BMJ Open Diabetes Research & Care

Three-protein signature is associated with baseline and persistently elevated or recurrent depressive symptoms in individuals with recent-onset diabetes

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To cite: Spagnuolo MC, Gottmann P, Sommer J, *et al*. Three-protein signature is associated with baseline and persistently elevated or recurrent depressive symptoms in individuals with recent-onset diabetes. *BMJ Open Diab Res Care* 2025;**13**:e004396. doi:10.1136/bmjdrc-2024-004396

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjdrc-2024-004396).

Received 11 June 2024
Accepted 5 January 2025

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ABSTRACT

Depression is associated with diabetes, but the underlying causes remain unclear. To better understand depression in diabetes, this study investigated associations between 135 inflammatory and neurological protein biomarkers and depressive symptoms in individuals with diabetes.

This cross-sectional study included 430 adults with a known diabetes duration <1 year from the German Diabetes Study (GDS), in whom biomarkers were measured in serum and depressive symptoms were evaluated at baseline and annually over 5 years using the Center for Epidemiological Studies Depression Scale (CES-D). Based on the information on depressive symptoms from the baseline and follow-up visits (n=305, ≥3 time points), we subdivided the sample into individuals with persistent or recurrent and transient or never depressive symptoms. We assessed the associations of each biomarker with baseline CES-D score (continuous) and persistent/recurrent depressive symptoms using multiple linear and logistic regression models, respectively.

After adjustment for covariates, we identified a three-protein signature associated with baseline CES-D score and persistent/recurrent depressive symptoms. CUB domain-containing protein 1 (CDCP1) and NAD-dependent protein deacetylase sirtuin-2 (SIRT2) were positively associated with baseline (β 1.24 (95% CI 0.19 to 2.29); β 0.89 (95% CI 0.06 to 1.72)), respectively) and persistent/recurrent depressive symptoms (OR 1.58 (95% CI 1.08 to 2.31); OR 1.32 (95% CI 1.03 to 1.71), respectively), whereas leptin receptor (LEPR) was inversely associated with baseline (β −0.99 (95% CI −1.87 to −0.11)) and persistent/recurrent depressive symptoms (OR 0.70 (95% CI 0.49 to 0.99)). However, results were not significant after adjustment for multiple testing.

In conclusion, the three-protein signature identified may provide insights into mechanisms underlying depressive symptoms in diabetes and might open new therapeutic avenues.

The trial registration number of the study is NCT01055093.

INTRODUCTION

Depression is common among individuals with diabetes,[1] with a twofold to threefold higher prevalence than in the general population.[1 2] Individuals with diabetes are at a higher risk of experiencing depressive symptoms shortly after disease diagnosis when initially faced with the self-management of their disease, recognizing complications or starting therapy.[3–5] Comorbid depression in diabetes is a burden because it has been associated with reduced treatment adherence and increased risk of diabetes-related complications.[6 7]

There are various treatment options available for depression, among which selective serotonin reuptake inhibitors are the most widely used antidepressants in the general population and in individuals with diabetes.[8 9] However, the low efficacy of conventional antidepressant treatments suggests that the pathogenesis of depression in diabetes involves more complex mechanisms beyond the serotonin reuptake mechanism. A growing body of evidence advocates chronic subclinical inflammation and slow neurodegenerative alterations, two distinguished features of diabetes, as potential pathogenic mechanisms of depression in diabetes.[10–12]

Subclinical inflammation in diabetes is associated with metabolic disturbances such as obesity and insulin resistance.[10] In diabetes, higher levels of circulating pro-inflammatory proteins can reach the brain, leading to neuroinflammation predisposing individuals to depression.[10 13 14] Several epidemiological studies measuring biomarkers of inflammation with single immunoassay methods showed a positive association between higher levels of inflammatory biomarkers and depression in individuals with recent-onset[15 16] and long-standing diabetes.[17–19] Interleukin-6 (IL-6), IL-1 receptor antagonist (IL-1RA) and C reactive protein (CRP) were the most promising

biomarkers identified.[20–22] Additionally, a meta-analysis showed that higher baseline levels of several inflammatory biomarkers and persistently elevated tumor necrosis factor-alpha levels were associated with resistance to conventional antidepressant treatment.[23] However, this meta-analysis did not specify the glycemic status of the participants. Thus, it remains unknown whether higher baseline subclinical inflammation is associated with persistent or recurrent depression in individuals with diabetes.

Slow progressive brain neurodegenerative alterations are increasingly recognised as a complication of diabetes,[24] with growing evidence associating diabetes with an increased risk for neurodegenerative diseases such as dementia.[25 26] Indeed, it has been hypothesized that brain alterations lead to the development of depression. However, only a few studies have examined the associations between neurology-specific biomarkers and depression, with most of them focusing on neurofilament light chain (NfL) as a biomarker of nerve damage and brain-derived neurotrophic factor (BDNF) as a biomarker of neuronal plasticity.[27 28]

Given the limited evidence for the association of inflammatory and neurological protein biomarkers with depression and its persistence or recurrence in individuals with diabetes, we aimed to investigate the associations of a large panel of protein biomarkers with prevalent and persistent/recurrent depressive symptoms in individuals with recent-onset diabetes.

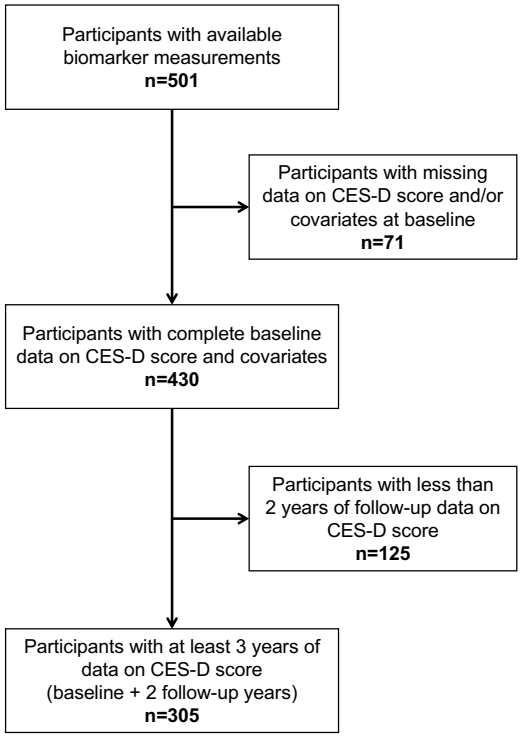

Figure 1 Flow chart of the study population. CES-D, Center for Epidemiological Studies Depression Scale.

MATERIALS AND METHODS

Study design and population

The data originated from the German Diabetes Study (GDS), an ongoing prospective observational study that evaluates the natural course of individuals recently diagnosed with diabetes and explores prognostic factors and mechanisms leading to the development of diabetes-related complications.[29] The GDS cohort includes individuals between 18 and 69 years of age at baseline examination with a known diabetes duration of <1 year. Diabetes is diagnosed according to the American Diabetes Association guidelines.[30] Individuals with other forms of diabetes, current pregnancy and acute or severe chronic cardiac, hepatic, renal or psychiatric disorders compromising the ability to comply with study procedures were excluded. At baseline, all participants undergo a comprehensive examination consisting of clinical tests, a face-to-face interview, standardized written questionnaires and detailed laboratory measurements. Participants are followed up at 5-year intervals through in-person examinations and annually with telephone interviews.

We used data from the consecutive sampling of 501 participants who entered the GDS between September 2005 and December 2011, from whom the measurement of protein biomarkers was available. In this cross-sectional analysis, we excluded participants with missing data on the depression score (n=62) and/or covariates for adjustment (n=24), leaving 430 participants for the complete case analysis. From this sample, we included participants with CES-D score follow-up data in 2 or more years for the analysis of persistent/recurrent depressive symptoms, leaving 305 participants (67, 70, 92 and 76 people with a total of 3, 4, 5 and 6 data points, respectively). This ensured all participants had at least 3 years of data, including baseline and two additional years. The flow chart of the study population is shown in figure 1.

Further details on the GDS design have been previously published.[29] The GDS was registered with ClinicalTrials.gov (registration no. NCT01055093).

Measurement of protein biomarkers

Blood samples were collected from overnight fasted participants and serum was analyzed with proximity assay extension (PEA) technology (OLINK, Uppsala, Sweden) using the OLINK Target Inflammation and Neuro Exploratory panels, each comprising 92 protein biomarkers. The inflammation panel includes pro-inflammatory and anti-inflammatory cytokines, chemokines, enzymes, receptors and growth factors covering biological processes of inflammation, angiogenesis, fibrosis and endothelial activation. The Neuro Exploratory panel includes biomarkers involved in neurological processes such as axon development, neurogenesis and synapse assembly. PEA technology allows the relative quantification of analyte concentrations, and results

are reported as normalized protein expression values (comparable to the log2-scaled values).

Of the 184 protein biomarkers included in the two panels, we excluded 49 due to values below the detection limit in ≥25% of all samples, inter-assay/intra-assay coefficients of variation >25% or technical problems with the assay, leaving 135 protein biomarkers for the analysis. The online supplemental table S1 gives a detailed description of all protein biomarkers.

Assessment of depressive symptoms

We evaluated depressive symptoms at baseline and 5-year follow-up through in-person interviews and telephone interviews after 1, 2, 3 and 4 years of follow-up (a total of six time points). Depressive symptoms were defined using the Center for Epidemiological Studies Depression Scale (CES-D) (the German translated version is the Allgemeine Depressionsskala, Langversion).[31] The CES-D score evaluates depressive symptoms in the previous week and consists of 20 questions on a 4-point scale with responses between 0 and 3 points.[32] The final score ranges from 0 to 60, with a higher score indicating elevated depressive symptoms. The CES-D score represents one of the most commonly used instruments in the clinical setting to screen for depressive symptoms in individuals with diabetes.[33] At baseline, the CES-D score was used as a continuous score to increase power. For the analysis of persistent or recurrent depressive symptoms, the CES-D score was used as a binary outcome with a cut-off of ≥16 indicating the presence of elevated depressive symptoms.[19 34 35] Studies have used different cut-off values for the CES-D score to define depressive disorder,[31] and we selected the relatively low cut-off of ≥16 because of our focus on elevated depressive symptoms rather than more severe forms of depression. We defined depressive symptoms as persistent (CES-D score ≥16 in two consecutive time points), recurrent (CES-D score ≥16 in two non-consecutive time points), transient episodes (CES-D score ≥16 in only one time point) or never (CES-D score <16 in all time points). Time points included baseline plus the 5 years of follow-up data.

Covariates

We collected demographic, anthropometric, metabolic and clinical data from each participant at baseline. We extracted participants' age and sex from the questionnaires and calculated body mass index (BMI) using baseline height (in cm) and weight (in kg) measurements. Total cholesterol, triglycerides and hemoglobin A1c (HbA1c) were measured at baseline using standardized laboratory procedures.[29] Use of non-steroidal anti-inflammatory drugs (NSAIDs), lipid-lowering drugs, antidepressive drugs and glucose-lowering drugs were all self-reported. We defined cardiovascular disease (CVD) as self-reported peripheral arterial disease, myocardial infarction, stroke and/or transient ischemic attack.

Statistical analysis

Descriptive statistics were presented using counts and percentages for categorical variables and means and SD for continuous variables. Baseline differences between individuals with persistent/recurrent depressive symptoms and transient/never depressive symptoms were tested using independent samples t-test or Wilcoxon test (depending on the variable distribution) or Pearson's χ^2 test. Triglyceride levels were log-transformed. Protein biomarker levels varied widely in their distributions (see online supplemental table S2). Therefore, all protein biomarkers were standardized (mean of zero and SD of 1) and analyzed per 1–SD increase to allow for the direct comparison of effect estimates between biomarkers, taking these differences in distribution into account.

First, we assessed the associations between each protein biomarker and baseline depressive symptoms (continuous CES-D score) with multivariable linear regression models. We built two models with increased complexity. Model 1 was adjusted for age and sex, and model 2 was additionally adjusted for BMI, HbA1c, triglycerides, total cholesterol, CVD, use of lipid-lowering drugs, antidepressive drugs, glucose-lowering drugs and NSAIDs. Results were reported as β estimates and 95% CIs.

Next, we assessed the associations between protein biomarkers and persistent/recurrent depressive symptoms, we combined 'persistent depressive symptoms' and 'recurrent depressive symptoms' in one group and 'transient depressive symptoms' together with 'never depressed' in another group to ensure higher statistical efficiency, similar to other studies.[36] We performed binary logistic regression (transient/never depressive symptoms as a reference) and reported the results as ORs and 95% CIs. In secondary analyses for both baseline CES-D score and persistent/recurrent depressive symptoms, we assessed for potential effect modification by diabetes type (type 1/type 2). All of the analyses examining the associations of biomarkers with depressive symptoms are exploratory and hypothesis-generating; therefore, no adjustment for multiple testing was performed as recommended.[37]

Statistical analyses were conducted using SAS V.9.4 (SAS Institute, Cary, North Carolina, USA) and data visualizations were created using R Foundation for Statistical Computing, R V.4.3.1 (Vienna, Austria). P values <0.05 were considered to indicate statistical significance. Adjustment for multiple testing was done using the Bonferroni correction (135 biomarkers, two outcomes with interaction analysis, ie, 540 tests) resulting in an adjusted p value of 0.00009.

RESULTS

Study population

The baseline characteristics of the study population (n=430) are presented in table 1. The mean age was

Table 1 Baseline characteristics of the total, persistent/recurrent and transient/never depressive symptoms study samples

Characteristic	Total (n=430)	Persistent/Recurrent depressive symptoms (n=69)	Transient/Never depressive symptoms (n=236)	P value
Age (years)	46.4 (14.2)	47.6 (12.0)	47.0 (14.8)	0.75
Males (n, %)	275 (64.0)	35 (50.7)	157 (66.5)	0.02
BMI (kg/m^2)	29.1 (6.5)	30.1 (6.3)	28.3 (6.4)	0.04
HbA1c (mmol/mol)	48.2 (12.3)	46.7 (10.2)	47.2 (11.6)	0.77
HbA1c (%)	6.6 (1.1)	6.4 (0.9)	6.5 (1.1)	0.77
Diabetes type (n, %)				0.11
Type 1	152 (35.4)	21 (30.4)	97 (41.1)	
Type 2	278 (64.6)	48 (69.6)	139 (58.9)	
Diabetes medication (n, %)				0.16
Metformin	118 (27.5)	19 (27.5)	67 (28.4)	
Insulin	151 (35.1)	19 (27.5)	95 (40.2)	
None	126 (29.3)	24 (34.8)	58 (24.6)	
Other medications	35 (8.1)	7 (10.2)	16 (6.8)	
Total cholesterol (mmol/L)	5.09 (1.10)	5.18 (1.09)	5.07 (1.02)	0.44
Triglycerides (mmol/L)	1.55 (1.24)	1.78 (1.30)	1.48 (1.13)	0.07
Cardiovascular disease* (n, %)	22 (5.1)	4 (5.8)	15 (6.4)	0.87
Hypertension (n, %)	235 (55.7)	33 (50.8)	137 (58.3)	0.28
Lipid-lowering drugs (n, %)	58 (13.5)	9 (13.0)	34 (14.4)	0.77
NSAIDs (n, %)	57 (13.4)	12 (17.4)	31 (13.1)	0.37
Antidepressive drugs (n, %)	10 (2.3)	4 (5.8)	5 (2.1)	0.11
CES-D score	10.6 (8.4)	--	--	--

Data are mean (SD) for continuous variables and count (percentages) for categorical variables.
Missing values were as follows: hypertension in the total sample=eight cases and hypertension in the persistent/recurrent sample=five cases.
*Cardiovascular disease includes peripheral arterial disease, myocardial infarction, stroke and/or transient ischemic attack.
BMI, body mass index; CES-D, Center for Epidemiological Studies Depression Scale; HbA1c, hemoglobin A1c; NSAIDs, non-steroidal anti-inflammatory drugs.

46 years, and 64% were men. Most participants had type 2 diabetes (64.6%), and the mean HbA1c was 6.6% (48.2 mmol/mol). At baseline, around 21% of individuals experienced depressive symptoms, and the mean (SD) CES-D score was 10.6 (8.4). Online supplemental table S2 presents the baseline characteristics stratified by diabetes type.

Individuals with persistent/recurrent depressive symptoms (n=69, 23%) were more likely to be women and have a higher BMI than individuals with transient depressive symptoms or no depressive symptoms (n=236, 77%). However, both groups did not differ in age, glycemic status or diabetes treatment.

Associations of protein biomarkers with baseline depressive symptoms

The overall associations between each protein biomarker and depressive symptoms are shown in online supplemental table S3, and biomarkers significantly associated with baseline depressive symptoms in model 1 or 2 are plotted in figure 2. In the age-adjusted and sex-adjusted model, 13 protein biomarkers were associated with depressive symptoms. After full adjustment for covariates, 10 protein biomarkers were significantly associated with depressive symptoms. Specifically, 4 biomarkers, namely CUB domain-containing protein 1 (CDCP1) (β: 1.24 (95% CI 0.19 to 2.29), p=0.0211), eukaryotic translation initiation factor 4B (β: 0.89 (95% CI 0.07 to 1.70), p=0.0325), matrix metalloproteinase 1 (MMP1) (β: 1.31 (95% CI 0.50 to 2.12), p=0.0015) and sirtuin 2 (SIRT2) (β: 0.89 (95% CI 0.06 to 1.72), p=0.0347) were positively associated with baseline depressive symptoms. In contrast, adhesion G protein-coupled receptor B3 (β: −0.93 (95% CI −1.74 to −0.12), p=0.0253), cadherin-15 (β: −1.45 (95% CI −2.44 to −0.47), p=0.0040), desmoglein-3 (β: −0.89 (95% CI −1.75 to −0.02), p=0.0440), Fms-related tyrosine kinase 3 ligand (β: −0.99 (95% CI −1.91 to −0.06), p=0.0360), leptin receptor (LEPR) (β: −0.99 (95% CI −1.87 to −0.11), p=0.0272) and stem cell factor (SCF) (β: −0.91 (95% CI −1.80 to −0.01), p=0.0477) were inversely associated with baseline depressive symptoms.

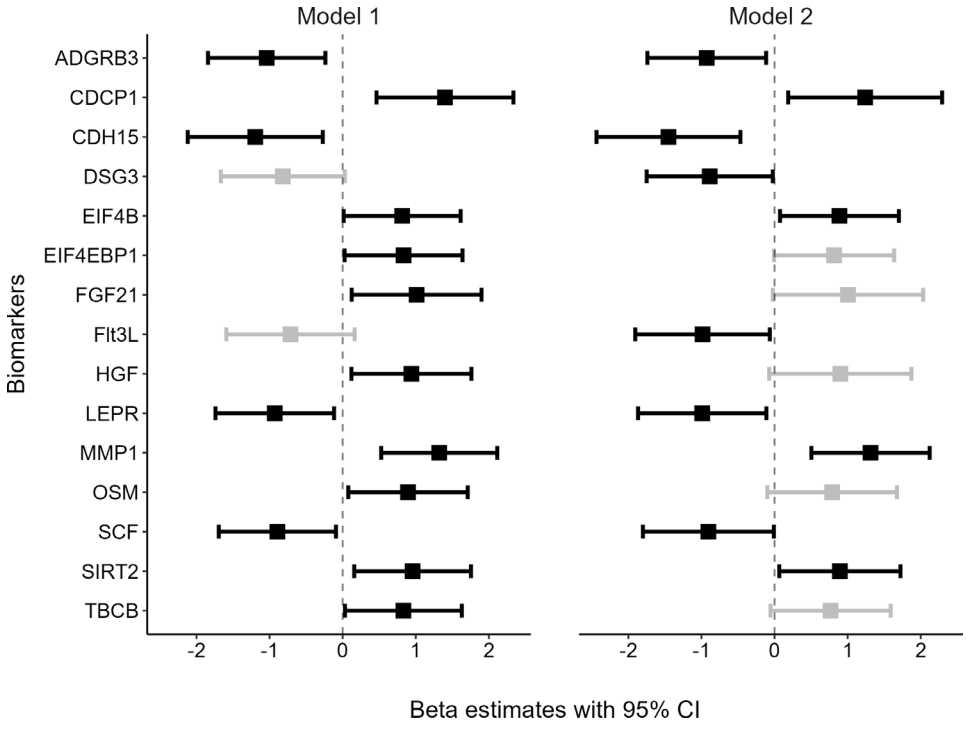

Figure 2 Associations of protein biomarkers with baseline depressive symptoms. Model 1 is adjusted for age and sex. Model 2 is additionally adjusted for BMI, cholesterol, triglycerides, CVD, HbA1c, NSAID use, lipid-lowering medication use, antidepressant medication use and glucose-lowering medication use. Biomarkers with significant associations in model 1 and model 2 are shown in black and biomarkers with non-significant from model 1 or model 2 are shown in gray. Biomarkers are ordered alphabetically. ADGRB3, adhesion G protein-coupled receptor B3; CDCP1, CUB domain-containing protein 1; CDH-15, caderin-15; DSG3, desmoglein-3; 4EBP1, eukaryotic initiation factor 4E-binding protein 1; EIF4B, eukaryotic translation initiation factor 4B; FGF21, fibroblast growth factor 21; Flt3L, Fms-related tyrosine kinase 3 ligand; HGF, human growth factor; LEPR, leptin receptor; MMP-1, matrix metalloproteinases 1; OSM, oncostatin-M; SCF, stem cell factor; SIRT-2, NAD-dependent protein deacetylase sirtuin-2; TBCB, tubulin-folding cofactor B.

The full results of this association by diabetes type are reported in online supplemental table S4. In diabetes type-specific stratification, there was significant interaction for 14 biomarkers. There was weak evidence of effect modification by diabetes type for LEPR, although the individual effect estimates for type 1 and type 2 diabetes were not significant. Additionally, there was evidence that the association between SIRT2 and depressive symptoms is more pronounced in individuals with type 2 diabetes (β: 1.33 (95% CI 0.15 to 2.51), p-interaction=0.0127). However, results from both the main analysis and the interaction tests were no longer statistically significant after adjustment for multiple testing.

Associations of protein biomarkers with persistent or recurrent depressive symptoms over 5-year follow-up

The overall associations of each protein biomarker with persistent/recurrent depressive symptoms are shown in online supplemental table S5, and biomarkers significantly associated with the outcome in model 1 or 2 are plotted in figure 3. There were 19 protein biomarkers associated with persistent/recurrent depressive symptoms in the age-adjusted and sex-adjusted model. In the fully adjusted model, 13 protein biomarkers were significantly associated with persistent/recurrent depressive symptoms, among which only higher levels of LEPR were associated with lower odds of persistent/recurrent depressive symptoms (OR 0.70 (95% CI 0.49 to 0.99), p=0.0444). In contrast, the remaining 12 biomarkers annexin A10 (ANXA10), AXIN1, eotaxin (CCL11), C-C motif chemokine 19 (CCL19), C-C motif chemokine 20 (CCL20), CDCP1, fibroblast growth factor 21 (FGF21), peptidyl-prolyl cis-trans isomerase FKBP5 (FKBP5), phosphomevalonate kinase, tyrosine-protein phosphatase non-receptor type 1, SIRT2 and tumor necrosis factor ligand superfamily member 14) were all associated with higher odds of persistent/recurrent depressive symptoms with ORs ranging from 1.32 to 1.73 (ie, SIRT2: OR 1.32 (95% CI 1.03 to 1.71), p=0.0316; CCL19: OR 1.73 (95% CI 1.28 to 2.35), p=0.0004).

The full results of this association by diabetes type is reported in online supplemental table S6. In diabetes type-specific stratification, there was significant interaction for nine biomarkers. Notably the p-interaction was significant for CDCP1, but only the effect estimate for type 1 diabetes was significant. Results both in the main analysis and in the interaction tests were not statistically significant anymore after adjustment for multiple testing.

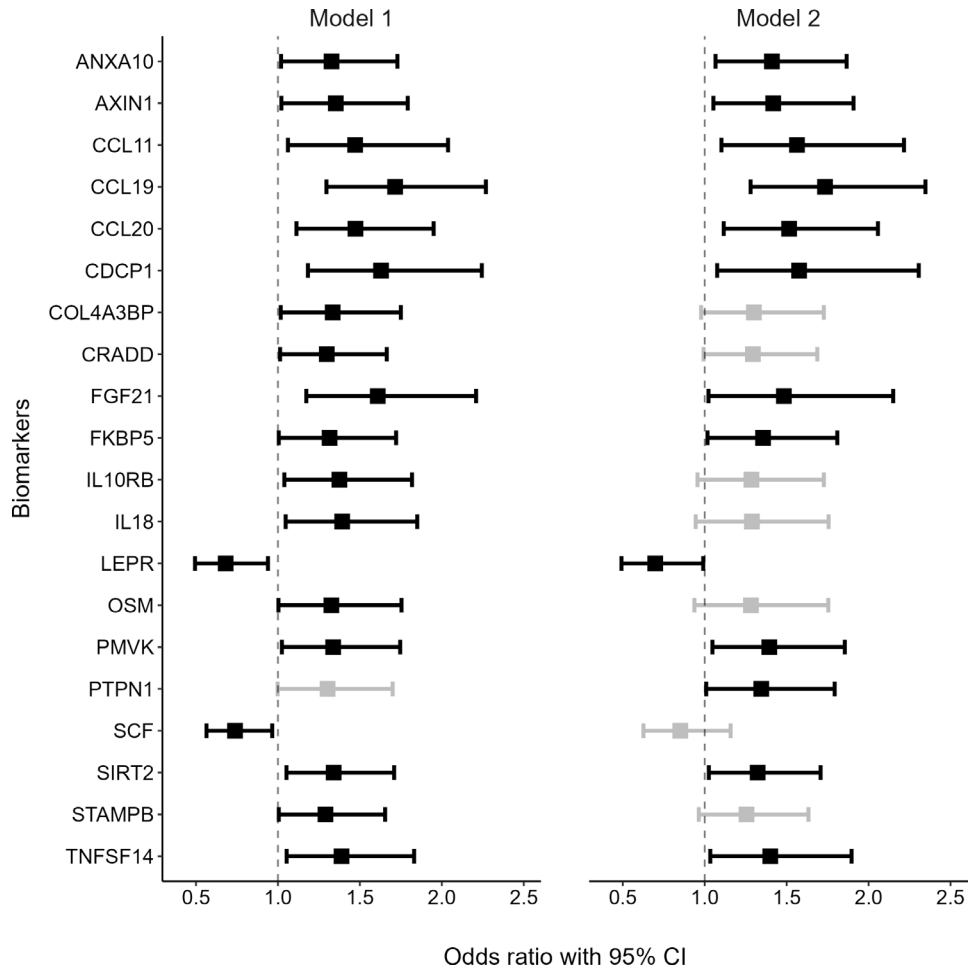

Figure 3 Associations of protein biomarkers with persistent/recurrent depressive symptoms. Model 1 is adjusted for age and sex. Model 2 is additionally adjusted for BMI, cholesterol, triglycerides, CVD, HbA1c, NSAID use, lipid-lowering medication use, antidepressant medication use and glucose-lowering medication use. Biomarkers with significant associations in model 1 and model 2 are shown in black and biomarkers with non-significant from model 1 or model 2 are shown in gray. Biomarkers are ordered alphabetically. ANXA10, annexin A10; AXIN1, axin1; CCL11, eotaxin; CCL19, C-C motif chemokine 19; CCL20, C-C motif chemokine 20; CDCP1, CUB domain-containing protein 1; COL4A3BP, ceramide transfer protein; CRADD, death domain-containing protein CRADD; FGF21, fibroblast growth factor 21; FKBP5, peptidyl-prolyl cis-trans isomerase FKBP5; IL10RB, interleukin-10 receptor subunit beta; IL18, interleukin-18; LEPR, leptin receptor; OSM, oncostatin-M; PMVK, phosphomevalonate kinase; PTPN1, tyrosine-protein phosphatase non-receptor type 1; SCF, stem cell factor; SIRT2, NAD-dependent protein deacetylase sirtuin-2; STAMPB, STAM-binding protein; TNFSF14, tumor necrosis factor ligand superfamily member 14.

Protein biomarkers associated with both baseline and persistent or recurrent depressive symptoms

Figure 4 shows a three-protein signature comprising CDCP1, SIRT2 and LEPR associated with baseline CES-D score and persistent/recurrent depressive symptoms after full adjustment for covariates. The direction of the associations was consistent for both outcomes, with LEPR inversely associated and CDCP1 and SIRT2 positively associated with baseline and persistent/recurrent depressive symptoms.

DISCUSSION

This high-throughput proteomics approach identified a three-protein signature, namely CDCP1, SIRT2 and LEPR, significantly associated with baseline CES-D score and persistently elevated or recurrent depressive symptoms in individuals with recent-onset diabetes. The positive association of CDCP1 and SIRT2 and the inverse association of LEPR with baseline and persistent/recurrent depressive symptoms were independent of confounding variables. In addition, our study revealed seven additional biomarkers mostly negatively associated with baseline CES-D score and 10 additional biomarkers all positively associated with persistent/recurrent depressive symptoms. However, statistical significance was lost after adjustment for multiple testing.

SIRT2 is an enzyme that is highly expressed in cerebral oligodendrocytes and also found in the circulation.[38] In the brain, SIRT2 plays a role in apoptosis, autophagy and neuroinflammation.[38] It is hypothesized that SIRT2 has a pro-inflammatory effect, opposite to the anti-inflammatory effect of SIRT1.[39] SIRT2 inhibition has been

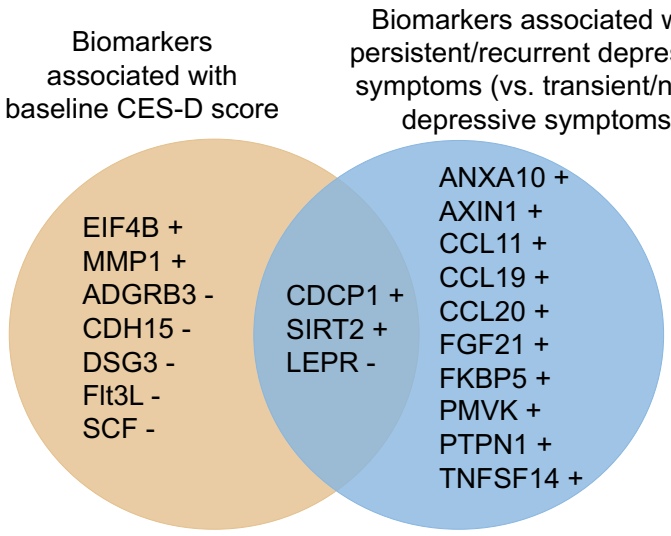

Figure 4 Venn diagram of biomarkers significantly associated with depressive symptoms. This figure shows biomarkers with significant associations in model 2 associated with baseline CES-D score and persistent/recurrent depressive symptoms. The (+) indicates a positive association, and the (–) indicates an inverse association. ADGRB3, adhesion G protein-coupled receptor B3; ANXA10, annexin A10; AXIN1, axin1; CCL11, eotaxin; CCL19, C-C motif chemokine 19; CCL20, C-C motif chemokine 20; CDCP1, CUB domain-containing protein 1; CDH-15, caderin-15; DSG3, desmoglein-3; EIF4B, eukaryotic translation initiation factor 4B; FGF21, fibroblast growth factor 21; FKBP5, peptidyl-prolyl cis-trans isomerase FKBP5; Flt3L, Fms-related tyrosine kinase 3 ligand; LEPR, leptin receptor; MMP-1, matrix metalloproteinases 1; PMVK, phosphomevalonate kinase; PTPN1, tyrosine-protein phosphatase non-receptor type 1; SCF, stem cell factor; SIRT-2, NAD-dependent protein deacetylase sirtuin-2; TNFSF14, tumor necrosis factor ligand superfamily member 14.

found to induce an antidepressant-like action[40] and has been suggested for the treatment of neurodegenerative diseases.[41] However, studies have also reported beneficial effects of SIRT2 on insulin resistance, fatty acid oxidation and inflammation in the context of type 2 diabetes.[42 43] These and other data suggest that SIRT2 action may be context-specific, and the potential of SIRT2 as pharmacological target appears difficult to assess in the absence of further knowlegde of the mechanistic links between this protein and both diabetes and depression.[44]

CDCP1 is part of the Inflammation panel, and higher levels were found to be associated with higher baseline and persistent/recurrent depressive symptoms. CDCP1 is a transmembrane protein involved in various processes, such as immune cell migration and chemotaxis.[45] As ligand for CD6 on T cells, it specifically contributes to T-cell-mediated inflammatory processes.[46] Currently, it is unknown if CDCP1 has a role in diabetes (type 1 and type 2) or depression. However, there are multiple studies that identified associations between CDCP1 and various other neurological conditions. One study found higher levels of CDCP1 to be associated with a higher risk of incident all-cause and Alzheimer's dementia.[47] Further studies reported associations between higher circulating CDCP1 levels and symptoms of psychotic disorder,[48] lower cognitive function[49] and smaller total cerebral brain volume.[50] Of note, CDCP1 is also upregulated in cancer cells from multiple different organs.[51] Taken together, these studies suggest a role of CDCP1 in neurological processes and pathways and in oncogenesis, which might be attributed to shared pro-inflammatory mechanisms.

Leptin is an adipokine produced and secreted from white adipose tissue.[52] It affects mood and cognition positively by causing structural and functional alterations in the brain.[52] Leptin binds to the leptin receptor in brain regions including the hypothalamus and hippocampus.[53] The inverse association between LEPR and baseline and persistent/recurrent depressive symptoms is in line with evidence from mouse models where a selective deletion of LEPR in the hippocampus was found to induce depression-like behavior. This provides evidence that the signaling of leptin in the hippocampus may be essential for maintaining positive mood states. Furthermore, this indicates that LEPR is involved in the molecular mechanism of leptin's antidepressant effect.[54] Signaling through LEPR is also essential in the crosstalk between adipose tissue and the central nervous system to regulate appetite and satiety, but in people with obesity and/or type 2 diabetes the sensitivity to leptin appears to be decreased.[55] Although leptin administration has been reported to lower excess weight and improve insulin resistance, these effects are only observed in people with leptin deficiency.[56] Leptin signaling plays multiple key physiological roles, such as in the regulation of neurological function and metabolism, but remains a challenging target for therapeutic intervention.

Our study revealed several other biomarkers to be associated with either baseline or persistent/recurrent depressive symptoms. For instance, MMP1 and SCF were previously found to be involved in various mechanisms related to the development and modulation of inflammatory processes and neurogenesis.[57 58] The chemokines

CCL11, CCL19 and CCL20 were positively associated with persistent/recurrent depressive symptoms. Chemokines and their receptors are widely expressed in the CNS and are involved in many roles, including immune surveillance, immune cell chemotaxis and blood-brain barrier permeability.[59] Despite differences in samples and methodologies, several human and animal studies have associated higher levels of chemokines with depressive behavioral symptoms.[59] Indeed, elevated levels of chemokines were found in individuals with major depressive disorder and other mental disorders, including bipolar disorder and schizophrenia.[59] Our study did not reveal a significant association between NfL and depression in contrast to other studies. We speculate that this is because neurodegenerative processes might be less pronounced in individuals with recent-onset diabetes with good glycemic control, or these processes are not a major hallmark of depression pathogenesis in patients with diabetes.

Most of the prior studies investigating associations of protein biomarkers with depression in individuals with diabetes have primarily focused on the inflammatory biomarkers IL-6, IL-1RA and CRP, among which only IL-6 was included in our panels. The lack of an association between IL-6 and depressive symptoms in the present study aligns with our and previous reports among individuals with diabetes.[15 16 19 34] These findings were consistent despite differing biomarker measurement techniques (ELISA and PEA technology) and diabetes duration.

Previous research has shown the potential for proteins to be used to help develop new treatments for depression.[60] Implementing proteomic-based techniques is crucial to examine the intricate intracellular networks concerning immune-inflammatory pathways.[60] By using omics-based technology to examine biomarkers, we can better assess these networks and pathways and therefore, research can be feasibly translated to patients with depression with or without comorbid disorders to help with drug discovery.[60] Furthermore, specifically for SIRT2, various SIRT2 functions have been identified in cells that show promise for it to be a potential target for drug development with therapeutic effects on several human diseases, including depression.[61] SIRT2 inhibitors have been found to induce an antidepressant-like action, and agents such as 33i, sirtinol and AGK2 are novel SIRT2 inhibitors that pose potential therapeutic options to treat depression.[61] However, the context-specific action of SIRT2 and potential adverse effects on other outcomes need to be considered.[44]

Strengths and limitations

The strength of our study is the repetitive measurement of depressive symptoms at up to six time points, allowing a reliable definition of persistent/recurrent depressive symptoms. Another study has previously assessed persistent/recurrent depressive symptoms in people with diabetes, but not in the context of biomarkers,[36] and defined persistent/recurrent depressive symptoms based on only three time points, a limited window to reliably define an individual as having persistent or recurrent depressive symptoms. Other strengths include investigating a diverse panel of protein biomarkers covering various inflammatory and neurological pathogenic processes measured with high-throughput proteomic technology. Most comparable studies concentrated on a single or few biomarkers, potentially overlooking the intricate complexity of biological mechanisms. The extensive phenotyping of our study cohort allowed for a comprehensive adjustment for covariates.

Our study has some limitations. The CES-D questionnaire evaluates recent self-reported depressive symptoms (current or last week's episode), which is not clinically validated. Nevertheless, the CES-D score represents a reliable tool used in the clinical setting among individuals with diabetes.[33] Different cut-offs for the CES-D score have been proposed to define depressive disorder,[31] however a lower cut-off was chosen in the present study since the outcome was focused on examining the presence of depressive symptoms. The analysis of persistent and recurrent depressive symptoms has a risk of misclassification for individuals with data on less than six time points. Our study includes individuals recently diagnosed with diabetes with relatively good glycemic control, which may limit the generalizability of the findings to individuals with long-standing diabetes or those with poor glycemic control. We did not perform a power calculation because we did not test a specific hypothesis as in a clinical trial but rather used all available cohort data for our study aims.[62] Therefore, the findings should be interpreted within this context. The potential limitations in sample size are reflected by the wide 95% CIs, which have been consistently reported throughout the manuscript, and by the fact that our results are not statistically significant after Bonferroni correction. Therefore, additional cohort studies and meta-analyses using these and our data will be desirable for more certain results. Previous studies have indicated associations between other biomarkers of inflammation and oxidative stress such as IL-1RA or isoprostane with depression, but these data were not available in the GDS. Lastly, given the known role of genetic susceptibility in the development of depression, our results based on individuals of primarily European descent may not be generalizable to non-European populations.

CONCLUSION

In conclusion, this study identified a three-protein biomarker signature comprising CDCP1, SIRT2 and LEPR associated with baseline CES-D score and persistently elevated or recurrent depressive symptoms in adults with recent-onset diabetes. The identified protein biomarkers associated with depressive symptoms could represent potential modulators in underlying inflammatory and neurological pathways involved in the pathogenesis of depression in individuals with diabetes. Future research

should focus on replicating these findings in a larger more heterogeneous population of individuals with recent-onset diabetes. These findings may provide novel insights for developing new treatments more tailored to treat depressive symptoms in individuals with diabetes and be hypothesis generating for future research.

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Acknowledgements We greatly appreciate the contribution of all study participants and thank the staff at the Clinical Research Center, Institute for Clinical Diabetology, German Diabetes Center, for their role in data collection.

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Contributors HM acquired funding. HM and MCS designed the study and drafted the analysis plan. JS, SOB, KS, WR, OPZ, ST, RW, AI, CH and MR contributed data. MCS performed the statistical analysis. HM, MCS and CH interpreted data. MCS wrote the manuscript. HM and CH contributed to the draft of the manuscript. All authors reviewed and edited the manuscript and approved its submission. MCS is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Funding This study was funded by a grant from EFSD/Novo Nordisk Foundation to HM (NNF22SA0079478). The German Diabetes Study (GDS) was initiated and financed by the German Diabetes Center (DDZ), which is supported by the German Federal Ministry of Health (Berlin, Germany), the Ministry of Culture and Science of the state of North Rhine-Westphalia (Düsseldorf, Germany), and grants from the German Federal Ministry of Education and Research (Berlin, Germany) to the German Center for Diabetes Research e.V. (DZD).

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study was approved by the ethics committee of Heinrich Heine University, Dusseldorf, Germany (ref. 4508), and was conducted in accordance with the Declaration of Helsinki. Participants provided informed consent before taking part in the study.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data may be obtained from a third party and are not publicly available. The data are subject to national data protection laws. Thus, data cannot be made freely available in a public repository. Nevertheless, data can be requested through an individual project agreement with the GDS Steering Committee (speaker: M. Roden, michael.roden@ddz.de).

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