Semi-quantitative analysis of serum proteome in patients with bipolar disorder

TOMSK NRMC

Mental Health Research Institute
Tomsk National Research Medical Center of the Russian Academy of Sciences
Tomsk, Russia

Seregin Alexander Alexandrovich
email: apocalips1991@mail.ru

Smirnova Lyudmila Pavlovna
email: lpsmirnova@yandex.ru

Simutkin German Gennadievich
email: ggsimutkin@gmail.com

Introduction

Bipolar disorder (BD) is a severe chronic recurrent mental illness that affects about 0.5 to 2% of the population (A.S. Clemente et al, 2015). The consequences of the disease are significant financial costs of health care for the treatment and rehabilitation of patients, in addition, the disease has a negative effect on patients and their relatives. This leads to a significant decrease in the quality of life of patients and increases the risk of suicidal behavior. The disease often has a similar clinical picture with many mental disorders. Significant difficulties are the differential diagnosis of BD with unipolar depression, as well as with schizophrenic spectrum disorders, and also high comorbidity with other mental disorders complicates the diagnosis. It is known that in some cases it takes about 10 years to establish the correct diagnosis (Hirschfeld R.M. et al., 2003). Which often leads to overdiagnosis of BAR, unreasonable long-term therapy with antipsychotics and antidepressants, aggravation of the disease, social maladaptation and disability of patients. The problem is complicated by the absence of paraclinical criteria for the differential diagnosis of these disorders (Marusin A.V., 2016) This is due to a lack of understanding of the molecular mechanisms of the pathogenesis of BD. In recent years, interest in proteomic studies of mental disorders has increased, and research in this direction has been intensively conducted (Dudley E., 2011). Identification of marker protein or regulatory proteins involved in the pathogenesis of BD in the biomaterial available for diagnostic purposes - blood serum - will bring us as close as possible to understanding the specific pathogenetic mechanisms of this disorder and can serve as the basis for new methods for the differential diagnosis and development of new pathogenetically based drugs.

Patients

In this work, the protein spectrum of blood serum of the following groups of patients was analyzed:

- 45 patients with bipolar disorder who were inpatients of the Department of Affective States of Mental Health Research Institute.

Patients

Distribution of patients with DB by gender and age

Feature	Value
age	32 [21;52]
disease duration	8 [5;11]

- The control group consisted of 24 mentally and somatically healthy volunteers matched to the study groups by gender and age.

Methods

Blood serum was taken from the ulnar vein in the morning on an empty stomach before starting therapy.

- **Affinity chromatography.** The serum was purified from 6 major proteins — albumin, immunoglobulin G, immunoglobulin A, antitrypsin, transferrin and haptoglobin (Chromatograph, Agilent Technologies 1200);
- **Concentration.** The purified proteins were concentrated to 1 ml using Amicon Ultra-0.5 ultrafilters (MILLIPORE) at 3 kDa.
- **Vertical protein electrophoresis** in PAGE using Laemmli U.K. using a 12% polyacrylamide gel (Protean II xi Cell from Bio-Rad, USA). Then, trypsinolysis of whites in a gel was carried out, followed by extraction;
- **Mass spectrometry.** Proteins were identified by HPLC / mass spectrometry on an LTQ Orbitrap Velos mass spectrometer (Thermo Scientific). The analysis was performed at IBMC, Moscow (Human Proteom Collective Use Center).
- **Data analysis.** Data was analyzed using MaxQuant (version 1.6.3.4). A false detection rate (FDR) of 0.01 for proteins and peptides and a minimum peptide length of 6 amino acids. Search system - Andromeda was used to search for spectra based on UniProt. Enzymatic specificity is trypsin. A maximum of two skipped cleavages were allowed. Only proteins quantified by at least two peptides were considered. LFQ intensities for proteins were log2 transformed and normalized to ensure equal median concentration. A two-sided unpaired t-test with an FDR of 0.05 and S0 = 2 was used to identify proteins whose content was significantly changed between the studied groups.

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RESULTS

As a result of mass spectrometric analysis, between 50 and 350 proteins in each lane were identified and about 1,600 proteins were identified for each person. Comparison of the proteome profiles of different groups revealed 18 proteins specific for BD. The most interesting of them from the point of view of possible participation in the pathogenesis of the disease are presented in the table.

The most interesting proteins uniquely found only in the blood serum of patients with BD

Uniprot id	Protein name	Gene	iBAQ	LFQ intensity
P15924	Desmoplakin	DSP	3,736,801	5,864,175
P17948	Vascular endothelial growth factor receptor 1	FLT1	4,665,292	6,331,822
P33151	Cadherin-5	CADH5	4,472,995	6,134,045
Q01538	Myelin transcription factor 1	MYT1	607,717	8,153,738

Proteins found in patients with BD, but missing from the control sample, are involved in the regulation of DNA and cell cycle synthesis, differentiation of progenitor cells, in the development of neurons and oligodendrocytes, such as **Myelin transcription factor 1** (MYT1), which regulates genes encoding myelin proteins and other central nervous system proteins (Singh S. M., 2010). This protein is a zinc finger DNA-binding protein that affects the proliferation, differentiation and transcription of myelin cell genes. MYT1 can regulate the critical transition point in oligodendrocyte lineage development by modulating oligodendrocyte progenitor proliferation relative to terminal differentiation and up-regulation of myelin gene transcription (Ryan, M., 2006). Ti Wang et al. Have shown that mutations in the MYT1L gene encoding a protein of the myelin transcription factor 1 family can increase the risks of major depressive disorder. However, a connection with pathogenesis has not yet been identified ([8] Ti Wang, 2010). A significant increase in the expression of the MYT1 gene in the prefrontal cortex of patients with BD is also known.

Desmoplakin (DSP) was also found in the serum of patients with BD, which participates in the organization of cadherin-placoglobin desmosome complexes, forming them in separate domains of the plasma membrane.

Cadherin 5 (CADH5) was also found in a group of patients with BD. The cadherin protein family not only provides mechanical contact between neighboring cells, but also participates in intracellular signal transduction, regulation of proliferation, migration, sorting, differentiation, and cell morphogenesis. In the tissues of adult organisms, cadherins regulate cell renewal, provide a physiological barrier between contacting tissues and selectivity of the transport of soluble substances.

Some mediators of the inflammatory response, such as the **vascular endothelial growth factor** (VEGFR1) we discovered, can interfere with the organization of contacts when their receptors are bound, thereby opening the barrier, and plasma proteins can pass through the endothelial barrier. In particular, VEGFR1 is involved in the initiation of autophosphorylation of cadherin 5 signaling cascades, which directly affect the development of endothelial dysfunction (Pang V, 2017). In addition, it is known that cadherins mediate cell sorting, migration, and segregation, morphogenesis, and axon growth in embryogenesis (Perez-Moreno M, 2003). Van et al. The involvement of genes encoding the CDH9 and CDH10 families have been demonstrated in autism (Wang K, 2009). However, there is no data yet, which indicates the participation of cadherin 5 in the pathogenesis of mental disorders, which requires further study.

Conclusions

The set of proteins in BD was mainly associated with the immune response, regulation of transport processes through the cell membrane and cellular communication. Many of these pathways are involved in the pathogenesis of mental disorders. They are interesting as potential markers. Cadherin-5 and vascular endothelial growth factor receptor 1 are a marker of endothelial dysfunction. The detection of these proteins indicates the presence of endothelial dysfunction in the pathogenesis of BD. Detection of desmoplakin may indirectly indicate a violation of the intercellular contacts of endotheliocytes. Identification of myelin 1 transcription factor and published data on the effect of mutations in the MYT1L gene on the risk of serious depressive disorder suggest that MYT1 protein is a potential BD biomarker. However, its role in the pathogenesis of BD remains to be seen. Further study of the proteins that we have identified as BD biomarkers may help uncover obscure aspects of pathogenesis and develop new paraclinical criteria for differential diagnosis of BD.

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