Atherosclerosis*. 2008;196:489–496.

24. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet*. 2012;380:572–580.

25. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area: a histopathologic correlative study. *Circulation*. 1995;92:2157–2162.

CLINICAL PERSPECTIVE

Atherosclerosis is a systemic vascular disease with considerable morbidity and mortality. It is known that lifestyle and environmental risk factors, such as hypercholesterolemia, overlap across different vessel beds. However, whether this shared cause also extends to genetic risk factors has not been elucidated yet. This is important because it has been shown that the burden of atherosclerosis differs across vessel beds. Over the years, genome-wide association studies have become a popular tool to identify common genetic variants of both diseases and risk factors of diseases. Using genome-wide association studies, recently important loci were identified that were associated with coronary artery calcification, a strong marker of atherosclerosis. Using computed tomography-based arterial calcification in a sample of community-dwelling elderly persons, we demonstrated that the genetic basis for aortic arch and carotid artery calcification overlaps with these loci of coronary artery calcification. Alternatively, the genetic basis of atherosclerosis can be investigated using the genetics of risk factors of atherosclerosis. Serum lipid levels (total cholesterol, low-density lipoprotein, high-density lipoprotein, and triglycerides) are among the most important risk factors of atherosclerosis, and multiple loci associated with the concentration of different lipid fractions have been identified. Using genetic risk scores of these lipid fractions, we demonstrated that serum lipids share a genetic predisposition with calcification in both the aortic arch and the carotid arteries. These findings add to the existing knowledge on the cause of atherosclerosis and may eventually contribute to the prevention of atherosclerosis.

Genetic Loci for Coronary Calcification and Serum Lipids Relate to Aortic and Carotid Calcification

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Circ Cardiovasc Genet. 2013;6:47-53; originally published online December 16, 2012;
doi: 10.1161/CIRCGENETICS.112.963934
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1942-325X. Online ISSN: 1942-3268

SUPPLEMENTAL MATERIAL

Supplementary table I. Single nucleotide polymorphisms (SNPs) for coronary calcification and serum lipid levels.

	RS-number
Coronary artery calcification	1333049, 9349379, 2026458
Serum lipid levels (total cholesterol, LDL, HDL, triglycerides)	7515577, 6759321, 2290159, 2814982, 9488822, 2285942, 2072183, 1961456, 2737229, 581080, 651007, 2255141, 10832963, 174550, 7941030, 11220463, 7206971, 7239867, 492602, 2277862, 2902940, 4297946, 11153594, 12670798, 217386, 649129, 1129555, 174583, 11220462, 2332328, 247616, 7225700, 2902941, 909802, 4660293, 1689800, 4846914, 12328675, 1515100, 13107325, 6450176, 2814944, 605066, 1084651, 17145738, 4731702, 9987289, 2293889, 10808546, 643531, 2923084, 3136441, 174601, 7115089, 7134375, 3741414, 7134594, 4759375, 4765127, 838880, 2652834, 16942887, 2925979, 881844, 4148008, 4082919, 7241918, 12967135, 7255436, 737337, 386000, 6065906, 181362, 2131925, 1321257, 10195252, 2943645, 645040, 442177, 9686661, 1553318, 2247056, 13238203, 7811265, 11776767, 1495743, 2954029, 10761731, 2068888, 174546, 11613352, 12310367, 2412710, 2929282, 261342, 11649653, 7205804, 439401, 4810479, 5756931, 12027135, 2479409, 3850634, 629301, 2807834, 514230, 1367117, 4299376, 12916, 6882076, 3757354, 1800562, 3177928, 1564348, 2126259, 1030431, 2954022, 11136341, 11065987, 1169288, 2000999, 6511720, 1883025, 1532085, 3764261, 4420638, 1800961, 1042034, 1260326, 12678919, 964184, 10401969

Supplementary table II. Vascular risk profiles across participants in the current study and total Rotterdam Study cohort.

Variable	Rotterdam Study-participants in present analyses	Other Rotterdam Study-participants*
Women	991 (49.9)	2572 (63.3)
Age (y)	69.8 ± 6.8	74.5 ± 7.8
BMI (kg/m^2)	27.6 ± 3.9	27.6 ± 4.3
Smoking (ever)	1351 (68.0)	2643 (65.1)
Systolic blood pressure (mmHg)	146.7 ± 20.4	151.5 ± 21.8
Diastolic blood pressure (mmHg)	80.1 ± 10.9	79.6 ± 11.0
Use of blood pressure-lowering medication	793 (39.9)	2060 (50.7)
Diabetes	213 (10.7)	601 (14.8)
Serum total cholesterol (mmol/l)	5.79 ± 0.96	5.85 ± 0.98
Serum HDL cholesterol (mmol/l)	1.38 ± 0.37	1.40 ± 0.40
Use of lipid-lowering medication	268 (13.5)	499 (12.3)

* These are all other Rotterdam Study-participants that participated in the follow-up visit [n = 6050 (all) – 1987 (current study) = 4063] from which the present study population (n=1987) was derived.

Supplementary table III. Correlations between calcification across the aortic arch, extracranial carotid and intracranial carotid artery

Location of calcification	Aortic arch	Extracranial carotid artery	Intracranial carotid artery
Aortic arch	-	0.57	0.53
Extracranial carotid artery	0.57	-	0.54
Intracranial carotid artery	0.53	0.54	-

Values represent Spearman's correlation coefficients ($P < 0.01$ for all correlations)

Supplementary table IV. Joint effect of all lipid SNPs and calcification in different vessel beds

GRS*	Aortic arch calcification	*P*	%EV	Extracranial carotid calcification	*P*	%EV	Intracranial carotid calcification	*P*	%EV
Model 1									
Combined risk score (LDL, HDL, triglycerides)	0.07(0.03;0.11)	0.002	0.5	0.10(0.05;0.14)	<0.001	1.0	0.05(0.01;0.10)	0.024	0.3
Combined risk score (total cholesterol, LDL, HDL, triglycerides)	0.08(0.03;0.12)	0.001	0.6	0.09(0.05;0.14)	<0.001	0.8	0.07(0.02;0.11)	0.003	0.4
Model 2									
Combined risk score (LDL, HDL, triglycerides)	0.05(0.01;0.09)	0.009	0.3	0.08(0.04;0.12)	<0.001	0.6	0.04(0.00;0.08)	0.056	0.1
Combined risk score (total cholesterol, LDL, HDL, triglycerides)	0.06(0.02;0.10)	0.003	0.4	0.08(0.04;0.12)	<0.001	0.6	0.06(0.02;0.10)	0.008	0.3
Model 3									
Combined risk score (LDL, HDL, triglycerides)	0.03(-0.01;0.07)	0.162	0.1	0.05(0.01;0.09)	0.028	0.2	0.02(-0.03;0.06)	0.434	0.0
Combined risk score (total cholesterol, LDL, HDL, triglycerides)	0.03(-0.02;0.07)	0.218	0.0	0.03(-0.02;0.07)	0.205	0.0	0.02(-0.02;0.06)	0.352	0.0

Values represent differences in standardized calcification volumes [Ln(calcification volume +1.0 mm^3)] with 95% confidence intervals, per SD increase in the combined genetic risk score (GRS). %EV = percent explained variance.

Model 1: unadjusted.

Model 2: adjusted for age, sex and cardiovascular risk factors (BMI, systolic and diastolic blood pressure, blood pressure lowering medication, diabetes mellitus and smoking status).

Model 3: as model 2, additionally adjusted for serum total cholesterol and the use of lipid-lowering medication.

Supplementary table V. Serum lipid levels and calcification in different vessel beds

Lipid fractions	Aortic arch calcification	*P*	Extracranial carotid calcification	*P*	Intracranial carotid calcification	*P*
Model 1						
Total cholesterol	0.11(0.07;0.16)	<0.001	0.12(0.08;0.17)	<0.001	0.07(0.03;0.12)	0.002
LDL cholesterol	0.15(0.11;0.20)	<0.001	0.13(0.08;0.18)	<0.001	0.08(0.03;0.13)	0.002
HDL cholesterol	-0.30(-0.41;-0.18)	<0.001	-0.13(-0.25;-0.01)	0.028	-0.13(-0.25;-0.01)	0.037
Triglycerides	0.10(0.04;0.16)	0.001	0.11(0.05;0.17)	0.001	0.09(0.02;0.15)	0.008
Model 2						
LDL cholesterol	0.14(0.09;0.19)	<0.001	0.12(0.07;0.17)	<0.001	0.07(0.02;0.12)	0.008
HDL cholesterol	-0.26(-0.39;-0.12)	<0.001	-0.01(-0.14;0.13)	0.945	-0.05(-0.19;0.09)	0.481
Triglycerides	0.01(-0.07;0.07)	0.893	0.08(0.01;0.15)	0.023	0.06(-0.02;0.13)	0.123

Values represent differences in standardized calcification volumes [Ln(calcification volume +1.0 mm^3)] with 95% confidence intervals, per unit increase of serum lipid concentration.

Model 1: Adjusted for age, sex and lipid-lowering medication.

Model 2: As model I, but for LDL: + HDL and triglycerides. For HDL: + LDL and triglycerides. For triglycerides: + HDL and LDL.

Supplementary table VI. Joint SNP-effect per lipid fraction and serum lipid levels

Genetic risk score*	Total cholesterol	*P*	LDL cholesterol	*P*	HDL cholesterol	*P*	Triglycerides	*P*
Total cholesterol	0.23(0.18;0.27)	<0.001	0.18(0.14;0.22)	<0.001	0.01(-0.01;0.02)	0.399	0.08(0.05;0.11)	<0.001
LDL cholesterol	0.20(0.16;0.24)	<0.001	0.19(0.15;0.23)	<0.001	-0.02(-0.03;0.01)	0.072	0.06(0.02;0.09)	0.001
HDL cholesterol	0.00(-0.04;0.04)	0.990	0.06(0.02;0.10)	0.002	-0.10(-0.11;-0.08)	<0.001	0.07(0.04;0.10)	<0.001
Triglycerides	0.09(0.05;0.13)	<0.001	0.04(0.01;0.08)	0.026	-0.04(-0.06;-0.02)	<0.001	0.17(0.14;0.20)	<0.001

Values represent differences in serum lipid concentrations with 95% confidence intervals, per SD increase in the genetic risk score of each lipid fraction.

* Based on weighted sum of the number of risk alleles of 52 SNPs for total cholesterol, 37 SNPs for LDL cholesterol, 47 SNPs for HDL cholesterol and 32 SNPs for triglycerides.