Arteriosclerosis, Thrombosis, and Vascular Biology

ORIGINAL RESEARCH

Plasma Proteins Associated With Psychosocial Factors and Heart Disease: The Jackson Heart Study

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BACKGROUND: Knowledge of proteomic mechanisms explaining the link between psychosocial stress and cardiovascular disease is limited. This study aimed to (1) identify plasma proteins associated with psychosocial factors and (2) assess associational pathways between psychosocial factors, identified proteins, and incident cardiovascular disease events in a discovery cohort, JHS (Jackson Heart Study), and 2 replication cohorts, the CHS (Cardiovascular Health Study), and the MESA (Multi-Ethnic Study of Atherosclerosis).

METHODS: JHS participants from exam 1 (2000–2004) with SomaScan 1.3k platform proteomics data were included (n=2143, mean age=55.3). Depressive symptoms and perceived stress scores were measured via the 20-item Centers for Epidemiological Studies scale and an 8-item perceived stress scale adapted for the JHS, respectively. Multivariable linear regression models were used to test the association between psychosocial factors and plasma proteins, controlling for age, sex, proteomics batch, and estimated glomerular function. Meta-analyses were also performed across cohorts, using Bonferroni correction for multiple testing ($P<3.782\times10^{-5}$). Mediation analyses with Cox proportional hazards models were used to evaluate potential proteomic pathways in the association between psychosocial factors and coronary heart disease, heart failure, and stroke in JHS.

RESULTS: Angiopoietin-2 (β=0.013, SE=0.002, $P<0.001$), contactin-5 (β=–0.013, SE=0.002, $P<0.001$), growth/differentiation factor 15 or macrophage inhibitory cytokine 1 (β=0.011, SE=0.002, $P<0.001$), neural cell adhesion molecule 120 (β=–0.012, SE=0.002, $P<0.001$), and KYNU (kynureninase; β=0.014, SE=0.003, $P<0.001$) were each significantly associated with depressive symptoms, with angiopoietin-2, contactin-5, macrophage inhibitory cytokine 1, and neural cell adhesion molecule 120 replicating in CHS and MESA. Leukotriene A-4 hydrolase was associated with perceived stress (β=–0.0235, SE=0.005, $P<0.001$). Macrophage inhibitory cytokine 1 partially accounted for the association between depressive symptoms and incident coronary heart disease in JHS (23%; P=0.0009).

CONCLUSIONS: Novel associations between psychosocial factors, plasma proteins, and cardiovascular disease were identified in JHS. Circulating proteomic profiles across 3 cardiovascular disease cohorts showed differences in protein concentrations by psychosocial measures. Future investigations should identify additional potentially targetable proteomic mechanisms by which psychosocial factors contribute to disease.

Key Words: cardiovascular disease ■ contactins ■ heart failure ■ inflammation ■ proteomics

Cardiovascular disease (CVD) disproportionately burdens Black communities in the United States, with Black individuals being 2 to 3× more likely to die of heart disease compared with White individuals.[1] Studies have found that psychosocial factors (high perceived stress and depressive symptoms) are significantly associated with cardiovascular health indicators among marginalized groups.[2–5] Due to the complex nature of the

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Nonstandard Abbreviations and Acronyms

CES-D	Centers for Epidemiological Studies Depression
CHD	coronary heart disease
CHS	Cardiovascular Health Study
CVD	cardiovascular disease
eGFR	estimated glomerular filtration rate
GDF15	growth differentiation factor 15
JHS	Jackson Heart Study
KYNU	kynureninase
LKHA4	leukotriene A4 hydrolase
MESA	Multi-Ethnic Study of Atherosclerosis
MIC-1	macrophage inhibitory cytokine 1
NCAM	neural cell adhesion molecule
NTproBNP	N-terminal pro-B-type natriuretic peptide

What Are the Clinical Implications?

This study conducted a proteome-wide analysis to identify circulating proteins associated with psychosocial factors and incident cardiovascular disease using a large cohort of Black adults from the Jackson Heart Study and replication in 2 additional multiethnic cohorts. We identified plasma proteins linked to depressive symptoms and perceived stress, many of which were involved in inflammation, vascular function, and cellular stress responses. Importantly, MIC-1 mediated the association between depressive symptoms and incident coronary heart disease, demonstrating a potential proteomic pathway by which stress is linked to heart disease. The findings move beyond traditional risk factors by showing that psychosocial stressors are linked to measurable molecular signals in the blood that are relevant to cardiovascular risk. In the future, stress-related proteomic profiles could help identify individuals at higher risk who may benefit from earlier or more intensive prevention, including behavioral, psychosocial, or anti-inflammatory interventions. The proteins identified here also represent targets for future studies to test whether improving mental and emotional health can favorably alter biological pathways and reduce cardiovascular disease, particularly in populations that experience a high burden of both psychosocial stress and cardiovascular disease.

pathophysiology of psychosocial factors on health and well-being, the mechanistic underpinnings and cause of incident CVD remain poorly understood across populations and in marginalized communities.[6] Unraveling the underlying psychosocial pathway of CVD risk and development will aid in public health interventions and efforts to prevent heart disease.[7]

Exposure to excessive psychosocial stress and having a high burden of depressive symptoms can influence CVD risk through dysregulation of central neuroendocrine pathways, including hyperactivity of the hypothalamic–pituitary–adrenal axis and sympathetic nervous system.[8] Dysregulation of these systems from excessive stress can lead to downstream alterations in immune, inflammatory, and metabolic signaling.[9] For instance, sustained elevations in glucocorticoids and catecholamines can promote endothelial dysfunction and oxidative stress characterized by increased cytokine production and perturbations in coagulation and vascular remodeling pathways.[9] These neuroimmune and inflammatory processes are increasingly recognized as key biological intermediates linking psychosocial exposures to atherosclerosis progression and incident cardiovascular events.[8]

Circulating plasma proteins can reflect the state of neuroendocrine and immune system dysfunction due to their roles in the immune system and hypothalamic nervous system regulation.[10] Studies that capture circulating proteins that are plausibly downstream from depressive symptoms and are linked to an increased risk of CVD have identified inflammatory biomarkers such as interleukin-6 and interleukin-10.[11] However, many of these proteomic studies assayed only a small number of candidate proteins, primarily traditional inflammation biomarkers.[12,13] The identification of proteomic biomarkers from more recent platforms could increase our understanding of the mechanisms that link psychosocial factors to CVD and lead to improvements in targeted interventions.[14] Additionally, while there is motivation to identify reproducible proteomic signatures associated with psychosocial stress, general proteomic-wide analysis with health-related risk factors has primarily been concentrated in predominantly White or European populations in previous investigations.[15–17] Current research on the association of depressive symptoms and perceived stress measures with a diverse set of plasma proteins is limited, especially in Black adults.

Our objective was to provide a comprehensive analysis of the association between psychosocial factors (depressive symptoms and perceived stress) and plasma proteins from the SomaScan platform in the JHS (Jackson Heart Study) cohort, one of the largest community-based cohorts of Black adults. We sought to identify targets for mediation analyses of biological pathways linking psychosocial factors to CVD outcomes and replicate the findings in 2 other multiethnic cohort studies that also had proteomics data on the SomaScan platform: the CHS (Cardiovascular Health Study) and the MESA (Multi-Ethnic Study of Atherosclerosis). Our study conducts a hypothesis-generating proteome-wide analysis of psychosocial factors in JHS and adds to the racial and ethnic diversity of proteomic research. Moreover, we

hypothesized that these proteomic analyses would reveal novel associations with psychosocial factors and incident CVD events.

METHODS

The data used for this study can be requested for purposes of reproducing results. Requests to access the JHS data set may be directed to the qualified researchers trained in human subject confidentiality within the JHS Coordinating Center at jhsccrc@umc.edu. Data used for replication can be requested from dbGaP and the cohorts named below.

Cohorts

The JHS is a community-based, single-site prospective study of the risk factors associated with CVD among Black adults from the Jackson, Mississippi metropolitan area. The on-going epidemiological investigation collected data from 5306 participants ages 21 and older beginning in 2000 (exam 1: 2000–2004, exam 2: 2005–2009, exam 3: 2009–2013, exam 4: 2021–ongoing).[18,19] Of these participants, proteomic profiling was performed at baseline using the SomaScan 1.3k platform on 2143 individuals with available blood samples and no history of CVD, with enrichment for individuals with genetic data consent and unrelated participants.[17] The study was approved by the institutional review boards of the University of Mississippi Medical Center, Jackson State University, and Tougaloo College. All participants provided written informed consent.

In replication and meta-analyses, we leveraged cohorts with similar available data with multiethnic representation who were part of the National Heart, Lung, and Blood Institute's TransOmics for Precision Medicine initiative. The MESA cohort recruited 6814 men and women aged 45 to 84 years, free of clinical CVD at baseline across 6 clinical centers in the United States.[20] The baseline exam was conducted from 2000 to 2002, with 5 additional subsequent exams completed (exam 7 is ongoing). Participants self-identified as belonging to one of 4 racial/ethnic groups: Black, Hispanic, Asian, or White. Proteomics profiling (SomaScan 1.3k, as described below) was performed in 978 randomly selected individuals, also at baseline. CHS is a population-based cohort study of risk factors for coronary heart disease (CHD) and stroke in adults 65 years and older recruited across 4 field centers. The original cohort of 5201 participants, predominantly non-Hispanic White, was recruited in 1989 to 1990 from random sampling of people on Medicare eligibility lists.[21,22] Subsequently, an additional predominantly Black cohort of 687 persons was enrolled. CHS proteomics data have been previously described[23] and were available for 3399 participants, 3237 of whom have matched phenotypic data in dbGaP. Blood plasma for proteomics data was obtained at Year 5 (1992–1993), at the same time as the psychosocial data. Thus, psychosocial factors and proteomic profiles were measured at the same assessment in all 3 cohorts.

Psychosocial Factors

Depressive symptoms and perceived stress were used as the predictor variables in all models in JHS. Depressive symptoms were captured at exam 1 (2000–2004) via a 20-item form in JHS, adapted from the Centers for Epidemiological Studies Depression (CES-D) scale.[24] Participants were asked to rate their depressive symptoms over the past week on a 4-point Likert scale (<1 day, 1–2 days, 3–4 days, or 5–7 days). The CES-D scale has demonstrated reliability and validity in Black English-speaking US adult populations and has a score range of 0 to 60, with scores >16 indicating high depressive symptoms.[25,26] CES-D was also used in CHS and MESA, as previously described[27,28]; of note, in CHS, a 10-item CES-D screening tool was used, with a maximum score of 30, not 60.[29]

Perceived stress was captured at exam 1 in JHS via an 8-item form, capturing stress (in relationships, caring for others, job, neighborhood, medical, meeting basic needs, mistreatment/discrimination, legal issues) over the past 12 months on a 4-point Likert scale (not stressful, mildly stressful, moderately stressful, or very stressful). The form was adapted from the perceived stress scale,[30] which has been validated in adult populations and found to be invariant across race and sex.[31] The range of the perceived stress score is 0 to 24. In MESA, chronic stress was assessed using the Chronic Burden scale,[28] and was similarly captured at the same time point as proteomic profiling (MESA baseline exam). In CHS, stress was assessed using the stressful life events score (range 0–10) obtained at year 5 (1992–1993).[22] Details on the stress and depressive symptoms scoring in each cohort are presented in Table S10.

Proteomic Profiling

Proteomic profiles were measured with the SomaScan platform, an aptamer-based proteomics assay that measures human protein analytes in plasma. Blood samples were collected at Exam 1 and were stored at −80 °C. Samples were assayed using the 1.3k platform in 3 batches for JHS participants and 1 batch for MESA participants, as previously described.[32] In CHS, participant samples were run in 2 batches using the 7k assay.[23] Although an expanded platform was used in CHS (≈7000 proteins assayed versus ≈1300 in JHS and MESA), proteins could be matched across cohorts using SomaScan aptamer IDs, ensuring the same assay aptamer was used to assay the target protein. Within the cohort, protein values (provided in relative fluorescence units) were log-transformed within batch (if samples were run in batches) and rank inverse normal transformed.

Incident CVD Events

Incident CVD event surveillance was performed through December 31, 2016, for 3 primary events in the JHS: CHD, stroke, and heart failure. CHD included myocardial infarction, angina, and ECG changes consistent with a CHD diagnosis. Stroke events were defined using *International Classification of Diseases*, *Ninth or Tenth* codes as neurological symptoms lasting more than 24 hours or leading to death. Stroke and CHD events were adjudicated beginning in 2000, and heart failure hospitalization surveillance was monitored since 2005, as previously described.[33–36]

Assessment of Covariates

All regression and mediation models controlled only for differences in proteomic profiles resulting from known nonpsychosocial factors: age, sex, batch, and estimated glomerular filtration (eGFR). Proteins are differentially expressed with both age and sex.[37] The JHS and CHS samples were assayed in

several batches that could confound analyses. MESA did not assay in batches. Further, kidney filtration has been connected to the regulation of circulating plasma proteins.[38] In previous studies of chronic kidney disease, levels of plasma proteins were significantly associated with eGFR, suggesting that the circulating proteome is impacted by a decline in kidney function.[39] Given that psychosocial stress could also impact kidney function, we considered kidney function a technical covariate that impacts accurate protein measurement and thus adjusted for it in our main models. We used the updated chronic kidney disease-epidemiology creatinine equations in all cohorts to capture eGFR, which no longer include race/race-ethnicity.[40]

We avoided adjusting for conventional cardiovascular risk factors in our main models, as we hypothesized that some of the effects of psychosocial stress on incident CVD could be mediated through risk factors such as smoking or body mass index. However, adjustment for traditional CVD factors (as described below) can be found in the supplement (Table S8).

Analyses

Covariate distributions in the JHS were captured by examining the median and range of continuous variables. Linear regression analyses on JHS and MESA data were run in R Studio Version 4.1.0. to identify associations between psychosocial factors and plasma proteins, controlling for age, sex, batch (if applicable), and eGFR. To explore the impact of established CVD risk factors, we tested another model adjusting for Framingham Risk Score components (high-density lipoprotein cholesterol, total cholesterol, smoking status, systolic blood pressure, and use of blood pressure medication).[41] CHS regression analyses were run using BioDataCatalyst.[42] In JHS, significant associations were identified using a Bonferroni corrected P value of α=3.782×10−5 (0.05/1322) to account for multiple testing for the 1322 proteins. METAL was used to run a sample-size weighted P value–based meta-analysis of the summary statistics from MESA and CHS to identify signals that replicated in external cohorts.[43] We considered a protein as replicated if it was directionally consistent and met a Bonferroni threshold based on the number of proteins carried forward for replication (6 proteins at α=0.05, or P<0.008). Since effect sizes cannot be interpreted identically in all cohorts (due to different scaling of the psychosocial stress and depressive symptoms variables), a sample size weighted P value based meta-analysis was used. We also present an exploratory meta-analysis across all 3 cohorts in Supplemental Materials (Tables S6 and S7), but only for proteins that are present in JHS. To explore potential interactions between psychosocial factors and sex, sex interaction models were estimated in JHS. Models adjusted for sex, age, eGFR, and batch and included an interaction term for the psychosocial variable (stress or depressive symptoms) and sex (Table S9).

A canonical correlation analysis of proteomics data in JHS and MESA previously identified strong associations between blood cell counts and protein abundance.[44] These findings suggest that adjusting for blood cell composition might affect proteomic association results; similar to eGFR, we would consider such relationships to be somewhat technical/related to accurate measurement, even though it is also true that blood cell counts may also be influenced by psychosocial factors.[45] Therefore, we performed a sensitivity analysis adjusting for measures of platelet (TH/CMM), red blood cell count (M/CMM), and white blood cell count (TH/CMM) in addition to age, sex, batch, and eGFR (Table S1).

We ran Cox proportional hazards models in JHS for both psychosocial factors to identify significant relationships with CVD events, adjusting for age, sex, batch, and eGFR (Bonferroni-adjusted P<0.05/6=0.008). For associations identified as statistically significant, additional Cox models were run to include psychosocial factors and the plasma proteins identified in our first set of analyses on the CVD event, adjusting for the same covariates. If there was a difference in the effect estimate between the Cox models of psychosocial factors versus psychosocial factors and proteins, a subsequent statistical mediation analysis was used to estimate the indirect effects of plasma proteins on psychosocial factors and the time- to-event CVD outcome,[46] controlling for age, sex, batch, and eGFR. The indirect effect estimates resulting from these models measured the extent to which plasma proteins have an indirect effect on the psychosocial factor and incident CVD association. All Cox models and mediation analyses were run in SAS Studio Version 3.81.

RESULTS

Summary Statistics for Characteristics of JHS Participants

In total, 2143 JHS participants had available proteomic profiling. These individuals were relatively similar across select characteristics at baseline compared with the full JHS cohort, as shown in Table 1. The median age of participants with proteomic profiling was 56 years, and the median total scores for depressive symptoms and perceived stress were 10 and 4, respectively. Additionally, 229 (11.66%) participants experienced CHD, 191 (10.40%) experienced heart failure, and 103 (5.09%) experienced a stroke event in the follow-up period. Time- to-event summary statistics are also provided in Table 1. Summary statistics for characteristics of MESA and CHS participants can be found in Tables 2 and 3.

Association Between Psychosocial Factors and Plasma Proteins

In JHS, 6 plasma proteins out of the 1322 tested were found to have a significant association with either depressive symptoms or perceived stress, after Bonferroni correction for multiple testing. There were no proteins associated with both depressive symptoms and perceived stress. As shown in Table 4, after adjustment for age, sex, eGFR, and batch effects, angiopoietin-2 (β=0.0142, SE=0.0032; P=1.3454×10−5), growth/differentiation factor 15 or MIC-1 (macrophage inhibitory cytokine 1; β=0.0137, SE=0.0025; P=3.9774×10−8), and KYNU (kynureninase; β=0.0143, SE=0.0032; P=1.2250×10−5) were positively associated with depressive symptoms. Contactin-5 (β=−0.0168, SE=0.0032; P=1.4922×10−7) and neural cell adhesion molecule

Table 1. Cohort Characteristics in Full Sample and the Proteomic Sample at Baseline: The JHS

	Proteomic sample (n=2143)		Full cohort (n=5306)	
	Median	Range	Median	Range
Age	56	20–93	55	20–95
eGFR	86.92	3.86–128.75	87.55	3.86–132.48
Depressive symptoms score	10	0–47	9	0–48
Perceived stress score	4	0–24	4	0–24
CHD follow-up years	13.52	0.02–16.27	13.72	0.02–16.27
Heart failure follow-up years	12.00	0.04–12.00	12.00	0.04–12.00
Stroke follow-up years	13.65	0.05–16.27	13.72	0.05–16.27
	Frequency (%)		Frequency (%)	
Sex (female)	1312 (61.2)		3367 (63.5)	

Note: 746 (34.8%) and 18 (0.8%) participants with proteomic data are missing a depressive symptoms total score or global stress total score, respectively. CHD indicates coronary heart disease; eGFR, estimated glomerular filtration rate; and JHS, Jackson Heart Study.

Table 2. Cohort Characteristics in Full Sample and the Proteomic Sample at Baseline: The MESA

	Proteomic Sample (n=978)		Full cohort (n=6452)	
	Median	Range	Median	Range
Age	59	44–83	62	44–84
eGFR	72.65	17.10–152.54	78.84	5.32–158.24
Depressive symptoms score	6	0–50	5	0–53
Perceived stress score	1	0–5	1	0–5
CHD follow-up years	17.93	5.44–19.41	17.58	0.11–19.46
Heart failure follow-up years	17.94	0.21–19.41	17.65	0.09–19.46
Stroke follow-up years	17.91	0.57–19.41	17.63	0.02–19.46
	Frequency (%)		Frequency (%)	
Sex (female)	521 (53.3)		3381 (52.4)	
Race/ethnicity				
White	414 (42.3)		2531 (39.2)	
Chinese	71 (7.3)		779 (12.1)	
Black	192 (19.6)		1689 (26.2)	
Hispanic/ Latino	301 (30.8)		1453 (22.5)	

Note: 3 (0.31%) and 8 (0.82%) participants with proteomic data are missing a depressive symptoms total score or global stress total score, respectively. CHD indicates coronary heart disease; eGFR, estimated glomerular filtration rate; and MESA, Multi-Ethnic Study of Atherosclerosis.

(NCAM) 120 (β=−0.0159, *SE*=0.0032, *P*=7.2374×10^{-7}) were negatively associated with depressive symptoms. LKHA4 (leukotriene A4 hydrolase) was positively associated with perceived stress (β=0.0235, *SE*=0.0050, *P*=2.8610×10^{-6}). Further adjustment for Framingham Risk Score components attenuated all associations, as shown in Table S8. Neither depressive symptoms nor stress had a significant interaction with sex in the association with these proteins (Table S9). Adjusting for complete blood count measures resulted in a modest attenuation (Table S1). Sensitivity analyses were not performed in MESA or CHS, as these cohorts did not have complete blood count measures available.

Table 5 shows a comparison of the proteins associated with psychosocial factors across cohorts and in the meta-analyses. Of the 6 proteins identified as statistically significant in JHS, 2 proteins (angiopoietin-2 and NCAM 120) were also associated with depressive symptoms in MESA and CHS. In the MESA-CHS meta-analyses, all proteins were statistically significant, but angiopoietin-2, contactin-5, MIC-1, and NCAM 120 successfully replicated in the meta-analyses due to their consistent direction and *P*<0.008. KYNU was directionally consistent but only nominally significant in the MESA/CHS meta-analysis (*P*=0.040), so it was not considered replicated. Statistics from the CHS and MESA meta-analysis are in Tables S4 and S5. Statistics from the full meta-analysis of proteins associated with depressive symptoms and perceived stress scores in JHS, MESA, and CHS are in Tables S6 and S7, as exploratory results for proteins that may be considered for replication in future analyses. Included in these tables are measures of heterogeneity (Cochran Q and I^2). We caution interpretation given that depressive symptoms and stress scores are on different scales across cohorts, which would be expected to lead to heterogeneity regardless of any actual heterogeneity in psychosocial factor-protein relationships by cohort.

Association Between Psychosocial Factors, Proteins, and Incident CVD Events in JHS

Table 6 shows the association between psychosocial factors and incident CVD events using a Bonferroni *P*<0.008 (0.05/6). Among JHS participants with proteomic data, depressive symptoms had a significant direct effect on the hazard of incident CHD (β=0.0301, *SE*=0.0101; *P*=0.0028), but this relationship was not significant for incident heart failure and stroke in the proteomics sample set. No statistically significant direct association was found between perceived stress and incident CVD events. The results from the time-to-event analyses of depressive symptoms and plasma proteins on CHD and heart failure, and the percent change in point estimates (total direct effects) between these models and the Cox models with depressive symptoms are in Table S3.

The 4 proteins associated with depressive symptoms in JHS and which replicated in the meta-analyses were tested in mediation models in the association between depressive symptoms and CVD, as shown in Table 7.

Table 3. Cohort Characteristics in Full Sample and the Proteomic Sample at Year 5: The CHS

	Proteomic sample (n=3206)		Full cohort (n=4589)	
	Median	Range	Median	Range
Age	73	65–98	74	65–103
eGFR	66.67	3.54–103.38	66.19	3.54–1032
Depressive symptoms score	4	0–29	4	0–29
Perceived stress score	1	0–6	1	0–6
CHD follow-up years	11.73	0.01–14.06	10.82	0–14.06
Heart Failure follow-up years	12.32	0.02–14.06	11.26	0.02–14.06
Stroke follow-up years	13.09	0.05–14.06	12.12	0.05–14.06
	Frequency (%)		Frequency (%)	
Sex (female)	1940 (60.5)		2668 (58.1)	
Race/ethnicity				
White	2644 (82.5)		3946 (13.47)	
Black	562 (17.5)		618 (86.00)	
Asian/Pacific islander	N/A		4 (0.09)	
American Indian/Alaskan native	N/A		10 (0.22)	
Other	N/A		11 (0.24)	

Note: 3 (0.0009%) and 2 (0.0006%) participants with proteomic data are missing a depressive symptoms total score or life events total score, respectively. eGFR indicates estimated glomerular filtration rate; CHD, coronary heart disease; and CHS, Cardiovascular Health Study.

Adjusting for multiple testing, only MIC-1 had a statistically significant indirect effect in the association between depressive symptoms and CHD, though Angiopoietin-2 was also nominally significant. MIC-1 resulted in the largest indirect effect estimate (β=1.0065 [95% CI, 1.0027–1.0103]; P=0.001) and percentage mediated (23.28%) for depressive symptoms and CHD.

DISCUSSION

The goal of this study was to identify plasma proteins associated with psychosocial factors and incident CVD events. Our analyses identified novel proteomic associations for depressive symptoms and perceived stress across multiple cohorts. In the discovery cohort, JHS, we found angiopoietin-2, contactin-5, MIC-1, NCAM 120, and KYNU to be cross-sectionally associated with depressive symptoms and LKHA4 to be cross-sectionally associated with perceived stress. When we performed replication and meta-analysis, angiopoietin-2, contactin-5, MIC-1, and NCAM 120 were also cross-sectionally associated with depressive symptoms in MESA and CHS meta-analysis, as was LKHA4 with perceived stress. Additionally, MIC-1, also known as GDF15 (growth differentiation factor 15), was significantly associated with both depressive symptoms and incident CHD in JHS.

Table 4. Associations Between Psychosocial Factors and Plasma Proteins: Results From Linear Regression Analyses in the Jackson Heart Study

Protein	Beta estimate	Standard error	*P* value
Depressive symptoms score			
Angiopoietin-2 (O15123)	0.0142	0.0032	1.3454×10^{-5}
Contactin-5 (O94779)	−0.0168	0.0032	1.4922×10^{-7}
MIC 1 (GDF-15, Q99988)	0.0137	0.0025	3.9774×10^{-8}
NCAM 120 (P13591)	−0.0159	0.0032	7.2374×10^{-7}
KYNU	0.0143	0.0032	1.2250×10^{-5}
Perceived Stress score			
LKHA4	0.0235	0.0050	2.8610×10^{-6}

Results are from linear regression. Covariates included age, sex, batch, and estimated glomerular filtration rate. The beta represents the SD change in depression or stress score for a 1 SD change in the higher level of protein. GDF-15 indicates growth differentiation factor 15; KYNU, kynureninase; LKHA4, luekotriene A-4 hydrolase; MIC, macrophage inhibitory cytokine; and NCAM, neural cell adhesion molecule 120.

In JHS, depressive symptoms were significantly and positively associated with incident CHD and heart failure, and this association is well-known across other cohorts. There are only a few studies, to our knowledge, that have conducted targeted proteome-wide analyses of psychosocial factors such as perceived stress and depressive symptoms, and none to our knowledge assessed as many proteins or included replication analyses similar to our study. In the English Longitudinal Study of Ageing, which included 3262 older adults, the authors found no association between depressive symptoms and 276 proteins measured using the Olink platform.[47] Another study tested the association between depressive symptoms and plasma proteins, also measured using a targeted Olink platform, among participants from the German Diabetes study.[48] Their results identified statistically significant associations between depressive symptoms and CDC137, SIRT2, and LEPR, but did not identify any of the proteins our study found from the SomaScan platform. In our study, only SIRT2 was common in all cohorts, and it was not statistically associated with depressive symptoms across cohort studies (Table S6: meta-analysis P>0.05). However, we note that prior studies did not include any participants from the United States, had different versions of the CES-D scale, and may have had differing cohort recruitment strategies. In the current study of US participants without CVD, angiopoietin-2, MIC-1, contactin-5, and NCAM120 met the Bonferroni threshold for being cross-sectionally associated with depressive symptoms across all cohorts. Angiopoietin-2 and MIC-1 are linked to cellular growth and inflammation[49,50] which could be important biomarkers for understanding the detrimental effects of psychosocial stress.

Table 5. Association Between Psychosocial Factors and Plasma Proteins That Replicated Across Cohorts: Results From Meta-Analysis

Protein	JHS beta estimate (*P* value)	MESA beta estimate (*P* value)	CHS beta estimate (*P* value)	Meta *P* value*
Depressive symptoms score				
Angiopoietin-2 (O15123)†	0.014 (P=1.345×10^{-5})	0.008 (P=0.015)	0.015 (P=7.659×10^{-5})	1.017×10^{-5}
Contactin-5 (O94779)†	−0.017 (P=1.492×10^{-7})	−0.013 (P=0.002)	−0.007 (P=0.080)	0.002
MIC-1 (O99988)†	0.014 (P=3.977×10^{-8})	0.004 (P=0.238)	0.013 (P=3.663×10^{-5})	2.863×10^{-5}
NCAM 120 (P13591)†	−0.016 (P=7.237×10^{-7})	−0.011 (P=0.010)	−0.008 (P=0.026)	0.001
KYNU (Q16719)	0.014 (P=1.225×10^{-5})	0.003 (P=0.469)	0.007 (P=0.015)	0.040
Perceived Stress Score				
LKHA4 (P09960)†	0.024 (P=2.861×10^{-6})	0.029 (P=0.241)	0.035 (P=0.015)	0.007

Results are from linear regression, and effect sizes cannot be interpreted identically in all cohorts- while proteins are inverse normalized in all cohorts, the scaling of psychosocial stress variables is not identical in each cohort, which is why a sample size weighted meta-analysis is used. CHS indicates Cardiovascular Health Study; JHS, Jackson Heart Study; KYNU, kynureninase; LKHA4, luekotriene A-4 hydrolase; MESA, Multi-Ethnic Study of Atherosclerosis; MIC, macrophage inhibitory cytokine; and NCAM, neural cell adhesion molecule 120.

*Unadjusted *P* value.

†Significant protein (directionally consistent with a Bonferroni-adjusted P<0.05/6=0.008).

Contactins and NCAMs are generally involved in nervous system development[51,52] and may play significant roles in anxiety and depression.[53–55] LKHA4 was also cross-sectionally associated with perceived stress in our analyses and replicated in MESA and CHS. The gene *LTA4H* is likely linked to the protein LKHA4, which has been reported to be associated with genetic variations of depression by sex.

To our knowledge, there is a paucity of studies that have attempted to map the proteomic pathway of psychosocial stress to incident CVD.[56] Although extensive prior studies have linked proteins like MIC-1/GDF-15 to CHD and other aging-related diseases,[57] our focus here on depressive symptoms and the association between depressive symptoms and CHD is more novel. Both angiopoietin-2 and MIC-1 had statistically significant indirect effects in the association between depressive symptoms and incident CHD. GDF-15 is induced under oxidative stress conditions and has potential immune regulatory functions,[50] which may help explain its association with CHD and all-cause mortality.[50,58] GDF-15 was highlighted as a key predictor of recurrent atherosclerotic cardiovascular disease in recent analyses of targeted Olink panels.[59] Recent analyses of contributors to variance in SomaScan measured proteins found that GDF-15[60] was in the top 1% of age-associated proteins (with >12% variance explained); while age has been adjusted for in our analyses, this fits with accumulating evidence that GDF-15 is a strong marker of accelerated aging. For example, GDF-15 has been included in accelerated aging protein clocks[61] and is strongly associated with DNA methylation-based accelerated aging clocks as well.[62] GDF-15 was also strongly influenced by current smoking status (>10% variance explained), which may mediate some of the observed effects of depressive symptoms.[63] Unfortunately, this ANOVA explained did not include psychosocial factors, which should be more comprehensively assayed with other potential upstream drivers of protein variance in future work.[60,64,65] Previous studies have similarly found Angiopoietin-2 to be associated with CVD risk, particularly for incident heart failure. Angiopoietin-2 has roles in endothelial cell function, proliferation, and in promoting postischemic cardiac hypoxia and inflammation.[66] Angiopoietin-2 has been associated with NT-proBNP (N-terminal-pro-B-type natriuretic peptide), a prognostic factor for heart failure, in patients with heart failure with preserved ejection fraction.[67] Additionally, angiopoietin-2 was one of the top 20 proteins (out of >4000 tested on the SomaScan panel) positively associated with the risk of death or heart failure-related hospital admission in the Penn Heart Failure Study.[68] We note that more detailed follow-up is needed to understand the exact mechanisms by which these proteins may be influenced by psychosocial factors, or vice versa.

Table 6. Associations Between Psychosocial Factors and Incident CVD Events: Results From Time-to-Event Analyses in the Jackson Heart Study

Incident cardiovascular disease event type	Hazard beta estimate	Standard error	*P* value
Depressive symptoms score			
CHD	0.0301	0.0101	0.0028
Heart failure	0.0230	0.0117	0.0503
Stroke	−0.0021	0.0172	0.9028
Perceived stress score			
CHD	0.0248	0.0166	0.1353
Heart failure	0.0263	0.0179	0.1412
Stroke	−0.0016	0.0252	0.9505

CHD indicates coronary heart disease; and CVD, cardiovascular disease.

*Total direct effects from the Cox proportional hazards model, adjusting for age, sex, and estimated glomerular filtration rate. Bonferroni-adjusted P<0.05/6=0.008.

Table 7. The Indirect Effect of Plasma Proteins in the Association Between Depressive Symptoms and Incident Coronary Heart Disease Events: Results From Mediation Analyses in the Jackson Heart Study

Incident CVD event	Protein	Hazard beta estimate (indirect effect)	SE	95% CI	*P* value	Percent mediated
CHD	Angiopoietin-2 (O15123)	1.0030	0.0015	1.0000–1.0060	0.0489	9.3315
	Contactin-5 (O94779)	1.0010	0.0017	0.9976–1.0043	0.5736	3.2383
	MIC-1 (Q99988)	1.0065	0.0019	1.0027–1.0103	0.0009	23.2836
	NCAM-120 (P13591)	1.0000	0.0014	0.9973–1.0028	0.9820	0.1070

Estimates represent the natural indirect effect of proteins in the association of depressive symptoms and incident CHD. Estimates include covariate adjustment for age, sex, batch, and estimated glomerular filtration rate. The Bonferroni-adjusted $P<0.05/4/0.0125$. CVD indicates cardiovascular disease; CHD, coronary heart disease; MIC, macrophage inhibitory cytokine; and NCAM, neural cell adhesion molecule.

The current study did not find a statistically significant association between psychosocial stress and incident CHD, stroke, or heart failure, nor did it find an association between depressive symptoms and stroke. We believe that JHS lacked statistical power in the proteomics analysis sample size. A power analysis confirmed that we were underpowered to identify a main effect (eg, with 2143 participants and 229 CHD cases, we had 0.07% power to detect a hazard ratio of 1.03). We note that prior work in the full JHS cohort did identify statistically significant associations between depressive symptoms and incident heart failure and stroke, though attenuation was observed on adjustment for lifestyle factors and self-reported coping strategies.[2,3] In JHS, the association between perceived stress and incident CVD has not been reported, but perceived stress has been associated with incident hypertension.[69] The number of proteins identified for perceived stress was also limited to LKHA4. Proteomic technologies enable the discovery of novel candidate biomarkers, and there are likely more proteins associated with psychosocial stress; future work should identify additional proteomic mediators in other cohorts with greater power. However, in the current study, we had confidence that LKHA4 was indeed a significant finding and applicable across samples, due to the conservative threshold used and the replication in CHS and MESA. Given the exploratory nature of this work, the proteins identified may help prioritize candidates for further follow-up. We hope to expand on this work as the number of participants with proteomics data and considerable follow-up time increases.

We acknowledge several limitations of our study. First, the proteomic platform available (SomaScan 1.3k) only covered a small portion of the human proteome. Although our work highlights potentially interesting biological findings, SomaScan protein measures are ultimately semi-quantitative and have limited ability to be translated directly to clinical biomarkers. Future work should prioritize follow-up with orthogonal platforms (eg, mass spectrometry). We would also like to note that our study is limited by differences in how the psychosocial factors were measured across cohorts, which may partially explain why KYNU was only associated with depressive symptoms in JHS.

There were a few limitations related to our methodological approaches. The Bonferroni correction approach used to identify significant associations between psychosocial factors and proteins fails to consider between-feature dependence, which can arise in proteomics because of the complex interactions between proteins, and in general, may have led to missed features of interest due to its conservative approach.[70] Also, the psychosocial factors and proteins were captured at the same time point, and the temporality assumption of the mediation analyses could not be fully satisfied. The assumption of temporal precedence of the predictor, mediator, and outcome in a mediation analysis expects changes in psychosocial factors to precede changes in protein measures, and changes in protein measures to precede incident CVD events.[71] Although psychosocial factors and protein measures were both collected at the same time point, we tested the mediation model under the assumption that psychosocial stress occurred first, as both the depressive symptoms and perceived stress forms inquired about past psychosocial experiences. Causal inference methods for mediation analysis also explicate additional assumptions for estimating natural indirect effects.[72] The models assume there is no unmeasured confounding between any of the associations tested in the mediation model. These assumptions were addressed to the extent possible by adjusting for the minimally sufficient set of covariates and limiting adjustment of other potential mediators; however residual confounding remains possible, particularly from socioeconomic status, lifestyle factors and health behaviors (eg, smoking, physical activity, and diet), and comorbidity-related factors, each of which may influence both psychosocial exposures and circulating protein levels. Although additional sensitivity analyses adjusted for traditional Framingham risk factors, these may not capture the complex social, behavioral, and clinical determinants that jointly shape psychosocial stress and proteins. Thus, our results should be interpreted with appropriate caution regarding potential unmeasured confounding. Lastly, there were differences in the psychosocial measures across cohorts, which influenced our ability to replicate and meta-analyze findings in CHS and MESA.

However, this study is not without strengths. Our discovery sample was comprised of a large sample

of Black participants from the Southeastern United States, where CVD burden and incidence are higher than in other areas in the United States. Participants were free of CVD, which allowed us to examine the true incidence of CVD events. Additionally, the majority of the significant proteins associated with depressive symptoms and stress showed consistent associations in other cohorts with participants from different areas in the US. This study also included mediation analyses, and our findings demonstrated evidence of a potential proteomic pathway from depressive symptoms to CHD. Future research should consider multiomic approaches to understand biological links between a broader array of social determinants of health and CVD, especially using validated multidimensional assessments of factors, such as stress, trauma, coping, and social support. We highlight that potentially combining omics could also be an additional method to explore a more complete biological pathway to CVD to help identify upstream regulators and potential intervention points. Future analyses in larger sample sizes could explore multi-mediator models and pathway or latent class analyses. Additionally, the use of longitudinal proteomics or repeated measures should be prioritized in future studies to address temporality. An important next step for this research is to develop better methods to explore such research questions and to include multiple racial/ethnic representations in cohort samples and external validation of findings. As studies continue to answer research questions about targetable biomarkers, it will be important for omics to be used to predict CVD beyond traditional models/risk scores to improve precision medicine based on psychosocial risk profiles.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S10

REFERENCES

1. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, Dai S, Ford ES, Fox CS, Franco S, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2014 update: a report from the American Heart Association. *Circulation*. 2014;129:e28–e292. doi: 10.1161/01.cir.0000441139.02102.80
2. Gaffey AE, Cavanagh CE, Rosman L, Wang K, Deng Y, Sims M, O'brien EC, Chamberlain AM, Mentz RJ, Glover LM, et al. Depressive symptoms and incident heart failure in the Jackson Heart Study: differential risk among black men and women. *J Am Heart Assoc*. 2022;11:e022514. doi: 10.1161/JAHA.121.022514
3. O'Brien EC, Greiner MA, Sims M, Hardy NC, Wang W, Shahar E, Hernandez AF, Curtis LH. Depressive symptoms and risk of cardiovascular events in blacks: findings from the Jackson Heart Study. *Circ Cardiovasc Qual Outcomes*. 2015;8:552–559. doi: 10.1161/CIRCOUTCOMES.115.001800
4. Hines AL, Albert MA, Blair JP, Crews DC, Cooper LA, Long DL, Carson AP. Neighborhood factors, individual stressors, and cardiovascular health among

Black and White adults in the US: the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study. *JAMA Netw Open*. 2023;6:e2336207–e2336207. doi: 10.1001/jamanetworkopen.2023.36207
5. Felix AS, Lehman A, Nolan TS, Sealy-Jefferson S, Breathett K, Hood DB, Addison D, Anderson CM, Cené CW, Warren BJ, et al. Stress, resilience, and cardiovascular disease risk among black women: Results from the Women's Health Initiative. *Circ Cardiovasc Qual Outcomes*. 2019;12:e005284. doi: 10.1161/CIRCOUTCOMES.118.005284
6. Dar T, Radfar A, Abohashem S, Pitman RK, Tawakol A, Osborne MT. Psychosocial stress and cardiovascular disease. *Curr Treat Options Cardiovasc Med*. 2019;21:1–17. doi: 10.1007/s11936-019-0724-5
7. Gallo LC. The reserve capacity model as a framework for understanding psychosocial factors in health disparities. *Appl Psychol*. 2009;1:62–72. doi: 10.1111/j.1758-0854.2008.01000.x
8. Feng L-S, Wang Y-M, Liu H, Ning B, Yu H-B, Li S-L, Wang Y-T, Zhao M-J, Ma J. Hyperactivity in the hypothalamic-pituitary-adrenal axis: an invisible killer for anxiety and/or depression in coronary artherosclerotic heart disease. *J Integr Neurosci*. 2024;23:222. doi: 10.31083/j.jin2312222
9. Webster JI, Tonelli L, Sternberg EM. Neuroendocrine regulation of immunity. *Annu Rev Immunol*. 2002;20:125–163. doi: 10.1146/annurev.immunol.20.082401.104914
10. Williams SA, Kivimaki M, Langenberg C, Hingorani AD, Casas JP, Bouchard C, Jonasson C, Sarzynski MA, Shipley MJ, Alexander L, et al. Plasma protein patterns as comprehensive indicators of health. *Nat Med*. 2019;25:1851–1857. doi: 10.1038/s41591-019-0665-2
11. Meyer T, Stanske B, Kochen MM, Cordes A, Yüksel I, Wachter R, Lüers C, Scherer M, Binder L, Pieske B, et al. Serum levels of interleukin-6 and interleukin-10 in relation to depression scores in patients with cardiovascular risk factors. *Behav Med*. 2011;37:105–112. doi: 10.1080/08964289.2011.609192
12. Osimo EF, Pillinger T, Rodriguez IM, Khandaker GM, Pariante CM, Howes OD. Inflammatory markers in depression: a meta-analysis of mean differences and variability in 5,166 patients and 5,083 controls. *Brain Behav Immun*. 2020;87:901–909. doi: 10.1016/j.bbi.2020.02.010
13. Lamers F, Milaneschi Y, Smit JH, Schoevers RA, Wittenberg G, Penninx BW. Longitudinal association between depression and inflammatory markers: results from the Netherlands study of depression and anxiety. *Biol Psychiatry*. 2019;85:829–837. doi: 10.1016/j.biopsych.2018.12.020
14. Corlin L, Liu C, Lin H, Leone D, Yang Q, Ngo D, Levy D, Cupples LA, Gerszten RE, Larson MG, et al. Proteomic signatures of lifestyle risk factors for cardiovascular disease: a cross-sectional analysis of the plasma proteome in the Framingham Heart Study. *J Am Heart Assoc*. 2021;10:e018020. doi: 10.1161/JAHA.120.018020
15. Sun BB, Suhre K, Gibson BW. Promises and challenges of populational proteomics in health and disease. *Mol Cell Proteomics*. 2024;23:100786. doi: 10.1016/j.mcpro.2024.100786
16. Sun BB, Chiou J, Traylor M, Benner C, Hsu Y-H, Richardson TG, Surendran P, Mahajan A, Robins C, Vasquez-Grinnell SG, et al; Alnylam Human Genetics. Plasma proteomic associations with genetics and health in the UK Biobank. *Nature*. 2023;622:329–338. doi: 10.1038/s41586-023-06592-6
17. Katz DH. Whole genome sequence analysis of the plasma proteome in black adults. *Circulation*. 2021;144:A9897–A9897. doi: 10.1161/circ.144.suppl_1.9897
18. Fuqua SR, Wyatt SB, Andrew ME, Sarpong DF, Henderson FR, Cunningham MF, Taylor HA. Recruiting African-American Research Participation in the Jackson Heart Study. *Ethn Dis*. 2005;15:18–29
19. Wilson JG, Rotimi CN, Ekunwe L, Royal CD, Crump ME, Wyatt SB, Steffes MW, Adeyemo A, Zhou J, Taylor HA. Study design for genetic analysis in the Jackson Heart Study. *Ethn Dis*. 2005;15:30–37
20. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, JacobsJr DR, Kronmal R, Liu K. Multi-Ethnic Study of Atherosclerosis: objectives and design. *Am J Epidemiol*. 2002;156:871–881. doi: 10.1093/aje/kwf113
21. Tell GS, Fried LP, Hermanson B, Manolio TA, Newman AB, Borhani NO. Recruitment of adults 65 years and older as participants in the Cardiovascular Health Study. *Ann Epidemiol*. 1993;3:358–366. doi: 10.1016/1047-2797(93)90062-9
22. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, Kuller LH, Manolio TA, Mittelmark MB, Newman A. The cardiovascular health study: design and rationale. *Ann Epidemiol*. 1991;1:263–276. doi: 10.1016/1047-2797(91)90005-w
23. Austin TR, McHugh CP, Brody JA, Bis JC, Sitlani CM, Bartz TM, Biggs ML, Bansal N, Buzkova P, Carr SA, et al. Proteomics and Population Biology in the Cardiovascular Health Study (CHS): design of a study with mentored access and active data sharing. *Eur J Epidemiol*. 2022;37:755–765. doi: 10.1007/s10654-022-00888-z
24. Radloff LS. Center for epidemiological studies depression scale. *Appl Psychol Meas*. 1977;1:385–401. doi: 10.1177/014662167700100306
25. Torres E. Psychometric properties of the center for epidemiologic studies depression scale in African American and Black Caribbean US adults. *Issues Ment Health Nurs*. 2012;33:687–696. doi: 10.3109/01612840.2012.697534
26. Sims M, Glover LSM, Gebreab SY, Spruill TM. Cumulative psychosocial factors are associated with cardiovascular disease risk factors and management among African Americans in the Jackson Heart Study. *BMC Public Health*. 2020;20:566. doi: 10.1186/s12889-020-08573-0
27. Schulz R, Beach SR, Ives DG, Martire LM, Ariyo AA, Kop WJ. Association between depression and mortality in older adults: the Cardiovascular Health Study. *Arch Intern Med*. 2000;160:1761–1768. doi: 10.1001/archinte.160.12.1761
28. Ranjit N, Diez-Roux AV, Shea S, Cushman M, Ni H, Seeman T. Socioeconomic position, race/ethnicity, and inflammation in the Multi-Ethnic Study of Atherosclerosis. *Circulation*. 2007;116:2383–2390. doi: 10.1161/CIRCULATIONAHA.107.706226
29. Barnes DE, Alexopoulos GS, Lopez OL, Williamson JD, Yaffe K. Depressive symptoms, vascular disease, and mild cognitive impairment: findings from the Cardiovascular Health Study. *Arch Gen Psychiatry*. 2006;63:273–279. doi: 10.1001/archpsyc.63.3.273
30. Cohen S, Kamarck T, Mermelstein R. Perceived stress scale. *Measuring stress: A guide for health and social scientists*. 1994;10:1–2.
31. Ezzati A, Jiang J, Katz MJ, Sliwinski MJ, Zimmerman ME, Lipton RB. Validation of the perceived stress scale in a community sample of older adults. *Int J Geriatr Psychiatry*. 2014;29:645–652. doi: 10.1002/gps.4049
32. Katz DH, Tahir UA, Bick AG, Pampana A, Ngo D, Benson MD, Yu Z, Robbins JM, Chen Z-Z, Cruz DE, et al; National Heart, Lung, and Blood Institute TOPMed (Trans-Omics for Precision Medicine) Consortium. Whole genome sequence analysis of the plasma proteome in black adults provides novel insights into cardiovascular disease. *Circulation*. 2022;145:357–370. doi: 10.1161/CIRCULATIONAHA.121.055117
33. Keku E, Rosamond W, Taylor HA, Garrison R, Wyatt SB, Richard M, Jenkins B, Reeves L, Sarpong D. Cardiovascular disease event classification in the Jackson Heart Study. *Ethn Dis*. 2005;15:62–70.
34. Bluemke DA, Kronmal RA, Lima JA, Liu K, Olson J, Burke GL, Folsom AR. The relationship of left ventricular mass and geometry to incident cardiovascular events: the MESA (Multi-Ethnic Study of Atherosclerosis) study. *J Am Coll Cardiol*. 2008;52:2148–2155. doi: 10.1016/j.jacc.2008.09.014
35. Psaty BM, Delaney JA, Arnold AM, Curtis LH, Fitzpatrick AL, Heckbert SR, McKnight B, Ives D, Gottdiener JS, Kuller LH, et al. Study of cardiovascular health outcomes in the era of claims data: the Cardiovascular Health Study. *Circulation*. 2016;133:156–164. doi: 10.1161/CIRCULATIONAHA.115.018610
36. Ives DG, Fitzpatrick AL, Bild DE, Psaty BM, Kuller LH, Crowley PM, Cruise RG, Theroux S. Surveillance and ascertainment of cardiovascular events: the Cardiovascular Health Study. *Ann Epidemiol*. 1995;5:278–285. doi: 10.1016/1047-2797(94)00093-9
37. Ignjatovic V, Lai C, Summerhayes R, Mathesius U, Tawfilis S, Perugini MA, Monagle P. Age-related differences in plasma proteins: how plasma proteins change from neonates to adults. *PLoS One*. 2011;6:e17213. doi: 10.1371/journal.pone.0017213
38. Jia L, Zhang L, Shao C, Song E, Sun W, Li M, Gao Y. An attempt to understand kidney's protein handling function by comparing plasma and urine proteomes. *PLoS One*. 2009;4:e5146. doi: 10.1371/journal.pone.0005146
39. Yang J, Brody EN, Murthy AC, Mehler RE, Weiss SJ, DeLisle RK, Ostroff R, Williams SA, Ganz P. Impact of kidney function on the blood proteome and on protein cardiovascular risk biomarkers in patients with stable coronary heart disease. *J Am Heart Assoc*. 2020;9:e016463. doi: 10.1161/JAHA.120.016463
40. Meeusen JW, Kasozi RN, Larson TS, Lieske JC. Clinical impact of the refit CKD-EPI 2021 creatinine-based eGFR equation. *Clin Chem*. 2022;68:534–539. doi: 10.1093/clinchem/hvab282
41. D'Agostino RB Sr, Pencina MJ, Massaro JM, Coady S. Cardiovascular disease risk assessment: insights from Framingham. *Glob Heart*. 2013;8:11–23. doi: 10.1016/j.gheart.2013.01.001
42. Ahalt S, Avillach P, Boyles R, Bradford K, Cox S, Davis-Dusenbery B, Grossman RL, Krishnamurthy A, Manning A, Paten B, et al. Building a collaborative cloud platform to accelerate heart, lung, blood, and sleep research. *J Am Med Inform Assoc*. 2023;30:1293–1300. doi: 10.1093/jamia/ocad048
43. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010;26:2190–2191. doi: 10.1093/bioinformatics/btq340

44. Jiang M-Z, Aguet F, Ardlie K, Chen J, Cornell E, Cruz D, Durda P, Gabriel SB, Gerszten RE, Guo X, et al; TOPMed Analysis Working Group. Uncovering cross-cohort molecular features with multi-omics integration analysis. *bioRxiv*. 2011;2022:2010–.515908.
45. Sealock JM, Lee YH, Moscati A, Venkatesh S, Voloudakis G, Straub P, Singh K, Feng YC, Ge T, Roussos P, Smoller JW. Use of the PsycheMERGE network to investigate the association between depression polygenic scores and white blood cell count. *JAMA Psychiatry*. 2021;78:1365–1374. doi: 10.1001/jamapsychiatry.2021.2959
46. Valente MJ, Rijnhart JJ, Smyth HL, Muniz FB, MacKinnon DP. Causal mediation programs in R, M plus, SAS, SPSS, and Stata. *Struct Equ Modeling*. 2020;27:975–984. doi: 10.1080/10705511.2020.1777133
47. Gong J, Scholes S, Cole S, Zaninotto P, Steptoe A. Associations between plasma proteins and psychological wellbeing: evidence from over 20 years of the English Longitudinal Study of Ageing. *medRxiv*. 2025. doi: 10.1101/2025.02.05.25321715
48. Spagnuolo MC, Gottmann P, Sommer J, Borgmann SO, Strassburger K, Rathmann W, Zaharia OP, Trenkamp S, Wagner R, Icks A, et al. Three-protein signature is associated with baseline and persistently elevated or recurrent depressive symptoms in individuals with recent-onset diabetes. *BMJ Open Diabetes Res Care*. 2025;13:e004396. doi: 10.1136/bmjdrc-2024-004396
49. Akwii RG, Sajib MS, Zahra FT, Mikelis CM. Role of angiopoietin-2 in vascular physiology and pathophysiology. *Cells*. 2019;8:471. doi: 10.3390/cells8050471
50. Wischhusen J, Melero I, Fridman WH. Growth/Differentiation Factor-15 (GDF-15): from biomarker to novel targetable immune checkpoint. *Front Immunol*. 2020;11:951. doi: 10.3389/fimmu.2020.00951
51. Weledji EP, Assob JC. The ubiquitous Neural Cell Adhesion Molecule (N-CAM). *Ann Med Surg*. 2014;3:77–81. doi: 10.1016/j.amsu.2014.06.014
52. Chatterjee M, Schild D, Teunissen CE. Contactins in the central nervous system: role in health and disease. *Neural Regener Res*. 2019;14:206–216. doi: 10.4103/1673-5374.244776
53. Cheng B, Yang X, Cheng S, Ce L, Zhang H, Liu L, Meng P, Jia Y, Wen Y, Zhang F. A large-scale polygenic risk score analysis identified candidate proteins associated with anxiety, depression and neuroticism. *Mol Brain*. 2022;15:66. doi: 10.1186/s13041-022-00954-3
54. Morris J, Leung SSY, Bailey ME, Cullen B, Ferguson A, Graham N, Johnston KJ, Lyall DM, Lyall LM, Ward J. Exploring the role of contactins across psychological, psychiatric and cardiometabolic traits within UK Biobank. *Genes*. 2020;11:1326. doi: 10.3390/genes11111326
55. Petrovska J, Coynel D, Fastenrath M, Milnik A, Auschra B, Egli T, Gschwind L, Hartmann F, Loos E, Sifalakis K, et al. The NCAM1 gene set is linked to depressive symptoms and their brain structural correlates in healthy individuals. *J Psychiatr Res*. 2017;91:116–123. doi: 10.1016/j.jpsychires.2017.03.007
56. Zhao J, Quyyumi AA, Patel R, Zafari AM, Veledar E, Onufrak S, Shallenberger LH, Jones L, Vaccarino V. Sex-specific association of depression and a haplotype in leukotriene A4 hydrolase gene. *Psychosom Med*. 2009;71:691–696. doi: 10.1097/PSY.0b013e3181b05c57
57. Chen Y-L, You J, Guo Y, Zhang Y, Yao B-R, Wang J-J, Chen S-D, Ge Y-J, Yang L, Wu X-R, et al. Identifying proteins and pathways associated with multimorbidity in 53,026 adults. *Metabolism*. 2025;164:156126. doi: 10.1016/j.metabol.2024.156126
58. Wiklund FE, Bennet AM, Magnusson PK, Eriksson UK, Lindmark F, Wu L, Yaghoutyfam N, Marquis CP, Stattin P, Pedersen NL, et al. Macrophage inhibitory cytokine-1 (MIC-1/GDF15): a new marker of all-cause mortality. *Aging Cell*. 2010;9:1057–1064. doi: 10.1111/j.1474-9726.2010.00629.x
59. Nurmohamed NS, Belo Pereira JP, Hoogeveen RM, Kroon J, Kraaijenhof JM, Waissi F, Timmerman N, Bom MJ, Hoefer IE, Knaapen P, et al. Targeted proteomics improves cardiovascular risk prediction in secondary prevention. *Eur Heart J*. 2022;43:1569–1577. doi: 10.1093/eurheartj/ehac055
60. Carrasco-Zanini J, Wheeler E, Uluvar B, Kerrison N, Koprulu M, Wareham NJ, Pietzner M, Langenberg C. Mapping biological influences on the human plasma proteome beyond the genome. *Nat Metab*. 2024;6:2010–2023. doi: 10.1038/s42255-024-01133-5
61. Sedaghat S, Park S, Walker RF, Wang S, Liu J, Hughes TM, Sabayan B, Tang W, Coresh J, Pankow JS, et al. Proteomics-based aging clocks in midlife or late-life and their associated risk of dementia. *Commun Med*. 2025;5:353. doi: 10.1038/s43856-025-01096-y
62. Torrens-Mas M, Navas-Enamorado C, Galmes-Panades A, Masmiquel L, Sanchez-Polo A, Capo X, Gonzalez-Freire M. GDF-15 as a proxy for epigenetic aging: associations with biological age markers, and physical function. *Biogerontology*. 2025;26:22. doi: 10.1007/s10522-024-10165-z
63. Weinberger AH, Kashan RS, Shpigel DM, Esan H, Taha F, Lee CJ, Funk AP, Goodwin RD. Depression and cigarette smoking behavior: a critical review of population-based studies. *Am J Drug Alcohol Abuse*. 2017;43:416–431. doi: 10.3109/00952990.2016.1171327
64. Peplinski BS, Houston BA, Bluemke DA, Kawut SM, Kolb TM, Kronmal RA, Lima JA, Ralph DD, Rayner SG, Steinberg ZL, et al. Associations of angiopoietins with heart failure incidence and severity. *J Card Fail*. 2021;27:786–795. doi: 10.1016/j.cardfail.2021.04.001
65. Benz AP, Hijazi Z, Lindbäck J, Connolly SJ, Eikelboom JW, Kastner P, Ziegler A, Alexander JH, Granger CB, Lopes RD, et al. Plasma angiopoietin-2 and its association with heart failure in patients with atrial fibrillation. *Europace*. 2023;25:euad200. doi: 10.1093/europace/euad200
66. Lee SJ, Lee CK, Kang S, Park I, Kim YH, Kim SK, Hong SP, Bae H, He Y, Kubota Y, et al. Angiopoietin-2 exacerbates cardiac hypoxia and inflammation after myocardial infarction. *J Clin Invest*. 2018;128:5018–5033. doi: 10.1172/JCI99659
67. Azzo JD, Dib MJ, Zagkos L, Zhao L, Wang Z, Chang CP, Ebert C, Salman O, Gan S, Zamani P, et al. Proteomic associations of N-terminal (NT)-pro hormone BNP (NT-proBNP) in Heart Failure with Preserved Ejection Fraction (HFpEF). *Circ Heart Fail*. 2024;17:e011146. doi: 10.1161/CIRCHEARTFAILURE.123.011146
68. Dib MJ, Levin MG, Zhao L, Diab A, Wang Z, Ebert C, Salman O, Azzo JD, Gan S, Zamani P, et al. Proteomic associations of adverse outcomes in human heart failure. *J Am Heart Assoc*. 2024;13:e031154. doi: 10.1161/JAHA.123.031154
69. Spruill TM, Butler MJ, Thomas SJ, Tajeu GS, Kalinowski J, Castañeda SF, Langford AT, Abdalla M, Blackshear C, Allison M, et al. Association between high perceived stress over time and incident hypertension in black adults: findings from the Jackson heart study. *J Am Heart Assoc*. 2019;8:e012139. doi: 10.1161/JAHA.119.012139
70. Karp NA, McCormick PS, Russell MR, Lilley KS. Experimental and statistical considerations to avoid false conclusions in proteomics studies using differential in-gel electrophoresis. *Mol Cell Proteomics*. 2007;6:1354–1364. doi: 10.1074/mcp.M600274-MCP200
71. Rijnhart JJ, Lamp SJ, Valente MJ, MacKinnon DP, Twisk JW, Heymans MW. Mediation analysis methods used in observational research: a scoping review and recommendations. *BMC Med Res Methodol*. 2021;21:226. doi: 10.1186/s12874-021-01426-3
72. VanderWeele TJ. Causal mediation analysis with survival data. *Epidemiology*. 2011;22:582–585. doi: 10.1097/EDE.0b013e31821db37e