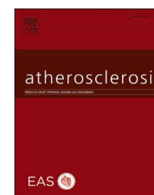
Atherosclerosis 398 (2024) 118613

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis



Relationship of proteins and subclinical cardiovascular traits in the population-based LIFE-Adult study

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A R T I C L E I N F O

Keywords:
Ankle-brachial index
Cardiovascular risk factors
Carotid intima media thickness
Carotid plaque
NT-pro-BNP
Pulse wave velocity

A B S T R A C T

Background and aims: Understanding molecular processes of the early phase of atherosclerotic cardiovascular disease conditions is of utmost importance for early prediction and intervention measures.

Methods: We measured 92 cardiovascular-disease-related proteins (Olink, Cardiovascular III) in 2024 elderly participants of the population-based LIFE-Adult study. We analysed the impact of 27 covariables on these proteins including blood counts, cardiovascular risk factors and life-style-related parameters. We also analysed protein associations with 13 subclinical cardiovascular traits comprising carotid intima media thickness, plaque burden, three modes of Vicorder-based pulse-wave velocities, ankle-brachial index and ECLIA-based N-terminal prohormone of brain natriuretic peptide (NT-proBNP).

Results: Estimated glomerular filtration rate, triglycerides and sex where the most relevant covariables explaining more than 1 % variance of 49, 22 and 20 proteins, respectively. A total of 43 proteins were significantly associated with at least one of the analysed subclinical cardiovascular traits. NT-pro-BNP, brachial-ankle pulse-wave velocity (baPWV) and parameters of carotid plaque burden accounted for the largest number of associations. Association overlaps were relatively sparse. Only growth/differentiation factor 15, low density lipoprotein receptor and interleukin-1 receptor type 2 are associated with these three different cardiovascular traits. We confirmed several literature findings and found yet unreported associations for carotid plaque presence (von-Willebrand factor, galectin 4), carotid intima-media thickness (carboxypeptidase A1 andB1), baPWV (cathepsin D) and NT-proBNP (cathepsin Z, low density lipoprotein receptor, neurogenic locus homolog protein 3, trem-like transcript 2). Sex-interaction effects were observed, e.g. for spondin-1 and growth/differentiation factor 15 likely regulated by androgen response elements.

Conclusions: We extend the catalogue of proteome biomarkers possibly involved in early stages of cardiovascular disease pathologies providing targets for early risk prediction or intervention strategies.

1. Introduction

Atherosclerotic cardiovascular disease (ASCVD) is a multifaceted disease condition with different types of manifestations such as coronary artery disease (CAD), peripheral artery disease (PAD), cerebrovascular disease, or aortic atherosclerosis [1]. Due to the long-term chronic progression, optimal risk management of patients is of utmost clinical importance [2,3]. Classical risk factors such as age, sex, diabetes,

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https://doi.org/10.1016/j.atherosclerosis.2024.118613
Received 23 October 2023; Received in revised form 6 September 2024; Accepted 19 September 2024
Available online 21 September 2024
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hypercholesterolemia, hypertension and smoking were combined to statistical risk models to assess ASCVD risk and to trigger dedicated intervention programs [4–6].

For prevention purposes, risk factors should detect early functional or morphological vascular pathologic changes. Indeed, statistical risk evaluation strategies are currently accompanied by sub-clinical disease phenotypes which could be assessed non-invasively such as intima media thickness, carotid plaque burden, pulse-wave velocities or ankle-brachial index [7–9].

The advent of high-throughput molecular measurement techniques greatly facilitated the search for easy to measure liquid biomarkers of ASCVD risk [6,10]. Numerous such biomarkers were identified to be associated with different ASCVD endpoints [6] or are combined to scores or models for improved risk prediction [11,12]. However, so far only a small number of biomarkers or related risk models were introduced into medical guidelines or clinical care, and biomarkers for the early phases of the ASCVD continuum remain scarce [13,14]. Most proteome studies were performed considering subjects with manifest disease or experiencing hard clinical endpoints [15–18] while associations with sub-clinical phenotypes were analysed to a smaller extent [19,20]. This, however, could be of high significance because subtle molecular changes may precede manifestation of cardiovascular disease. Moreover, proteins could not only be of value as predictive biomarkers of manifest disease but could also serve as therapy or prevention targets if there is evidence that these proteins are involved, for example, in early disease development.

Some of these studies used the high-throughput technique of specific proximity extension assays (PEA) to analyse associations between selected proteins and selected atherosclerotic endpoints [19–23]. With the aim to complement these findings, we here applied another PEA-based array containing a panel of 92 proteins with putative cardiovascular function and associated these proteins with a set of sub-clinical cardiovascular traits to provide evidence that these proteins may be involved in early disease development. To achieve this goal, we analysed protein associations and their overlaps for a more comprehensive set of subclinical cardiovascular traits in a large population-based cohort of middle-aged and elderly subjects. We also analysed in detail the impact of covariables potentially affecting the levels of the measured proteins to identify possible confounders of the observed relationships with cardiovascular traits. By our study, we aim to contribute to the understanding of the diverse roles of the considered cardiovascular biomarker panel in the pathophysiology of sub-clinical cardiovascular phenotypes. Our study design is displayed in Fig. 1.

2. Patients and methods

2.1. Study population

Subjects analysed in this study were recruited in the framework of the LIFE-Adult study – a population-based study of 10,000 residents of the city of Leipzig, Germany [24,25]. The study was performed in adherence with the ethical standards of the Declaration of Helsinki. The protocol was approved by the local Ethics Committee (Reg. No 263-2009-14122009). Written informed consent was collected from all

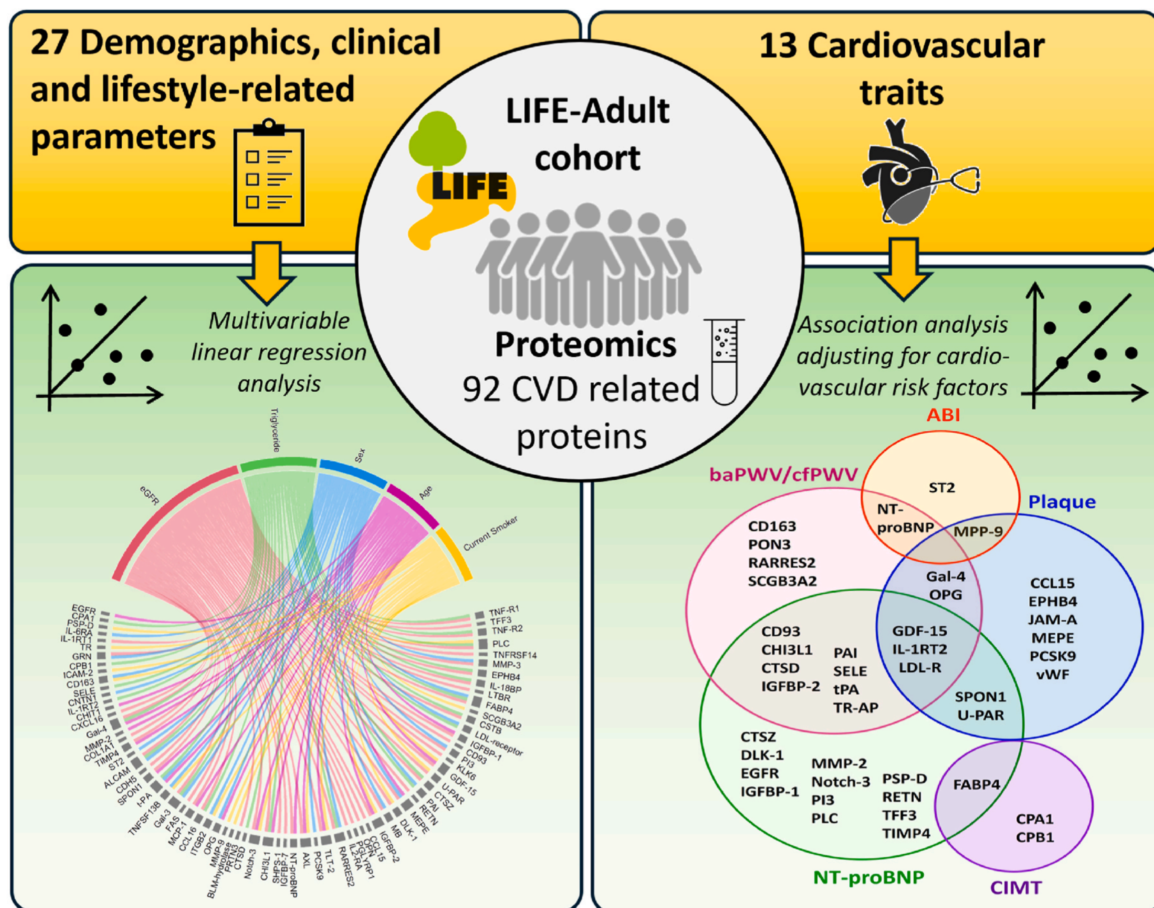

Fig. 1. Graphical abstract.

We measured 92 proteins in 2024 subjects of the population-based LIFE-Adult study and analysed their association with 27 clinical and life-style related parameters. The five most important factors are glomerular filtration rate, triglycerides, sex, age and current smoking (left panel). We also analysed the association of the proteins with 13 cardiovascular traits. Significant associations and their overlaps between the different cardiovascular traits are presented at the right panel.

study participants prior to enrolment.

2.2. Basic assessments

Anthropometric assessments to determine body-mass index (BMI) and waist-to-hip ratio (WHR) was performed using standard operating procedures. Amount of alcohol consumption and smoking was assessed by standardized questionnaires. We dichotomized smoking status by never or former smokers vs. active smokers.

Medication was categorized by the Anatomical Therapeutic Chemical (ATC) Classification System. We defined the following types of specific medication: hypoglycaemic treatment (A10), anti-hypertensive drugs (C02, C03, C07-C09), lipid lowering medication (C10), thyroid therapy (H03), and sexual hormones (G03).

Blood sampling was performed in the morning after a recommend overnight fasting. Fasting duration was dichotomized on the basis of total fasting hours (<8h vs. ≥ 8h). Standard laboratory assessments (including blood count) were performed as described in Ref. [24]. The standardised serum preparation for all samples was completed within 120 min after a clotting time of 30–45 min. Thus, preanalytical variables of platelet activation are assumed to play no major role. We defined type 2 diabetes by positive anamnesis, medication with ATC A10 or HbA1c level ≥6.5 %. We additionally calculated the glomerular filtration rate (GFR) based on cystatin C using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [26] using the R package “nephron”.

2.3. Cardiovascular assessments

All LIFE-Adult participants underwent a comprehensive assessment of subclinical cardiovascular traits. Blood pressure was measured with an automatic oscillometric blood pressure monitor as previously described [27]. Hypertension was defined as a systolic blood pressure >140 mmHg, antihypertensive treatment (medication with one of the ATC codes C02, C03, C07, C08 or C09) or anamnestic information.

Carotid ultrasound was performed to measure carotid intima media thickness (CIMT) of the far wall of the common carotid artery CCA and to determine plaque status. Standard operating procedures were described in detail elsewhere [28,29]. Briefly, subjects were placed in a supine position and high-resolution B-mode ultrasound images were acquired using the GE Vivid ultrasound platform with a 12.0-MHz linear-array transducer (GE Healthcare). Analysis of images was performed by trained study nurses. CIMT was measured with a semi-automated border detection program (EchoPAC Dimension 06, GE Medical Systems, Munich, Germany) following Mannheim consensus [30] as described before [28]. The detecting area of CIMT was defined as the distal 1 cm of the common carotid arteries, proximal to the origin of the bulb. CIMT measurements of both sides were averaged.

Plaque status was determined bilaterally at the following four regions: common carotid artery (CCA), bulb (Bulb), internal carotid artery (ICA) and external carotid artery (ECA). Plaque was defined by echogenic thickening of the intima by at least 0.5 mm, or more than 50 % of the surrounding thickness or a CIMT>1.5 mm. Plaque status was set to missing in case of insufficient image quality. We defined plaque scores (PS) as the number of plaques at the single regions, the number of plaques at CCA and bulb (PS4) and the total number of plaques (PS8). Plaque scores were set to missing if more than 25 % of the required single plaque assessments were missing.

We considered pulse-wave velocities (PWV) as operationalization of central vascular stiffness. The oscillometric Vicorder device (SMT Medical, Würzburg Germany) was used for that purpose. All Vicorder assessments were performed in a quiet, well-aired and temperature-controlled room by trained examiners. Participants were in a supine position, asked to be quiet for at least 10min before assessments and to breathe calmly during the evaluations.

Three modes of PWV were assessed as described in Refs. [27,31]: carotid-femoral PWV (cfPWV), brachial-ankle PWV (baPWV) and brachial-femoral PWV (bfPWV). Blood pressure cuffs with photoplethysmographic sensors were placed at the right common carotid artery, right arm and right upper thigh. Waves were recorded and pulse transit time was determined from foot-to-foot real time shift between simultaneous two-point-recorded pulse waves using an in-built cross-correlation algorithm. Travel distances were determined separately for each PWV modus using a flexible tape. For cfPWV the distance was measured from the suprasternal notch to the middle of the femoral cuff. For baPWV and bfPWV the distances were measured directly from the center of the brachial to the center of the ankle and femoral cuff, respectively.

Ankle-brachial index (ABI) is used as operationalization of subclinical signs of peripheral artery disease. ABI determination was carried out by automated photoplethysmography (PPG)-based method employed in the Vicorder device. Procedures for ABI determination have been previously described in detail [32,33].

Finally, NT-proBNP measured with electro-chemi-luminescent immunoassay (ECLIA) was considered as proxy for (sub-clinically) reduced heart function [34]. This is also justified by the fact that even a small increase of this parameter is associated with inferior long-term outcome [35]. Since this biomarker was also included in the proteome array, we analysed associations of our ECLIA-based NT-proBNP with all proteomic features except for PEA-based NT-proBNP.

2.4. Proteomics profiling

From the 10,000 subjects recruited in LIFE-Adult, we selected 2024 participants with complete as possible major assessments (in particular magnetic resonance imaging and cardiovascular profiling) and genotype information for proteomic profiling (see Ref. [36] for details). This selection was not representative for LIFE-Adult but enriched elderly subjects. Sex-stratified characteristics of study population is shown in Table 1.

Serum samples were processed by the Leipzig Medical Biobank using standard operating procedures within 2 h and stored at −80 °C. Protein abundance was measured using a proximity extension assay (PEA), namely the Target96 Multiplex Cardiovascular III panel (CVD III, Olink Proteomics, Uppsala, Sweden). Briefly, the method relies on oligonucleotide-linked antibodies and qPCR for protein detection and quantification. Upon target binding, probes come into contact with the material and a DNA polymerase extends the two oligonucleotides pair, generating a unique PCR target sequence [37]. The assay read-out are normalized proteins expression (NPX) value on a log2 scale. Our samples were analysed within 23 analytical batches including the same two quality control samples for normalization in order to minimize technical variation between batches. Eight samples failed quality criteria and were removed. To assess remaining batch effects, we conducted a one-way ANOVA analysis of protein expressions and batch number. Across proteins, batches explained <2 % variance, i.e. no relevant batch effects were found.

Each assay had an experimentally determined lower limit of detection (LOD) defined as three standard deviations above noise level. All samples with valid measurements were included in the analysis. For Chitotriosidase-1 (CHIT1), we replaced values below LOD by the respective LOD since given values represent strong outliers of the continuum of values above the LOD. Such an issue was not observed for the other proteins. Assay characteristics including LOD calculations, assay performance and validations are available from the manufacturer’s website (http://www.olink.com). In our hands, the 92 assays showed

Table 1
Sex stratified baseline characteristics of study participants.

Variables	Overall (n = 2014)	Female (n = 974)	Male (n = 1040)	*p*-values
Age (years)	62.49 (11.48)	62.08 (11.35)	62.89 (11.59)	**2.47E-02**
Body mass index (kg/m^2)	27.66 (4.47)	27.24 (4.78)	28.05 (4.13)	**1.02E-06**
Waist-to-hip ratio	0.94 (0.09)	0.88 (0.07)	1.00 (0.07)	**6.65E-205**
Fasting period (hours)	12.84 (1.95)	12.87 (1.86)	12.81 (2.03)	5.89E-01
Prolonged fasting ≥ 8-h (yes/no)	2006 (99.6)	973 (99.9)	1033 (99.3)	9.31E-02
Alcohol (g/day)	12.08 (17.44)	5.16 (8.15)	18.90 (21.09)	**9.64E-70**
Current smoker (yes/no)	320 (16.7)	147 (16.2)	173 (17.3)	5.49E-01
Total cholesterol (mmol/l)	5.70 (1.06)	5.87 (1.06)	5.53 (1.04)	**3.35E-13**
HDL-C (mmol/l)	1.62 (0.46)	1.81 (0.46)	1.44 (0.39)	**2.86E-73**
LDL-C (mmol/l)	3.58 (0.95)	3.59 (0.96)	3.57 (0.95)	5.88E-01
Triglyceride (mmol/l)	1.41 (0.93)	1.28 (0.69)	1.54 (1.10)	**4.28E-09**
Interleukin 6 (pg/ml)	3.82 (5.03)	3.58 (5.27)	4.04 (4.80)	**1.09E-04**
hsCRP (mg/l)	2.82 (6.01)	3.14 (6.89)	2.51 (5.04)	**2.69E-08**
Erythrocytes	4.67 (0.40)	4.49 (0.33)	4.85 (0.39)	**6.59E-99**
Lymphocytes (%)	30.13 (7.89)	31.23 (7.41)	29.09 (8.18)	**8.26E-11**
Monocytes (%)	8.30 (2.11)	7.74 (1.99)	8.83 (2.08)	**4.69E-35**
Reticulocytes	12.08 (3.94)	12.01 (3.71)	12.15 (4.15)	7.63E-01
Platelets	236.47 (57.46)	254.06 (55.96)	219.96 (53.87)	**2.77E-47**
Antihypertensives (a) (yes/no)	1011 (50.2)	460 (47.3)	551 (53.0)	**1.27E-02**
Lipid modifying agents (b) (yes/no)	319 (15.9)	124 (12.8)	195 (18.8)	**2.99E-04**
Thyroid therapy (c) (yes/no)	316 (15.7)	236 (24.3)	80 (7.7)	**3.08E-24**
Drugs used in diabetes (d) (yes/no)	217 (10.8)	78 (8.0)	139 (13.4)	**1.52E-04**
Sex hormones/Modulators of genital system (e) (yes/no)	133 (6.6)	128 (13.2)	5 (0.5)	**6.88E-30**
Hypertension (yes/no)	1166 (58.7)	496 (51.9)	670 (65.1)	**2.91E-09**
Diabetes (yes/no)	479 (23.8)	214 (22.0)	265 (25.5)	7.25E-02
GFR (ml/min/1.73m^2)	80.01 (17.72)	79.13 (17.82)	80.84 (17.59)	**2.96E-02**

For continuous parameters, arithmetic means and standard deviations are provided. For binary variables, total numbers and percentages are shown. Differences between sexes were tested with Mann-Whitney *U* test for continuous parameters and Chi-squared test for binary parameters. Significant differences were marked in bold. HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; hsCRP: highly sensitive C-reactive protein. GFR: glomerular filtration rate estimated with CKD-EPI formula.
*ATC-code beginning with (a) C02, C03 or C07-09, (b) C10, (c) H03, (d) A10, (e) G03.

mean intra-assay (within run) and inter-assay (between run) variations of 8.1 % and 11.5 %, respectively. Analysed proteins are described in details in Supplementary Supplementary Table S1. To detect sample outliers within the proteomic data we calculate the Mahalanobis distance using the function *mahalanobis* from the R-package *stats*. Two outliers were detected and removed resulting in a total samples size of 2014.

2.5. Data analysis

Baseline characteristics are compared between sexes using Mann-Whitney *U* test (continuous variables), χ^2-Test (binary variables) or proportional odds logistic regression (variables with ordered categories such as plaque scores). Prior to regression analyses, distribution assumptions were tested visually using quantile-quantile (QQ) plots. We used raw untransformed NPX values for analyses except for trefoil factor 3 (TFF3) and Azurocidin (AZU1), which were log-transformed. This was decided based on the evaluation of quantile-quantile plots.

Laboratory parameters and PWV were log-transformed to approximate normal distributions. Thereafter, associations between proteins (dependent variable) and 27 relevant clinical and lifestyle parameters were calculated separately for each protein by fitting univariable and multivariable linear regression models using the *lm*

function of R. Effect sizes of associations were assessed by the explained variance (r^2) of the considered factor in the univariable models, or as partially explained variance, in the multivariable regression models considering all 27 factors in parallel (function *etasq* from the *heplots* library). To account for multiple testing (27 clinical or life-style factors x 92 proteins = 2484 tests), we employed control of the global false discovery rate (FDR) at 5 % as previously described [38]. Factors associating with protein levels were considered significant if their FDR-adjusted p-values (q-values) were below 0.05.

Vascular phenotypes (i.e. CIMT, PS, PWV, ABI and serum NT-proBNP) were tested for associations with protein levels using appropriate regression models (linear regression for CIMT, ABI, log-transformed PWV and log-transformed NT-proBNP, logistic regression for plaque presence and proportional-odds logistic regression for plaque scores). Binary logistic regression and POLR models were performed using the *glm* and *polr* function in R, respectively. Strengths of effects were assessed by the respective regression coefficients (beta values) of the linear models or by log-odds-ratios (OR) for the logistic models representing risk modifications per unit of the protein levels. In order to assess the added value of the measured proteins in order to predict the subclinical traits, all models were adjusted for established cardiovascular risk factors, namely sex, age, BMI, LDL-cholesterol, diabetes, smoking status, GFR and hypertension status. To account for multiple comparisons (13 cardiovascular phenotypes x 92 proteins – 1 (combination of ECLIA and PEA-based NT-proBNP) = 1195 tests), we again controlled the global FDR at 5 %, i.e. q-values below 0.05 were considered significant. Since cardiovascular traits express pronounced sexual dimorphisms, we tested for sex differences of protein effect sizes by formal sex interaction analysis. The same control of FDR was applied as for the main protein effects.

For pathway enrichment analysis, proteins were mapped to Entrez IDs which was successful for all proteins defining the background of the enrichment analysis. As foreground, we considered associations with $q < 0.05$ per cardiovascular phenotype. Gene-sets from KEGG, GO, Reactome and DOSE were considered. Only intersections comprising more than one protein feature were tested for enrichment (over-representation). Multiple testing correction was performed per cardiovascular parameter and gene-set data base using Benjamini-Hochberg correction. The following R-libraries and tools were used for this purpose: *clusterProfiler* [39], *DOSE* [40], *ReactomePA* [41].

Unsupervised hierarchical clustering of protein NPX (or respective transformed) values and cardiovascular parameters was performed using the R-package *hclust* using the distance function of one minus absolute value of Spearman's correlation coefficient. The distance between clusters was defined by the default method of *hclust*, i.e. the

Table 2
Sex-stratified subclinical cardiovascular traits of study participants.

Variables	Overall (n = 2014)	Female (n = 974)	Male (n = 1040)	*p*-values
NT-proBNP (pg/ml)	125.34 (186.68)	133.77 (156.02)	117.43 (211.24)	**2.55E-24**
baPWV (m/s)	12.81 (2.17)	12.42 (1.99)	13.18 (2.27)	**6.33E-14**
bfPWV (m/s)	18.52 (7.42)	19.59 (8.22)	17.51 (6.41)	**8.23E-06**
cfPWV (m/s)	10.74 (3.09)	10.85 (3.16)	10.65 (3.02)	2.77E-01
ABI	1.08 (0.12)	1.05 (0.11)	1.11 (0.13)	**1.66E-33**
CIMT (mm)	0.78 (0.14)	0.77 (0.14)	0.80 (0.15)	**3.47E-05**
Carotid plaque (yes/no)	1082 (57.5)	446 (48.4)	636 (66.3)	**5.09E-15**
Plaques at ACC (%)				**6.74E-08**
0	1667 (86.2)	854 (90.6)	813 (82.1)	
1	167 (8.6)	62 (6.6)	105 (10.6)	
2	99 (5.1)	27 (2.9)	72 (7.3)	
Plaques at ECA (%)				**2.18E-14**
0	1544 (93.3)	815 (95.9)	729 (90.7)	
1	92 (5.6)	30 (3.5)	62 (7.7)	
2	18 (1.1)	5 (0.6)	13 (1.6)	
Plaques at ACI (%)				**1.73E-15**
0	1307 (78.3)	738 (86.2)	569 (70.0)	
1	232 (13.9)	81 (9.5)	151 (18.6)	
2	130 (7.8)	37 (4.3)	93 (11.4)	
Plaques at bulb (%)				**3.34E-05**
0	866 (46.7)	498 (54.7)	368 (39.1)	
1	454 (24.5)	220 (24.1)	234 (24.8)	
2	533 (28.8)	193 (21.2)	340 (36.1)	
Plaque score PS4 (%)				**6.38E-16**
0	885 (46.0)	511 (54.4)	374 (38.1)	
1	432 (22.5)	206 (21.9)	226 (23.0)	
2	408 (21.2)	158 (16.8)	250 (25.5)	
3	120 (6.2)	49 (5.2)	71 (7.2)	
4	77 (4.0)	16 (1.7)	61 (6.2)	
Plaque score PS8 (%)				**4.74E-18**
0	799 (44.4)	476 (52.7)	323 (36.0)	
1	344 (19.1)	176 (19.5)	168 (18.7)	
2	292 (16.2)	136 (15.1)	156 (17.4)	
3	154 (8.6)	46 (5.1)	108 (12.0)	
4	96 (5.3)	37 (4.1)	59 (6.6)	
5	59 (3.3)	22 (2.4)	37 (4.1)	
6	38 (2.1)	7 (0.8)	31 (3.5)	
7	12 (0.7)	2 (0.2)	10 (1.1)	
8	6 (0.3)	1 (0.1)	5 (0.6)	

For continuous parameters, arithmetic means and standard deviations are provided. For categorical and binary variables, total number and percentages are shown. Differences between sexes were tested with Mann-Whitney *U* test for continuous parameters and Chi-squared test for binary parameters. Plaque scores were compared with proportional odds logistic regression. Significant differences are marked in bold. NT-proBNP: N-terminal pro-B-type natriuretic peptide; baPWV: brachial-ankle pulse wave velocity; bfPWV: brachial-femoral pulse wave velocity; cfPWV: carotid-femoral pulse wave velocity; CIMT: carotid intima-media thickness; CCA: common carotid artery; ECA: external carotid artery; ICA: internal carotid artery; PS4: sum of plaques in CCA and bulb; PS8: sum of plaques over all eight anatomical regions considered.

complete linkage method was applied considering the maximum distance between the individual components of two clusters. Dendrograms were combined with heatmaps of -log10 p-values between proteins and cardiovascular parameters. To assess stability of both, clusters of cardiovascular parameters and proteins, we performed bootstrapping with 1000 replicates and determined the average Jaccard index. Clusters with average Jaccard values > 0.5 were considered as stable. The R-package *fpc* was used for that purpose.

All statistical analyses and data visualizations were performed using the statistical software package *R version 4.0.2*.

3. Results

Subclinical cardiovascular traits of study participants are shown in Table 2 and compared between sexes. While male study participants showed higher CIMT and carotid plaque burden, female study participants showed less favourable parameters regarding peripheral artery disease (ABI) and heart function (NT-proBNP).

3.1. Associations of proteome profiles with clinical and lifestyle-related factors

We analysed 27 parameters regarding association with our 92 proteins. Results of univariable and multivariable association analyses are displayed in Fig. 2 allowing comparisons of raw associations and fully adjusted associations. All statistics are provided as Supplementary Table S2. In multivariable linear regression analysis, we found 89 proteins relevantly and significantly associated with at least one factor after correction for multiple hypothesis testing. Only, the proteins aminopeptidase N (AP-N), CC-chemokine ligand 24 (CCL-24), and von-Willebrand factor (vWF) showed no such associations. Factors relevant for the highest number of proteins were GFR, triglycerides, sex, age and smoking explaining more than 1 % of variance for 49, 22, 20, 18 and 17 proteins, respectively. Prolonged fasting, IL-6, hypertension, and thyroid therapy showed no independent effects.

Partial explained variances by factor are displayed in Supplementary Fig. S1, numbers are provided in Supplementary Table S2. The relative impact of individual clinical and lifestyle factors on protein abundance differed considerably between the 92 proteins. It revealed that GFR has a particularly strong impact on several proteins with a partial explained variance of up to 25 % (for tumor necrosis factor receptor superfamily member 1A (TNF-R1) and other members of this superfamily). Another strong independent effect was that of sex hormones on trefoil factor 3 (TFF3) levels (partial r^2 = 23 %).

Fig. 2. Distribution of uni- and multivariable explained variance of protein levels by clinical and lifestyle-related factors.
Boxplots show the distribution of explained variances from univariable (left) and partial explained variance from multivariable models (right) of 27 clinical and lifestyle-related factors on our 92 proteins. The strongest two relevant and significant associations (r^2>1 %, q-value<0.05) by parameter are labeled with the respective protein names. Complete statistics can be found in Supplementary Table S2. WHR: waist-to-hip ratio; BMI: body mass index; hsCRP: highly sensitive C-reactive protein; GFR: estimated glomerular filtration rate.

3.2. Associations of proteome profiles with subclinical cardiovascular traits

We next analysed the association of the proteins with our subclinical cardiovascular traits. In all analyses, we adjusted for the cardiovascular risk factors sex, age, body mass index, LDL-cholesterol, diabetes, smoking status, estimated glomerular filtration rate and hypertension. Associations significant after correcting for multiple testing are shown in Table 3. Volcano plots of beta estimates are displayed in Fig. 3 for the major traits. Results of other traits are shown as Supplementary Fig. S2. Numerous associations were found involving a total of 43 proteins.

Exemplarily, seven proteins were found to be positively associated with presence of plaque (OR ranging from 1.22 [1.07,1.40] to 1.78 [1.35–2.35] see Table 3, Fig. 3). Higher plasma concentrations of matrix metalloproteinase-9 (MMP-9), growth/differentiation factor 15 (GDF-15), proprotein convertase subtilisin/kexin type 9 (PCSK9), junctional adhesion molecule A (JAM-A), galectin-4 (Gal-4), interleukin-1 receptor type 2 (IL-1RT2) and vWF were associated with higher chance of carotid

Table 3
Associations between proteins and subclinical cardiovascular traits.

Phenotype	Protein	Beta	SE	q-value
Linear regression				
NT-proBNP	IGFBP-2	0.285	0.032	1.13E-15
	IGFBP-1	0.189	0.022	1.43E-14
	Notch-3	0.307	0.051	3.90E-07
	MMP-2	0.313	0.052	3.90E-07
	TR-AP	−0.310	0.054	1.05E-06
	TIMP4	0.249	0.044	1.27E-06
	LDL-receptor	−0.206	0.037	3.25E-06
	TFF3	1.910	0.355	7.58E-06
	DLK-1	−0.167	0.032	2.46E-05
	PAI	−0.139	0.028	5.12E-05
	CD93	0.269	0.060	3.71E-04
	SPON1	0.326	0.074	4.86E-04
	U-PAR	0.229	0.052	5.00E-04
	SELE	−0.140	0.032	5.24E-04
	PSP-D	0.107	0.026	1.07E-03
	GDF-15	0.167	0.041	1.13E-03
	FABP4	0.134	0.035	2.67E-03
	CTSZ	−0.191	0.050	2.78E-03
	EGFR	−0.324	0.089	5.15E-03
	t-PA	−0.123	0.034	6.56E-03
	PI3	0.132	0.038	9.83E-03
	CHI3L1	0.074	0.022	1.42E-02
	PLC	0.239	0.075	1.85E-02
	IL-1RT2	−0.166	0.055	2.83E-02
	RETN	0.113	0.040	4.52E-02
	CTSD	−0.128	0.046	4.86E-02
baPWV	SELE	0.032	0.005	9.57E-08
	CTSD	0.044	0.007	3.53E-07
	CD93	−0.042	0.009	3.95E-04
	Gal-4	0.027	0.006	4.71E-04
	CHI3L1	0.015	0.004	6.26E-04
	OPG	0.037	0.009	7.03E-04
	TR-AP	0.036	0.008	7.68E-04
	PON3	−0.020	0.005	3.31E-03
	IGFBP-2	−0.019	0.005	4.80E-03
	GDF-15	0.023	0.006	5.80E-03
	PAI	0.015	0.004	1.04E-02
	LDL-receptor	0.019	0.006	1.54E-02
	NT-proBNP	−0.010	0.003	1.67E-02
	t-PA	0.017	0.005	1.70E-02
	CD163	0.019	0.006	2.58E-02
	RARRES2	0.028	0.009	3.43E-02
	IL-1RT2	0.025	0.009	4.22E-02
	SCGB3A2	−0.010	0.003	4.31E-02
cfPWV	NT-proBNP	−0.022	0.006	8.90E-03
	SELE	0.027	0.010	4.98E-02
ABI	MMP-9	−0.015	0.003	9.08E-04
	NT-proBNP	−0.011	0.003	7.21E-03
	ST2	−0.015	0.005	4.36E-02
CIMT	FABP4	−0.019	0.005	4.66E-03
	CPB1	−0.013	0.004	2.51E-02
	CPA1	−0.012	0.004	2.80E-02
Binary logistic regression				
Carotid plaque	vWF	0.304	0.072	8.37E-04
	PCSK9	0.577	0.142	1.31E-03
	GDF-15	0.394	0.120	1.43E-02
	MMP-9	0.221	0.069	1.69E-02
	IL-1RT2	0.514	0.160	1.76E-02
	Gal-4	0.359	0.115	2.16E-02
	JAM-A	0.199	0.068	3.67E-02
Proportional odds logistic regression				
PS-Bulb	PCSK9	0.526	0.126	9.14E-04
	Gal-4	0.423	0.101	9.29E-04
	MMP-9	0.232	0.061	3.09E-03
	LDL-receptor	0.357	0.094	3.44E-03
	vWF	0.212	0.064	1.49E-02
	GDF-15	0.321	0.102	2.17E-02
	U-PAR	0.422	0.135	2.17E-02
	SPON1	0.571	0.185	2.44E-02
	JAM-A	0.174	0.060	3.98E-02

(continued on next page)

Table 3 (*continued*)

Phenotype	Protein	Beta	SE	q-value
PS-CCA	PCSK9	0.712	0.191	3.97E-03
	OPG	0.715	0.207	9.23E-03
	vWF	0.326	0.096	1.11E-02
	Gal-4	0.474	0.145	1.58E-02
	U-PAR	0.586	0.200	3.55E-02
PS-ICA	Gal-4	0.511	0.135	3.38E-03
	MMP-9	0.244	0.080	2.75E-02
	PCSK9	0.513	0.172	3.12E-02
	EPHB4	−0.666	0.225	3.29E-02
	MEPE	−0.450	0.155	3.84E-02
	vWF	0.245	0.088	4.77E-02
PS4	Gal-4	0.460	0.097	1.28E-04
	PCSK9	0.568	0.121	1.69E-04
	MMP-9	0.244	0.058	8.86E-04
	vWF	0.228	0.061	3.97E-03
	U-PAR	0.465	0.130	6.29E-03
	LDL-receptor	0.319	0.090	7.21E-03
	OPG	0.466	0.135	9.36E-03
	GDF-15	0.333	0.099	1.18E-02
	CCL15	0.287	0.093	2.55E-02
	SPON1	0.526	0.179	3.51E-02
PS8	PCSK9	0.586	0.124	1.41E-04
	Gal-4	0.459	0.098	1.64E-04
	vWF	0.269	0.063	6.96E-04
	MMP-9	0.237	0.059	1.58E-03
	U-PAR	0.409	0.133	2.47E-02
	IL-1RT2	0.409	0.138	3.31E-02
	GDF-15	0.292	0.101	3.85E-02
	JAM-A	0.163	0.059	4.99E-02

Associations in multivariable linear regression after correction for multiple testing are shown (FDR<0.05). Models were adjusted for sex, age, BMI, LDL-C, diabetes, smoking status, GFR and hypertension, throughout. Complete results are shown in Supplementary Table S3. baPWV: brachial-ankle pulse wave velocity; CIMT: carotid intima-media thickness; ABI: ankle-brachial index; NT-proBNP: N-terminal pro-B-type natriuretic peptide (ELICA-based); PS-Bulb: plaque score at bulb; PS-CCA: plaque score at common carotid artery; PS-ICA: plaque score at internal carotid artery; PS4: sum of plaques on the right and left CCA and bulb; PS8: sum of plaques on the right and left CCA, bulb, ICA and ECA. NT-proBNP values and baPWV values were logarithmized prior to analysis.

plaque occurrence. Analysing associations with the different carotid plaque scores revealed additional seven significant proteins (Supplementary Table S3, Supplementary Fig. S2). PCSK9, vWF and Gal-4 were positively associated with the plaque score at three carotid areas with the exception of ECA. In contrast, osteoprotegerin (OPG) was solely associated with the plaque score at CCA while matrix extracellular phosphoglycoprotein (MEPE) and ephrin type-B receptor 4 (EPHB4) were exclusive and negatively associated with the plaques score at ICA. Positive associations only found for carotid bulb comprised LDL-R, GDF-15, JAM-A and spondin-1 (SPON1). Of note, all seven proteins associated with carotid plaque presence, were also significantly associated with PS8. Among the plaque scores, only ECA showed no associations.

Regarding CIMT, three significant and negative associations (carboxypeptidase A1 (CPA1), carboxypeptidase B1 (CPB1) and fatty-acid binding protein 4 (FABP4)) were observed (see Table 3, Fig. 3B). Of note, the sets of proteins significant for plaque parameters and CIMT were disjoint.

We analysed three traits of vascular stiffness, namely baPWV, bfPWV and cfPWV. A total of 20 associations were detected, 18 with baPWV and two with cfPWV (see Table 3, Fig. 3D, Supplementary Figs. S2B and C). The two proteins associated with cfPWV were also associated with baPWV, thus a total of 18 proteins were associated, five with negative effect direction, 13 with positive direction. Strongest associations were found for E-selectin (SELE) and cathepsin D (CTSD).

Ankle-brachial index was analysed as subclinical operationalization of peripheral artery disease. Three proteins were significantly and negatively correlated with ABI, namely MMP-9, PEA-based NT-proBNP and ST2 protein (ST2).

Finally, we analysed ECLIA-based NT-proBNP as a proxy trait of heart function. As expected, ECLIA-based NT-proBNP values were in strong correlation with PEA-based NT-proBNP values (Pearson's correlation: r = 0.91). Thus, we primarily considered the other proteins for association analysis. A total of 26 proteins were associated with levels of ECLIA-based NT-proBNP, ten with negative effect direction, 16 with positive effect direction (Table 3, Fig. 4). Strongest positive associations were observed for insulin-like growth factor-binding proteins 1 and 2 (IGFBP-1, IGFBP-2), matrix metalloproteinase-2 (MMP-2), and negatively, with tartrate-resistant acid phosphatase type 5 (TR-AP).

We analysed the overlap of associations between our five cardiovascular trait entities. It revealed that none of the proteins was associated with all five or four of the entities. A total of 15 proteins were associated with exactly two traits while only three proteins (GDF-15, LDL-R, IL-1RT2) were associated with the three entities plaque, pulse-wave velocity and NT-proBNP.

To test for sex differences between protein effect sizes, we added an interaction term of sex and protein to our regression models. A total of 21 interaction terms were significant after multiple testing correction. Complement component C1q receptor (CD93) showed the most pronounced sex interactions with several plaque phenotypes. Effects in males were much smaller suggesting a female-specific effect. Another strong sex-interaction was observed for SPON1 regarding NT-proBNP with an effect in males only. Complete interaction results are presented in Supplementary Table S4.

We also analysed whether there is an enrichment of protein associations in gene-sets of the different pathway entities KEGG, GO, Reactome and DOSE. This analysis is performed trait-wise using the associations with q-values<0.05 as respective foregrounds. Only pathway overlaps of more than one protein were tested for enrichment. After correction for multiple testing, only two KEGG pathways showed significant enrichment for association with CIMT, namely "Pancreatic secretion" and "Protein digestion and absorption", both due to fact that these pathways comprise the associated proteins CPA1 and CPB1. Results are summarized in Supplementary Table S5.

Finally, we performed a two-fold clustering of the association

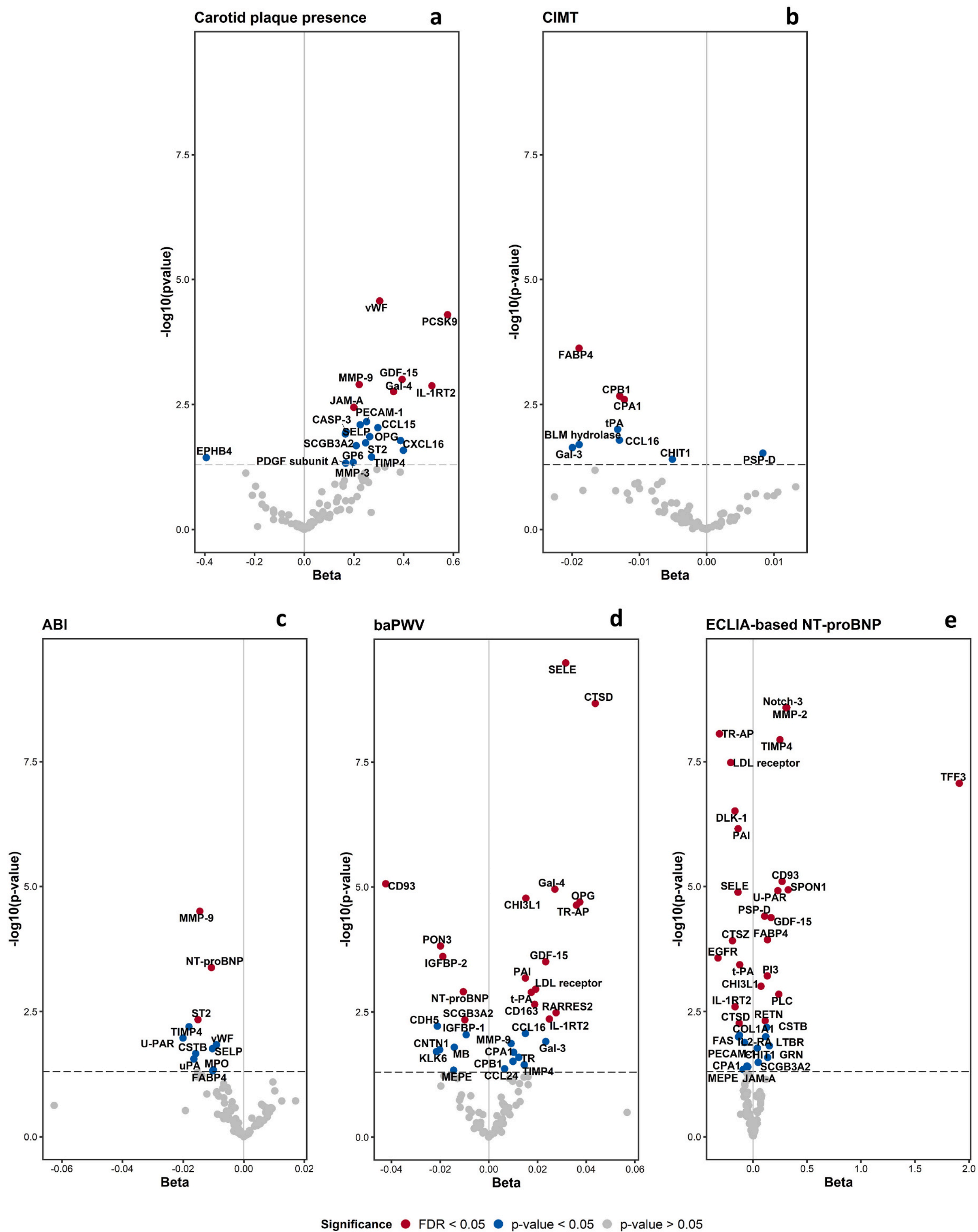

Fig. 3. Volcano Plots of associations of proteins with major subclinical cardiovascular traits.
We present association results for (A) presence of carotid plaque, (B) carotid intima media thickness (CIMT), (C) ankle-brachial index (ABI), (D) brachial-ankle pulse wave velocity (baPWV), and (E) ECLIA-based NT-proBNP. Beta estimates correspond to respective regression models adjusting for sex, age, BMI, LDL-C, diabetes, smoking status, GFR and hypertension. Points are colored according to raw p-values (grey $p > 0.05$, blue $p \leq 0.05$, i.e. nominally significant associations). Associations significant after multiple testing correction (q-value<0.05) are shown as red points. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

findings (Fig. 5). The dendrogram structure of cardiovascular traits revealed three clusters. Cluster 1 only contains ABI, cluster two comprises NT-proBNP and the three pulse wave parameters while cluster 3 represents the parameters of carotid ultrasound investigation. Bootstrapping analysis showed that all three clusters are stable (Supplementary Table S6A). Regarding proteins, we detected four major clusters comprising 13, 44, 30, and 5 proteins, respectively. By bootstrapping analysis, we found that clusters 2 and 4 were stable (Supplementary Table S6B). Cluster 2 contains associations for all subclinical cardiovascular traits except for bfPWV and PS-ECA showing no

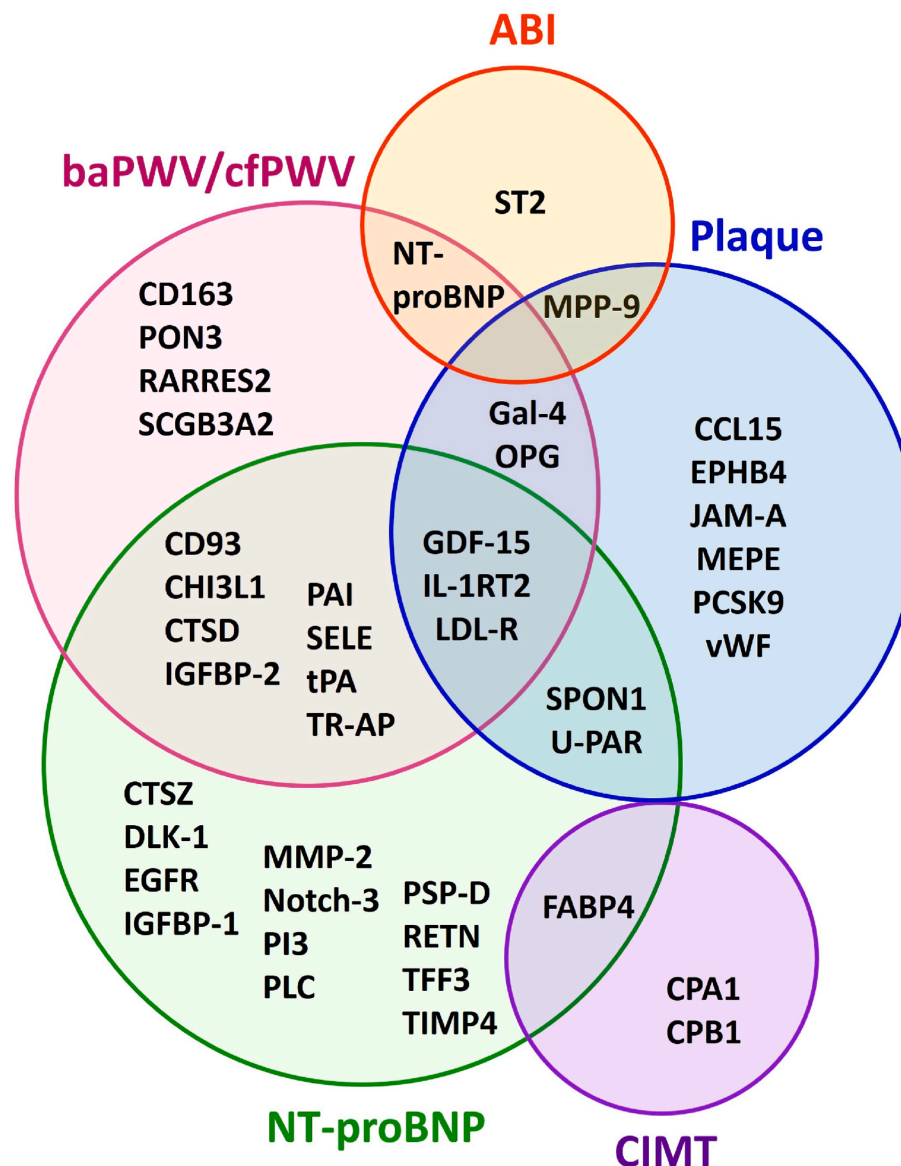

Fig. 4. Overlap of proteins associated with five cardiovascular traits.
We determined the overlap of significant protein associations between the five traits carotid plaque status, carotid intima media thickness (CIMT), ankle-brachial index (ABI), brachial-ankle/carotid-femur pulse-wave velocity (baPWV/cfPWV) and ECLIA-based N-terminal pro-B-type natriuretic peptide (NT-proBNP).

associations at all. Cluster 3 shows a similar association profile while Cluster 1 is dominated by NT-proBNP and PWV associations. Cluster 4 shows associations with NT-proBNP and baPWV only but contains the two strongest associations with NT-proBNP.

4. Discussion

In this study, we analysed the relationship of PEA-based measurements of 92 proteins previously reported in a cardiovascular context and 27 covariables comprising blood counts, life-style related parameters and cardiovascular risk factors in the large population-based study LIFE-Adult to detect possible confounding factors of the measured protein levels likely explaining inconsistent findings in the literature. We then analysed the relationships of the proteins with 13 sub-clinical cardiovascular parameters to understand possible pathophysiological differences between different facets of ASCVD development comprising carotid atherosclerosis, peripheral atherosclerosis, vascular stiffness and reduced heart function. As major results, we found that almost all proteins (89/92) showed relevant associations (partial explained variance >1 %) with the analysed covariables but the relevance of single factors differed widely among the proteins. Conversely, 24 of the analysed 27 covariables showed a significant and relevant association with at least one protein in multivariable analysis demonstrating that the spectrum of possible confounders is large. Regarding the considered sub-clinical cardiovascular phenotypes, we observed associations with a total of 43 proteins independent of major cardiovascular risk factors. Most associations were observed regarding NT-proBNP, carotid plaque status and baPWV, a marker of arterial stiffness. Associating proteins only showed a limited overlap between these entities. A few sex-differential protein associations were observed suggesting sex-specific pathomechanisms.

Association of cardiovascular risk factors and CVD-related biomarkers were studied comprehensively in the literature, identifying age, sex, BMI, blood cell counts, hypertensive treatment and hsCPR as major factors in agreement with our findings [42,43]. However, associations observed with different lipid parameters [44], diabetes [45] or hypertension [46] appeared to be less relevant in our multivariable analysis except for triglyceride levels accounting e.g. for a large percentage of the

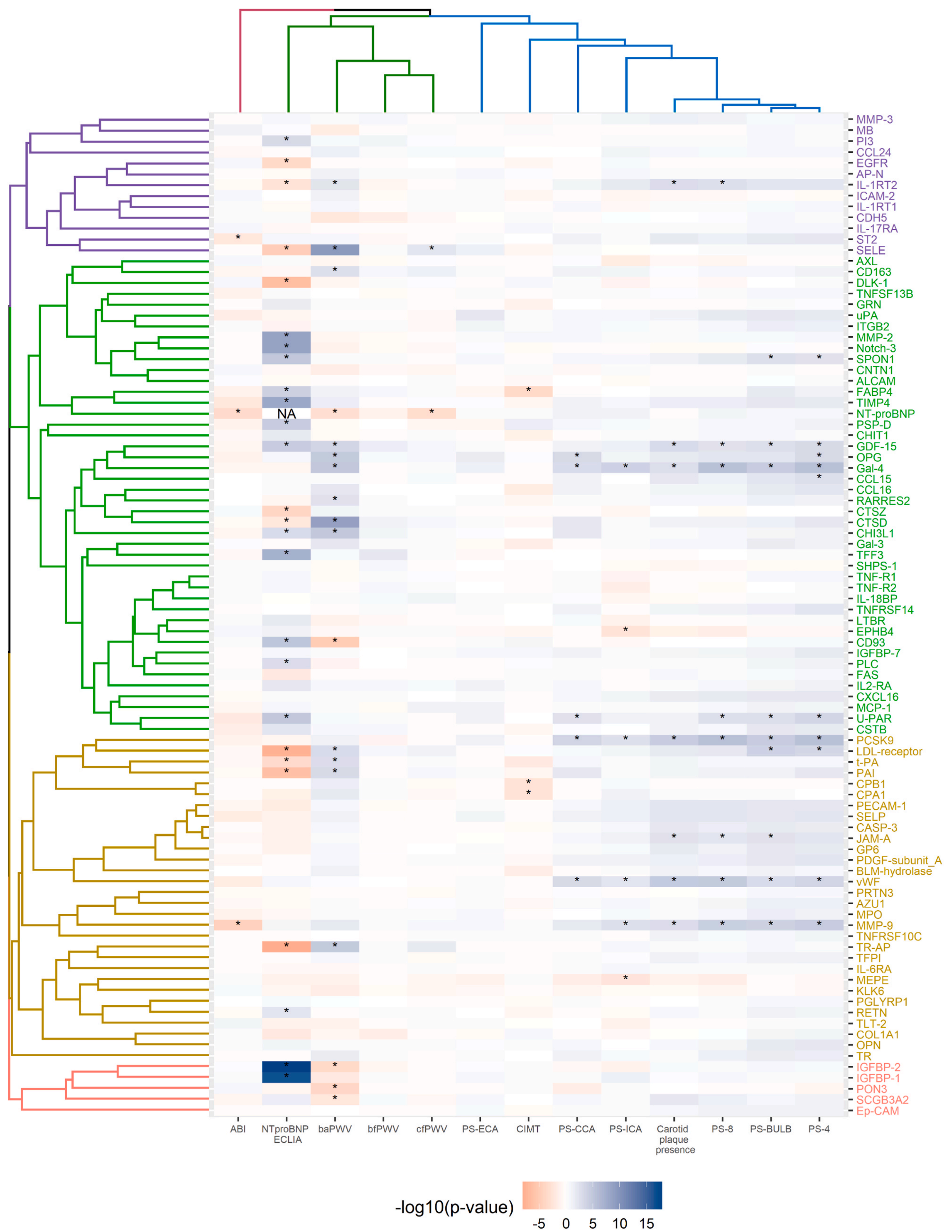

Fig. 5. Heatmap of protein associations and hierarchical clustering.

We show a heatmap of -log10 transformed *p*-values of protein associations with cardiovascular traits signed by effect directions (red = negative correlation, blue = positive correlation). Hierarchical clustering is performed using (transformed) values of both, proteins and cardiovascular traits. Identified clusters are color-coded. *Significant associations after multiple testing correction ($q < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

variance of LDL-R. A possible explanation is that kidney function (GFR) strongly affected several of the proteins, qualifying this parameter as a major confounding factor to be considered in proteome association analyses [47].

Another frequently and relevantly associated factor was platelet count with the strongest associations observed for platelet glycoprotein VI (GP6), platelet-derived growth factor subunit A (PDGF-A) and caspase-3 (CASP-3), respectively. To the best of our knowledge, the association with GP6 was not yet described but is plausible because GP6 is a transmembrane glycoprotein found exclusively on platelets and their precursors. Proteolytic cleavage of GP6 could be triggered by platelet activation [48]. Indeed, five of the proteins associated with platelet counts, namely GP6, P-selectin (SELP), JAM-A, trem-like transcript 2 (TLT-2), and platelet endothelial cell adhesion molecule (PECAM), were described as soluble membrane proteins derived from activated platelets [49] in agreement with the observed positive association with platelet count. One might speculate that these observations could be triggered by pre-analytic treatment of samples because platelets could be activated by the sampling process. We analysed, whether this effect could impact our findings by performing additional adjustments for platelet counts during protein association analysis of cardiovascular traits. Results are very similar suggesting no relevant confounding of cardiovascular trait associations (Supplementary Fig. S3, Supplementary Table S7).

We analysed the measured proteins with several types of sub-clinical cardiovascular phenotypes, which could be assess non-invasively in large population-based cohorts. We expected to find several associations due to the selection of CVD-related biomarkers included into the Cardiovascular III Olink panel. However, we also expected differences in associations between the considered entities of cardiovascular traits.

First, we considered parameters of carotid atherosclerosis derived by carotid ultrasound, namely CIMT and different plaque scores. Carotid plaque assessments was found to be a better predicter of cardiovascular events than CIMT [50] because plaques are detectable at a later state of atherosclerosis formation. Moreover, plaque burden is correlated with coronary artery disease [29]. We considered different operationalizations of plaque burden by counting the numbers of carotid segments with plaque.

CIMT was associated with three proteins (CPA1, CPB1, FABP4), interestingly without overlaps with plaque associations. There are contradicting results regarding the association with FABP4 [51,52], probably due to small sample sizes of reporting studies. The association with CPA1 was found in a small study of Africans but notwithstanding adjustment for ethnicity [53]. Thus, the associations with CPA1 and CPB1 represent novel findings.

Regarding plaque status or burden, we observed a total of 14 significant protein associations. Strongest associations comprised vWF, Gal-4, MMP-9 and PCSK9. The latter two findings are in line with the literature [54–56]. Here, we provided new evidence for a positive association of vWF levels with plaque presence, which is in line with a positive association observed for calcification volume in carotid arteries [57] and with carotid plaque area [58]. Increase in vWF plasma levels was considered as a marker of endothelial dysfunction representing an early step of atherosclerosis formation [59]. Gal-4 was not directly linked to carotid plaque, but this was observed for another member of the Galectin family, namely Gal-3 [56]. A strong sex-interaction for plaque parameters was observed for CD93 with stronger effects in females, which could be explained by a high confidence androgen response element at the transcription termination site of the CD93 gene [60].

Pulse-wave velocities were used as parameters of vascular stiffness. In our study, PWV was assessed between three anatomical regions, namely brachial-ankle (baPWV), brachial-femur (bfPWV) and carotid-femur (cfPWV). We previously observed that the three PWV modes are only moderately correlated possibly representing different pathomechanistic changes [27]. Although cfPWV was most often assessed in studies, and therefore considered as gold-standard, we observed a stronger correlation with age and blood pressure for baPWV proposing this mode as an alternative proxy of vascular stiffness [27]. Indeed, most of the protein associations were observed with this PWV mode.

Strongest associations with baPWV were observed for SELE and CTSD, both with positive effect direction. In agreement with previous findings, we confirmed the association between SELE and arterial stiffness, and the lack of association with other measured cell adhesion molecules such as SELP and intracellular adhesion molecule 2 (ICAM-2) [61]. Since SELP and ICAM-2 are produced by other sources, our data support an assumed relationship between endothelial dysfunction and arterial stiffness [62]. CTSD is assumed to be a marker of atherosclerosis due to its increased expression in atherosclerotic plaques and a possible involvement in apoptosis of macrophages and vascular smooth muscle cells [63]. Moreover, an association with CIMT was observed [64]. In our study, we could not find associations of CTSD and carotid ultrasound parameters but observed a strong, possibly novel association with baPWV. This is plausible because another member of the cathepsin family, namely Cathepsin L, was already found to be associated with arterial stiffness [65].

Regarding other sub-clinical cardiovascular phenotypes, we considered ABI as a proxy for PAD. We found only three associations with the proteins MMP-9, ST2 and PEA-based NT-proBNP. Association of MMP-9 with PAD status was already found by other groups [66,67]. Other associations found in Dakhel et al. [67] did not withstand multiple testing correction in our study. ST2 was found to be elevated in PAD patients compared to controls, in agreement with our observed effect direction with ABI [68]. The association with NT-proBNP was also expected due to observed higher levels in PAD patients [69].

We here considered ECLIA-based NT-proBNP as a proxy for (sub-clinically) reduced heart function. NT-proBNP is a well-established marker for heart function [70] also available at the PEA array considered in our study. For the purpose of association analysis, we used the ECLIA-based NT-proBNP measurements of our study. NT-proBNP showed the largest number of significant associations among the analysed cardiovascular traits. Associated proteins involve several processes known to be related to heart failure, namely cardiometabolism (IGFBP-1, IGFBP-2), myocardial injury (FABP4), inflammation (GDF-15, urokinase plasminogen activator surface receptor (U-PAR)), cardiac remodeling (MMP-2, metalloproteinase inhibitor 4 (TIMP-4)), and immune response (spondin 1 (SPON1), TR-AP) [71,72]. On the other hand, we could not confirm associations of interleukin 1 receptor-like 1 (ST-2) or Gal-3, currently considered as diagnostic markers of heart failure [14]. We observed the strongest (positive) associations with IGFBP-1 and IGFBP-2. These associations were also observed by others [73]. Regarding our positive association with MMP-2, there are conflicting results claiming either no association [74], a negative association [75] or a positive association [76]. Several other associations were also confirmations of literature results but four associations, namely those of cathepsin Z (CTSZ), LDL-R, neurogenic locus notch homolog protein 3 (Notch-3) and TLT-2 were not yet described to the best of our knowledge. Among those, the positive association with Notch-3 represented the strongest finding. Notch-3 is expressed in vascular smooth muscle cells and involved in pulmonary vascular remodeling [77]. In sex interaction analysis, we identified a male-specific effect of SPON1, which again could be explained by androgen response elements described up- and downstream of this gene [60].

For a more integrated view on our findings, we performed pathway enrichment analysis and hierarchical clustering. Pathway enrichment was mostly negative despite analysing a large number of gene sets of four different ontologies. This was not unexpected because the feature selection for the Cardiovascular III Olink is not optimized for pathway coverage. A more comprehensive list of proteins should be measured and analysed to identify possible disease-related pathways. With respect to hierarchical clustering, we identified three clusters of our sub-clinical cardiovascular traits. Not unexpectedly, ABI and parameters of carotid ultrasound clustered separately representing different facets of the

cardiovascular disease spectrum, namely peripheral vs. carotid atherosclerosis. NT-proBNP and pulse-wave parameters were summarized in another cluster. This is plausible due to the known patho-mechanistic relationship between arterial stiffness and heart failure [78,79]. Regarding proteins, we identified four clusters but cluster assignment was unstable. Larger sample sizes are required to corroborate the clustering of the analysed protein spectrum and its interpretation.

Our study has some limitations. We analysed a highly selected collection of proteins with putative functions in cardiovascular disease represented by the content of the Cardiovascular III Olink panel. Thus, a large number of associations were expected. On the other hand, we searched for associations with sub-clinical cardiovascular parameters in a population-based study of elderly subjects while several of the proteins were selected due to associations with hard cardiovascular endpoints or due to their predictive potential in cardiovascular disease patients, which we did not consider in the present study. We only analysed associative relationships between proteins and different cardiovascular traits. Although we controlled for major cardiovascular risk factors, causality cannot be concluded from our analysis.

Strengths of the study are the relatively large sample size allowing stringent multiple-testing correction and the comprehensive sub-clinical assessment of cardiovascular parameters allowing cross-phenotype comparisons.

In summary, we comprehensively analysed the impact of different types of covariables on the considered cardiovascular-related proteins identifying relevant potential confounders. We performed association analyses of the protein levels with a broad set of sub-clinical cardiovascular traits representing different pathologies and allowing cross-trait comparisons. We confirmed several associations described in the literature but also found several new once. Possible differences in the underlying molecular pathologies of the analysed traits are mirrored by a limited overlap of the found associations across traits. Sex-interactions observed for proteins likely regulated by androgen response elements point towards sex-specific patho-mechanisms. By our analysis, we extend the catalogue of proteins possibly involved in early stages of cardiovascular disease pathologies. Further research is required to better understand the possible patho-mechanistic role of the identified proteins, to assess their potential for early risk prediction and to derive targets for intervention strategies.

Conflict of interest

Markus Scholz received funding from Pfizer Inc. for a project not related to this research. Markus Scholz also received funding from Owkin for a project related to heart-failure research. The other authors have nothing to disclose.

Financial support

LIFE-Adult is funded by the Leipzig Research Center for Civilization Diseases (LIFE). LIFE is an organizational unit affiliated to the Medical Faculty of the University of Leipzig. LIFE is funded by means of the European Union, by the European Regional Development Fund (ERDF) and by funds of the Free State of Saxony within the framework of the excellence initiative. Olink measurements were funded by the HIMAG project "Serum proteome biomarkers as mediators of cardiometabolic disease development" of the Medical Faculty of the University Leipzig and the Helmholtz Zentrum München. Data analysis was supported by the German Federal Ministry of Education and Research (BMBF) within the framework of the e:Med research and funding concept (SYMPATH, grant #01ZX1906B).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

Study recruitment: KW, SH, ML, MS; Measurements: AP, SMH, RB; Conceived the study: MS; Data analyses: TG, HK; Interpretation of findings: TG, HK, MS; Manuscript writing: TG, MS; Contribution to discussion and to manuscript writing: AP, SMH, RB, KW, JP, AT, SH, ML, AP. All authors read and approved the final version of the manuscript.

Acknowledgements

We thank all study participants of the LIFE-Adult study whose personal dedication and commitment have made this project possible.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2024.118613.

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