Journal of Proteomics 223 (2020) 103814

Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

Digging deeper in the proteome of different regions from schizophrenia brains

G. Reis-de-Oliveira[a,1], G.S. Zuccoli[a,1], M. Fioramonte[a,1], A. Schimitt[e,f], P. Falkai[e], V. Almeida[a], D. Martins-de-Souza[a,b,c,d,*]

[a] Lab of Neuroproteomics, Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas, Brazil
[b] Experimental Medicine Research Cluster (EMRC), University of Campinas, Campinas, Brazil
[c] D'Or Institute for Research and Education (IDOR), São Paulo, Brazil
[d] Instituto Nacional de Biomarcadores em Neuropsiquiatria (INBION), Conselho Nacional de Desenvolvimento Científico e Tecnológico, São Paulo, Brazil
[e] Department of Psychiatry and Psychotherapy, Ludwig Maximilian University (LMU), Munich, Germany
[f] Laboratory of Neurosciences (LIM-27), Institute of Psychiatry, University of São Paulo, São Paulo, Brazil

ARTICLE INFO

Keywords:
Caudate nucleus
Cerebellum
Posterior cingulate cortex
Postmortem

ABSTRACT

Schizophrenia is a psychiatric disorder that affects 21 million people worldwide. Despite several studies having been shown that some brain regions may play a critical role in the pathophysiology of schizophrenia, the molecular basis to explain this diversity is still lacking. The cerebellum (CER), caudate nucleus (CAU), and posterior cingulate cortex (PCC) are areas associated with negative and cognitive symptoms in schizophrenia. In this study, we performed shotgun proteomics of the aforementioned brain regions, collected postmortem from patients with schizophrenia and compared with the mentally healthy group. In addition, we performed a proteomic analysis of nuclear and mitochondrial fractions of these same regions. Our results presented 106, 727 and 135 differentially regulated proteins in the CAU, PCC, and CER, respectively. Pathway enrichment analysis revealed dysfunctions associated with synaptic processes in the CAU, transport in the CER, and in energy metabolism in the PCC. In all brain areas, we found that proteins related to oligodendrocytes and the metabolic processes were dysregulated in schizophrenia.

Significance: Schizophrenia is a complex and heterogeneous psychiatric disorder. Despite much research having been done to increase the knowledge about the role of each region in the pathophysiology of this disorder, the molecular mechanisms underlying it are still lacking. We performed shotgun proteomics in the postmortem cerebellum (CER), caudate nucleus (CAU) and posterior cingulate cortex (PCC) from patients with schizophrenia and compared with healthy controls. Our findings suggest that each aforementioned region presents dysregulations in specific molecular pathways, such as energy metabolism in the PCC, transport in the CER, and synaptic process in the CAU. Additionally, these areas presented dysfunctions in oligodendrocytes and metabolic processes. Our results may highlight future directions for the development of novel clinical approaches for specific therapeutic targets.

1. Introduction

Schizophrenia is a severe psychiatric disorder that affects 21 million people around the world [1]. It is characterized by the presence of positive (e.g. hallucinations, delusions) and negative symptoms (disorganized speech, social withdraw, anhedonia), and cognitive deficits [2]. Schizophrenia is a neurodevelopmental disorder, in which *N*-methyl-D-aspartate receptor (NMDAr) hypofunction and dysfunction in dopaminergic activity in mesolimbic and mesocortical pathways are associated with symptoms development [3,4]. In addition, several brain imaging studies have described the role of each brain area in the pathophysiology of this disorder.

The cerebellum (CER) is a well-known brain region related to motor coordination, as well as functions in language, emotions, sleep, and visceral responses [5,6]. Increasing recognition of the importance of the cerebellum has pointed to a potential role of this area in the

* Corresponding author at: Lab of Neuroproteomics, Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas, Brazil.
E-mail address: dmsouza@unicamp.br (D. Martins-de-Souza).
[1] These author contributed equally to this work.

https://doi.org/10.1016/j.jprot.2020.103814
Received 24 January 2020; Received in revised form 21 April 2020
Available online 07 May 2020

pathophysiology of schizophrenia [7]. A recent mega-analysis showed that patients with schizophrenia present a decrease in gray matter in posterior regions of the cerebellum, which has functional connectivity with associative areas, such as the cortex [8]. In addition, evidence suggests a correlation between the positive symptoms and cerebello-thalamo-cortical disconnectivity [9,10].

The posterior cingulate cortex (PCC) plays a pivotal role in cognitive functions and in the default mode network [11]. Patients with schizophrenia show alterations in the default mode network when presented passive tasks [12,13]. Despite the PCC exhibiting a high metabolic rate, imaging studies have described disturbances in glucose metabolic rate in the PCC for patients with schizophrenia [14–16]. Rencently, Kirino and colleagues have found that this patients present an enhanced functional connectivity between cortical areas, such as the PCC and caudate nucleus, suggesting an additional disruption in cortico-striatal networks [17].

The caudate nucleus (CAU), which plays a pivotal role in learning and reward processes, is a basal nucleus and a part of the striatum [18]. Dysfunctions in the CAU are related to negative symptoms, such as deficits in reward processes, which is hypothesized to be due to a dysregulation of dopamine pathways in the striatum [19–22]. In addition, the CAU was also associated with cognitive symptoms, since this region and cortical areas connected to the CAU present abnormal hemispheric specialization, which can affect memory and language functions [23].

Despite all these efforts to understand schizophrenia, a molecular basis related to the brain regions mentioned above is still lacking. Proteomics is a powerful approach to investigate complex and multifactorial diseases such as schizophrenia and other psychiatric disorders [24–26]. Several investigations have been done using postmortem brain tissue from patients with schizophrenia, mainly with the prefrontal cortex [27,28], anterior temporal lobe [29], orbitofrontal cortex [30], corpus callosum [31], and thalamus [32]. Proteomic approaches even allow for the investigation of subcellular fractions, such as the nucleus and mitochondria. Studies have shown that nuclei from white and gray matter are differentially regulated in patients with schizophrenia [33], as well as mitochondria presenting aberrant size, location, and number among the brain areas [34].

Here, we performed shotgun proteomics to characterize the proteome of postmortem Posterior Cingulate Cortices, Caudate Nuclei, and Cerebella from patients with schizophrenia and compared them with a mentally healthy control group. Additionally, we carried out a subcellular fractionation of mitochondria and nuclei from the same brain regions, performing each respective proteomic analysis. Therefore, this approach allows a whole, mitochondrial and nuclear analysis to hypothesize about novel molecular features associated with schizophrenia spectrum disorder.

2. Materials and methods

2.1. Brain extraction and storage

Brain samples were collected postmortem and provided by the Brain Net Europe Consortium. Patient samples (n = 5, for each region) came from the State Mental Hospital, Wiesloch, Germany. The individuals were chronic patients with residual symptoms, diagnosed antemortem according to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria and were medicated. The mentally healthy group (n = 5, for each region) did not present any brain disorder or somatic disease, neither were they medicated with antidepressants or antipsychotics. Control samples came from the Institute of Neuropathology, Heidelberg University, Heidelberg, Germany. Both groups were represented by German Caucasians with no history of alcohol or drug abuse. Supplementary Table 1 shows individual aspects of patients and healthy subjects, as well as the respective brain regions collected. Additional information about patients can be found in Saia-Cereda, 2015 [35].

2.2. Subcellular fractionation, protein extraction, and digestion

Subcellular fractionation, protein extraction, and digestion were carried out following the protocol described by Reis-de-Oliveira [36]. Approximately 45 mg of brain tissue was weighed out in duplicate; the first sample was used for subcellular fractionation and the second was for whole-proteome analysis. The portion used for subcellular fractionation was submitted to cell lysis and a sucrose gradient, followed by differential centrifugation to isolate high membranous organelles (e.g. nuclei) and mitochondria. Both enrichment and whole tissue samples were added in denaturing buffer(Tris-SDS, 2-mercaptoethanol, glycerol, and bromophenol blue), followed by mechanical lysis with pestle and ultrasonication. The samples were then heated at 95 °C and centrifuged at 21,000 $\times g$. Samples were desalted using electrophoresis on a 12% polyacrylamide gel. This experiment was carried out on a short electrophoresis run and a unique gel spot was generated and digest. Each gel lane was trimmed and submitted to in-gel trypsin digestion. The peptides were dried and stored at −80 °C [36].

2.3. LC-MS/MS analyses

Peptides were resuspended in ammonium formate (50 mM, pH 10), quantified by a spectrophotometer (DS-11, DeNovix), and analyzed by liquid chromatography tandem-mass spectrometry (LC-MS/MS). Samples were injected into an ACQUITY UPLC M-Class (Waters, Co) coupled to a Synapt G2-Si mass spectrometer (Waters, Co). The peptides were separated using an HSS T3 1.8 μm × 75 μm × 150 mm column, carrying out a binary gradient from 3 to 40% acetonitrile (ACN) with 0.1% formic acid, followed by a binary gradient from 40 to 85% ACN at a flow rate of 400 nL/min. When performing two-dimensional liquid chromatography (2D), the peptides were first loaded on an XBridge BEH130 C18 5 μm × 300 μm × 50 mm column and fractionated in five elutions before the stepwise gradient across the analytical column, using 11.4%, 14.7%, 17.4%, 20.7%, and 50.0% ACN. The chromatographic conditions were based on previous work from our group [31,36]. Details about each experiment are described in Table 1.

NanoElectrospray ionization was set to positive mode and Data-Independent Acquisition was performed using ion mobility separation and fragmented using collision-induced fragmentation (CID) (HDMSe, Waters Co.). The acquisition range utilized was 50 to 2000 m/z, using a collision energy fragmentation ramp of 19 to 53 eV. Glu-fibrinopeptide B was used as reference lock mass compound, at a flow rate of 500 nL/min and 100 fmol/uL. CER analyses were enhanced by optimizing precursor fragmentation efficiency based on drift time-specific collision energy profiles (UDMSe).

Table 1
Chromatographic and mass spectrometry methods for each experiment performed.

Brain region	Sample type	UPLC system	Fractions/ sample	Gradient time (min)	MS acquisition mode
CAU	WP	1D	–	95	HDMSE
CAU	MIT	1D	–	95	HDMSE
CAU	NUC	1D	–	95	HDMSE
PCC	WP	1D	–	120	HDMSE
PCC	MIT	1D	–	54	HDMSE
PCC	NUC	1D	–	54	HDMSE
CER	WP	2D	5	54	UDMSE
CER	MIT	2D	5	54	UDMSE
CER	NUC	2D	5	54	UDMSE

CAU = Caudate Nucleus; PCC = Posterior Cingulate Cortex; CER = Cerebellum; WP = Whole-proteomic; MIT = Mitochondrial-enrichment; NUC = Nucleus-enrichment.

2.4. Data processing and statistical analysis

Progenesis QI for Proteomics® (version 3.0) was used to analyze MS raw files. MS/MS spectra were searched against the *Homo sapiens* database (Uniprot Reviewed database, March 2019), using the Ion Accounting algorithm (Version 4.0) [37]. Cysteine carbamidomethylation was applied as fixed modification and methionine oxidation as variable one. Trypsin was set as the digestion enzyme with a maximum of 1 missed cleavage and 600 kDa as the maximum protein mass. The ion set requirements were 2 or more fragments/peptide, 5 or more fragments/protein, 1 or more peptides/protein, and a false discovery rate (FDR) of 1% for peptide and protein identification. Quantitation was performed using the 3 most abundant peptides for each protein [38]. Proteins with absolute mass error of 20 ppm were deleted.

Progenesis QI for proteomics performs a variation of the one-factor ANOVA calculation, which assumes that the conditions are independent and the means of the conditions are all equal. Therefore, the tests return a *p*-value that takes into account the mean difference and the variance and also the sample size [39]. Proteins differentially regulated (ANOVA < 0.05) were used to perform the in silico analyses in Database for Annotation, Visualization and Integrated Discovery (DAVID) [40] and Ingenuity Pathway Analysis® (IPA, QIAGEN). Data visualization was performed in an RStudio [41] and Cytoscape [42].

3. Results

3.1. Proteomic analysis

The *postmortem* caudate nucleus (CAU), cerebellum (CER), and posterior cingulate cortex (PCC) samples from patients with schizophrenia and the control group were submitted to differential centrifugation in order to perform subcellular fractionation of high-density organelles (e.g. nuclei) and low-density mitochondria. The proteins from all experiments, whole tissue and its enrichments, were extracted and digested in-gel with trypsin. The peptides were loaded in the liquid-chromatographic system coupled to a Q-TOF mass spectrometer and the data were analyzed in Progenesis® QI for Proteomics (Fig. 1A).

The CER analyses represented the highest number of proteins quantified, with 1518, 1599, and 1703 proteins from mitochondrial enrichment (CERMIT), nuclear enrichment (CERNUC), and the whole proteome (CERWP), respectively. For the CAU samples, 1518 (CAUMIT), 1599 (CAUNUC), and 1703 (CAUWP) proteins were quantified among the experiments. Finally, the PCC was the region with the lowest number of quantitated proteins: 788 proteins in mitochondrial enrichment (PCCMIT), 1097 in nuclear enrichment (PCCNUC), and 1473 in the whole proteome (PCCWP) analysis (Fig. 1B).

We also calculated Pearson's correlation coefficient between the patient and control datasets (Fig. 1C). The hierarchical clustering suggests a switching in the proteomic similarities among the brain areas of schizophrenia patients when compared with control subjects. This data suggests that patients with schizophrenia share more similarities in proteome profiling between the PCC and CAU than the control group, in which the PCC is closer to the CER.

3.2. Cerebellum

Proteomic analysis of the CER revealed 135 proteins to be differently regulated in patients with schizophrenia compared to controls (ANOVA < 0.05, Fig. 2A and Supplementary Table 2). Functional enrichment analyses carried out using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) suggest these proteins are mainly related to cellular junctions and cytoskeleton processes (Fig. 2B).

The network analysis performed in IPA (Fig. 2C) presented dysfunction in cellular organization, which highlighted the role of these proteins in the over-migration of neurons (p = 1.40e-06) and Purkinje cells (p = 3.14E-05), and other cellular movements (p = 1.97e-06). Additionally, deregulated proteins in these networks can lead to dysfunctions in the proteasome, NfkB complex, caspase, and ERK signaling pathways (Fig. 2D).

3.3. Caudate nucleus

The proteomic analysis of the CAU showed 106 to be proteins differentially regulated in patients with schizophrenia when compared with control subjects (ANOVA < 0.05, Fig. 3A, Supplementary Table 3). The in silico functional analysis carried out using GO and KEGG revealed that the CAU region from patients presented dysregulations in vesicle-mediated transport (p = 2.0e-04), and the synaptic vesicle cycle (p = 3.11e-04, Fig. 3B).

The network analysis performed in IPA reinforced the dysregulation of transport synaptic vesicles (p = 2.55e-08) in the top scoring networks. Additionally, IPA revealed that patients with schizophrenia presented disturbances in the apoptotic process in neurons (p = 4.80e-05, Fig. 3C). Proteins related to these networks can also affect key molecules of the immunological process (e.g. TCR, NFkB complex), signaling (ERK1/2), and hormonal pathways (LSH, growth hormone, Fig. 3D).

3.4. Posterior cingulate cortex

The posterior cingulate cortex showed a total of 727 proteins to be differentially regulated in patients compared to controls (ANOVA < 0.05, Fig. 4A-B, Supplementary Table 4). The in silico analysis revealed these proteins are related to the tricarboxylic acid cycle (p = 2.96e-15), carbon metabolism (p = 9.24e-25), oxidative phosphorylation (p = 2.92e-22), and neuronal processes, such as synaptic vesicle cycle (p = 2.61e-11) and dopaminergic synapses (p = 6.55e-07).

The further network analyses carried out in IPA uncovered that proteins deregulated in the merged top1 networks were related to transport of proteins (p = 1.20e-07), vesicles (p = 6.38e-06), and molecules (p = 1.16e-05); mitochondrial disorders (p = 3.73e-05); and neuritogenesis (p = 1.18e-04, Fig. 4C). In addition, this merged network presented proteins related to energy metabolism (e.g. phosphatases, ATPases), as well as signaling pathways (e.g. ERK1/2, 14–3-3) and the immunological process (e.g. NFkB, TCR).

3.5. All brain regions

According to the proteomic analyses, patients with schizophrenia presented 897 differentially regulated proteins. Of the three regions, the PCC was the region that was most affected, while the CAU was the least affected(Fig. 5A). Although there is no shared dysregulated protein among all three brain regions, the PCC shared 35 proteins with both the CAU and CER, while the CAU and CER just presented 1 protein in common (VPS35).

The enriched pathways generated from differentially regulated proteins were submitted to the Enrichment Map [43] and MCODE [44] plugins in Cytoscape in order to select the regions with higher connections in the pathway networks (Fig. 5B). This analysis showed a relationship between the brain areas and their main affected pathways: the PCC was highly represented by energy metabolism and signaling pathways, the CAU was most deregulated in neurotransmission processes, and the CER presented a dysregulation in transport-related proteins. In addition, translational processes were affected in all the brain regions.

As mentioned above, 35 differentially regulated proteins were identified in the CAU and PCC areas, as well as in the CER and PCC (Fig. 5C). We performed a pathway enrichment analysis of just these proteins that were deregulated in the PCC and other regions (Fig. 5D). Despite the absence of shared proteins among all three regions, the

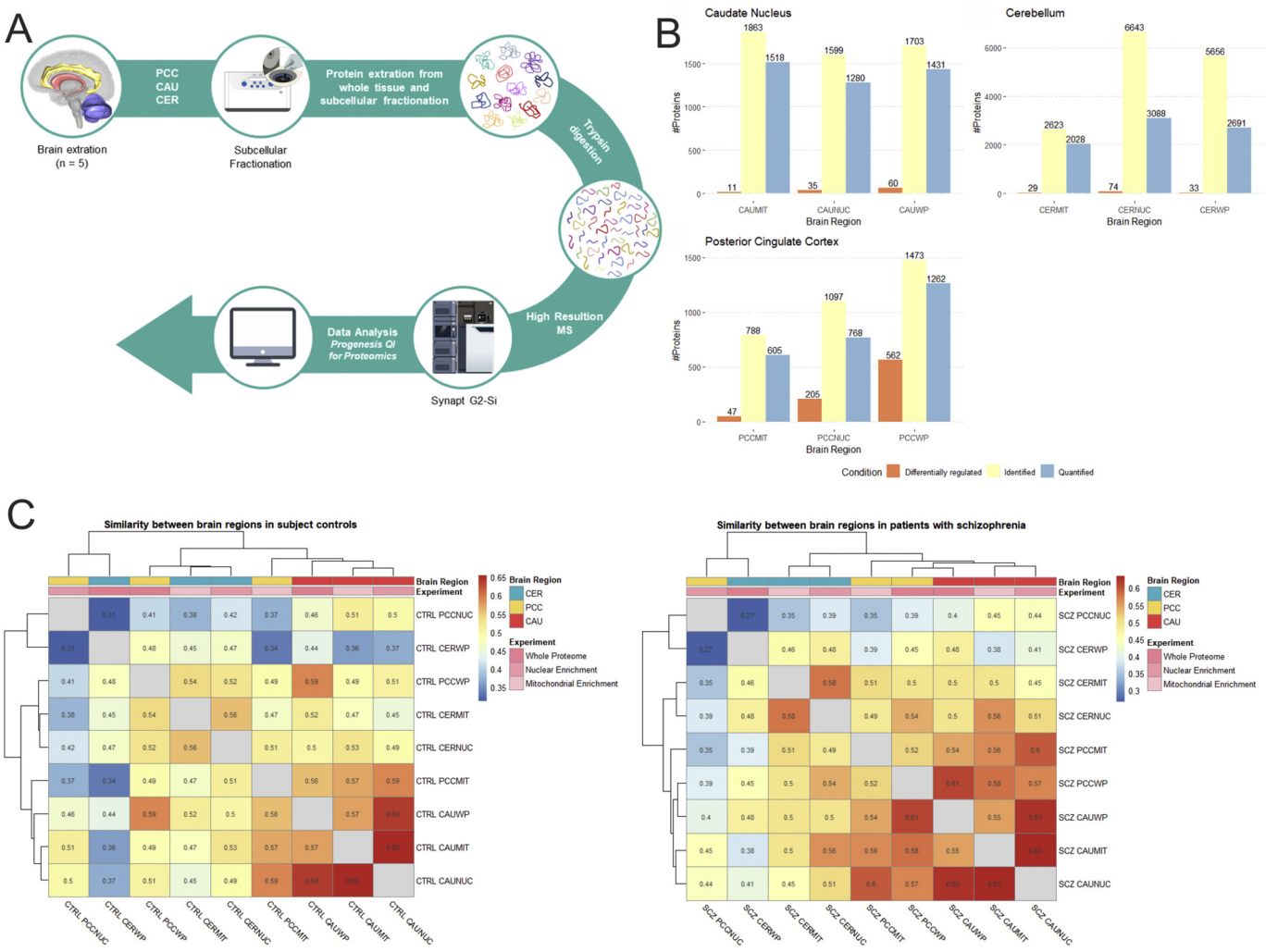

Fig. 1. Proteomic analysis of postmortem brain tissue from patients with schizophrenia and controls. (A) Experimental workflow for sample preparation and quantitative mass spectrometry. (B) Number of proteins identified, quantified, and differentially regulated for the three brain regions and enrichments. (C) Efficiency of mitochondrial and nuclear enrichment. (D) Pearson correlation between analyses of control subjects and patients with schizophrenia.

proteins deregulated in more than one region were related to metabolic pathways, oxidative phosphorylation, neuron development, the myelin sheath, and the MAPK signaling pathway. Taken together, despite our data suggesting compartmentalization of cellular dysfunctions throughout the brain of patients with schizophrenia, these regions also share some dysregulations in their biological processes.

4. Discussion

As far as we are aware, this is the first study using shotgun proteomics to investigate multiple postmortem brain areas from patients with schizophrenia, areas which are known to be associated with different functions, such as movement coordination, default mode network, and reward processes. These divergent functions were reflected in our proteomic results, in which each brain region presented specific dysregulated pathways, such as the transport process in the CER, synaptic processes in the CAU, and energy metabolism in the PCC of patients when compared to controls.

Proteomic analyses of all brain areas suggested that the proteome of patients with schizophrenia share different profiles in each region compared to healthy subjects. These data suggest that functional dysconnectivity observed in imaging studies with schizophrenia patients [45,46] may be associated with the molecular changes observed in our study.

4.1. Cerebella from patients present dysregulation in transport-related processes

The cerebellum has emerged as a pivotal brain region in schizophrenia development, such as the cortico-cerebellar-thalamic-cortical circuits highlighted in gene expression analyses [7,47]. Our findings showed that patients with schizophrenia present a dysfunction in proteins related to cytoskeleton processes, such as cell over-migration of Purkinje cells (Fig. 2C). Purkinje cells are GABAergic interneurons present in the cerebellum and they were found reduced in patients with schizophrenia and bipolar disorder [48]. G Protein Subunit Alpha 12 (GNA12) and G Protein Subunit Alpha 13 (GNA13) are transducer proteins associated with migration [49] and cell-adhesion processes [50]. Studies have shown that these genes are affected in schizophrenia [51] and their partial ablation in a mouse model affects Purkinje cell migration and the development of the cerebellum [52]. Disruption in neuronal migration was reported in patients with schizophrenia, playing a crucial role in the neurodevelopment of cerebellum and other brain regions [53,54]. In addition, our data also showed upregulation of the AIF1 protein, which is an activation marker for microglial cells, suggesting a potential inflammatory status in the brains of patients. The inflammation has critical relevance to schizophrenia [55]. A recent postmortem study has shown that the cerebellum of patients with schizophrenia presented an over-activation of the TLR4 pathway, which

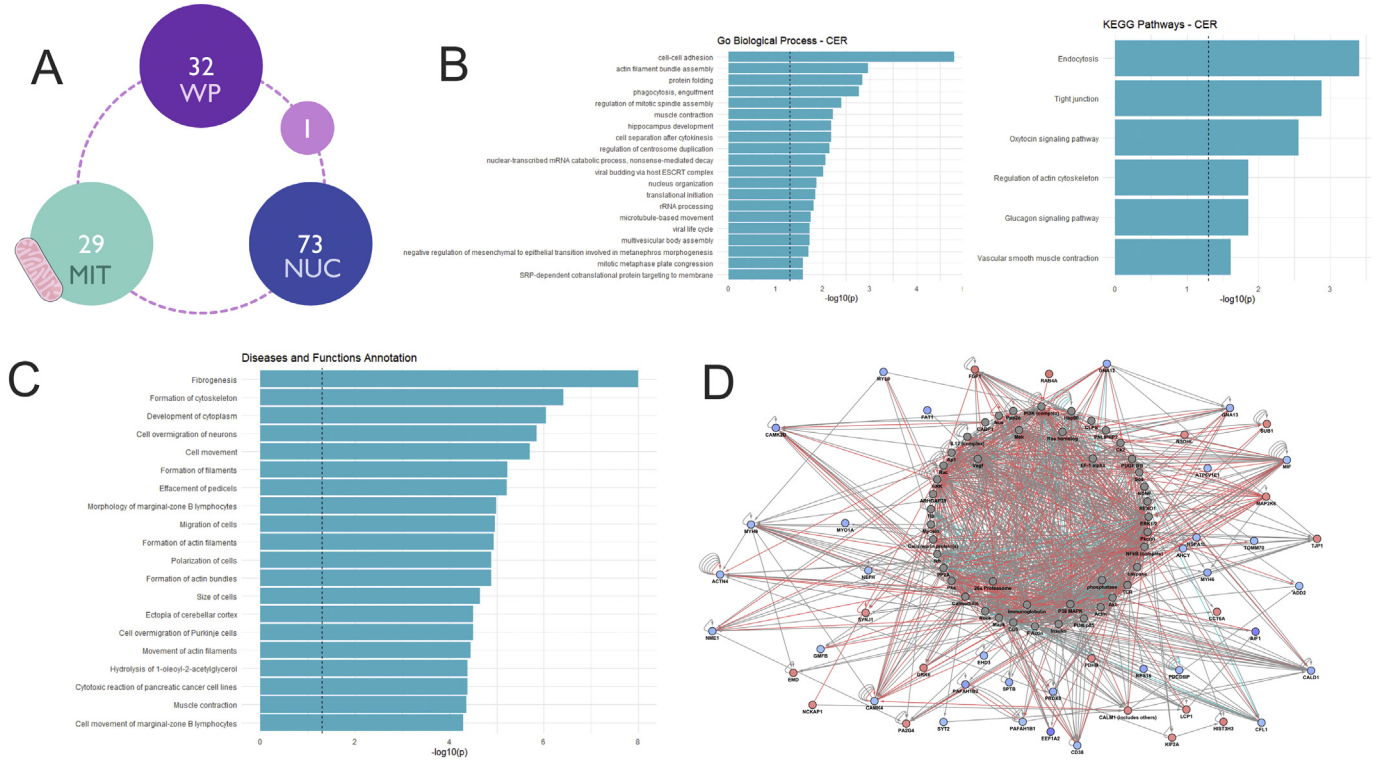

Fig. 2. Proteomic analysis of the cerebellum. (A) Diagram with numbers of proteins found differentially regulated in different fractions. (B) Pathway enrichment analysis performed in KEGG pathway database under Gene Ontology (GO) Biological Process. (C) Diseases and functional annotation performed using IPA. (D) Merging of network analysis from each experiment, performed with IPA. Red arrow: activation; blue arrow: inhibition; red circles: proteins found upregulated in the proteomic analysis; blue circles: proteins found downregulated in the proteomic analysis. WP = Whole proteome; MIT = Mitochondrial enrichment; NUC = Nuclear enrichment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

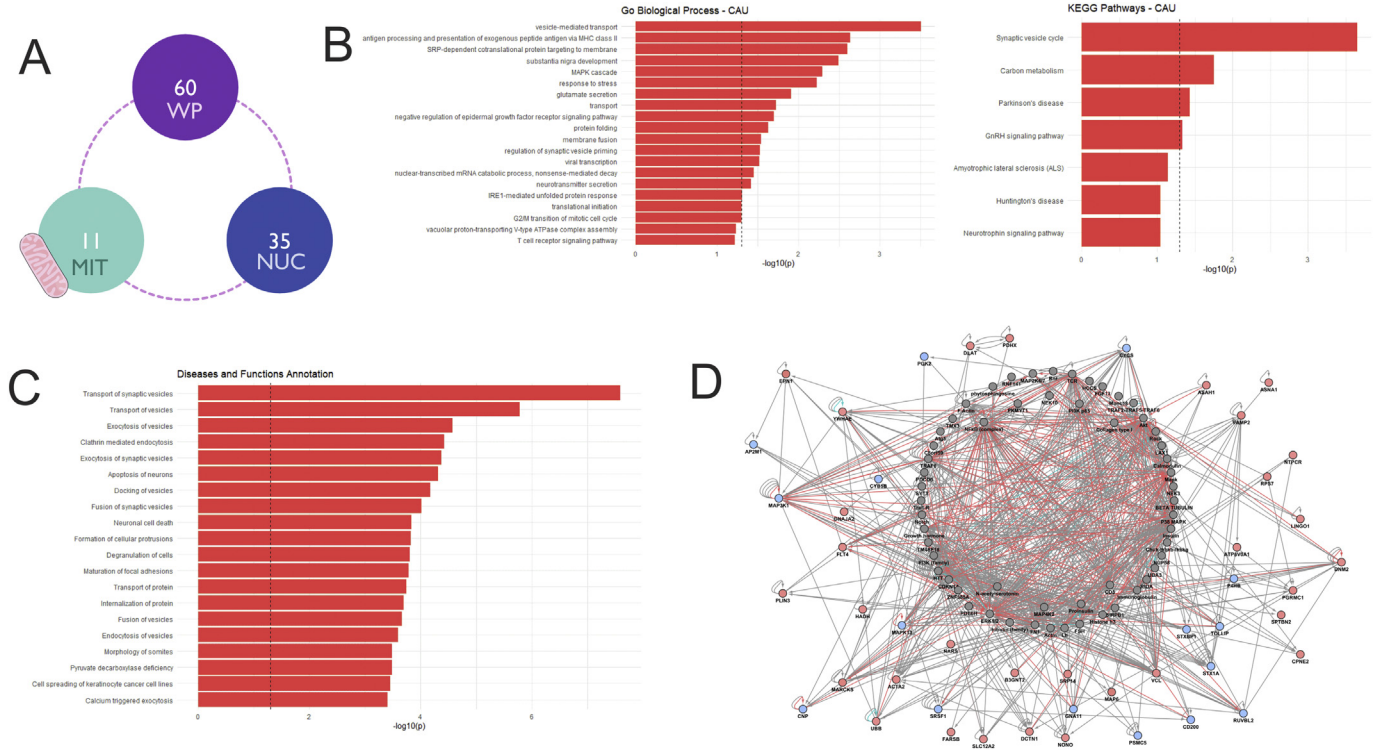

Fig. 3. Proteomic analysis of the caudate nucleus. (A) Diagram with numbers of proteins found differentially regulated in different fractions. (B) Pathway enrichment analysis performed in KEGG pathway database under Gene Ontology (GO) Biological Process. (C) Diseases and functional annotation performed using IPA. (D) Merging of network analysis from each experiment, performed with IPA. Red arrow: activation; blue arrow: inhibition; red circles: proteins found upregulated in the proteomic analysis; blue circles: proteins found downregulated in the proteomic analysis. WP = Whole proteome; MIT = Mitochondrial enrichment; NUC = Nuclear enrichment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

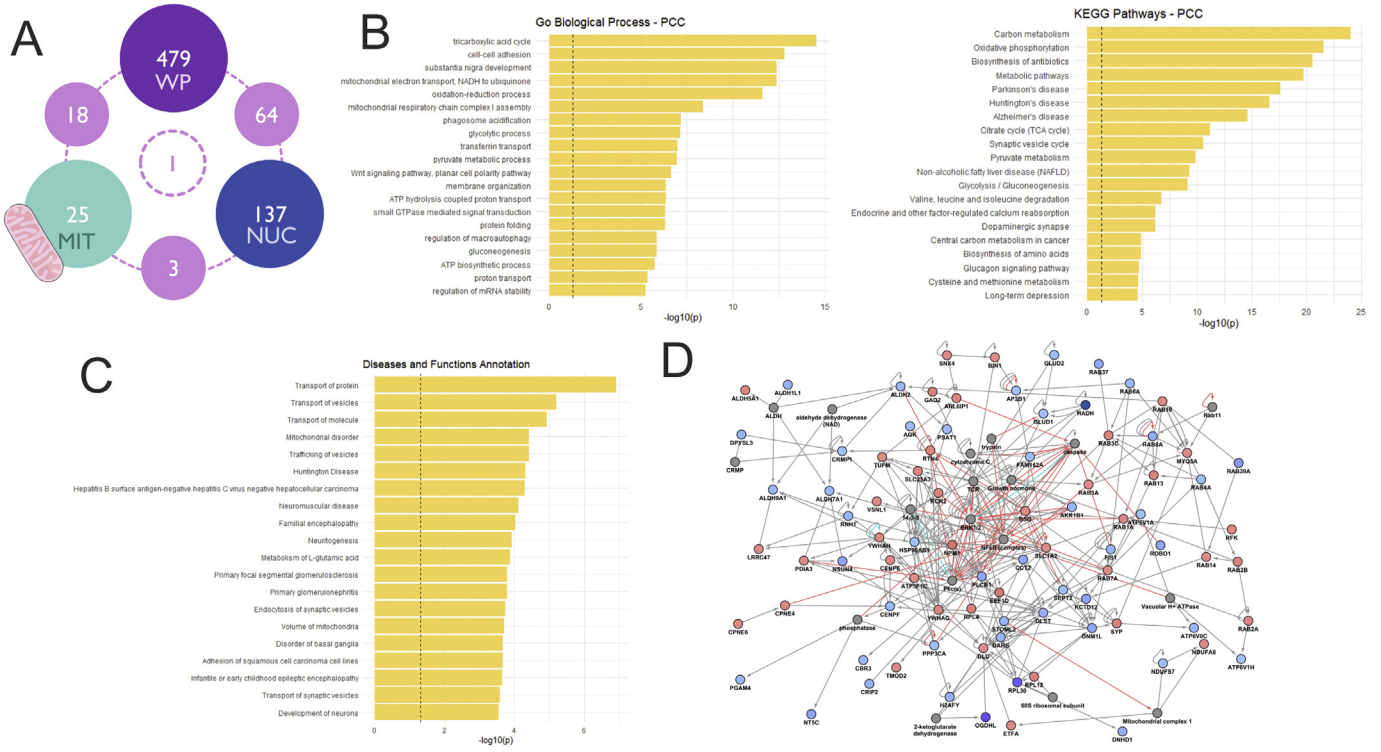

Fig. 4. Proteomic analysis of the posterior cingulate cortex. (A) Diagram with numbers of proteins found differentially regulated in different fractions. (B) Pathway enrichment analysis performed in KEGG pathway database under Gene Ontology (GO) Biological Process. (C) Diseases and functional annotation performed using IPA. (D) Merging of network analysis from each experiment, performed with IPA. Red arrow: activation; blue arrow: inhibition; red circles: proteins found upregulated in the proteomic analysis; blue circles: proteins found downregulated in the proteomic analysis. WP = Whole proteome; MIT = Mitochondrial enrichment; NUC = Nuclear enrichment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

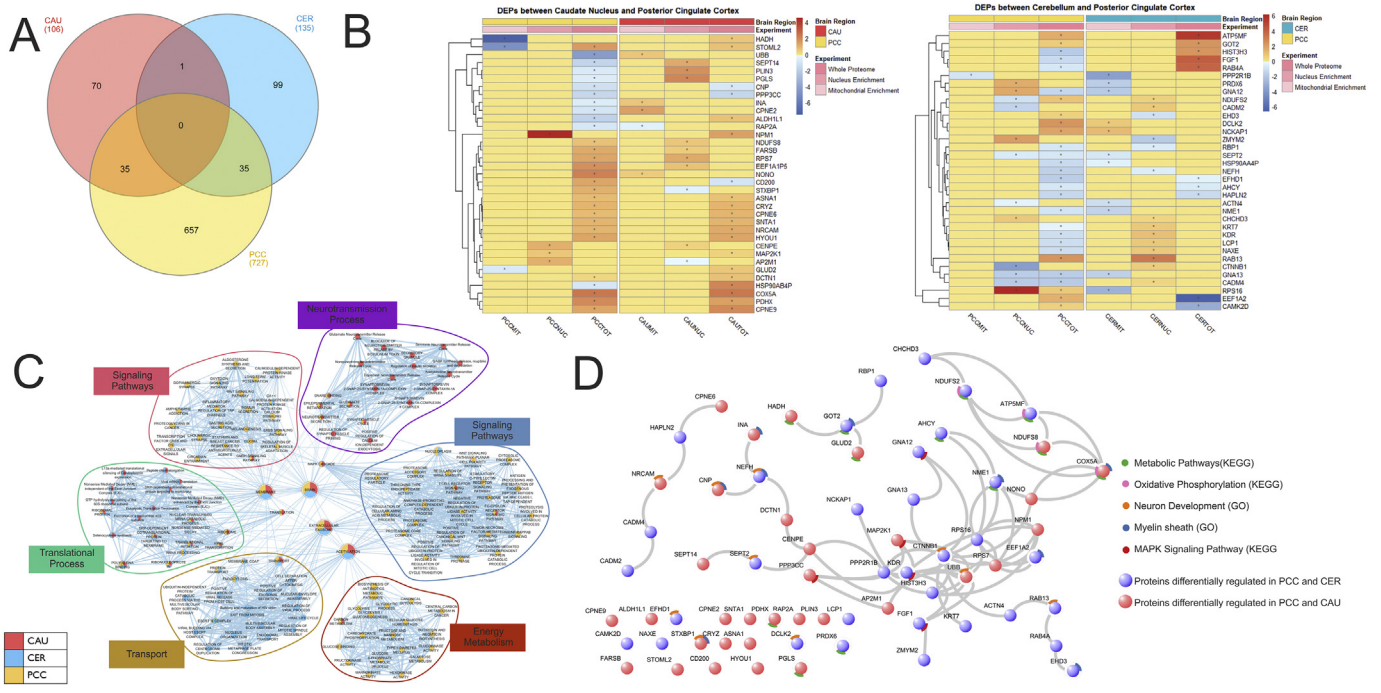

Fig. 5. Association between brain areas. (A) Venn diagram with proteins found differentially regulated in each cerebral region. (B) Network of pathways deregulated according to proteomic analysis, showing clusters associated with individual cerebral regions. (C) Heatmaps of proteins found differentially regulated in more than one brain region. (D) Protein-protein interactions of proteins deregulated in the posterior cingulate cortex and other brain regions. WP = Whole proteome; MIT = Mitochondrial enrichment; NUC = Nuclear enrichment; CAU = Caudate nucleus; PCC = Posterior Cingulate Cortex; CER = Cerebellum.

is directly associated with inflammatory conditions [56].

4.2. Caudate nuclei from patients present dysregulation in synaptic pathways

The CAU is a region rich in D2 receptors [57], which are pivotal receptors in schizophrenia pathophysiology [58]. Our data showed that proteins dysregulated in the CAU are related to transport in synaptic vesicles (AP2M1, DNM2, EPN1, SPTBN2, STX1A, VAMP2). Accordingly, dysfunctions in synaptic organization have been reported in the CAU from patients with schizophrenia, which present an increase in synaptic density in striatal areas [59]. In addition, proteomic analyses also showed a dysregulation in apoptosis of neurons (ASAH1, CD200, CYCS, LINGO1, MAP3K1, MAPK13, P4HB, STXBP1), which could explain the reduced number of neurons found in the CAU in patients with schizophrenia [60]. Leucine-rich repeat and immunoglobulin-like domain-containing nogo receptor-interacting protein 1 (LINGO-1) was also found to be upregulated in schizophrenia in the dorsolateral prefrontal cortex and hippocampus [61]. LINGO-1 plays a role in brain development, regulating the maturation of oligodendrocytes and neuronal outgrowth, and whose upregulation could lead to a reduction of myelin-related processes (reviewed in [62]). Our data also showed that proteins associated with carbon metabolism and pyruvate decarboxylase deficiency (PDHX, PGLS, DLAT, PGK2, GLUD2, ALDOB) were deregulated, showing a disturbance in energy metabolism of the CAU in schizophrenia. These results are in line with alterations in mitochondrial density, size, and distribution already described in neurons and glial cells from the CAU of patients [34,63–65].

4.3. Posterior cingulate cortices from patients present dysregulation in energy metabolism

The PCC was the most affected brain region in patients with schizophrenia. Although the PCC presents a high metabolic rate at default states [66], some studies have shown a reduction in its metabolic rate in patients with schizophrenia during verbal working memory tasks [14,16]. This is in line with our proteomic analysis, since it revealed a disturbance in carbon and energy metabolism, such as glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation. These processes were already associated with other brain areas in patients with schizophrenia, with dysregulations in protein and metabolite levels [27,67,68]. Proteomic analyses of the anterior cingulate cortex revealed a dysfunction in energy metabolism proteins in schizophrenia and bipolar disorder [69,70]. It was already described that patients with schizophrenia present anterior cingulate cortex decreased mitochondrial and synaptic density, which could suggest a correlation between energy process and synaptic function [71]. In addition, our data also showed that PCC presented a downregulation in superoxide dismutase 1 (SOD1). SOD1 is antioxidant metalloprotein already described to be downregulated in cerebrospinal fluid [72] and plasma [73,74] from patients with schizophrenia. Positive symptoms presented in schizophrenia has been inversely associated with SOD levels [75], as well as cognitive deficits related to antioxidant status [76].

Proteins related to synaptic vesicles and the transport of proteins were also found differentially regulated in the PCC. Synaptophysin (SYP) is a presynaptic protein directly associated to synaptic density [77] and was linked to intellectual disabilities [78]. Although our results have pointed to an upregulation of SYP, a recent meta-analysis found this protein to be downregulated in patients with schizophrenia in the anterior cingulate cortex and hippocampus [79]. Dysregulation in the synaptic process was already described in metabolomic analysis of anterior cingulate cortex, which presents lower GABA levels in patients with schizophrenia than healthy subjects [80]. Taken together, these data could suggest that synaptic deficits have regional specificities, affecting each area in different ways compared to others.

4.4. Immunological system and signaling pathways are dysfunctioned among all brain regions

In all brain regions, the Nuclear Factor-κB Transcriptional Complex (NFκB) were enriched in IPA network analysis. NFκB plays a key role in the immune system, controlling the regulation of inflammatory molecules, such as cytokines [81]. Recently, Volk and colleagues have shown that the prefrontal cortex from patients with schizophrenia presents higher levels of NFκB transcripts, suggesting an immune activation in these patients [82]. In addition, several reports have shown that patients with schizophrenia present dysfunctions in immune systems, including high levels of cytokines cortical areas [83], overactivation of microglial cells [84], and genomic variants associated with Major Histocompatibility Complex (MHC) [85].

The extracellular-regulated protein kinase (ERK) also were enriched in IPA networking analysis for all the brain regions. This pathway was already found dysregulated in schizophrenia, being associated with a reduction in synaptic plasticity in these patients [86]. In addition, a recent proteomic analysis using the prefrontal cortex from patients with schizophrenia has shown that the suppression in GNA13-ERK signaling affects the synaptic plasticity in the patients [87] . Since synaptic plasticity is a biological process directly associated with learning and memory, dysfunction in this process are associated with the negative and cognitive symptoms found in patients with schizophrenia [88].

4.5. Schizophrenia patients present a brain compartmentalization of protein dysfunctions

Taking these dysregulations together, it can be concluded that our results showed that the differences among the brain regions were of both proteins and molecular pathways. The changes in the CER were related to dysfunctions in transport processes, while the CAU had associations with synaptic processes and the PCC with energy metabolism and signaling pathways. These results agree with studies that showed divergences of patterns among brain regions in schizophrenia [89,90]. On the other hand, our data also showed proteins that were differentially regulated in more than one region, which were related to the myelin sheath (STXBP1, EHD3, CNP, NEFH, INA, EEF1A2, NME1, COX5A, GOT2), metabolic pathways (PDHX, PRDX6, PGLS, GLUD2, GOT2, NME1, COX5A, NDUFS8, ATP5MF, HADH, AHCY), and neuron development (CNP, NRCAM, NEFH, SERT2, EFHD1, STXBP1, DCLK2, RAB13, UBB, CTNNB1, Fig. 5 C-D). 2′,3’-Cyclic-nucleotide 3′-phosphodiesterase (CNP) is a oligodendrocyte marker associated with the size of the oligodendrocyte population in tissue. Our results showed lower levels of CNP in the CAU and PCC in schizophrenia, suggesting a decrease in the oligodendrocyte population in schizophrenia. Studies have shown decreased oligodendrocyte density in the CAU [91] and prefrontal cortex [92], and in the anterior frontal cortex, anterior cingulate cortex, and hippocampus [93,94] of patients with schizophrenia. Since oligodendrocytes play a pivotal role in myelination and energy support to neuron axons [95], we suggest that dysfunctions in both processes are intrinsically related to the whole-brain dysfunctions seen in schizophrenia.

4.6. Limitations

When working with postmortem brain tissues in psychiatric disorders, the main limitations are associated with the inherent characteristics of this model, such as the sample size, methods of collection, and storage conditions. In this study, the major limitation is indeed the modest sample size used in our analysis. In addition, the samples used in this study were collected from patients that were treated with antipsychotics. Although the treatment may affect the identification of novel proteins associated with schizophrenia, Chan and colleagues showed that antipsychotics normalize the effects of disease according to lifetime medication [96]. Finally, each brain region was analyzed with

different chromatographic and MS acquisition methods, which affect protein identification and comparison between the regions. Despite these limitations, postmortem brain tissue is a unique model to perform molecular analysis directly in the brains of patients with schizophrenia. In addition, our findings are supported by several other results in the literature and for all experiments, we applied the same statistical parameters to obtain the highest proteome coverage and trust in the data as possible.

5. Conclusion

This study provides a differential pattern of proteins in the CER, CAU and PCC from patients with schizophrenia compared with healthy controls. Our results pointed to 135, 106 and 727 deregulated proteins in the aforementioned regions, respectively. In addition, pathway enrichment analysis showed dysfunctions in synaptic processes in the CAU, transport in the CER, and energy metabolism in the PCC. Our study also reinforces the role of oligodendrocytes and metabolic processes in these areas in schizophrenia. Further analysis using other Omic approaches, such as lipidomics and metabolomics, are needed to complement our results and show if these changes can be seen among the brain regions also in metabolic levels.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jprot.2020.103814.

Contribution

DMS conceived and organized the study. GRO, DMS, and VA wrote the paper. GRO, GSZ, MF performed the subcellular fractionation; protein extraction, and digestion. GRO and MF performed the mass spectrometry experiments. AS and PF provide the post mortem brain tissues from patients and healthy control. GRO performed the statistical analysis and data visualization. GRO, MF, GSZ contributed equally to this work.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

GRO, GSZ, MF, VA, and DMS would like to thank Sao Paulo Resarch Foundation (FAPESP) for funding (under grant numbers 2014/10068-4, 2018/01410-1, 2018/03673-0, 2017/25588-1, 2019/00098-7, 2016/18715-4, 2017/18242-1, 2018/14666-4, 2014). Authors thank Bradley Smith for proofreading (2016/07948-8).

References

[1] Schizophrenia, 2019 https://www.who.int/news-room/fact-sheets/detail/schizophrenia, (2020) , Accessed date: 1 May 2019.
[2] R. Freedman, Schizophrenia, N. Engl. J. Med. 349 (2003) 1738–1749.
[3] O.D. Howes, R. McCutcheon, M.J. Owen, R.M. Murray, The role of genes, stress, and dopamine in the development of schizophrenia, Biol. Psychiatry 81 (2017) 9–20.
[4] J.T. Kantrowitz, D.C. Javitt, N-methyl-d-aspartate (NMDA) receptor dysfunction or dysregulation: the final common pathway on the road to schizophrenia? Brain Res. Bull. 83 (2010) 108–121.
[5] S.L. Reeber, T.S. Otis, R.V. Sillitoe, New roles for the cerebellum in health and disease, Front. Syst. Neurosci. 7 (2013) 83.
[6] O. Baumann, R.J. Borra, J.M. Bower, K.E. Cullen, C. Habas, R.B. Ivry, M. Leggio, J.B. Mattingley, M. Molinari, E.A. Moulton, M.G. Paulin, M.A. Pavlova, J.D. Schmahmann, A.A. Sokolov, Consensus paper: the role of the cerebellum in perceptual processes, Cerebellum. 14 (2015) 197–220.
[7] N.C. Andreasen, R. Pierson, The role of the cerebellum in schizophrenia, Biol. Psychiatry 64 (2008) 81–88.
[8] T. Moberget, N.T. Doan, D. Alnæs, T. Kaufmann, A. Córdova-Palomera, T.V. Lagerberg, J. Diedrichsen, E. Schwarz, M. Zink, S. Eisenacher, P. Kirsch, E.G. Jönsson, H. Fatouros-Bergman, L. Flyckt, KaSP, G. Pergola, T. Quarto, A. Bertolino, D. Barch, A. Meyer-Lindenberg, I. Agartz, O.A. Andreassen, L.T. Westlye, Cerebellar volume and cerebellocerebral structural covariance in schizophrenia: a multisite mega-analysis of 983 patients and 1349 healthy controls, Mol. Psychiatry 23 (2018) 1512–1520.
[9] J.A. Bernard, J.M. Orr, V.A. Mittal, Cerebello-thalamo-cortical networks predict positive symptom progression in individuals at ultra-high risk for psychosis, Neuroimage Clin. 14 (2017) 622–628.
[10] H. Cao, O.Y. Chén, Y. Chung, J.K. Forsyth, S.C. McEwen, D.G. Gee, C.E. Bearden, J. Addington, B. Goodyear, K.S. Cadenhead, H. Mirzakhanian, B.A. Cornblatt, R.E. Carrión, D.H. Mathalon, T.H. McGlashan, D.O. Perkins, A. Belger, L.J. Seidman, H. Thermenos, M.T. Tsuang, T.G.M. van Erp, E.F. Walker, S. Hamann, A. Anticevic, S.W. Woods, T.D. Cannon, Cerebello-thalamo-cortical hyperconnectivity as a state-independent functional neural signature for psychosis prediction and characterization, Nat. Commun. 9 (2018) 3836.
[11] R. Leech, D.J. Sharp, The role of the posterior cingulate cortex in cognition and disease, Brain 137 (2014) 12–32, https://doi.org/10.1093/brain/awt162.
[12] S. Whitfield-Gabrieli, H.W. Thermenos, S. Milanovic, M.T. Tsuang, S.V. Faraone, R.W. McCarley, M.E. Shenton, A.I. Green, A. Nieto-Castanon, P. LaViolette, J. Wojcik, J.D.E. Gabrieli, L.J. Seidman, Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 1279–1284.
[13] R.L. Buckner, The brain's default network: origins and implications for the study of psychosis, Dialogues Clin. Neurosci. 15 (2013) 351–358.
[14] M.M. Haznedar, M.S. Buchsbaum, E.A. Hazlett, L. Shihabuddin, A. New, L.J. Siever, Cingulate gyrus volume and metabolism in the schizophrenia spectrum, Schizophr. Res. 71 (2004) 249–262.
[15] S.A. Mitelman, W. Byne, E.M. Kemether, E.A. Hazlett, M.S. Buchsbaum, Metabolic disconnection between the Mediodorsal nucleus of the thalamus and cortical Brodmann's areas of the left hemisphere in schizophrenia, Am. J. Psychiatr. 162 (2005) 1733–1735, https://doi.org/10.1176/appi.ajp.162.9.1733.
[16] J.-H. Kim, J.-H. Kim, Y.-D. Son, Y.-H. Joo, S.-Y. Lee, H.-K. Kim, M.-K. Woo, Altered interregional correlations between serotonin transporter availability and cerebral glucose metabolism in schizophrenia: a high-resolution PET study using [C]DASB and [F]FDG, Schizophr. Res. 182 (2017) 55–65.
[17] E. Kirino, S. Tanaka, M. Fukuta, R. Inami, R. Inoue, S. Aoki, Functional connectivity of the caudate in schizophrenia evaluated with simultaneous resting-state functional MRI and electroencephalography recordings, Neuropsychobiology 77 (2019) 165–175.
[18] B. Knutson, J.C. Cooper, Functional magnetic resonance imaging of reward prediction, Curr. Opin. Neurol. 18 (2005) 411–417.
[19] J.M. Gold, G.P. Strauss, J.A. Waltz, B.M. Robinson, J.K. Brown, M.J. Frank, Negative symptoms of schizophrenia are associated with abnormal effort-cost computations, Biol. Psychiatry 74 (2013) 130–136.
[20] A.E. Whitton, M.T. Treadway, D.A. Pizzagalli, Reward processing dysfunction in major depression, bipolar disorder and schizophrenia, Curr. Opin. Psychiatry 28 (2015) 7–12.
[21] S. Saperia, S. Da Silva, I. Siddiqui, O. Agid, Z.J. Daskalakis, A. Ravindran, A.N. Voineskos, K.K. Zakzanis, G. Remington, G. Foussias, Reward-driven decision-making impairments in schizophrenia, Schizophr. Res. (2018), https://doi.org/10.1016/j.schres.2018.11.004.
[22] M.Ø. Nielsen, E. Rostrup, B.V. Broberg, S. Wulff, B. Glenthøj, Negative symptoms and reward disturbances in schizophrenia before and after antipsychotic monotherapy, Clin. EEG Neurosci. 49 (2018) 36–45.
[23] S. Mueller, D. Wang, R. Pan, D.J. Holt, H. Liu, Abnormalities in hemispheric specialization of caudate nucleus connectivity in schizophrenia, JAMA Psychiatry 72 (2015) 552–560.
[24] J.M. Nascimento, D. Martins-de-Souza, The proteome of schizophrenia, NPJ Schizophr. 1 (2015) 14003.
[25] J.M. Nascimento, S. Garcia, V.M. Saia-Cereda, A.G. Santana, C. Brandao-Teles, G.S. Zuccoli, D.G. Junqueira, G. Reis-de-Oliveira, P.A. Baldasso, J.S. Cassoli, D. Martins-de-Souza, Proteomics and molecular tools for unveiling missing links in the biochemical understanding of schizophrenia, Proteomics Clin. Appl. 10 (2016) 1148–1158.
[26] V.M. Saia-Cereda, J.S. Cassoli, D. Martins-de-Souza, J.M. Nascimento, Psychiatric disorders biochemical pathways unraveled by human brain proteomics, Eur. Arch. Psychiatry Clin. Neurosci. 267 (2017) 3–17.
[27] D. Martins-de-Souza, W.F. Gattaz, A. Schmitt, G. Maccarrone, E. Hunyadi-Gulyás, M.N. Eberlin, G.H.M.F. Souza, S. Marangoni, J.C. Novello, C.W. Turck, E. Dias-Neto, Proteomic analysis of dorsolateral prefrontal cortex indicates the involvement of cytoskeleton, oligodendrocyte, energy metabolism and new potential markers in schizophrenia, J. Psychiatr. Res. 43 (2009) 978–986.
[28] S. Prabakaran, J.E. Swatton, M.M. Ryan, S.J. Huffaker, J.T.-J. Huang, J.L. Griffin, M. Wayland, T. Freeman, F. Dudbridge, K.S. Lilley, N.A. Karp, S. Hester, D. Tkachev, M.L. Mimmack, R.H. Yolken, M.J. Webster, E.F. Torrey, S. Bahn, Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress, Mol. Psychiatry 9 (2004) 684–697 (643).
[29] D. Martins-de-Souza, W.F. Gattaz, A. Schmitt, C. Rewerts, S. Marangoni, J.C. Novello, G. Maccarrone, C.W. Turck, E. Dias-Neto, Alterations in oligodendrocyte proteins, calcium homeostasis and new potential markers in schizophrenia anterior temporal lobe are revealed by shotgun proteome analysis, J. Neural Transm. 116 (2009) 275–289.
[30] E. Velásquez, F.C.S. Nogueira, I. Velásquez, A. Schmitt, P. Falkai, G.B. Domont, D. Martins-de-Souza, Synaptosomal proteome of the orbitofrontal cortex from schizophrenia patients using quantitative label-free and iTRAQ-based shotgun proteomics, J. Proteome Res. 16 (2017) 4481–4494.
[31] V.M. Saia-Cereda, J.S. Cassoli, A. Schmitt, P. Falkai, D. Martins-de-Souza, Differential proteome and phosphoproteome may impact cell signaling in the corpus callosum of schizophrenia patients, Schizophr. Res. 177 (2016) 70–77.

[32] D. Martins-de-Souza, G. Maccarrone, T. Wobrock, I. Zerr, P. Gormanns, S. Reckow, P. Falkai, A. Schmitt, C.W. Turck, Proteome analysis of the thalamus and cerebrospinal fluid reveals glycolysis dysfunction and potential biomarkers candidates for schizophrenia, J. Psychiatr. Res. 44 (2010) 1176–1189.

[33] V.M. Saia-Cereda, A.G. Santana, A. Schmitt, P. Falkai, D. Martins-de-Souza, The nuclear proteome of white and gray matter from schizophrenia postmortem brains, Mol. Neuropsychiatry 3 (2017) 37–52.

[34] R.C. Roberts, Postmortem studies on mitochondria in schizophrenia, Schizophr. Res. 187 (2017) 17–25.

[35] V.M. Saia-Cereda, J.S. Cassoli, A. Schmitt, P. Falkai, J.M. Nascimento, D. Martins-de-Souza, Proteomics of the corpus callosum unravel pivotal players in the dysfunction of cell signaling, structure, and myelination in schizophrenia brains, Eur. Arch. Psychiatry Clin. Neurosci. 265 (2015) 601–612.

[36] G. Reis-de-Oliveira, M. Fioramonte, D. Martins-de-Souza, A complete proteomic workflow to study brain-related disorders via postmortem tissue, Methods Mol. Biol. 1916 (2019) 319–328.

[37] G.-Z. Li, J.P.C. Vissers, J.C. Silva, D. Golick, M.V. Gorenstein, S.J. Geromanos, Database searching and accounting of multiplexed precursor and product ion spectra from the data independent analysis of simple and complex peptide mixtures, Proteomics 9 (2009) 1696–1719.

[38] J.C. Silva, M.V. Gorenstein, G.-Z. Li, J.P.C. Vissers, S.J. Geromanos, Absolute quantification of proteins by LCMSE, Mol. Cell. Proteomics 5 (2006) 144–156, https://doi.org/10.1074/mcp.m500230-mcp200.

[39] T. Clough, M. Key, I. Ott, S. Ragg, G. Schadow, O. Vitek, Protein quantification in label-free LC-MS experiments, J. Proteome Res. 8 (2009) 5275–5284.

[40] D.W. Huang, B.T. Sherman, R.A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, Nat. Protoc. 4 (2009) 44–57.

[41] Website, RStudio Team (2015), RStudio: Integrated Development for R. RStudio, Inc, Boston, MA, 2020 URL http://www.rstudio.com/ , Accessed date: 20 January 2020(n.d.).

[42] P. Shannon, A. Markiel, O. Ozier, N.S. Baliga, J.T. Wang, D. Ramage, N. Amin, B. Schwikowski, T. Ideker, Cytoscape: a software environment for integrated models of biomolecular interaction networks, Genome Res. 13 (2003) 2498–2504.

[43] D. Merico, R. Isserlin, O. Stueker, A. Emili, G.D. Bader, Enrichment map: a network-based method for gene-set enrichment visualization and interpretation, PLoS One 5 (2010) e13984.

[44] G.D. Bader, C.W.V. Hogue, BMC Bioinformatics, vol. 4, (2003), p. 2, https://doi.org/10.1186/1471-2105-4-2.

[45] G.D. Honey, E. Pomarol-Clotet, P.R. Corlett, R.A.E. Honey, P.J. McKenna, E.T. Bullmore, P.C. Fletcher, Functional dysconnectivity in schizophrenia associated with attentional modulation of motor function, Brain 128 (2005) 2597–2611.

[46] T.E. Mwansisya, A. Hu, Y. Li, X. Chen, G. Wu, X. Huang, D. Lv, Z. Li, C. Liu, Z. Xue, J. Feng, Z. Liu, Task and resting-state fMRI studies in first-episode schizophrenia: a systematic review, Schizophr. Res. 189 (2017) 9–18.

[47] T. Sundararajan, A.M. Manzardo, M.G. Butler, Functional analysis of schizophrenia genes using GeneAnalytics program and integrated databases, Gene 641 (2018) 25–34.

[48] E. Maloku, I.R. Covelo, I. Hanbauer, A. Guidotti, B. Kadriu, Q. Hu, J.M. Davis, E. Costa, Lower number of cerebellar Purkinje neurons in psychosis is associated with reduced reelin expression, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 4407–4411.

[49] X. Gan, J. Wang, C. Wang, E. Sommer, T. Kozasa, S. Srinivasula, D. Alessi, S. Offermanns, M.I. Simon, D. Wu, PRR5L degradation promotes mTORC2-mediated PKC-δ phosphorylation and cell migration downstream of Gα12, Nat. Cell Biol. 14 (2012) 686–696.

[50] T.E. Meigs, M. Fedor-Chaiken, D.D. Kaplan, R. Brackenbury, P.J. Casey, Galpha12 and Galpha13 negatively regulate the adhesive functions of cadherin, J. Biol. Chem. 277 (2002) 24594–24600.

[51] P. Jia, L. Wang, A.H. Fanous, C.N. Pato, T.L. Edwards, International Schizophrenia Consortium, Z. Zhao, Network-assisted investigation of combined causal signals from genome-wide association studies in schizophrenia, PLoS Comput. Biol. 8 (2012) e1002587.

[52] A. Moers, A. Nürnberg, S. Goebbels, N. Wettschureck, S. Offermanns, Galpha12/Galpha13 deficiency causes localized overmigration of neurons in the developing cerebral and cerebellar cortices, Mol. Cell. Biol. 28 (2008) 1480–1488.

[53] H. Jaaro-Peled, A. Hayashi-Takagi, S. Seshadri, A. Kamiya, N.J. Brandon, A. Sawa, Neurodevelopmental mechanisms of schizophrenia: understanding disturbed postnatal brain maturation through neuregulin-1-ErbB4 and DISC1, Trends Neurosci. 32 (2009) 485–495.

[54] J.A. Bernard, V.A. Mittal, Cerebellar-motor dysfunction in schizophrenia and psychosis-risk: the importance of regional cerebellar analysis approaches, Front. Psychiatry 5 (2014) 160.

[55] N. Müller, E. Weidinger, B. Leitner, M.J. Schwarz, The role of inflammation in schizophrenia, Front. Neurosci. 9 (2015) 372.

[56] K.S. MacDowell, R. Pinacho, J.C. Leza, J. Costa, B. Ramos, B. García-Bueno, Differential regulation of the TLR4 signalling pathway in post-mortem prefrontal cortex and cerebellum in chronic schizophrenia: relationship with SP transcription factors, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 79 (2017) 481–492.

[57] R.A. Lahti, R.C. Roberts, C.A. Tamminga, D2-family receptor distribution in human postmortem tissue: an autoradiographic study, Neuroreport 6 (1995) 2505–2512.

[58] O.D. Howes, S. Kapur, The dopamine hypothesis of schizophrenia: version III–the final common pathway, Schizophr. Bull. 35 (2009) 549–562.

[59] E. Perez-Costas, M. Melendez-Ferro, R.C. Roberts, Basal ganglia pathology in schizophrenia: dopamine connections and anomalies, J. Neurochem. 113 (2010) 287–302.

[60] P. Kreczmanski, H. Heinsen, V. Mantua, F. Woltersdorf, T. Masson, N. Ulfig, R. Schmidt-Kastner, H. Korr, H.W.M. Steinbusch, P.R. Hof, C. Schmitz, Volume, neuron density and total neuron number in five subcortical regions in schizophrenia, Brain 130 (2007) 678–692.

[61] F. Fernandez-Enright, J.L. Andrews, K.A. Newell, C. Pantelis, X.F. Huang, Novel implications of Lingo-1 and its signaling partners in schizophrenia, Transl. Psychiatry 4 (2014) e348.

[62] J.L. Andrews, F. Fernandez-Enright, A decade from discovery to therapy: Lingo-1, the dark horse in neurological and psychiatric disorders, Neurosci. Biobehav. Rev. 56 (2015) 97–114.

[63] N.A. Uranova, M.F. Casanova, N.M. DeVaughn, D.D. Orlovskaya, D.V. Denisov, Ultrastructural alterations of synaptic contacts and astrocytes in postmortem caudate nucleus of schizophrenic patients, Schizophr. Res. 22 (1996) 81–83.

[64] N. Uranova, D. Orlovskaya, O. Vikhreva, I. Zimina, N. Kolomeets, V. Vostrikov, V. Rachmanova, Electron microscopy of oligodendroglia in severe mental illness, Brain Res. Bull. 55 (2001) 597–610.

[65] S.M. Somerville, R.R. Conley, R.C. Roberts, Mitochondria in the striatum of subjects with schizophrenia, World J. Biol. Psychiatry 12 (2011) 48–56.

[66] M.E. Raichle, A.M. MacLeod, A.Z. Snyder, W.J. Powers, D.A. Gusnard, G.L. Shulman, A default mode of brain function, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 676–682.

[67] D. Martins-de-Souza, W.F. Gattaz, A. Schmitt, J.C. Novello, S. Marangoni, C.W. Turck, E. Dias-Neto, Proteome analysis of schizophrenia patients Wernicke's area reveals an energy metabolism dysregulation, BMC Psychiatry 9 (2009) 17.

[68] B. Dean, N. Thomas, E. Scarr, M. Udawela, Evidence for impaired glucose metabolism in the striatum, obtained postmortem, from some subjects with schizophrenia, Transl. Psychiatry 6 (2016) e949.

[69] M. Föcking, P. Dicker, L.M. Lopez, M. Hryniewiecka, K. Wynne, J.A. English, G. Cagney, D.R. Cotter, Proteomic analysis of the postsynaptic density implicates synaptic function and energy pathways in bipolar disorder, Transl. Psychiatry 6 (2016) e959.

[70] D. Clark, I. Dedova, S. Cordwell, I. Matsumoto, A proteome analysis of the anterior cingulate cortex gray matter in schizophrenia, Mol. Psychiatry 11 (2006) 459–470 (423).

[71] R.C. Roberts, K.A. Barksdale, J.K. Roche, A.C. Lahti, Decreased synaptic and mitochondrial density in the postmortem anterior cingulate cortex in schizophrenia, Schizophr. Res. 168 (2015) 543–553.

[72] J.M. Coughlin, L.N. Hayes, T. Tanaka, M. Xiao, R.H. Yolken, P. Worley, F.M. Leweke, A. Sawa, Reduced superoxide dismutase-1 (SOD1) in cerebrospinal fluid of patients with early psychosis in association with clinical features, Schizophr. Res. 183 (2017) 64–69.

[73] Ö. Akyol, H. Herken, E. Uz, E. Fadıllıoğlu, S. Ünal, S. Söğüt, H. Özyurt, H.A. Savaş, The indices of endogenous oxidative and antioxidative processes in plasma from schizophrenic patients, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 26 (2002) 995–1005.

[74] C. Gonzalez-Liencres, C. Tas, E.C. Brown, S. Erdin, E. Onur, Z. Cubukcoglu, O. Aydemir, A. Esen-Danaci, M. Brüne, Oxidative stress in schizophrenia: a case-control study on the effects on social cognition and neurocognition, BMC Psychiatry 14 (2014) 268.

[75] Z. Wu, X.Y. Zhang, H. Wang, W. Tang, Y. Xia, F. Zhang, J. Liu, Y. Fu, J. Hu, Y. Chen, L. Liu, D.C. Chen, M.H. Xiu, T.R. Kosten, J. He, Elevated plasma superoxide dismutase in first-episode and drug naive patients with schizophrenia: inverse association with positive symptoms, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 36 (2012) 34–38.

[76] X.Y. Zhang, D.C. Chen, M.H. Xiu, W. Tang, F. Zhang, L. Liu, Y. Chen, J. Liu, J.K. Yao, T.A. Kosten, T.R. Kosten, Plasma total antioxidant status and cognitive impairments in schizophrenia, Schizophr. Res. 139 (2012) 66–72.

[77] M.E. Calhoun, M. Jucker, L.J. Martin, G. Thinakaran, D.L. Price, P.R. Mouton, Comparative evaluation of synaptophysin-based methods for quantification of synapses, J. Neurocytol. 25 (1996) 821–828.

[78] P.S. Tarpey, R. Smith, E. Pleasance, A. Whibley, S. Edkins, C. Hardy, S. O'Meara, C. Latimer, E. Dicks, A. Menzies, P. Stephens, M. Blow, C. Greenman, Y. Xue, C. Tyler-Smith, D. Thompson, K. Gray, J. Andrews, S. Barthorpe, G. Buck, J. Cole, R. Dunmore, D. Jones, M. Maddison, T. Mironenko, R. Turner, K. Turrell, J. Varian, S. West, S. Widaa, P. Wray, J. Teague, A. Butler, A. Jenkinson, M. Jia, D. Richardson, R. Shepherd, R. Wooster, M.I. Tejada, F. Martinez, G. Carvill, R. Goliath, A.P.M. de Brouwer, H. van Bokhoven, H. Van Esch, J. Chelly, M. Raynaud, H.-H. Ropers, F.E. Abidi, A.K. Srivastava, J. Cox, Y. Luo, U. Mallya, J. Moon, J. Parnau, S. Mohammed, J.L. Tolmie, C. Shoubridge, M. Corbett, A. Gardner, E. Haan, S. Rujirabanjerd, M. Shaw, L. Vandeleur, T. Fullston, D.F. Easton, J. Boyle, M. Partington, A. Hackett, M. Field, C. Skinner, R.E. Stevenson, M. Bobrow, G. Turner, C.E. Schwartz, J. Gecz, F.L. Raymond, P.A. Futreal, M.R. Stratton, A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation, Nat. Genet. 41 (2009) 535–543.

[79] E.F. Osimo, K. Beck, T. Reis Marques, O.D. Howes, Synaptic loss in schizophrenia: a meta-analysis and systematic review of synaptic protein and mRNA measures, Mol. Psychiatry 24 (2019) 549–561.

[80] R.C. Ramaker, K.M. Bowling, B.N. Lasseigne, M.H. Hagenauer, A.A. Hardigan, N.S. Davis, J. Gertz, P.M. Cartagena, D.M. Walsh, M.P. Vawter, E.G. Jones, A.F. Schatzberg, J.D. Barchas, S.J. Watson, B.G. Bunney, H. Akil, W.E. Bunney, J.Z. Li, S.J. Cooper, R.M. Myers, Post-mortem molecular profiling of three psychiatric disorders, Genome Med. 9 (2017) 72.

[81] B. Hoesel, J.A. Schmid, The complexity of NF-κB signaling in inflammation and cancer, Mol. Cancer 12 (2013) 86.

[82] D.W. Volk, A.E. Moroco, K.M. Roman, J.R. Edelson, D.A. Lewis, The role of the nuclear factor-κB transcriptional complex in cortical immune activation in schizophrenia, Biol. Psychiatry 85 (2019) 25–34.

[83] S.G. Fillman, N. Cloonan, V.S. Catts, L.C. Miller, J. Wong, T. McCrossin, M. Cairns, C.S. Weickert, Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia, Mol. Psychiatry 18 (2013) 206–214.
[84] P.S. Bloomfield, S. Selvaraj, M. Veronese, G. Rizzo, A. Bertoldo, D.R. Owen, M.A. Bloomfield, I. Bonoldi, N. Kalk, F. Turkheimer, P. McGuire, V. de Paola, O.D. Howes, Microglial activity in people at ultra high risk of psychosis and in schizophrenia: an [(11)C]PBR28 PET brain imaging study, Am. J. Psychiatry 173 (2016) 44–52.
[85] Schizophrenia Working Group of the Psychiatric Genomics Consortium, Biological insights from 108 schizophrenia-associated genetic loci, Nature 511 (2014) 421–427.
[86] P. Yuan, R. Zhou, Y. Wang, X. Li, J. Li, G. Chen, X. Guitart, H.K. Manji, Altered levels of extracellular signal-regulated kinase signaling proteins in postmortem frontal cortex of individuals with mood disorders and schizophrenia, J. Affect. Disord. 124 (2010) 164–169.
[87] M. Hirayama-Kurogi, Y. Takizawa, Y. Kunii, J. Matsumoto, A. Wada, M. Hino, H. Akatsu, Y. Hashizume, S. Yamamoto, T. Kondo, S. Ito, M. Tachikawa, S.-I. Niwa, H. Yabe, T. Terasaki, M. Setou, S. Ohtsuki, Downregulation of GNA13-ERK network in prefrontal cortex of schizophrenia brain identified by combined focused and targeted quantitative proteomics, J. Proteome 158 (2017) 31–42.
[88] L. Mei, W.-C. Xiong, Neuregulin 1 in neural development, synaptic plasticity and schizophrenia, Nat. Rev. Neurosci. 9 (2008) 437–452.
[89] P. Fusar-Poli, J. Perez, M. Broome, S. Borgwardt, A. Placentino, E. Caverzasi, M. Cortesi, P. Veggiotti, P. Politi, F. Barale, P. McGuire, Neurofunctional correlates of vulnerability to psychosis: a systematic review and meta-analysis, Neurosci. Biobehav. Rev. 31 (2007) 465–484.
[90] J. van Os, S. Kapur, Schizophrenia, Lancet 374 (2009) 635–645.
[91] V.M. Vostrikov, N.A. Uranova, Reduced density of oligodendrocytes and oligodendrocyte clusters in the caudate nucleus in major psychiatric illnesses, Schizophr. Res. (2019), https://doi.org/10.1016/j.schres.2019.10.027.
[92] N.A. Uranova, V.M. Vostrikov, O.V. Vikhreva, I.S. Zimina, N.S. Kolomeets, D.D. Orlovskaya, The role of oligodendrocyte pathology in schizophrenia, Int. J. Neuropsychopharmacol. 10 (2007) 537–545.
[93] S. Dracheva, K.L. Davis, B. Chin, D.A. Woo, J. Schmeidler, V. Haroutunian, Myelin-associated mRNA and protein expression deficits in the anterior cingulate cortex and hippocampus in elderly schizophrenia patients, Neurobiol. Dis. 21 (2006) 531–540.
[94] S.W. Flynn, D.J. Lang, A.L. Mackay, V. Goghari, I.M. Vavasour, K.P. Whittall, G.N. Smith, V. Arango, J.J. Mann, A.J. Dwork, P. Falkai, W.G. Honer, Abnormalities of myelination in schizophrenia detected in vivo with MRI, and post-mortem with analysis of oligodendrocyte proteins, Mol. Psychiatry 8 (2003) 811–820.
[95] A.S. Saab, I.D. Tzvetanova, K.-A. Nave, The role of myelin and oligodendrocytes in axonal energy metabolism, Curr. Opin. Neurobiol. 23 (2013) 1065–1072.
[96] M.K. Chan, T.M. Tsang, L.W. Harris, P.C. Guest, E. Holmes, S. Bahn, Evidence for disease and antipsychotic medication effects in post-mortem brain from schizophrenia patients, Mol. Psychiatry 16 (2011) 1189–1202.