IJC Heart & Vasculature 58 (2025) 101667

Contents lists available at ScienceDirect

IJC Heart & Vasculature

journal homepage: www.sciencedirect.com/journal/ijc-heart-and-vasculature

Sex differences in proteomics of cardiovascular disease – *Results from the Yale-CMD registry*

Yihan Liu [a,b], Zuoheng Wang [a,b,c], Sean P. Collins [d], Jeffery Testani [e], Basmah Safdar [f,*]

[a] *Program in Computational Biology & Biomedical informatics, Yale University, New Haven, CT, USA*
[b] *Department of Biostatistics, Yale University School of Public Health, New Haven, CT, USA*
[c] *Department of Biomedical Informatics & Data Science, Yale University School of Medicine, New Haven, CT, USA*
[d] *Department of Emergency Medicine, Vanderbilt University Medical Center, and Veterans Affairs Tennessee Valley Healthcare System, Geriatric Research, Education and Clinical Center (GRECC), Nashville, TN, USA*
[e] *Section of Cardiovascular Medicine, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA*
[f] *Department of Emergency Medicine, Yale University School of Medicine, New Haven, CT, USA*

ARTICLE INFO

Keywords:
Proteomics
Sex differences
Coronary artery disease
Coronary microvascular dysfunction
Olink
Precision medicine

ABSTRACT

Aims This study assessed sex-specific proteomic profiles by cardiovascular disease (CVD) phenotype (coronary artery disease [CAD] vs coronary microvascular dysfunction [CMD]) and describe their role in sex-specific pathways. Methods: In a secondary biobank analysis of the Yale-CMD registry, adults with ischemic symptoms who underwent cardiac positron emission test/computed tomography were categorized as a) controls (normal coronary flow reserve (CFR) > 2 without perfusion defect or coronary calcification), b) having CMD (CFR < 2 without defect or calcification), or c) having CAD (known CAD or new perfusion defect). Using proximity extension assays (Olink® Explore 3072), we examined 2944 proteins. Differential protein expression was assessed using linear regression models, adjusting for age, race, body mass index, diabetes, dyslipidemia, hypertension, or smoking. Results: Of 190 patients, 91 provided blood samples (mean age, 56 years; 66 %, females; 48 %, controls; 24 %, CAD; 27 %, CMD). Among controls, 15 proteins showed sex differences (5 proteins upregulated in females, 10 in males; false discovery rate [FDR < 0.05]). Upregulated in CAD patients were FSHB in females and INSL3 and EDDM3B in males (FDR < 0.05). Among CMD patients, SCGB3A1 and HGFAC were higher in females; INSL3, SPINT3, EDDM3B, and KLK3 were higher in males (FDR < 0.05). Per pathway analysis, females showed upregulation of immune pathways in CAD and lipid and glucose metabolism pathways in CMD. Males showed upregulated endothelial regulation of blood flow in CAD and increased angiogenesis in CMD. Conclusions: Sex differences exist in the proteomic profiles of CAD and CMD patients, highlighting a need for precision medicine.

1. Introduction

Cardiovascular disease (CVD) remains the leading cause of mortality in the United States.[1] Sexual dimorphism exists in the onset, progression, and outcomes of CVD, highlighting a need for improved understanding of sex-specific underlying mechanisms. Males show a higher prevalence of ischemic heart disease at younger ages, primarily due to ischemia from obstructive coronary artery disease (CAD) involving one of the *epicardial* arteries. In contrast, females present at later ages, and show a higher prevalence of heart failure and ischemia due to the coronary microvascular disease (CMD) involving the microvasculature.[2] Even attribution of conventional cardiac risk factors varies between sexes. Females with diabetes, obesity, or smoking are at higher risk for CVD, whereas males with hypertension or dyslipidemia demonstrate greater risk. Genetic factors (such as sex chromosomes) and sex hormones explain some of these differences, independently and through a complex interplay; for instance, estrogen imparts a protective effect against ischemic heart disease in premenopausal females.[3] However, genetics does not fully explain CVD differences between postmenopausal females and similarly aged males, implicating the role of environmental factors such as lifestyle choices, social factors, and medications.[4] Novel tools are need that allow a unified platform to study the influence of both factors on CVD expression in males and females.

* Corresponding author.
E-mail address: basmah.safdar@yale.edu (B. Safdar).

https://doi.org/10.1016/j.ijcha.2025.101667
Received 5 January 2025; Received in revised form 20 March 2025; Accepted 22 March 2025

Proteomics investigates dynamic biological states of proteins, influenced by both genetics and environmental factors, to explain the sex differences in CVD phenotypes.[5] Blood proteins behave differently in males and females.[6] Proteomics in population-based cohorts indicate sex differences in incident development of CAD,[7] and heart failure,[8] with sex-specific variations in biological pathways.[9] However, sex comparisons in acute ischemia remain scarce, or report conflicting results. In a female cohort, Prescott et al.[10] found inflammation to be independently associated with CMD, whereas Chandramouli et al.[11] found the opposite, with inflammation playing a greater role in males with CMD in heart failure patients. Thus, a discovery-based assessment of sex differences in proteomics is needed to better elucidate the mechanism by phenotype.

Here, we used large-scale blood proteomics in emergency department (ED) patients with symptoms of acute ischemia to understand sex-specific pathways pertaining to CAD and CMD.

2. Methods

2.1. Study population

This study was a secondary analysis of patients enrolled in the Yale-CMD registry, a prospective observational cohort study. Patients were eligible if they met the following inclusion criteria: Adults presenting to the Yale ED with chest pain or an angina equivalent and admitted to the Yale observation unit between May 2014 and February 2020 for a cardiac positron emission test (PET) with computed tomography (CT). We excluded patients who had acute myocardial infarction, hemodynamic instability, or acute heart failure; were currently receiving dialysis; had a febrile illness; or were unable to consent. This study was approved by the Yale Institutional Review Board (HIC 2000022866) and conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

2.2. Data sources

A trained research associate (RA) interviewed consented patients for sociodemographic characteristics, family history, symptoms, reproductive history (in females), height, weight, medications, and reviewed medical records for test results.

2.3. Blood sample collection, processing, and storage

Between 9am-12 pm, 10 ml of peripheral blood was collected after overnight fasting in non-EDTA tubes, centrifuged at 3000 RPM (~1200 g) for 15 min into serum, aliquoted in 500-ul labeled cryovials, and stored in a − 80 °C freezer within 2 h of collection.

2.4. Protein biomarker measurements

Deidentified blood samples (100 ul) were shipped to O-link by standardized shipping procedures (overnight on dry ice) for proteomics using Olink's high-throughput Proximity Extension Assay (PEA) technology.[12] Olink uses a proximity-dependent DNA polymerization event, which generates a unique PCR target sequence. Subsequently, the resulting DNA sequence is detected and quantified using a microfluidic real-time PCR instrument (Biomark HD, Fluidigm Corporation, CA, USA). Data were quality controlled and normalized using an internal extension control and an inter-plate control to adjust for intra- and inter-run variation. The extension control is composed of an antibody coupled to a unique pair of DNA tags that serve as a synthetic control added to every sample well. This approach adjusts for technical variation introduced in the extension step and hence reduces intra-assay variability. Protein biomarker levels are recorded as normalized protein expression values on a log2 scale. Olink® Explore 3072 incorporating 2944 plasma proteins for eight panels (Cardiometabolic, Cardiometabolic II, Inflammation, Inflammation II, Neurology, Neurology II, Oncology, and Oncology II) were analyzed. All assay validation data for the proteins for these panels (detection limits, intra- and inter-assay precision data, accuracy, reproducibility, and validity) are available on O-link website.[12–15] Quality control process were applied both per manufacturer recommendation and with coefficient of variation analysis for both inter- and intra-assays. We excluded biomarkers with >15 % of values below the detection limit. Patients with samples that failed quality control were excluded. A complete list of 2944 plasma proteins is provided in Supplemental Table 1.

2.5. Disease phenotype classification

Disease phenotyping was based on quantitative analysis of dynamic 3-dimensional rubidium-82 cardiac PET/CT. We defined disease phenotypes in accordance with classification by the Coronary Vasomotor Disorders International Study (COVADIS) Group:[16] 1) controls (normal coronary flow reserve [CFR ≥ 2]) without perfusion defect or coronary calcification, 2) having coronary microvascular dysfunction (CMD, CFR < 2 without defect, calcification or cardiomyopathy, or if confirmed by vasoreactivity testing on follow up angiogram), or 3) having CAD (prior CAD or revascularization, new perfusion defect).[17] We reported this methodology in detail previously.[17].

2.6. Statistical analysis

Baseline characteristics of patients were compared between males and females within each group using Wilcoxon rank sum tests for continuous variables and chi-squared tests for categorical variables. Baseline characteristics were compared among the three groups (control, CAD, and CMD) using Kruskal-Wallis tests for continuous variables and chi-squared tests for categorical variables.

To assess differential protein expression between males and females, we used linear regression models, adjusting for age, race, body mass index (BMI), comorbidities (history of diabetes, dyslipidemia, or hypertension), and smoking. Assumptions of linear regression models, including normality, linearity, and homoscedasticity, were assessed and no significant violations were found. For each protein biomarker, the regression coefficient of sex scaled by the standard deviation of biomarker expression was reported. This scaling allows for more interpretable results by reporting changes in units of standard deviation rather than raw values, which varied between proteins. Multiple hypothesis testing was corrected using the false discovery rate (FDR). Proteins with an FDR < 0.05 were considered significant. Differential protein expression analyses were performed separately for the CAD, CMD, and control patients. All statistical analyses were conducted using R (version 4.4.0).

2.7. Pathway and network analyses

To understand the biological functions of proteins that are differentially expressed in males versus females, functional pathway analysis was performed using ingenuity pathway analysis (IPA).[18] Differentially expressed proteins with P-value < 0.05 and $|\beta| > 1$ were included in the analysis. Upregulated proteins in males and females were analyzed separately to identify enriched biological pathways. For the CAD and CMD groups, proteins were excluded from pathway analysis if they were also differentially expressed in the control group. All biomarkers measured on the protein assay in this study were used as the enrichment background. Fisher's exact test was used to determine the overrepresentation of a specific functional pathway among the selected differentially expressed proteins. Pathways with P-value < 0.05 were considered significant. The STRING database[19] was used to identify protein–protein interactions among biomarkers that were upregulated in males and females separately. The Markov cluster (MCL) algorithm [20] was used to identify clusters in the interaction networks with the

parameter values: granularity = 4.0 and array source = combined_score. Proteins in each cluster were used to determine their associated biological processes.[21,22].

2.8. Weighted correlation network analysis of proteomics

We performed weighted correlation network analysis (WGCNA) to construct protein correlation networks and to identify modules of highly correlated proteins in the CAD, CMD, and control patients separately. [23] WGCNA is an unsupervised method for identifying clusters of highly correlated biomarkers. Network was constructed using a minimum module size of 30 biomarkers. The protein expression profiles of modules were summarized by eigenproteins, the first principal component of the biomarker expression. Spearman correlation was used to assess the correlation between each module's eigenprotein and sex. Proteins in the top modules with P-value < 0.05 were selected for pathway analysis. Pathways with P-value < 0.05 were considered significant.

3. Results

3.1. Baseline characteristics

Of the 189 eligible patients in the Yale-CMD registry, 91 contributed blood samples for proteomic analysis. These included 22 patients with CAD, 25 patients with CMD, and 44 patients with normal flow. This sample selection was made to maintain an approximate 1:2 ratio of CAD or CMD patients (cases) to normal flow patients (controls). Patients who provided blood samples were similar to those who participated in the Yale-CMD registry (median age 56 vs 57 years; 66 % versus 67 % female; with 48 % versus 50 % controls, respectively). Baseline characteristics indicate no significant sex difference in sociodemographic or clinical characteristics (Table 1), similar to the full cohort (Supplemental Table 2). Patients with CMD were predominantly females compared with those with CAD (88 % versus 50 %, $P = 0.012$), younger (52 versus 62 years, $P = 0.002$), and more obese (mean BMI 41 versus 32, $P < 0.001$). Female patients across all three groups, CAD, CMD, and control, did not show significant differences in reproductive factors associated with CVD risk including status of menopause, polycystic ovary syndrome (PCOS), an history of pregnancy loss. The test results and p-values for all factors comparing across groups are provided in Supplemental Table 3.

3.2. Sex differences in protein biomarker expression

We assessed sex differences in plasma protein biomarker expression. Table 2 lists the 20 proteins with the smallest FDR in each group. Whereas expression of reproductive proteins was the highest across all three groups, CAD patients had higher upregulation of inflammatory proteins (CXCL9, ICAM2, SELL, CD80, SLAMF7), while proteins from multiple mechanisms were higher in CMD patients ranging from inflammation (SCGB3A1), rho kinase activity (OPHN1), extracellular matrix remodeling (MFAP5, XG, CDCP1, IGDCC4), to cell metabolism (ANGPTL3). Results for all proteins are shown in Supplemental Table 4.

In the control group, 15 proteins were differentially expressed between males and females (FDR < 0.05; Fig. 1A). Among them, five proteins had higher expression in females, with the largest difference observed in PZP ($\beta = 1.69$), followed by XG, FSHB, LEP, and GFAP ($\beta > 0.99$). These proteins are involved in the following biological functions: pregnancy and fertility (PZP – Pregnancy zone protein, FSHB – Follitropin subunit beta), blood homeostasis and immune processes (XG – Xg glycoprotein), hormonal regulation (LEP – Leptin), and neurological function (GFAP – Glial fibrillary acidic protein). The other 10 proteins had higher expression in males, with the largest difference observed in INSL3 (β = -2.04), followed by SPINT3, KLK3, EDDM3B, ACRV1, TEX101, CALCA, OBP2B, MYLPF, and PROK1 (β < -1.15). These proteins are related to hormone response (INSL3 – Insulin-like 3 A chain, EDDM3B – Epididymal secretory protein E3-beta, KLK3 – Prostate-specific antigen, ACRV1 – Acrosomal vesicle protein 1, TEX101 – Testis-expressed protein 101, PROK1 – Prokineticin-1, CALCA – Calcitonin), enzymatic inhibition (SPINT3 – Serine peptidase inhibitor, Kunitz type 3), muscle contraction (MYLPF – Myosin light chain, phosphorylatable, fast skeletal muscle), and olfactory processes (OBP2B – Odorant-binding protein 2b).

Sex differences were also observed in the CVD phenotypes. In the CAD group, three proteins were differentially expressed between males and females (FDR < 0.05; Fig. 1B). Among them, FSHB had higher expression in females ($\beta = 1.76$), whereas INSL3 (β = -1.77) and EDDM3B (β = -1.44) were higher in males. All three proteins are hormone related and were also differentially expressed in the control group (Fig. 1D). In the CMD group, six proteins were differentially expressed between males and females (FDR < 0.05; Fig. 1C), all with at least a 2.5-SD difference. Among them, secretoglobin family 3A member 1 (SCGB3A1; $\beta = 2.70$) and hepatocyte growth factor activator short chain (HGFAC; $\beta = 2.67$) had higher expression in females. SCGB3A1 is involved in secretion processes and HGFAC is involved in liver-associated growth modulation. The other four proteins had higher expression in males, with the largest difference observed in EDDM3B (β = -2.85), followed by INSL3, SPINT3, and KLK3 (β < -2.56). All four proteins upregulated in males are hormone related and are also differentially expressed in the control group (Fig. 1D).

3.3. Pathway enrichment analyses

In the control group, 25 proteins had a P-value < 0.05 and $|\beta| > 1$. Among them, 10 were upregulated in females and 15 were upregulated in males. No pathway was found to be enriched in these proteins.

The pathway enrichment analysis for CAD samples showed sex differences. After excluding proteins that were also upregulated in females in the control group (Supplemental Fig. 1A), the 112 proteins upregulated in females in the CAD group were enriched for pathways associated with immune system processes, such as cell adhesion, immune cell communication, and inflammation (Fig. 2A). Multiple chemokines (CCL14, CCL15, CCL16, CCL19, CCL20, CCL25, CCL7, CXCL9, XCL1), adhesion molecules (ICAM1, ICAM2), and T-lymphocyte activation antigens (CD80, CD86) were upregulated and enriched in the top pathways. These proteins also highly interact with each other in the protein–protein interactions (PPI) network. Moreover, four clusters were identified in the PPI network (Fig. 2C). Pathways related to immune response were enriched in protein cluster 1. In cluster 2, proteins were enriched for pathways related to immune response in addition to cytokine- and chemokine-mediated signaling. In contrast, after excluding proteins that were also upregulated in males in the control group (Supplemental Fig. 1B), the 11 proteins upregulated in males were enriched for pathways involved in the endothelial regulation of blood flow (Fig. 2B). However, due to the limited number of proteins upregulated in males, there were not enough interactions to construct a PPI network for this group.

For the CMD group, in the 57 proteins upregulated in females, after excluding proteins that were also upregulated in females in the control group (Supplemental Fig. 1A), there was a substantial emphasis on lipid and glucose metabolism, as seen in the enriched pathways related to liver X receptor/ farnesoid X receptor/ retinoid X receptor (LXR/FXR/RXR) activation, Clathrin-mediated endocytosis signaling, 3β-hydroxysterol Δ24-reductase (DHCR24) signaling, and atherosclerosis signaling (Fig. 3A). The upregulated proteins in these pathways, especially apolipoproteins (APOA1 and APOD), suggest an active regulatory mechanism related to cholesterol transport and synthesis. In contrast, after excluding proteins that were also upregulated in males in the control group (Supplemental Fig. 1B), the 59 proteins upregulated in males were enriched for pathways involved in angiogenesis and some lipid pathways (Fig. 3B).

Table 1
Baseline characteristics of patients for proteomic analysis.

	Control (n = 44)		CAD (n = 22)	CMD (n = 25)					
	Male	Female	P-value	Male	Female	P-value	Male	Female	P-value
N	17	27		11	11		3	22	
Social demographics									
Age, years	52.0 (42.0—59.0)	54.0 (51.0—57.5)	0.188	61.0 (59.0—67.0)	62.0 (60.0—74.5)	0.621	49.0 (45.5—50.0)	55.5 (49.5—62.8)	0.143
Ethnicity, Hispanic (%)	1 (5.9 %)	4 (14.8 %)	0.312	2 (18.2 %)	2 (18.2 %)	1	0 (0 %)	2 (9.1 %)	1
Race, White (%)	11 (64.7 %)	18 (66.7 %)	1	6 (54.5 %)	6 (54.5 %)	1	2 (66.7 %)	12 (54.5 %)	1
Insurance, Private Insured (%)	11 (64.7 %)	19 (70.4 %)	0.952	7 (63.6 %)	6 (54.5 %)	1	3 (100 %)	9 (40.9 %)	0.192
Educational level, Less than college (%)	8 (47.1 %)	19 (70.4 %)	0.713	8 (72.7 %)	10 (90.9 %)	0.58	2 (66.7 %)	17 (77.3 %)	1
Clinical risk factors									
Hypertension	8 (47.1 %)	17 (63.0 %)	0.469	10 (90.9 %)	10 (90.9 %)	1	3 (100 %)	17 (77.3 %)	0.878
Hyperlipidemia	9 (52.9 %)	15 (55.6 %)	1	8 (72.7 %)	10 (90.9 %)	0.58	1 (33.3 %)	14 (63.6 %)	0.706
Hyperglycemia	10 (58.8 %)	13 (48.1 %)	0.704	5 (45.5 %)	6 (54.5 %)	1	2 (66.7 %)	12 (54.5 %)	1
Known CAD	0 (0 %)	0 (0 %)	NA	8 (72.7 %)	7 (63.6 %)	1	0 (0 %)	3 (13.6 %)	1
Cerebral Vascular Accident (CVA)	0 (0 %)	1 (3.7 %)	1	1 (9.1 %)	1 (9.1 %)	1	0 (0 %)	4 (18.2 %)	1
Dementia	3 (17.6 %)	3 (11.1 %)	0.87	1 (9.1 %)	1 (9.1 %)	1	1 (33.3 %)	8 (36.4 %)	1
Pulmonary Embolism (PE) & Deep Vein Thrombosis (DVT)	0 (0 %)	1 (3.7 %)	1	1 (9.1 %)	2 (18.2 %)	1	0 (0 %)	1 (4.5 %)	1
Thyroid	2 (11.8 %)	11 (40.7 %)	0.087	3 (27.3 %)	0 (0 %)	0.214	0 (0 %)	7 (31.8 %)	0.641
Autoimmune	0 (0 %)	4 (14.8 %)	0.26	1 (9.1 %)	0 (0 %)	1	0 (0 %)	3 (13.6 %)	1
Thrombolysis in myocardial infarction (TIMI) score			0.631			0.497			0.878
0	9 (52.9 %)	11 (40.7 %)		2 (18.2 %)	1 (9.1 %)		0 (0 %)	5 (22.7 %)	
1–3	8 (47.1 %)	16 (59.3 %)		8 (72.7 %)	7 (63.6 %)		3 (100 %)	17 (77.3 %)	
4	0 (0 %)	0 (0 %)		1 (9.1 %)	3 (27.3 %)		0 (0 %)	0 (0 %)	
Smoker (%)	8 (47.1 %)	13 (48.1 %)	1	8 (72.7 %)	6 (54.5 %)	0.658	2 (66.7 %)	15 (68.2 %)	1
BMI, kg/m2	40.5 (37.3—44.5)	38.6 (33.9—43.3)	0.555	31.1 (28.5—35.9)	32.6 (28.8—36.9)	0.748	36.0 (34.4—47.5)	40.9 (36.3—49.6)	0.783
Family History of Myocardial Infarction (MI)	5 (29.4 %)	10 (37.0 %)	0.847	2 (18.2 %)	5 (45.5 %)	0.36	2 (66.7 %)	4 (18.2 %)	0.261
Chest pain type			0.126			0.801			0.575
Atypical angina	11 (64.7 %)	10 (37.0 %)		5 (45.5 %)	4 (36.4 %)		2 (66.7 %)	13 (59.1 %)	
Typical angina	2 (11.8 %)	10 (37.0 %)		5 (45.5 %)	5 (45.5 %)		0 (0 %)	4 (18.2 %)	
Non-anginal	4 (23.5 %)	7 (25.9 %)		1 (9.1 %)	2 (18.2 %)		1 (33.3 %)	3 (13.6 %)	
Female-specific risk factors									
Menopause	/	21 (77.8 %)		/	11 (100 %)		/	15 (68.2 %)	
Polycystic ovary syndrome (PCOS)	/	2 (7.4 %)		/	2 (18.2 %)		/	0 (0 %)	
Pregnancy loss	/	12 (44.4 %)		/	7 (63.6 %)		/	9 (40.9 %)	
Physiological data									
Coronary Flow Reserve (CFR)	3.62 (2.68—3.88)	2.79 (2.51—3.25)	0.085	2.51 (2.37—2.91)	2.12 (1.63—2.57)	0.173	1.84 (1.83—1.93)	1.81 (1.40—1.96)	0.359
Average heart rate, beats/min	93.3 (85.1—100)	96.5 (84.8—109)	0.448	88.5 (79.3—95.8)	89.0 (86.0—98.0)	0.39	113 (111—115)	96.0 (90.0—112)	0.19
Average systolic blood pressure, mmHg	128 (116—139)	126 (113—150)	0.95	128 (105—137)	131 (128—140)	0.39	175 (147—178)	122 (109—143)	0.138
Medications									
Angiotensin-converting enzyme (ACE) inhibitor	9 (52.9 %)	13 (48.1 %)	1	4 (36.4 %)	5 (45.5 %)	1	3 (100 %)	8 (36.4 %)	0.143
Aspirin	6 (35.3 %)	7 (25.9 %)	0.746	8 (72.7 %)	9 (81.8 %)	1	2 (66.7 %)	9 (40.9 %)	0.823
Beta blocker	2 (11.8 %)	6 (22.2 %)	0.635	8 (72.7 %)	8 (72.7 %)	1	2 (66.7 %)	12 (54.5 %)	1
Calcium channel blocker (CCB)	2 (11.8 %)	7 (25.9 %)	0.453	5 (45.5 %)	3 (27.3 %)	0.658	0 (0 %)	10 (45.5 %)	0.379
Antiplatelet	0 (0 %)	0 (0 %)	NA	4 (36.4 %)	5 (45.5 %)	1	0 (0 %)	0 (0 %)	NA
Diabetes medications	6 (35.3 %)	5 (18.5 %)	0.371	3 (27.3 %)	3 (27.3 %)	1	2 (66.7 %)	9 (40.9 %)	0.823
Lipid lowering drugs	6 (35.3 %)	11 (40.7 %)	0.965	9 (81.8 %)	11 (100 %)	0.458	1 (33.3 %)	12 (54.5 %)	0.941

Continuous variables are shown as median (interquartile range), and categorical variables are shown as n (%). P-values were derived from Wilcoxon rank sum test and chi-squared test for continuous and categorical variables respectively; CVA: Cerebral Vascular Accident; PE: Pulmonary Embolism; DVT: Deep Vein Thrombosis; TIMI: Thrombolysis in myocardial infarction; PCOS: Polycystic ovary syndrome; CFR: Coronary Flow Reserve; ACE: Angiotensin-converting enzyme; CCB: Calcium channel blocker;

Missing values are quantified as a percentage of the cohort totals. For variables applicable to all participants: Educational Level (6.59%), Chest pain type (2.20%), Coronary Flow Reserve (3.30%), Average Heart Rate (8.79%), and Average Systolic Blood Pressure (5.49%). For female-specific variables, percentages reflect missing data within the female cohort subset: Menopause (1.67%) and Pregnancy Loss (8.33%).

Table 2
Top 20 plasma protein biomarkers that are differentially expressed between males and females in three groups.

Control Biomarker	Protein Name	Scaled β	SE	FDR
INSL3	Insulin-like 3	−2.039	0.171	1.81E-25
SPINT3	Kunitz-type protease inhibitor 3	−1.985	0.319	3.04E-17
EDDM3B	Epididymal secretory protein E3-beta	−1.850	0.182	3.30E-12
KLK3	Prostate-specific antigen	−1.853	0.308	3.02E-09
PZP	Pregnancy zone protein	1.685	0.357	3.48E-06
FSHB	Follitropin subunit beta	1.378	0.193	3.57E-04
XG	Glycoprotein Xg	1.476	0.128	4.57E-04
ACRV1	Acrosomal protein SP-10	−1.465	0.317	5.08E-04
LEP	Leptin	1.163	0.261	6.19E-04
TEX101	Testis-expressed protein 101	−1.390	0.259	9.61E-04
PROK1	Prokineticin-1	−1.146	0.231	1.19E-03
CALCA	Calcitonin	−1.176	0.238	1.59E-02
MYLPF	Myosin regulatory light chain 2, skeletal muscle isoform	−1.146	0.615	4.41E-02
GFAP	Glial fibrillary acidic protein	1.000	0.191	4.41E-02
OBP2B	Odorant-binding protein 2b	−1.152	0.226	4.41E-02
SPESP1	Sperm equatorial segment protein 1	−1.231	0.247	5.08E-02
PAFAH2	Platelet-activating factor acetylhydrolase 2, cytoplasmic	1.094	0.268	5.40E-02
PSPN	Persephin	−1.099	0.393	7.93E-02
PTPRR	Receptor-type tyrosine-protein phosphatase R	1.111	0.158	9.99E-02
CLMP	CXADR-like membrane protein	0.916	0.090	1.76E-01
CAD Biomarker	**Protein Name**	**Scaled β**	**SE**	**FDR**
INSL3	Insulin-like 3	−1.766	0.667	5.98E-03
FSHB	Follitropin subunit beta	1.764	0.217	7.60E-03
EDDM3B	Epididymal secretory protein E3-beta	−1.435	0.339	4.26E-02
SPINT3	Kunitz-type protease inhibitor 3	−1.563	0.884	8.82E-02
KLK3	Prostate-specific antigen	−1.382	0.800	9.10E-02
PTPRC	Receptor-type tyrosine-protein phosphatase C	1.456	0.144	9.10E-02
CTSZ	Cathepsin Z	1.148	0.168	9.10E-02
CXCL9	C-X-C motif chemokine 9	1.087	0.275	1.57E-01
BPIFB2	BPI fold-containing family B member 2	1.176	0.275	1.71E-01
ICAM2	Intercellular adhesion molecule 2	1.157	0.137	1.92E-01
SELL	L-selectin	1.454	0.099	2.11E-01
SOX9	Transcription factor SOX-9	1.381	1.090	2.11E-01
CD80	T-lymphocyte activation antigen CD80	1.311	0.190	2.11E-01
TJP3	Tight junction protein ZO-3	1.242	0.264	2.11E-01
INHBB	Inhibin beta B chain	1.293	0.348	2.17E-01
GGH	Gamma-glutamyl hydrolase	1.194	0.125	2.17E-01
MMP7	Matrilysin	1.299	0.068	2.24E-01
SLAMF7	SLAM family member 7	1.447	0.319	2.24E-01
OSCAR	Osteoclast-associated immunoglobulin-like receptor	1.121	0.150	2.29E-01
ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2	1.118	0.182	2.63E-01
CMD Biomarker	**Protein Name**	**Scaled β**	**SE**	**FDR**
INSL3	Insulin-like 3	−2.797	0.311	1.72E-08
SPINT3	Kunitz-type protease inhibitor 3	−2.603	0.515	9.62E-06
EDDM3B	Epididymal secretory protein E3-beta	−2.854	0.301	2.43E-04
KLK3	Prostate-specific antigen	−2.565	0.422	2.98E-03
SCGB3A1	Secretoglobin family 3A member 1	2.698	0.161	9.21E-03
HGFAC	Hepatocyte growth factor activator	2.670	0.140	9.21E-03
SNCG	Gamma-synuclein	1.302	0.278	6.16E-02
PZP	Pregnancy zone protein	2.467	0.877	2.12E-01
FOXJ3	Forkhead box protein J3	−1.685	0.411	2.12E-01
OPHN1	Oligophrenin-1	−2.231	0.607	2.56E-01
ANGPTL3	Angiopoietin-related protein 3	2.094	0.274	2.95E-01
CDCP1	CUB domain-containing protein 1	−1.904	0.355	3.57E-01
IGDCC4	Immunoglobulin superfamily DCC subclass member 4	1.958	0.191	4.82E-01
CA9	Carbonic anhydrase 9	−2.085	0.365	4.92E-01
COL9A2	Collagen alpha-2(IX) chain	1.947	0.464	5.12E-01
MFAP5	Microfibrillar-associated protein 5	1.822	0.327	5.49E-01
PSAPL1	Proactivator polypeptide-like 1	1.629	0.387	6.58E-01
XG	Glycoprotein Xg	1.593	0.294	6.58E-01
ATP5PO	ATP synthase subunit O, mitochondrial	−2.020	0.489	6.58E-01
SERPINF2	Alpha-2-antiplasmin	2.035	0.344	6.58E-01

3.4. Weighted correlation network analysis

Protein correlation network was constructed to identify modules of highly correlated proteins using WGCNA. The identified protein modules and their correlation with sex were assessed in both CAD and CMD groups. Eleven protein modules were identified in the CAD group, among which the green module (Supplemental Fig. 2A) was positively correlated with sex ($r = 0.49$, $P = 0.02$). The proteins in the green module were significantly enriched for pathways related to immune response and inflammation, including granulocyte and agranulocyte

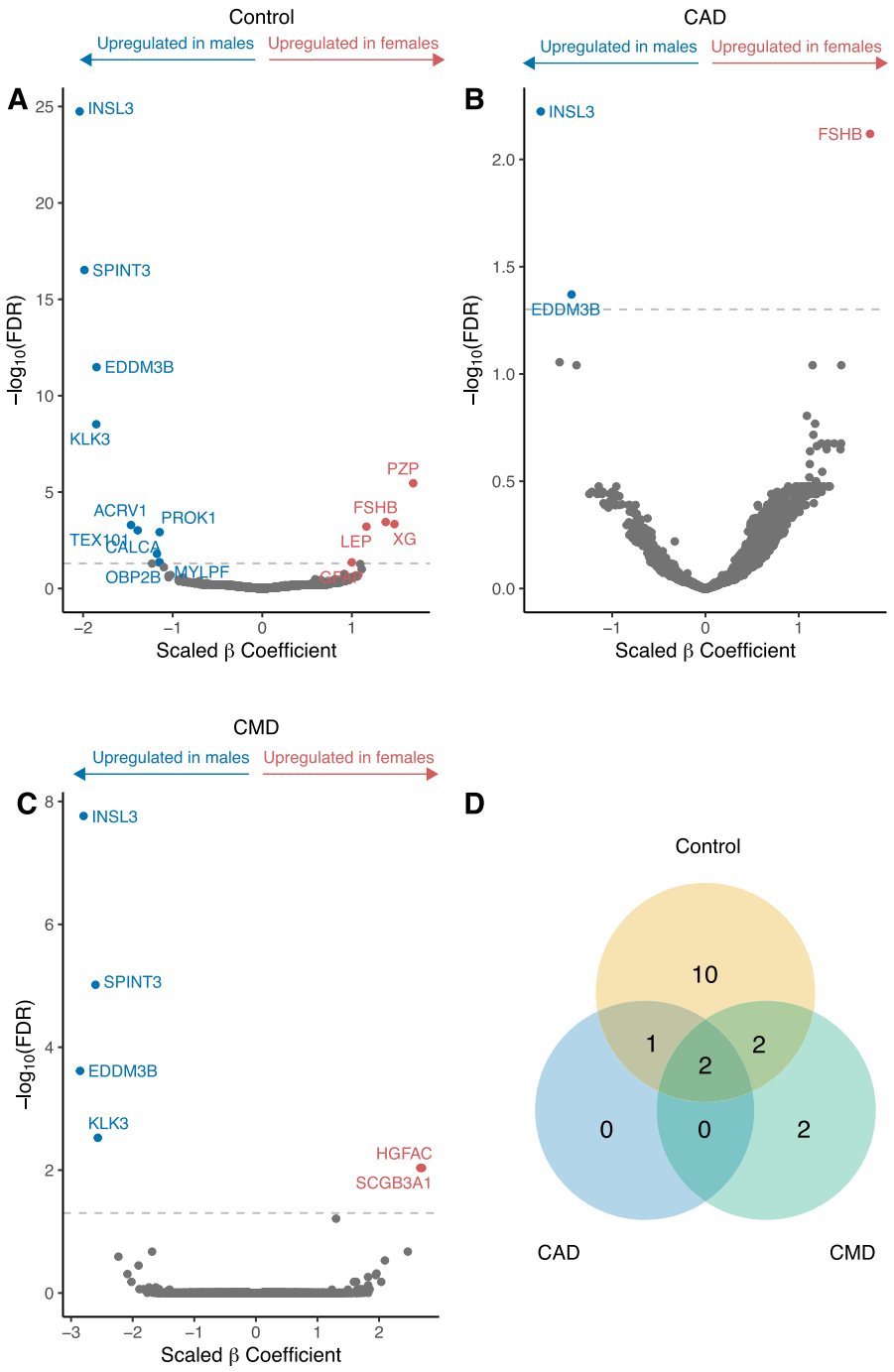

Fig. 1. Differential protein expression between males and females in three groups. (A-C) Volcano plots of relative protein expressions in the control (A), CAD (B), and CMD (C) patients. Positive x-values represent proteins that are higher in females (red points), and negative x-values represent proteins that are higher in males (blue points). The dashed line represents FDR < 0.05. (D) Venn diagram showing the intersection of differentially expressed proteins in the control, CAD, and CMD groups. Overlapping regions indicate proteins that show sex differences, either upregulated in males or females, across groups.

adhesion and diapedesis, the complement system, phagosome formation (Supplemental Fig. 2B). In contrast, no module was significantly correlated with sex in the CMD group.

4. Discussion

In a novel proof-of-concept study, we used an unbiased, discovery-based plasma proteomics approach to compare sex differences by CVD phenotype for acute ischemia. We noted important differential protein expression in reproductive hormones between male and female patients across all groups, as well as multiple biological pathways that shed light onto sexual divergence in CVD development. Among CAD patients, females showed upregulation of inflammatory and immune pathways while males showed upregulation of proteins related to vascular flow. Among CMD patients, females had upregulation of glucose and lipid pathways while males had upregulation of angiogenesis and endothelial flow. To our knowledge, this is the first study to identify sex differences in proteomics of acutely symptomatic patients being assessed for cardiac ischemia in the ED.

While proteomics, particularly untargeted genome-wide approaches,

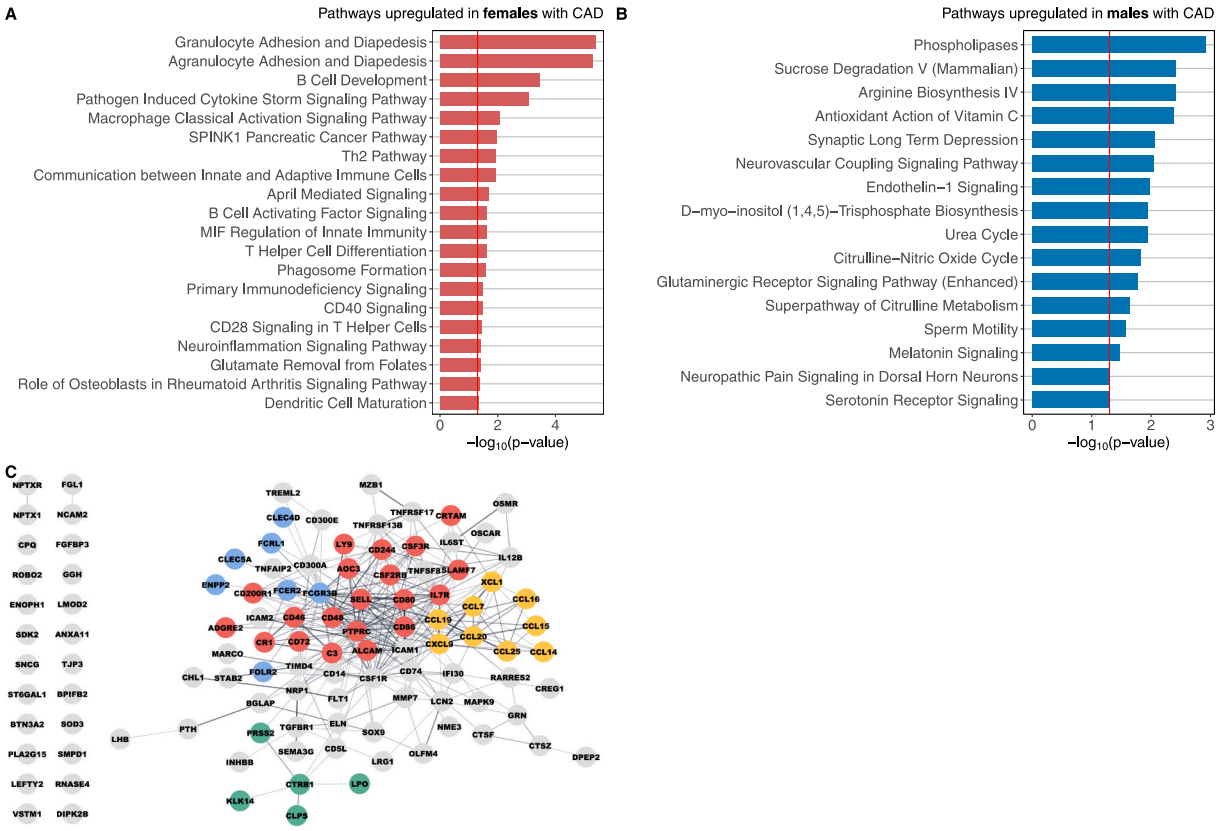

Fig. 2. Pathway overrepresentation in sex-specific protein upregulation in the CAD group. (A) Overrepresented pathways upregulated in females among CAD patients. The red line represents $p < 0.05$. (B) Overrepresented pathways upregulated in males among CAD patients. The red line represents $p < 0.05$. (C) Protein-protein interaction network for biomarkers that are higher in females with CAD. Red represents protein cluster 1, yellow represents protein cluster 2, blue represents protein cluster 3, and green represents protein cluster 4.

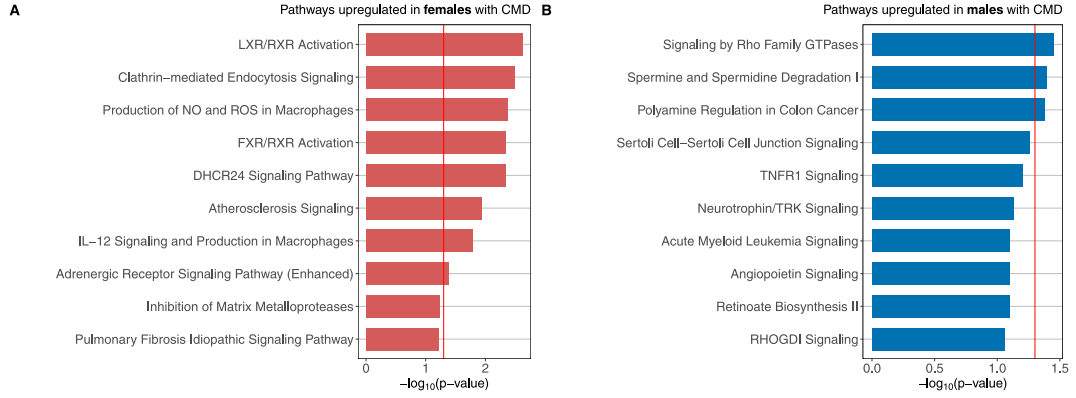

Fig. 3. Pathway overrepresentation in sex-specific protein upregulation in the CMD group. (A) Overrepresented pathways upregulated in females among CMD patients. The red line represents $p < 0.05$. (B) Overrepresented pathways upregulated in males among CMD patients. The red line represents $p < 0.05$.

is well-established in chronic cardiovascular research, its application in acute ischemic events remains limited. Previous work, such as Mazidi et al,[24] demonstrated the integration of proteomics and genetic data in ischemic heart disease, these studies focused primarily on chronic stable state of ischemia and population-level analyses. We extended this approach to an acute clinical setting, specifically examining sex-based proteomic differences among symptomatic patients being assessed for acute cardiac ischemia in the ED and builds upon existing proteomic research by investigating a broader panel of proteins.[7,9] We identified a much larger number of upregulated proteins among female patients than among males with CVD, confirming the findings of a previous study.[25] Experimental rat models of ischemia shed light into the underlying mechanism of this observation.[26] Compared with males, female rats after ischemia show significantly higher upregulation of proteins linked with extracellular matrix remodeling and inflammation – an estrogen led effect leading to faster replenishment of contractile structures and more rapid healing.[26] This finding aligns with De Bakker et al.[27], who reported extracellular matrix organization as dominant in females and regulation of apoptotic processes as prevalent in males with heart failure with reduced ejection fraction. These consistent observations suggest shared biological mechanisms involving tissue remodeling, immune regulation, and vascular maintenance across different cardiovascular conditions.

We also identified upregulated immune and cytokine-mediated

proteins in CAD patients most pronounced among females. Our findings are consistent with the functional pathways noted in the UK Biobank registry as well as the Framingham Heart Registry.[7,28] Although the specific proteins in the UK study were different from those found in our study, reflecting the dynamic nature of a changing proteome in acute versus chronic ischemic states, we found similar biological pathways involved in CAD among females. Immune activation has been shown to accelerate plaque development and atherosclerosis.[29] In females, immune pathways appear to affect plaque through dyslipidemia pathways[30], effects that could be related to hormonal changes. For instance, we observed elevated FSHB in females with CAD and among controls, which could reflect the high proportion of menopausal females in both groups. FSH has been implicated in the development of CAD through its role in the development of dyslipidemia in perimenopausal and menopausal females, even with similar estrogen levels.[25] In fact, the blockade of FSH signaling helps to lower serum cholesterol levels by 30 %, potentially offering a sex-specific therapeutic target after menopause.[31].

We also observed upregulation of proteins in males with CAD, primarily in relation to vascular flow. Arginine and endothelin (ET) pathways, unregulated in our study, are related to vascular flow and blood pressure regulation and might explain increased risk for CAD in younger males.[32] Sex differences have been noted in the endothelin (ET) pathway of the vasculature, heart, and kidneys of humans and experimental animals with higher levels noted in males compared to females.[33] In addition to the effects of sex chromosomes, testosterone positively modulates ET-1 expression, whereas estrogen downregulates it.[34] Our finding corroborates the multiple shared biological pathways observed with CAD in both males and females in the UK biobank, with male-specific upregulation of vascular flow and hormonal response (insulin-like growth factor response) pathways.[25].

Another key finding is that in CMD, proteins with a primarily sex-linked heterogeneous profile of mechanisms were upregulated. In females, we observed upregulation in glucose and LXR pathways, which maintain cholesterol homeostasis for protein targets, such as APOA1 and APOD. Postmenopausal females, due to declining estrogen production, experience disruptions in their evolutionarily optimized lipid and cholesterol metabolism, increasing their susceptibility to obesity-related diseases, including CVD.[35,36] Two thirds of our female CMD group was postmenopausal, and majority morbidly obese (median BMI of 40.9), hence upregulation of these pathways underscores the well-known connection among menopausal status, obesity, and CMD.[37] Schindler[38] enriches this argument by explaining the role of different adipose tissue types in obesity and suggests that increases in visceral adipose tissue, as opposed to subcutaneous fat, lead to greater metabolic abnormalities and CMD. Abnormal lipid metabolism may cause endothelial dysfunction in CMD patients.[39] This effect is particularly relevant to postmenopausal females, who tend to lose the protective benefits of subcutaneous fat accumulation with age, thus potentially increasing CMD risks. The Mayo Clinic Proteomic Markers of Arteriosclerosis Study reported sex differences with the HDL component of ApoA-1 to be higher in females with CMD.[40] Obesity status has also been associated with higher levels of free fatty acids in circulating blood, likely due to increased sympathetic drive, causing activation of lipolysis in adipose tissue.[41] High levels of free fatty acid reduce cardiac glucose utilization, leading to increased oxygen consumption and making the heart more susceptible to ischemic events.

Our findings regarding the upregulation of the LXR/RXR pathway align with prior evidence of sex-dimorphic regulation in lipid metabolism, as described by Rando and Wahli.[42] Nuclear receptors such as PPARα, LXR, and ERα modulate lipid and glucose metabolism through hormone-sensitive regulatory networks, with females exhibiting more robust compensatory mechanisms. This overlap suggests that the observed upregulation of LXR/RXR pathways in CMD females could reflect both intrinsic sex-based differences and disease-specific metabolic disruption. This convergence emphasizes the complex interplay between biological sex and CVD pathophysiology, supporting further investigation into sex-specific regulatory networks.

Two additional pathways in relation to CMD deserve attention. First, we noted a differential upregulation of HGFAC in females with CMD as compared to controls and CAD patients. Hepatocyte growth factor has long been identified as a potent mitogen for hepatocytes promoting angiogenic growth. Furthermore, high HCF levels counter response to endothelial dysfunction, offering both a prognostic and therapeutic target.[43,44] Second, SCGB3A1, a protein in females with CMD, is implicated in the immune response to ischemia. Interestingly, nocarandil, a smooth muscle vasodilator, suppresses SCGB3A1 in acute ischemia,[45] offering a potential biological pathway for improved microvascular flow. Together, these findings support further research to identify sex-specific molecular targets by CVD phenotype as an important step in advancing personalized CVD care.

The rationale for using WGCNA, rather than individual protein-level statistical tests, is its ability to uncover biologically meaningful modules of co-expressed proteins that function collectively within the same biological pathways. This network-based approach can reveal hidden relationships among proteins that may be missed by conventional univariate statistical methods, enhancing interpretation of sex-specific molecular mechanisms. Importantly, what we found through WGCNA aligns with the findings from individual protein-level analysis. Specifically, proteins involved in immune and inflammatory pathways were upregulated in females with CAD in both approaches. This convergence supports the robustness of our findings, it suggests that proteins within the same biological processes are not only individually significant but also co-regulated, strengthening the case for these pathways' involvement in sex-specific CAD mechanisms.

5. Limitations

This was a single site study limited to a single time point assessment. As such, the results show only an association without evidence of causality. We also did not find diagnostic differentiation of proteomic profile between controls and CVD phenotype. This could also be due to the heterogeneity of mechanisms among controls who were symptomatic patients with overlapping CVD comorbidities as opposed to healthy individuals. However, consistent with prior investigations that established sex differences in proteome profiles among healthy individuals, we identified differential expression of proteins in males and females in our controls as well as among diseased groups, making a sex-specific comparison valid.[6].

The limited sample size in this single-site study restricted the statistical power to detect differentially expressed proteins. For example, under the threshold FDR < 0.05, we identified fewer differentially expressed proteins in CVD phenotypes compared to controls, likely due to the control group being about twice as large as the CAD or CMD groups. However, in the pathway analysis using the criteria P-value < 0.05 and $|\beta| > 1$, we observed a greater number of proteins in CAD and CMD than in the control group, suggesting that sex-specific proteomic differences can persist or even become more pronounced in the presence of disease. Additionally, we observed differences in medication use, such as antiplatelets and aspirin, across groups. Differences in medications could influence protein expression and pathways. While we did not find a statistically significant difference in medication use between males and females, we acknowledge that the overall differences in medication use across groups may influence the proteomic comparisons. Also, the inclusion or exclusion of posttranslational modifications (PTMs) of proteins presents a methodological challenge. PTMs can substantially alter protein function and interactions, impacting the interpretability of proteomic data in the context of CVD phenotypes. Our analysis did not account for PTMs, which may affect the comprehensiveness and accuracy of our findings regarding protein function and regulation.

We used unadjusted p-values for pathway enrichment analysis in exploratory proteomics to increase sensitivity in detecting pathways of

interest, though this might increase the risk of false positives. While differentially expressed proteins were identified using FDR control, the same adjustment was not applied in pathway analysis to avoid missing potentially important biological pathways. Future studies could incorporate adjusted p-values or validate key findings in independent samples. Finally, we acknowledge that tissue-based proteomics is more sensitive in capturing local physiology of myocardial changes or vascular function related to cardiac ischemia. While tissue samples provide greater insights, they are invasive and difficult to obtain especially in less sick patients limiting its generalizability. A study of circulating protein biomarkers in patients with ischemia as we and others have done,[10,11] should be considered hypothesis generating for insights into the biological pathways for ischemia. It also provides a relative distribution of proteins and thus interpretation of pathways should consider both upregulation as well as downregulation of proteins relative to sexes. Further investigation of proteomics in cardiac tissue is necessary to gain deeper mechanistic insight into CVD phenotypes as well as absolute differences.

6. Conclusions

In this exploratory analysis, we demonstrated that sex differences exist in the proteome of CVD phenotypes. We revealed sex differences in proteomics and biological pathways for acutely symptomatic CMD and CAD, illustrating they are distinct diseases with potential for different therapeutic targets. Further validation studies that incorporate omics into clinical pathways would help advance a precision-based approach for personalized CVD care.

CRediT authorship contribution statement

Yihan Liu: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Zuoheng Wang:** Writing – review & editing, Formal analysis. **Sean P. Collins:** Writing – review & editing, Conceptualization. **Jeffery Testani:** Writing – review & editing, Conceptualization. **Basmah Safdar:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Funding

Dr. Safdar is supported by NIH grants (1OTHL56812-01; U24NS129500, 1OT2HL162110-01), a CDC grant (75D30121F0002), as well as institutional grants from Comprehensive Research Associates. Dr. Wang is supported by NIH grant (R01LM014087). This publication was made possible by CTSA Grant Number UL1 TR001863 or KL2 TR001862 or TL1 TR001864 (as appropriate) from the National Center for Advancing Translational Science (NCATS), a component of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NIH.

Declaration of competing interest

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

Acknowledgement

We thank Dr. Sharese Terrell Willis and Noah Brazer for editing and submission of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcha.2025.101667.

References

[1] C.W. Tsao, A.W. Aday, Z.I. Almarzooq, C.A.M. Anderson, P. Arora, C.L. Avery, C. M. Baker-Smith, A.Z. Beaton, A.K. Boehme, A.E. Buxton, Y. Commodore-Mensah, M.S.V. Elkind, K.R. Evenson, C. Eze-Nliam, S. Fugar, G. Generoso, D.G. Heard, S. Hiremath, J.E. Ho, R. Kalani, D.S. Kazi, D. Ko, D.A. Levine, J. Liu, J. Ma, J. W. Magnani, E.D. Michos, M.E. Mussolino, S.D. Navaneethan, N.I. Parikh, R. Poudel, M. Rezk-Hanna, G.A. Roth, N.S. Shah, M.-P. St-Onge, E.L. Thacker, S. S. Virani, J.H. Voeks, N.-Y. Wang, N.D. Wong, S.S. Wong, K. Yaffe, S.S. Martin, Heart Disease and Stroke Statistics—2023 Update: A Report From the American Heart Association, *Circulation*. 147 (2023) e93–e621, https://doi.org/10.1161/CIR.0000000000001123.

[2] N. Williams, How Sex and Gender Impact Clinical Practice: An Evidence-Based Guide to Patient Care, *Occup Med-Oxford*. 71 (2021) 307, https://doi.org/10.1093/occmed/kqab056.

[3] L. Aryan, D. Younessi, M. Zargari, S. Banerjee, J. Agopian, S. Rahman, R. Borna, G. Ruffenach, S. Umar, M. Eghbali, The Role of Estrogen Receptors in Cardiovascular Disease, *Int. J. Mol. Sci.*. 21 (2020) 4314.

[4] V. Regitz-Zagrosek, G. Kararigas, Mechanistic Pathways of Sex Differences in Cardiovascular Disease, *Physiol Rev*. 97 (2017) 1–37, https://doi.org/10.1152/physrev.00021.2015.

[5] R.M. Hoogeveen, J.P.B. Pereira, N.S. Nurmohamed, V. Zampoleri, M.J. Bom, A. Baragetti, S.M. Boekholdt, P. Knaapen, K.T. Khaw, N.J. Wareham, A.K. Groen, A. L. Catapano, W. Koenig, E. Levin, E.S.G. Stroes, Improved cardiovascular risk prediction using targeted plasma proteomics in primary prevention, *Eur Heart J*. 41 (2020) 3998–4007, https://doi.org/10.1093/eurheartj/ehaa648.

[6] K. Miike, M. Aoki, R. Yamashita, Y. Takegawa, H. Saya, T. Miike, K. Yamamura, Proteome profiling reveals gender differences in the composition of human serum, *Proteomics*. 10 (2010) 2678–2691, https://doi.org/10.1002/pmic.200900496.

[7] E.S. Lau, S.M. Paniagua, J.S. Guseh, V. Bhambhani, M.V. Zanni, P. Courchesne, A. Lyass, M.G. Larson, D. Levy, J.E. Ho, Sex Differences in Circulating Biomarkers of Cardiovascular Disease, *J Am Coll Cardiol*. 74 (2019) 1543–1553, https://doi.org/10.1016/j.jacc.2019.06.077.

[8] A. Raafs, J. Verdonschot, J.P. Ferreira, P. Wang, T. Collier, M. Henkens, J. Bjorkman, A. Boccanelli, A.L. Clark, C. Delles, J. Diez, A. Gonzalez, N. Girerd, J. W. Jukema, F. Pinet, P. Rossignol, T. Thum, N. Vodovar, R.A. de Boer, V. van Empel, J.A. Staessen, M. Hazebroek, J. Cleland, F. Zannad, S. Heymans, Identification of sex-specific biomarkers predicting new-onset heart failure, *ESC Heart Fail.* 8 (2021) 3512–3520, https://doi.org/10.1002/ehf2.13476.

[9] Lew J, Sanghavi M, Ayers CR, McGuire DK, Omland T, Atzler D, Gore MO, Neeland I, Berry JD, Khera A, Rohatgi A, de Lemos JA. Sex-Based Differences in Cardiometabolic Biomarkers. *Circulation*. 2017;135:544-+. doi: 10.1161/Circulationaha.116.023005.

[10] E. Prescott, K.B. Bove, D.F. Bechsgaard, B.H. Shafi, T. Lange, J. Schroder, H. E. Suhrs, R.L. Nielsen, Biomarkers and Coronary Microvascular Dysfunction in Women With Angina and No Obstructive Coronary Artery Disease, *JACC Adv*. 2 (2023), https://doi.org/10.1016/j.jacadv.2023.100264.

[11] C. Chandramouli, T.W. Ting, J. Tromp, A. Agarwal, S. Svedlund, A. Saraste, C. Hage, R.S. Tan, L. Beussink-Nelson, M.L. Fermer, L.M. Gan, L. Lund, S.J. Shah, C. S.P. Lam, Sex differences in proteomic correlates of coronary microvascular dysfunction among patients with heart failure and preserved ejection fraction, *Eur J Heart Fail*. 24 (2022) 681–684, https://doi.org/10.1002/ejhf.2435.

[12] E. Assarsson, M. Lundberg, G. Holmquist, J. Björkesten, S.B. Thorsen, D. Ekman, A. Eriksson, E.R. Dickens, S. Ohlsson, G. Edfeldt, A.C. Andersson, P. Lindstedt, J. Stenvang, M. Gullberg, S. Fredriksson, Homogenous 96-Plex PEA Immunoassay Exhibiting High Sensitivity, Specificity, and Excellent Scalability, *PLoS One*. 9 (2014), https://doi.org/10.1371/journal.pone.0095192.

[13] Olink. https://olink.com/. Accessed 3/25/24.

[14] A. Petrera, C. von Toerne, J. Behler, C. Huth, B. Thorand, A. Hilgendorff, S. M. Hauck, Multiplatform Approach for Plasma Proteomics: Complementarity of Olink Proximity Extension Assay Technology to Mass Spectrometry-Based Protein Profiling, *J Proteome Res*. 20 (2021) 751–762, https://doi.org/10.1021/acs.jproteome.0c00641.

[15] L. Wik, N. Nordberg, J. Broberg, J. Bjorkesten, E. Assarsson, S. Henriksson, I. Grundberg, E. Pettersson, C. Westerberg, E. Liljeroth, A. Falck, M. Lundberg, Proximity Extension Assay in Combination with Next-Generation Sequencing for High-throughput Proteome-wide Analysis, *Mol Cell Proteomics*. 20 (2021) 100168, https://doi.org/10.1016/j.mcpro.2021.100168.

[16] P. Ong, P.G. Camici, J.F. Beltrame, F. Crea, H. Shimokawa, U. Sechtem, J.C. Kaski, C.N.B. Merz, C.V. Disorders, International standardization of diagnostic criteria for microvascular angina, *Int J Cardiol*. 250 (2018) 16–20, https://doi.org/10.1016/j.ijcard.2017.08.068.

[17] B. Safdar, G. D'Onofrio, J. Dziura, R.R. Russell, C. Johnson, A.J. Sinusas, Prevalence and characteristics of coronary microvascular dysfunction among chest pain patients in the emergency department, *Eur Heart J Acute Cardiovasc Care*. 9 (2020) 5–13, https://doi.org/10.1177/2048872618764418.

[18] A. Krämer, J. Green, J. Pollard, S. Tugendreich, Causal analysis approaches in Ingenuity Pathway Analysis, *Bioinformatics*. 30 (2014) 523–530, https://doi.org/10.1093/bioinformatics/btt703.

[19] D. Szklarczyk, R. Kirsch, M. Koutrouli, K. Nastou, F. Mehryary, R. Hachilif, A. L. Gable, T. Fang, N.T. Doncheva, S. Pyysalo, P. Bork, L.J. Jensen, C. von Mering, The STRING database in 2023: protein-protein association networks and functional

enrichment analyses for any sequenced genome of interest, *Nucleic Acids Res*. 51 (2023) D638, https://doi.org/10.1093/nar/gkac1000.
[20] S. Van Dongen, Graph clustering via a discrete uncoupling process, *Siam J Matrix Anal A*. 30 (2008) 121–141, https://doi.org/10.1137/040608635.
[21] M. Ashburner, C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, J.T. Eppig, M.A. Harris, D.P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J.C. Matese, J.E. Richardson, M. Ringwald, G.M. Rubin, G. Sherlock, Gene Ontology: tool for the unification of biology, *Nat. Genet.*. 25 (2000) 25–29, https://doi.org/10.1038/75556.
[22] S.A. Aleksander, J. Balhoff, S. Carbon, J.M. Cherry, H.J. Drabkin, D. Ebert, M. Feuermann, P. Gaudet, N.L. Harris, D.P. Hill, R.Y. Lee, H.Y. Mi, S. Moxon, C. J. Mungall, A. Muruganugan, T. Mushayahama, P.W. Sternberg, P.D. Thomas, K. Van Auken, J. Ramsey, D.A. Siegele, R.L. Chisholm, P. Fey, M.C. Aspromonte, M. V. Nugnes, F. Quaglia, S. Tosatto, M. Giglio, S. Nadendla, G. Antonazzo, H. Attrill, G. dos Santos, S. Marygold, V. Strelets, C.J. Tabone, J. Thurmond, P.L. Zhou, S. H. Ahmed, P. Asanitthong, D.L. Buitrago, M.N. Erdol, M.C. Gage, M.A. Kadhum, K. Y.C. Li, M. Long, A. Michalak, A. Pesala, A. Pritazahra, S.C.C. Saverimuttu, R.Z. Su, K.E. Thurlow, R.C. Lovering, C. Logie, S. Oliferenko, J. Blake, K. Christie, L. Corbani, M.E. Dolan, D. Sitnikov, C. Smith, A. Cuzick, J. Seager, L. Cooper, J. Elser, P. Jaiswal, P. Gupta, S. Naithani, M. Lera-Ramirez, K. Rutherford, V. Wood, J.L. De Pons, M.R. Dwinell, G.T. Hayman, M.L. Kaldunski, A.E. Kwitek, S. J.F. Laulederkind, M.A. Tutaj, M. Vedi, S.J. Wang, P. D'Eustachio, L. Aimo, K. Axelsen, A. Bridge, N. Hyka-Nouspikel, A. Morgat, S.R. Engel, K. Karra, S. R. Miyasato, R.S. Nash, M.S. Skrzypek, S. Weng, E.D. Wong, E. Bakker, T. Z. Berardini, L. Reiser, A. Auchincloss, G. Argoud-Puy, M.C. Blatter, E. Boutet, L. Breuza, C. Casals-Casas, E. Coudert, A. Estreicher, M.L. Famiglietti, A. Gos, N. Gruaz-Gumowski, C. Hulo, F. Jungo, P. Le Mercier, D. Lieberherr, P. Masson, I. Pedruzzi, L. Pourcel, S. Poux, C. Rivoire, S. Sundaram, A. Bateman, E. Bowler-Barnett, H. Bye-A-Jee, P. Denny, A. Ignatchenko, R. Ishtiaq, A. Lock, Y. Lussi, M. Magrane, M.J. Martin, S. Orchard, P. Raposo, E. Speretta, N. Tyagi, K. Warner, R. Zaru, A.D. Diehl, J.C.R. Chan, S. Diamantakis, D. Raciti, M. Zarowiecki, M. Fisher, C. James-Zorn, V. Ponferrada, A. Zorn, S. Ramachandran, L. Ruzicka, M. Westerfield, The Gene Ontology knowledgebase in 2023, *Genetics*. 224 (2023), https://doi.org/10.1093/genetics/iyad031.
[23] P. Langfelder, S. Horvath, WGCNA: an R package for weighted correlation network analysis, *BMC Bioinformatics*. 9 (2008) 559, https://doi.org/10.1186/1471-2105-9-559.
[24] M. Mazidi, N. Wright, P. Yao, C. Kartsonaki, I.Y. Millwood, H. Fry, S. Said, A. Pozarickij, P. Pei, Y. Chen, D. Avery, H. Du, D.V. Schmidt, L. Yang, J. Lv, C. Yu, J. Chen, M. Hill, M.V. Holmes, J.M.M. Howson, R. Peto, R. Collins, D.A. Bennett, R. G. Walters, L. Li, R. Clarke, Z. Chen, China Kadoorie Biobank Collaborative G. Plasma Proteomics to Identify Drug Targets for Ischemic Heart Disease, *J Am Coll Cardiol*. 82 (2023) 1906–1920, https://doi.org/10.1016/j.jacc.2023.09.804.
[25] Sier VQ, van Dijk KW, van Heemst D, Quax PHA, Jukema JW, Noordam R, de Vries MR. Sex-Specific Associations of the Plasma-Proteome with incident Coronary Artery Disease. *medRxiv*. 2024:2024.2002.2017.24302702. doi: 10.1101/2024.02.17.24302702.
[26] B.A. Barta, M. Ruppert, K.E. Fröhlich, M. Cosenza-Contreras, A. Oláh, A.A. Sayour, K. Kovács, G.B. Karvaly, M. Biniossek, B. Merkely, O. Schilling, T. Radovits, Sex-related differences of early cardiac functional and proteomic alterations in a rat model of myocardial ischemia, *J Transl Med*. 19 (2021), https://doi.org/10.1186/s12967-021-03164-y.
[27] M. de Bakker, T.B. Petersen, K.M. Akkerhuis, M. Harakalova, V.A. Umans, T. Germans, K. Caliskan, P.D. Katsikis, P.J. van der Spek, N. Suthahar, R.A. de Boer, D. Rizopoulos, F.W. Asselbergs, E. Boersma, I. Kardys, Sex-based differences in cardiovascular proteomic profiles and their associations with adverse outcomes in patients with chronic heart failure, *Biol Sex Differ*. 14 (2023) 29, https://doi.org/10.1186/s13293-023-00516-9.
[28] Y.F. Huang, Q. Hui, M. Gwinn, Y.J. Hu, A.A. Quyyumi, V. Vaccarino, Y.V. Sun, Sexual Differences in Genetic Predisposition of Coronary Artery Disease, *Circ-Genom Precis Me*. 14 (2021), https://doi.org/10.1161/CIRCGEN.120.003147.
[29] T.R. Sakkers, M. Mokry, M. Civelek, J. Erdmann, G. Pasterkamp, E. Diez Benavente, H.M. den Ruijter, Sex differences in the genetic and molecular mechanisms of coronary artery disease, *Atherosclerosis*. 384 (2023) 117279, https://doi.org/10.1016/j.atherosclerosis.2023.117279.
[30] D.J. Jiang, D.W. Zheng, L.Y. Wang, Y. Huang, H.B. Liu, L.T. Xu, Q. Liao, P.P. Liu, X. B. Shi, Z.Y. Wang, L.B. Sun, Q.Y. Zhou, N. Li, L.M. Xu, Y.P. Le, M. Ye, G.F. Shao, S. W. Duan, Elevated Gene Promoter Methylation as a Gender-Specific Marker of Aging Increases the Risk of Coronary Heart Disease in Females, *PLoS One*. 8 (2013), https://doi.org/10.1371/journal.pone.0059752.
[31] Y. Guo, M. Zhao, T. Bo, S. Ma, Z. Yuan, W. Chen, Z. He, X. Hou, J. Liu, Z. Zhang, Q. Zhu, Q. Wang, X. Lin, Z. Yang, M. Cui, L. Liu, Y. Li, C. Yu, X. Qi, Q. Wang, H. Zhang, Q. Guan, L. Zhao, S. Xuan, H. Yan, Y. Lin, L. Wang, Q. Li, Y. Song, L. Gao, J. Zhao, Blocking FSH inhibits hepatic cholesterol biosynthesis and reduces serum cholesterol, *Cell Res.*. 29 (2019) 151–166, https://doi.org/10.1038/s41422-018-0123-6.
[32] H.L. Gornik, M.A. Creager, Arginine and endothelial and vascular health, *J Nutr*. 134 (2004) 2880s, https://doi.org/10.1093/jn/134.10.2880S.
[33] E.Y. Gohar, F.R. Giachini, D.M. Pollock, R.C. Tostes, Role of the endothelin system in sexual dimorphism in cardiovascular and renal diseases, *Life Sci*. 159 (2016) 20–29, https://doi.org/10.1016/j.lfs.2016.02.093.
[34] K.H. Polderman, C.D.A. Stehouwer, G.J. Vankamp, G.A. Dekker, F.W.A. Verheugt, L.J.G. Gooren, Influence of Sex-Hormones on Plasma Endothelin Levels, *Ann Intern Med*. 118 (1993) 429–432, https://doi.org/10.7326/0003-4819-118-6-199303150-00006.
[35] R. Baetta, M. Pontremoli, A.M. Fernandez, C.M. Spickett, C. Banfi, Proteomics in cardiovascular diseases: Unveiling sex and gender differences in the era of precision medicine (Reprinted from Journal of Proteomics, vol 173, pg 62-76, 2018), *J Proteomics*. 178 (2018) 57–72, https://doi.org/10.1016/j.jprot.2018.03.017.
[36] S. Della Torre, A. Maggi, Sex Differences: A Resultant of an Evolutionary Pressure? *Cell Metab*. 25 (2017) 499–505, https://doi.org/10.1016/j.cmet.2017.01.006.
[37] J.C. Watso, T.G. Bissen, Vascular dysfunction in females post-menopause with obesity: an ultrasonic view, *Int J Obesity*. (2024), https://doi.org/10.1038/s41366-024-01561-z.
[38] T.H. Schindler, Coronary Microvascular Dysfunction A Preferred Risk Marker in Obesity? *J Am Coll Cardiol*. 72 (2018) 718–720, https://doi.org/10.1016/j.jacc.2018.05.050.
[39] Y. Zhang, J. Zhao, C. Ren, B. Hu, R. Ding, Z. He, C. Liang, Y. Zhang, J. Zhao, C. Ren, B. Hu, R. Ding, Z. He, C. Liang, Free fatty acids induce coronary microvascular dysfunction via inhibition of the AMPK/KLF2/eNOS signaling pathway, *Int J Mol Med*. 51 (2023) 34, https://doi.org/10.3892/ijmm.2023.5237.
[40] C.X. Kim, K.R. Bailey, G.G. Klee, A.A. Ellington, G.H. Liu, T.H. Mosley, H. Rehman, I.J. Kullo, Sex and Ethnic Differences in 47 Candidate Proteomic Markers of Cardiovascular Disease: The Mayo Clinic Proteomic Markers of Arteriosclerosis Study, *PLoS One*. 5 (2010), https://doi.org/10.1371/journal.pone.0009065.
[41] Hendrickson SC, St. Louis JD, Lowe JE, Abdel-aleem S. Free fatty acid metabolism during myocardial ischemia and reperfusion. *Molecular and Cellular Biochemistry*. 1997;166:85-94. doi: 10.1023/A:1006886601825.
[42] G. Rando, W. Wahli, Sex differences in nuclear receptor-regulated liver metabolic pathways, *Biochim Biophys Acta*. 1812 (2011) 964–973, https://doi.org/10.1016/j.bbadis.2010.12.023.
[43] Y. Chen, J. Shen, H. Nilsson Anna, I. Goncalves, A. Edsfeldt, G. Engström, S. Zaigham, O. Melander, M. Orho-Melander, U. Rauch, M. Venuraju Shreenidhi, A. Lahiri, C. Liang, J. Nilsson, Circulating Hepatocyte Growth Factor Reflects Activation of Vascular Repair in Response to Stress. *JACC: Basic to Translational, Science*. 7 (2022) 747–762, https://doi.org/10.1016/j.jacbts.2022.03.013.
[44] R. Morishita, M. Aoki, Y. Yo, T. Ogihara, Hepatocyte growth factor as cardiovascular hormone: Role of HGF in the pathogenesis of cardiovascular disease, *Endocr J*. 49 (2002) 273–284, https://doi.org/10.1507/endocrj.49.273.
[45] K.Q. Hu, X.Q. Wang, H.Y. Hu, Z.Y. Xu, J.X. Zhang, G.P. An, G.H. Su, Intracoronary application of nicorandil regulates the inflammatory response induced by percutaneous coronary intervention, *J Cell Mol Med*. 24 (2020) 4863–4870, https://doi.org/10.1111/jcmm.15169.