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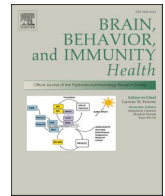
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## Proteomic profiles of cytokines and chemokines in moderate to severe depression: Implications for comorbidities and biomarker discovery

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### ABSTRACT

**Objective:** This study assessed the proteomic profiles of cytokines and chemokines in individuals with moderate to severe depression, with or without comorbid medical disorders, compared to healthy controls. Two proteomic multiplex platforms were employed for this purpose.

**Methods:** An immunofluorescent multiplex platform and an aptamer-based method were used to evaluate 32 protein analytes from 153 individuals with moderate to severe major depressive disorder (MDD) and healthy controls (HCs). The study focused on determining the level of agreement between the two platforms and evaluating the ability of individual analytes and principal components (PCs) to differentiate between the MDD and HC groups. Additionally, the study investigated the relationship between PCs consisting of chemokines and cytokines and comorbid inflammatory and cardiometabolic diseases.

**Findings:** Analysis revealed a small or moderate correlation between 47% of the analytes measured by the two platforms. Two proteomic profiles were identified that differentiated individuals with moderate to severe MDD from HCs. High eotaxin, age, BMI, IP-10, or IL-10 characterized profile 1. This profile was associated with several cardiometabolic risk factors, including hypertension, hyperlipidemia, and type 2 diabetes. Profile 2 is characterized by higher age, BMI, interleukins, and a strong negative loading for eotaxin. This profile was associated with inflammation but not cardiometabolic risk factors.

**Conclusion:** This study provides further evidence that proteomic profiles can be used to identify potential biomarkers and pathways associated with MDD and comorbidities. Our findings suggest that MDD is associated with distinct profiles of proteins that are also associated with cardiometabolic risk factors, inflammation, and obesity. In particular, the chemokines eotaxin and IP-10 appear to play a role in the relationship between MDD and cardiometabolic risk factors. These findings suggest that a focus on the interplay between MDD and comorbidities may be useful in identifying potential targets for intervention and improving overall health outcomes.

### 1. Background

Major Depressive Disorder (MDD) has emerged as a prominent global health concern, affecting approximately 264 million individuals worldwide (Murray and Lopez, 1997; Steel et al., 2018; Subramaniapillai et al., 2021). Characterized by a complex interplay of symptoms and variable responses to standard therapies, the clinical management of MDD frequently poses significant challenges,

necessitating a deeper and more nuanced approach to research (Rush et al., 2006; Saveanu et al., 2015).

In the ongoing pursuit to unravel the complexities of MDD, significant attention has been directed towards understanding its potential inflammatory underpinnings (Akil et al., 2018; Strawbridge et al., 2017; Sokolowska et al., 2015). Early research in this domain has underscored the involvement of various individual biomarkers, paving the way for an analysis of the interplay of cytokines and chemokines in the

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pathophysiology of MDD (Miller et al., 2009). This study seeks to build upon this foundational knowledge, examining inflammatory and immune proteomic biomarker patterns across different platforms and exploring their correlation with the clinical and psychiatric phenotypes of MDD.

Numerous studies have consistently highlighted the significant role of inflammatory markers in the disorder. For instance, two decades ago, there was a surge in studies illustrating the involvement of cytokines in MDD, with a particular focus on markers like interleukin (IL)-6 and C-reactive protein (CRP) (Milaneschi et al., 2021). These markers were found to have a differential presence between MDD patients and healthy controls (HC), laying the groundwork for further in-depth analyses. Subsequent studies explored other markers, such as IL-8, which demonstrated a consistent differential expression between MDD patients and HCs irrespective of the duration or severity of the depressive episode (Vogelzangs et al., 2016). However, results have varied, with some markers like tumor necrosis factor (TNF- $\alpha$ ), MCP-1, and IL-1 $\beta$  showcasing inconsistent associations with MDD (Strawbridge et al., 2017; Lehto et al., 2010). This complex history of research points to a need to shift from individual biomarkers to a more integrated approach, exploring patterns and clusters of chemokines and cytokines to provide insight into the role of immune biomarkers in moderate to severe MDD.

To facilitate this shift, our study leverages the plasma proteomic technologies available in the SomaLogic and Luminex platforms (Lasserter et al., 2020). The SomaLogic platform utilizes aptamer-based technology, which allows for the simultaneous analysis of thousands of proteins, providing a broad overview of the proteomic landscape. In contrast, the Luminex platform relies on a multi-analyte profiling system using xMAP technology, offering the advantage of analyzing multiple biomarkers in a single sample. However, these platforms are primarily research tools, not commonly used in clinical practice. Still, these platforms can be central in identifying recurrent proteomic patterns across multiple platforms, providing a more reliable molecular understanding of MDD. It is acknowledged, however, that achieving consistency remains a challenge due to factors including variability across platforms and differences in protein concentrations among biological samples (Lehallier et al., 2019; Katz et al., 2022; Pietzner et al., 2021).

Building upon this technological foundation, we aim to examine the recurrent proteomic patterns identified and assess their association with distinct clinical phenotypes of moderate to severe MDD. By focusing on patterns rather than isolated biomarkers, we anticipate gaining insights into the immune and inflammatory manifestations of MDD. This approach represents a step forward in crafting more effective diagnostic and treatment strategies. Further, we plan to evaluate how these recurrent proteomic patterns correlate with the clinical and psychiatric characteristics of the study participants. Guided by these insights, our investigation seeks to answer the following questions:

Do proteomic patterns of chemokines and cytokine identified through the SomaLogic and Luminex platforms differentiate moderate to severe MDD patients from HCs, and do these patterns predict depressive symptoms one year after the initial assessment? Are these pro-inflammatory and immune biomarker patterns reliable across the two proteomic platforms? How are these identified patterns associated with specific clinical and psychiatric phenotypes observed in MDD, particularly focusing on inflammatory and cardiometabolic indicators?

## 2. METHODS

### 2.1. Participant Selection

Participants in this study were between 18 and 70 years old. MDD patients met the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) criteria for a current major depressive episode without psychotic features, diagnosed using the Structured Clinical Interview for DSM (SCID) (American Psychiatric Association, 2013; First et al., 2015). In addition, MDD patients were required to have a

minimum score of 21 on the 21-item Hamilton Depression Rating Scale (HAM-D) to ensure at least a moderate level of severity (Hamilton, 1960). To ensure homogeneity in endogenous depressive symptoms, there was a minimum required score on the 7-item Thase Core Endomorphemic Scale, a subscale derived from the HAM-D (Thase et al., 1983). HC participants were required to score less than six on the HAM-D and have no psychotic symptoms. Additionally, according to the SCID, they were required to have no current or past psychiatric disorders.

Exclusion criteria for the MDD and HC groups included autoimmune disease (e.g., systemic lupus erythematosus), current pregnancy, or postpartum status. The authors allowed for other comorbid medical illnesses if they were not thought to be the primary cause of the depressive episode. Other exclusions included a history of manic or hypomanic episodes and primary pre-existing obsessive-compulsive disorder. Participants were allowed to continue their psychiatric medications. MDD patients were taking antidepressants, antipsychotics, anxiolytics, and mood stabilizers.

Recruitment of participants at all three sites, Cornell University, Stanford University, and University of California Irvine (UCI), was conducted via two approaches: 1) internal and external recruitment via physician referrals and 2) institutional review board (IRB) approved flyers at each university's medical campus as well as distributing flyers in the local community and health clinics and online ads.

### 2.2. Study procedure

An IRB at each of the three study locations approved the study, and all participants gave written informed consent before participation. Study screening procedures included the SCID, HAM-D, Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1961), comprehensive metabolic panel, urine drug screening, and urine pregnancy screening for female participants. Participants who met inclusion criteria at the eligibility screening returned for baseline procedures.

Medical comorbidities were measured using the Medical History Form at the screening visit, including past and present comorbidities. The Medical History Form is designed to comprehensively gather information about an individual's past and current health conditions. It offers a structured dialogue regarding specific health conditions, allowing the respondent to indicate whether they've been diagnosed with each condition by selecting "Yes," "No," or "Unknown." Additionally, there is space for detailed descriptions for further clarity on each illness. The form covers a range of medical conditions spanning various bodily systems such as the endocrine, cardiovascular, renal, nervous, hepatic, respiratory, reproductive, and immune systems.

The presence of inflammatory disease was classified in each participant as the presence of one or more of the following conditions: type 2 diabetes, hypertension, hyperlipidemia, coronary artery disease, myocardial infarction, kidney disease, hepatitis, thyroid disease, asthma, autoimmune disease, cancer, and polycystic ovarian syndrome.

At the baseline visit, vital signs and physical assessments were performed. Blood samples were collected in the morning from participants fasting for  $\geq 8$  h and processed for clinical use. Clinical raters evaluated participants using psychiatric rating scales, such as HAM-D and BPRS, self-report ratings, and neuropsychological assessments at baseline.

At the 3, 6, and 9-month time points, a phone visit assessed changes in mood and medications, and a one-year follow-up assessment was also completed. Mood at interim visits was evaluated using the Longitudinal Interval Follow-up Evaluation (LIFE), an integrated system for assessing the longitudinal course of psychiatric disorders (Keller et al., 1987).

The procedures for 12-month visit were identical to those of the baseline visit with the addition of the Medical History Form that was initially conducted at the screening visit.

### 2.3. Biospecimen Storage and processing

Blood samples were collected as 10 mL samples in K2 EDTA tubes (BD) Vacutainer # 366643) inverted 8–10 times and kept on ice for no longer than 30 min before spinning. Tubes were spun for 15 min at 1300×g in a 4 °C (refrigerated) centrifuge and kept on ice at all other times. The plasma was carefully collected on ice from above, not including the cell pellet or buffy coat layer. Plasma was divided into aliquots (tubes were pre-chilled and kept on ice until freezing) and stored at –80 °C until use.

Frozen biospecimens collected at Cornell and UCI, stored at –80 °C to maintain their integrity, were packaged in insulated containers with ample dry ice to prevent temperature fluctuations during transit to the Stanford site. Samples were stored at –80 °C at Stanford until plasma samples were packed in dry ice and delivered to the Human Immune Monitoring Center (HIMC) for Luminex analysis and to Alkahest in San Carlos, CA, for SomaLogic's SomaScan assay. Upon arrival at these respective laboratories, the samples were processed according to established protocols to ensure accurate and reliable analysis.

Immune function data were assayed via two platforms: Luminex-EMD Millipore Human 41 Plex and SomaScan Assay. In addition, plasma was collected to determine fasting glucose, insulin, and lipids.

### 2.4. Luminex-EMD Millipore Human 41 Plex assay

The Human Immune Monitoring Center at Stanford University Immunoassay Team performed the assay. Kits were purchased from EMD Millipore Corporation, Burlington, MA, and run according to the manufacturer's recommendations with modifications: the HCYTMAG-60K-PX41 assay setup followed the recommended protocol: plasma samples were diluted 3-fold. 25ul of the diluted sample was mixed with antibody-linked magnetic beads in a 96-well plate and incubated overnight at 4 °C with shaking. Cold and room temperature incubation steps were performed on an orbital shaker at 500–600 rpm. Plates were balanced considering case/control status and study site with longitudinal samples loaded on the same plate, and samples run in duplicate. These controls acted as an internal standard, pivotal in validating our normalization process and providing a reliable baseline for comparative analysis. By including these controls, we enhanced the methodological robustness of our study, ensuring a more accurate representation of the observed biomarker trends. Custom Assay Chex control beads were used as a standard.

Plates were washed twice with wash buffer in a BioTek ELx405 washer (BioTek Instruments, Winooski, VT). Following 1-h incubation at room temperature with a biotinylated detection antibody, streptavidin-PE was added for 30 min with shaking. Plates were washed as described above, and PBS was added to wells for reading in the Luminex Flex-Map3D Instrument with a lower bound of 50 beads per sample per cytokine. Each sample was measured in duplicate. Custom Assay Chex control beads were purchased and added to all wells (Radix Bio-Solutions, Georgetown, TX). Wells with a bead count <50 were flagged, and data with a bead count <20 were excluded. Data were corrected across plates in R using methods described in [Maecker et al. \(2020\)](#). This will be referred to as the Luminex assay. Samples were run in duplicate.

We employed Luminex kits as a standard for assessing the SomaScan method. Our aim was to compare unprocessed signal data across different platforms to discern patterns among the subjects. To counter-balance site-specific differences and other variables that we could not control, we applied comprehensive normalization techniques. This normalization was vital for comparing data across platforms and relied on direct measurements of biomarkers, allowing for a nuanced understanding of subject-specific trends.

To ensure consistency across our assays and minimize batch effects, we used the same lot of kits for all analyses. An R utility was used to correct for plate/batch/lot artifacts and nonspecific binding, resulting in a detrended preprocessed median fluorescence intensity (dpMFI), as

described in [Maecker et al. \(2020\)](#). HC and MDD samples were analyzed simultaneously.

### 2.5. SomaLogic SomaScan Assay

In collaboration with Alkahest, San Carlos, CA, baseline plasma samples were processed using the latest SomaLogic (Boulder, CO) SomaScan proteomics assay ([Maecker et al., 2020](#)). This aptamer-based assay has been optimized to quantify >7000 human proteins accurately.

These assays have been widely used to quantify relative levels of proteins involved in several processes, such as intercellular signaling, extracellular proteolysis, and metabolism. The SomaScan technology uses Slow Off-rate Modified Aptamers (SOMAMers), modified, short, single-stranded deoxyoligonucleotides that fold into compact structures and have specific binding affinity to a single protein. Assay details were previously described by Gold et al. ([Gold et al. \(2010\)](#)). The SomaLogic data was processed with normalization and calibration steps that utilized QC and calibrator samples to remove bias within and across plates ([Gold et al., 2010](#)). Each plasma protein's relative fluorescence units (RFUs) were log-transformed for analysis. Samples were run as singletons.

## 3. Statistical analysis

The demographic and clinical characteristics of individuals with moderate to severe MDD and HCs were summarized by the mean and standard deviation or frequency and percent according to variable type.

Analyses included 32 chemokines and cytokines that were shared between the two proteomic platforms and had a minimum of 80% protein data available. Five analytes did not have the necessary sample size and were thus excluded from data analysis; they were the CD-40 Ligand, IFN- $\alpha$ 2, IL-2, IL-8, and MIP-1 $\alpha$ . Among the selected analytes, some proteomic values were absent due to insufficient volume in the assay. The missing proteomic and covariate values were then imputed using the HPIMPUTE procedure in SAS 9.4. Proteins were compared across platforms using Pearson correlation coefficients. All proteins were log-transformed before analysis.

Each proteomic chemokine and cytokine for the two platforms was evaluated separately for their cross-sectional association with moderate to severe MDD compared to HCs using logistic regression. The Benjamini-Hochberg procedure was applied to account for multiple tests with a false discovery rate of 20%. Models were adjusted for sex, age, ethnicity and race, education, and study site.

### 3.1. Principal components analysis

Principal component analyses (PCAs) using Varimax rotation were conducted for Luminex and SomaLogic analytes separately. Each PCA included age, body mass index (BMI), and the 32 proteomic analytes from a given proteomic platform. Age and BMI were included in the PCA as clinical predictors because they form an integral part of the profile for inflammatory MDD.

Heat maps summarized the standardized scoring coefficients PCs. We selected the number of PCs based on the number that included eigenvalues  $\geq 1$ . The association of PCs between the two assays was compared via Pearson correlation.

Our objective was to examine the impact of proteomic principal components (PCs) on two psychiatric outcomes: 1) the distinction between MDD status and healthy controls (HC), and 2) the severity of depression after one year.

PCs from each platform were added to a logistic regression model to assess the association between proteomic profile and moderate to severe MDD status. These models were adjusted for sex, education, race and ethnicity, and study site. BMI and age were not adjusted in logistic regression models as they were included in the PCA as independent variables. Discriminatory PCs were defined as those that differentiated

individuals with MDD from HCs.

Adjusted mixed model analyses evaluated the association between all PCs (i.e., between MDD cases and HCs) and one-year depression severity scores among the participants with moderate to severe MDD. These models were adjusted for sex, education, race and ethnicity, study site, and baseline use of antidepressant or anxiolytic medication.

### 3.2. Analysis of cardiometabolic risk factors with PCs

Pearson correlation assessed the association between discriminatory PCs and cardiometabolic risk factors, including the homeostatic model of insulin resistance (HOMA-IR), BMI, fasting plasma glucose (FPG), fasting plasma insulin (FPI), and C-reactive protein (CRP). CRP was available from two of the three sites. The strength and direction of the correlations were calculated using statistical software, and significance was considered at  $p < 0.05$ .

### 3.3. Analysis of medical comorbidities with PCs

T-tests were conducted to assess differences in mean discriminatory PC values and medical comorbidities. Comorbidity frequency at baseline was tallied. Any illness present in  $\geq 5$  participants was included in this analysis: hypertension, hyperlipidemia, type 2 diabetes, thyroid disease, migraine, asthma, and the presence of any inflammatory disease. The significance level was set at  $p < 0.05$ .

### 3.4. Supplemental analyses

Discriminatory PCs (those associated with moderate to severe MDD) were evaluated for site effects using scatterplots. In addition, to assess the consistency of effect size and directionality across the three study sites, a supplemental analysis modeled the PCs associated with moderate to severe MDD ( $p < 0.05$ ) in separate logistic regression models for each study site. These models were adjusted for sex and study site only, as sample sizes were too small to adjust for race and ethnicity, and education.

In additional supplemental analyses, multinomial logistic regression models evaluated the association between discriminating PCs from the primary analysis ( $p < 0.05$ ) and moderate to severe MDD when stratified by antidepressant medication use. This analysis divided the study population into three groups: 1) individuals with MDD who were taking antidepressant medication, 2) individuals with MDD who were not taking antidepressant medication, and 3) HCs (the reference group for this analysis). A second, identical analysis was undertaken but compared individuals with 1) moderate to severe MDD who were taking antidepressants or anxiolytic medication, 2) individuals who take neither antidepressants nor anxiolytics, and 3) HC. All models were adjusted for sex, education, race and ethnicity, and study site.

Finally, logistic regression analyses of the association between PCs and moderate to severe MDD compared to HC were replicated in the subset of individuals without a diagnosis of inflammatory disease.

All analyses were conducted using SAS 9.4 and R 4.2.2 statistical software.

## 4. Results

Demographic and clinical characteristics are summarized by study group in Table 1. The MDD and HC groups were similar in their distributions of sex, education, and BMI. MDD patients were significantly older than HCs. MDD patients had significantly greater rates of inflammatory disease, hyperlipidemia, and hypertension but not type 2 diabetes.

The mean 21-item HAM-D score in the MDD group was 26.6, signifying depression scores in the moderate to severe range. There were differences across the three participating sites in the number of MDD participants recruited, with Stanford recruiting most MDD participants.

**Table 1**

Demographic and clinical characteristics of study participants by depression status.

	Not Depressed (n = 81)	Depressed (n = 72)	p-Value	Missing
<b>Age, Mean (SD)</b>	38.9 (14.8)	46.0 (15.6)	0.005	0
<b>Sex</b>				
Female	53 (65.4%)	49 (68.1%)	0.73	0
Male	28 (34.6%)	23 (31.9%)		
<b>Study Site</b>				
Cornell	27 (33.3%)	16 (22.2%)	0.007	0
Stanford	28 (34.6%)	43 (59.7%)		
University of California Irvine	26 (32.1%)	13 (18.1%)		
<b>Hamilton Depression Rating Scale-21, Mean (SD)</b>	0.80 (1.3)	26.51 (4.6)	<0.001	0
<b>Education</b>				
High School Diploma or GED	7 (8.6%)	8 (11.1%)	0.55	0
Technical School, Associate's Degree, or Some College	24 (29.6%)	15 (20.8%)		
College Diploma	23 (28.4%)	26 (36.1%)		
Graduate or Professional Degree	27 (33.3%)	23 (31.9%)		
<b>Ethnicity</b>				
Hispanic or Latino	6 (7.4%)	6 (8.3%)	0.003	1
Asian	14 (17.3%)	7 (9.7%)		
African American	18 (22.2%)	3 (4.2%)		
White	30 (37.0%)	45 (62.5%)		
Other	12 (14.8%)	11 (15.3%)		
<b>Body Mass Index, Mean (SD)</b>	25.8 (5.5)	27.1 (6.5)	0.21	31
<b>Any Inflammatory Disease, N (%)</b>	28 (34.6%)	45 (62.5%)	<0.001	0
<b>Asthma, N (%)</b>	7 (8.8%)	11 (15.3%)	0.07	1
<b>Hyperlipidemia, N (%)</b>	10 (12.5%)	23 (31.9%)	0.007	1
<b>Hypertension, N (%)</b>	6 (7.6%)	17 (23.6%)	0.006	2
<b>Migraine, N (%)</b>	3 (3.8%)	22 (30.6%)	<0.001	1
<b>Type 2 Diabetes, N (%)</b>	5 (6.3%)	4 (5.6%)	0.85	1
<b>Thyroid Disease, N (%)</b>	7 (8.8%)	12 (16.9%)	0.17	1
<b>Antidepressant Medication, N (%)</b>	1 (1.2%)	50 (69.4%)	<0.001	0
<b>Antipsychotic Medication, N (%)</b>	0 (0%)	8 (11.1%)	0.002	0
<b>Anxiolytic Medication, N (%)</b>	0 (0%)	21 (29.2%)	<0.001	0
<b>Any Psychiatric Medication, N (%)</b>	1 (1.2%)	52 (72.2%)	<0.001	0

Most MDD patients took at least one class of psychiatric medication (72%) (Table 1).

There were low or moderate cross-platform correlations between equivalent proteins (Table 2). There was moderate agreement for five chemokines and growth factors, with the strongest correlations ( $r > 0.40$ ) found in the case of eotaxin, endothelial growth factor (EGF), IL-7, platelet-derived growth factor AA (PDGF-AA), and GRO- $\alpha$ . On the other hand, weaker correlations ( $>0.2$ ,  $p < 0.01$  uncorrected) were observed for IL-4, IL-5, IP-10, Macrophage Derived Chemokine (MDC), TNF- $\alpha$ , and tumor necrosis factor- $\beta$  (TNF- $\beta$ ).

Several interleukins (IL-3, IL-6, IL-15, IL-17, IL-12, IL-1 $\alpha$ , IL1 $\beta$ , and IL-1RA) did not demonstrate agreement across the two assays, along with fractalkine, PDGF-AA, granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF), and IFN- $\gamma$  (Table 2).

Adjusted logistic regressions found that none of the individual proteins were significantly associated with MDD case status after adjustment for multiple comparisons.

**Table 2**  
Pearson correlations between Luminex and SomaLogic assays (n = 153).

Luminex Assay Protein	EGF	Eotaxin	FGF-2	Flt-3L	Fractalkine	G-CSF	GM-CSF	GRO- $\alpha$	IFN- $\gamma$
SomaLogic Assay	0.63**	0.64**	0.07	-0.04	0.04	-0.02	0.05	0.42**	0.03
SomaLogic Secondary Analyte <sup>a</sup>	0.46**	-	-	-	-	0.05	-	-	0.18
-	<b>IL-1<math>\alpha</math></b>	<b>IL-1<math>\beta</math></b>	<b>IL1-RA</b>	<b>IL-3</b>	<b>IL-4</b>	<b>IL-5</b>	<b>IL-6</b>	<b>IL-7</b>	<b>IL-9</b>
SomaLogic Assay	0.09	0.09	0.15	0.10	0.25**	0.22**	0.02	0.44**	0.19*
SomaLogic Secondary Analyte	-	-	-	-	0.18*	0.22**	-0.05	0.37**	-
-	<b>IL-10</b>	<b>IL-12 P40</b>	<b>IL-13</b>	<b>IL-15</b>	<b>IL-17</b>	<b>IP-10</b>	<b>MCP-1</b>	<b>MCP-3</b>	<b>MDC</b>
SomaLogic Assay	0.17*	0.05	0.06	0.07	0.06	0.26**	0.12	0.13	0.21**
SomaLogic Secondary Analyte	0.01	-	-	-	-	-	-	0.19	-
-	<b>PDGF-AA</b>	<b>RANTES</b>	<b>TNF<math>\alpha</math></b>	<b>TNF<math>\beta</math></b>	<b>VEGF</b>	-	-	-	-
SomaLogic Assay	0.59**	-0.12	0.24**	0.22**	0.19*	-	-	-	-
SomaLogic Secondary Analyte	-	-0.12	0.27**	-	-	-	-	-	-

\*p < 0.05 \*\*p < 0.01.

<sup>a</sup> Some Luminex analytes had two distinct corresponding analytes in the SomaLogic Assay. EGF = Epidermal Growth Factor; FGFb = Fibroblast Growth Factor 2; Flt-3L = FMS-like Tyrosine Kinase 3 Ligand; G-CSF = Granulocyte Colony-Stimulating Factor; GM-CSF = Granulocyte/Macrophage Colony-Stimulating Factor; GRO- $\alpha$  = Growth-regulated protein alpha; IFN- $\gamma$  = Interferon Gamma; IFN- $\alpha$ 2 = Interferon alpha-2; IL = Interleukin; IL1-RA = Interleukin 1 Receptor Antagonist; MCP-1 = Monocyte Chemoattractant Protein 1; MCP-3 = Monocyte Chemoattractant Protein 3; MDC = Macrophage Derived Chemokine; MIP-1A = Macrophage Inflammatory Protein 1A; PDGF-AA = Platelet Derived Growth Factor AA; TNF $\alpha$  = Tumor Necrosis Factor Alpha; TNF $\beta$  = Tumor Necrosis Factor Beta; VEGF = Vascular Endothelial Growth Factor.

#### 4.1. PCAs of multiple proteins for separating moderate to severe MDD and healthy controls

Two PCAs – one for each proteomic platform – produced seven PCs with eigenvalues  $\geq 1$  (Supplemental Table 1). PCs were standardized and centered before interpretation. The standardized scoring coefficients for PCs were summarized by heat maps (Fig. 1a and b). The Luminex and SomaLogic PCs were compared via Pearson correlation (Table 3).

Logistic regression models assessed the association between each proteomic PC and moderate to severe MDD status with HC as the reference group. SomaLogic PC3 was associated with increased odds of moderate to severe MDD compared to being an HC (Odds Ratio: 1.65, 95% CI: 1.05, 2.59), as was SomaLogic PC4 (odds ratio: 1.54, 95% CI: 1.01, 2.34). Luminex PC4 showed a similar association (odds ratio: 1.86, 95% CI: 1.22, 2.83). Thus, the PCs that predicted MDD case status correlated well across platforms. See Table 4 for a description of logistic regression models.

Adjusted mixed models evaluated the association between PCs and one-year HAM-D scores among the participants with moderate to severe MDD. Baseline Luminex PC4 was significantly, negatively associated depression severity score over a one-year follow-up ( $\beta$ : 2.46, 95% confidence interval: 0.29, 4.63). Baseline SomaLogic PC3 and PC4 scores were not associated with change in depression severity over the same period.

#### 4.2. Comparing principal components across proteomic platforms

The most influential standardized coefficient loadings (those exceeding 0.20 or falling below -0.20) for various analytes on three distinct discriminating principal components (PCs) were as follows: 1) Luminex PC4: eotaxin, IP-10, IL-7, and MCP-1, 2) SomaLogic PC4: IL-6, IL-12 P40, and RANTES, 3) SomaLogic PC3: eotaxin and IL-10.

We conducted a comparison of the standardized coefficients of analytes on these discriminatory PCs using a threshold of  $>0.10$  or  $<-0.10$ . On Luminex PC4, nine analytes met this criterion: eotaxin, IP-10, MCP-1, EGF, Flt-3L, IL-7, IL-17, GRO- $\alpha$ , and TNF- $\alpha$ . Out of these, five

analytes also exhibited loadings exceeding  $>0.10$  or  $<-0.10$  on one or both of the SomaLogic discriminatory PCs: eotaxin, IP-10, IL-7, GRO- $\alpha$ , and IL-17.

As a result, these five proteomic analytes played a role in distinguishing between individuals with moderate to severe MDD and healthy controls on both proteomic platforms. Among these, the first four analytes showed a moderate correlation between the assays (with correlation coefficients of  $r = 0.64$ ,  $r = 0.26$ ,  $r = 0.44$ , and  $r = 0.42$ , respectively, as shown in Table 2).

#### 4.3. Differential Contribution of interleukins to discriminatory principal components in MDD analysis

The involvement of ILs in the discriminatory PCs was more pronounced in the SomaLogic platform than in Luminex. Specifically, SomaLogic PCs, such as PC3 and PC4, included several ILs that displayed loadings exceeding  $>0.10$  or  $<-0.10$ . In contrast, when examining Luminex PC4, standardized scoring coefficients for only two ILs met this threshold (namely, IL-7 and IL-17) as illustrated in Fig. 1a and b.

Notably, SomaLogic PC4 featured several contributory ILs, indicating a potential profile characterized by both elevated body mass index (BMI) and heightened inflammatory activity in individuals with moderate to severe MDD.

#### 4.4. Analysis of cardiometabolic risk factors with PCs

Table 5 presents the correlation coefficients among SomaLogic PC3, SomaLogic PC4, Luminex PC4, and various cardiometabolic measures, including HOMA-IR, FPI, BMI, FPG, and CRP.

All three PCs exhibit positive correlations with HOMA-IR, FPI, and FPG, with the strength of these correlations ranging from small to moderate. Additionally, all three discriminatory PCs display positive correlations with BMI. SomaLogic PC4 exhibits a strong correlation, while SomaLogic PC3 and Luminex PC4 exhibit smaller to moderate correlations.

Lastly, SomaLogic PC4 displays a moderate positive correlation with

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Age	0.00	-0.01	0.31	0.14	-0.13	0.27	-0.40
BMI	0.00	-0.02	0.13	0.53	0.27	0.21	0.06
EGF	0.04	0.09	-0.07	0.04	-0.01	0.03	0.06
EGF V2	0.03	0.11	0.01	-0.18	-0.02	0.04	0.03
Eotaxin	0.02	-0.02	0.29	-0.26	-0.11	-0.08	-0.10
FGF-2	0.03	0.10	-0.05	0.08	-0.24	0.01	0.10
FLT-3L	0.04	-0.05	-0.07	0.08	0.01	-0.06	0.02
Fractalkine	0.03	-0.12	0.14	-0.03	-0.14	0.02	0.31
G-CSF	0.02	0.02	-0.01	0.10	0.04	0.21	0.59
G-CSF V2	0.03	-0.13	-0.03	0.00	0.10	-0.04	-0.09
GM-CSF	0.03	0.03	0.00	-0.08	0.09	0.12	-0.02
GRO- $\alpha$	0.02	0.03	-0.03	-0.04	0.23	0.06	-0.02
IFN- $\gamma$	0.03	0.15	-0.02	-0.05	0.02	0.03	0.09
IFN- $\gamma$ V2	0.03	-0.14	0.00	-0.13	0.02	0.01	0.07
IL-1a	0.03	-0.03	-0.06	0.02	0.10	-0.06	-0.16
IL-1b	0.01	0.03	0.01	0.28	0.31	-0.65	0.00
IL-1RA	0.04	0.04	0.04	0.15	-0.03	0.11	0.05
IL-3	0.03	0.04	-0.06	-0.02	-0.02	0.06	0.07
IL-4	0.04	-0.11	-0.03	-0.03	-0.03	0.02	0.14
IL-4 V2	0.01	-0.06	0.07	-0.15	0.55	0.42	-0.01
IL-5	0.04	-0.09	-0.05	-0.05	-0.04	-0.08	0.08
IL-5 V2	0.04	-0.08	-0.03	-0.11	0.07	0.01	0.03
IL-6	0.03	0.16	-0.06	0.06	-0.04	0.08	0.11
IL-6 V2	0.03	-0.05	0.00	0.13	-0.08	0.09	-0.02
IL-7	0.04	-0.03	-0.08	-0.01	-0.04	-0.03	-0.10
IL-7 V2	0.04	0.04	-0.07	-0.02	-0.05	0.05	-0.11
IL-9	0.04	0.00	-0.07	0.03	-0.06	0.02	-0.13
IL-10	0.02	0.00	0.28	-0.08	0.03	-0.17	0.14
IL-10 V2	0.03	-0.06	0.08	-0.05	-0.01	-0.10	0.21
IL-12 P40	0.03	-0.01	0.11	0.10	-0.18	-0.03	0.23
IL-13	0.03	-0.10	-0.05	-0.04	0.16	-0.10	-0.09
IL-15	0.03	0.13	-0.12	0.14	0.00	0.09	-0.07
IL-17	0.03	-0.04	-0.07	0.10	-0.06	0.03	-0.15
IP-10	0.03	0.08	0.09	0.15	-0.19	0.08	-0.08
MCP-1	0.03	0.01	0.00	0.10	-0.14	-0.03	-0.23
MCP-3	0.04	-0.01	0.01	0.01	0.00	0.01	-0.18
MCP-3 V2	0.04	-0.06	-0.01	0.01	0.04	-0.01	-0.09
MDC	0.02	0.07	0.19	0.09	0.15	-0.18	0.02
PDGF-AA	0.03	0.12	-0.01	-0.12	0.08	-0.01	-0.10
RANTES	0.03	0.21	0.07	-0.14	0.10	-0.04	-0.02
RANTES V2	0.03	0.21	0.08	-0.13	0.13	-0.06	-0.02
TNF- $\alpha$	0.04	-0.08	-0.02	0.08	-0.05	0.01	-0.07
TNF- $\alpha$ V2	0.04	-0.10	-0.02	-0.05	0.06	-0.02	0.00
TNF- $\beta$	0.04	-0.09	-0.05	0.00	-0.02	0.00	-0.10
VEGF	0.03	-0.03	0.10	0.01	-0.06	-0.09	0.02

Standardized scoring coefficients represent principal component values.

**Fig. 1a.** Principal Component Analysis of Log-Transformed SomaLogic Proteomic Analytes Standardized scoring coefficients represent principal component values.

inflammatory biomarker, CRP, whereas the other two PCs do not.

#### 4.5. Analysis of medical comorbidities with PCs

Table 6 illustrates differences in the associations between SomaLogic PC3, SomaLogic PC4, and Luminex PC4 scores and medical comorbidities. SomaLogic PC3 is positively associated with type 2 diabetes, hypertension, hyperlipidemia, thyroid disease, and inflammatory diseases.

Luminex PC4 shows positive associations with the same comorbidities as SomaLogic PC3, as well as with migraine. On the other hand, SomaLogic PC4 is only positively associated with the presence of inflammatory disease.

#### 4.6. Supplemental analyses

A supplemental analysis did not find differences in PC distribution by



	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Age	0.00	0.00	-0.04	0.30	0.30	0.23	-0.27
BMI	0.00	-0.01	0.00	0.11	0.14	0.54	0.48
EGF	0.04	0.01	0.22	-0.12	0.12	0.00	0.02
Eotaxin	0.03	0.06	0.12	0.20	-0.06	0.12	-0.40
FGF-2	0.05	0.12	-0.02	-0.07	-0.07	0.01	0.00
FIT-3L	0.04	0.07	-0.09	0.14	0.12	-0.14	-0.21
Fractalkine	0.04	0.09	-0.04	0.00	-0.10	-0.13	-0.04
G-CSF	0.05	0.07	0.01	0.00	-0.01	-0.15	0.08
GM-CSF	0.06	0.09	-0.02	0.01	0.06	-0.12	0.11
GRO- $\alpha$	0.01	0.01	0.28	-0.12	0.17	-0.01	0.04
IFN- $\gamma$	0.05	0.12	-0.02	-0.06	-0.22	0.06	-0.05
IL-1a	0.06	-0.13	0.01	0.05	-0.08	-0.05	0.00
IL-1b	0.06	0.07	-0.04	-0.08	0.06	-0.02	0.00
IL-1RA	0.06	-0.10	0.01	0.05	-0.01	-0.01	0.05
IL-3	0.05	0.09	-0.08	-0.02	0.29	-0.09	0.00
IL-4	0.05	-0.08	0.13	-0.05	-0.01	-0.09	0.11
IL-5	0.06	-0.12	-0.02	-0.01	0.00	-0.03	-0.03
IL-6	0.06	-0.11	-0.01	0.06	-0.17	0.07	-0.06
IL-7	0.06	0.03	0.00	-0.11	0.19	0.15	-0.07
IL-9	0.06	-0.11	0.00	0.02	-0.04	0.00	0.00
IL-10	0.06	0.01	-0.05	0.06	0.15	-0.02	0.11
IL-12 P40	0.06	0.04	-0.07	0.00	0.10	-0.03	0.14
IL-13	0.06	-0.12	-0.01	0.01	-0.04	0.01	0.01
IL-15	0.07	0.05	-0.05	-0.01	0.10	-0.05	0.10
IL-17	0.04	0.09	-0.06	-0.11	-0.28	0.22	-0.19
IP-10	-0.01	0.05	0.03	0.35	0.02	-0.29	0.29
MCP-1	0.01	0.04	0.14	0.30	-0.21	0.10	-0.12
MCP-3	0.06	-0.14	-0.01	0.03	-0.07	-0.02	0.00
MDC	0.01	0.09	0.16	0.08	-0.31	-0.04	0.34
PDGF-AA	0.01	-0.01	0.30	-0.06	0.15	0.14	-0.12
RANTES	0.00	0.03	0.16	0.04	0.07	-0.34	-0.13
TNF- $\alpha$	0.05	0.12	0.05	0.11	0.00	0.13	0.12
TNF- $\beta$	0.06	-0.12	0.00	0.05	-0.01	-0.02	0.00
VEGF	0.06	0.07	0.01	-0.08	-0.06	0.20	-0.06

Standardized scoring coefficients represent principal component values.

**Fig. 1b.** Principal Component Analysis of Log-Transformed Luminex Proteomic Analytes Standardized scoring coefficients represent principal component values.

**Table 3**

Pearson correlations between SomaLogic and Luminex principal components.

	SomaLogic PC1	SomaLogic PC2	SomaLogic PC3	SomaLogic PC4	SomaLogic PC5	SomaLogic PC6	SomaLogic PC7
Luminex PC1	0.21*	-0.01	-0.10	-0.06	-0.05	0.02	-0.13
Luminex PC2	-0.04	0.02	0.02	0.02	-0.02	0.06	0.02
Luminex PC3	<b>0.38*</b>	0.21*	-0.19	-0.17	0.12	0.19	0.01
Luminex PC4	-0.18	-0.24*	<b>0.51*</b>	0.24*	-0.14	0.17	-0.16
Luminex PC5	0.16	0.27	0.18	0.17	-0.01	0.15	-0.20
Luminex PC6	<b>0.35*</b>	-0.10	<b>0.33*</b>	<b>0.35*</b>	0.11	0.18	-0.02
Luminex PC7	-0.05	0.02	-0.15	<b>0.53*</b>	0.18	0.01	0.29*

\*Denotes  $p \leq 0.01$ ; bold indicates correlations  $\geq 0.30$ .

study site (Supplemental Fig. 1). In addition, to assess the consistency of effect size and directionality across the three study sites, a supplemental analysis modeled the PCs associated with moderate to severe MDD ( $p < 0.05$ ) in separate logistic regression models for each study site. Similar directionality and effect sizes were observed across study sites (Supplemental Table 2).

Multinomial logistic regression models evaluated the association between discriminatory PCs ( $p < 0.05$ ) and moderate to severe MDD when stratified by antidepressant medication use. There was a stronger

association between proteomic PC and moderate to severe MDD among individuals who used antidepressants than those who did not. Findings were similar when grouping MDD participants by antidepressants or anxiolytic use (Supplemental Table 3)

Finally, logistic regression analyses of the association between PCs and participants with moderate to severe MDD compared to HCs were replicated in the subset of individuals without a diagnosis of inflammatory disease; effect sizes remained positive but were reduced in size in these models when compared to those that included the entire study

**Table 4**

Adjusted logistic regressions of moderate/severe MDD status compared to controls using proteomic principal components.

	Point Estimate	95% CI
SomaLogic PC1	1.00	0.65, 1.52
SomaLogic PC2	1.04	0.72, 1.52
SomaLogic PC3*	1.65	1.05, 2.59
SomaLogic PC4*	1.54	1.01, 2.34
SomaLogic PC5	0.81	0.55, 1.19
SomaLogic PC6	0.85	0.58, 1.25
SomaLogic PC7	1.17	0.80, 1.70
	Point Estimate	95% CI
Luminex PC1	1.09	0.75, 1.58
Luminex PC2	0.81	0.57, 1.17
Luminex PC3	0.88	0.58, 1.33
Luminex PC4*	1.86	1.22, 2.83
Luminex PC5	1.08	0.75, 1.55
Luminex PC6	1.20	0.81, 1.78
Luminex PC7	1.14	0.77, 1.68

\*Denotes  $p \leq 0.05$ . Each PC was added to a separate logistic regression model (14 total models). Age and BMI were included in PCs. All models were adjusted for sex, ethnicity, education, and study site.

population.

## 5. Discussion

This study aimed to explore the proteomic profiles of moderate to severe MDD patients and HCs using two proteomic platforms, alongside an investigation of associated comorbidities. The sample size employed in this research surpassed previous studies (Lehto et al., 2010; de la Pena et al., 2020; Simon et al., 2008).

Several studies have been instrumental in advancing our understanding of circulating biomarkers in MDD by focusing on combinations of these biomarkers. Zhang et al. utilized machine learning and antibody array technology to examine 440 cytokines in 8 MDD patients and matched controls, effectively pinpointing MDD by combining circulating cytokines (Zhang et al., 2023). In a larger study, Gao et al. harnessed machine learning techniques to propose serum CC chemokines, including CCL2, CCL3, and CCL4, as potential diagnostic biomarkers for MDD (Gao et al., 2022).

These studies have significantly enriched our understanding of MDD biomarkers, highlighting the diagnostic potential of combined biomarker profiles. Our current research builds upon these foundations by incorporating a larger sample size, dual-platform proteomic analysis, and an assessment of comorbidities.

Our study found low to moderate agreement between the platforms; cross-platform correlations between interleukins were generally weak. None of the individual analytes significantly discriminated between individuals with moderate to severe MDD and HCs after adjusting for covariates. A more defined separation between the groups emerged

**Table 5**

Pearson correlations between proteomic PCs associated with moderate/severe MDD and cardiometabolic risk factors.

	SomaLogic PC3	SomaLogic PC4	Luminex PC4	HOMA-IR	FPI	BMI	FPG	CRP	HAM-D
SomaLogic PC3	1.00	0.00	0.51*	0.35*	0.33*	0.29*	0.38*	0.08	0.25*
SomaLogic PC4		1.00	0.24*	0.40*	0.40*	0.69*	0.28*	0.28*	0.08
Luminex PC4			1.00	0.38*	0.34*	0.23*	0.42*	0.01	0.32*
HOMA-IR				1.00	0.96*	0.52*	0.68*	0.23*	0.21*
FPI					1.00	0.54*	0.48*	0.26*	0.26*
BMI						1.00	0.27*	0.39*	0.10
FPG							1.00	0.10	0.08
CRP								1.00	-0.10
HDRS-21									1.00

There was no missing data for SomaLogic PC3, SomaLogic PC4, Luminex PC4, and the Hamilton Depression Rating Scale (HDRS-21) (N = 153). The N of body mass index (BMI) = 122, c-reactive protein (CRP) = 150, homeostatic model of insulin resistance (HOMA-IR) = 125, fasting plasma insulin (FPI) = 126, fasting plasma glucose (FPG) = 130.

upon applying PCA.

A pivotal discovery in this study was the identification of two distinctive protein profiles associated with moderate to severe MDD, each characterized by unique comorbidity patterns. These profiles not only correlated with the presence of moderate to severe MDD but also demonstrated associations with insulin resistance. Profile 1, characterized by high scores on either SomaLogic PC3 or Luminex PC4, displayed strong associations with cardiometabolic risk factors, including hypertension, hyperlipidemia, and type 2 diabetes, as well as inflammatory diseases. Furthermore, an increase in Luminex PC4 was linked with a noticeable reduction or worsening in depression severity scores at one year.

Conversely, Profile 2, linked to high scores on SomaLogic PC4, was primarily characterized by age, BMI, and various interleukins with a moderate negative loading for eotaxin. While this PC showed positive associations with C-reactive protein and inflammatory diseases, it did not correlate significantly with cardiometabolic risk factors. These findings suggest that Profile 2 is primarily characterized by obesity and inflammation, with limited relevance to one-year depression severity scores.

The study found five proteomic analytes that loaded  $>0.10$  or  $<-0.10$  (in standardized coefficient score) in discriminatory PCs on both platforms. Eotaxin and IP-10 are chemokines implicated in aging disorders and cardiometabolic function. Some have also been reported in studies on major depression or bipolar disorders (Strawbridge et al., 2018; Misiak et al., 2020). Eotaxin is crucial in mobilizing eosinophile responses, participating in various processes such as neurogenesis, neurodegeneration, insulin resistance, and, recently, depression (de la Pena et al., 2020; Simon et al., 2008; Ivanovska et al., 2020; Nazarinia et al., 2022; Teixeira et al., 2018). High levels of eotaxin were linked with progression from insulin resistance to type 2 diabetes and related complications (Chang et al., 2015; Baker et al., 2021; Boccardi et al., 2022; Pan et al., 2021).

IP-10 was found to possess pro-thrombotic and pro-inflammatory properties. It is positively correlated with insulin resistance and the onset of type 2 diabetes and was found elevated in a study focusing on adolescent depression (de la Pena et al., 2020; Chang et al., 2015).

Furthermore, the elevated combination of IP-10 and eotaxin (as shown in Luminex PC4) has been found in several studies of type 2 diabetes, coronary artery disease, and risk for major adverse cardiovascular events (Novo et al., 2015; Meikle, 2012). In bipolar disorder, a specific combination of IP-10 and eotaxin has been associated with bipolar depression (Teixeira et al., 2018; Klaus FS et al., 2020; Brietzke et al., 2009).

This study included three academic sites that collected and processed the samples using a common protocol. There was no site effect observed for the study findings. The current study also included MDD participants who were not taking any psychiatric medication at study enrollment. A stronger association between proteomic PC and moderate to severe MDD was observed among individuals using antidepressants compared to

**Table 6**  
Mean differences in proteomic PCs associated with moderate/severe MDD by disease comorbidity.

SomaLogic PC3	N (%) Comorbidity Present	Comorbidity Absent	Comorbidity Present	t-Value	p-Value
		Mean PC Score	Mean PC Score		
Any Inflammatory Disease	73 (48%)	-0.36	0.40	-5.10	<.0001
Hyperlipidemia	33 (22%)	-0.14	0.52	-4.16	<.0001
Hypertension	23 (15%)	-0.07	0.70	-4.12	<.0001
Type 2 Diabetes	9 (6%)	-0.06	1.02	-3.22	0.0003
Thyroid Disease	19 (13%)	-0.09	0.68	-3.19	0.002
Asthma	18 (12%)	-0.01	0.08	-0.36	0.72
Migraine	25 (17%)	0.06	0.29	-1.59	0.11
SomaLogic PC4		Mean PC Score	Mean PC Score	t-Value	p-Value
Any Inflammatory Disease	73 (48%)	-0.19	0.20	-2.45	0.02
Hyperlipidemia	33 (22%)	-0.05	0.20	-1.66	0.10
Hypertension	23 (15%)	-0.05	0.29	-1.50	0.14
Type 2 Diabetes	9 (6%)	-0.01	0.27	-0.80	0.42
Thyroid Disease	19 (13%)	-0.04	0.33	-1.52	0.13
Asthma	18 (12%)	-0.03	0.36	-1.55	0.12
Migraine	25 (17%)	-0.004	0.15	-0.70	0.49
Luminex PC4		Mean PC Score	Mean PC Score	t-Value	p-Value
Any Inflammatory Disease	73 (48%)	-0.33	0.36	-4.50	<.0001
Hyperlipidemia	33 (22%)	-0.11	-0.40	-2.60	0.01
Hypertension	23 (15%)	-0.11	0.67	-3.53	0.001
Type 2 Diabetes	9 (6%)	-0.06	1.03	-3.27	0.001
Thyroid Disease	19 (13%)	-0.08	-0.64	-2.99	0.003
Asthma	18 (12%)	-0.04	0.20	-0.96	0.34
Migraine	25 (17%)	-0.08	0.37	-2.08	0.04

The study sample comprised 153 participants; <3% of data were missing for any self-reported disease comorbidity. Inflammatory disease was defined as the presence of one or more of the following conditions: type 2 diabetes, hypertension, hyperlipidemia, coronary artery disease, myocardial infarction, kidney disease, hepatitis, thyroid disease, asthma, autoimmune disease, cancer, and polycystic ovarian syndrome.

those who did not.

Our findings were in line with the study conducted by Syed et al., where the use of antidepressant treatment seemed to have an impact on inflammatory markers that varied among different patient subgroups (Syed et al., 2018). Non-responders demonstrated further increases in pro-inflammatory markers, whereas responders demonstrated stability pre-to post-treatment. In the current study, it is unclear if we are observing the effect of depression medications on protein levels, as Syed and colleagues described, or some other clinical feature (Syed et al., 2018). However, in our sample, the medicated patients did not differ significantly from the unmedicated in the severity of depression, anxiety, or degree of refractoriness.

This study has several limitations. The sample size is small for a discovery and exploratory analysis. Secondly, though the study attempted to include participants with a wide range of medical conditions, excluding individuals with autoimmune disease, pregnancy, and postpartum status inherently limits generalizability. The potential impact of psychiatric medications on cytokine and chemokine levels remains uncertain and requires future study. Finally, the imputation of missing proteomic data carries potential bias. Addressing these limitations in future research can refine our understanding of MDD-associated proteomic profiles.

Overall, this study provides further evidence that proteomic profiles can be used to identify potential biomarkers and pathways associated with MDD and comorbidities. Our findings suggest that MDD is associated with distinct profiles of proteins that are also associated with cardiometabolic risk factors, inflammation, and obesity. In particular, the chemokines eotaxin and IP-10 may play a role in the relationship between MDD and cardiometabolic risk factors. These findings suggest that a focus on the interplay between MDD and comorbidities may be useful in identifying potential targets for intervention and improving overall health outcomes.

#### CRedit authorship contribution statement

**Kathleen T. Watson:** Writing – review & editing, Writing – original

draft, Software, Methodology, Formal analysis, Data curation. **Jennifer Keller:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Caleb M. Spiro:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Isaac B. Satz:** Writing – review & editing, Writing – original draft, Data curation. **Samantha V. Goncalves:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Heather Pankow:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Data curation. **Idit Kost:** Writing – review & editing, Validation, Data curation, Conceptualization. **Benoit Lehallier:** Writing – review & editing, Validation, Methodology, Data curation. **Adolfo Sequeira:** Writing – review & editing, Writing – original draft, Methodology. **William E. Bunney:** Writing – review & editing, Project administration, Methodology. **Natalie L. Rasgon:** Writing – review & editing, Writing – original draft. **Alan F. Schatzberg:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

#### Declaration of Competing interest

There is no conflict of interest among all the authors with either Luminex or Somalogic.BL and IK are a full-time employees of the biopharmaceutical company Alkahest, Inc.

#### Data availability

The authors do not have permission to share data.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbih.2024.100731>.

## References

- Akil, H., Gordon, J., Hen, R., Javitch, J., Mayberg, H., McEwen, B., Meaney, M.J., Nestler, E.J., 2018. Treatment resistant depression: a multi-scale, systems biology approach. *Neurosci. Biobehav. Rev.* 84, 272–288.
- American Psychiatric Association, 2013. *Diagnostic and Statistical Manual of Mental Disorders*, fifth ed.
- Baker, J.F., England, B.R., George, M., Cannon, G., Sauer, B., Ogdie, A., Hamilton, B.C., Hunter, C., Duryee, M.J., Thiele, G., Mikuls, T.R., 2021. Disease activity, cytokines, chemokines and the risk of incident diabetes in rheumatoid arthritis. *Ann. Rheum. Dis.* 80, 566–572.
- Boccardi, V., Mancinetti, F., Baroni, M., Cecchetti, R., Bastiani, P., Ruggiero, C., Mecocci, P., 2022. Metabolic score for insulin resistance (METS-IR) and circulating cytokines in older Persons: the role of Gender and body mass index. *Nutrients* 14.
- Brietzke, E., Kauer-Sant'Anna, M., Teixeira, A.L., Kapczinski, F., 2009. Abnormalities in serum chemokine levels in euthymic patients with bipolar disorder. *Brain Behav. Immun.* 23, 1079–1082.
- Chang, C.C., Wu, C.L., Su, W.W., Shih, K.L., Tarn, D.C., Chou, C.T., Chen, T.Y., Kor, C.T., Wu, H.M., 2015. Interferon gamma-induced protein 10 is associated with insulin resistance and incident diabetes in patients with nonalcoholic fatty liver disease. *Sci. Rep.* 5, 10096.
- de la Pena, F.R., Cruz-Fuentes, C., Palacios, L., Giron-Perez, M.I., Medina-Rivero, E., Villanueva, M.D., Alvarez-Herrera, S., Perez-Sanchez, G., Becerril-Ponce, E., Maldonado-García, J.L., Jimenez-Martinez, M.C., Pavon, L., 2020. Serum levels of chemokines in adolescents with major depression treated with fluoxetine. *World J Psychiatry* 10, 175–186.
- First, M.B., Williams, J.B.W., Karg, R.S., Spitzer, R.L., 2015. *Structured Clinical Interview for DSM-5—Research Version (SCID-5 for DSM-5, Research Version; SCID-5-RV)*. American Psychiatric Association, Arlington, VA.
- Gao, W., Xu, Y., Liang, J., et al., 2022. Serum CC chemokines as potential biomarkers for the diagnosis of major depressive disorder. *Psychol. Res. Behav. Manag.* 15, 2971–2978. <https://doi.org/10.2147/PRBM.S384267>.
- Gold, L., Ayers, D., Bertino, J., Bock, C., Bock, A., Brody, E.N., Carter, J., Dalby, A.B., Eaton, B.E., Fitzwater, T., Flather, D., Forbes, A., Foreman, T., Fowler, C., Gawande, B., Goss, M., Gunn, M., Gupta, S., Halladay, D., Heil, J., Heilig, J., Hicke, B., Husar, G., Janjic, N., Jarvis, T., Jennings, S., Katilius, E., Keeney, T.R., Kim, N., Koch, T.H., Kraemer, S., Kroiss, L., Le, N., Levine, D., Lindsey, W., Lollo, B., Mayfield, W., Mehan, M., Mehler, N., Nelson, S.K., Nelson, M., Nieuwandt, D., Nikrad, M., Ochsner, U., Ostroff, R.M., Otis, M., Parker, T., Pietrasiewicz, S., Resnicow, D.I., Rohloff, J., Sanders, G., Sattin, S., Schneider, D., Singer, B., Stanton, M., Sterkel, A., Stewart, A., Stratford, S., Vaught, J.D., Vrkljan, M., Walker, J.J., Watrobka, M., Waugh, S., Weiss, A., Wilcox, S.K., Wolfson, A., Wolk, S. K., Zhang, C., Zichi, D., 2010. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One* 5, e15004.
- Hamilton, M., 1960. A rating scale for depression. *J. Neurol. Neurosurg. Psychiatry* 23, 56–62.
- Ivanovski, M., Abdi, Z., Murdjeva, M., Macedo, D., Maes, A., Maes, M., 2020. CCL11 or eotaxin-1: an immune marker for ageing and Accelerated ageing in Neuro-psychiatric disorders. *Pharmaceuticals* 13.
- Katz, D.H., Robbins, J.M., Deng, S., Tahir, U.A., Bick, A.G., Pampana, A., Yu, Z., Ngo, D., Benson, M.D., Chen, Z.Z., Cruz, D.E., Shen, D., Gao, Y., Bouchard, C., Sarzynski, M. A., Correa, A., Natarajan, P., Wilson, J.G., Gerszten, R.E., 2022. Proteomic profiling platforms head to head: Leveraging genetics and clinical traits to compare aptamer- and antibody-based methods. *Sci. Adv.* 8, eabm5164.
- Keller, M.B., Lavori, P.W., Friedman, B., Nielsen, E., Endicott, J., McDonald-Scott, P., Andreasen, N.C., 1987. The Longitudinal Interval Follow-up Evaluation. A comprehensive method for assessing outcome in prospective longitudinal studies. *Arch Gen Psychiatry* 44 (6), 540–548. <https://doi.org/10.1001/archpsyc.1987.01800180050009>. PMID: 3579500 Jun.
- Klaus FS, A., Soontornniyomkij, B., Benitez, R., Elyer, L., 2020. Inter-relationship of inflammatory biomarker levels in bipolar disorder. *Biol Psychiatry* 87, S189.
- Lasseter, H.C., Provost, A.C., Chaby, L.E., Daskalakis, N.P., Haas, M., Jeromin, A., 2020. Cross-platform comparison of highly sensitive immunoassay technologies for cytokine markers: platform performance in post-traumatic stress disorder and Parkinson's disease. *Cytokine X* 2, 100027.
- Lehallier, B., Gate, D., Schaum, N., Nanasi, T., Lee, S.E., Yousef, H., Moran Losada, P., Berdnik, D., Keller, A., Verghese, J., Sathyan, S., Franceschi, C., Milman, S., Barzilai, N., Wyss-Coray, T., 2019. Undulating changes in human plasma proteome profiles across the lifespan. *Nat Med* 25, 1843–1850.
- Lehto, S.M., Niskanen, L., Herzig, K.H., Tolmunen, T., Huotari, A., Viinamäki, H., Koivumaa-Honkanen, H., Honkalampi, K., Ruotsalainen, H., Hintikka, J., 2010. Serum chemokine levels in major depressive disorder. *Psy Neuro En* 35, 226–232.
- Maecker, H.T., Rosenberg-Hasson, Y., Kolstad, K.D., Steen, V.D., Chung, L.S., 2020. A novel utility to correct for plateplate/batch/lot and nonspecific binding artifacts in Luminex data. *J. Immunol.* 204, 3425–3433.
- Meikle, F., 2012. Identifying inflammatory markers in Vascular disease. In: *Vascular Research*. University of Otago.
- Milaneschi, Y., Kappelmann, N., Ye, Z., Lamers, F., Moser, S., Jones, P.B., Burgess, S., Penninx, B.W.J.H., Khandaker, G.M., 2021. Association of inflammation with depression and anxiety: evidence for symptom-specificity and potential causality from UK Biobank and NESDA cohorts. *Mol. Psychiatr.* 26, 7393–7402.
- Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 65, 732–741.
- Misiak, B., Bartoli, F., Carra, G., Malecka, M., Samochowiec, J., Jarosz, K., Banik, A., Stanczykiewicz, B., 2020. Chemokine alterations in bipolar disorder: a systematic review and meta-analysis. *Brain Behav. Immun.* 88, 870–877.
- Murray, C.J., Lopez, A.D., 1997. Alternative projections of mortality and disability by cause 1990–2020: global burden of disease study. *Lancet* 349, 1498–1504.
- Nazarinia, D., Behzadifard, M., Gholampour, J., Karimi, R., Gholampour, M., 2022. Eotaxin-1 (CCL11) in neuroinflammatory disorders and possible role in COVID-19 neurologic complications. *Acta Neurol. Belg.* 122, 865–869.
- Novo, G., Bellia, C., Fiore, M., Bonomo, V., Pugliesi, M., Giovino, M., Sasso, B.L., Meraviglia, S., Assennato, P., Novo, S., Dieli, F., Ciaccio, M., 2015. A risk score derived from the analysis of a cluster of 27 serum inflammatory cytokines to predict Long term outcome in patients with acute myocardial infarction: a Pilot study. *Ann. Clin. Lab. Sci.* 45, 382–390.
- Overall, J.E., Gorham, D.R., 1961. The Brief psychiatric rating scale. *Psychol. Rep.* 10, 799–812.
- Pan, X., Kaminga, A.C., Wen, S.W., Liu, A., 2021. Chemokines in Prediabetes and type 2 diabetes: a meta-analysis. *Front. Immunol.* 12, 622438.
- Pietzner, M., Wheeler, E., Carrasco-Zanini, J., Kerrison, N.D., Oerton, E., Koprulu, M., Luan, J., Hingorani, A.D., Williams, S.A., Wareham, N.J., Langenberg, C., 2021. Synergistic insights into human health from aptamer- and antibody-based proteomic profiling. *Nat. Commun.* 12, 6822.
- Rush, A.J., Trivedi, M.H., Wisniewski, S.R., Nierenberg, A.A., Stewart, J.W., Warden, D., Niederehe, G., Thase, M.E., Lavori, P.W., Lebowitz, B.D., McGrath, P.J., Rosenbaum, J.F., Sackeim, H.A., Kupfer, D.J., Luther, J., Fava, M., 2006. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR\*D report. *Am J Psychiatry* 163, 1905–1917.
- Saveanu, R., Etkin, A., Duchemin, A.M., Goldstein-Piekarski, A., Gyurak, A., Debattista, C., Schatzberg, A.F., Sood, S., Day, C.V., Palmer, D.M., Rekshan, W.R., Gordon, E., Rush, A.J., Williams, L.M., 2015. The international Study to Predict Optimized Treatment in Depression (iSPOT-D): outcomes from the acute phase of antidepressant treatment. *J. Psychiatr. Res.* 61, 1–12.
- Simon, N.M., McNamara, K., Chow, C.W., Maser, R.S., Papakostas, G.I., Pollack, M.H., Nierenberg, A.A., Fava, M., Wong, K.K., 2008. A detailed examination of cytokine abnormalities in Major Depressive Disorder. *Eur. Neuropsychopharmacol* 18, 230–233.
- Sokolowska, I., Ngounou Wetie, A.G., Wormwood, K., Thome, J., Darie, C.C., Woods, A. G., 2015. The potential of biomarkers in psychiatry: focus on proteomics. *J. Neural. Transm.* 122 (Suppl. 1), S9–S18.
- Steel, N., Ford, J.A., Newton, J.N., Davis, A.C.J., Vos, T., Naghavi, M., Glenn, S., Hughes, A., Dalton, A.M., Stockton, D., Humphreys, C., Dallat, M., Schmidt, J., Flowers, J., Fox, S., Abubakar, I., Aldridge, R.W., Baker, A., Brayne, C., Brughna, T., Capewell, S., Car, J., Cooper, C., Ezzati, M., Fitzpatrick, J., Greaves, F., Hay, R., Hay, S., Kee, F., Larson, H.J., Lyons, R.A., Majeed, A., McKee, M., Rawaf, S., Rutter, H., Saxena, S., Sheikh, A., Smeeth, L., Viner, R.M., Vollset, S.E., Williams, H. C., Wolfe, C., Woolf, A., Murray, C.J.L., 2018. Changes in health in the countries of the UK and 150 English local Authority areas 1990-2016: a systematic analysis for the global burden of disease study 2016. *Lancet* 392, 1647–1661.
- Strawbridge, R., Young, A.H., Cleare, A.J., 2017. Biomarkers for depression: recent insights, current challenges and future prospects. *Neuropsychiatr Dis Treat* 13, 1245–1262.
- Strawbridge, R., Young, A.H., Cleare, A.J., 2018. Biomarkers for depression: recent insights, current challenges and future prospects. *Focus* 16, 194–209.
- Subramaniapillai, M., Chen, V.C., McIntyre, R.S., Yang, Y.H., Chen, Y.L., 2021. Added burden of major depressive disorder on cardiovascular morbidity and mortality among patients with cardiovascular disease and the modifying effects of antidepressants: a national retrospective cohort study. *J. Affect. Disord.* 294, 580–585.
- Syed, S.A., Beurel, E., Loewenstein, D.A., Lowell, J.A., Craighead, W.E., Dunlop, B.W., Mayberg, H.S., Dhabhar, F., Dietrich, W.D., Keane, R.W., de Rivero Vaccari, J.P., Nemeroff, C.B., 2018. Defective inflammatory pathways in Never-treated depressed patients are associated with poor treatment response. *Neuron* 99, 914–924 e913.
- Teixeira, A.L., Gama, C.S., Rocha, N.P., Teixeira, M.M., 2018. Revisiting the role of eotaxin-1/CCL11 in psychiatric disorders. *Front Psychiatry* 9, 241.
- Thase, M.E., Hersen, M., Bellack, A.S., Himmelhoch, J.M., Kupfer, D.J., 1983. Validation of a Hamilton subscale for endogenomorphic depression. *J. Affect. Disord.* 5, 267–278.
- Vogelzangs, N., de Jonge, P., Smit, J.H., Bahn, S., Penninx, B.W., 2016. Cytokine production capacity in depression and anxiety. *Transl Psychiat* 6.
- Zhang, E., Huang, Z., Zang, Z., Qiao, X., Yan, J., Shao, X., 2023. Identifying circulating biomarkers for major depressive disorder. *Front Psychiatry* 14, 1230246. <https://doi.org/10.3389/fpsy.2023.1230246>.