Chapter 24

Proteomic biomarkers for bipolar disorder

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24.1 Introduction

Bipolar disorder (BD), a chronic and recurrent mood disorder has its onset in teenage years or early adulthood and its course is characterized by repeated affective exacerbations which could be manic/hypomanic or depressive in nature (Joslyn, Hawes, Hunt, & Mitchell, 2016). BD is conceptualized as a spectrum disorder and classical presentations with recurrent manic and depressive episodes constitute only one-fourth of the cases (Muneer & Mazommil, 2018). It is useful to define the term "Biomarker" at the beginning of the discussion. A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological response to a therapeutic intervention (Califf, 2018). With the advent of biological psychiatry there has been an ever-growing interest in employing biomarkers in psychiatric practice. In this regard, initial efforts consisted of hypothesis-driven and genomic investigations, but in spite of decades of research clinically applicable biomarkers were not identified. The field of biomarker discovery was driven toward investigation of cellular protein expression and biochemical pathways, essential data which needed to be incorporated with the existing genetic information. Studies aiming at protein expression levels were likely to inform about the functional role of genetic risk loci and outline how these links charted on to specific endophenotypes of the disorder. Since proteins performed the great majority of functions in all organisms, this made them more appropriate for clinical purposes as compared to genomics and transcriptomics, which were nevertheless, complemented by proteomic studies (Preece, Han, & Bahn, 2018).

The proteome is the complete array of proteins produced by an organism and varies with time, biological requirements, stress, and other environmental factors. Proteomics denote a large-scale inclusive analysis of proteins in a

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biological system, at a precise point in time and under a fixed condition. Its target is to obtain an overall and unified understanding of biology by studying all the proteins of a cell, instead of each one independently. Hence, protein profiling may better delineate active pathophysiological processes (Adhikari et al., 2020). In the past couple of decades, the advancement of high-throughput technologies of proteomic analysis has hosted a fresh period of biomarker discovery. For complex, multifactorial disorders such as BD such examination has the potential to increase diagnostic accuracy by differentiating between closely related disease phenotypes (Muneer, 2020). It helps in the generation of extrapolative representations, with the inclusion and integration of other "OMICS" data, with the process driven by a hypothesis-free paradigm. No doubt, proteomics holds the potential for predicting the onset of the disorder, while envisaging the disease trajectory and outcome (Patel, 2014).

24.2 The development of proteomic techniques

The term "proteomics" was first used in the late 90 and since then a number of methods have been employed to study proteins. On the whole, protein detection comprises of two techniques, namely antibody-based methods (immunoassays) or mass spectrometry (MS) (Geyer, Holdt, Teupser, & Mann, 2017). The former is exemplified by enzyme-linked immunosorbent assay (ELISA) and Western blot which have been used for a long time as validation tools to detect and quantify candidate proteins. More recently, higher-throughput multiplex immunoassay panels have been developed to simultaneously identify and quantify hundreds of proteins (Stephen, Schwarz, & Guest, 2017). Although immunoassay methods have advanced over time, these face inherent limitations with respect to multiplexing, specificity for protein isoforms and incompatibility with hypothesis-free investigations. In contrast, MS-based methods have taken precedence as technological advancements over the past years have radically improved proteomics. As such, MS-based techniques can now characterize human plasma proteomes with unparalleled precision (Aslam, Basit, Nisar, Khurshid, & Rasool, 2017; Manes & Nita-Lazar, 2018).

MS-based proteomics has many methods of protein separation, visualization and analysis, and in comparison, to immunoassays, proteins are identified using MS instruments. Initially, in the first decade of MS-based proteomics gel-based two-dimensional electrophoretic methods were employed for relative measurement of proteins in samples. However, these techniques were labor-intensive, limited by poor separation of certain protein groups (especially membrane proteins) and mostly only identified a small number of proteins. As such, gel-based methods did not accomplish the purpose of large-scale proteome characterization (Calderón-Celis, Encinar, & Sanz-Medel, 2018). Nowadays, the most widespread method for discovery proteomics is termed "shotgun proteomics." In this technique, the first step

is sample preparation during which a complex protein mixture is enzymatically digested into peptides. This is followed by a combination of a liquid chromatography system that allows the separation of peptides over time (Li, Wang, & Chen, 2017). Next the peptides are ionized by the electrospray ionization technology. This allows the formation of charged molecules, followed by their analysis in the mass spectrometer. Finally, the MS spectra are run through sequence databases by "search engines" to identify peptides using statistically defined criteria.

In contrast, in "targeted proteomics" information on a comparatively smaller number of peptides of interest, usually far less than hundred, is obtained with high specificity and sensitivity. The most common targeted MS method is defined as "multiple reaction monitoring" in which, for a selected set of peptides, a higher sensitivity and throughput may be possible compared to shotgun proteomics (Vidova & Spacil, 2017). For both shotgun and targeted proteomics, absolute protein quantification is achieved by including heavy isotope labeled peptides as reference standards for endogenous proteins of interest. In summary, mass spectrometry with liquid chromatography-mass spectrometry (LC-MS/MS) and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF/TOF) are extensively applied tools and are principal among contemporary protein analysis techniques.

24.3 Proteome characterization of the peripheral blood

From the standpoint of clinical practice proteomic methodologies using blood plasma or serum should be the preferred techniques for biomarker profiling of psychiatric disorders. In clinical practice such specimens had proven diagnostic value, and for the purpose of research these were easily available through biological sample banks across thousands of human studies. Hence, a comprehensive approach was taken to systematically review the extant literature describing such minimally invasive proteomics studies in BD from the recent past. In order to obtain an overall view, closely related phenotypes, that is, schizophrenia (SZ) and major depressive disorder (MDD) were also referred to in the discussion that followed. The purpose of this exercise was twofold, firstly, to ascertain what proteomic investigations had been able to accomplish so far and secondly, to determine the limiting factors of this research in psychiatry. Table 24.1 gives a summary of the selected studies in BD beginning with the most recent (Alsaif et al., 2012; Chen et al., 2015; Coppens et al., 2020; de Jesus et al., 2017; Frye et al., 2015; Guest et al., 2010; Haenisch et al., 2014, 2015; Herberth et al., 2011; Kim et al., 2021; Kittel-Schneider et al., 2020; Lee et al., 2021; Ren et al., 2017; Rhee et al., 2020; Schwarz et al., 2012; Smirnova et al., 2019; Song et al., 2015).

The following areas were given special consideration: (i) first author and year of study, (ii) study country, (iii) aim, (iv) number and sources of

TABLE 24.1 Blood serum and plasma-based investigations of proteomic biomarkers in bipolar disorder.

Study	Subjects; diagnosis	Sample	Technique	Proteins with altered abundance	Biological pathways	Conclusion
Kim et al. (2021)	42 MDD, 45 BD, 51 HC	Plasma	Liquid chromatography–tandem mass spectrometry (LC-MS/MS)	In MDD 14 proteins dysregulated compared to controls. Six proteins altered in BD compared to HC. F13A1, PPBP, PF4, GAPDH, and TMSB4X were dysregulated in both disorders.	Coagulation pathway, glucose metabolism, and immune response.	For proteins dysregulated in both, except F13A1, higher fold changes were observed in MDD than in BD patients. The findings may help in discovering biomarkers for major mood disorders. In addition, these could assist in explaining their pathophysiology and underlying biochemical abnormalities.
Lee et al. (2021)	BD type II ($n = 185$), HC ($n = 186$)	Plasma	Isobaric tags for relative and absolute quantification (iTRAQ), followed by analysis with LC-MS/MS.	The expression levels (at least twofold) of five candidate proteins—MMP9, FARSB, PRDX2, CA-1, and PCSK9—in the plasma of BPII and HC were assessed using ELISA kits.	Gene Ontology analysis of upregulated proteins identified pathways involved in the regulation of lipoproteins, response to oxidative stress, and innate immune response.	Logistic regression analysis was used to form the composite probability score of the five proteins. ROC-AUC showed good diagnostic validity, such that plasma levels of PRDX2, CA-1, FARSB, MMP9, and PCSK9 may be associated with BD-II as potential biomarkers.

Kittel-Schneider et al. (2020)	70 BD (BDI and II), 42 MDD.	Serum	Multiplex Immunoassay, Myriad Rules-Based Medicine (Myriad RBM). Human Discovery MAP. A multivariate predictive model was developed to distinguish between BD and MDD.	Based on the various proteomic profiles, the algorithm generated could discriminate depressed BD patients from MDD with an accuracy of 67%.	Identified proteins were not mapped to discover associated biological pathways.	Based on the various proteomic profiles, the specific algorithm could discriminate depressed BD patients from MDD with an accuracy of 67%. In patients experiencing a depressive episode, MDD can be differentiated from BD with the help of the peripheral proteome.
Rhee et al. (2020)	MDD ($n = 15$), BD ($n = 10$). All patients were psychotropic drug-free.	Serum	Liquid chromatography-tandem mass spectrometry (LC-MS/MS) and label-free quantification	14 proteins differentially expressed between MDD and BD. Ras-related protein Rab-7a and Rho-associated protein kinase 2 significantly overexpressed in MDD. Exportin-7 significantly overexpressed in BD.	Bioinformatics analysis showed that cellular functions and inflammation/ immune pathways were significantly different.	Ras-related protein Rab-7a, Rho-associated protein kinase 2, and Exportin-7 identified as potential biomarkers to distinguish MDD from BD. Further large sample studies with longitudinal designs and validation processes were warranted.

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TABLE 24.1 (Continued)

Study	Subjects; diagnosis	Sample	Technique	Proteins with altered abundance	Biological pathways	Conclusion
Coppens et al. (2020)	MDD ($n = 5$), BD − depressed ($n = 3$), BD − manic ($n = 4$), SZ ($n = 4$), HC ($n = 6$)	PBMCs	State-of-the-art MS	PBMC proteome was analyzed yielding 4271 proteins. For discrimination between MDD and BD-D, 66 candidate biomarkers were found. 72 proteins might harbor a biomarker capacity to differentiate between BD-M and SZ.	Examination of the entire proteome. No specific biological pathway investigated.	A register of candidate biomarkers with the potential to objectively discriminate between MDD and BD-M and BD-M and SZ. A proof of concept study limited by small sample size.
Smirnova et al. (2019)	33, SZ; 23, BD; 24, HC	Blood serum	Quantitative MS-based on one-dimensional Laemmli polyacrylamide gel (PAG) electrophoresis	27 proteins being specific for SZ, and 18 for BD.	Distinct protein sets in SZ and BD associated with diverse processes involving immune response, cell communication/ transport, cell growth, and maintenance.	Specific protein sets useful for discovering new pathways in major psychiatric disorders.

Ren et al. (2017)	30, MDD; 30, BD; 30, HC.	Blood plasma	Isobaric tags for relative and absolute quantification (iTRAQ) technology combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) with bioinformatics analysis	9 proteins significantly altered between MDD and BP. B2RAN2, B4E1B2, APOA1, ENG, SBSN, and QSOX2 upregulated; ORM1, MRC2 and SLP1 downregulated.	Most identified proteins related to the immune system. Bioinformatics analysis showed that B2RAN2 and ENG may serve as biomarkers for discriminating between MDD and BP.	Further investigation required to illuminate the roles of B2RAN2 and ENG in MDD and BP. The study identified a novel biomarker panel that may serve as state markers for BD.
de Jesus et al. (2017)	14, BD using Li; 4 other psychiatric (OD) patients using Li; 23, SZ; 12, HC.	Blood serum	2-D DIGE and nano LC-MS/MS analysis	In the serum of patients vs controls, 37 protein spots found differentially abundant exhibiting ≥ 2.0-fold change in value.	From detected spots, 13 different proteins identified including ApoA1, ApoE, ApoC3, ApoA4, Samp, SerpinA1, TTR, IgK, Alb, VTN, TR, C4A, and C4B.	Proteomic analysis allowed discrimination of BD from SZ. The findings contribute toward pathophysiology/ biomarker discovery for main psychiatric conditions.
Haenisch et al. (2015)	29, BD (manic), 17, BD (mixed); 53 HC	Plasma	Multiplex immunoassay analysis	C-peptide, progesterone, insulin, and antigen 125 altered in both manic/mixed states. Peptide YY and sortilin changed only in mania.	Mania and mixed mood patients revealed similar changes in proteins related to insulin signaling (trait markers). Mania patients showed specific changes in	Further studies can increase the understanding of systemic biological pathways affected in different BD mood states, leading to the identification of novel biomarkers and potential new drug targets.

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TABLE 24.1 (Continued)

Study	Subjects; diagnosis	Sample	Technique	Proteins with altered abundance	Biological pathways	Conclusion
				Haptoglobin, CC4 and matrix metalloproteinase 7 (MMP-7) altered specifically in mixed states.	hormonal and growth factor values, while mixed mood patients had a higher number of alterations in inflammation-related molecules.	
Chen et al. (2015)	20, BD-II; 30, MDD; 30, HC.	Plasma	2-DE coupled with MALDI-TOF MS/MS; ELISA for validation.	Twofold differences for at least 25 distinct protein spots in BD-II vs MDD; 3 proteins significantly differentially expressed in cases vs controls. Results: C3, MDD > bipolar II > HC; CFI and C4BPα, HC > MDD > bipolar II.	Enriched biological processes in BD-II relative to MDD were immune regulatory, acute inflammatory and innate immune responses. To determine the altered biological pathways, all differentially expressed proteins were mapped on	The findings provided evidence that autoimmune dysregulation was involved in the pathophysiology of bipolar II/ MDD.

					to the KEGG database (Kyoto Encyclopedia of Genes and Genome). This revealed that the significantly enriched pathways were those of complement and coagulation cascades.	
Frye et al. (2015)	52, Unipolar; 49, BD-II; 46, BD-I; 141, HC	Blood serum	Multiplex profiling of 320 proteins utilizing the Myriad RBM Discovery Multi-Analyte Profiling platform (MAP).	73 proteins showed nominally significant differences; 6 proteins different after Bonferroni correction.	Series of statistical analysis showed that matrix metalloproteinase-7 significantly different in mood disorder patients vs HC. MMP-7, GDF-15, HPN significantly different in bipolar cases (BP-I + BP-II) vs controls. GDF-15, HPX, HPN, RBP-4 and TTR proteins significantly different in BP-I vs controls.	Good diagnostic accuracy (ROC-AUC $\geqslant 0.8$) obtained for GDF-15, RBP-4, and TTR when comparing BP-I vs controls. This discovery sample suggested applicability of proteomic panels in identifying and distinguishing mood disorders, in particular bipolar I disorder.

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TABLE 24.1 (Continued)

Study	Subjects; diagnosis	Sample	Technique	Proteins with altered abundance	Biological pathways	Conclusion
Song et al. (2015)	10, euthymic BD-I; 20, depressed BD-I; 15, manic BD-I; 20, HC	Plasma	2-D electrophoresis and MALDI-TOF/ MS-MS. Proteomic results validated by immunoblotting.	32 proteins identified with fivefold change in expression compared with HC.	16 proteins perturbed in BD independent of mood state, while 16 proteins specifically associated with particular BD mood states. Two mood-independent differentially expressed proteins were apolipoprotein (Apo) A1 and Apo L1.	Results suggested that BD pathophysiology was associated with early perturbations in lipid metabolism. Downregulation of one mood-dependent protein, carbonic anhydrase 1, implied that it may be involved in the pathophysiology of BP.
Haenisch et al. (2014)	17, BD out-patients; 46, HC	Plasma	Human Discovery MAP multiplexed immunoassay	190 proteins measured. Identification of 26 dysregulated proteins in BD patients compared to controls.	Detected proteins comprised mostly of growth factors, hormones, lipid transport and inflammatory proteins. Decreased apolipoprotein A1 previously associated with BD, was confirmed in the study.	Future studies were needed to increase understanding of BD pathophysiology, helping in patient stratification/ better treatment outcomes.

Schwarz et al. (2012)	250, first/ recent onset SZ; 35, MDD; 32, euthymic BD; 329, controls	Blood serum	Human MAP multiplex immunoassay.	Identification of a signature that comprised of 34 analytes in a cohort of closely matched SZ ($n = 71$) and control ($n = 59$) subjects.	Partial least squares discriminant analysis using this signature gave a separation of 60%–75% of SZ subjects from controls across cohorts. The same analysis also gave a separation of ~50% of MDD patients and 10%–20% of BD subjects vs HC.	A biological signature for SZ identified in the patient serum. It laid the basis for a diagnostic test used for distinguishing SZ from controls, as well as patients with overlapping psychiatric symptoms.
Schwarz et al. (2012)	75, pre-proximal SZ; 110, BD; 75 + 110, controls	Blood serum	Discovery MAP immunoassay.	Samples drawn within 1 month before estimated onset of illness. Identification of 20 altered molecules in pre-SZ and 14 in pre-BD compared to controls.	Only two of those molecular changes were identical in both data sets and predictive testing confirmed that the biomarker signatures for pre-SZ and pre-BD were dissimilar. Identified molecules related to inflammation and immune response.	Distinct serum alterations occurred before clinical manifestation of schizophrenia and BD. Helpful in the development of diagnostic tests for early identification and categorization of predisposed patients, allowing for preemptive and more effective therapeutic interventions.

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TABLE 24.1 (Continued)

Study	Subjects; diagnosis	Sample	Technique	Proteins with altered abundance	Biological pathways	Conclusion
Alsaif et al. (2012)	24, BD; 21, HC	Blood serum and plasma	Multiplex immunoassay	Total of 190 proteins measured in serum and plasma.	In the disease cohort the researchers identified six proteins that changed significantly in serum and ten in plasma with an overlap of two proteins.	Expressed differences were found in proteome coverage/reliability of measurement when comparing serum and plasma. This could have significant impact on identifications made in biomarker studies.
Herberth et al. (2011)	16, euthymic BD-I outpatients (remitted); 16, euthymic BD-II outpatients (remitted); 32, HC Validation cohort: 7, BD-I; 7, BD-II; 14, controls	Blood serum (and PBMCs)	Liquid chromatography-mass spectrometry and multi-Analyte Profiling (Human Map)	Identification of approximately 60 differentially expressed molecules involved predominantly in cell death/survival pathways.	In PBMCs, this was manifested in cytoskeletal and stress response-associated proteins, whereas most serum analytes were associated with the inflammatory response. The predicted effect of serum analytes on	A peripheral fingerprint was detected that had detrimental effects on cell functioning and could be used to distinguish BD patients from HC despite being in a remission phase. Additional studies of BD patients in the manic and depressed phases could lead to the identification of a molecular fingerprint that

					physiological systems was tested by treating PBMCs with serum obtained from the same patients, resulting in reduced cellular survival.	could be used for predicting episodic switching and guiding treatment strategies.
Guest et al. (2010)	6, first-onset/ acutely psychotic SZ; 10 euthymic BD; 78 HC.	Blood serum	Fluorescence assays and immunoassay.	5 molecules differentially expressed in the cohorts	Identified proteins involved in dysregulated glucose metabolic pathways.	Results suggest insulin-related molecules and potentially other cosecreted insulin-secretory granule proteins may be potential biomarkers.

Abbreviations: *BD,* bipolar disorder; *BP,* bipolar depression; *HC,* healthy controls; *MDD,* major depressive disorder; *SZ,* schizophrenia.

participants, (v) sources of information on exposure, (vi) medication use, (vii) information on comorbidities of included participants, (viii) biological sample used, (ix) method for protein profiling, (x) confounding factors controlled for, (xi) statistical analysis methods, (xii) blinding, (xiii) major findings, and (xiv) outlook. The included studies gave data on up- and downregulated proteins, and for differentially expressed proteins, outcome measures of interest included: (i) whether or not statistically significant, (ii) fold change and direction of differential abundance, and (iii) associated pathways and processes. For BD the proteomics data were cross-referenced with genetic loci identified in genome-wide association studies (GWAS) and top enriched canonical pathways were identified via ingenuity pathway analysis (IPA).

24.4 Current status

Scrutiny of the proteomic data revealed that a range of methodologies was used for biomarker discovery in psychiatric disorders. In the studies sample sizes varied from less than 10 to more than 600 (Bot et al., 2015), while majority of the investigations included healthy controls (HC). Various techniques were used for protein depletion and quantification; for instance, in some studies high abundant proteins were depleted by antibody-based methods prior to proteomic analysis. Indeed, such procedures could result in loss of reproducibility of protein quantification, particularly for low abundant proteins (Ignjatovic et al., 2019). In most of the investigations label-free quantification was used, however in some studies chemical labeling techniques such as iTRAQ (Isobaric tags for relative and absolute quantitation) or TMT (tandem mass tag) were applied which permitted multiplexing and consequently a higher sample throughput, though at the cost of introducing bias in the analysis. As far as statistical procedures were concerned, it was found that some studies employed matched case-control methodologies and did simple t-tests for group comparisons, whereas others used regression modeling which allowed inclusion of confounding variables. While, a number of studies applied multiple testing, some did not do so which caused false identification of certain proteins as being differentially abundant. While some studies applied stricter significance thresholds ($P < .01$), others used ($P < .05$) without correcting for multiple testing. Moreover, some studies only reported those proteins that exceeded a certain fold-change threshold and consequently, data on proteins with smaller effect sizes might have been overlooked. In addition, proteins with lower fold-change thresholds could be contributing to false-positive results. Inconsistencies in defining patient populations and varied selection criteria further contributed to difficulties in comparing results of different studies. In addition, several studies did not reveal whether key confounding variables such as diet, smoking, and exercise were given consideration. Blood was highly active in nature and got

into contact with every tissue of the body such that a number of external factors must be controlled for. This task was attainable, and more recently it has been demonstrated that vigorous workflows could be developed, which aptly took these factors into account (Paulo, Kadiyala, Banks, Steen, & Conwell, 2012; Zhou, Petricoin, & Longo, 2017). There was evidence that the above mentioned caveats were more applicable to older studies, since the most recent investigations had employed technically sound workflows and robust methodologies (Kim et al., 2021; Kittel-Schneider et al., 2020; Lee et al., 2021; Rhee et al., 2020).

24.5 Differentially abundant proteins

While interpreting data on BD, it was essential that information on MDD and SZ be also given consideration, as these were interrelated conditions. Analysis of the proteomic data showed that roughly 202 peptides were specifically abundant in SZ, 141 in MDD, and 99 in BD. For SZ, increased circulating levels of insulin-related peptides were often reported (Çakici et al., 2019), whereas, interleukins including IL-10, IL-12β, IL-17α, IL-5 and growth factors such as BDNF, were differentially expressed in SZ patients (Herberth et al., 2014). A number of original studies reported a decrease of apolipoproteins, and at least two found dysregulation of APO A_1, APO A_2, APO A_4, and APO C_1 (Knöchel et al., 2017).

Two studies examined the possibility of assay panels for the distinction of SZ patients from controls. In one of these studies, Schwarz et al. identified a set of analytes reproducibly altered in SZ patients, compared to HC. Their technique, the refined 51-plex immunoassay had a sensitivity of 83% and specificity of 83% with a receiver operative characteristic area under the curve (ROC-AUC) of 89% (Schwarz et al., 2010). Another study by the same group recognized a set of 34 analytes; the investigators performed a partial least squares discriminant analysis which provided a separation of 60%–75% of SZ patients from controls across five independent cohorts. The same analysis gave a separation of about 50% of MDD patients and 10%–20% of BD subjects from controls (Schwarz et al., 2012).

A number of MDD studies discovered distinct abundance of proinflammatory cytokines and oxidative stress response proteins. Stelzhammer et al. reported an association of ACE, acute phase proteins, BDNF, C4B, cortisol, cytokines, growth hormone, and SOD1 with symptom severity in major depression (Stelzhammer et al., 2014). Chen et al. described three differentially expressed complement proteins validated with ELISA; C3, MDD > BD-II > HC; CFI and C4BPA, HC > MDD > BD-II subjects (Chen et al., 2015). Diniz et al. investigated proteins related to cognitive impairment in later life depression and reported higher levels of chemokines CCL13, CXCL11, CCL18, and lower levels of IL-12β, reduced levels of KIT-ligand (KITLG), and deceased levels of insulin-like growth factor binding protein

($IGFBP_3$ and $IGFBP_5$) (Diniz et al., 2015). Xu et al. also found altered proteins implicated in immunoregulation and lipid metabolism (Xu et al., 2012). Bot et al. found analytes related to cell communication and signal transduction, immune response (CXCL1), and coagulation (VWF). These alterations were related to acute depression symptomatology (Bot et al., 2015). One study found insulin to be the marker with the highest significance statistically—increased in MDD cases compared to control (Domenici et al., 2010). Lee et al. identified a serum biomarker panel of six proteins, namely APO D, APO B, Gc protein-derived macrophage-activating factor (GcMAF), complement proteins (CP), S100 protein (HRNR), and Profilin-1 (PFN1) which could separate MDD patients from HC with a 68% diagnostic accuracy (Lee et al., 2016).

For BD, a recent study employing LC-MS/MS compared peripheral plasma proteome of MDD and bipolar subjects. Compared to controls, 14 and 6 proteins in MDD and BD patients, respectively, were differentially expressed. Among these coagulation factor XIII A chain (F13A1), platelet basic protein (PPBP), platelet factor 4 (PF4), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and thymosin beta-4 (TMSB4X) were altered in both disorders. For proteins dysregulated in both, except F13A1, higher fold changes were observed in MDD than in BD patients. These results could identify candidate biomarkers of mood disorders and elucidate their underlying pathophysiology and biochemical abnormalities (Kim et al., 2021). An older study reported intriguing findings of a combination of 20 significantly altered proteins/metabolites, including cortisol, connective tissue growth factor (CTGF), serum amyloid P component (APCS), and Trefoil factor 3 (TFF3) in BD cases prior to clinical manifestations. From this study Schwarz et al. concluded that their findings could serve as potential biomarkers for detecting vulnerable patients early in the course of BD (Schwarz et al., 2012). A study by Frye et al. assessed the feasibility of mitochondria associated proteins in distinguishing bipolar patients from HC and differentiating subgroups of mood disorders. They found that growth/differentiation factor 15 (GDF15), retinol-binding protein 4 (RBP4), and transthyretin (TTR) were good predictors of BD type I with an ROC AUC of 0.81. Protein levels of GDF15, Hemopexin (HPX), Nephrocan (NPN), Matrix metalloproteinase-7 (MMP7), RBP4, and TTR were higher in BD-I versus unipolar and BD-II patients, as well as controls (Frye et al., 2015). Of note, one study of BD reported differential abundance of molecules involved in cell death/survival pathways (Herberth et al., 2011), whereas another concluded that BD pathophysiology may be associated with perturbations in lipid metabolism. Notably, in the later study APO A_1 and APO L_1 were differentially expressed independent of mood state (Song et al., 2015). Please refer to Table 24.1 for a wide-ranging overview of peripheral proteomic studies in BD.

There was a slight overlap of differentially expressed proteins across studies and 21 altered proteins, and small molecules overlapped across all

three diagnoses. These included A2M, APOA1, APOA2, APOB, APOC1, APOH, C4BPA, C3, CSF2, IgM, KNG1, KITLG, LH, MIF, progesterone, TF, APCS, TTR, CD40, GC, and PROS1. Three of these proteins involved in immune response, namely, Complement C3 (up), Macrophage Migration Inhibitory Factor (up), and Immunoglobulin M (down), were differentially high in the same direction across all three disorders. Many more proteins showed overlap between two disorders or were changed in abundance in equal measures explicitly for one disorder. These findings were in agreement with the broadly believed notion that no single biomarker existed for the diagnosis of major psychiatric disorders. Instead, a panel of biomarkers was required for clinical application.

24.6 Biological processes and pathways

Comes et al. (2018), utilizing the IPA determined the five foremost enriched canonical pathways for each diagnosis of BD, MDD, and SZ. The topmost involved pathways were Farnesoid X receptor/Retinoid X receptor (FXR/RXR) Activation ($P = 2.89E{-}30$), acute phase response signaling ($P = 2.90E{-}31$), and Liver X receptor/Retinoid X receptor (LXR/RXR) activation ($p = 1.62E{-}23$) for BD, SZ, and MDD, respectively. They also performed bioinformatic enrichment analyses with Gene Ontology annotations to confirm IPA results using PANTHER GO-Slim Biological Process, PANTHER Protein Class and PANTHER Pathways analysis. This analysis found commonality in enhanced biological processes across diagnoses while confirming IPA results of enrichment of pathways implicated in immune and inflammatory responses. The principal biological processes across all three disorders involved response to interferon-gamma, the cytokine-mediated signaling pathway, locomotion, blood coagulation, and complement activation. Pathways involved in all three diagnoses were the blood coagulation, plasminogen-activating cascade, and interleukin-signaling pathways. Enrichment of protein classes included the complement component, chemokine, and growth factors classes for all three diagnoses (Hendrickx, van Gastel, Leysen, Martin, & Maudsley, 2020; van Gastel et al., 2019).

24.7 The future of "OMICS"

The framework of cross-sectional criteria for diagnosis as provided by DSM-5 and ICD-11 had grave limitations with regard to precision and this flaw could only be rectified by a classification system that incorporated benchmarks ranging from cellular/molecular markers to behavioral and clinical manifestations. A paradigm shift was required to move away from the "atheoretical" criteria-based diagnoses to biological marker-based entities that had real clinical value (Rush & Ibrahim, 2018). Such a model based on unique pathophysiological mechanisms could inform the type, time, and sequence of

clinical interventions needed, thus leading to a biologically grounded and specified approach to psychiatric treatments. Biomarkers that could be consistently identified in the bloodstream of the patients allow minimally invasive and cost-effective monitoring at different disease stages and treatment courses. Hence, high-throughput "omics" approaches, in which data were integrated from genomics, transcriptomics, and proteomics, offered a powerful tool for future research in severe mental illness (Fernandes et al., 2017; Wium-Andersen, Vinberg, Kessing, & McIntyre, 2017).

Currently, some of the most effective appreciation of the complexity inherent in biologic disease and drug response intricacy was achieved using high-dimensionality (H-D) data streams from the "omics" pipelines (Shih, 2017). Multiple H-D data sets were now common and freely accessible for complex diseases such as metabolic disorders, cardiovascular diseases, and neuropsychiatric conditions. Over the last decade, the ability to analyze these high-dimensionality data streams had been profoundly enhanced through the development and implementation of vastly effective bioinformatics platforms. Employing these computational approaches to grasp the complexity of multifactorial diseases provided a way of integrating therapeutic-mediated signaling. It must be clearly understood that all diseases, as well as their treatment, were far more complex than supposed previously. With the advent of commonplace access to technologies that produced large volumes of high-dimensionality "omics" data, it was now essential to develop effective tools which could help in the appreciation of this highly nuanced data (Passos et al., 2019; Tai et al., 2019).

24.8 Future directions

The development of modern proteomic workflows that allowed high-throughput studies with large cohorts of well-defined samples is highly promising. Proteomics advanced from routine discovery and identification of proteins to integrated multiomics projects associating specific proteins to their genes and metabolites. Using additional information, such as assessment of biological pathways, will enable the utilization of targeted protein quantitation for monitoring fold changes in expression, as well as, biomarker discovery (Chu, Miller, Gieschen, & Fischer, 2017).

In order for proteomic investigations to exactly determine biomarkers, it was essential to select an accurately defined clinical population, employ adequate sampling technique and use standardized procedures for sample processing, as seen in more recent studies. Also, it was necessary to have a large enough sample size to permit adequate statistical power after stratifying for potential confounding factors (i.e., medication use, lifestyle factors). Future studies should preferably be longitudinal in design, allowing for repeated measures while accounting for interindividual differences in protein

expression levels. Furthermore, results from smaller discovery samples should be verified in larger cohorts. As was true in GWAS, proteomic studies with sufficient discovery as well as validation cohorts would have a significant lead in correctly detecting and confirming biomarkers (Patel, 2012, 2014).

Certainly, for progress to take place in the area of psychiatric proteomics, more improvements were needed in methodology. Clearly, the field had been stalled because of the need for replicable, robust, and high-throughput workflows. Nevertheless, more recent investigations demonstrated that it was possible to have rapid and technically sound proteome profiling pipelines. In this vein, for the sake of absolute protein quantification, progress was required in targeted proteomic methodologies, for example, by incorporating heavy isotope-labeled standards in the pipelines (Bittremieux et al., 2018; Scifo et al., 2017).

In addition, the use of standardized nomenclature and integration with other databases such as genomics and transcriptomics were likely to further enhance these efforts. Presently, even protein reporting sometimes lacked agreed terminology for gene symbols, making between-study comparisons problematic. This obstacle could be overcome by applying a universal nomenclature for entry codes and recommended names. While methodological challenges are still present, recent improvements with more rapid and robust workflows are highly promising. Minimally invasive samples such as plasma and serum were still unexploited sources of possible biomarkers which could greatly improve the clinical care of the patients. In addition, modern machine learning approaches may enhance model performance by evaluating multivariate patterns. The endeavors to incorporate peripheral blood profiling data with other laboratory and clinical endpoints could lead to the identification of omics-based novel "multidimensional" markers and provide a new understanding of complex diseases, based ultimately on pathophysiological mechanisms.

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