Review
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Potential Candidates for Biomarkers in Bipolar Disorder: A Proteomic Approach through Systems Biology

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Bipolar disorder (BD) is one of the most disabling diseases characterized by severe humor fluctuation. It is accompanied by cognitive and functional impairment in addiction to high suicide rates. BD is often underdiagnosed and treated incorrectly because many of the reported symptoms are not exclusive to the disorder. Once the diagnosis is exclusively clinical, it is not possible to state precisely. From that, proteomic approaches were used to identify, in a large scale, all proteins involved in cellular or tissue processes. This review aggregate data from blood proteomes, by using protein association network, of subjects with BD and healthy controls to suggest dysfunctional molecular pathways involved in disease. Original articles containing proteomic analysis were searched in PubMed. Seven studies were selected and data were extracted for posterior analysis. A protein-protein interaction network was created by STRING database. A final set of proteins in this network were employed as input in ClueGO and, the main biological process was visualized using R package pathview. The analysis revealed proteins associated with many biological processes, including growth and endocrine regulation, iron transportation, protease inhibition, protection against pathogens and cholesterol transport. Moreover, pathway analysis indicated the association of uncovered proteins with two main metabolic pathways: complement system and coagulation cascade. Thus, a better understanding on the pathophysiology of psychiatric disorders and the identification of potential biomarker candidates are essential to improve diagnostic, prognostic and design pharmacological strategies.

KEY WORDS: Blood; Biomarkers; Bipolar disorder; Proteomics; Systems biology.

INTRODUCTION

Bipolar disorder (BD) is a chronic psychiatric illness characterized by recurrent and alternating episodes of mood that are often separated by periods of remission, also called "euthymia" (the Diagnostic and Statistical Manual of Mental Disorders 5th edition, DSM-V) [1]. BD affects almost 3% of the North American population and is associated with long-term cognitive and psychosocial impairment [2,3]. It is also associated with high rates of mortality by both natural causes and suicide [4].

BD is often underdiagnosed and untreated because many of the reported symptoms, including irritability, sleep disturbance, impulsive behavior, alcohol and substance abuse, are not exclusive to the disorder. Indeed, the mood fluctuations as well as the chronic and heterogeneous course of BD make it one of the most challenging illnesses to diagnose and treat [5,6]. The diagnosis of mood disorders is made by assessing symptoms through clinical interviews and based on the criteria established in the DSM-V or the International Statistical Classification of Diseases and Health-Related Problems version 10 (10th revision, ICD-10) [7]. However, the establishment of well-defined boundaries between distinct diagnostic catego-

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ries can be challenging, especially among psychiatric disorder that share some biological aspects or have overlapping of symptoms such as BD and major depressive disorder (MDD) [8-11]. According to the literature, about 40% of BD patients are initially misdiagnosed as MDD, as most cases present with depressive episode at onset and seek medical assistance when depressed and not hypomanic. Consequently, BD patients might receive inadequate treatment which can aggravate the course of the illness and worsen the outcome. Therefore, the development of valid biomarkers for BD is critical to improve the diagnostic accuracy as well as to prognosis and treatment response in psychiatry [12].

The advances in omics approaches have created novel opportunities for identifying molecular signatures and/or biomarkers in various medical specialties, including psychiatry. For instance, proteomics is the science that allows the identification, in a large scale, of all proteins involved in cellular or tissue processes (i.e., proteomes) and seems to be essential for understanding the biological processes underlying health and disease [13,14]. As proteomics represents the translated and transcribed genetic information after epigenetic changes, it has been suggested that this analysis more reliably reflects the pathophysiology of a disease and the current state of the patient than genomic and transcriptomic analysis [15].

Therefore, a number of researchers throughout the world have preferred proteomics-based technologies in order to identify potential biomarker candidates for disease diagnosis, prognosis, and treatment response prediction. Within this rationale, some studies were also performed in BD [10,13,16-20]. The current review aimed to compare the plasma and serum proteomes of subjects with BD and health individuals using protein-protein interaction, in order to identify and propose associated dysfunctional biological pathways involved in BD.

METHODS

Studies Eligibility Criteria

For this review, we selected original articles reporting protein identification in the blood of individuals diagnosed with BD according to the following inclusion criteria:

- Studies including subjects with BD type I or II as confirmed by ICD-10, DSM-IV or DSM-V criteria;
- Comparative studies evaluating levels of protein in the peripheral blood (plasma or serum) of BD patients and healthy controls;
- Studies assessing protein levels in treated or drug-free patients with BD;
- Studies assessing protein levels in BD patients during euthymia or mood episodes;
- Studies using liquid chromatography and two-dimensional electrophoresis methods to separate proteins in peripheral blood of BD patients that differentiate or not the mood states and compared to healthy subjects;
- Studies that performed mass spectrometry or immunoassay multiplex analysis of proteins to identify the expression of proteins.

Search Strategy and Study Selection

Publications were searched on PubMed in July, 2020 using the following search strategy: ("Proteomic" OR "Proteomic biomarker" OR "proteome") AND ("Serum" OR "plasma" OR "Blood") AND ("Bipolar disorder" OR "bipolar" OR "psychiatric illness"). The search yielded a total of 35 original studies on proteomic analysis in patients with BD. Four authors (PRZ, JGF, JFG, and ARR) revised the abstracts and methodologies to identify studies that match the inclusion criteria. The studies reporting major depressive disorder, schizophrenia, psychotic episodes and dementia were excluded (n = 31) as shown in Figure 1. Three relevant studies found in the reference list of selected articles were also included. Finally, seven articles were included, and the extracted data included information on proteins accession numbers (Uniprot Consortium)

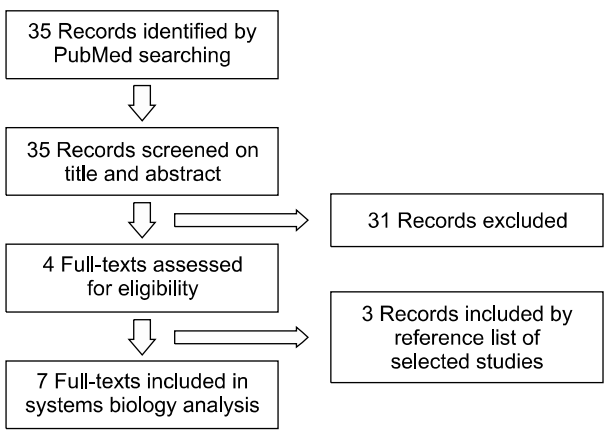

Fig. 1. Flowchart of eligibility criteria and research information.

and fold change (when available), BD diagnosis, and treatment (if applicable).

Proteins Selection and Pathway Analysis

The protein-protein interaction data was downloaded from STRING database [21]. The data was imported to R 3.6 and the interactions with combined score equal to or less than 0.7 were removed. This procedure keeps only interaction with high confidence. Based on the list of differentially expressed proteins, a network was generated. Then, proteins that were found to be differentially expressed in three or more studies were selected in the

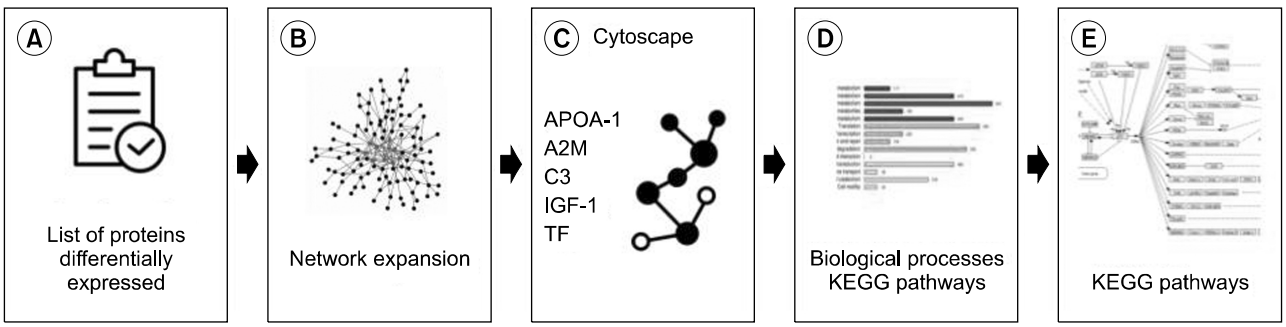

Fig. 2. Systems biology protocol. (A) A list of proteins differentially expressed was organized. (B) The list was imported to R 3.6.1 and the networks were created. (C) Based on the networks generated, the neighbors of the proteins differentially expressed found by three authors or more were selected. (D) This set of proteins was used as input to ClueGO. (E) The main biological process was visualized using the pathview R package as well as differentially expressed proteins.
APOA-1, apolipoprotein A-1; A2M, alpha-2-macroglobulin; C3, third component of complement; IGF-1, insulin growth factor-1; TF, transferrin; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 1. Proteomic studies identifying BD peripheral biomarkers

Author	Blood fraction	Subjects	Sample size	Proteomic technique	Results
Herberth *et al.* [19] (2011)	Serum	BD	32	LC-MS	22 differentially expressed analytes compared HC
		HC	32		
		BD	16	Multiplex	
		HC	15		
Alsaif *et al.* [16] (2012)	Serum	BD	24	Multiplex	6 (serum) and 10 (plasma) differentially expressed proteins in BD compared to HC
		HC	21		
	Plasma	BD	24		
		HC	21		2 changed proteins in both fluids
Haenisch *et al.* [18] (2014)	Plasma	BD	17	Multiplex	26 differentially expressed analytes compared HC
		HC	46		
Chen *et al.* [10] (2015)	Plasma	BD	20	2-DE/MS	3 differentially expressed proteins in BD compared to HC
		HC	30	Multiplex	
Song *et al.* [13] (2015)	Plasma	Euthymic	10	2-DE/MS	32 differentially expressed proteins in BD compared to HC;
		HC	20		
		Depressed HC	20		16 differentially expressed proteins in BD independently of mood;
		Manic	20		
		HC	15		16 proteins associated with particular mood.
			20		
de Jesus *et al.* [17] (2017)	Serum	BD	14	LC-MS/MS	6 differentially expressed proteins in BD compared to HC
		HCFN	9		
		BD	14		
		HCF	3		
Ren *et al.* [20] (2017)	Plasma	BD	30	LC-MS/MS	54 differentially expressed proteins in BD compared to HC
		HC	30		

BD, bipolar disorder; HC, healthy control; HCFN, non familiar healthy control; HCF, familiar healthy control; LC, liquid chromatography; MS, mass spectrometry; 2-DE, two-dimensional electrophoresis.

Table 2. Summary of diagnostic biomarkers in BD

Author	Groups	Up-regulated	Down-regulated
Herberth *et al.* [19] (2011)	BD × HC	C-C motif chemokine 16; tumor necrosis factor receptor superfamily member 5; CD40 ligand; connective tissue growth factor; endothelin-1; pro-epidermal growth factor; tumor necrosis factor ligand superfamily member 6; macrophage migration inhibitory factor; lymphotactin; luteinizing hormone; progesterone; testosterone; glutathione S-transferase A1; insulin-like growth factor-binding protein 2	Apolipoprotein A-I; C-C motif chemokine 26; immunoglobulin A; immunoglobulin M; interleukin-13; kit ligand; tumor necrosis factor; apolipoprotein- C-III
Alsaif *et al.* [16] (2012)	BD × HC (plasma)	Lipoprotein-A; adrenocorticotropic hormone	Alpha-2-macroglobulin; macrophage migration inhibitory factor; macrophage inflammatory protein-3a; sex hormone-binding globulin; tenascin-C; apolipoprotein A; insulin-like growth factor I; monocyte chemotactic protein-4; platelet-derived growth factor subunit B; stem cell factor; superoxide dismutase; transferrin
Alsaif *et al.* [16] (2012)	BD × HC (serum)	Lipoprotein-A; macrophage migration inhibitory factor; insulin-like growth factor I; stem cell factor; superoxide dismutase	Alpha-2-macroglobulin; macrophage inflammatory protein-3a; sex hormone-binding globulin; tenascin-C; apolipoprotein A; monocyte chemotactic protein-4; platelet-derived growth factor subunit B; transferrin
Haenisch *et al.* [18] (2014)	BD × HC	S100 calcium binding protein B; interferon gamma induced protein 10; clusterin; complement C3; granulocyte colony stimulating factor; osteopontin; prostatic acid phosphatase; TIMP-1; C-peptide; hepatocyte growth factor; insulin; insulin like growth factor I; intact proinsulin; total proinsulin; vascular endothelial growth factor; peptide YY; chromogranin A; alpha 1 microglobulin; beta 2 microglobulin; matrix metalloproteinase 7; vitamin K dependent protein S; cystatin-C; apolipoprotein H	Apolipoprotein AI; myoglobin; sex hormone binding globulin
Chen *et al.* [10] (2015)	BD × HC	-	Complement component 3 isoform CRA_a; C4b-binding protein alpha chain; complement factor 1
Song *et al.* [13] (2015)	BD × HC	Haptoglobin; apolipoprotein L1; afamin; pigment epithelium-derived factor; complement C4-B; vitamin D-binding protein; complement C4A3; carboxypeptidase B2; serotransferrin; fibrinogen beta chain; fibrinogen gamma chain; complement C3; hemopexin; keratin; complement subcomponent subunit C; complement factor I heavy chain; mannose-binding protein C; complement C4 gamma chain	Apolipoprotein A-I; carboxypeptidase N catalytic chain; N-acetylmuramoyl-L-alanine amidase; inter-alpha-trypsin inhibitor heavy chain H1; serum amyloid P-component; inter-alpha-trypsin inhibitor heavy chain H4; CD5 antigen-like; C4b-binding protein alpha chain; carbonic anhydrase 1; alpha-2-macroglobulin; complement factor H-related protein 1; complement C1r subcomponent; fibrinogen alpha chain
de Jesus *et al.* [17] (2017)	BD × HC	Albumin; apolipoprotein A-I	Complement C4-A; alpha-1-antitrypsin; apolipoprotein E; transferrin
Ren *et al.* [20] (2017)	BD × HC	Immunoblobulin light chain; full-length cDNA clone; immunoglobulin J chain; C4b-binding protein beta chain; hemoglobin beta subunit variant; myosin-reactive immunoglobulin heavy chain variable region; catalase; mutant hemoglobin alpha 2 globin chain; peroxiredoxin-2; carbonic anhydrase 1; superoxide dismutase; cDNA FLJ57106; haptoglobin; hemoglobin beta; alpha-2-macroglobulin; flavin reductase; Ig heavy chain variable region; beta globin; Ig kappa chain V-IV region Len; cDNA FLJ35079; insulin growth factor 1; IGL@; coagulation factor V; hemoglobin beta chain; protein S100; peroxiredoxin-1; alpha-1-acid glycoprotein 1; apolipoprotein A-I; anti-(ED-B) scFV; alpha-hemoglobin-stabilizing protein; delta-aminolevulinic acid dehydratase; immunglobulin heavy chain variable region; ATP-binding cassette sub-family B member 9; epididymis secretory protein; selenium-binding protein 1	cDNA FLJ60397; brain acid soluble protein 1; Rab GDP dissociation inhibitor alpha; secreted phosphoprotein 24; amyloid beta A4 protein; endoglin; cDNA, FLJ95014; cathepsin S; thyroid peroxidase; ATP synthase subunit alpha; eukaryotic translation elongation factor 1 alpha; suprabasin; protein crumbs homolog 1; platelet endothelial cell adhesion molecule; sulfhydryl oxidase; trans-Golgi network integral membrane protein 2; multimerin-1; platelet basic protein; keratin-associated protein

BD, bipolar disorder; HC, healthy control.

network. From this set, neighboring proteins of up to two degrees were selected, since proteins that are close tend to take part in similar biological processes. Each identified protein was converted and mapped onto its corresponding gene object. The final set of proteins was employed as input in ClueGO v2.5.7 (a Cytoscape v3.8 plug-in) [22,23] with the following parameters: two-sided hypergeometric (statistical test for the enrichment), Bonferroni step down correction, and kappa score of 0.4. In order to visualize the main biological process, the R package pathview version 1.24 [24] was used. Systems biology protocol was illustrated in Figure 2.

RESULTS

The characteristics of all included studies are shown in Table 1. The set of proteins found to be differentially expressed between BD and healthy subjects in each study is listed in Table 2.

Study-protein Network

We generated two types of networks: study-protein (Figs. 3 and 4) and protein-protein (Fig. 5) interaction networks. Study-protein networks have two types of nodes, one that represents each study and another that represents the proteins identified as differentially expressed in the studies. In order to maintain the accuracy of the results obtained, our analysis was made through the presentation of each author and the type of sample (serum or plasma). Furthermore, it has a comparison made by mood state (depression, euthymia, or mania) which was described in one study [13]. Usually, most sets of differentially expressed proteins found by each study are exclusive, unique, and specific. It is possible to observe that protein expression can differ within and between studies. For instance, Alsaif *et al.* [16] found that insulin growth factor-1 (IGF-1), superoxide dismutase 1, KIT ligand, and macrophage migration inhibitory factor displayed an opposite expression profile in plasma (underexpressed) compared to the serum (overexpressed).

Overall, we observed heterogeneity among studies re-

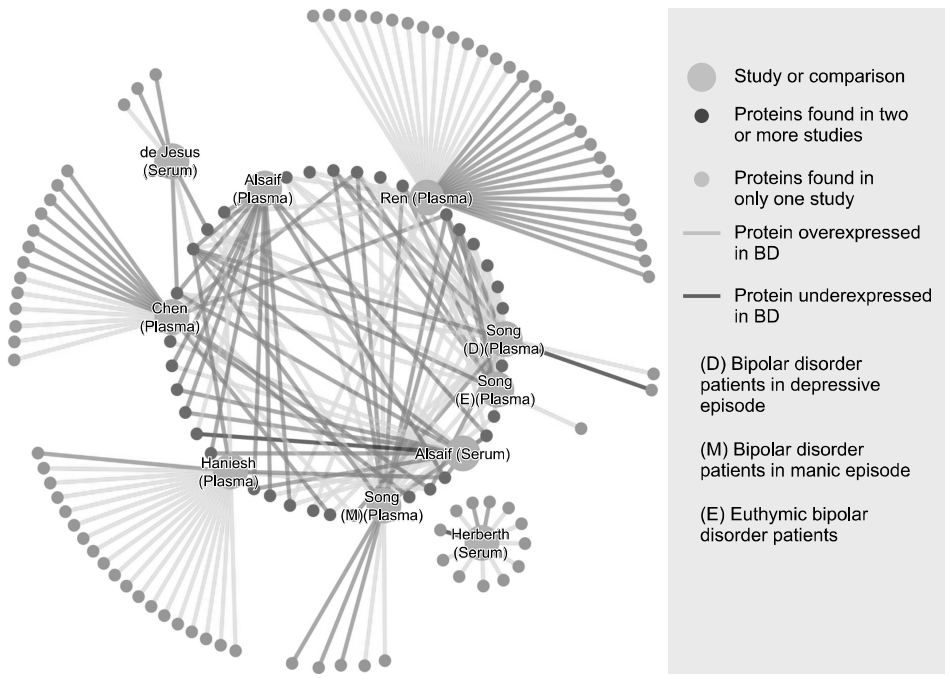

Fig. 3. Study-protein interaction network. The seven studies selected presented individual and shared proteins. Alsaif *et al.* [16] found a shared set of proteins between plasma and serum. The proteomics profile found by Song *et al.* [13] was very similar between different disease states and shared some proteins with the profile found by Ren *et al.* [20] and Chen *et al.* [10]. Alsaif presented results based on sample type (serum and plasma), meanwhile, Song stratifies studies based on different disease stages (depression, euthymia, and mania). The rest of the authors did not differentiate the disease stage. All studies, except Alsaif, found uniquely expressed proteins. One study (Herberth *et al.* [19]) showed 12 uniquely expressed proteins, therefore there were no proteins in common with other studies and it was not bound to the main network.
BD, bipolar disorder.

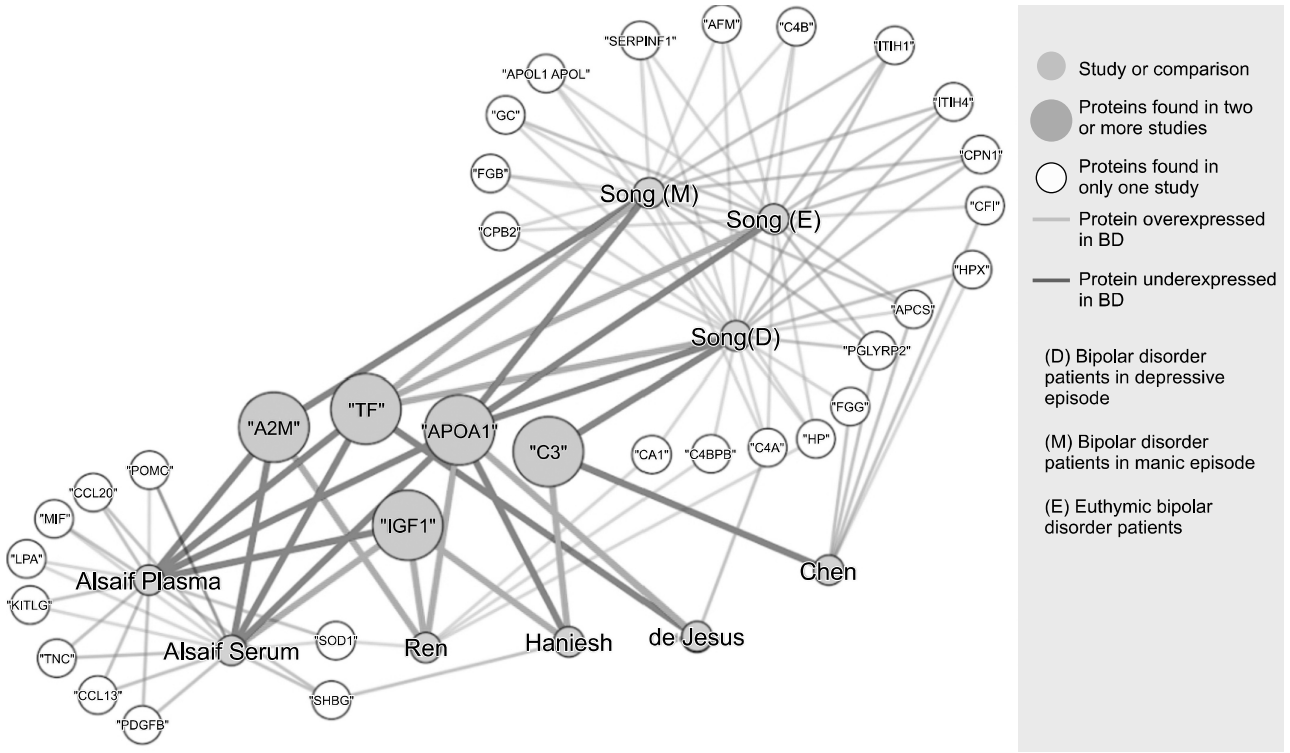

Fig. 4. Study-protein network analysis of differentially expressed proteins. Five proteins that presented significant change in expression between bipolar disorder and healthy control samples were included in the analysis. These network proteins are involved in growth regulation, endocrine system, iron transportation, protease inhibition, defense against pathogens and cholesterol transport.
Song, Song *et al.* [13]; Chen, Chen *et al.* [10]; de Jesus, de Jesus *et al.* [17]; Haenisch, Haenisch *et al.* [18]; Alsaif, Alsaif *et al.* [16]; Ren, Ren *et al.* [20]. APOA-1, apolipoprotein A-1; A2M, alpha-2-macroglobulin; C3, third component of complement; IGF-1, insulin growth factor-1; BD, bipolar disorder.

garding the number of proteins differentially expressed between individuals with BD and controls, which may be as a result of sample characteristic and methodological differences between studies. For instance, patients were on pharmacological treatment in five studies, while only one study included drug-free BD patients [19]. Also, most studies included chronic BD subjects, except for one that recruited patients in the first mood episode [10]. All experiments used plasma or serum as blood fraction. Furthermore, there were methodological differences regarding of protein depletion and quantification techniques, sample size, mass spectrometry database, and data and statistical analysis used in each study.

We identified 123 differentially expressed proteins from the seven articles included [10,13,16-20]. A total of 112 (91.1%) proteins were found differentially expressed by at least one study, 6 (4.9%) by two studies, and 3 (2.4%) by three studies. In particular, transferrin (TF) and apolipoprotein A-1 (APOA-1) were identified in four and five different studies, respectively (Fig. 4). Therefore, to identify potential biomarkers in BD, we decided to focus on the proteins identified in three or more studies. As a result, our analysis revealed the following five proteins as relevant in BD: TF, APOA1, alpha-2-macroglobulin (A2M), complement C3 (C3), and IGF-1 (Fig. 4). Based on the interactions from STRING database, we were able to found proteins directly or indirectly connected with them (PPBP, CXCL8, INS, CXCL10, PC, B2M, POMC, DCTN1, MMP10, CD1E, PAI1, PAI2, PDGFD, GNG5, RANBP2). This set of proteins was used as input in ClueGO, which showed that the proteins that interact closely are associated with the coagulation cascade.

Main KEGG Pathway-related Selected Proteins and Neighbors

The most significant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was the complement and coagulation cascade (p-adj. Bonferroni = 1.0×10^{-13}). This

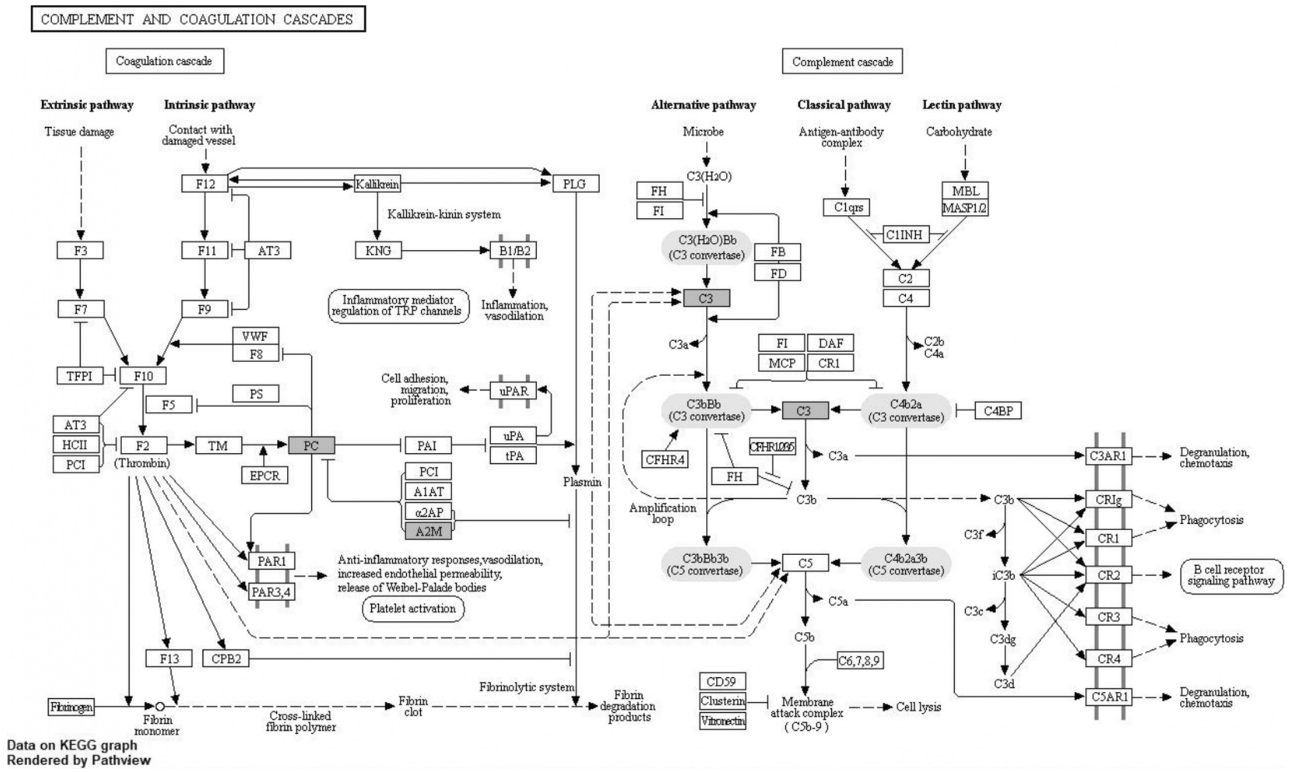

Fig. 5. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Complement and coagulation cascade (p-adj. Bonferroni = 1.0×10^{-13}) were the two main signaling pathways involved in bipolar disorder biological processes. Grey squares represent differentially expressed proteins found by three or more authors. A2M forms a complex with PCI, alpha a1AT, alpha 2AP. This complex of proteins inhibits PC. Furthermore, alpha2AP and A2M complex inhibits plasmin activity. C3 is part of the alternative pathway and integrates this pathway with classical and lectin pathways. This figure can be visualized in more details here (https://www.kegg.jp/kegg-bin/highlight_pathway?scale=1.0&map=map04610&keyword=coagulation).
A2M, alpha-2-macroglobulin; PCI, protein C inhibitor; α1AT, alpha-1-antitrypsin; α2AP, alpha-2-antiplasmin; C3, third component of complement.

pathway has plasmin as the common molecule. A2M forms a complex with protein C inhibitor, alpha-1-antitrypsin and alpha-2-antiplasmin (α2AP). This complex of proteins inhibits PC. Furthermore, α2AP and A2M complex inhibits plasmin activity. C3 is part of the alternative pathway and integrates this pathway with classical and lectin pathways. Haenisch *et al.* [18] found PC (Protein C, Inactivator Of Coagulation Factors Va And VIIIa) was overexpressed in blood of BD patients.

DISCUSSION

In the present study, we gathered data on differentially expressed proteins in the plasma and serum proteomes of subjects with BD compared to healthy controls. Hence, we sought to identify potential biomarkers for discriminating between patients and controls and dysfunctional molecular pathways underlying the pathophysiology of BD. Here, we identified five proteins: IGF-1, TF, A2M, C3, and APOA1. Overall, these proteins are involved in common biological process such as growth regulation, endocrine system, free iron transportation, protease inhibition, defense against pathogens and cholesterol transport. Specifically, to understand the high-level functions and utilities of the uncovered proteins, KEGG pathway analysis revealed that proteins were associated with two main metabolic pathways, the complement system and coagulation cascade. The relevance of each protein and potential interaction mechanisms in the context of BD are described below.

C3

C3 is a protein of the human complement system involved in host defense against pathogens in the bloodstream. It acts stimulating both the innate and adaptive immune systems, eliminating apoptotic cells and cell de-

bris [25]. C3 is mainly produced in the liver [26], however, it is also expressed in adipose tissue of obese man [27] and in the brain of humans and mice [28]. The complement activation triggered by different stimuli converges to C3 activation and cleavage by proteases into effectors molecules such as C3b (mainly involved in the pathogen opsonization and further elimination), C3a and C5a (which are potent anaphylatoxins that promote inflammation), and C5b-9-membrane attack complex (MAC) (mainly involved in the lysis of target pathogens) [29]. Some studies have shown C3 alterations in serum from bipolar patients. For instance, C3 levels were lower in BD patients, independent of mood state, compared to healthy controls [30]. Similar results were found in patients with chronic BD treated with lithium [31]. However, Reginia *et al.* [32] demonstrated an increase in C3a, C5a, C5b-9 concentrations in blood of euthymic bipolar disorder patients who were not treated with lithium in the past 5 years. Our review showed that C3 was differentially expressed in three studies while underexpressed levels were found in two of them. Despite this evidence, the role of the complement system in the etiology of BD is still unclear. One possible explanation is that the complement system is involved in neuroinflammation process that it is also part of the etiology of BD [32]. The main suggested mechanism by which the peripheral immune system interacts with the central nervous system (CNS) is the increased permeability of the blood-brain barrier (BBB), which has been shown to be disrupted in patients with BD. Consequently, the complement components may penetrate the CNS [32]. Specific receptors of complement are present in neurons (such as C5aR C3aR) and in oligodendrocytes (C5b-9) and may trigger antiapoptotic signaling. Additionally, the over activation of the complement system, including C3, and microglia is involved in early synaptic pruning in the hippocampus of humans/mice [33], which may promote the secretion of proinflammatory cytokines by glial cells and induce neuronal damage and death. This mechanism explains, in part, the neurodegenerative process found in CNS diseases such as Alzheimer disease [34].

APOA-1

The APOA-1 is a protein involved in the high-density lipoprotein (HDL) maturation, cholesterol efflux from artery wall cells, and reverse transport of cholesterol [35,36]. APOA-1 is mainly synthesized by hepatocytes and duodenojejunal mucosa cells [37,38], and released as lipid-free or lipid-poor APOA-1 and small HDL [38]. In individuals with schizophrenia (SZ), APOA-1 protein concentration seems to be reduced in the cerebrospinal fluid (CSF), red blood cells, post-mortem liver, dorsolateral prefrontal cortex, and serum [39]. Lower level of APOA-1 was also found in BD I patients compared to healthy controls [40], and a negative correlation between APOA-1 levels and patients treated with lithium has been demonstrated [41]. On the other hand, APOA-1 levels may not be altered in BD patients before the onset of symptoms [42], suggesting that changes occur later in the course of the disorder. In our review, APOA-1 was underexpressed in three studies while two others showed that this protein overexpressed. According to literature, APOA-1 is involved in HDL-accepting cholesterol process from macrophages, resulting in a smaller amount of cholesterol to be oxidized and consequent lower local inflammation [43]. Thus, there may be an interesting relationship between peripheral and central APOA-1 levels that could be mediated by inflammatory mechanisms. Furthermore, APOA-1 is able to act as an anti-inflammatory molecule, in part by limiting the macrophage cholesterol efflux or preventing macrophage chemotaxis towards chemokines coagulation cascade (CC) [44]. In addition to *in vitro* studies, there is evidence that BD is associated with inflammatory processes, suggesting the influence of blood protein alteration on the brain. A recent study showed an upregulation of pro-inflammatory cytokines decreased chemokines secretion in macrophages exposed to serum of patients during manic and depressive episodes compared to those in euthyma [45]. Moreover, dysfunction in macrophage activity occurs in the late stage of BD, due to low cytokine secretion by macrophages in response to inflammatory environment [46]. Many signaling proinflammatory molecules are upregulated during acute episodes of BD supporting the concept of a chronic low-grade inflammatory state in BD [47,48]. An interesting meta-analysis suggests that BD is accompanied by dysregulation of the immune response by demonstrating elevated levels of interleukin, its receptors, and tumor necrosis factor-alpha (IL-2R, sIL-6R, TNF-α, sTNFR1, IL-4) in patients compared with healthy controls [49].

TF

TF is an iron transporter glycoprotein that plays important roles in human physiology. Iron, in turn, has relevant functions in biological systems such as DNA metabolism, oxygen transport, and energy production [50]. However, free iron can be toxic inducing oxidative damage, thus TF acts on the safe transportation through the body [51]. Iron can be carried from blood to the brain through TF receptors located in the endothelial cells of the BBB, internalize the protein-iron complex releasing ferrous iron to the CNS [51,52]. Synthesized predominantly by hepatocytes, TF is expressed in several tissues including the brain [52]. A study using separation methods showed that TF in the CSF can be derived from blood [52]. There is evidence that altered TF is associated with pathologies including MDD [53] and schizophrenia [54]. A study involving antidepressant drug-naive patients with MDD showed a decrease in serotransferrin levels suggesting a relationship between the initial state of disease and immune response [53]. Tsai *et al.* [55] also found increased TF receptors in BD patients during acute mania and in subsequent remission. We also showed an overexpression of TF levels in three disease stages. Song *et al.* [13] author, in contrast to two authors, that presented TF down-regulated levels. Interestingly, another study found lower coagulation measures for fibrinogen and plasminogen activator inhibitor, and higher levels of plasmin-α2-antiplasmin complex in anxiety or depressed patients on serotonergic antidepressant treatment than in patients without these agents [56]. These findings indicate an activation of coagulation factors in the direction of a hypercoagulable state in patients with psychiatric disorders. This hypercoagulable state may explain, in part, the higher risk for cardiovascular diseases associated with anxiety and mood disorders. Depressed patients have been demonstrated increased baseline platelet activation, suggesting a mechanism by which depression is a key risk factor in vascular disease [57]. A recent study showed the ability of TF to potentiate thrombin and FXIIa activity, two important coagulation enzymes. Elevated levels of TF found in atherosclerotic plasma are related to the maintenance of coagulation balance [58]. Moreover, there is evidence showing a relationship between TF alterations and cardiovascular diseases [59,60]. It is possible to find a variety of studies relating to central nervous system diseases and coagulation cascade [61-63].

IGF-1

IGF-1 is a protein similar in molecular structure to insulin which plays an important role in growth regulation and endocrine system through increased glucose uptake and decreased hepatic glycogenolysis and gluconeogenesis, thus improving insulin sensitivity [64,65]. IGF-1 belongs to a group of polypeptides where most of the mRNA is detected in the liver, kidney, brain, and myocardium. IGF-1 gene expression is stimulated by growth hormone production, which in turn is suppressed by high levels of IGF-1 suggesting a feedback compensatory mechanism [66]. Many factors such as age and gender influence these protein levels. Higher levels of IGF-1 are produced in the initial phases of life while a decrement is common during aging [66,67]. As IGF-1 is present in critical brain regions such as olfactory bulb and hippocampus [68], this peptide exerts modulatory effects including synaptic plasticity [69], neuronal excitability [70], cognitive function, and behavior [71,72]. In BD, Kim and collaborators have suggested that IGF-1 can be a trait marker for BD due to its relevant roles in the pathophysiology of the condition [73]. An in vitro experiment demonstrated that IGF-1 increased lithium sensitivity in lymphoblastoid cell lines from non-responders BD patients [74]. Corroborating with previous data, we showed elevated peripheral levels of IGF-1 in euthymic BD patients compared to healthy controls [75]. Not only BD but also MDD patients presented high levels of IGF-1 when compared to healthy controls [76]. Our review found an overexpression of IGF-1 levels in serum from patients with BD while underexpression was found in plasma suggesting that these alterations may be tissue-specific. Several studies have associated IGF-1 levels alterations with inflammatory diseases such as obesity [77], and diabetes [78]. It has been proposed that obesity promotes chronic low-grade inflammation in periphery, and IGF-1 resistance. Inflammation, on the other hand, enhances IGF-1 resistance. Both factors play a relevant role in triggering CNS disorder [77,79]. Evidence with animal models showed that central administration of IGF-1 decreased the expression of inflammatory markers, suggesting a reduction in depressive-like behavior [80]. This evidence leads us to believe that IGF-1 collaborates positively in inflammatory changes that psychiatric illnesses can cause.

A2M

As part of a glycoproteins group, A2M is present in vertebrates body fluids with diversified roles. One of the most important functions is the inhibition of proteases without directly blocking protease active site. It is widely involved in body protection against proteolytic activity [81]. Moreover, A2M has the ability to connect to several non-protease ligands such as cytokines, growth factors, and apolipoproteins [82]. Evidence shows that A2M is altered in a variety of illnesses including Alzheimer's disease [83], Parkinson's disease, and schizophrenia [84]. Also, there is data demonstrating three new genes predicting depression in response to stress including the A2M gene through "omics" approach [85]. Further, patients predisposed to develop depression have high levels of A2M [86], besides patients diagnosed with depression have elevated levels of A2M [87,88], in parallel with our data, that showed A2M up-regulated in only one study. Also, our analysis presented A2M levels down-regulated specifically in the mania group of Song study [13]. Interestingly, recent research showed altered levels of A2M in patients on the first episode psychosis, suggesting that acute phase proteins are involved with schizophrenic illness [89]. Acute-phase proteins are changed in response to inflammatory status and have presented a relationship with a mental disorder [89-91]. Although there is little evidence of A2M presence in BD patients blood, other mental illnesses have reported this change, like mentioned above.

Coagulation Cascade and Complement System in BD

From the analysis of proteins differentially expressed in BD, we identified the involvement of two main signalling pathways. Although the precise mechanisms underlying the interaction between the complement system (CS) and the CC are still not fully elucidated, current research has indicated a bidirectional modulation between these systems. The CS seems to be derived from the serine protease reaction cascade, which is encoded by the same ancestor genes as the coagulation factors [92,93]. Besides a common origin, these systems also share similar roles, including promoting the first defense line against infections and tissue repairing, while potentially contributing to either homeostasis or the development of pathological conditions [94]. Here, we observed that A2M, from the CC, and C3, a component of the CS, have been found differentially expressed in BD. Such finding corroborates previous evidence supporting the crosstalk between CC and CS and further implicates this interaction in the pathophysiology of the disorder.

Like the CS, the CC is characterized by a highly regulated and coordinated event that culminates in clot formation and, when combined with the fibrinolytic system and platelets, constitutes the hemostasis system (Fig. 5). The activation of the CC comprises primary and secondary hemostasis, and it is usually accompanied by the activation of inflammatory mechanisms [95]. The primary hemostasis is characterized by the activation and aggregation of platelets and culminates with the formation of fibrin by thrombin. This event is also accompanied by an acute inflammatory response to control tissue damage, stop loss of blood and prevent microbial infection. During the second hemostasis, plasmin dissolves fibrin along with reparative inflammatory cells in a combined effort to remodel and repair damaged tissue [96]. Hence, under physiological conditions, a strictly controlled hemostasis system confers minimal risk of complications or failed response.

On the other hand, the dysregulation of the acute phase response, as a result of a disproportionate CC activation of inflammatory signalling, can be detrimental to tissue repair and homeostasis. Therefore, the proper regulation of this pathway relies on modulatory anticoagulant mechanisms such as the protein C pathway (PC), the tissue factor pathway inhibitor and the antithrombin-heparin pathway [97]. Overall, these mechanisms inhibit most of the factors that become activated throughout the CC, being the PC considered the major one [98]. As a coordinated mechanism is also essential for the thrombus resolution and wound healing, circulating α2AP, and A2M represents the main modulators of the fibrinolytic system. Thus, the downregulation of A2M in BD, as observed by most of the studies included in this review, results in a lack of control of both PC pathway and plasmin activity; thus, upregulating the anticoagulation capacity and fibrinolytic system, respectively. Specifically, activated PC impairs the procoagulant effects of thrombin [95], while enhanced plasmin activity increases CS activation (Fletcher-Sandersjöö *et al.* [99], discussed below). The possible overregulation of coagulation by anticoagulation mechanisms can result in abnormal bleeding, which is not usually reported in psychiatric disorders. However, pharmacological treatment has been suggested to interfere in the balance of the

CC causing haematological side effects [100].

Throughout the CC, there are several steps involving the activation of the CS components. For instance, activated platelets present surface molecules, such as P-selectin and C1q receptor, that activate the alternative and classical pathways of the CS, respectively [93,94]. Fibrinogen is a potent acute phase reactant and inflammatory mediator [101]. Also, thrombin and plasmin can activate C3 and C5 in the coagulation site independently of C3 conversion (Fletcher-Sandersjöö *et al.* [99]; Fig. 5, dashed line). Besides chemo attractant properties, activated CS components C3a and C5a induce the activation, aggregation and degranulation of platelets, promote calcium influx and enhance procoagulant activity [102]. Also, the formation of C5a favours neutrophils and monocytes recruitment, while C5b contributes to MAC formation, which further augments platelet activation and aggregation [93]. Thus, the crosstalk between these systems is suggested to generate a self-strengthening cycle.

CS activity is directly related to an increased prothrombotic and antifibrinolytic state. For instance, mannan-binding lectin serine proteases initiate the lectin pathway of the CS and form activated thrombin, suggesting that the CS can generate the end product of the CC [99]. Also, the modulatory mechanisms of the CS, including the C1 inhibitor and C4-binding protein, play a dual role as regulatory proteins in both systems. In the CC, these proteins ultimately cleave activated factors V and VIII, reducing the activation of procoagulant mechanisms [93]. On the contrary, C1 inhibitor has also been shown to inhibit plasmin, which consequently reduces fibrinolytic activity and increases thrombogenesis [103]. Therefore, dysfunctional CS activation has been implicated in pathogenic mechanisms underlying hemolytic and thrombotic diseases, as hyperactivation of this pathway is associated with both systemic inflammation and thrombosis [94, 104,105]. Such events have been extensively reported and characterized in sepsis, whereas a chronic low-grade inflammatory state is commonly observed in BD [48].

Albeit the crosstalk between CS and CC has not been fully understood yet, the CS is considered the primary mediator, while C3 represents the common component of this interaction [94]. Accordingly, our results indicate an altered expression of C3 in BD, which seems to be downregulated in the disorder [32,106]. Reduced levels of C3 may be a result of higher consumption and activation of CS components, being compatible with a peripheral and central proinflammatory milieu. As previously discussed, inflammation can promote coagulation and, specifically, IL-6 has been shown to increase platelet activation and aggregation, which further augments the secretion of other inflammatory markers [107,108]. Peripheral proinflammatory cytokines are known to be increased in BD, especially during mood episodes [48,109], and to downregulate the PC pathway–the main anticoagulant system [98]. In sum, the dysregulation of the complement system triggers the activation of hemostatic factors, consequently leading to thrombosis and intravascular coagulation. On the other hand, thrombosis or tissue damage activates the CS amplifying the inflammatory response and promoting additional local tissue injury.

Due to this interplay, extant literature supports the implication of CC and its regulatory mechanism—including the CS—in BD. Interestingly, in sepsis, the levels of active CS components seem to be higher in the serum than in the plasma, and evidence shows a better correlation between CS and hemostasis parameters than with other inflammatory markers [110]. Then, increased activation of CS during coagulation might be mediated by the platelets, which are considered a non-specific first line inflammatory marker and suggested to play a role in psychiatric disorders [111]. Razouki *et al.* [112,113] have found that BD, among others, might be a predictive factor associated with more time below the target therapeutic range for treatment with warfarin, which points out to an impaired anticoagulation control among BD patients. Moreover, more recent evidence of the involvement of these systems in BD implicates other signalling pathways. For instance, C5a induces the upregulation of the plasminogen activator inhibitor-1 (PAI-1), a regulator of the fibrinolytic pathway, which inhibits the tissue plasmin activator (tPA) [94]. Besides promoting a procoagulant effect, the inhibition of tPA may contribute to impair its activity in converting proBDNF to BDNF [114]. The role of BDNF in BD and other psychiatric disorders has been extensively investigated [115,116]. For instance, the tPA-BDNF pathway has been implicated in MDD. Specifically, tPA, BDNF, and BDNF/proBDNF ratio were lower in MDD, while 8-week antidepressant treatment rescued those levels [117]. In BD, peripheral BDNF levels are commonly found to be reduced during mania and depression [118], as well as tPA, proBDNF, TrkB and p75NTR [119].

Hence, combined peripheral levels of these markers—but not each marker individually—have been proposed to present a good accuracy of diagnosis and differentiation among SZ, BD at different episodes, MDD and healthy controls. Interestingly, authors have found a good diagnostic efficacy for differentiating mania from depression in BD. As both CC and CS seem to be involved in BD, the investigation of these pathways may be informative of pathophysiological mechanisms involved in the disorder and potentially indicative of a marker of state (e.g., mood episode) [120].

Limitations

This review presented a number of limitations. First, methodological differences such as protein levels assay (multiplex assay and chromatography) may have contributed to the heterogeneous results. Immunoassay is based in a specific interaction between antibody and target, whereas chromatography separates molecules according to their solubility, size, and mass. Considering that immunoassay has a high specificity it is likely that chromatography does not present all proteins detected by multiplex assay. On the other hand, multiplex is characterized by being selective, not covering all proteins separated in chromatographic analysis. This leads us to believe that different methods of analysis can generate heterogeneous results. Second, sample characteristics including demographic variables (such as ethnicity, gender and age), mood state, symptom severity, chronicity, and comorbidities among others are also factors that may influence the protein expression. Additionally, we can not rule out biomarkers differences according to patients with first episodes versus those with chronic course [121]. Third, it is possible to find a variety of physiological alterations in patients according to the drug therapy [122,123]. Since interferences related to weight gain, gastrointestinal disturbances, neural tube defects, up until cytochrome P450 enzymes induction [123], may be a precursor of proteomic modification.

It is possible to note a great heterogeneity among studies in both uniquely expressed proteins and a number of proteins. Those differences may be explained to some extent by biological sample type, subjects characteristics, and proteomic technique carried out.

CONCLUSION

In sum, this review demonstrates a potential biological signature in BD patients based on proteomic analysis. We compared blood proteomes, by using protein association network, of subjects with BD and healthy controls to suggest dysfunctional molecular pathways involved in disease. The results revealed proteins associated with several biological processes, including growth and endocrine regulation, iron transportation, protease inhibition, protection against pathogens and cholesterol transport. Moreover, pathway analysis indicated the association of uncovered proteins with two main metabolic pathways: complement system and coagulation cascade. Many of these physiological processes are related to psychiatric disorders. Therefore, it is important to identify possible biomarkers for mental illnesses differentiation. Since psychiatry still strongly relies on clinical judgment, there is a risk for misdiagnosis and, consequently, inadequate/erroneous treatment. Thus, it is essential to improve the current knowledge on the pathophysiology of psychiatric disorders and underlying molecular patterns.

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■ **Conflicts of Interest**

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

■ **Author Contributions**

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