

ORIGINAL ARTICLE



Plasma Protein Profile Associated With a Family History of Early-Onset Coronary Heart Disease

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BACKGROUND: Proteins linked to heritable coronary heart disease (CHD) could uncover new pathophysiological mechanisms of atherosclerosis. We report on the protein profile associated with a family history of early-onset CHD and whether the relation between proteins and coronary atherosclerotic burden differs according to family history status, as well as inferences from Mendelian randomization.

METHODS: Data on coronary atherosclerotic burden from computed tomography angiography and Olink proteomics were retrieved for 4521 subjects, free of known CHD, from SCAPIS (the Swedish Cardiopulmonary Bioimage Study). Records of myocardial infarction and coronary revascularization therapies in any parent or sibling of subjects were retrieved from national registers. Linear associations between family history and proteins were adjusted for age, sex, and study site. Statistical interactions between proteins and family history for the association between proteins and the coronary atherosclerotic burden were also studied. Mendelian randomization for causal associations between proteins and CHD was performed with genome-wide association study summary data from UKB-PPP (UK Biobank Pharma Proteomics Project), CARDIoGRAMplusC4D, and FinnGen.

RESULTS: Of 4251 subjects, family history of early-onset CHD was present in 9.5%. Thirty-eight proteins, with biological features of inflammation, lipid metabolism, and vascular function, were associated with family history using a false discovery rate of 0.05. The strongest associations were observed with cathepsin D, paraoxonase 3, renin and follistatin, neither of which was attenuated by adjusting for cardiovascular risk factors. Eighteen proteins were statistical interactors with family history in the association between each protein and the coronary atherosclerotic burden, most notably the LDL (low-density lipoprotein) receptor, transferrin receptor protein 1, and PECAM1 (platelet endothelial cell adhesion molecule 1). In 2-sample Mendelian randomization, a novel association was found for follistatin and myocardial infarction, and previous associations for PCSK9 (proprotein convertase subtilisin/kexin type 9) and PECAM1 were repeated.

CONCLUSIONS: These findings highlight new potential mechanisms for heritable and general atherosclerosis.

Key Words: cardiovascular diseases ■ coronary artery disease ■ follistatin ■ Mendelian randomization analysis ■ myocardial infarction

Having a family history of coronary heart disease (CHD) is a well-established risk factor for CHD, believed to represent the composite effect of familial genetic and environmental risk factors.^{1–3} Although a proportion of CHD heritability can be explained by risk factors, such as dyslipidemias or CHD polygenic risk

scores, a significant proportion is yet unexplained and may uncover new pathophysiological mechanisms in CHD. Recent advances in large-scale analysis of proteomics have led to new insights into mechanisms of atherosclerosis.⁴ We sought to identify circulating proteins associated with a family history of early-onset CHD

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Nonstandard Abbreviations and Acronyms	
CACS	coronary artery calcium score
CCL16	C-C motif chemokine 16
CCTA	coronary computed tomography angiography
CHD	coronary heart disease
CTSZ	cathepsin Z
CTSD	cathepsin D
FDR	false discovery rate
GDF-15	growth/differentiation factor 15
HDL	high-density lipoprotein
IL	interleukin
IL-1RA	interleukin-1 receptor antagonist protein
IV	instrumental variable
LDL	low-density lipoprotein
LPL	lipoprotein lipase
MGR	Multi-Generation Register
MMP	matrix metalloproteinase
MR	Mendelian randomization
NPX	normalized protein expression
PCSK9	proprotein convertase subtilisin/kexin type 9
PECAM1	platelet endothelial cell adhesion molecule
PON3	PARAOXONASE 3
pQTL	protein quantitative trait loci
REN	renin
SBP	systolic blood pressure
SCAPIS	Swedish Cardiopulmonary Bioimage Study
SCF	stem cell factors
SIS	segment involvement score
tPA	tissue-type plasminogen activator
TR	transferrin receptor protein 1
UKB-PPP	UK Biobank Pharma Proteomics Project

and to further study the relationship between identified proteins and coronary atherosclerotic burden in subjects with and without a family history of CHD. In addition, we aimed to identify causal roles of associated proteins in coronary atherosclerosis by using 2-sample Mendelian randomization (MR).

METHODS

Population

This was a study of the circulating plasma proteome in a middle-aged cohort from SCAPIS (the Swedish Cardiopulmonary Bioimage Study).⁵ Individuals aged between 50 and 64 years were invited to participate in the prospective SCAPIS cohort, based on a random sample from the Swedish census register

between 2013 and 2018, at 6 participating University sites in Sweden (Gothenburg, Linköping, Malmö/Lund, Stockholm, Umeå, and Uppsala). The only exclusion criterion for SCAPIS was inability to understand spoken and written Swedish for the informed consent and study questionnaire. In total, 30 154 individuals were ultimately included in SCAPIS and underwent a comprehensive clinical examination, including physical anthropometry, health questionnaire, fasting blood sampling, and coronary computed tomography angiography (CCTA). We included subjects from the SCAPIS cohort who gave explicit consent for linkage of their data to other registers and who had fulfilled the CCTA protocol, as well as the Olink biomarker analysis. We also excluded subjects reporting previous CHD in the form of myocardial infarction, percutaneous coronary intervention, or coronary artery bypass grafting in the questionnaire. The implementation of the original SCAPIS study, as well as subsequent analyses in this article, were approved by the Regional Ethical Board at Umeå University (2010-228-21M, 2017-183-31) and the Swedish Ethical Review Authority (2021-02951, 2022-04143-02). Because of the sensitive nature of the data collected for this study, requests to access the data sets from qualified researchers trained in human subject confidentiality protocols may be sent to the SCAPIS organization at <https://scapis.org>.

Clinical Covariates

At the SCAPIS core clinical examination, body weight and height were measured with light clothing. Systolic blood pressure was assessed with an automatic device, and the mean value of 2 measurements was reported in the study protocol. Smoking status was assessed with an oral question before laboratory sampling, classified as either smoker or nonsmoker. Previous medical history, as well as usage of lipid-lowering and antihypertensive medications, were also self-reported in the questionnaire. A venous blood sample was drawn after an overnight fast for immediate analysis as well as for biobank storage for all SCAPIS participants. Analyses of total, HDL (high-density lipoprotein), and LDL (low-density lipoprotein) cholesterol were made at the local laboratory of each participating site. For a subgroup of 5075 participants, samples were sent for commercial proteomic biomarker analysis by Olink Proteomics (Uppsala, Sweden), with 2 predefined target panels comprising 184 biomarkers. Each participating site in the SCAPIS study included 825 to 850 samples for proteomic analysis. Participant samples were eligible for proteomic analysis only if complete informed consents regarding biobanking, register linkage and data use for future studies were present, smoking data from the questionnaire was completed and if the participant had performed computed tomography imaging of the chest, ultrasound of the carotid arteries, body accelerometry and CCTA (noncontrast CCTA due to an estimated glomerular filtration rate <50 mL/min was also considered eligible). The first participants to complete the full examination protocol were consecutively included from each site until samples from 850 (825 for Stockholm) participants were collected for proteomic analysis. Baseline characteristics of the SCAPIS cohort according to Olink analysis participation are presented in Table S1. Participants included for the Olink analysis had a marginally lower BMI and slightly higher systolic blood pressure than the remaining participants from the

SCAPIS cohort; otherwise, the groups were similar. The full scope of clinical examinations in SCAPIS has been described previously.⁵

Coronary Imaging

In study participants without contraindication to iohexol contrast administration, an oral or intravenous β -blocker, as well as sublingual nitroglycerin, was administered to study subjects with a heart rate above 60 bpm or a systolic blood pressure above 110 mmHg before scanning. Coronary imaging was performed using a dual-source computed tomography scanner (Somatom Definition Flash, Siemens Healthcare, Germany) with iohexol contrast medium at 325 mg I/kg body weight. In noncontrast image sets, coronary artery calcium was identified and scored in Agatston Units using syngo.via software (Volume Wizard; Siemens). The coronary artery calcium score (CACS) was categorized into groups of 0, 1 to 10, 11 to 100, 101 to 400, and >400. Five different CCTA scanning protocols were used, chosen according to baseline CACS, heart rate, and subject body weight. Contrast CCTA image sets were visually examined for coronary atherosclerosis by trained radiologists or cardiologists at the 6 participating sites and reported per segment using a model of 18 anatomical coronary segments.⁶ The degree of coronary atherosclerosis was reported according to the degree of luminal stenosis as well as according to the presence of plaques, or nonassessable due to either the degree of plaque calcification or technical artifacts, for each coronary segment. A segment involvement score (SIS) was calculated as the sum of diseased coronary segments, regardless of the degree of luminal stenosis.⁷

Analysis of Circulating Proteins

Analyses were performed by Olink Proteomics (Uppsala, Sweden), using 2 predefined Target panels (cardiovascular disease II and cardiovascular disease III), comprising 184 protein biomarkers selected by the vendor. Olink provides a proximity extension assay, where pairs of proximity extension assay probes with specific affinity for each target protein are equipped with an oligonucleotide sequence. When 2 probes bind to the target protein, the oligonucleotides hybridize and form a DNA template, which is then amplified by a DNA polymerase and quantified using quantitative real-time polymerase chain reaction. The quantitative real-time polymerase chain reaction readout for each target protein is proportional to the initial concentration in the sample. Biomarker quantitative real-time polymerase chain reaction readouts are provided as Normalized Protein Expression (NPX) data on the log₂ scale, an arbitrary unit derived from quantitative real-time polymerase chain reaction cyclic threshold values that are compared with sample means from quality control markers in each sample plate. Four internal controls were added to each sample plate. Quality control was not passed if the standard deviations of internal controls in a sample plate exceeded 0.2 NPX, as were samples for which the deviation from the median value of the controls exceeded 0.3 NPX. Samples that did not pass these quality controls were not included in the statistical analyses. As the NPX value is normalized for each protein signal, high NPX values correspond to higher concentrations of the target protein; however, absolute NPX values cannot be compared across samples of different proteins.

Register Linkages and Exposure Definition

By means of unique personal identification numbers, issued to residents by the Swedish Tax Agency,⁸ data on study participants were linked to national registers of kinship and diseases. First-degree relatives of study participants were identified in the Swedish MGR (Multi-Generation Register), containing information on relatives of a majority of individuals born in 1932 and later, resident in Sweden from January 1, 1962, and onward.⁹ Data on relatives were linked to the National Patient Register and the Cause of Death Register, managed by the Swedish National Board of Health and Welfare, to identify registered manifestations of CHD in relatives. These registers include data on hospital admissions with registered main diagnoses according to the *International Classification of Diseases*, as well as registered causes of death according to the *International Classification of Diseases*. Subjects missing information from any parent in the MGR were excluded from further analyses. Family history of early-onset CHD was defined as having at least 1 parent or sibling with a register-verified hospitalization or death due to a composite of either myocardial infarction or angina pectoris with any coronary revascularization procedure, occurring before the age of 65 years,¹⁰ in both sexes.^{11,12} The corresponding *International Classification of Diseases, Tenth Revision*, and historical codes for CHD manifestations in relatives are provided in [Table S2](#).

Statistical Analysis in Biomarker Identification

Data management and statistical analyses were performed in STATA 16.1 (StataCorp, College Station, TX) and R (R version 4.3.1, The R Foundation). Baseline characteristics were tested for equality between individuals with and without a family history of early-onset CHD with Pearson's χ^2 test for categorical variables, independent *t* tests for normally distributed continuous variables, and Mann-Whitney *U* test for non-normally distributed continuous variables. Associations between a family history of early-onset CHD as the independent variable and plasma levels of each protein as the dependent variable were tested using linear regression with covariate adjustment for age, sex, and study site. To account for multiple testing, the Benjamini-Hochberg method of a false discovery rate (FDR) was used. The Benjamini-Hochberg critical values were calculated using an FDR of 0.05, and a family history of early-onset CHD was considered to have a significant effect on a protein only if the regression coefficient's *P* value was lower than the respective critical value. It is henceforth noted as at FDR<0.05 if this condition was met. Regression and correlation coefficients for associations significant at FDR<0.05 were illustrated in forest plots, volcano plots, and heat maps. The model was then subsequently adjusted for other cardiovascular risk factors, including linearly for HDL-cholesterol, LDL-cholesterol, systolic blood pressure, and BMI, as well as for the use of lipid-lowering or antihypertensive medication, smoking status, and presence of diabetes. Thereafter, the linear association between each biomarker as the independent variable and SIS as well as CACS as dependent variables were assessed with linear regression, adjusted for age, sex, and study site in a basic model and in an adjusted model with other cardiovascular risk factors. Additionally, a third model included statistical interaction terms between family history of early-onset CHD and each biomarker. The association between biomarkers and SIS was also examined in subgroups of family

history status. Lastly, the association between a family history of early-onset CHD as the independent variable and SIS as the dependent variable was examined with linear regression. The same analyses were performed for CACS. The power for tests of interactions in the regression models for SIS and CACS was estimated using the InteractionPowerR package in R,¹³ and presented in [Figures S1 and S2](#).

Two-Sample MR for CHD

A 2-sample MR approach was used to explore possible causal relationships between biomarkers and CHD to further examine the pathophysiology of heritable CHD. For the proteins that were significantly associated with family history of early-onset CHD or that were among the top 20 proteins associated with SIS in subjects with positive family history, or those that were significant statistical interactors in the relationship between family history and SIS, we systematically searched for genetic instrumental variables (IV) for MR. Protein quantitative trait loci (pQTL) for each of the selected proteins, with an F statistic of at least 50, were identified from the UKB-PPP (UK Biobank Pharma Proteomics Projects), in which GWAS for an abundance of circulating proteins has been performed in 54 219 individuals.¹⁴ We used only the sentinel cis-pQTL with the lowest *P* value ($P < 1.7 \times 10^{-11}$) from the complete UKB-PPP cohort for each protein as IVs to reduce the risk of horizontal pleiotropy. For proteins of which the sentinel cis-pQTL was a multiallelic variant in the complete cohort, the meta-analysis statistic from the discovery and replication cohort was used if it was a biallelic variant. If the cis-pQTL was also multiallelic in the discovery and replication cohorts, a biallelic single nucleotide polymorphism with the lowest *P* value within a 1 Mb window of the coding gene from the combined cohort was used. Outcome data on CHD and myocardial infarction from the CARDIoGRAMplusC4D 1000 Genomes project have been contributed by CARDIoGRAMplusC4D investigators and have been downloaded from www.CARDIOGRAMPLUSC4D.ORG, including 60 801 cases and 123 504 controls.¹⁵ Furthermore, outcome data on myocardial infarction were also retrieved from the FinnGen R10 populations study release, including 26 060 cases and 360 108 controls.¹⁶ The Wald ratio was used to calculate a causal estimate of the association between each protein and myocardial infarction. Causal estimates of association between genetic determinants of proteins and myocardial infarction from CARDIoGRAMplusC4D and FinnGen R10 outcome data were subsequently combined in meta-analysis using a fixed-effects model. The limit of significance was Bonferroni adjusted to $P = 0.001389$. The pQTLs included as IVs in the analysis are provided in [Table S3](#).

RESULTS

Biomarker Identification

In total, 4251 subjects free of self-reported CHD were included from SCAPIS. A family history of early-onset CHD was present in 405 (9.5%) subjects. The exclusion process is visualized in [Figure 1](#). Baseline characteristics according to family history status are presented in [Table 1](#). Subjects with a family history of early-onset CHD were younger and more commonly female. The levels of LDL-cholesterol were largely the same across family

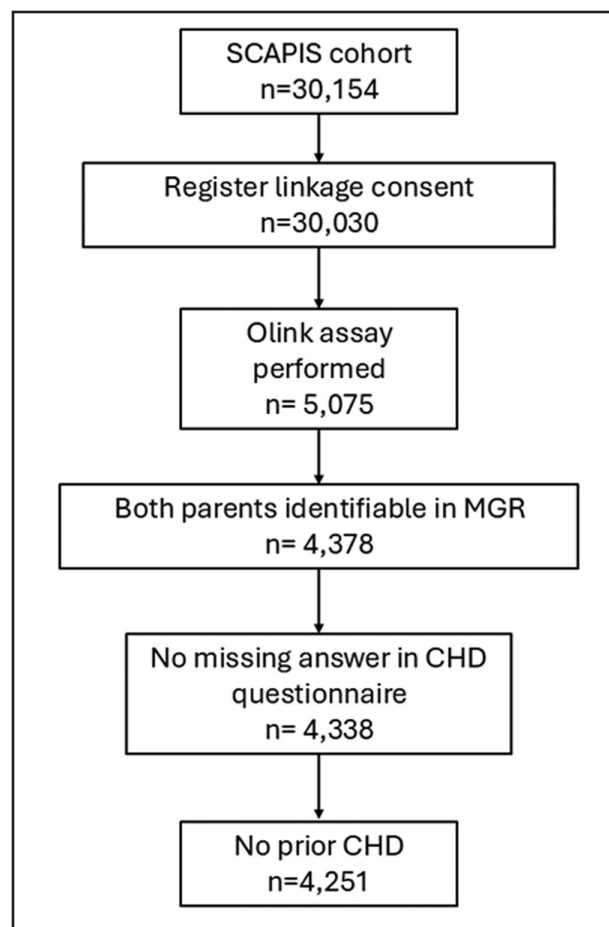


Figure 1. The exclusion process.

Flow chart of participants in each step of the exclusion process. CHD indicates coronary heart disease. MGR, Multi-Generation Register; and SCAPIS, Swedish Cardiopulmonary Bioimage Study.

history groups; however, family history positive subjects were more often on a lipid-lowering therapy, which could explain the lack of differences in LDL-cholesterol levels. Descriptives of subjects excluded due to known CHD are presented in [Table S4](#).

In a basic model adjusted for age, sex, and study site, a total of 38 biomarkers were significantly associated with family history of early-onset CHD using an $FDR < 0.05$, presented in [Figure 2A](#) and [Table 2](#). All protein associations with a family history of early-onset CHD are listed in [Table S5](#). After adjusting for cardiovascular risk factors, 26 biomarkers remained significantly associated with family history of early-onset CHD at $P < 0.05$, as presented in [Figure 2B](#). The beta coefficients of cardiovascular risk factors in the fully adjusted model are illustrated in a heatmap in [Figure 2C](#). PON3 (paraoxonase 3), SCF (stem cell factor), and LPL (lipoprotein lipase) were negatively associated with family history of early-onset CHD, whereas some of the biomarkers with the strongest positive correlation with family history were CTSD (cathepsin D), REN (renin), IL-1RA (interleukin-1 receptor antagonist protein), and follistatin.

Table 1. Subject Characteristics According to Family History Status

	Family history of early-onset CHD (n=405)	No family history of early-onset CHD (n=3846)	P value
Female	233 (57.5%)	1945 (50.6%)	0.008
Age, y	57.1 (4.3)	57.6 (4.3)	0.050
Site			0.13
Göteborg	62 (15.3%)	626 (16.3%)	
Linköping	88 (21.7%)	678 (17.6%)	
Malmö	61 (15.1%)	574 (14.9%)	
Stockholm	56 (13.8%)	607 (15.8%)	
Umeå	82 (20.2%)	689 (17.9%)	
Uppsala	56 (13.8%)	672 (17.5%)	
Smoking status			0.82
Former smoker	143 (35.3%)	1413 (36.7%)	
Never smoked	207 (51.1%)	1968 (51.2%)	
Current smoker	49 (12.1%)	410 (10.7%)	
Missing	6 (1.5%)	55 (1.4%)	
History of diabetes	27 (6.7%)	127 (3.3%)	<0.001
BMI, kg/m ²	27.17 (4.57)	26.59 (4.16)	0.008
HDL cholesterol, mg/dL	61.9 (18.9)	65.7 (18.9)	0.38
LDL cholesterol, mg/dL	131.4 (37.9)	131.4 (36.3)	1.00
Total cholesterol, mg/dL	216.5 (41.8)	216.5(40.6)	0.75
Triglycerides, mg/dL	106.3 (38.1)	97.4 (34.5)	0.001
Lipid-lowering medication	53 (13.1%)	266 (6.9%)	<0.001
Antihypertensive medication	93 (23.0%)	728 (18.9%)	0.050
Systolic blood pressure, mm Hg	129 (16.7)	127 (16.7)	0.11
SIS	1.56 (2.23)	1.05 (1.78)	<0.001
Moderate to severe coronary atherosclerosis (SIS≥4)	83 (20.5)	379 (9.8)	<0.001

Values are n (%) or mean (SD). BMI indicates body mass index; CHD, coronary heart disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and SIS, segment involvement score.

Mean SIS was 1.56 (±2.23 SD) in subjects with a family history of early-onset CHD, and 1.04 (±1.78 SD) in subjects without a family history. The distribution of SIS scores is shown in [Table S6](#). Family history of early-onset CHD was significantly associated with SIS, with an age, sex, and site-adjusted SIS of 0.64 units higher in subjects with family history as compared with those without family history [95% CI, 0.46–0.81; $P=1.4\times10^{-12}$]. Overall, 79 proteins were associated with SIS in a basic model at $FDR<0.05$ out of which 28 were also associated with a family history of early-onset CHD. The associations between each protein and SIS were also modeled in subgroups according to family history status, presented using a standardized P value¹⁷ in [Figure 3](#) and [Table S7](#). In subjects with a family history of early-onset CHD, a strong negative association with SIS was

observed for PON3, whereas strong positive associations were observed for LDL receptor, MMP (matrix metallo-proteinase) 12, and tPA (tissue-type plasminogen activator). There was also a positive relationship between TR (transferrin receptor protein 1) and SIS in subjects with a family history, whereas the association was nonsignificant with a negative coefficient for subjects without a family history. Notably, among the proteins associated with family history of early-onset CHD, some were, however, not significantly associated with SIS in subjects with positive family history, such as SCF, LPL, REN, and GDF-15 (growth/differentiation factor 15). The 20 proteins with the strongest association with SIS at $FDR<0.2$ in each subgroup are shown in [Figure S3](#). Similarly, 85 proteins were associated with CACS adjusted for age and sex at $FDR<0.05$, presented in [Table S8](#) and [Figure S4](#). There was substantial overlap with the analysis of SIS, also in analyses according to family history status, with largely the same proteins identified.

To test whether the associations between circulating proteins and coronary atherosclerotic burden differed between subjects according to family history status, interaction tests between family history and each protein in the association between proteins and SIS were performed. Significant statistical interactions were observed for 18 proteins at $P<0.05$, shown in [Figure 4](#), [Figure S5](#), and [Table S9](#). In subjects with family history, IL (interleukin)-1, LPL, and PON3 had a steeper inverse association with SIS, whereas circulating LDL receptor, TR, tPA, CTSD, CTSZ (cathepsin Z), and PECAM1 (platelet endothelial cell adhesion molecule 1) showed a steeper positive association with SIS. A corresponding interaction analysis was performed for proteins, family history, and CACS, of which 6 proteins were significant interactors with family history, presented in [Figure S6](#). Aside from the protein brother of cell adhesion molecule-related/down-regulated by oncogenes, showing a steeper inverse association with CACS in subjects with family history, all identified interactors were also interactors with family history in the association with SIS.

Two-Sample MR for CHD

Proteins that were associated with family history of early-onset CHD, or among the top 20 proteins most strongly associated with SIS in subjects with family history, or that were significant interactors with family history in the relationship with SIS were selected for 2-sample MR. In total, 36 proteins that had strong genetic instruments available in data from the UKB-PPP¹⁴ were used in the analysis. At a Bonferroni adjusted $P<0.001389$, 3 proteins showed potential causal associations with myocardial infarction in the meta-analyzed Mr MR odds ratios for myocardial infarction are shown in [Figure 5](#) and [Table S10](#). PECAM1, PCSK9 (proprotein convertase subtilisin/kexin type 9), and follistatin showed evidence of

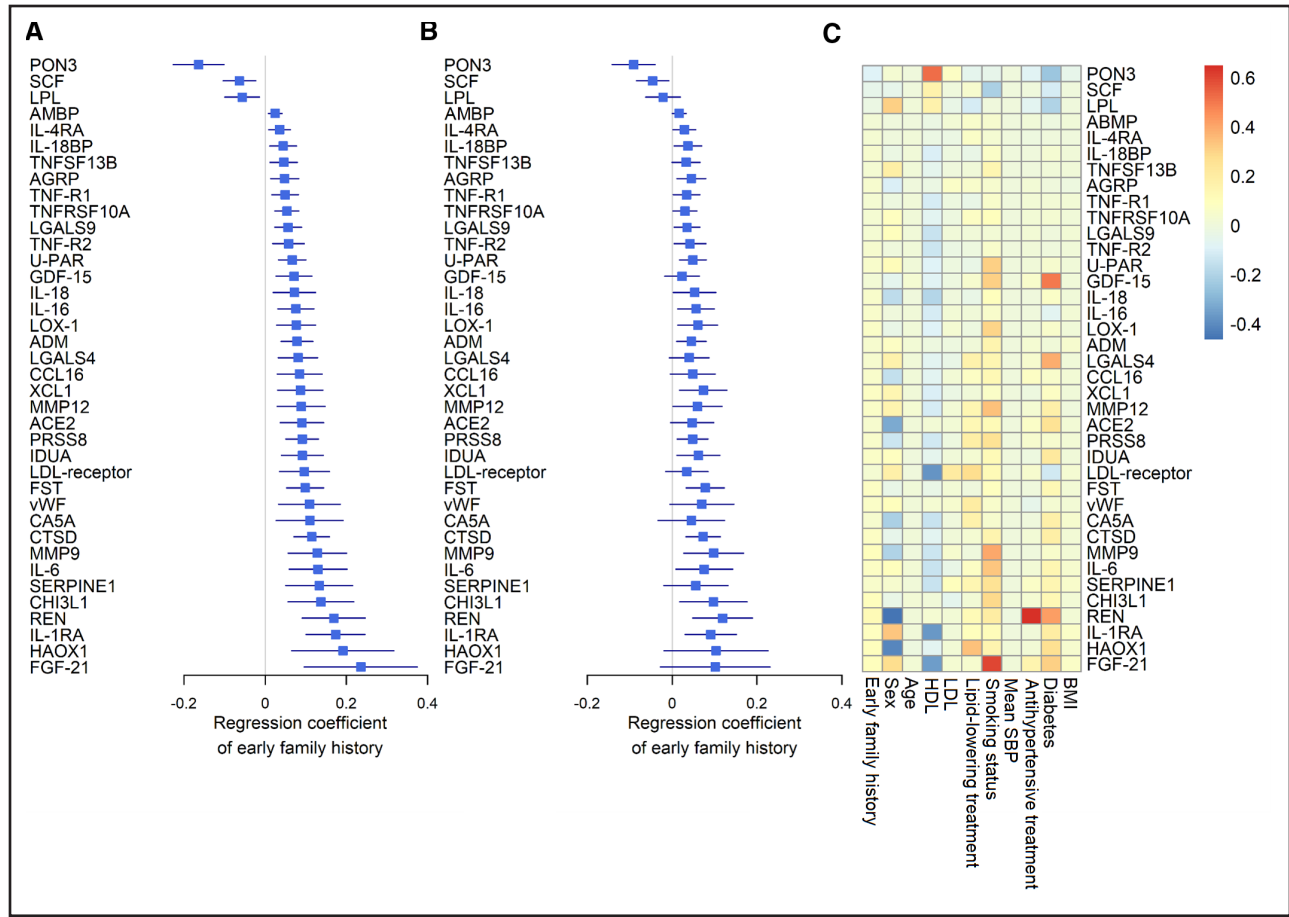


Figure 2. Proteins significantly associated with a family history of early-onset coronary heart disease. **A**, Regression coefficients and 95% CIs of family history associated with each protein, adjusted for age, sex, and study site, significant using a false discovery rate of <0.05. **B**, Regression coefficients and 95% CIs of family history associated with each protein, additionally adjusted for body mass index (BMI), diabetes, antihypertensive treatment, mean systolic blood pressure (SBP), smoking status, lipid-lowering treatment, LDL (low-density lipoprotein)-cholesterol, and HDL (high-density lipoprotein)-cholesterol. **C**, Heat map of the beta coefficients of each cardiovascular risk factor in the fully adjusted model. AMBP indicates protein alpha-1-microglobulin/bikunin precursor; ACE2, angiotensin-converting enzyme 2; ADM, proadrenomedullin; AGRP, Agouti-related protein; CA5A, carbonic anhydrase 5A, mitochondrial; CCL16, C-C motif chemokine 16; CHI3L1, chitinase-3-like protein 1; CTSD, cathepsin D; FDR, false discovery rate; FGF-21, fibroblast growth factor 21; FST, follistatin; GDF-15, growth/differentiation factor 15; HAOX1, hydroxyacid oxidase 1; IDUA, alpha-L-iduronidase; IL-6, interleukin-6; IL-16, prointerleukin-16; IL-18, interleukin-18; IL-18BP, interleukin-18-binding protein; IL-1RA, interleukin-1 receptor antagonist protein; IL-4RA, interleukin-4 receptor subunit alpha; LDL, low-density lipoprotein; LGALS4, galectin-4; LGALS9, galectin-9; LOX-1, lectin-like oxidized LDL receptor 1; LPL, lipoprotein lipase; MMP-12, matrix metalloproteinase 12; MMP9, matrix metalloproteinase 9; PON3, paraoxonase; PRSS8, prostatic serine protease 8; REN, renin; SCF, stem cell factor; SERPINE1, plasminogen activator inhibitor 1; TNF-R1, tumor necrosis factor receptor 1; TNF-R2, tumor necrosis factor receptor 2; TNFRSF10A, tumor necrosis factor receptor superfamily member 10A; TNFRSF13B, tumor necrosis factor receptor superfamily member 13B; U-PAR, urokinase plasminogen activator surface receptor; vWF, von Willebrand Factor; and XCL1, lymphotactin.

causality, of which PECAM1 conferred the greatest combined odds ratio for CHD of 2.52 ([95% CI, 1.81–4.00]; $P=1.57\times10^{-6}$). We also noted a trend towards significance for additional proteins, such as LPL and MMP-12, both with negative directions of effect in relation to myocardial infarction.

DISCUSSION

In this article, we identify circulating proteins associated with a family history of early-onset CHD and describe how these proteins are associated with coronary atherosclerotic burden in subjects of the general population,

free of previously reported CHD. Furthermore, we study how a family history of early-onset CHD may influence the way in which these proteins associate with coronary atherosclerotic burden. By using 2-sample MR, we report potential causal effects of several proteins on the risk of myocardial infarction, a detrimental manifestation of CHD. In summary, out of 184 tested proteins, 38 were significantly associated with a family history of early-onset CHD and 79 with the coronary atherosclerotic burden. Furthermore, a significant statistical interaction with family history was observed for 18 proteins in their association with the coronary atherosclerotic burden, with steeper positive associations between circulating LDL receptor, TR,

Table 2. Proteins Significantly Associated With Family History of Early-Onset Coronary Heart Disease at FDR<0.05, in a Crude Model Adjusted for Age, Sex, and Site and Then Additionally Adjusted for Cardiovascular Risk Factors

Protein	Regression coefficient	P value	Adjusted regression coefficient	Adjusted P value
CTSD	0.1145	2.88×10 ^{−7}	0.0731	4.04×10 ^{−4}
PON3	−0.1638	3.22×10 ^{−7}	−0.0907	4.39×10 ^{−4}
IL-1RA	0.1734	2.70×10 ^{−6}	0.0910	0.0031
PRSS8	0.0911	8.03×10 ^{−6}	0.0483	0.0089
REN	0.1690	2.11×10 ^{−5}	0.1188	9.67×10 ^{−4}
FST	0.0985	2.58×10 ^{−5}	0.0778	6.99×10 ^{−4}
ADM	0.0784	8.92×10 ^{−5}	0.0452	0.0105
U-PAR	0.0668	9.21×10 ^{−5}	0.0486	0.0024
TNFRSF10A	0.0536	3.33×10 ^{−4}	0.0300	0.0352
IL-6	0.1305	3.43×10 ^{−4}	0.0756	0.0266
IDUA	0.0917	4.70×10 ^{−4}	0.0618	0.0169
MMP9	0.1285	4.82×10 ^{−4}	0.0981	0.0063
IL-16	0.0757	8.21×10 ^{−4}	0.0563	0.0103
LGALS9	0.0565	8.44×10 ^{−4}	0.0349	0.0256
CHI3L1	0.1372	8.67×10 ^{−4}	0.0973	0.0157
FGF-21	0.2357	9.28×10 ^{−4}	0.1018	0.1229
ACE2	0.0907	9.40×10 ^{−4}	0.0471	0.0699
LGALS4	0.0807	0.0012	0.0402	0.0941
SERPINE1	0.1332	0.0015	0.0558	0.1486
GDF-15	0.0711	0.0016	0.0234	0.2623
LOX-1	0.0765	0.0016	0.0604	0.0110
SCF	−0.0634	0.0020	−0.0460	0.0183
LDL receptor	0.0966	0.0021	0.0346	0.1752
XCL1	0.0867	0.0023	0.0734	0.0098
CCL16	0.0851	0.0024	0.0485	0.0713
HAOX1	0.1917	0.0028	0.1034	0.0986
TNF-R1	0.0493	0.0032	0.03392	0.0336
MMP-12	0.0887	0.0033	0.05596	0.0426
TNF-R2	0.0574	0.0037	0.0420	0.0281
AMBP	0.0249	0.0042	0.01662	0.0426
vWF	0.1093	0.0048	0.0697	0.06991
AGRP	0.0481	0.0062	0.0449	0.0091
IL-18	0.0719	0.0069	0.0529	0.0389
TNFRSF13B	0.0458	0.0078	0.0327	0.0540
IL-18BP	0.0444	0.0086	0.0371	0.0259
CA5A	0.1100	0.0089	0.0453	0.2585
IL-4RA	0.0355	0.0090	0.0289	0.0337
LPL	−0.0567	0.0095	−0.0216	0.3019

AMBP indicates protein alpha-1-microglobulin/bikunin precursor; ACE2, angiotensin-converting enzyme 2; ADM, proadrenomedullin; AGRP, Agouti-related protein; CA5A, carbonic anhydrase 5A, mitochondrial; CCL16, C-C motif chemokine 16; CHI3L1, chitinase-3-like protein 1; CTSD, cathepsin D; FDR, false discovery rate; FGF-21, fibroblast growth factor 21; FST, follistatin; GDF-15, growth/differentiation factor 15; HAOX1, hydroxyacid oxidase 1; IDUA, alpha-L-iduronidase; IL-6, interleukin-6; IL-16, prointerleukin-16; IL-18, interleukin-18; IL-18BP, interleukin-18-binding protein; IL-1RA, interleukin-1 receptor antagonist protein; IL-4RA, interleukin-4 receptor subunit alpha; LDL, low-density lipoprotein; LGALS4, galectin-4; LGALS9, galectin-9; LOX-1, lectin-like oxidized LDL receptor 1; LPL, lipoprotein lipase; MMP-12, matrix metalloproteinase 12; MMP9, matrix metalloproteinase 9; PON3, paraoxonase; PRSS8, prostasin; REN, renin; SCF, stem cell factor; SERPINE1, plasminogen activator inhibitor 1; TNF-R1, tumor necrosis factor receptor 1; TNF-R2, tumor necrosis factor receptor 2; TNFRSF10A, tumor necrosis factor receptor superfamily member 10A; TNFRSF13B, tumor necrosis factor receptor superfamily member 13B; U-PAR, urokinase plasminogen activator surface receptor; vWF, von Willebrand Factor; and XCL1, lymphotactin.

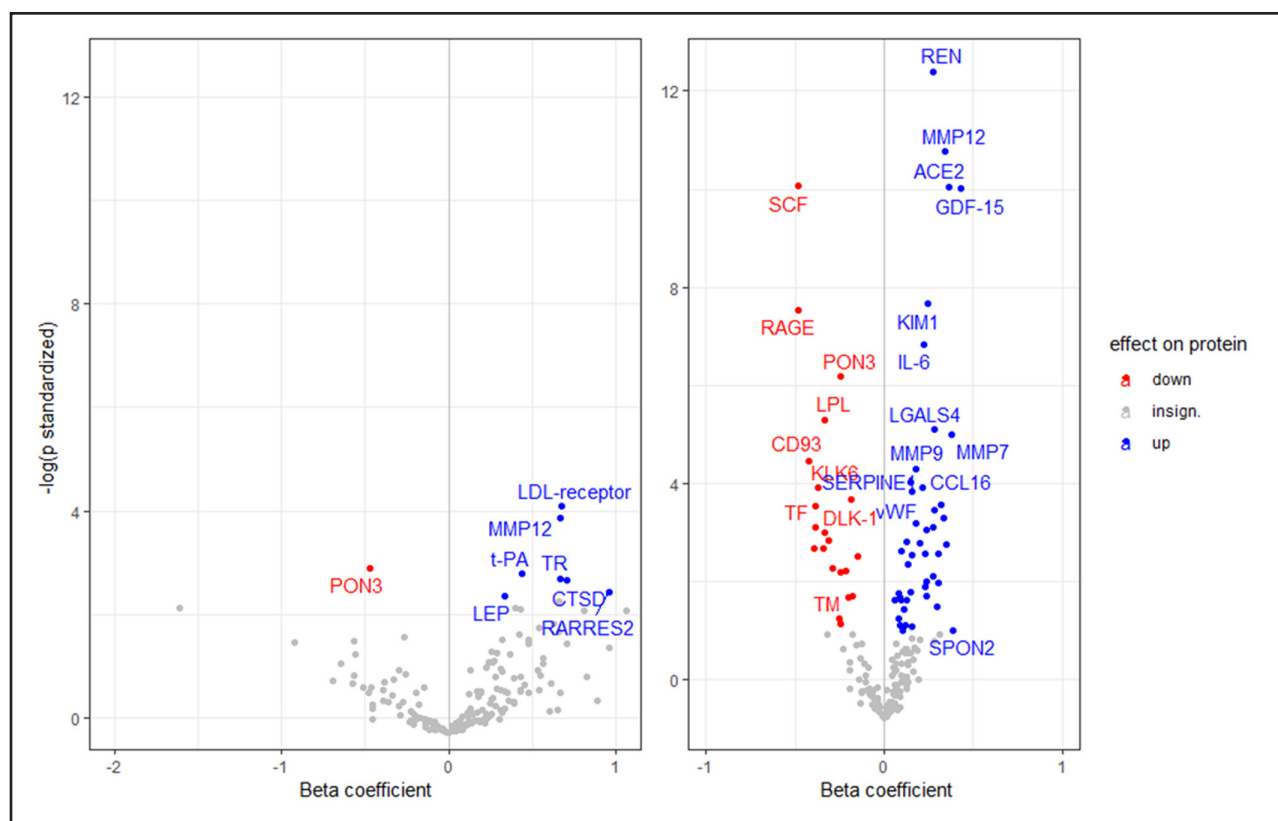


Figure 3. Volcano plot of beta coefficients and the standardized *P* value for the associations between each protein and the segment involvement score.

Proteins in color were identified using a false discovery rate of <0.05 . **A** depicts proteins significant among subjects with a family history of early coronary heart disease, whereas **B** depicts proteins significant among subjects without such a family history. The standardized *P* value was calculated using the crude *P* value for each protein-SIS association, multiplied by the square root of $n/100^{17}$. ACE indicates angiotensin-converting enzyme; CCL16, C-C motif chemokine 16; CD93, complement component C1q receptor; CTSD, cathepsin D; DLK-1, protein delta homolog 1; GDF, growth/differentiation factor; IL, interleukin; KIM1, kidney injury molecule; LDL, low-density lipoprotein; LEP, leptin; LGAL, galectin; LPL, lipoprotein lipase; MMP, matrix metalloproteinase; PON, paraoxonase 3; RAGE, receptor for advanced glycosylation end products; RARRES2, retinoic acid receptor responder protein 2; REN, renin; SCF, stem cell factor; SERPINE, plasminogen activator inhibitor 1; SPON2, spondin-2; TF, tissue factor; TM, thrombomodulin; tPA, tissue-type plasminogen activator; TR, transferrin receptor protein 1; and vWF, von Willebrand Factor.

tPA, and coronary atherosclerosis in those with a family history of early-onset CHD. We establish follistatin as a novel addition to proteins involved in familial coronary atherosclerosis, and in a 2-sample MR analysis, for the first time, we report a potential causal relationship between follistatin and myocardial infarction. Moreover, we confirm previous reports on the causal effects of proteins involved in lipid metabolism and the risk of myocardial infarction, for PCSK9 and PECAM1. Our findings give new insights into the specific proteome of individuals with a family history of CHD and its relevance to disease mechanisms of coronary atherosclerosis. Furthermore, MR results provide further evidence of potential biological plausibility, which may contribute to new prediction tools or treatment targets.

For decades, CHD has been known as a familial disease,¹⁸ and although metabolic risk factors may aggregate within families, independent familial effects on CHD risk have repeatedly been reported.¹⁹ Serum lipid levels are highly heritable, and elevated LDL-cholesterol is established as a causal factor in CHD pathophysiology.^{20,21}

Elevated circulating PCSK9 levels escalate LDL receptor degradation and thus increase circulating LDL-cholesterol. We confirm the results of previous MR studies, showing a causal effect of PCSK9 on the risk of myocardial infarction.²² Furthermore, levels of soluble LDL receptor were strongly associated with atherosclerotic burden, as measured by SIS, in subjects with a family history of early-onset CHD, and were also an interactor with family history in the relationship between soluble LDL receptor and SIS. Although there was no strong genetic instrument for the soluble LDL receptor in our MR analysis to estimate causal effects, our findings further underline the role of LDL-turnover in the development of heritable CHD. LPL was negatively associated with family history of early-onset CHD, and in subjects with family history, there was a steeper negative relationship between LPL and coronary atherosclerotic burden as compared with subjects without family history. In MR, LPL showed a trend towards an inverse causal relationship with CHD, in line with previous studies indicating a higher risk of CHD in subjects with

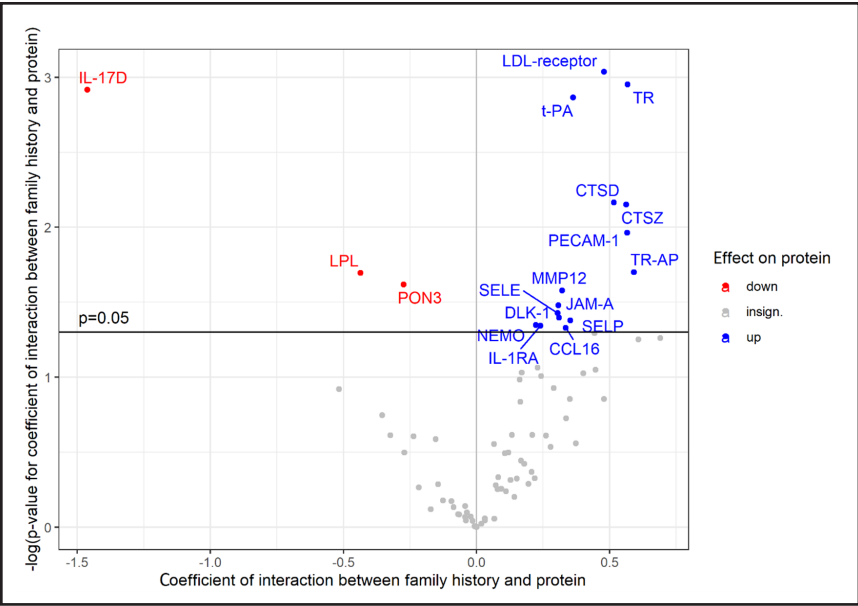


Figure 4. Volcano plot of interaction coefficients and the *P* value for interactions. Interaction coefficients and the crude *P* value for the interaction between family history of early-onset coronary heart disease and each protein, in the associations between proteins and the segment involvement score. CCL16 indicates C-C motif chemokine 16; CTSD, cathepsin D; CTSZ, cathepsin Z; DLK-1, protein delta homolog 1; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist protein; JAM-A, junctional adhesion molecule A; LDL, low-density lipoprotein; LPL, lipoprotein lipase; MMP, matrix metalloproteinase; NEMO, NF-kappa-B essential modulator; PECAM1, platelet endothelial cell adhesion molecule 1; PON, paraoxonase 3; SELE, E-selectin; SELP, P-selectin; tPA, tissue-type plasminogen activator; TR, transferrin receptor protein 1 and TR-AP, tartrate-resistant acid phosphatase type 5.

loss-of-function variants of LPL, as well as a protective relationship between triglyceride-lowering LPL variants and CHD in MR analyses.²³ PON3 inhibits LDL-oxidation and has been shown to slow atherosclerosis progression.²⁴ In this study, PON3 was strongly negatively associated with both family history of CHD and SIS; however, it did not meet the corrected significance level in our MR analysis. Nevertheless, the negative associations between PON3 and family history as well as coronary atherosclerotic burden were strong and independent of traditional cardiovascular risk factors, accentuating the importance of LDL-oxidation in CHD development, as well as the importance of familial factors influencing such oxidation.

PECAM1 is involved in cell adhesion and the patency of the vascular endothelial barrier, as well as in signaling between endothelial cells and migrating cells involved in inflammation and hemostasis. Increased PECAM1 levels in response to shear stress of the vessel wall have been associated with the development of atherosclerotic plaques in lesion-prone regions of the aorta in mice,²⁵ in which an elevated expression of PECAM1 is observed locally in the vessel wall of such aortic sections. In this study, PECAM1 exhibited a steeper association curve with SIS in subjects with a family history of CHD and showed a causal, positive relationship with myocardial infarction in MR. However, PECAM1 was not significantly associated with a family history of early-onset CHD, nor

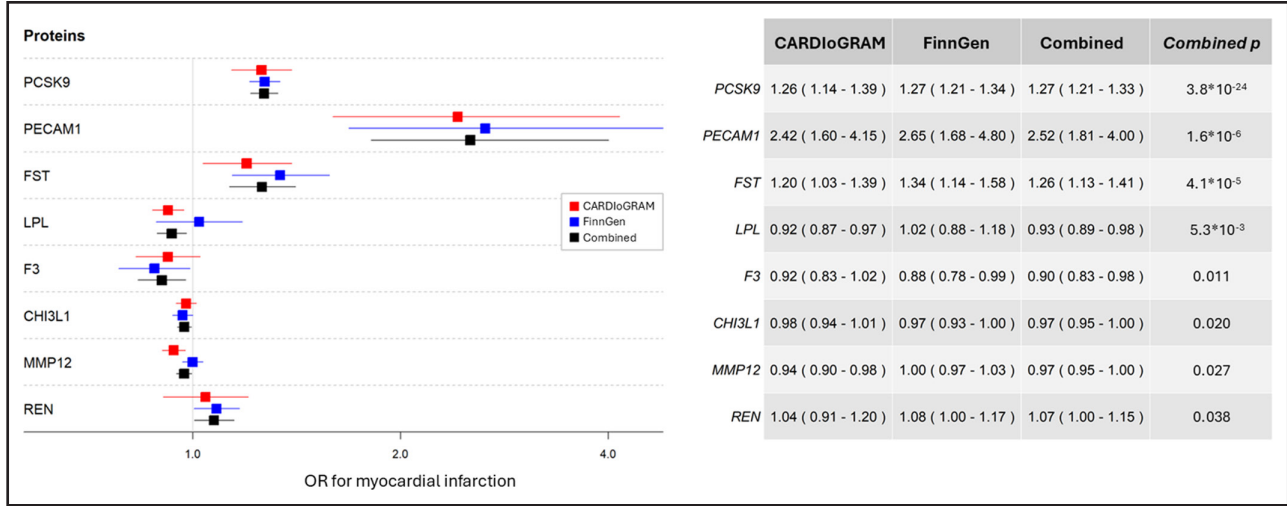


Figure 5. Mendelian randomization odds ratios (ORs) for selected proteins and myocardial infarction. OR and 95% CIs for every genetically predicted \pm SD of the normalized protein level of myocardial infarction, for the 8 strongest associations of tested proteins. Results shown for outcome data from CARDIoGRAM, FinnGen, and the meta-analysis, respectively. CHI3L1, chitinase-3-like protein 1; F3, tissue factor; FST, follistatin; LPL, lipoprotein lipase; MMP, matrix metalloproteinase; PCSK9, proprotein convertase subtilisin/kexin type 9; PECAM1, platelet endothelial cell adhesion molecule 1; and REN, renin.

the coronary atherosclerotic burden as measured by SIS. This suggests that while a family history of CHD is not necessarily predictive of PECAM1 levels, there may be modulating effects on PECAM1 functions. In addition, as PECAM1 was not associated with coronary atherosclerotic burden in subjects without previously known CHD in this study, there may instead be modulating effects of PECAM1 in late-stage atherosclerosis, possibly influenced by other familial factors. We replicate the findings of Lind et al,²⁶ reporting an association between PECAM1 and myocardial infarction from 2-sample MR, along with strong support of causal variants shared between PECAM1 and CHD in colocalization analyses.

Follistatin was positively associated with a family history of early-onset CHD, independent of traditional risk factors, and associated with coronary atherosclerotic burden in the overall analysis of all subjects. The results from 2-sample MR further suggest that follistatin may play a causal role in CHD, and potentially contribute to the development of heritable coronary atherosclerosis. Follistatin is known to modulate the inflammatory response and muscle growth signaling, indicating a role in tissue repair and remodeling. Our findings suggest potential familial effects on follistatin expression or function, as well as a role of follistatin in the development of atherosclerosis. Circulating follistatin has previously been linked to mortality due to heart failure and stroke, partly mediated through diabetes.²⁷ Previous MR analyses have established a potential causal association between follistatin and diabetes²⁸; however, to our knowledge, no causal relationship between follistatin and CHD has previously been published until now.

Whereas not meeting the Bonferroni corrected *P* value for causality in the MR analysis, several proteins involved in inflammation exhibited intriguing associations with family history. MMP (matrix metalloproteinase)-12, a regulator of vascular macrophage infiltration, has previously been positively associated with heart failure, ischemic stroke, and CHD^{28,29} and was associated with both family history of early-onset CHD and SIS in this study. However, our MR findings were borderline indicative of an inverse causal relationship between MMP-12 and CHD. This has been observed previously in works of Lind et al,²⁸ where MMP-12 was positively associated with ischemic stroke and CHD, while exhibiting an inverse causal association in MR analysis. Similarly, MMP-12 has been positively associated with peripheral artery disease, while in the same material, a negative causal relationship between MMP-12 and peripheral artery disease was observed in MR.³⁰ Reverse MR by Li et al³¹ showed signs of reverse causality of cardiovascular disease on MMP-12, indicating a possible feedback mechanism of CHD on MMP-12 expression. The clinical significance of this potential feedback mechanism is still unknown.

CTSD is a regulator of lysosomal degradation, and elevated levels in plasma and myocardial cells have been associated with myocardial injury and coronary events.^{32,33}

It has been suggested that dysfunctional autophagy may lead to atherosclerotic plaque formation, and that advanced atherosclerosis may lead to overexpression of CTSD, as autosomal systems cannot manage the burden of oxidative stress. In this study, CTSD, similar to MMP-12, was strongly connected to both family history of early-onset CHD and overall atherosclerotic burden. CTSD also demonstrated a steeper association curve with SIS in subjects with a positive family history of early-onset CHD. However, a causal association between CTSD and CHD was not found in MR, which may emphasize the theory that CTSD is elevated as a response to extensive oxidative stress rather than causative of atherosclerosis.

CCL16 (C-C motif chemokine 16) is a chemotactic agent found in plasma and liver tissue involved in inflammatory response; however, little is known about its role in disease development. In this study, CCL16 was positively associated with family history of early-onset CHD, as well as with overall SIS, and was an interactor with family history in the association with SIS. However, no evidence of a causal association with CHD was found. Previously, CCL16 has been associated with incident myocardial infarction³⁴ and microvascular dysfunction in nonobstructive angina in women³⁵; however, the mechanism of CCL16 in CHD is still unclear.

The inverse association between IL-17D and coronary atherosclerotic burden in this study was significantly steeper in subjects with a family history of early-onset CHD. The IL-17 family has been proposed to be involved in coronary atherosclerosis, reportedly associated with instability of coronary plaques.³⁶ IL-17D has also been associated with all-cause mortality in patients with heart failure³⁷; however, the pathophysiological importance of each member of the IL-17 family in atherosclerosis remains unsettled.

Soluble TR is upregulated in iron deficiency. We have shown that TR is associated with coronary atherosclerotic burden in subjects with a family history of early-onset CHD, whereas it is not associated with SIS in subjects without such a history. Thereto, TR was a potent interactor with family history in the association with SIS. It has been suggested that relative iron deficiency, even before hemoglobin levels are affected, may influence the inflammation, remodeling, and survival of cardiomyocytes in myocardial infarction and iron deficiency as measured with the soluble TR has been associated with cardiovascular mortality and myocardial infarction in subjects with known CHD.³⁸ In addition, TR has been associated with an increased risk of aortic valve replacement due to aortic stenosis, only in subjects with established CHD.³⁹ Our findings highlight that iron metabolism may play a particular role in the development of heritable CHD.

Strengths and Limitations

The greatest strength of this study is in the unique way that data on proteins, cardiovascular risk factors, and

CCTA-verified coronary atherosclerotic burden in the general population are combined with national registers of kinship and diseases. The use of national register data to ascertain family history of CHD provides a more objective and comprehensive assessment of familial disease compared with self-reports and increases the robustness of disease history, particularly for early manifestations of familial disease. Furthermore, we included data from international GWAS data sets to infer causal effects of proteins related to heritable CHD on coronary atherosclerotic burden, triangulating evidence from different methods. The meta-analysis of GWAS data sets improves the power to infer such causal associations. There are, however, limitations pertinent to the inference of this study. First, due to the unique features of the SCAPIS cohort that combines cardiac imaging and proteomic data in a population-based setting, with the availability for extensive register linkages that enable the identification of register-based familial disease with nationwide coverage, there is no similar cohort currently available for replication. As the first 825 to 850 consecutive participants to complete the full examination protocol from each site were included for Olink analysis, some healthy volunteer recruitment bias might be present; however, as the Olink cohort did not differ significantly in baseline characteristics from the remaining participants, we expect this to be minor. Due to the limited number of subjects in the SCAPIS study undergoing testing for proteomic markers, the cohort was too small to purposefully divide into separate discovery and validation cohorts. Second, the group size according to family history status was unbalanced. The statistical power of the interaction analyses was estimated to be around 70% to 80% for analyses of SIS, and 60% to 80% for analyses of CACS. Thus, some statistically significant interactions may not have been detected. Third, genomic data were not available, limiting the use of polygenic risk scores to capture familial disease risk. Fourth, although genomic data were not included in this study, as parental coverage in Swedish registers of kinship was required for inclusion, we expect the diversity of ethnic origin to be lower than for the full SCAPIS cohort. Fifth, the strength of the genetic instruments used in MR is the determining factor in finding causal effects of proteins on a designated outcome, in this case, myocardial infarction. Including several instruments per protein would further increase the power to find proteins causally involved in atherosclerosis. However, we used only the strongest pQTL available as the IV for each protein, increasing robustness and strength of instruments while also reducing the risk of horizontal pleiotropy pertinent to including several weaker variants in IVs. Lastly, the inclusion of additional cis-pQTLs as well as trans-pQTLs could possibly identify other proteins causally associated with CHD, for which we did not find strong enough instruments with our approach, but could also increase the risk of horizontal pleiotropy and thus compromise the core assumption of exclusion restriction of MR.

Conclusions

In this study, we identify several plasma proteins strongly associated with a family history of early-onset CHD with biological features of inflammation, lipid metabolism, and vascular function. Further, family history of early-onset CHD strongly influenced how different proteins associated with the coronary atherosclerotic burden, suggesting a specific proteomic profile of heritable coronary atherosclerosis. Notably, through MR analysis, we have established a possible causal relationship between PCSK9, PECAM1, and follistatin and myocardial infarction, which may have implications for further understanding of the pathophysiology of CHD.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S10

Figures S1–S6

REFERENCES

1. Chow CK, Islam S, Bautista L, Rumboldt Z, Yusufali A, Xie CC, Anand SS, Engert JC, Rangarajan S, Yusuf S. Parental history and myocardial infarction risk across the world the INTERHEART study. *J Am Coll Cardiol*. 2011;57:619–627. doi: 10.1016/j.jacc.2010.07.054
2. Khera AV, Kathiresan S. Genetics of coronary artery disease: discovery, biology and clinical translation. *Nat Rev Genet*. 2017;18:331–344. doi: 10.1038/nrg.2016.160
3. Knuuti J, Wijns W, Saraste A, Capodanno D, Barbato E, Funck-Brentano C, Prescott E, Storey RF, Deaton C, Cuisset T, et al; ESC Scientific Document Group. 2019 ESC Guidelines for the diagnosis and management of chronic

- coronary syndromes: the task force for the diagnosis and management of chronic coronary syndromes of the European Society of Cardiology (ESC). *Eur Heart J*. 2020;41:407–477. doi: 10.1093/eurheartj/ehz425
4. Nurmohamed NS, Kraaijenhof JM, Mayr M, Nicholls SJ, Koenig W, Catapano AL, Stroes ESG. Proteomics and lipidomics in atherosclerotic cardiovascular disease risk prediction. *Eur Heart J*. 2023;44:1594–1607. doi: 10.1093/eurheartj/ehad161
5. Bergström G, Berglund G, Blomberg A, Brandberg J, Engström G, Engvall J, Eriksson M, Faire U, Flinck A, Hansson MG, et al. The Swedish cardiopulmonary bioimage study: objectives and design. *J Intern Med*. 2015;278:645–659. doi: 10.1111/joim.12384
6. Bergström G, Persson M, Adiels M, Björnson E, Bonander C, Ahlström H, Alfredsson J, Ångerås O, Berglund G, Blomberg A, et al. Prevalence of subclinical coronary artery atherosclerosis in the general population. *Circulation*. 2021;144:916–929. doi: 10.1161/CIRCULATIONAHA.121.055340
7. Bergström G, Hagberg E, Björnson E, Adiels M, Bonander C, Strömberg U, Andersson J, Brunström M, Carlhäll CJ, Engström G, et al. Self-report tool for identification of individuals with coronary atherosclerosis: the Swedish Cardiopulmonary Bioimage Study. *J Am Heart Assoc*. 2024;13:e034603. doi: 10.1161/JAHA.124.034603
8. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekblom A. The Swedish personal identity number: possibilities and pitfalls in health-care and medical research. *Eur J Epidemiol*. 2009;24:659–667. doi: 10.1007/s10654-009-9350-y
9. Ekblom A. The Swedish multi-generation register. *Methods Mol Biol*. 2011;675:215–220. doi: 10.1007/978-1-59745-423-0_10
10. Wahrenberg A, Kuja-Halkola R, Magnusson PKE, Häbel H, Warnqvist A, Hambraeus K, Jernberg T, Svensson P. Cardiovascular family history increases the risk of disease recurrence after a first myocardial infarction. *J Am Heart Assoc*. 2021;10:e022264–e022264. doi: 10.1161/JAHA.121.022264
11. Sniderman AD, Thanassoulis G, Williams K, Pencina M. Risk of premature cardiovascular disease vs the number of premature cardiovascular events. *JAMA Cardiol*. 2016;1:492–494. doi: 10.1001/jamacardio.2016.0991
12. Vasan RS, Song RJ, van den Heuvel ER. Temporal trends in incidence of premature cardiovascular disease over the past 7 decades: the Framingham Heart Study. *J Am Heart Assoc*. 2022;11:e026497. doi: 10.1161/JAHA.122.026497
13. Baranger DAA, Finsaas MC, Goldstein BL, Vize CE, Lynam DR, Olino TM. Tutorial: power analyses for interaction effects in cross-sectional regressions. *Adv Methods Pract Psychol Sci*. 2023;6:25152459231187531. doi: 10.1177/25152459231187531
14. Sun BB, Chiou J, Traylor M, Benner C, Hsu YH, Richardson TG, Surendran P, Mahajan A, Robins C, Vasquez-Grinnell SG, et al; Alnylam Human Genetics. Plasma proteomic associations with genetics and health in the UK Biobank. *Nature*. 2023;622:329–338. doi: 10.1038/s41586-023-06592-6
15. Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, Saleheen D, Kyriakou T, Nelson CP, Hopewell JC, et al. A comprehensive 1000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet*. 2015;47:1121–1130. doi: 10.1038/ng.3396
16. Kurki MI, Karjalainen J, Palta P, Sipilä TP, Kristiansson K, Donner KM, Reeve MP, Laivuori H, Aavikko M, Kaunisto MA, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature*. 2023;613:508–518. doi: 10.1038/s41586-022-05473-8
17. Good IJ. C140. Standardized tail-area probabilities. *J Stat Comput Simul*. 1982;16:65–66. doi: 10.1080/00949658208810607
18. Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. *N Engl J Med*. 1994;330:1041–1046. doi: 10.1056/NEJM199404143301503
19. Lloyd-Jones DM, Nam BH, D'Agostino RB, Levy D, Murabito JM, Wang TJ, Wilson PWF, O'Donnell CJ. Parental cardiovascular disease as a risk factor for cardiovascular disease in middle-aged adults—a prospective study of parents and offspring. *JAMA*. 2004;291:2204–2211. doi: 10.1001/jama.291.18.2204
20. Heller Debra A, de Faire U, Pedersen Nancy L, Dahlen G, McClearn Gerald E. Genetic and environmental influences on serum lipid levels in twins. *N Engl J Med*. 1993;328:1150–1156. doi: 10.1056/NEJM199304223281603
21. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, Hegele RA, Krauss RM, Raal FJ, Schunkert H, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J*. 2017;38:2459–2472. doi: 10.1093/eurheartj/ehx144
22. Pott J, Schlegel V, Teren A, Horn K, Kirsten H, Bluecher C, Kratzsch J, Loeffler M, Thiery J, Burkhardt R, et al. Genetic regulation of PCSK9 (proprotein convertase subtilisin/kexin type 9) plasma levels and its impact on atherosclerotic vascular disease phenotypes. *Circ Genom Precis Med*. 2018;11:e001992–e001992. doi: 10.1161/CIRCGEN.117.001992
23. Ference BA, Kastelein JJP, Ray KK, Ginsberg HN, Chapman MJ, Packard CJ, Laufs U, Oliver-Williams C, Wood AM, Butterworth AS, et al. Association of triglyceride-lowering LPL variants and LDL-C-lowering LDLR variants with risk of coronary heart disease. *JAMA*. 2019;321:364–373. doi: 10.1001/jama.2018.20045
24. Priyanka K, Singh S, Gill K. Paraoxonase 3: structure and its role in pathophysiology of coronary artery disease. *Biomolecules*. 2019;9:817. doi: 10.3390/biom9120817
25. Harry BL, Sanders JM, Feaver RE, Lansey M, Deem TL, Zarbock A, Bruce AC, Pryor AW, Gelfand BD, Blackman BR, et al. Endothelial cell PECAM-1 promotes atherosclerotic lesions in areas of disturbed flow in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol*. 2008;28:2003–2008. doi: 10.1161/ATVBAHA.108.164707
26. Lind L, Mazidi M, Clarke R, Bennett DA, Zheng R. Measured and genetically predicted protein levels and cardiovascular diseases in UK Biobank and China Kadoorie Biobank. *Nat Cardiovasc Res*. 2024;3:1189–1198. doi: 10.1038/s44161-024-00545-6
27. Pan J, Nilsson J, Engström G, De Marinis Y. Elevated circulating follistatin associates with increased risk of mortality and cardiometabolic disorders. *Nutr Metab Cardiovasc Dis*. 2024;34:418–425. doi: 10.1016/j.numecd.2023.09.012
28. Lind L, Gigante B, Borné Y, Feldreich T, Leppert J, Hedberg P, Östgren CJ, Nyström FH, Sundström J, Årnlöv J, et al. Plasma protein profile of carotid artery atherosclerosis and atherosclerotic outcomes. *Arterioscler Thromb Vasc Biol*. 2021;41:1777–1788. doi: 10.1161/ATVBAHA.120.315597
29. Yuan S, Titova OE, Zhang K, Chen J, Li X, Klarin D, Åkesson A, Damrauer SM, Larsson SC, VA Million Veteran Program. Circulating proteins and peripheral artery disease risk: observational and Mendelian randomization analyses. *Eur Heart J Open*. 2023;3:oead056. doi: 10.1093/ehjopen/oead056
30. Macvanin MT, Rizzo M, Radovanovic J, Sonmez A, Paneni F, Isenovic ER. Role of Chemerin in Cardiovascular Diseases. *Biomedicines*. 2022;10:2970. doi: 10.3390/biomedicines10112970
31. Li Y, Liu B, Chen Y, Liu Z, Ye D, Mao Y, Sun X. Genetic evidence for the causal association of circulating cytokines and growth factors with coronary artery disease. *J Am Heart Assoc*. 2024;13:e030726. doi: 10.1161/JAHA.123.030726
32. Gonçalves I, Hultman K, Dunér P, Edsfieldt A, Hedblad B, Fredrikson GN, Björkbacka H, Nilsson J, Bengtsson E. High levels of cathepsin D and cystatin B are associated with increased risk of coronary events. *Open Heart*. 2016;3:e000353–e000353. doi: 10.1136/openhr-2015-000353
33. Wu P, Yuan X, Li F, Zhang J, Zhu W, Wei M, Li J, Wang X. Myocardial upregulation of cathepsin D by ischemic heart disease promotes autophagic flux and protects against cardiac remodeling and heart failure. *Circ Heart Fail*. 2017;10:e004044. doi: 10.1161/circheartfailure.117.004044
34. Lind L, Titova O, Zeng R, Zanetti D, Ingelsson M, Gustafsson S, Sundström J, Årnlöv J, Elmstahl S, Assimes T, et al. Plasma protein profiling of incident cardiovascular diseases: a multisample evaluation. *Circ Genom Precis Med*. 2023;16:e004233–e004233. doi: 10.1161/CIRCGEN.123.004233
35. Schroder J, Mygind ND, Frestad D, Michelsen M, Suhrs HE, Bove KB, Gustafsson I, Kastrup J, Prescott E. Pro-inflammatory biomarkers in women with non-obstructive angina pectoris and coronary microvascular dysfunction. *Int J Cardiol Heart Vasc*. 2019;24:100370. doi: 10.1016/j.ijcha.2019.100370
36. Su SA, Ma H, Shen L, Xiang MX, Wang JA. Interleukin-17 and acute coronary syndrome. *J Zhejiang Univ Sci B*. 2013;14:664–669. doi: 10.1631/jzus.BQ1CC701
37. Baumhove L, Bomer N, Tromp J, van Essen BJ, Dickstein K, Cleland JG, Lang CC, Ng LL, Samani NJ, Anker SD, et al. Clinical characteristics and prognosis of patients with heart failure and high concentrations of interleukin-17D. *Int J Cardiol*. 2024;396:131384–131384. doi: 10.1016/j.ijcard.2023.131384
38. Weidmann H, Bannasch JH, Waldeyer C, Shrivastava A, Appelbaum S, Ojeda-Echevarria FM, Schnabel R, Lackner KJ, Blankenberg S, Zeller T, et al. Iron metabolism contributes to prognosis in coronary artery disease: prognostic value of the soluble transferrin receptor within the atherogene study. *J Am Heart Assoc*. 2020;9:e015480. doi: 10.1161/jaha.119.015480
39. Ljungberg J, Janiec M, Bergdahl IA, Holmgren A, Hultdin J, Johansson B, Näslund U, Siegbahn A, Fall T, Söderberg S. Proteomic biomarkers for incident aortic stenosis requiring valvular replacement. *Circulation*. 2018;138:590–599. doi: 10.1161/CIRCULATIONAHA.117.030414