Mass Spectrometry Proteomics in Neuropsychiatric Disorder Biomarkers Assessment

Contributor: João Rodrigues, Bruno Manadas, Ana Martinho, Cátia Santa, Nuno Madeira, Manuel Coroa, Vítor Santos, Maria J. Martins, Carlos N. Pato, António Macedo

The success of mass spectrometry (MS) in proteomics is mainly due to its specificity and sensitivity, which are attributable to advances in liquid chromatography coupled to tandem MS (LC-MS/MS) approaches, and the development of statistical tools that allow the use of Big Data analysis strategies to extract meaningful biological information obtained by MS-based methods. This type of technology can reveal proteome insights at the composition, structure, and function level. Proteomics tools make it possible to evaluate the proteins in complex biological samples qualitatively and quantitatively (either relative or absolute). Based on the meta-analysis results, the upregulation of FCN3 and downregulation of APOA1, APOA2, APOC1, and APOC3 in schizophrenia (SCZ) patients is suggested. Despite the proven ability of MS proteomics to characterize SCZ, several confounding factors contribute to the heterogeneity of the findings.

1. Introduction

1.1. Neuropsychiatric Disorders

Psychiatric disorders (PD) comprise a wide range of mental health problems that can severely impact the well-being of those affected [1][2]. This set of clinical conditions can affect people of all ages and be a leading cause of morbidity, even in childhood and adolescence [3][4]. The effects of PD on public health are profoundly adverse and hugely contribute to the world's burden of the disease [1][3]. About 10% of the world population is affected, with mental disorders making up 30% of the global burden of non-fatal disease (WHO 2016) overcoming cancer and cardiovascular disease, while 1 million people worldwide die annually from suicide [5].

Thus, the global situation is bleak, with more than 450 million people worldwide living with some form of mental illness; in the European Union, the number of individuals affected per year is around 165 million [6][7]. Moreover, it is estimated that one-quarter of the world's population will manifest at least one mental disorder in some period of their life [7][8]. Unfortunately, for several reasons, progress in understanding PD has been slow [1][9].

1.2. Schizophrenia

The genetic architecture of schizophrenia is highly complex and heterogeneous. It is characterized by rare mutations that recently emerged with relatively high risk and common variants with individually minor effects on the disease [10]. Genes implicated by both common and rare alleles operate in crucial pathways for brain development, including histone modification, neuronal migration, transcriptional regulation, immune function, and synaptic plasticity [11].

People living with this disease have a significantly reduced average life expectancy, ~20 years lower than the general population. Nonetheless, the mortality rates are high across all age groups [8][12]. The current diagnosis of schizophrenia is mainly based on phenomenological observation and clinical descriptions using the standard operational criteria defined in systematic classifications, namely the Diagnostic and Statistical Manual of Mental Disorders, edition five (DSM-5), and International Classification of Diseases, version 11 (ICD-11), published by the American Psychiatric Association and WHO, respectively [3][13][14]. The main problem is that these diagnostic definitions have relatively good reliability but no established validity [15].

Epidemiologic studies show that it can take up to several years between symptom onset and diagnosis; evidence suggests that the earlier the diagnosis, the better the prognosis, by decreasing the duration of untreated psychosis [16][17].

The symptoms, which typically arise during adolescence or early adulthood, are defined as: (i) positive, such as hallucinations, delusions, and thought disorder; (ii) negative, such as poverty of speech or alogia, lack of motivation and social withdrawal; and (iii) cognitive symptoms, such as attention and learning deficits. While positive symptoms can stabilize throughout the course of the illness, negative symptoms tend to increase and become chronic along with cognitive impairments [18][19][20], although currently available interventions, such as antipsychotics and cognitive remediation, can reduce negative and cognitive symptomatology [21][22].

Psychotic symptoms, which integrate positive symptoms, are a defining feature of SCZ spectrum disorders, and their onset defines the first episode of psychosis [23][24]. Despite being considered the main feature for disease onset and diagnostic recognition, psychotic disorders are characterized by an earlier stage, a pre-psychotic stage termed prodrome, which is usually missed by clinicians [25][26].

The pathophysiology of SCZ remains unclear, lacking a comprehensive view of the underlying neurobiological mechanisms, although some aspects are beginning to be clarified. Dopaminergic dysfunction has been one of the pathophysiological hypotheses defended for decades, under various formulations, and is supported by genetic findings [27].

Hypo and hyperactivities of the dopaminergic system are seen in SCZ patients, and both are linked to the symptoms previously described [28][29]. Additionally, other dysfunctions underlying the pathophysiology of SCZ, such as neurotransmitter signaling of glutamate, hypothalamic-pituitary-axonal (HPA) axis signaling, immune system dysregulation and synaptic plasticity anomalies have been reported [19][29][30]. Changes in brain structures, which have also been proposed as etiologically relevant, are correlated with some of these alterations [30].

1.3. The Search for Biomarkers

To improve knowledge about these complex disorders, "omics" approaches have emerged to shed light on disease pathogenesis and support a trustworthy way of predicting and diagnosing PD [20][31]. With a vast potential associated, high-throughput omics technologies can be a solution to predict clinical endpoints, with the improvement of patient care and outcomes as the ultimate goal. However, the translation from research to a successful clinical omics-based test is far from the great potential of these approaches [32][33].

The search for candidate biomarkers is one of the outputs of -omic studies. According to the National Institute of Health (NIH), a biological marker, generally just termed as a biomarker, is a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [34]. The study of the brain and the associated disorders is complex since it presents a high degree of inter- and intra-cellular heterogeneity; so, different locations may have a distinct proteome due to modifications in different cell types and cellular networks. The CNS proteome can change even with minimal alterations in the normal course of its development and/or function [35][36]. Proteomics can be a powerful tool since it can give a real-time evaluation of an individual state, health vs. disease, and, in an ideal scenario, predict the susceptibility to develop a specific mental disorder [4][35]. The possibility of identifying and quantifying the proteins makes the proteomic approach more reliable for evaluating psychiatric diseases at different stages. Moreover, protein-based tests can offer the nearest view of the pathophysiological process behind PD since their expression and function are the results of what happens during post-transcriptional (e.g., alternative mRNA splicing) and post-translational events (e.g., phosphorylation, glycosylation, oxidation), as well as the interactions between them [3][4][37].

2. Mass Spectrometry

Since its development, mass spectrometry (MS)-based technologies have been improved and, in recent decades, became a well-suited method for biomarker discovery, supporting the expansion of the proteomics field [38][39]. The success of MS in proteomics is due to its specificity and sensitivity, which are mainly attributable to advances in liquid chromatography coupled to tandem MS (LC-MS/MS) approaches. This type of technology can reveal proteome insights at the composition, structure, and function level. Proteomics tools make it possible to evaluate the proteins in complex biological samples qualitatively and quantitatively (either relative or absolute) [40][41].

In the beginning, successes in proteomics approaches were supported by two-dimensional gel electrophoresis (2-DE), with complex protein mixtures being separated by their molecular charge (isoelectric point) and mass (molecular weight) in the first and second dimensions, respectively. This approach calculates protein abundances based on stained protein spots' intensities, followed by MS analysis for protein identification [23][42][43]. Although improvements were made, other methodologies emerged to circumvent some of the previous technical drawbacks, namely to face the dynamic range

limitations and the unsuitable separation and detection of some protein subtypes, such as membrane proteins [23][40]. Throughout the years, improvements in proteomics approaches were achieved, and a variety of more in-depth MS-based methods were quickly applied to compare protein profiles, usually between control versus disease states. Considering this, there are two main groups within quantitative proteomics methods: (i) labeling techniques, which involve different isotopic labeling of samples, including chemical, enzymatic or metabolic labeling, followed by MS analysis; and (ii) label-free techniques, where the sample is individually analyzed without the addition of any other chemical compound. The newest quantitative approaches are regarded as versatile and cost-effective alternatives to labeled quantitation, having gained significant interest in recent years, mainly due to the development of more sensitive and reliable methods. Additionally, some techniques capable of detecting either relative or absolute peptide levels can provide a targeted MS approach and be used as a validation method [43][44][45].

The absence of molecular biomarkers being used in the clinical environment and the increasing use of large proteomics screenings to search for SCZ biomarkers, allowed scholars to study on the use of MS-based methods in proteomic studies to assess biomarkers or a panel of biomarkers associated with SCZ based only on the analysis of peripheral fluids.

3. Mass Spectrometry Proteomics in Neuropsychiatric Disorder Biomarkers Assessment

To evaluate the efficacy of MS proteomics applied to human peripheral fluids to assess SCZ biomarkers and identify relevant networks of biological pathways, a systematic review (following PRISMA Guidelines) and meta-analysis were performed. To do so, a search for studies using MS proteomics to identify proteomic differences between SCZ patients and healthy controls was performed. Overall, nineteen articles fulfilled the inclusion criteria, allowing a total of 217 proteins to be identified as altered between SCZ and healthy control groups in peripheral fluids, including serum, plasma, PBMCs, sweat, and saliva.

Apolipoproteins (APOs) were the group of proteins mostly reported in SCZ vs. control studies as differentially expressed. In fact, ten studies reported the dysregulation of apolipoproteins [46][47][48][49][50][51][52][53][54][55]. APOs are very important in lipid homeostasis by transporting cholesterol and lipids between cells, having a well-established role in the transport and metabolism of lipids, and in inflammatory and immune response regulation [56][57]. This group of compounds has been indicated as potential candidates for psychiatric biomarkers, with several studies reporting altered levels of cholesterol and APOs in psychiatric disorders [57][58][59]. Accordingly, APOs alterations were associated with inflammatory response [49][54][55], immune system [46][53], lipid metabolism [49][53], cardiovascular system [48], retinoid transport [55], and cognitive decline and underlying morphological changes [50].

APOA1 is the major protein component of the HDL fraction in plasma. Together with APOA2, APOA4, APOC1, and APOD, APOA1 is recognized for regulating the plasma levels of free fatty acids, having an important role in HDL and triglyceride-rich lipoprotein metabolism in the reverse cholesterol transport pathway [60]. APOA1 is also reported as having pro-immune and anti-inflammatory potential [56]. In all selected studies where it was identified as altered, ApoA1 level was reported to be reduced in schizophrenia patients compared to healthy subjects [50][53][54][55].

APOA2, the second most abundant protein in HDL fraction, is a key regulator of HDL metabolism [60], although its inflammation role is not clearly defined, with different studies reporting it as having pro- and anti-inflammatory effects [61]. APOA2 was identified as differentially expressed in four studies, being downregulated in SCZ patients in all studies [49][50][53][54].

APOA4, a lipid-binding protein, is known to be involved in a broad spectrum of biological processes, including lipid metabolism, reverse cholesterol transport, atherosclerosis protection, and glucose hemostasis [62]. APOA4 was identified as differentially expressed in four studies; however, it showed a heterogeneous behavior: downregulated in three studies [49][50][54] and upregulated in only one study [51].

The apolipoproteins APOC1, APOC2, APOC3, APOD, and APOE were identified in three studies as differently expressed, showing a general tendency of downregulation in SCZ patients except for APOE, which has a trend for upregulation. Of these, only for APOD, a soluble carrier protein of lipophilic molecules that is mostly expressed in neurons and glial cells within the central and peripheral nervous system [63], the results were consistent in all three studies, and it was identified as decreased in SCZ patients [64][47][65]. A trend of downregulated behavior was identified for APOC1 (the smallest of all APOs, participating in lipid transport and metabolism) [49][54], APOC2 (a small exchangeable apolipoprotein found on triglyceride-rich lipoprotein particles) [51][54], and APOC3 (an APO capable of inhibiting lipoprotein lipase and hepatic lipase) [49][51], in two out of three studies.

APOF [50][52], APOH [47][49], and APOL1 [50][52] had a similar behavior: upregulated in the two studies. For APOB, no clear trend was observed, with one study reporting its increase [48] and another a decrease [50] in SCZ patients.

RET4 is mainly expressed in the liver with a primary function of transporting retinol (vitamin A) from the liver to peripheral tissues, with retinol being essential for the brain to facilitate learning, memory, and cognition [66]. Retinoid signaling plays a vital role in immune cell function. Accordingly, it is suggested that factors that affect this system could have important implications for SCZ and other psychiatric disorders-associated inflammatory stress [67].

ANT3, a glycoprotein anticoagulant mainly produced in the liver that exerts anticoagulant and anti-inflammatory effects by targeting activated thrombin and other blood coagulation factors [68], was identified as being increased in SCZ patients [49] [50][55].

FCN3 is a ficolin, a protein containing both a collagen-like domain and a fibrinogen-like domain with a specific binding affinity for N-acetylglucosamine. FCN3 can complex with mannose-associated serine proteases to activate the complement pathway [69], being ficolins' activation already reported as a potential biomarker of the severity of schizophrenia [70]. In the selected studies, FCN3 was also identified in three studies as upregulated FC [49][50][71].

The immune system and inflammatory response were the most identified biological processes altered in SCZ patients [46] [49][72][73][71][54][74]. These results agree with current knowledge about SCZ, associating the immune system and inflammatory response with the SCZ pathophysiology [75][76][77]. In fact, a wide range of immune alterations has been reported in SCZ patients, such as elevated levels of cytokines and inflammation markers, abnormalities of the blood-brain barrier, CNS inflammation, and increased autoantibody reactivity [76].

Several other mechanisms have also been linked to SCZ, including mitochondrial dysfunction, energy metabolism processes, complement and coagulation cascades, oxidative stress, transport, morphological changes, cognitive impairment, lipid metabolism, and hypothalamic–pituitary–adrenal (HPA) axis over-activation [77][78].

4. Directions for Future Research

The recent advances in MS proteomics strategies applied to human peripheral fluids allow the establishment of a robust platform for proteome profiling of clinical samples with an unprecedented depth. In fact, the MS's ability to generate different levels of information about the individual proteome may lead to the comprehensive characterization of the biological network of pathways involved in SCZ, seeking the identification of reliable biomarkers of the disorder to improve prediction and diagnosis towards the ultimate goal of improving patient care and outcome.

However, a standardization of the studies' characteristics is required for more specific clinical proteomics studies. In fact, a precise definition of the study's objectives and standardization of sociodemographic, clinical, and cognitive variables across the studied groups would make them more objective and specific, allowing a more comprehensive understanding of SCZ pathophysiology and increasing the possibility of identifying specific biomarkers of SCZ. This will minimize the confounding factors, leading to improvements in the statistical power and, consequently, the efficiency of translating biomarker candidates and drug targets to the clinical application associated with the disorder.

The use of MS proteomics pipelines combining (i) standardized conditions; (ii) high-throughput sample preparation techniques; (iii) high computational power for data processing and analysis will lead to a rapid expansion of clinical cohort sizes and consequently to more robust studies. An extra effort should be made to provide data in an open format so the community can re-analyze and perform more extensive studies based on data analysis from multiple centers. After full implementation of those proteomics pipelines, their application in extended clinical cohorts will allow taking into account the different variables (such as gender, comorbidities, illness duration, and treatment), leading to a more comprehensive understanding of SCZ pathophysiology and, consequently, increasing the possibility of identifying specific biomarkers of SCZ, seeking to improve prediction and diagnosis towards the ultimate goal of improving patient care and outcome.

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