Cerebrospinal Fluid Proteome Evaluation in Major Depressive Disorder by Mass Spectrometry

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Abstract

Introduction: Depression affects approximately 7.1% of the United States population every year and has an economic burden of over $210 billion dollars annually. Several recent studies have attempted to investigate the pathophysiology of depression utilizing cerebrospinal fluid (CSF) and serum analysis. Inflammation and metabolic dysfunction have been strongly implicated as potential etiological factors. Inflammatory proteins such as IL-12, TNF, IL-6, IFN-γ, IL-9, IL-17A, and IL-10 have been found to be significantly correlated with depression.

Methods: CSF samples were obtained from patients diagnosed with major depressive disorder and matched for age and gender with non-psychiatric controls. CSF protein profiles were obtained using quantitative mass spectrometry. The data were analyzed by Progenesis QI proteomics software to identify significantly dysregulated proteins. The results were subjected to bioinformatics analysis using the Ingenuity Pathway Analysis suite to obtain mechanistic insight into biologically relevant interactions and pathways.

Results: Several dysregulated proteins were identified. Bioinformatics analysis indicated that the potential disorder/disease pathways include inflammatory response, metabolic disease, and organismal injury and abnormalities. Molecular and cellular functions that were affected are cellular compromise, cell-to-cell signaling & interaction, cellular movement, protein synthesis, and cellular development. The major canonical pathway that was regulated was acute phase response signaling. Endogenous upstream regulators that may influence depression are interleukin-6 (IL-6), signal transducer and activator of transcription 3 (STAT3), oncostatin M, PR domain zinc finger protein 1 (PRDM1), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A).

Conclusions: The proteome profiling data in this report identifies several potential biological functions that may be disrupted in major depressive disorder’s pathophysiology. Future research into how the differential expression of these proteins is involved in the etiology and severity of depression will be important.

1. Introduction

According to the National Institute of Mental Health (NIMH), 17.3 million U.S. adults had at least one
episode of Major Depressive Disorder (MDD) in 2017(1). This number represents 7.1% of the United States population and that number is only expected to rise. The total economic burden of MDD in the U.S. was estimated to be $210.5 billion dollars in 2010. (2) Recent research has pointed to possible correlations of depression with processes such as inflammation (3, 4) and metabolic disease (5). Inflammatory proteins such as IL–12, TNF, IL–6, IFN-γ, IL–9, IL–17A, and IL–10 have been reported as being elevated in MDD patients (6) and studies regarding the role of metabolism in the disease are underway. Some researchers have investigated biomarkers circulating in the peripheral blood (7, 8), which could easily be used in routine screening. However, cerebrospinal fluid (CSF) may be a more promising biomarker source because of its proximity to and direct interactions with brain tissue (9). Traditionally, CSF proteomics studies have employed 2D-gel electrophoresis, which is quantitative but requires relatively large quantities of protein that can only be identified post-analysis (10). Mass spectrometry methods are ideal for CSF studies because their high sensitivity requires relatively low protein concentrations (11). In this study, CSF samples from MDD patients and matching non-psychiatric patients were analyzed by quantitative mass spectrometry. The resulting data was subjected to bioinformatic analyses with Ingenuity Pathway Analysis to determine potential pathways involved in the pathophysiology of MDD.

2. Materials And Methods
2.1. Cerebrospinal Fluid Collection
Ten adult outpatients fulfilling DSM-IV criteria for unipolar MDD and ten healthy control subjects, selected to age- and gender-match the patient group, provided voluntary written informed consent to participate in this study. The protocol was approved by the institutional review boards of Yale University (New Haven, CT) and Butler Hospital (Providence, RI), and conducted at both institutions. Healthy subjects were recruited by postings around college/university campuses and on community bulletin boards. Depressed patients were recruited from those who presented seeking treatment in one of several clinical research trials for MDD. While several different versions of the Hamilton Depression Rating Scale (Hamilton 1960) were used to determine eligibility for the clinical trials, all required the equivalent of a baseline score greater than 17. Semi-structured diagnostic interviews
were used to determine the presence of unipolar MDD (patient group) or the absence of any current and lifetime DSM-IV Axis I disorder (controls). Individuals with any other major Axis I comorbidity were excluded. While Axis II disorders were not formally assessed, those patients who presented with symptoms or characteristics suggestive of prominent personality pathology were excluded from entry into the CSF and depression treatment protocols. Also excluded were subjects with any other clinically significant medical disorder, based on history, physical examination, and laboratory studies, which included a complete blood count, serum chemistries, liver and thyroid function tests, urine toxicology, electrocardiogram, and pregnancy test for women. All subjects were medication-free for at least two weeks by the time of participation and were remunerated for participating. MDD subjects underwent CSF sampling within two weeks prior to starting their clinical trial antidepressant treatment. All subjects agreed to comply with instructions for a modified diet and activity schedule in the 24 hours preceding the lumbar puncture (LP).

Specific measures were taken to reduce anxiety and HPA axis arousal associated with the LP procedure. Subjects did not disrobe or change into hospital gowns for the procedure but wore loose clothes that were simply rolled away from the lumbar area. Subjects did not enter the procedure room until immediately before the LP began, and medical supplies such as needles and equipment used in the LP were kept out of subjects’ view throughout the protocol. Subjects were placed in a comfortable leaning-forward seated position on a bed and repeatedly encouraged to give feedback about their comfort so the physician could achieve a relatively pain-free LP through use of comfortable positioning and liberal application of local anesthetic. Casual conversation between the subject and physician performing the LP continued throughout the procedure. If the procedure was not successful in producing a CSF sample by 30 minutes after the start of sterile preparations, the procedure was terminated.

Following intradermal injection of 1% lidocaine, a 20-gauge introducer needle was used to penetrate skin and superficial tissue. The introducer is necessary because the Sprotte® 24-gauge pencil point spinal needle is very thin and has a relatively dull tip. The spinal needle was then inserted, through the introducer, to the L4-L5 interspace. A total of 12 ml of CSF was collected, immediately divided into
0.5 ml aliquots, and frozen at -80° C until assayed.

2.2. Mass Spectrometry Detection and Quantification of Proteins

Data Acquisition on an LTQ Orbitrap Elite Mass Spectrometer: 100 µg of protein per sample were taken and detergent was removed by chloroform/methanol extraction, and the protein pellet was re-suspended in 100 mM ammonium bicarbonate and digested with MS-grade trypsin (Pierce) overnight at 37°C with. Peptides cleaned with PepClean C18 spin columns (Thermo) were re-suspended in 2% acetonitrile (ACN) and 0.1% formic acid (FA) and 500 ng of each sample was loaded onto trap column Acclaim PepMap 100 75µm x 2 cm C18 LC Columns (Thermo Scientific™) at flow rate of 4 µl/min then separated with a Thermo RSLC Ultimate 3000 (Thermo Scientific™) on a Thermo Easy-Spray PepMap RSLC C18 75µm x 50cm C-18 2 mm column (Thermo Scientific™) with a step gradient of 4–25% solvent B (0.1% FA in 80 % ACN) from 10–130 min and 25–45% solvent B for 130–145 min at 300 nL/min and 50oC with a 180 min total run time. Eluted peptides were analyzed by a Thermo Orbitrap Fusion Lumos Tribrid (Thermo Scientific™) mass spectrometer in a data dependent acquisition mode. A survey full scan MS (from m/z 350–1800) was acquired in the Orbitrap with a resolution of 120,000. The AGC target for MS1 was set as 4 × 105 and ion filling time set as 100 ms. The most intense ions with charge state 2–6 were isolated in 3 s cycle and fragmented using HCD fragmentation with 35 % normalized collision energy and detected at a mass resolution of 30,000 at 200 m/z. The AGC target for MS/MS was set as 5 × 104 and the ion filling time was set at 60 ms while the dynamic exclusion was set for 30 s with a 10 ppm mass window. The data were processed with Progenesis QI (version 4.1; Nonlinear Dynamics, LLC.) and protein identification was searched using Mascot search algorithm (version 2.3.0) (Matrix Science). See details below.

Protein identification: MS/MS data were searched against the Swiss-Prot human protein database downloaded on Feb 13, 2019 using the in-house Mascot 2.6.2 (Matrix Science) search engine. The search was set up for full tryptic peptides with a maximum of two missed cleavage sites. Acetylation of protein N-termini and oxidized methionines were included as variable modifications and carbamidomethylation of cysteine was set as a fixed modification. The precursor mass tolerance threshold was set at 10 ppm and the maximum fragment mass error was 0.02 Da. The significance
threshold of the ion score was calculated based on a false discovery rate of \( \leq 1\% \). Qualitative analysis was performed using Progenesis QI proteomics 4.1 (Nonlinear Dynamics). After removal of degraded proteomic samples, nine female and six male samples that were age and gender matched were used for bioinformatics analysis.

2.3. Ingenuity Pathway Analysis
Analysis of LTQ Orbitrap Elite mass spectrometry derived proteomics data was performed using Ingenuity Pathways Analysis (IPA) software. The fold expression change data linked to each protein was uploaded as an Excel document to the IPA servers. A core analysis was performed to identify any potentially interesting relationships in the dataset. Overlap with canonical pathways or specific biological functions was calculated algorithmically by the software using its statistical formulas.

3. Results
3.1. Biological functions altered in Major Depressive Disorder (MDD)
After mass spectrometry was completed on the cerebrospinal fluid (CSF) samples, statistical analysis was performed using the Progenesis QI software. This resulted in identifying 43 proteins that were differentially expressed with 23 upregulated and 20 downregulated in MDD. These results are shown in Figure 1 and Figure 2. Table 1 shows the top ten proteins for both upregulation and downregulation. An Ingenuity Pathway Analysis core analysis was performed on the complete dataset to elucidate any biological functions related to the dataset. This analysis resulted in the list of disorders/diseases shown in Table 2. This includes inflammatory response, metabolic disease, and organismal injury and abnormalities. Several molecular and cellular functions were also significantly implicated in this dataset. The affected functions listed in Table 3 are cellular compromise, cell-to-cell signaling & interaction, cellular movement, protein synthesis, and cellular development.

3.2. Canonical pathways related to Major Depressive Disorder as generated by IPA
The IPA core analysis also identified several canonical pathways that had a substantial overlap with the dataset (Fig. 3). The activated pathways include acute phase response signaling, coagulation system, intrinsic prothrombin activation pathway, and glycoprotein VI (GP6) invasiveness signaling. The sole downregulated pathway was LXR/RXR activation. The most significantly regulated pathway was acute phase response signaling (Fig. 4).
3.3. Upstream regulators generated by IPA software

Shown in Table 4 is a list of upstream regulators that can regulate the processes connected to the dataset. These included cytokines interleukin-6 (IL-6) and oncostatin M (OSM); chemical drugs phenacetin and carboplatin; transcription regulators PR domain zinc finger protein 1 (PRDM1), signal transducer and activator of transcription 3 (STAT3), and PPARG coactivator 1 alpha (PPARGC1A); and the chemical toxicant thioacetamide.

Excluding exogenous regulators from this list leaves interleukin-6, oncostatin M, PRDM1, STAT3, and PPARGC1A. As seen in Fig. 5, three of these molecules are interconnected in one pathway leading to the activation of STAT3. This correlates with the data in Fig. 4 as many of the molecules downstream of STAT3 are upregulated.

4. Discussion

We performed a proteomic analysis of CSF from MDD and matched controls and further analyzed the data for functional significance using Ingenuity Pathway Analysis software. This revealed altered molecular and cellular functions such as: cellular compromise, cell-to-cell signaling & interaction, cellular movement, protein synthesis, and cellular development. Disease/disorder processes related to MDD were also statistically significant, including inflammatory response, metabolic disease, and organismal injury and abnormalities.

Previous research has shown that MDD patients have elevated levels of inflammatory proteins including those revealed in our study such as alpha-1-antitrypsin (12, 13) and proteins involved in CNS development such as protein cordon-bleu (14). Downregulated proteins found in our study and in the literature include energy metabolism proteins such as triosephosphate isomerase (15, 16). Studies examining the comorbidity of depression and metabolic dysregulation have been supported by reports that have focused on poor glycemic control (17), diabetes (18), and metabolic syndrome (19). The specific link between these conditions has not been sufficiently elucidated. Also, the directionality of the relationship is still being debated.

The role of inflammation in depression has attracted significant attention and there is substantial evidence to indicate that it is important to disease pathophysiology (20–23). Several chemokines are
dysregulated in the blood of depressive patients, including elevated C-Reactive Protein (CRP) levels (24). It is interesting to note that the reward pathway is strongly influenced by inflammatory cytokines such as interferons, interleukin-1β, and tumor necrosis factor (25). This modulation of the reward pathway by pro-inflammatory signaling could emerge as a causal link between proteomics data and disease phenotype. In addition to highlighting the acute phase response signaling system, our dataset also implicated several upstream regulators that could have a role in depression. These signaling molecules are oncostatin M (OSM), interleukin-6, and STAT3. IL-6 and STAT3 have been previously shown to be involved in serotonin transporter function and depression-like behavior (26). OSM has been shown to have various effects in the body including inflammation, but it has not been well studied with regard to depression (27). Several ongoing clinical studies (ClinicalTrials.gov) are investigating the role of IL-6 as a biomarker or causative molecule in depression, but none are investigating STAT3 or oncostatin M. It should be noted that STAT3 is activated by elevated IL6 and oncostatin M belongs to the IL6 family. Future research focused on manipulating levels of these molecules in preclinical models can shed light on whether they play important roles in depressive behavior.

Fibrinogen has been shown in previous studies to be positively correlated with MDD (28, 29). In patients with high CSF levels of fibrinogen, significant white matter tract abnormalities were also observed (30). Haptoglobin has also been implicated in MDD over the past few decades (13) and more recent research has focused on investigating the effects of different haptoglobin genotypes (31, 32). Our work further suggests that these proteins are likely to be related to the MDD phenotype via their identification in CSF.

5. Conclusion

The proteome profiling data in this report identified several potential biological functions that may be disrupted as part of the pathophysiology of MDD. These include inflammatory response, metabolic disease, and organismal injury/abnormalities. Additionally, several biological functions including cellular compromise, cell-to-cell signaling and interaction, cellular movement, protein synthesis, and cellular development were also suggested to be involved in MDD. Acute phase response was
identified as a significantly impacted canonical pathway by this analysis. Finally, several endogenous upstream regulators including interleukin-6, oncostatin M, STAT3, PRDM1, and PPARGC1A were identified by statistical analyses of the proteome profiling data.

**Declarations**

*Ethics approval and consent to participate:* IRB approval was given by both Brown University and Yale University for this study. Informed consent was given by all participants.

*Consent for publication:* Not applicable.

*Availability of data and materials:* Data may be found on Mendeley (Franzen, Avery; Sathyanesan, Samuel; Duman, Ronald; Williams, Kenneth; Nairn, Angus; Lam, Tukiet; Carpenter, Linda; Kumar, Vikas (2020), “Proteomic Analysis of Cerebrospinal Fluid in Major Depressive Disorder”, Mendeley Data, v1, http://dx.doi.org/10.17632/th4h8988d4.1)

*Competing Interest:* RSD has received research funding or been a consultant for Allergan, Johnson and Johnson, Taisho, Sumitomo Dainippon, Lilly, Naurex, Aptynx, Relmada, Navitor, and Heptares.

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*Authors’ Contributions:* ADF performed the bioinformatic analysis and prepared the manuscript. TTL and VK performed the mass spectrometry techniques and analyzed the proteomic data. LLC performed the lumbar punctures. KRW and AN reviewed the mass spectrometry data and methods. MS analyzed processing of CSF for MS studies. RSD and SSN designed the experiment and provided intellectual input. All authors reviewed and approved the final manuscript.

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*References*

1. Health NIoM. Major Depression 2017 [updated February 2019. Available from: https://www.nimh.nih.gov/health/statistics/major-depression.shtml.

2. Greenberg PE, Fournier A-A, Sisitsky T, Pike CT, Kessler RC. The economic burden of adults with
major depressive disorder in the United States (2005 and 2010). J Clin Psychiatry. 2015;76(2):155–62.

3. Maes M. The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. Neuroendocrinology Letters. 2008;29(3).

4. Kelly K, Mezuk B. M41 - DEPRESSION, INFLAMMATION, AND METABOLIC RISK: A GENETICALLY-INFORMED EXPLORATORY STUDY. European Neuropsychopharmacology. 2019;29:S976-S7.

5. Danese A, Moffitt TE, Harrington H, Milne BJ, Polanczyk G, Pariante CM, et al. Adverse Childhood Experiences and Adult Risk Factors for Age-Related Disease: Depression, Inflammation, and Clustering of Metabolic Risk Markers. Adverse Childhood Experiences and Disease Risk. JAMA Pediatrics. 2009;163(12):1135–43.

6. Syed SA, Beurel E, Loewenstein DA, Lowell JA, Craighead WE, Dunlop BW, et al. Defective Inflammatory Pathways in Never-Treated Depressed Patients Are Associated with Poor Treatment Response. Neuron. 2018;99(5):914–24 e3.

7. Wan Y, Liu Y, Wang X, Wu J, Liu K, Zhou J, et al. Identification of differential microRNAs in cerebrospinal fluid and serum of patients with major depressive disorder. PLoS One. 2015;10(3):e0121975.

8. Akpinar A, Yaman GB, Demirdas A, Onal S. Possible role of adrenomedullin and nitric oxide in major depression. Prog Neuropsychopharmacol Biol Psychiatry. 2013;46:120-5.

9. Kroksveen AC, Opsahl JA, Aye TT, Ulvik RJ, Berven FS. Proteomics of human cerebrospinal fluid: discovery and verification of biomarker candidates in neurodegenerative diseases using quantitative proteomics. J Proteomics. 2011;74(4):371–88.

10. Ditzen C, Tang N, Jastorff AM, Teplytska L, Yassouridis A, Maccarrone G, et al. Cerebrospinal fluid biomarkers for major depression confirm relevance of associated pathophysiology. Neuropsychopharmacology. 2012;37(4):1013–25.

11. Dayon L, Hainard A, Licker V, Turck N, Kuhn K, Hochstrasser DF, et al. Relative Quantification of Proteins in Human Cerebrospinal Fluids by MS/MS Using 6-Plex Isobaric Tags. Analytical Chemistry. 2008;80(8):2921–31.
12. Papakostas GI, Shelton RC, Kinrys G, Henry ME, Bakow BR, Lipkin SH, et al. Assessment of a multi-assay, serum-based biological diagnostic test for major depressive disorder: a pilot and replication study. Mol Psychiatry. 2013;18(3):332–9.

13. Maes M, Scharpe S, Grootel LV, Uyttenbroeck W, Cooreman W, Cosyns P, et al. Higher α1-antitrypsin, haptoglobin, ceruloplasmin and lower retinol binding protein plasma levels during depression: Further evidence for the existence of an inflammatory response during that illness. Journal of Affective Disorders. 1992;24(3):183–92.

14. Xie P, Kranzler HR, Yang C, Zhao H, Farrer LA, Gelernter J. Genome-wide association study identifies new susceptibility loci for posttraumatic stress disorder. Biol Psychiatry. 2013;74(9):656–63.

15. English JA, Dicker P, Focking M, Dunn MJ, Cotter DR. 2-D DIGE analysis implicates cytoskeletal abnormalities in psychiatric disease. Proteomics. 2009;9(12):3368–82.

16. Marais L, Hattingh SM, Stein DJ, Daniels WM. A proteomic analysis of the ventral hippocampus of rats subjected to maternal separation and escitalopram treatment. Metab Brain Dis. 2009;24(4):569–86.

17. Lustman PJ, Anderson RJ, Freedland KE, Groot MD, Carney RM, Clouse RE. Depression and Poor Glycemic Control. Epidemiology/Health Services/Psychosocial Research. 2000;23(7).

18. Talbot F, Nouwen A. A review of the relationship between depression and diabetes in adults: is there a link? Diabetes Care. 2000;10.

19. Pan A, Keum N, Okereke OI, Sun Q, Kivimaki M, Rubin RR, et al. Bidirectional association between depression and metabolic syndrome: a systematic review and meta-analysis of epidemiological studies. Diabetes Care. 2012;35(5):1171–80.

20. Oddy WH, Allen KL, Trapp GSA, Ambrosini GL, Black LJ, Huang RC, et al. Dietary patterns, body mass index and inflammation: Pathways to depression and mental health problems in adolescents. Brain Behav Immun. 2018;69:428–39.

21. Dooley LN, Kuhlman KR, Robles TF, Eisenberger NI, Craske MG, Bower JE. The role of inflammation in core features of depression: Insights from paradigms using exogenously-induced inflammation. Neurosci Biobehav Rev. 2018;94:219–37.
22. Osimo EF, Baxter LJ, Lewis G, Jones PB, Khandaker GM. Prevalence of low-grade inflammation in depression: a systematic review and meta-analysis of CRP levels. Psychol Med. 2019;1–13.

23. Leighton SP, Nerurkar L, Krishnadas R, Johnman C, Graham GJ, Cavanagh J. Chemokines in depression in health and in inflammatory illness: a systematic review and meta-analysis. Mol Psychiatry. 2018;23(1):48–58.

24. Chamberlain SR, Cavanagh J, de Boer P, Mondelli V, Jones DNC, Drevets WC, et al. Treatment-resistant depression and peripheral C-reactive protein. Br J Psychiatry. 2019;214(1):11–9.

25. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol. 2016;16(1):22–34.

26. Kong E, Sucic S, Monje FJ, Savalli G, Diao W, Khan D, et al. STAT3 controls IL6-dependent regulation of serotonin transporter function and depression-like behavior. Sci Rep. 2015;5:9009.

27. Richards CD. The enigmatic cytokine oncostatin m and roles in disease. ISRN Inflamm. 2013;2013:512103.

28. Hattori K, Ota M, Sasayama D, Yoshida S, Matsumura R, Miyakawa T, et al. Increased cerebrospinal fluid fibrinogen in major depressive disorder. Sci Rep. 2015;5:11412.

29. Toker S, Shirom A, Shapira I, Berliner S, Melamed S. The association between burnout, depression, anxiety, and inflammation biomarkers: C-reactive protein and fibrinogen in men and women. J Occup Health Psychol. 2005;10(4):344–62.

30. Wang L, Leonards CO, Sterzer P, Ebinger M. White matter lesions and depression: A systematic review and meta-analysis. Journal of Psychiatric Research. 2014;56:56–64.

31. Livny A, Schnaider Beeri M, Heymann A, Schmeidler J, Moshier E, Tzukran R, et al. The Association of Depressive Symptoms With Brain Volume Is Stronger Among Diabetic Elderly Carriers of the Haptoglobin 1–1 Genotype Compared to Non-carriers. Front Endocrinol (Lausanne). 2019;10:68.

32. Ravona-Springer R, Livny A, Heymann A, Bendlin BB, Johnson SC, Schmeidler J, et al. Structural Brain Abnormalities Associated with Depression in Elderly with Type 2 Diabetes Differ by Haptoglobin Genotype. Alzheimer’s & Dementia. 2016;12(7).
Due to technical limitations, Tables 1-4 are provided in the Supplementary Files section.

Legends:

**Table 1.** Top ten upregulated and downregulated proteins in MDD cerebrospinal fluid. Columns show UniProt ID, gene symbol, fold change, description, molecular function and cellular localization. Molecular function and cellular localization are from Ingenuity Pathway Analysis (IPA) software.

**Table 2.** Disorders and diseases identified by Ingenuity Pathway Analysis software as being implicated in MDD. p-value ranges were calculated for this dataset for the involvement of including inflammatory response, metabolic disease, and organismal injury and abnormalities. # proteins indicate the number of proteins from this dataset that were implicated as being involved in each of the indicated disorders and diseases.

**Table 3.** Molecular and cellular functions dysregulated by MDD. Functions include cellular compromise, cell-to-cell signaling & interaction, cellular movement, protein synthesis, and cellular development. # proteins indicate the number of proteins from this dataset that were implicated as being involved in each of the indicated molecular and cellular functions.

**Table 4.** Upstream regulators with a predicted state of activation or inhibition. Based on the dataset, IPA generated a list of upstream regulators and determined their predicted activation state, activation z-score, and p-value of overlap with the dataset.

**Supplementary Figure Caption**

**Supplementary Figure 1.** Complete acute phase response signaling. Red nodes are upregulated and green nodes are down regulated.

**Figures**
Volcano plot showing the distribution of proteins (307) with relative protein abundance (log2 MDD vs CONT) plotted against its significance level (negative log10 P-value), showing significantly (P < 0.05) increased (> 1.5; Red) and decreased (< - 1.5; Green) proteins in MDD.
Heat map representation of 6 individual samples abundances for 49 significantly altered proteins after unsupervised hierarchical clustering, segregating samples into CONT (left) and MDD (right) and proteins into up-regulated (bottom) and down-regulated (top) proteins in MDD.
Complete list of canonical pathways associated with the dysregulated proteins identified in this dataset. Y-axis lists the canonical pathway and the x-axis is the log of the corresponding p-value for each. Orange coloring means the pathway is activated and blue coloring means the pathway is inhibited. If there is no coloring than there is insufficient data in the dataset or the IPA knowledge base to determine if the pathway is activated or inhibited.
Activation of the acute phase response. Red nodes indicate upregulation and green nodes indicate downregulation. The intensity of the color relates to the extent of regulation with darker meaning greater. An arrow means activation whereas a perpendicular line indicates inhibition.
Figure 5

Downstream effects of OSM regulation. Orange nodes mean upregulation, blue nodes mean downregulation, orange arrows mean activation, and blue arrows mean inhibition.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

Tables.pdf
figS1.png