Proteomic Analysis Reveals sympathetic system and immune-inflammation biomarkers in serum of drug-free obsessive-compulsive disorder

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Article

Keywords:

Posted Date: May 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1650133/v1

Abstract

Obsessive-compulsive disorder (OCD) is a debilitating mental disorder, of which the mechanism is unclear. Biomarker detection will help in the mechanism exploration, early diagnosis and targeted treatment. Therefore, we conducted an iTRAQ-based proteomic analysis to compare serum proteome profile between OCD patients and healthy control, in order to find out the possible biomarkers of OCD and their underlying mechanisms. 81 drug-free OCD patients and 78 healthy controls were enrolled. A total of 479 proteins were identified and only those with a fold change ≥ 1.2 and p value 0.05 were accepted as differentially expressed proteins (DEPs). 7 up-regulated proteins, and 28 down-regulated ones were discovered. Besides dopamine beta-hydroxylase that plays a part in sympathetic system, lots of other DEPs are involved in immuno-inflammatory process. These DEPs were enriched in 44 gene ontology (GO) terms. However, none of KEGG pathway was detected significantly enriched. Our study suggested these sympathetic system and immuno-inflammation related proteins as potential biomarkers of OCD. Especially, immuno-inflammation may play an important role in OCD pathophysiology. Further researches employing larger sample sizes, longitudinal design and multi-omics methodology will be needed to verify our results and clarify the role of DEPs in OCD.

Introduction

Obsessive-compulsive disorder (OCD) is a debilitating mental disorder characterized by recurrent, unwanted thoughts (obsessions) and repetitive behaviors (compulsions). The lifetime prevalence of OCD is about 2–3% over the world and 2.4% in China(1, 2). Currently, our limited understanding about its pathophysiology led to the difficulties in the diagnosis and treatment. On the one hand, diagnoses of OCD based on clinical interviews, lacking of objective assay-based evidence. On the other hand, up to 40–60% of OCD patients didn't response to clinical treatment(3). If reliable biomarkers of OCD were available, early detection, early diagnosis and targeted treatment would be possible.

Researchers have been working hard to explore those possible biomarkers of mental disorders by genomics(4), transcriptomics(5), lipidomics(6) and proteomics(7) in the past years. However, most of results were inconsistent, but they highlighted the importance of pathways and circuits rather than single biomarkers(8). As proteins execute majority of functions in every organism as the downstream performers, proteomics is becoming a more promising tool in discovering potential biomarkers(9).

Proteomics can be detected either in post-mortem brain or in peripheral tissues. However, whether proteins of post-mortem brain can reflect the state in vivo is still controversial. With the advantages of minimal invasion and reflecting features in vivo, blood-based proteomic studies have been attracting more and more attention. There have been lots of proteomic researches in schizophrenia(10), depression(11), bipolar disorder(12) and so on. Nonetheless, studies of proteomics were scarce and were with extremely small sample sizes in the field of OCD(13, 14). Therefore, the aims of this study were to determine 1) whether there were specific proteomic biomarkers in serum in OCD patients compared with

healthy controls, and 2) whether those biomarkers were related to any certain functional pathways or mechanisms.

Materials And Methods

Subjects

81 drug-free OCD patients were recruited consecutively at the mental health clinics of Shanghai Mental Health Center (China) in 2015. OCD participants were included in the study if they 1) met the DSM-IV diagnostic criteria for OCD; 2) had a Yale-Brown Obsessive-compulsive Scale (Y-BOCS) total score(15)≥16; 3) were between 18 and 54 years old; 4) had at least a junior high school education; 5) never be treated with any psychiatric medicine or had been treated irregularly and had already stopped taking medicine for at least eight weeks; and 6) were in sufficient health to complete the research. Individuals were excluded if they: 1) met any other DSM-IV axis I diagnosis; 2) had obsessive and/or compulsive symptoms which were too heavy such they could not complete the research; 3) had any prior or current suicide attempts; 4) were pregnant or lactating; 5) were in physical health condition such that they could not complete the study; or 6) had any prior or current history of drug abuse.

78 healthy people were recruited as control participants from the staff of the Shanghai Mental Health Center and from community individuals recruited from advertisement. Individuals who met DSM-IV criteria for any Axis I psychiatric disorder were excluded. Healthy controls were also required to meet all the inclusion and exclusion criteria described above with the exception of the items relating to OCD status.

This study was approved by Ethics Committee of Shanghai Mental Health Center. Written informed consent was obtained from each participant.

Clinical assessment

DSM-IV Axis I psychiatric diagnoses were screened using International Neuropsychiatric Interview (MINI) (16), a structured diagnostic psychiatric interview for DSM-IV and ICD-10. All the OCD patients were diagnosed by senior attending psychiatrists or chief psychiatrists. Socio-demographic and additional clinical information were collected using a semi-structured interview with a format we developed. The Y-BOCS was used to assess OCD symptom severity. All the assessment work was performed by researchers who had been trained specially for this study.

Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) Quantitative Proteomic Analysis

iTRAQ proteomic analysis process including sample collection, removal of high abundant proteins, iTRAQ labeling and SCX-based fractionation, LC-MS/MS analysis and MS data analysis were presented in supplementary methods.

Data analysis

Demographic data and clinical characteristics were analyzed using Chi-squared or t-test. Statistical analyses were conducted using SPSS 26 (SPSS Inc., Chicago, IL, USA).

Proteins with p□0.05 and fold changes≥1.2 were considered as differentially expressed proteins (DEPs). All peptide ratios were normalized by the median protein ratio that should be 1 after the normalization.

Bioinformatic analyses were perform on all DEPs. GO mapping and annotations were made via Blast2GO software (Version 2.7.0). GO enrichment analysis and KEGG pathway analysis was conducted using R package “clusterProfiler”. The threshold was set as p□0.05 and q value≤0.2. Clustering analysis was made using R package “pheatmap” via average linkage method. The STRING Database v11.5(http://stringdb.org/) was used to analyze the protein–protein interactions.

Results

Comparison of socio-demographic characteristics

The socio-demographic characteristics were presented in Table 1. The age, sex and education years between two groups were well-matched generally.

Table 1
Socio-demographic data and clinical characteristics of obsessive-compulsive disorder group and healthy control group

	OCD(n = 81)	HC(n = 78)	t/χ^2	p
Age (years: mean ±SD)	29.15 ± 7.22	27.85 ± 5.73	1.26	0.21
Gender				
Male (n, %)	43(53.1%)	44(56.4%)	0.18	0.67
Female (n, %)	38(46.9%)	34(43.6%)		
Age of onset (years)	19.35 ± 8.14	-	-	-
Duration of disease(months)	103.94 ± 73.45	-	-	-
Education years	14.74 ± 2.49	15.33 ± 2.42	1.52	0.13
YBOCS	25.35 ± 5.39	-	-	-
HAMA	8.33 ± 3.514	-	-	-
HAMD	12.71 ± 6.146	-	-	-

Quantitative LC-MS/MS Analysis and clustering analysis

Totally 169146 spectra were detected, containing 11918 peptide-matched spectra. Totally 3587 peptides, and among which 3446 unique peptides were detected. These peptides were referred to 479 proteins (Supplementary Table 1). There were 35 DEPs between two groups, among which 8 proteins were up-regulated and 27 were down-regulated in OCD (fold of change ≥ 1.2, p□0.05) (Table 2). Results of clustering analysis showed that OCD group and healthy controls were well divided by DEPs (Fig. 1).

Table 2
Analysis results of differential protein expression

Accession	Gene name	Description	average fold change	*p* value
P55056	APOC4	Apolipoprotein C-IV	1.21313	0.0010328
P01591	JCHAIN	Immunoglobulin J chain	1.3564	0.0054881
Q7L8C5	SYT13	Synaptotagmin-13	1.3534833	0.0187794
P09172	DBH	Dopamine beta-hydroxylase	1.2941067	0.0294702
P05090	APOD	Apolipoprotein D	1.2706	0.0368922
P01023	A2M	Alpha-2-macroglobulin	1.3257867	0.038175
P01880	IGHD	Ig delta chain C region	1.5334033	0.0470741
Q9NP70	AMBN	Ameloblastin	1.3646133	0.0487134
Q9Y2K9	STXBP5L	Syntaxin-binding protein 5-like	0.434693	0.0001628
Q6UX71	PLXDC2	Plexin domain-containing protein 2	0.693374	0.0003151
Q9C0D2	CEP295	Centrosomal protein of 295 kDa	0.376205	0.0003249
P14618	PKM	Pyruvate kinase PKM	0.7741803	0.0015627
P03950	ANG	Angiogenin	0.8031237	0.0024506
P33151	CDH5	Cadherin-5	0.8327187	0.0033146
Q9BYK8	HELZ2	Helicase with zinc finger domain 2	0.430094	0.0037792
P34096	RNASE4	Ribonuclease 4	0.5779657	0.00467421
Q8NEN0	ARMC2	Armadillo repeat-containing protein 2	0.4490583	0.00508739
P28799	GRN	Progranulins	0.6227103	0.00616178
B9A014	C21orf140	Uncharacterized protein C21orf140	0.8009367	0.00835616
P58166	INHBE	Inhibin beta E chain	0.8204983	0.00841598
P08581	MET	Hepatocyte growth factor receptor	0.7227637	0.011765
Q15828	CST6	Cystatin-M	0.767616	0.0144019
P29401	TKT	Transketolase	0.7682063	0.0182818
Q12841	FSTL1	Follistatin-related protein 1	0.8248383	0.0214975
Q13822	ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2	0.6769353	0.0241561

Accession	Gene name	Description	average fold change	*p* value
Q12907	LMAN2	Vesicular integral-membrane protein VIP36	0.8034757	0.0244395
P22692	IGFBP4	Insulin-like growth factor-binding protein 4	0.6872093	0.0248987
A1L4H1	SSC5D	Soluble scavenger receptor cysteine-rich domain-containing protein SSC5D	0.6653747	0.0250695
Q9HCL0	PCDH18	Protocadherin-18	0.6770457	0.0309427
Q8TER0	SNED1	Sushi, nidogen and EGF-like domain-containing protein 1	0.5444963	0.030944
Q07075	ENPEP	Glutamyl aminopeptidase	0.7636663	0.0374732
Q99623	PHB2	Prohibitin-2	0.7632467	0.0395655
Q4LDE5	SVEP1	Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1	0.712105	0.040202
P08833	IGFBP1	Insulin-like growth factor-binding protein 1	0.789259	0.040754
Q8WWQ8	STAB2	Stabilin-2	0.7520403	0.0453012

Gene Ontology (Go) Analysis

These DEPs between two groups were analyzed using annotations in GO database. Results showed that these DEPs were involved in biological processes (30 proteins in 223 biological processes), molecular functions (33 proteins in 84 molecular functions) and cellular components (32 proteins in 80 cellular components) (Supplementary Table 2). At level 2, the DEPs were enrolled in 16 biological processes, 8 molecular functions and 8 cellular components (Fig. 2).

Enrichment analysis revealed that the proteins were enriched in 24 significant cellular component categories and 20 significant molecular function categories (Supplementary Table 3). The top 10 enriched GO terms in both categories were presented in Fig. 3, respectively.

Kegg Pathway Analysis

Detected KEGG pathways contained carbon metabolism, cytokine-cytokine receptor interaction and so on (Supplementary Table 4). However, no pathway was found significant in enrichment analysis.

Protein-protein Interaction Analysis

Four up-regulated proteins (A2M, APOC4, APOD, AMBN) and ten down-regulated proteins (ANG, CDH5, ENPEP, GRN, IGFBP1, IGFBP4, MET, PKM, TKT) were involved in the Protein-protein interaction (PPI) network (Fig. 4). The parameters used to construct the network were shown in Supplementary Table 5.

Discussion

This study shows discovered 35 DEPs between OCD patients and healthy controls, among which 8 are up-regulated and 27 are down-regulated in OCD. Two proteins (DBH, GRN) were previously thought to be possibly related to OCD. Three proteins were widely discussed in other mental disorders but not reported in OCD before (A2M, APOD, IGFBP1). Among these proteins, DBH functions as a vital molecule in sympathetic system, others are important part in immuno-inflammation system. However, we didn't detect significantly enriched KEGG pathways, which limited our power to illuminate the mechanism of OCD. Nevertheless, results of GO enrichment still provided some clues. Several immuno-inflammatory related GO terms were enriched in our results. Concerning the protein functions and PPI network, our results are in favor of the sympathetic alteration and the existence of immune-inflammatory mechanism in the pathophysiology in OCD.

We found dopamine beta-hydroxylase (DBH) protein expressed higher in OCD. DBH is an enzyme critical in the process of synthesizing norepinephrine (NE) from dopamine, of which the serum level was thought to be an indicator of sympathetic activity (17). Studies proved that DBH played a meaningful role in schizophrenia, depression, attention deficit hyperactivity disorder and so on (18, 19). There was a study in mice suggesting that Dbh-/- mice virtually lacked obsessive-compulsive behaviors seen in control mice, indicating a potential role of DBH and NE in stress-induced compulsive behaviors(20). Though the abnormal sympathetic activity could be interpreted as the heightened autonomic arousal symptoms in OCD, previous researches regarding sympathetic activity in OCD patients yielded inconsistent results (21–23). Gender differences may be a source of the heterogeneity(24). Therefore, though our study suggested an overall elevation of serum DBH level in OCD, stratification by sex will be needed in future study. Also, the role of norepinephrine in OCD pathology and clinical treatment strategy using anti-adrenergic drugs needs further ascertainment.

Other proteins in our study are mostly related to immuno-inflammation. In fact, researchers have been paying more and more attention on the inflammation mechanism in mental disorders. It was suggested that inflammatory inducers induced by environmental triggers activate the nervous system inflammation. Exposure to inflammatory cytokines may lead to CNS inflammation, affecting key brain areas and driving to clinical symptoms. It may be a vicious spiral that clinical symptoms are also the risk factors of inflammation(25). Inspired by the discovery of the Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal infection/ Pediatric Acute onset Neuropsychiatric Syndrome (PANDAS/PANS), the inflammation and autoimmunity hypothesis of OCD has attracted researchers' attention (26). Evidence of serology(27), radiology (28) and therapeusis(26) cumulated. Previous serology clues focused on the cytokines including TNF-α, IL-6, IL-1β and so on(27). There was also a proteomic study in OCD highlighting haptoglobin and hemopexin, which were both immuno-inflammation

related proteins. However, the small sample size of that study posed challenge to its power(14). In our study, we provided support for the immuno-inflammatory hypothesis of OCD again.

Progranulins (GRN, or PGRN) is a protein we found decreased in OCD. GRN is a key regulator of inflammation widely expressed in microglia and neurons(29), and also a neurotrophic factor whose deficiency may result in frontotemporal dementia (FTD), a kind of dementia frequently manifesting obsessive-compulsive behaviors(30). In previous work, we supposed some shared pathophysiological factors between OCD and FTD and made a gene*environment analysis, revealing the interaction between GRN gene and the early trauma in OCD (31). There was also a study reported that Grn−/- mice could develop excessive self-grooming behavior (32). Our result was in accordance with previous studies, revealing a complementarity between genomic and proteomic studies and suggesting that GRN may be an important molecule and a promising biomarker in OCD, worthy of further exploration.

Besides GRN, we also found A2M, APOD, IGFBP1 and IGFBP4 significantly altered in serum level of OCD. These proteins are connected intactly in the PPI network that may imply the existence of immuno-inflammatory mechanism.

Alpha-2-macroglobulin (A2M), one of the most important acute phase proteins, was found increased in OCD. A2M was mostly studied in Alzheimer's disease as a biomarker indicating systematic inflammation(33). There were literatures reporting the elevated serum level of A2M in schizophrenia(34) and depression(35). However, there was also another study reporting its lower serum level in drug-naïve schizophrenia and even lower level in those at-risk-of-psychosis(36). It's uncertain whether drug usage have any effect on the altered level of A2M. A2M was also a candidate biomarker for treatment response(37, 38). Furthermore, there were researches studying about A2M at genetic or transcriptive level, both were in favor of the role A2M playing in mental disorders(39, 40). Our study reported the elevated level of A2M as a possible biomarker in OCD patients for the first time. Nevertheless, inflammation influenced by other factors such as depressive symptoms was not ruled out in our study. Further studies taking other confounding factors into consideration will be needed. Longitudinal cohort study is also required to outline the trajectory of A2M level during the development of disease, which is vital to tell the inflammatory mechanism of OCD.

We found the elevated serum level of Apolipoprotein D (APOD) in OCD patients for the first time. APOD is an atypical apolipoprotein transporting extracellular lipid, which highly expresses in the nervous system(41) and is the major protein in cerebrospinal fluid(42). Besides, APOD is associated with membrane phospholipid signal transduction and metabolism(43). It has been reported as a potential biomarker in several mental disorders. However, the results were mixed. There was a study found APOD plasma level higher in drug-naive schizophrenia patients(44). This alteration may be explained by the compensatory neuroprotective effects of APOD by attenuating lipid peroxidation. Conversely, other studies found APOD serum level lower in schizophrenia patients(43, 45–47). This contradiction may be owing to the effect of atypical antipsychotics usage. Studies of major depressive disorder (MDD) also met with mixed results (48–51). Nevertheless, it was noteworthy that APOD level was found decreased in

serum but elevated in central nervous system in schizophrenia and bipolar disorder patients (43, 45). It may suggest the region-specific effect of APOD compensatory to the systemic lipid metabolism insufficiencies in mental disorder. In our study, we found serum level of APOD higher in OCD. As previous studies also reported the elevation of lipid peroxidation in OCD(52, 53), it is possible that APOD alteration represents a nonspecific response to the disturbed lipid metabolism shared by OCD and other mental disorders. As our samples were from drug-free patients, the effects of drug usage can be ruled out. Though few studies focus on it, the abnormal lipid peroxidation induced area-specific neuronal cell damage may play a part in OCD pathophysiology(52). Due to the essential role of lipid in brain, lipid metabolism will be a promising direction in OCD research. However, the cross-sectional design limited our power to detect the causal relationship. Longitudinal studies and multi-omics analysis will help to understand the pathophysiological role of APOD in OCD.

Insulin-like growth factor-binding protein 1(IGFBP1) and Insulin-like growth factor-binding protein 4 (IGFBP4) we found down-regulated in OCD were both important members in the insulin-like growth factor (IGF) system, which consists of growth peptides (IGF1/2), receptors (IGF1R/2R), and circulating binding proteins (IGFBPs 1–6). IGF1 plays a critical regulatory role in many human tissues. Furthermore, it is famous for its far-reaching effect on immunological system(54). When it comes to mental disorders, IGF1 has been documented as a mood regulator(55) of which the serum level was altered in affective disorders and schizophrenia (56). IGFBPs bind to IGF1 in circulation (57), acting as dynamic regulators of IGF bioactivity by transporting IGF1 and regulating IGF metabolism (58). IGFBP1 was found inversely correlated with the level of free IGF1 in healthy participants(59). Furthermore, IGFBP1 also took part in the immunological system by manifesting positive bilateral regulatory effect with proinflammatory cytokines (60). IGFBP4 mRNA expression was also found increased in hippocampus of schizophrenia patients, indicating the activation of immuno-inflammation-related pathways(61). Studies about IGF system in OCD were scarce. There was one study suggesting a higher serum level of IGF1 in OCD(62), while higher serum level in OCD patients was found correlated with better SSRI responses in another study(63). None of literatures about IGFBPs in OCD were available. In our study, we found IGFBP1 and IGFBP4 downregulated in OCD, but none of significant difference was observed between groups with regards to IGF1, which was different from previous studies. IGFBP1 is rapidly regulated by metabolic status (57). Therefore, we can’t rule out the possibility that the different expression of IGFBP1 was misted by metabolic difference between groups. Also, it was uncertain that whether IGFBP1 and IGFBP4 function in IGF-dependent pathways in OCD. Alternatively, it may also imply a dynamic cooperation mechanism to maintain the balance of IGF system. As shown in interaction network, A2M may also interact with IGFBPs in IGF regulation via IGFBP1/A2M combination, though the exact mechanism was unknown(64). Longitudinal and targeted studies towards IGF systems in OCD will be needed to clarify the pathophysiological role of IGF system molecules.

Furthermore, our study also reported the elevated level of Ig delta chain C (IGHD) and immunoglobulin J chain (JCHAIN). There was a study reporting that Ig kappa chain C was decreased in drug-naïve OCD but was elevated after treatment(13). These results may imply some alteration of secretory immunology in OCD, but the exact pathological meaning was uncertain. There are still other promising proteins in our

study, whilst the lack of literatures made it hard to tell their pathophysiological roles. For example, ribonuclease 4 (RNASE4) and angiogenin (ANG) that promote neurogenesis by stimulating angiogenesis(65, 66), which were closely related and both were found downregulated in our samples; syntaxin-binding protein 5-like (STXBP5L) that regulates the neurotransmission presynaptically and may play an important role in mental disorders(67, 68), which was detected with extremely low expression and extremely small p in our result; pyrophosphatase/phosphodiesterase family member 2 (ENPP2) that regulates myelin formation and maintenance, which was detected decreased in the postmortem interval delay of MDD(69).

Limitations

Our study had limitations. Firstly, we didn't rule out some of metabolism-related confounded factors such as BMI, depression status and so on. Secondly, due to the cross-sectional study design, we can't make conclusion on the causal relation between OCD and protein alterations. Thirdly, we enrolled only 81 OCD patients and 78 healthy controls in our study. Studies with larger sample sized will help to validate our results and explore more clues.

Our study was conducted based on serum samples. It still needs further research to determine that to what extent the serum proteins alteration can represent the biological changes in central nervous system. Although proteomics is a promising approach to find out the biomarkers of OCD, it only manifested a cross-section in the downstream biological process and only reflect a one-sided perspective of OCD pathophysiology. It's crucial to conduct multi-omics study to clarify the biological mechanism of OCD.

Conclusion

We made an iTRAQ-based proteomics analysis between drug-free OCD patients and healthy controls. DEPs were discovered. Besides DBH that reflecting sympathetic activity, other DEPs reported in mental disorders before were mostly enrolled in immuno-inflammatory process. Our study suggested these sympathetic-system-related or immuno-inflammation-related proteins as potential biomarkers of OCD. However, further researches employing larger sample sizes, longitudinal design and multi-omics methodology are warranted to validate our results and clarify the role of these DEPs in OCD.

Declarations

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81971261), Hospital Project of Shanghai Mental Health Center (2019-YJ15) in the sample collection and proteomic sequencing.

We thanked for the substantial support from all the patients and healthy volunteers. Also, we thanked every member in our group exploring the mechanism of OCD and better strategy of OCD treatment.

Conflict of interest

The authors declared none of conflict of interest.

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Figures

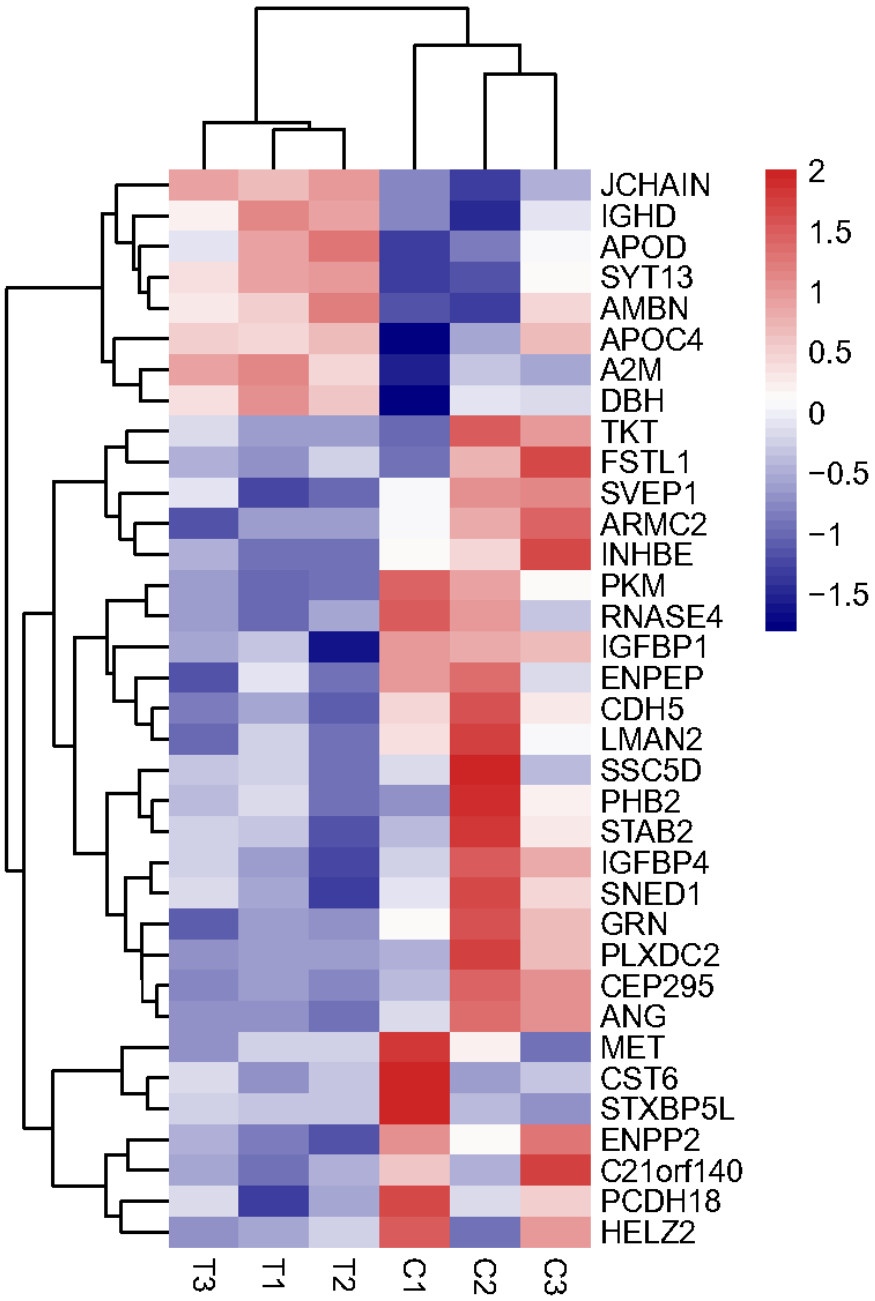

Figure 1

Heat maps of differentially expressed proteins (DEPs). T1/T2/T3 represented the merged 3 samples in obsessive-compuslsive disorder (OCD) group while C1/C2/C3 represented the merged 3 samples in healthy control group mentioned in methodology.

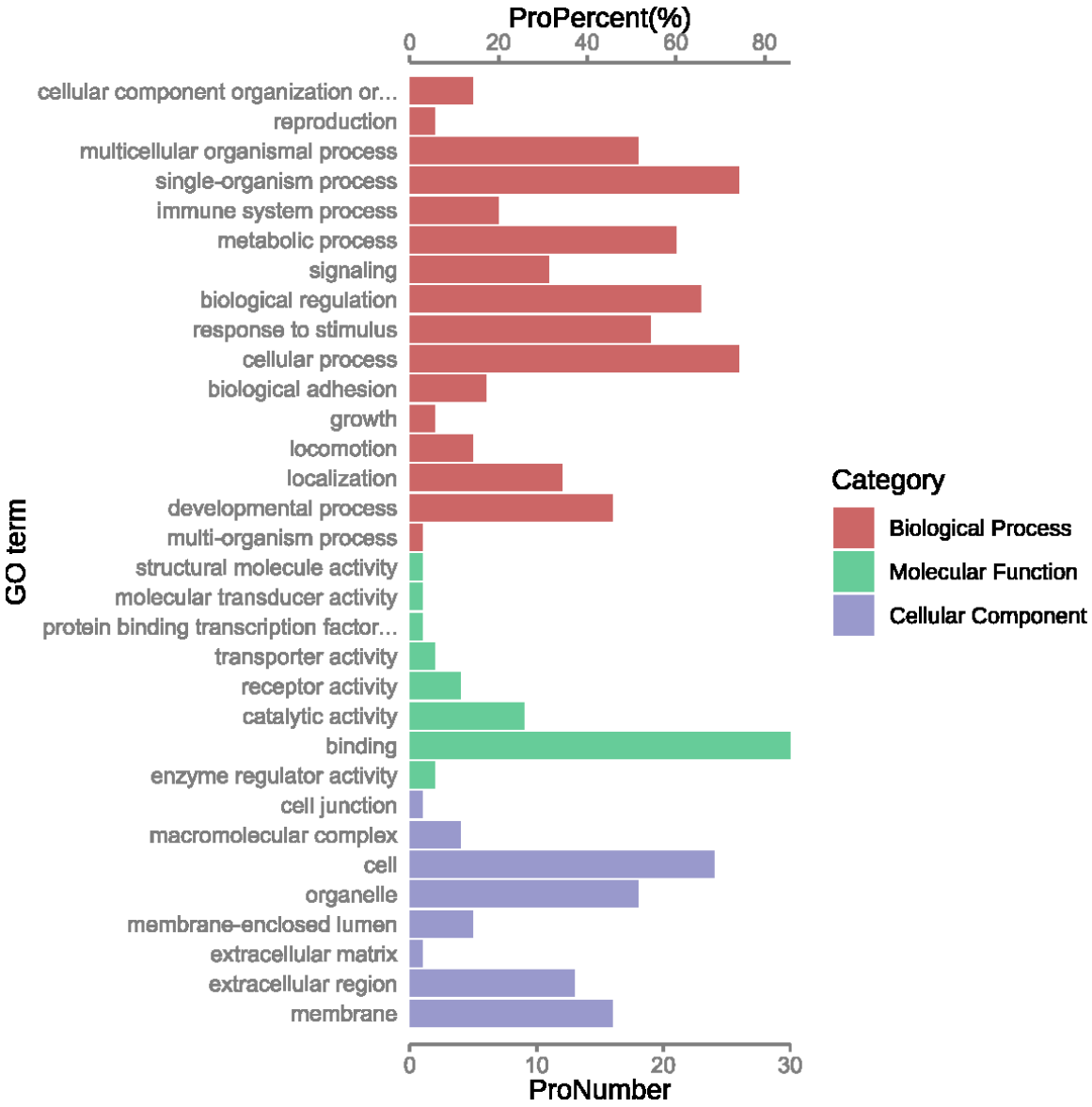

Figure 2

Distribution of gene ontology (GO) functional categories in obsessive-compulsive disorder (OCD) and healthy controls at level 2.

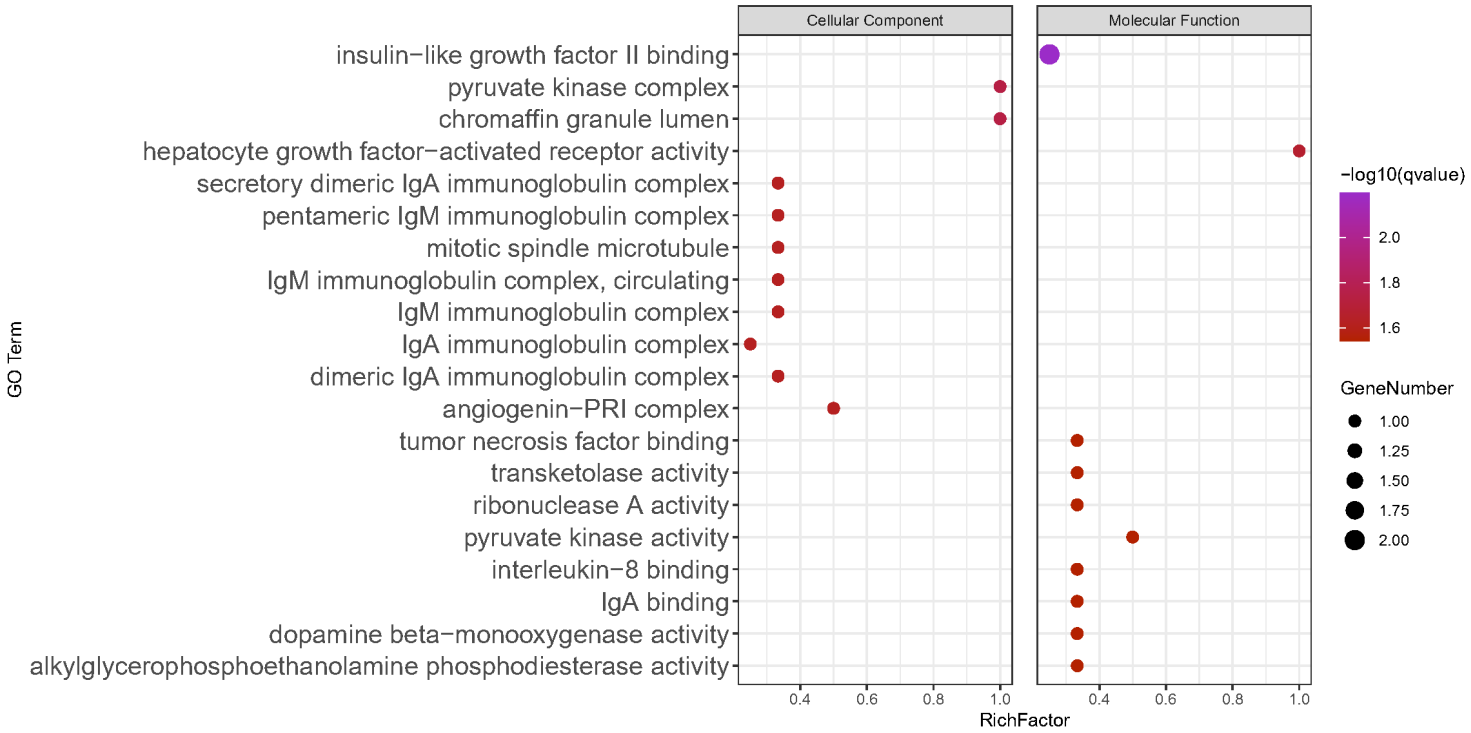

Figure 3

Bubble plot presenting the top10 significant gene ontology (GO) terms in different functional categories in GO enrichment results

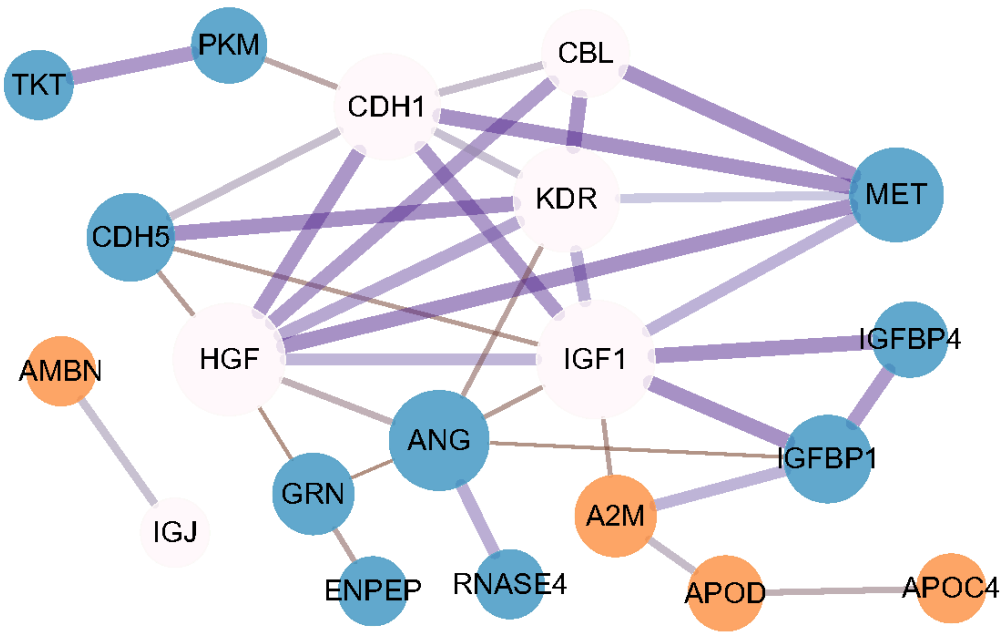

Figure 4

Protein-protein interaction (PPI) network based on STRING database. Proteins that were not detected with PPI in our differentially expressed protein (DEP) dataset were not presented. Nodes in orange represented the up-regulated proteins, nodes in blue represented the down-regulated proteins. Nodes in white represented those proteins not existed in our DEP dataset, but may serve as a cross convergence in the network. The sizes of the nodes represented the degree (the number of links between the node and other nodes) of the node. The strength of data supporting the connection between nodes (combined score) was mapping to the width and the color gradient from purple to brown of the line.

Supplementary Files

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

- SupplementaryMethods.docx
- SupplementaryTable1.xlsx
- SupplementaryTable2.xlsx
- SupplementaryTable3.xlsx
- SupplementaryTable4.xlsx
- SupplementaryTable5.xlsx