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# Proteomic Insights into Biology of Bipolar Disorder: Implications for Health Complexity and Mortality

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**Abstract**

**Objective:** The present study has the following objectives: 1) identify differentially expressed proteins and pathways in blood samples of BD compared to healthy controls by employing high-throughput proteomics and bioinformatics and 2) characterize disease-related molecular signatures through in-depth analysis of the differentially expressed proteins and pathways.

**Methods:** Blood samples from BD patients (n=10) classified into high (BD+) or poor functioning (BD-), based on functional and cognitive status, and healthy controls (n=5)

were analyzed using mass spectrometry-based proteomic analysis. Bioinformatics was performed to detect biological processes, pathways, and diseases related to BD.

**Results:** Eight proteins exclusively characterized the molecular profile of patients with BD+ compared to HC, while 26 altered proteins were observed in the BD- group. These altered proteins were mainly enriched in biological processes related to lipid metabolism, complement system and coagulation cascade, and cardiovascular diseases; all these changes were more prominent in the BD- group.

**Conclusion:** These findings may represent systemic alterations that occur with the progression of the illness and a possible link between BD and medical comorbidities. Such comprehensive understanding provides valuable insights for targeted interventions, addressing mental and physical health aspects in subjects with BD. Despite these promising findings, further research is warranted, encompassing larger sample cohorts and incorporating biological validation through molecular biology methods.

**Keywords:** Mass spectrometry, molecular signatures, bioinformatics, biomarkers, complement system, coagulation cascade.

## 1. Introduction

Bipolar disorder (BD) is a highly disabling disease associated with elevated rates of premature mortality.[1,2] Patients with BD died nine years younger than the general population.[3] This occurs not only by suicide but also due to a high prevalence of medical comorbidities.[4,5] A recent meta-analysis of mortality in subjects with BD showed that they have a twofold increased risk of dying prematurely due to medical diseases when compared to the general population.[6] The Task Force of the International Society for Bipolar Disorders also suggested a strong association

between BD and cardiovascular disease, raising questions regarding the reasons for this link. Unhealthy lifestyle factors may contribute to the high rates of medical comorbidities in BD.[5] In addition to concerns about physical well-being, these risk factors lead to more severe manifestations of BD. For example, individuals with both BD and obesity are at a higher risk of encountering more severe mood symptoms, increased resistance to treatment, and cognitive and functional impairment.

Multifactorial models, including inflammation, oxidative stress, mitochondrial dysfunction, and neurotrophic factors, partially explain BD's accelerated aging and premature mortality.[7–9] Despite these known mechanisms, our understanding of the systemic changes observed throughout the BD remains incomplete. The existing literature has limitations, such as focusing on isolated mechanisms, emphasis on behavioral factors, and limited approaches. There is a tendency to analyze factors individually without considering the complex interaction between different molecular pathways that contribute to the pathophysiology of BD. The influence of unhealthy lifestyle factors on medical comorbidities of BD is frequently studied, but this does not fully explain the relationship between the disease and early mortality, and current methodologies may not capture the full spectrum of molecular changes associated with BD. To overcome these limitations, we need new approaches that analyze the interplay between multiple molecular pathways and search underlying biological markers that help explain the relationship between BD and medical diseases.

In this sense, to address the limitations of current understanding and explore the multifaceted molecular mechanisms underlying BD and its associated medical comorbidities, the present study has two key objectives: 1) identify differentially expressed proteins and pathways in blood samples of BD compared to healthy controls by employing high-throughput proteomics and bioinformatics and; 2)

characterize disease-related molecular signatures through in-depth analysis of the differentially expressed proteins and pathways.

## 2. Methods

Subjects

Ten female participants were recruited by convenience from the Bipolar Disorders Program (PROTHABI) at the Hospital de Clínicas de Porto Alegre. The inclusion criteria were: (1) diagnosis of BD according to DSM-5 (SCID) and (2) age between 18 and 70 years. Exclusion criteria were clinical diagnosis of intellectual disability and/or Alzheimer's disease, uncontrolled medical conditions (as determined by medical records, self-report, or routine blood tests), autoimmune diseases, cancer, substance use, alcohol abuse or dependence, and pregnancy or lactation and (4) electroconvulsive therapy within the past year. All patients received pharmacological treatment according to the Program's protocols.

The control group comprised five healthy volunteers and blood donors from the Hemocenter at HCPA. These participants had no current or previous history and no first-degree family history of a major psychiatric disorder, including dementia or intellectual disability, assessed by the non-patient version of the Structured Clinical Interview for DSM—5 (SCID). They also had no uncontrolled medical conditions (as determined through self-report or routine blood tests) and no autoimmune diseases.

Participants were matched according to gender and age. Before participating in the study, all patients provided written informed consent. The study protocol was approved by the Research Ethics Committee of the HCPA (2019-0640).

Clinical Assessment

We obtained patients' sociodemographic, clinical, and pharmacological data through a structured interview and from medical records. In particular, the clinical assessment encompassed the following scales: Montgomery-Asberg Depression Rating Scale (MADRS), Young Mania Rating Scale (YMRS), Patient-Reported Outcomes Measurement Information System for depression (PROMIS Depression), Patient-Reported Outcomes Measurement Information System for anxiety (PROMIS Anxiety), Cognitive Dysfunction in Bipolar Disorder Rating Scale (COBRA) and Functioning Assessment Short Test (FAST).

The FAST assessed the overall functional outcome, an instrument widely used in patients with BD. This scale encompasses 24 items evaluating six functional domains (autonomy, occupational functioning, cognitive functioning, financial issues, interpersonal relationships, and leisure time). The overall FAST score varies from 0–72 (the cut-off value is 11); the higher the score, the greater the disability.[10] Cognitive difficulties in daily life situations were assessed with COBRA. The COBRA is a 16-item self-report instrument with satisfactory psychometric properties developed to assess cognitive complaints experienced by bipolar patients. The COBRA total score is obtained when the scores of each item are added up. The COBRA total score ranges from 0–48 (the cut-off value is 10); a higher score means a more significant dysfunction.[11,12] For the present study, patients were also classified into high (BD+) or poor (BD-) functioning based on their functional and cognitive status, which was assessed using the Functioning Assessment Short Test (FAST) and Cognitive Bipolar Rating Assessment (COBRA).[10–13] Therefore, BD patients were classified into BD+ if they met FAST scores < 11 and COBRA < 10, while those with FAST scores greater than 40 and COBRA greater than 25 were classified into BD-.

Sample Collection and Depletion of High Abundant Proteins

The blood samples (10 ml) were collected in non-anticoagulant tubes from all participants and then centrifuged at 1500 g for 15 minutes at room temperature to separate the serum. The serum was aliquoted and stored at -80°C for further analysis. The most abundant proteins were removed with the Top14 Abundant Protein Depletion Mini Spin Columns following the manufacturer's specifications (High Select™ Depletion Spin Columns - Thermo Scientific™). Each column contains 400 µL of a 50% slurry in 10mM PBS, 0.02% sodium azide, pH 7.4, and is optimized to bind up to 10 µL of serum. This process enhances the detection of low-abundance proteins when using mass spectrometry analysis.

LC-MS/MS Analysis

Depleted serum was inserted into a Microcon® 5 kDa centrifugal filter and washed with Tris-HCl and the retained volume. A portion corresponding to 40 µg of protein was then dried, and disulfide bonds were ruptured by dithiothreitol and urea. After iodoacetamide incubation, urea dilution, and trypsin digestion, the resulting peptides were desalted using C18 Stage Tips. Identification of serum proteins was performed using a Dionex Ultimate 3000 RSLC nanoUPLC system coupled to an Orbitrap Fusion Lumos Mass Spectrometer. Peptides were separated through reversed-phase chromatography, and mass spectrometry analysis was conducted using high-resolution Orbitrap mass analyzers. Data-dependent scans facilitated the isolation and fragmentation of selected peptides, providing a detailed analysis of the serum protein composition.

Data Analysis

Demographic data and clinical characteristics were expressed as medians and interquartile ranges ([IQR], percentiles 25th and 75th), and group comparisons were conducted using the Kruskal-Wallis test with Dunn post hoc analysis. Statistical analyses were performed using SPSS 18 for Windows, provided by SPSS Inc. in Chicago, IL, USA.

The Peptide Spectrum Match (PSM) analysis was conducted using MaxQuant version 2.2.0.0, with searches performed against the UniProt Human database. Specific parameters, including trypsin-specific digestion, variable modifications such as oxidation (M) and protein N-acetylation, and fixed modifications like carbamidomethyl (C), were employed. Additional settings included a peptide tolerance of 7 ppm, MS/MS tolerance of 0.1 Da, allowance for two missed cleavages, and a 1% False Discovery Rate (FDR) for both PSM and protein assignment. Relative quantification of proteins was accomplished using MaxLFQ intensity values.

Protein-Protein Interaction Network, Biological Processes, and KEGG Pathways

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a database and online resource that contains information on known and predicted protein-protein interactions. The Enrichr software application[14] was utilized to attribute functional significance to the identified proteins. The analysis focused on the differentially expressed proteins to identify biological processes and KEGG pathways associated with BD. Subsequently, the bar chart generated by Enrichr was sorted based on the ranking of combined scores. The combined score is calculated by taking the logarithm of the p-value derived from the Fisher exact test and multiplying it by the z-score,

representing the deviation from the expected rank. Terms with higher combined scores are considered the most relevant in the analysis.

## Weighted Correlation Network Analysis

In this study, we used the R software package Weighted Gene Co-expression Network Analysis (WGCNA) to perform weighted correlation network analysis aspects. WGCNA was employed to identify clusters (modules) of highly interconnected genes and to summarize these clusters using the module eigengene, which is considered representative of the gene expression profiles within a module. The module eigengenes were assessed for correlation with external phenotypes using Pearson's correlation coefficient.[15] We utilized BD groups as the primary outcome phenotype. Then, we performed analyses to investigate whether confounding variables such as the number of medications, number of hospitalizations, number of suicide attempts, and COBRA score also exhibited significant correlations with module eigengenes.

## 3. Results

The demographic and clinical characteristics of the participants are summarized in Table 1. Gender homogeneity was maintained among the groups, as all participants were women. No significant differences in age between the groups were observed (p = 0,897). As expected from the selection criteria, BD- subjects had a higher mean FAST score (p < 0.001) and COBRA score (p < 0.001) when compared to BD+ and HC. Considering the number of medications used, BD- exhibited a higher number than HC (p = 0,004).

Table 1: Clinical and Demographic Data.

| Variable | Controls (n = 5) | BD- (n = 5) | BD+ (n = 5) | *p-value |
|---|---|---|---|---|
| Age, years | 47 [45 – 55] | 54 [52 – 55] | 57 [36 – 60] | 0.954 |
| Weight, kg | 73.5 [63 – 78.5] | 82 [81 – 103] | 72 [62 – 75] | 0.132 |
| BMI, kg/m² | 27.48 [27.18 – 28.72] | 32.03 [32.64 – 41.40] | 26.47 [26.49 – 26.77] | 0.065 |
| Number of medications | 0 [0 – 3] A | 5 [4 – 6] B | 2 [2 – 3] AB | *0.013* |
| Number of hospitalizations | 0 [0 – 0] A | 5 [2 – 6] B | 2 [1 – 2] AB | *0.005* |
| Number of suicide attempts | 0 [0 – 0] A | 2 [1.5 – 6] B | 0 [0 – 0] A | *0.005* |
| MADRS score | 2 [0- 6] AB | 27 [26 – 32] A | 0 [0 – 1] B | *0.006* |
| YRMS score | 0 [0 – 1] AB | 4 [3 – 6] A | 0 [0 – 0] B | *0.008* |
| COBRA score | 12 [4 – 20] AB | 29 [28 – 44] A | 2 [2 – 3] B | *0.005* |
| FAST score | 1 [1 – 5] A | 49 [48 – 52] B | 5 [3 – 5] B | *0.007* |

Data expressed as median and interquartile range ([IQR], percentiles 25th and 75th). Italic values indicate a *p-value* < 0.05. *Kruskal-Wallis test with Dunn *post hoc* analysis. AB Different letters indicate significant differences among groups. Legend: kg – kilogram; m – meter; BMI – body mass index; BD+ = bipolar disorder with high functioning; BD- = bipolar disorder with poor functioning; MADRS = Montgomery-Asberg Depression Rating Scale; YMRS = Young Mania Rating Scale; FAST = Functioning Assessment Short Test; COBRA = Cognitive Complaints in Bipolar Disorder Rating Scale. Significance set at 5% for all analyses.

The present study was conducted to identify differentially expressed proteins between BD and HC based on Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) technology. In total, 3506440 MS/MS spectra were obtained from 30 LC-MS/MS runs, of which 128163 peptide sequences were identified using 1% FDR. The LC-MS/MS detected 234 proteins corresponding to the identified peptides for the 15 participants. Figure 1 presents a protein-protein interaction network generated using STRING that illustrates the interactions between proteins in the BD and HC groups.
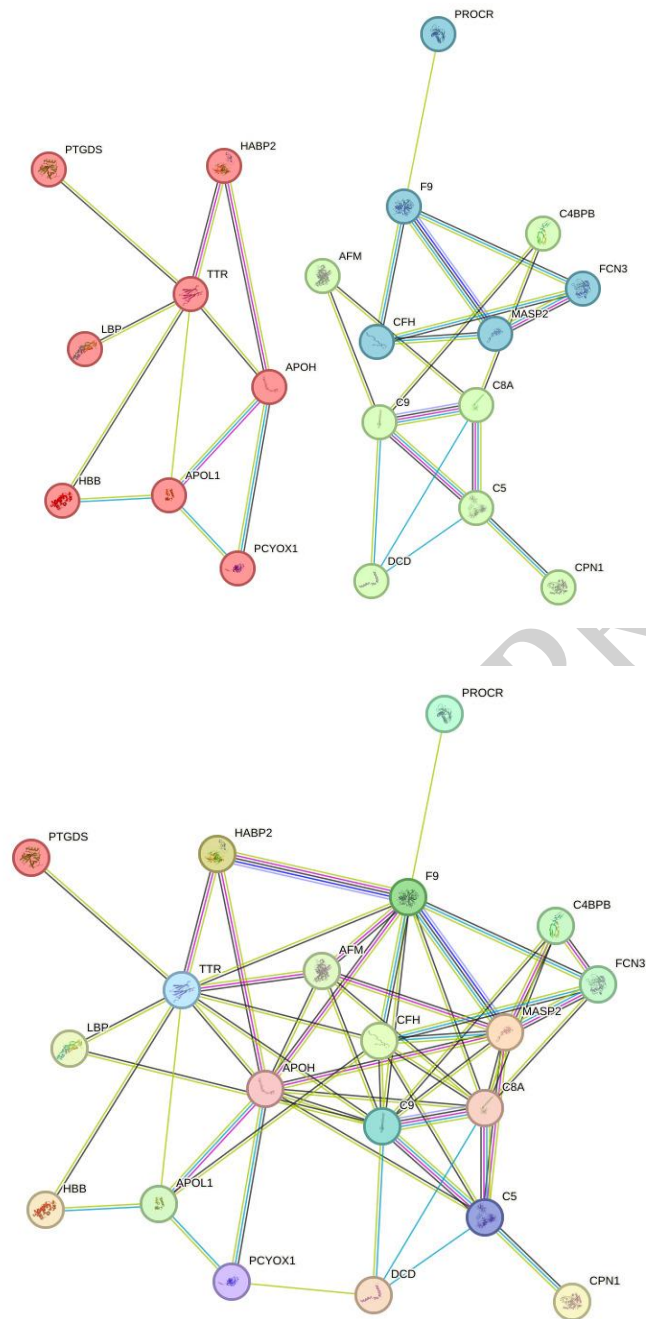
Figure 1: Protein-protein interaction network. The differentially expressed proteins in the BD vs HC comparison revealed the following interaction network and their respective clusters.

After statistical analysis, we identified 21 differentially expressed proteins in BD vs. HC, 12 in BD+ vs. HC, and 30 in BD- vs. HC, as shown in Table 2. The Venn Diagram displays the intersection of proteins identified in the three comparison groups. Furthermore, four proteins—C4BPB, C5, C9, and DCD—were consistently present across all comparisons (Supplementary Material 1). Most proteins identified as significantly differential in BD were involved in the lipid metabolism, complement, and coagulation cascade (e.g., Apolipoproteins, Complement factors, and Coagulation factors).

Table 2: Molecular signatures from patients with BD and HC blood samples.

| Gene names | Protein IDs | Protein names | Log FC | | |
|---|---|---|---|---|---|
| | | | BD / HC | BD+ / HC | BD- / HC |
| AFM | P43652 | Afamin | 0.21 | | |
| APOA1 | P02647 | Apolipoprotein A1 | | | 0.24 |
| APOA2 | V9GYM3 | Apolipoprotein A2 | | | 0.53 |
| APOF | Q13790 | Apolipoprotein F | | | 0.59 |
| APOH | P02749 | Apolipoprotein H | 0.31 | 0.37 | |
| APOL1 | O14791 | Apolipoprotein L1 | -0.61 | | |
| B2M | P61769 | Beta-2-microglobulin | | | -0.63 |
| C1QC | P02747 | Complement C1q C chain | | | 0.47 |
| C3 | P01024 | Complement C3 | | | -0.17 |
| C4BPB | P20851 | Complement component 4 binding protein beta | -0.36 | -0.42 | -0.29 |
| C5 | P01031 | Complement factor C5 | -0.43 | -0.50 | -0.36 |
| C8A | P07357 | Complement C8 alpha chain | -0.25 | | -0.30 |
| C9 | P02748 | Complement factor C9 | -0.91 | -0.83 | -0.99 |
| CFD | P00746 | Complement factor D | | 0.65 | |

| | | | | | |
|---|---|---|---|---|---|
| CFH | P08603 | Complement factor H | -0.35 | | -0.41 |
| CPN1 | P15169 | Carboxypeptidase N subunit 1 | 0.37 | 0.45 | |
| CSF1R | P07333 | Colony stimulating factor 1 receptor | | | -0.71 |
| DCD | P81605 | Dermcidin | -1.21 | -0.61 | -1.8 |
| F12 | P00748 | Coagulation factor XII | | | 0.56 |
| F9 | P00740 | Coagulation factor IX | -0.27 | | -0.38 |
| FCN3 | O75636 | Ficolin 3 | -0.35 | | -0.38 |
| HABP2 | Q14520 | Hyaluronan binding protein 2 | -0.21 | | |
| HBB | P68871 | Hemoglobin subunit beta | -1.37 | -1.98 | |
| IGF1 | P05019 | Insulin-like growth factor 1 | | | 0.86 |
| IGF2 | P01344 | Insulin-like growth factor 2 | | | 0.53 |
| IGFALS | P35858 | Insulin-like growth factor binding protein acid labile subunit | | | 0.72 |
| IGHA1 | P01876 | Immunoglobulin heavy constant alpha 1 | | | 0.95 |
| IGHG2 | P01859 | Immunoglobulin heavy constant gamma 2 | | | 1.29 |
| IGHG3 | P01860 | Immunoglobulin heavy constant gamma 3 | | | 1.90 |
| IGLL5 | P0CG04 | Immunoglobulin | | | 1.92 |
| LBP | P18428 | Lipopolysaccharide binding protein | -0.88 | -1.08 | |
| LDHB | P07195 | Lactate dehydrogenase B | -0.54 | -0.69 | |
| LGALS3BP | Q08380 | Galectin 3 binding protein | | | -1.49 |
| MASP2 | O00187 | MBL associated serine protease 2 | -0.92 | | |
| ORM2 | P19652 | Orosomucoid 2 | | | -0.70 |
| PCYOX1 | Q9UHG3 | Prenylcysteine oxidase 1 | -0.66 | -0.66 | |
| PROCR | Q9UNN8 | Protein C receptor | 0.70 | | 0.79 |
| PTGDS | H0Y5A1 | Prostaglandin D2 synthase | 0.39 | | 0.43 |
| Q6NSD3 | Q6NSD3 | Complement factor H related 3 | | | -0.18 |
| SELL | P14151 | Selectin L | | -0.40 | |

| THBS4 | P35443 | Thrombospondin 4 | | -0.68 |
| TTR | P02766 | Transthyretin | 0.39 | |
| VWF | P04275 | Von Willebrand factor | | -1.13 |

BD=bipolar disorder; HC=healthy control; BD+= bipolar disorder with high functioning; BD- = bipolar disorder with poor functioning. Comparisons considering unadjusted p-value < 0.05 and a fold change over 15%.

We utilized the Enrichr software[14] to detect KEGG pathways and diseases associated with BD. In all comparisons, the prominent signaling pathway identified through KEGG is the complement and coagulation cascade (Figure 2). Notably, the identified proteins in the BD- group were associated with cardiovascular diseases (Figure 3).
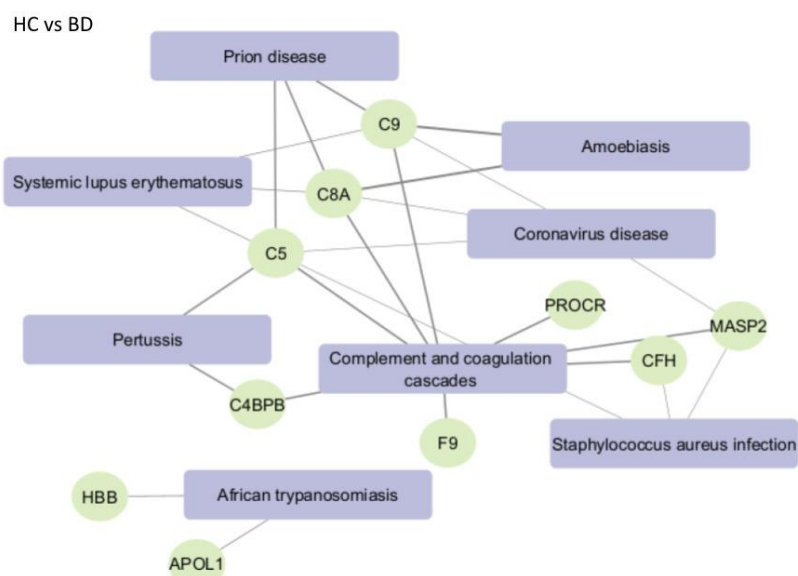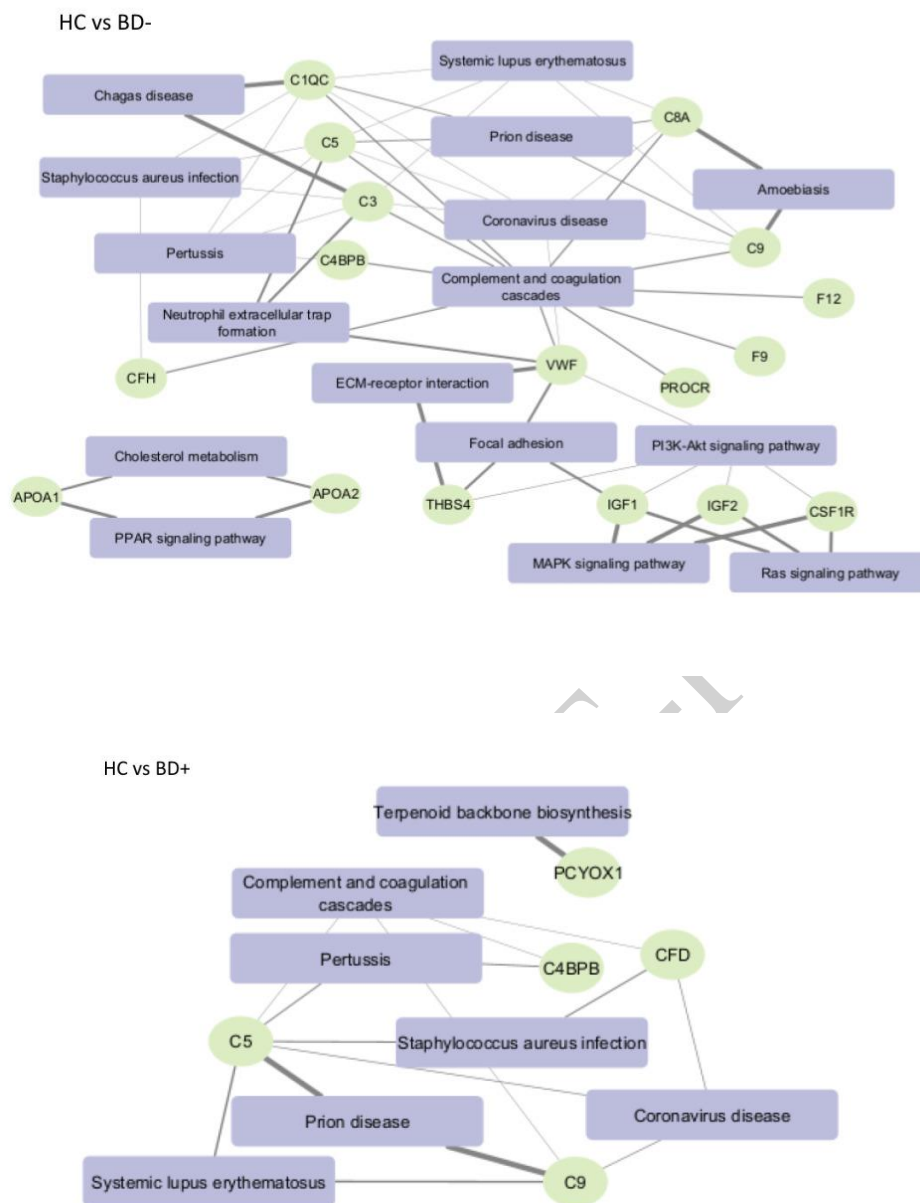
Figure 2: KEGG pathway analysis. Complement and coagulation cascade were the two main signaling pathways involved in BD, BD+ and BD-.

Legend: HC = healthy control; BD = bipolar disorder; BD+ = bipolar disorder with high functioning; BD- = bipolar disorder with poor functioning. We considered an adjusted p-value < 0.00001 computed using the Benjamin-Hochberg method.
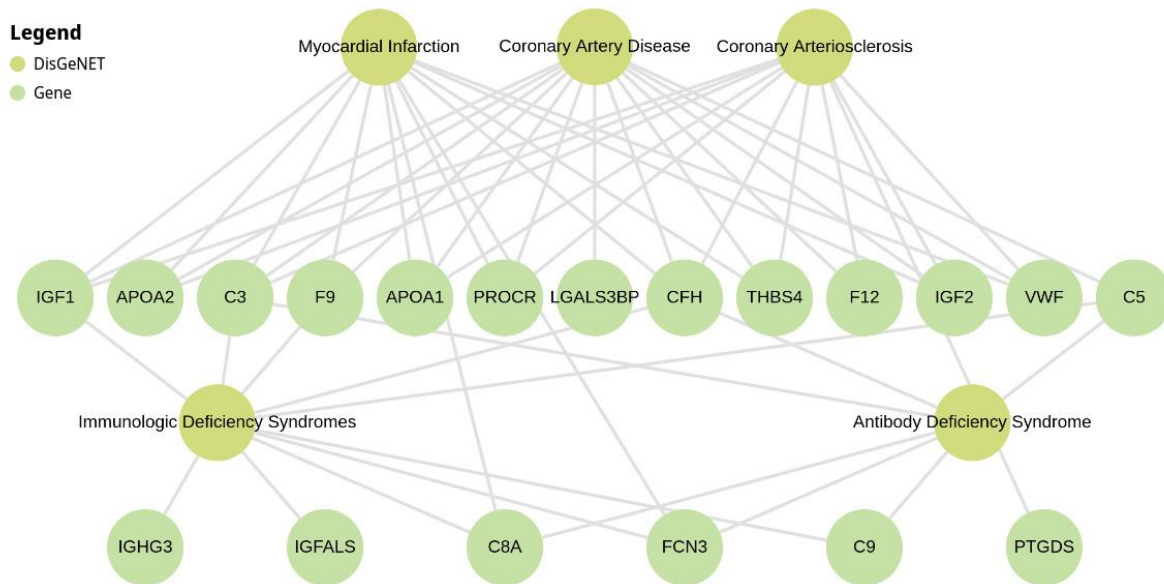
Figure 3: Enrichr analysis illustrating gene enrichment results using DisGeNET data. The figure highlights the association between BD- genes and cardiovascular diseases.

Legend: BD- = bipolar disorder with poor functioning. We considered an adjusted p-value < 0.00001 computed using the Benjamin-Hochberg method.

WCGNA identified a co-expression network comprising four distinct modules in our sample (Fig. 4 A). Only one of these four modules, consisting of 36 co-expressed genes and denoted the "blue module," was significantly correlated with BD (r = 0.67). The blue module was also correlated (r = 0.74) with the number of medications used by patients, suggesting this module might be involved in biological processes related to BD and its treatment response (Figure 4 D).
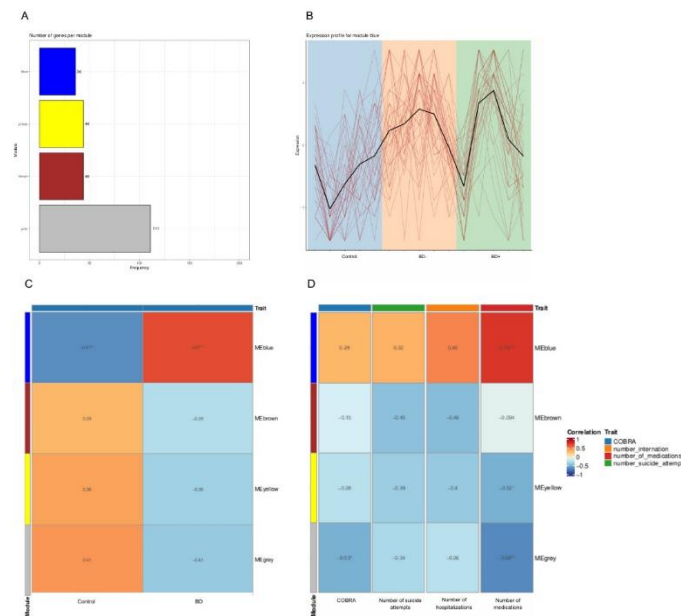
Figure 4: Weighted Gene Co-expression Network Analysis for the blue module. A) Number of proteins per module. B) A gene expression pattern in the HC group shows a negative distinction from the BD- and BD+ groups. C) Positive correlation between the blue module (y-axis) and the BD group (x-axis) and a negative correlation between the blue module and the HC group. The brown, yellow, and gray modules did not correlate significantly. D) The blue module exhibits a robust positive correlation with the quantity of medications our patients use. The remaining modules only display negative correlations with the covariates.

Legend: BD = bipolar disorder; BD+ = bipolar disorder with high functioning; BD- = bipolar disorder with poor functioning; COBRA = Cognitive Bipolar Rating Assessment.

## 4. Discussion

Using a combined proteomic approach and bioinformatics, the current study revealed molecular signatures and pathways correlated with BD. Eight proteins exclusively characterized the molecular profile of patients with BD+ compared to HC, while 26 proteins, that is, three times more altered proteins, were observed in the BD- group.

The altered proteins were mainly enriched in biological processes related to the complement system, coagulation cascade, lipid profile, and cardiovascular diseases; all these changes were most prominent in the BD- group. Thus, this is the first study to show, through mass spectrometry-based proteomic analysis, molecular patterns, pathways, and primary medical diseases related to different stages of BD. Despite these encouraging discoveries, further research is warranted, encompassing larger sample cohorts and incorporating biological validation through molecular biology methods.

The differential proteins and biological processes identified in this study can represent systemic changes that occur with the progression of the disease or even the treatment response. The "blue module" identified by the WGCNA analysis showed a correlation between BD diagnosis and the number of medications. Polypharmacotherapy can be regarded as an indicator of disease complexity, suggesting the presence of various health conditions and a challenge in managing these conditions. We showed that medical comorbidities, in particular, cardiovascular diseases, were more preeminent in BD- suggesting this subgroup of patients may experience a more chronic course of the illness and poor prognosis. An interesting study showed that premature mortality in BD is linked to various causes, including myocardial infarction, diabetes, chronic obstructive pulmonary disease, pneumonia, and suicide.[16] Metabolic, respiratory, and cardiovascular diseases are among the top 10 causes of death worldwide and are notably highly prevalent comorbidities in BD. Therefore, medical comorbidities in individuals with BD can exacerbate the mortality disparity between them and the general population.[17] Many of these comorbidities are closely linked to the complement system,[18] coagulation cascade,[19] and lipid metabolism. [20]

Regarding biological processes and pathways, our findings support previous studies[21–24], indicating that the interplay between the complement system and coagulation cascade is involved in the etiology and progression of BD. The complement system originates from the serine protease reaction cascade, sharing a common ancestry with the coagulation factors encoded by the same ancestor genes.[25,26] Beyond their common origin, these systems exhibit similar roles in promoting the initial defense against infections and tissue repair, potentially contributing to homeostasis or the onset of pathological conditions. Analogous to the complement system, the coagulation cascade is a tightly regulated and coordinated process culminating in clot formation, constituting the hemostasis system when combined with the fibrinolytic system and platelets. The coagulation cascade activation involves primary and secondary hemostasis, often coinciding with the activation of inflammatory mechanisms.[27] Primary hemostasis involves platelet activation and aggregation, leading to fibrin formation by thrombin. This event triggers an acute inflammatory response to control tissue damage, halt blood loss, and prevent microbial infection. During secondary hemostasis, plasmin dissolves fibrin and reparative inflammatory cells, collectively working to remodel and repair damaged tissue.[28] Under normal conditions, a tightly regulated hemostasis system poses minimal risk of complications or failed responses. However, dysregulation can lead to overactive platelet responses, abnormal fibrin formation, and impaired fibrinolysis, creating an environment conducive to cardiovascular events. Furthermore, the intercommunication between the complement and coagulation systems can amplify inflammatory processes within the vascular system, heightening the risk of cardiovascular events.

Like the complement and coagulation system, lipid metabolism is also related to comorbidities with high mortality rates. Lipid metabolism refers to the biochemical processes involving the organism's production, transport, storage, and utilization of lipids. Dysregulation of this process can result in conditions such as elevated lipid levels (hyperlipidemia), the development of atherosclerosis, and various cardiovascular diseases.[29] Atherosclerosis is a condition driven by lipoproteins, resulting in the development of plaques along the arterial tree through processes such as inflammation and necrosis. Increased concentrations of lipoproteins containing apolipoprotein B, mainly low-density lipoprotein (LDL), are often a primary factor in the independent development of atherosclerosis. Furthermore, the disease often develops at lower levels of LDL in combination with additional risk factors such as smoking, hypertension, and diabetes mellitus.[30] Literature also shows that lipid metabolic pathways undergo evident changes in respiratory illnesses such as chronic obstructive pulmonary disease. The lipid modifications enable lung tissue to activate anabolic pathways that initiate the synthesis of active molecules directly involved in inflammation. There is evidence suggesting that obesity is linked to decreased lung function and heightened morbidity in individuals with chronic obstructive pulmonary disease.[31] Also, lipid metabolism is traditionally considered important in the risk factors of ischemic stroke, encompassing elevated levels of total cholesterol, triglycerides, LDL cholesterol, and reduced high-density lipoprotein cholesterol levels. Additionally, managing hyperlipidemia is essential not only for reducing the risk of cardiovascular disease but also for preventing strokes.[32] In individuals diagnosed with BD, a prevalent occurrence of these comorbidities may contribute to an elevated mortality rate. Several of these comorbidities are a consequence of a sedentary lifestyle, tobacco use, heightened alcohol consumption, and chronic exposure to stress.[5] A comprehensive

understanding of the molecular fundaments involving lipid metabolism, complement activation, and coagulation cascade abnormalities establishes an essential link between cellular mechanisms and comorbidities clinical manifestations in BD. Thus, complement and coagulation systems and lipid metabolism may be potential mediators of BD's early onset of medical comorbidities. These findings suggest that collaborative care interventions focusing on the treatment of these comorbidities can significantly decrease early death among individuals with BD.[3]

Furthermore, we cannot rule out the possible effect of psychotropic medications on our results since all patients in this study were under pharmacological treatment. Mood stabilizers, as well as many atypical antipsychotics commonly used for the treatment of BD, can promote weight gain and, thus, the entire cascade of metabolic effects that lead to long-term cardiovascular complications. Nevertheless, it's crucial to acknowledge that, despite the apparent heightened risk of cardiovascular diseases linked to numerous psychotropic medications, there is no conclusive evidence that these medications autonomously amplify the risk of cardiovascular disease in individuals with BD. Some evidence opposes this hypothesis, suggesting that enhanced symptom control might facilitate improved health-related behaviors, increased utilization of health services, and, indirectly, better physical health.[5]

Understanding the molecular pathways and processes identified in the study opens avenues for targeted interventions, including molecular mechanisms underlying BD and its associated medical comorbidities. Specifically, targeting proteins associated with lipid metabolism, the complement system, and the coagulation cascade could mitigate the systemic alterations observed in BD patients. Early identification of systemic alterations enables the development of preventive strategies, reducing the risk of medical comorbidities and ultimately reducing mortality rates

among BD patients. Pharmaceutical interventions or lifestyle modifications to modulate these pathways could be explored to prevent or alleviate medical comorbidities. Ultimately, our findings underscore the importance of integrated care models that address BD patients' mental and physical health needs. Collaborative care approaches involving mental health professionals, primary care physicians, and specialists in relevant medical fields could ensure holistic management of BD and its associated comorbidities, improving patient outcomes. By adopting personalized, mechanism-based interventions and integrating mental and physical health care, clinicians can strive towards more effective and integral care for individuals with BD.

Some limitations should be considered when interpreting the results of this study. The sample size was relatively small, so the Type I error cannot be ruled out. Convenience sampling was used, which may have limited the generalizability of the results. All patients were on pharmacological treatment. The polypharmacy introduces additional complexities that warrant thorough analysis since the pharmacological agents can directly influence the investigated biological responses and generate synergistic or antagonistic effects in signaling pathways and observed outcomes. Also, individual variability in treatment response is a significant factor, making it challenging to draw definitive conclusions about the associations between identified proteins and comorbid health conditions. Overall, the medications taken by the patients are likely to have multifaceted effects on the results, influencing the observed biological responses. Therefore, they must be considered limiting factors in interpreting the study's findings. Also, the present study employed a cross-sectional design, which inherently limits our ability to establish causal relationships between the observed variables.  Some factors, like diet and infections, may have short-term effects on the technique used, introducing noise into the data and potentially leading to biased

results. Future research utilizing a longitudinal design with repeated measurements would be beneficial in strengthening the causal inferences drawn from this study. Algorithms and analysis methods can significantly influence the results. Different algorithms may yield distinct interpretations from the same datasets, and the biological interpretation of results obtained through bioinformatic approaches can be challenging. Due to this, we partnered with an experienced bioinformatician to work with the data. Furthermore, a proteomic profile was obtained from a blood sample, potentially not fully capturing the complex molecular changes associated with BD throughout the brain.

## 5. Conclusion

In summary, despite the exploratory analysis, this is the first study to identify protein profile, using LC-MS/MS, within the peripheral blood across various stages of BD patients. Our findings underscore specific proteins primarily linked to lipid metabolism, the complement system, and the coagulation cascade. These findings may represent systemic alterations that occur with the progression of the illness and a possible link between BD and medical comorbidities. This comprehensive understanding provides valuable insights for targeted interventions, addressing mental health aspects and mechanisms driving comorbidities in BD. While our findings offer valuable insights, several recommendations for future research directions emerge: a) Conduct larger-scale studies with increased sample sizes, including a more diverse sample population, to enhance statistical power and improve the generalizability of findings; b) Implement longitudinal study designs to assess how changes in protein expression correlate with clinical outcomes following pharmacological or psychosocial interventions; c) Utilize multi-omics approaches, integrating data from genomics,

transcriptomics, proteomics, and metabolomics, to investigate the molecular pathways involved in BD comprehensively; d) Conduct functional studies to validate the biological relevance of identified proteins and elucidate their roles in disease pathogenesis.

## Competing interests

No potential conflict of interest relevant to this article was reported.

## Author's Approval

All authors have seen and approved the manuscript.

## Authors' contributions

**PRZ:** Investigation, Resources, Writing - Original Draft. **MAB:** Software, Formal Analysis. **PPA:** Investigation, Resources. **PHR:** Investigation, Writing - Original Draft. **TS:** Investigation, Writing - Original Draft. **GM:** Investigation, Writing - Original Draft.

**ES:** Resources, Writing - Original Draft. **FK:** Conceptualization, Supervision. **ARR:** Conceptualization, Methodology, Supervision, Writing - Review & Editing.

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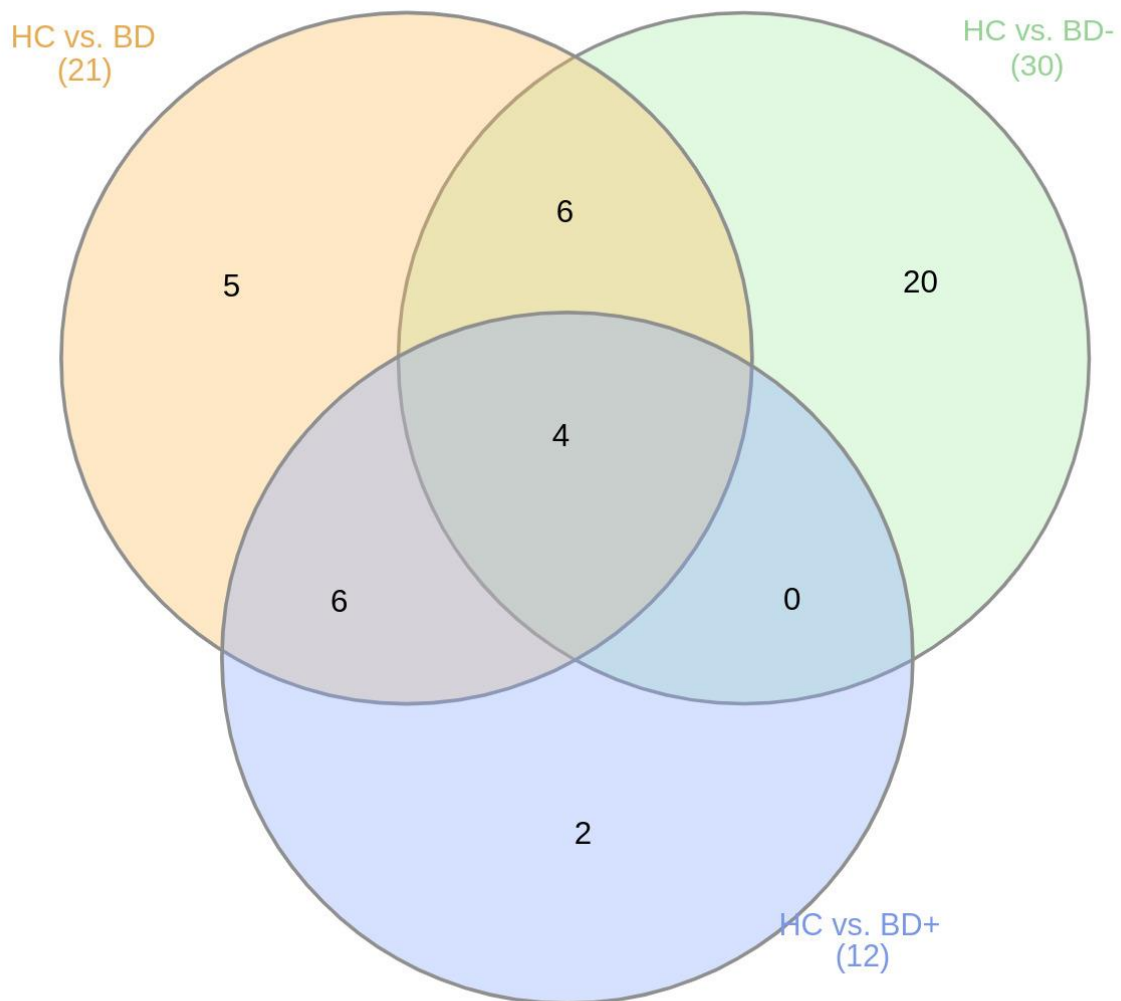
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Supplementary Material



Supplementary Material 1: Protein intersection in comparison groups. Venn Diagram displaying proteins identified in BD, BD+, and BD- comparison groups.

Supplementary Material 2: Proteins Identified in the Blue Module. A list of proteins found within the Blue Module and relevant KEGG pathways.

| Gene names | Protein IDs | Protein names | KEGG Pathway |
|---|---|---|---|
| CD163 | Q86VB7 | Scavenger receptor cysteine-rich type 1 protein M130 | |
| CFP | P27918 | Properdin | |
| ATRN | O75882 | Attractin | |
| FCN3 | O75636 | Ficolin 3 | |
| C8B | P07358 | Complement component C8 beta chain | |
| HABP2 | Q14520 | Hyaluronan-binding protein 2 | |
| MASP2 | O00187 | Mannan-binding lectin serine protease 2 | |
| C8A | P07357 | Complement component C8 alpha chain | |
| CSF1R | P07333 | Macrophage colony-stimulating factor 1 | |
| LDHB | P07195; | L-lactate dehydrogenase B chain | |
| MASP1 | P48740 | Mannan-binding lectin serine protease 1 | |
| OLFM1 | Q99784 | Noelin | Complement and coagulation cascades |
| APOC2 | P02655 | Apolipoprotein C-II | |
| BTD | P43251 | Biotinidase | |
| PCYOX1 | Q9UHG3 | Prenylcysteine oxidase 1 | |
| AFM | P43652 | Afamin | |
| DCD | P81605 | Dermcidin | |
| C4BPB | P20851 | C4b-binding protein beta chain | |
| APOM | O95445 | Apolipoprotein M | |
| HSPA5 | P11021 | Endoplasmic reticulum chaperone BiP | |
| APOC4 | P55056 | Apolipoprotein C-IV | |
| PTPRJ | Q12913 | Receptor-type tyrosine-protein phosphatase eta | |
| CTBS | Q01459 | Di-N-acetylchitobiase | |
| SERPINA6 | P08185 | Corticosteroid-binding globulin | |
| APOC1 | P02654 | Apolipoprotein C-I | |

| APOB | P04114 | Apolipoprotein B-100 | |
|------|--------|----------------------|---|
| CLU | P10909 | Clusterin | |
| APOC3 | P02656 | Apolipoprotein C-III | |
| LGALS3BP | Q08380 | Galectin-3-binding protein | |
| DPP4 | P27487 | Dipeptidyl peptidase 4 | |
| CD5L | O43866 | CD5 antigen-like | |
| IL1RAP | Q9NPH3 | Interleukin-1 receptor accessory protein | |
| COMP | G3XAP6 | Cartilage oligomeric matrix protein | |
| GPLD1 | P80108 | Phosphatidylinositol-glycan-specific phospholipase D | |
| QSOX1 | O00391 | Sulfhydryl oxidase 1 | |
| THBS4 | P35443 | Thrombospondin-4 | |