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# Family history and polygenic risk of cardiovascular disease: Independent factors associated with secondary cardiovascular events in patients undergoing carotid endarterectomy

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## HIGHLIGHTS

- Positive family history (FHx) is independently associated with higher 3-year risk of secondary cardiovascular events (sCVE).
- Higher genetic risk (MetaGRS) is associated with higher 3-year risk of sCVE, independent of FHx and risk factors.
- Higher MetaGRS was associated with more vulnerable plaque characteristics suggesting putative underlying mechanisms.
- Future studies should further unravel exact underlying pathophysiological mechanisms.
- Future studies should explore the value of MetaGRS and FHx in individual risk prediction for sCVE.

## ARTICLE INFO

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## ABSTRACT

*Background and aims:* Family history (FHx) of cardiovascular disease (CVD) is a risk factor for CVD and a proxy for cardiovascular heritability. Polygenic risk scores (PRS) summarizing >1 million variants for coronary artery disease (CAD) are associated with incident and recurrent CAD events. However, little is known about the influence of FHx or PRS on secondary cardiovascular events (sCVE) in patients undergoing carotid endarterectomy (CEA).

*Methods:* We included 1788 CEA patients from the Athero-Express Biobank. A weighted PRS for CAD including 1.7 million variants was calculated (MetaGRS). The composite endpoint of sCVE during three years of follow-up included coronary, cerebrovascular and peripheral events and cardiovascular death. We assessed the impact of FHx and MetaGRS on sCVE and carotid plaque composition.

*Results:* Positive FHx was associated with a higher 3-year risk of sCVE independent of cardiovascular risk factors and MetaGRS (adjusted HR 1.40, 95%CI 1.07–1.82, $p = 0.013$). Patients in the highest MetaGRS quintile had a higher 3-year risk of sCVE compared to the rest of the cohort independent of cardiovascular risk factors including FHx (adjusted HR 1.35, 95%CI 1.01–1.79, $p = 0.043$), and their atherosclerotic plaques contained more fat (adjusted OR 1.59, 95%CI, 1.11–2.29, $p = 0.013$) and more macrophages (OR 1.49, 95%CI 1.12–1.99, $p = 0.006$).

*Conclusions:* In CEA patients, both positive FHx and higher MetaGRS were independently associated with increased risk of sCVE. Moreover, higher MetaGRS was associated with vulnerable plaque characteristics. Future studies should unravel underlying mechanisms and focus on the added value of PRS and FHx in individual risk prediction for sCVE.

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## 1. Introduction

Family history of cardiovascular disease (FHx) is a major risk factor for primary cardiovascular disease (CVD) and serves as a surrogate for genetic predisposition [1,2]. Risk prediction for secondary cardiovascular events remains challenging as traditional risk factors have limited discriminative performance [3]. The main underlying mechanism of CVD is atherosclerosis, and atherosclerotic plaque composition, exemplified by intraplaque haemorrhage (IPH), has been associated with adverse secondary cardiovascular events (sCVE) [4]. Yet, the relevance of FHx for secondary outcome of cardiovascular events is still unclear [5–9].

Large-scale genome-wide association studies (GWAS) have identified hundreds of common genetic variants (single-nucleotide polymorphisms or SNPs) robustly associated with coronary artery disease (CAD) [10–14] and ischemic stroke [15–17] predisposition, albeit with small individual effects. Exact pathobiological mechanisms leading to cardiovascular symptoms are still poorly understood, but CAD- and ischemic stroke genetic variants were previously associated with atherosclerotic plaque composition [18].

Polygenic risk scores (PRS) summarize the small individual genetic effects into a quantitative measure of genetic disease susceptibility. CAD-PRS were strongly correlated with prevalent and incident CAD independent of traditional risk factors including family history in the UK Biobank population [19,20]. For example, individuals with higher scores of the MetaGRS (a PRS for CAD including 1.7 million SNPs) were at 1.7–4.2 fold higher risk for a first coronary event compared to individuals with lower MetaGRS scores. Two recent studies in CAD patients showed that CAD-PRS was also associated with an increased risk of recurrent CAD events [21,22]. However, no studies have investigated this relation in patients undergoing carotid endarterectomy (CEA), nor its impact on plaque composition. Therefore, we aimed to investigate the association between MetaGRS and sCVE in patients undergoing CEA and explore possible underlying pathophysiological mechanisms by studying the impact of MetaGRS on carotid histological plaque characteristics. Given that FHx is used in clinical practice as a derivative of genetic background, we also examined the association between FHx, sCVE and plaque characteristics.

## 2. Materials and methods

### 2.1. Athero-Express Biobank

All patients in this study were included in the Athero-Express Biobank (www.atheroexpress.nl), a prospective cohort study of consecutive patients with severe carotid artery stenosis undergoing CEA in two large tertiary referral hospitals in The Netherlands, the University Medical Centre Utrecht (inclusion is ongoing) and the St. Antonius Hospital Nieuwegein (inclusion until 2014) [23]. The study design has been published before [23]. In short, patient characteristics, such as demographics, cardiovascular risk factors, including medical history, medication use, and FHx for cardiovascular disease, were obtained through standardized questionnaires and checked in electronic health records. Preoperative blood samples were drawn. The atherosclerotic plaque obtained during surgery was collected and immunohistochemically analysed for plaque characteristics. Patients were followed up for three years after surgery for the occurrence of secondary cardiovascular events through standardized questionnaires and by checking electronic health records. General practitioners were consulted in case of no response to questionnaires or in order to obtain further information regarding reported cardiovascular events. Patients operated for restenosis (6% of 2044 eligible patients for this study) were excluded because these differ in future cardiovascular event risk [24]. Thus for the current study, a total of 1788 patients operated from March 2002 until July 2016 had available 3-year follow-up data and FHx data, and were included for analysis. Of these, 1551/1788 (87%) patients had available histological carotid plaque data. A total of 1319/1788 (74%) patients had available genotype data of whom 1301 (98%) also had histological carotid plaque data. This study was performed according to the Declaration of Helsinki and was approved by the local ethics committee of both hospitals. Patients provided written informed consent before study participation.

### 2.2. Definitions

A positive FHx was defined as having a first-degree relative (either a parent or sibling) with onset of cardiovascular disease (myocardial infarction (MI), coronary artery stenosis, stroke, abdominal aortic aneurysm (AAA), or cardiovascular death including sudden death) before the age of 60 years. The primary outcome of this study was defined as a composite secondary cardiovascular event (sCVE) within three years of follow-up including fatal or non-fatal MI, fatal or non-fatal stroke, ruptured AAA, fatal cardiac failure, coronary or peripheral interventions (either percutaneous or bypass surgery), leg amputation due to cardiovascular causes and cardiovascular death. Secondary outcomes were histological atherosclerotic carotid plaque characteristics.

### 2.3. Genotyping

Methods for genotyping, quality control and imputation in the Athero-Express biobank have been published elsewhere [25,26]. Briefly, DNA was extracted from EDTA whole blood samples or if not present from atherosclerotic plaque tissue according to validated protocols. Genotyping was performed with two commercially available chips: the first batch by Affymetrix Genome-Wide Human SNP array 5.0 (previously used in the Athero-Express Genomics Study 1 (AEGS1), covering samples obtained in 2002–2007) and the second batch by Affymetrix Axiom GW CEU 1 array (previously used in Athero-Express Genomics Study 2 (AEGS2), covering samples obtained in 2002–2013). Procedures for data quality control and data cleaning were in accordance with global standards [27]. After genotype calling according to Affymetrix' specification, data was filtered on 1) individual call rate > 97%, 2) genotype call rate > 97%, 3) minor allele frequencies >3%, 4) average heterozygosity rate ± 3.0 standard deviations, 5) relatedness (pi-hat > 0.20), 6) Hardy–Weinberg equilibrium $p < 1.0 \times 10^{-6}$), and 7) population stratification (based on HapMap 2, release 22, b36) by excluding samples deviating more than 6 standard deviations from the average in 5 iterations during principal component analysis and by visual inspection [25].

After pre-phasing using SHAPEIT2 v2.644, a combined dataset of 1000 Genome (phase 3, version 5) and The Genome of the Netherlands Project release 5 was used as a reference for imputation with IMPUTE2 v2.3.0 to impute missing genotypes for 88,784,475 variants [28].

### 2.4. Polygenic risk score (MetaGRS)

To estimate the polygenic cardiovascular disease susceptibility for included patients in our cohort, we used the previously published polygenic risk score for CAD (MetaGRS) [19]. Its construction was described elsewhere [19]. Briefly, the MetaGRS comprises 1,745,179 genetic variants with a minor allele frequency (MAF) > 0.1% associated with CAD and was constructed through meta-analysis of three genomic risk scores: GRS46K (comprising 46,000 cardiometabolic genetic variants), FDR202 (including 202 genetic variants associated with CAD at false discovery rate $p < 0.05$ in the recent GWAS CARDIoGRAMplusC4D), and the 1000Genomes genetic score also created with CARDIoGRAMplusC4D. The MetaGRS was internally and externally validated for the primary risk of prevalent and incident CAD in the UK Biobank [19].

We matched the 1.7 million variants from the MetaGRS to 1,742,593 variants in our data (2586 variants were not present in our data). Given that the median imputation quality was high

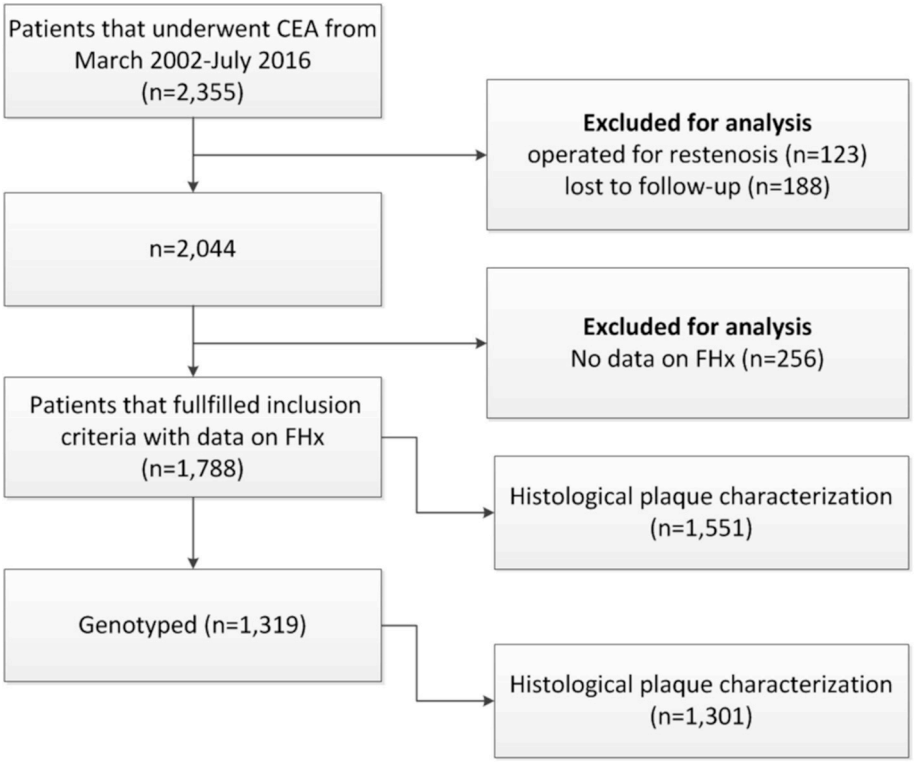


**Fig. 1.** Flowchart.

(INFO = 0.978 [IQR 0.945–0.991]), and the variants included in the MetaGRS have MAF >0.1% as described in the Supplemental Material of the original publication [19], we did not further filter on imputation quality. Moreover, since we used the imputed genotype probabilities to calculate the MetaGRS, rather than the hard-coded genotypes, bias arising from imputation error, i.e. low imputation quality, will only reduce predictive accuracy. Thus, we calculated the MetaGRS for each included patient in this study using PRSice-2 [29]. We standardized the MetaGRS to mean-zero and unit-variance for each genotyping batch separately, i.e. AEGS1 and AEGS2, respectively.

### 2.5. Sample handling

After CEA, the atherosclerotic plaque was directly processed in the laboratory following standardized protocols [4,23,30,31]. The plaque was cut in cross-sectional segments of 5 mm. The segment with largest plaque burden was chosen as the culprit lesion and immunohistochemically analysed for macrophages, smooth muscle cells (SMC), lipid core, calcification, collagen, intraplaque haemorrhage (IPH) and microvessel content. Extensive description of the standardized protocol for atherosclerotic plaque processing and analysis of plaque characteristics has been previously reported and is added to the Supplemental Materials [4,23,30,31]. To assess the overall vulnerability of the atherosclerotic plaque, a vulnerability score was created ranging from 0 to 5 with 1 point for plaque characteristics that are considered hallmarks of a vulnerable plaque (moderate/heavy macrophages, no/minor collagen, no/minor SMC, lipid core>10% and presence of IPH), based on a previous publication [32].

### 2.6. Statistical analysis

Baseline characteristics were compared between patient groups (FHx and MetaGRS) by chi-square test for categorical variables and Student's t-test for continuous variables (lipid levels were log-transformed). We analysed the association between FHx and MetaGRS and sCVE by Cox-proportional hazard regression models and the associations with plaque characteristics through logistic or linear regression models. To fully unravel the genetic association, the association of MetaGRS with sCVE and plaque characteristics was analysed in three ways: (1) MetaGRS as a continuous quantification of genetic CAD susceptibility, (2) patients in the top 20% of the MetaGRS distribution compared to the remaining 80%, and (3) patients in the top 20% of the MetaGRS distribution compared to those in the bottom 20% of the distribution. Kaplan-Meier curves were constructed to graphically illustrate univariate associations. Confounders for multivariable analyses were selected based on literature [19,32,33] (for sCVE these were age, sex, diabetes, BMI, smoking and hypercholesterolemia and for plaque characteristics these were age, sex, surgery year and type of cerebrovascular symptoms). Additional confounders were added when showing an association of $p < 0.20$ with the determinant (FHx or MetaGRS) and outcome of interest (sCVE or plaque characteristics). For MetaGRS models, genotype array and principal components 1–4 were also added. Full model description is shown in the Supplemental Tables S1 and S2. Because a previous study in our biobank showed that IPH is associated with sCVE [4], IPH was added to multivariable models of FHx, MetaGRS and sCVE to explore whether IPH could be one possible underlying mechanism. Sex-stratified analyses were performed to unravel sex-dependent differences in associations. Values with $p < 0.05$ were considered statistically significant. All analyses were performed in IBM SPSS Statistics version 25.0.

## 3. Results

Patient selection from the Athero-Express Biobank and characteristics of the study population are displayed in Fig. 1 and Table 1. Patients had a mean age of 69 years and 70% were men. The cohort represented a typically severe atherosclerotic cohort with high prevalence of traditional risk factors and atherosclerotic manifestations in other vascular beds (coronary or peripheral arteries, respectively 30% and 20%). Baseline characteristics were similar between the total cohort

**Table 1**
Baseline characteristics.

| | Cohort with data on FHx | | | | Subcohort with genotype data | | | |
|---|---|---|---|---|---|---|---|---|
| | Total (n = 1788) | Negative FHx (n = 1044) | Positive FHx (n = 744) | *p*-value | Total (n = 1319) | Remaining 80% MetaGRS (n = 1055) | Upper 20% MetaGRS (n = 264) | *p*-value |
| Age (mean, SD) | 68.9 [9.1] | 70.1 [9.0] | 67.1 [8.9] | **$2.754 \times 10^{-12}$* [b]** | 68.7 [9.3] | 69.1 [9.1] | 67.1 [9.7] | **0.002* [b]** |
| Male | 1244 (69.6) | 745 (71.4) | 499 (67.1) | 0.052[b] | 914 (69.3) | 748 (70.9) | 166 (62.9) | **0.012* [b]** |
| BMI (mean, SD) | 26.4 [3.9] | 26.2 [3.7] | 26.8 [4.2] | **0.002* [b]** | 26.3 [3.8] | 26.2 [3.6] | 26.7 [4.3] | 0.103[b] |
| GFR (mean, SD) | 73 [20.8] | 72.6 [21.0] | 74.1 [20.5] | 0.146[b] | 72.3 [20.1] | 72.2 [20.1] | 72.7 [20.2] | 0.744 |
| Smoking | 604 (34.0) | 341 (33.0) | 263 (35.4) | 0.286 | 456 (35.2) | 359 (34.6) | 97 (37.5) | 0.387 |
| Diabetes | 390 (21.8) | 220 (21.1) | 170 (22.8) | 0.370 | 303 (23.0) | 255 (24.2) | 48 (18.2) | **0.039* [b]** |
| Hypertension | 1306 (74.4) | 728 (71.4) | 578 (78.4) | **0.001* [b]** | 921 (72.2) | 729 (71.8) | 192 (74.1) | 0.445 |
| Hypercholesterolemia | 1162 (65.0) | 637(64.0) | 525 (73.9) | **$1.500 \times 10^{-5}$*[b]** | 806 (65.9) | 634 (65.1) | 172 (69.1) | 0.237 |
| History of CAD | 535 (29.9) | 248 (23.8) | 287 (38.6) | **$1.327 \times 10^{-11}$* [b]** | 389 (29.5) | 306 (29.0) | 83 (31.6) | 0.422 |
| History of stroke | 574 (32.1) | 365 (35.0) | 209 (28.1) | **0.002* [b]** | 437 (33.1) | 348 (33.0) | 89 (33.7) | 0.823 |
| History of PAD | 355 (19.9) | 185 (17.7) | 170 (22.9) | **0.006* [b]** | 270 (20.5) | 216 (20.5) | 54 (20.5) | 0.989 |
| Stenosis ipsilateral | | | | 0.083[b] | | | | 0.990 |
| Φ 50–70% | 132 (7.5) | 70 (6.9) | 62 (8.5) | | 83 (6.4) | 66 6.4) | 17 (6.6) | |
| Φ 70–99% | 1609 (91.9) | 941 (92.3) | 668 (91.4) | | 1200 (93.2) | 962 (93.2) | 238 (93.0) | |
| Contralateral stenosis of 50–100% | 714 (39.9) | 406 (43.1) | 308 (45.4) | 0.362 | 543 (45.4) | 425 (44.4) | 118 (49.4) | 0.168[b] |
| Presenting symptoms | | | | **0.010* [b]** | | | | 0.497 |
| Φ Asymptomatic | 228 (12.8) | 120 (11.6) | 108 (14.6) | | 178 (13.5) | 135 (12.8) | 43 (16.3) | |
| Φ Ocular | 302 (16.9) | 174 (16.8) | 128 (17.3) | | 203 (15.4) | 163 (15.5) | 40 (15.2) | |
| Φ TIA | 774 (43.3) | 437 (42.2) | 337(45.5) | | 580 (44.1) | 466 (44.3) | 114 (43.3) | |
| Φ Stroke | 472(26.4) | 304 (29.4) | 168 (22.7) | | 354 (26.9) | 288 (27.4) | 66 (25.1) | |
| Total cholesterol (median, IQR) | 4.4 [1.6] | 4.4 [1.6] | 4.4 [1.7] | 0.140[b] | 4.4 [1.6] | 4.4 [1.6] | 4.4 [1.7] | 0.716 |
| LDL cholesterol (median, IQR) | 2.4 [1.3] | 2.4 [1.3] | 2.5 [1.4] | 0.101[b] | 2.4 [1.3] | 2.4 [1.3] | 2.5 [1.4] | 0.330 |
| HDL cholesterol (median, IQR) | 1.1 [0.4] | 1.1 [0.4] | 1.1 [0.4] | 0.785 | 1.1 [0.4] | 1.1 [0.4] | 1.1 [0.4] | 0.384 |
| Triglycerides (median, IQR) | 1.5 [1.0] | 1.4 [0.9] | 1.6 [1.0] | **0.001* [b]** | 1.5 [1.0] | 1.5 [1.0] | 1.5 [0.9] | 0.491 |
| Lipid lowering drug use | 1379 (77.1) | 779 (74.6) | 600 (80.8) | **0.002* [b]** | 999 (75.9) | 795 (75.5) | 204 (77.3) | 0.547 |
| Antiplatelet drug use | 1583 (88.5) | 926 (88.8) | 657(88.5) | 0.876 | 1164 (88.5) | 928 (88.3) | 236 (89.4) | 0.617 |
| Surgery year | | | | 0.612 | | | | 0.820 |
| Φ 2002–2003 | 215 (12) | 129 (11.6) | 86 (11.6) | | 192 (14.6) | 151 (14.3) | 41 (15.5) | |
| Φ 2004–2005 | 327 (18.3) | 197 (18.9) | 130 (17.5) | | 307 (23.3) | 243 (23.0) | 64 (24.2) | |
| Φ 2006–2007 | 288 (16.1) | 156 (14.9) | 132 (17.7) | | 270 (20.5) | 214 (20.3) | 56 (21.2) | |
| Φ 2008–2009 | 241 (13.5) | 146 (14.0) | 95 (12.8) | | 232 (17.6) | 185 (17.5) | 47 (17.8) | |
| Φ 2010–2011 | 265 (14.8) | 155 (14.8) | 110 (14.8) | | 224 (17.0) | 182 (17.3) | 42 (15.9) | |
| Φ 2012–2013 | 260 (14.5) | 157 (15.0) | 103 (13.8) | | 94 (7.1) | 80 (7.6) | 14 (5.3) | |
| Φ 2014–2015 | 154 (8.6) | 82 (7.9) | 72 (9.7) | | – | – | | |
| Φ 2016 | 38 (2.1) | 22 (2.1) | 16 (2.2) | | – | – | | |

Values are displayed as n (%), unless otherwise specified. Values indicated as bold* are statistically significant. Values below $p < 0.20$ are indicated with (b).
BMI, body mass index; CAD, coronary artery disease; PAD, peripheral artery disease; eGFR, estimated glomerular filtration rate; TIA, transient ischemic attack; SD, standard deviation. When multiple cerebrovascular symptoms occurred in the six months prior to the operation, the most serious symptom counts in the following order: stroke > TIA > ocular. Ocular symptoms include transient or permanent retinal ischemia.
History of any stroke includes ipsilateral or contralateral stroke. Antiplatelet drug use includes use of aspirin, dipyridamole or any ADP-inhibitor. Lipid lowering drug use includes any lipid-lowering drug. Diabetes, hypercholesterolemia and hypertension were defined as diagnosed by a medical doctor or medication use for the specific comorbidity.

with FHx data and the sub-cohort with genotyped data (Table 1).

### 3.1. Patients with positive FHx have a higher risk of sCVE

Patients with a positive FHx (744/1788, 41.6%) were younger and had on average more cardiovascular risk factors (Table 1). During a median follow-up of 2.9 years, 418 patients (23.4%) reached the composite endpoint of sCVE (Fig. 2A) of whom 105 (5.9%) had stroke or fatal stroke, 119 (6.7%) had MI or fatal MI, 29 (1.6%) had cardiovascular death due to other causes (fatal cardiac failure, AAA rupture or sudden death) and 165 (9.2%) had a peripheral intervention or leg amputation.

Patients with positive FHx had an increased risk of sCVE compared to those without (absolute 3-year risks of 26.5% *versus* 21.2% respectively, hazard ratio (HR) 1.292, 95% confidence interval (CI), 1.066–1.566, $p = 0.009$) (Figs. 2A and 3; Supplemental Table S3). This association remained significant after correction for confounders with adjusted HR 1.287, 95%CI 1.033–1.604, $p = 0.024$ (Fig. 3 and Supplemental Table S3) and was independent of genetic predisposition as measured by MetaGRS (adjusted HR 1.397, 95%CI 1.074–1.819, $p = 0.013$, Fig. 3 and Supplemental Table S3). Sex-stratified analyses confirmed results in men (with adjusted HR after correction for confounders of 1.380, 95%CI 1.068–1.783, $p = 0.014$; adjusted HR after correction for confounders including MetaGRS of 1.513, 95%CI 1.115–2.052, $p = 0.008$). However, in women the univariate association between FHx and sCVE was not significant (unadjusted HR 1.187, 95%CI 0.822–1.171, $p = 0.360$) but multivariable analyses could not be performed because of limited power (Supplemental Table S3).

### 3.2. Patients with higher MetaGRS have a higher risk of sCVE

The MetaGRS, standardized to mean-zero and unit-variance, approximated a normal distribution in the study population (Supplemental Fig. S1). Patients in the top 20% of MetaGRS were relatively more often females, younger of age and had less often diabetes compared to the remaining 80% of the cohort (Table 1). Also, high genetic risk patients (highest quintile of MetaGRS) had higher LDL cholesterol levels compared to low genetic risk patients (lowest quintile of MetaGRS), see Supplemental Table S4. In the 3-year follow-up, a total of 326/1319 (24.7%) patients reached the composite endpoint of sCVE of whom 96 (7.3%) had stroke or fatal stroke, 85 (6.4%) had MI or fatal MI, 21 (1.6%) died of other cardiovascular causes (fatal cardiac

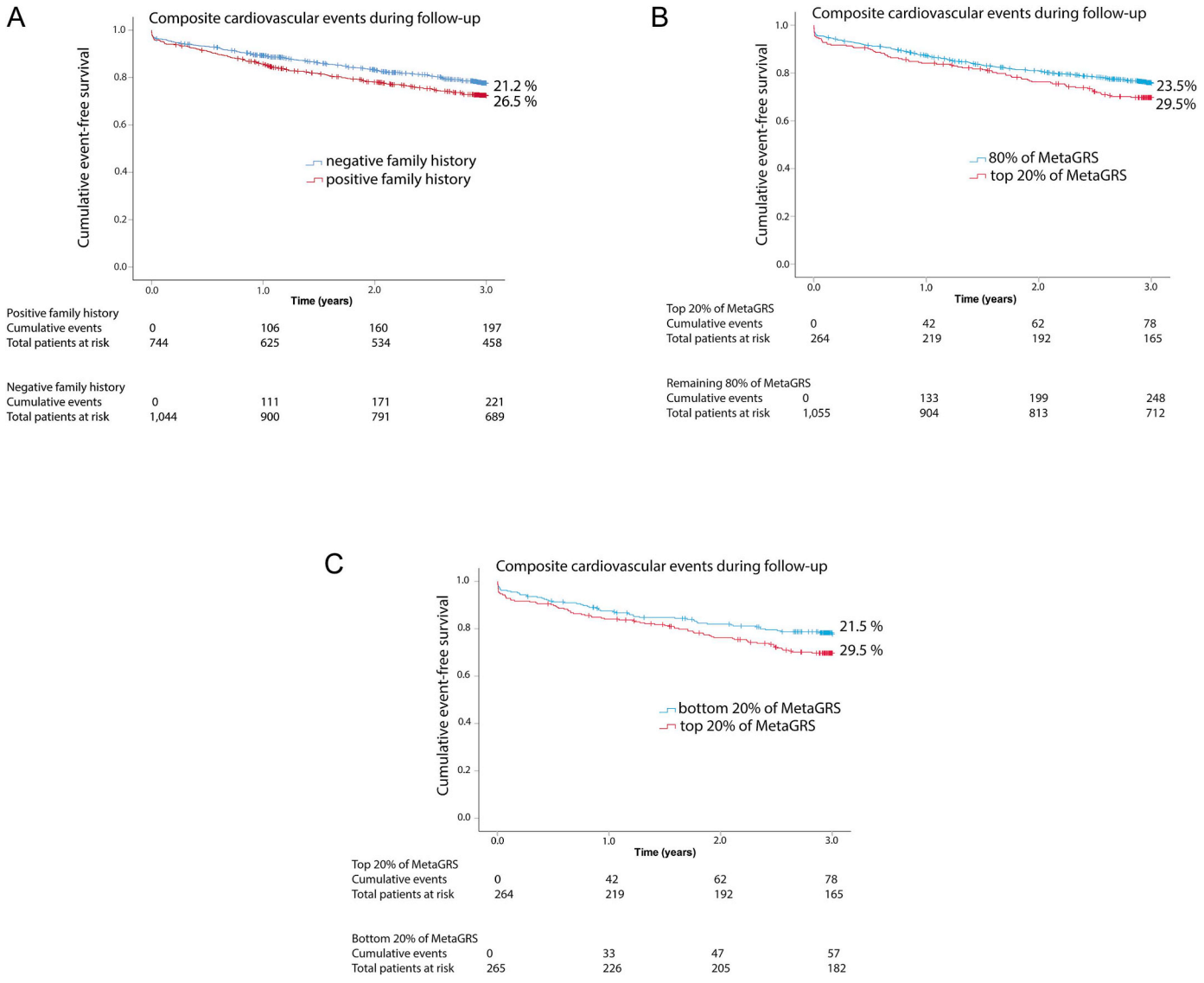


**Fig. 2.** Kaplan-Meier graphs for 3-year risk of sCVE after CEA.
The cumulative event-free survival from sCVE in years is shown for (A) patients with positive FHx compared to those without, (B) patients in the top 20% of MetaGRS compared to the rest of the patients (remaining 80%), (C) patients in the top 20% of MetaGRS compared to those in the bottom 20% of MetaGRS. Vertical lines indicate censoring.

failure, AAA rupture or sudden death) and 124 (9.4%) had a peripheral intervention or leg amputation.

Patients in the top 20% of MetaGRS had 1.4 fold increased risk of developing sCVE within the three years of follow-up when compared to the remaining 80% of the cohort (Fig. 2B, absolute 3-year risks of 29.5% *versus* 23.5% respectively, HR 1.353, 95%CI 1.047–1.749, $p = 0.021$). After adjustment for possible confounders including FHx, this association remained statistically significant (HR for top 20% 1.345, 95%CI 1.009–1.792, $p = 0.043$). We found similar results when we compared patients in the top 20% to the bottom 20% of MetaGRS (in univariate analysis with HR 1.539, 95%CI 1.086–2.181, $p = 0.015$ (Fig. 2C) and for multivariable analysis adjusted HR including FHx 1.583, 95%CI, 1.066–2.351, $p = 0.023$), and when analysing MetaGRS as a continuous quantity (adjusted for confounders with HR 1.150 per one SD increase in MetaGRS, 95%CI 1.022–1.293, $p = 0.021$, adjusted HR including FHx 1.112 per one SD increase in MetaGRS, 95% CI, 0.983–1.259, $p = 0.091$). Results are illustrated in Fig. 2B and C and 3 and in Supplemental Table S3. Confounders added to multivariable models are displayed in Supplemental Tables S1 and S2. Similar results were found in men (adjusting for confounders including FHx showed a HR 1.219 per one SD increase in MetaGRS, 95%CI 1.056–1.408, $p = 0.006$, Supplemental Table S3). In women, univariate analyses showed no significant associations between MetaGRS and sCVE (HR 0.916 per one SD increase in MetaGRS, 95% CI 0.743–1.129, $p = 0.413$), yet multivariable analysis was not possible due to lack of power (Supplemental Table S3).

### 3.3. MetaGRS is associated with vulnerable carotid plaque characteristics

To unravel possible underlying pathophysiological mechanisms of the associations between MetaGRS, FHx and CVD, we explored the impact of FHx and the MetaGRS on atherosclerotic plaque characteristics. We found no associations between histological plaque characteristics and FHx in the total cohort or in women although not all multivariable analyses could be performed (Supplemental Tables S5 and S6). However, carotid plaques from men with a positive FHx contained less collagen and less SMC compared to men with negative FHx (Supplemental Table S6). MetaGRS was associated with significantly higher overall plaque vulnerability score (regression coefficient β of 0.198 for top 20% of MetaGRS compared to the rest, 95%CI,

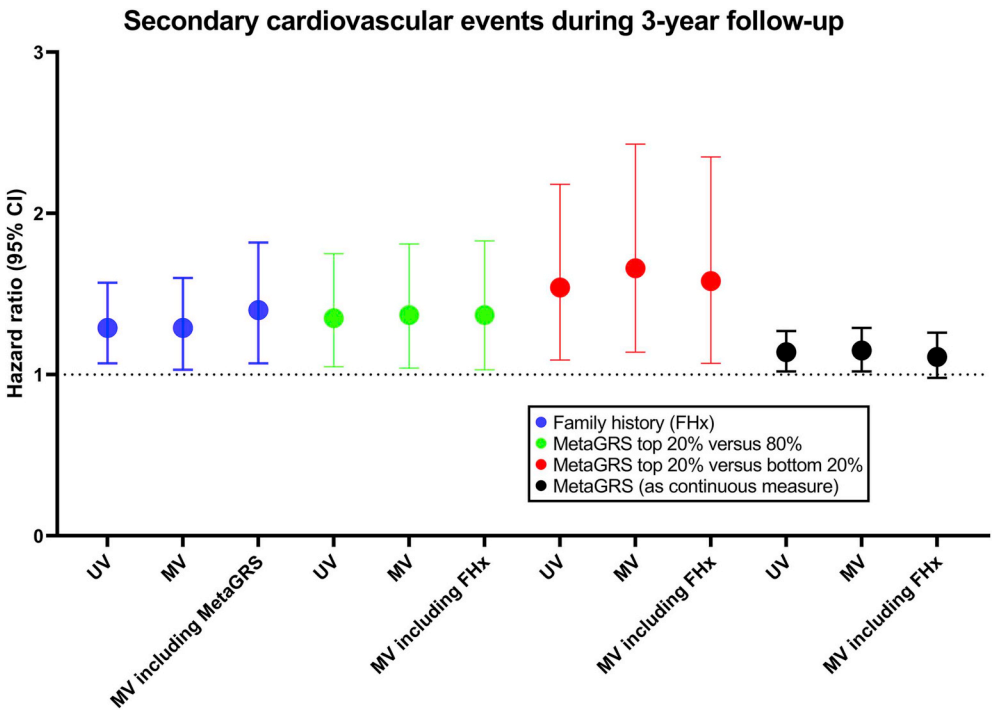


**Fig. 3.** Cox-regression analyses of FHx and MetaGRs for sCVE after CEA.
Hazard ratios (HR) and 95% confidence intervals (95% CI) for the different univariate and multivariable Cox-regression models of FHx or MetaGRS for sCVE. HR for MetaGRS as a continuous quantity indicates HR per one SD increase in MetaGRS.
UV, univariate model. The univariate model for MetaGRS included age, sex, PC1-4 and genotype array. MV, multivariable model. The multivariable model for FHx was corrected for traditional risk factors (age, sex, hypercholesterolemia, diabetes, hypertension, BMI and smoking) and additional confounders (history of CAD, history of PAD, cerebrovascular symptoms and eGFR). The univariate model for MetaGRS included age, sex, PC1-4 and genotype array. For multivariable analyses of MetaGRS, traditional risk factors (hypercholesterolemia, diabetes, hypertension, BMI and smoking) were added.

0.003–0.364, $p = 0.004$, Table 2). To determine the plaque characteristics on which this association was based, plaque characteristics were analysed separately. High genetic risk patients (in top 20% of MetaGRS) had more frequently a lipid core > 10% of total plaque area (adjusted odds ratio (OR), 1.591, 95% CI 1.105–2.291, $p = 0.013$) and more macrophage infiltration (adjusted OR 1.490, 95% CI 1.118–1.986, $p = 0.006$) compared to patients with MetaGRS in the remaining 80% (Table 2). For a lipid core > 10%, we found the same association when comparing the top 20% with the bottom 20% of the MetaGRS (adjusted HR 1.887, 95% CI, 1.188–2.997, $p = 0.007$, Supplemental Table S7). Analyses of MetaGRS as a continuous quantity confirmed the association with lipid core > 10% (adjusted OR 1.171 per SD increase in MetaGRS, 95% CI 1.026–1.337, $p = 0.019$, Supplemental Table S8). Sex-stratified analyses revealed a significant association of MetaGRS with macrophages in women (adjusted OR per SD increase in MetaGRS 1.238, 95%CI, 1.007–1.521, $p = 0.043$) while a significant association of MetaGRS with IPH was found in men (adjusted OR per SD increase 1.220, 95%CI 1.050–1.418, $p = 0.010$ Supplemental Table S9).

Because IPH has been associated with increased risk of sCVE [4], we added IPH to the multivariable models of FHx, MetaGRS and sCVE to unravel whether the association between FHx, MetaGRS and sCVE could be explained by IPH. We found that all associations of FHx and MetaGRS were independent of IPH given that adding IPH to multivariable models of sCVE did not alter the effect sizes (Supplemental Table S3).

## 4. Discussion

We validated the polygenic risk score for CAD (MetaGRS) for sCVE in a severe atherosclerotic cohort of carotid artery stenosis patients undergoing CEA. We report two key findings. First, in CEA patients FHx and MetaGRS were both independently associated with an increased risk of sCVE. Second, high MetaGRS was associated with more vulnerable atherosclerotic plaque characteristics suggesting possible underlying pathobiological mechanisms through which genetic variants could affect CVD.

Although positive FHx is a well-known risk factor for primary CVE [1,2], previous studies assessing FHx and secondary outcome are inconsistent [5,7–9,34]. In patients with first-MI [7,8,34], studies have reported a protective effect of FHx on all-cause mortality, whereas others showed an increased risk of CVE [5,9]. One can assume that patients with positive FHx are identified earlier as at-risk individuals through screening programs resulting in more intensive surveillance and preventive strategies leading to the benefit in overall survival [7]. Indeed, in our cohort, patients with positive FHx were also younger at timing of CEA. Of note, it is known that the sensitivity of self-reported FHx can be low (50%–70%) and might therefore be an unreliable estimate [35].

Our results are in line with two recent studies in CAD-patients that showed that high PRS was associated with an elevated risk of recurrent CAD events, of which one study used MetaGRS [21,22]. We now validate that MetaGRS is associated with an increased risk of sCVE in a different population consisting of CEA patients with high prevalence of other CVD comorbidities either in coronary or peripheral vascular beds. Our results therefore underscore the concept of atherosclerosis as a complex and systemic disease underlying CVD. Indeed, a 300-SNP-CAD-GRS has been previously associated with the development of stroke, peripheral artery disease (PAD) and AAA indicating shared genetic roots [36]. Furthermore, we provide mechanistic insights by showing associations of MetaGRS with plaque characteristics (lipid core and macrophages content) indicative of an unstable plaque morphology.

Interestingly, the association of MetaGRS as continuous quantity became insignificant after addition of FHx but remained significant when the highest quintile of MetaGRS was compared with the remaining patients. Although one could argue that such cut-off limits may be arbitrary, it rather indicates that the effect of PRS on sCVE is not linear but either exponential. Indeed, the exponential relationship between PRS and CVD-risk has already been shown for primary CAD-event risk [20].

Previous studies have demonstrated that PRS was associated with first- and recurrent CAD-events independent of FHx [19–21,37,38]. We now show that FHx is associated with increased risk of sCVE independent of MetaGRS. Several reasons could be hypothesized for the non-overlapping associations. MetaGRS includes common genetic variants associated with an increased risk for CAD in the general population, whilst CVD in families may arise in part from more rare genetic mutational events; thus a positive FHx captures individual yet family specific rare variation. Another explanation could be that FHx reflects

**Table 2**
Associations of carotid plaque characteristics from patients in top 20% of MetaGRS compared to the rest of the patients (80% of MetaGRS).

| Categorical plaque characteristics | 80% of MetaGRS (n = 1040) | Top 20% of MetaGRS (n = 261) | Unadjusted OR (95% CI) | Unadjusted *p*-value | Adjusted OR (95% CI) | Adjusted *p*-value |
|---|---|---|---|---|---|---|
| Moderate/heavy calcifications | 525/1038 (50.6) | 135/261 (51.7) | 1.047 (0.798–1.374) | 0.741 | 1.001 (0.754–1.330) | 0.995 |
| Moderate/heavy collagen | 826/1039 (79.5) | 211/261 (80.8) | 1.088 (0.772–1.534) | 0.629 | 1.091 (0.755–1.577) | 0.644 |
| Presence of lipid core >10% | 752/1040 (72.3) | 209/261 (80.1) | 1.539 (1.104–2.147) | **0.011*** | 1.591 (1.105–2.291) | **0.013*** |
| Presence of IPH | 624/1039 (60.1) | 160/261 (61.3) | 1.054 (0.798–1.392) | 0.713 | 1.112 (0.821–1.506) | 0.493 |
| Moderate/heavy smooth muscle cells | 736/1037 (71.0) | 185/260 (71.2) | 1.009 (0.747–1.362) | 0.954 | 0.908 (0.652–1.265) | 0.570 |
| Moderate/heavy macrophages | 545/1036 (52.6) | 161/260 (61.9) | 1.465 (1.109–1.936) | **0.007*** | 1.490 (1.118–1.986) | **0.006*** |
| **Continuous plaque characteristics** | | | **Unadjusted beta (95% CI)** | **Unadjusted *p*-value** | **Adjusted beta (95% CI)** | **Adjusted *p*-value** |
| Percentage of macrophage staining (median, IQR) | 0.3 [1.0] | 0.5 [1.1] | 0.058 [-0.006-0.123] | 0.077 | 0.055 (−0.012–0.121) | 0.107 |
| Percentage of SMC staining (median, IQR) | 1.4 [2.3] | 1.3 [2.3] | −0.010 [-0.095-0.076] | 0.822 | −0.056 (−0.143–0.031) | 0.209 |
| Microvessels (median, IQR) | 7.0 [7.0] | 7.3 [7.0] | 0.088 [-0.021-0.197] | 0.114 | 0.072 (−0.037–0.182) | 0.196 |
| Plaque vulnerability score | 2.0 [2.0] | 3 [1] | 0.216 (0.048–0.385) | **0.012*** | 0.198 (0.003–0.364) | **0.004*** |

Values are displayed as n/total (%), unless otherwise specified. Values indicated as bold* are statistically significant.OR, odds ratio; IPH, intraplaque haemorrhage.
Unadjusted OR indicates univariate analysis, adjusted OR indicates multivariable analysis. Multivariable analyses were corrected for age, sex, surgery year, cerebrovascular symptoms and additional confounders showing $p < 0.20$ association with MetaGRS and the plaque characteristics of interest. Confounders included in various multivariable models can be found in Supplemental Table S2.

not only genetic factors but also non-genetic factors. Although we corrected for traditional risk factors, other environmental factors that were not taken into account such as social economic status or nutrition patterns could still be attributable to the risk of secondary events.

We found that MetaGRS was associated with a more rupture-prone atherosclerotic plaque displayed by a higher plaque vulnerability score caused by more fat, IPH (in men) and macrophages (predominantly in women). Moreover, carotid plaques from men with positive FHx were associated with less SMC and less collagen, whereas the association in women remains unclear. Similarly, a previous AE study showed that PRSs constructed based on summary statistics from a GWAS on CAD using increasingly liberal *p*-value thresholds were correlated with more fat, whereas large-artery stroke-PRSs were correlated with more IPH and SMC [18]. However, the current MetaGRS is the result of a meta-analytic approach to identify 1.7 million variants capturing information from the full genome for CAD and was internally and externally validated making the MetaGRS more generalizable [19,22]. Thus, the results presented here provide more evidence supporting the view that genetic variants could mediate their effect on CVD by influencing atherosclerotic plaque composition and morphology. Interestingly, IPH has been associated with sCVE in men independent of other clinical risk factors in our biobank [4,39], yet we found that the associations of MetaGRS, FHx and sCVE were independent of IPH. It therefore remains unclear which is the exact underlying mechanism through which MetaGRS and FHx exert their increased sCVE risk. A previous study investigating a 50-CAD-SNP-GRS and positive FHx found that only small proportions (<8%) of the effects were mediated through known metabolic pathways such as blood lipids and hypertension while the majority (>80%) was not [40]. Although CAD variants have been linked to pathways involved in atherosclerosis [13], most CAD variants are situated outside protein-coding regions with unknown functions, making them hard to map to pathophysiological mechanisms [13,41]. Future studies should explore exact pathophysiological mechanisms of how the genetic variants of MetaGRS influence plaque destabilization and CVE, for example through deep-phenotyping of atherosclerotic plaque characteristics by quantitative computerized analysis [42] and mapping MetaGRS loci to specific CVE (MI, stroke or PAD). In addition, potential sex-differences should be investigated.

Although PRS could identify high-risk patients for CVE, one could conclude that due to their unfavourable genetic risk, the CVE risk is unchangeable. However, previous studies have suggested that high genetic risk is modifiable by lifestyle interventions or medication, thus not deterministic *per se* [21,43]. Among individuals with high genetic risk (top 20% of CAD-GRS), those adhering to a healthy lifestyle had lower risk of a first CAD event compared to those with an unfavourable lifestyle [43]. Moreover, *post-hoc* analyses of secondary prevention trials investigating statins [37,44] and PCSK-9 inhibitor [21] showed that high genetic risk patients had a greater absolute and relative risk reduction of recurrent CAD events than those with lower genetic risk, despite equal LDL-level reductions. Prospective studies and implementation studies are needed to confirm PRS as a useful tool to predict the benefit of preventive medications and for selecting those patients that benefit most from such add-on therapies.

Admittedly, our study has several limitations. First, although our results may suggest interesting sex differences as we only observed the independent association of MetaGRS and FHx for sCVE in men, the association in women remains unclear because we were underpowered for multivariable analysis. Second, we did not have data regarding medication use during follow-up nor therapy compliance, which could have interfered with observed sCVE rates. Second, owing to limited power, we were unable to assess associations with separate CVE or determine the predictive value of MetaGRS above clinical risk factors. Last, most included patients are of European ancestries and generalizability to other ethnicities needs further attention. Yet, the Athero-Express Biobank is unique in its scope and major strengths are the unique population that is relatively unexplored in the field of FHx and

PRS, and the extensive data on plaque morphology that enable us to identify putative pathological mechanisms.

In the future, PRS may be a useful tool for personalized risk prediction for primary or secondary CVE. Adding MetaGRS to a model with traditional risk factors improved prediction of first CAD events, although model improvement was modest [19]. The clinical utility of PRS for sCVE is still unknown because the incremental value of PRS above clinical factors still needs to be established. One study suggested an added predictive value of PRS above clinical factors but did not include FHx [45], whereas other studies failed to demonstrate this [46–48]. The power of these studies may have been limited due to the limited number of CVE. Pooling data of several cohorts, including detailed data on preventive strategies and medications during follow-up, together with use of uniform outcome definitions for sCVE and uniform PRS composition, could help elucidate the clinical value of PRS, for example within international collaborations such as the GENIUS-CHD Consortium [49]. Furthermore, possible sex differences in the role of risk prediction with PRS need to be further elucidated.

In conclusion, both higher MetaGRS and positive FHx were independently associated with increased risk of sCVE in CEA patients. Higher MetaGRS was also associated with more vulnerable atherosclerotic plaque characteristics indicating possible underlying mechanisms of how genetic variants influence CVD. PRS could identify high-risk individuals and may help select future study populations when investigating new therapeutic CVD prevention strategies.

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## CRediT authorship contribution statement

**Nathalie Timmerman:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Dominique P.V. de Kleijn:** Writing - review & editing, Funding acquisition, Supervision. **Gert J. de Borst:** Writing - review & editing, Funding acquisition, Supervision. **Hester M. den Ruijter:** Writing - review & editing. **Folkert W. Asselbergs:** Writing - review & editing. **Gerard Pasterkamp:** Investigation, Resources, Writing - review & editing, Funding acquisition. **Saskia Haitjema:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Sander W. van der Laan:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Supervision, Writing - original draft, Writing - review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2020.04.013.

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