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Label-free proteomics differences in the dorsolateral prefrontal cortex between bipolar disorder patients with and without psychosis

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Abstract

Background: Psychosis is common in bipolar disorder (BD) and is related to more severe cognitive impairments. Since the molecular mechanism of BD psychosis is elusive, we conducted this study to explore the proteomic differences associated with BD psychosis in the dorsolateral prefrontal cortex (DLPFC; BA9).

Methods: Postmortem DLPFC gray matter tissues from five pairs of age-matched male BD subjects with and without psychosis history were used. Tissue proteomes were identified and quantified by label-free liquid chromatography tandem mass spectrometry and then compared between groups. Statistical significance was set at $q < 0.40$ and Log_2 fold change (Log_2FC) $\geq |1|$. Protein groups with differential expression between groups at $p < 0.05$ were subjected to pathway analysis.

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Author contributions

AMC, DSC, and MV designed the experiments. CMC conducted the proteomics experiments while CJH performed the proteomics data and statistical analyses. AMC and ACA performed validation experiments. GM, GR, and CAS collected and managed the *postmortem* human tissues and related clinical information. AMC and MV prepared the manuscript. All authors contributed to the intellectual content and commented on the manuscript.

Declaration of Competing Interest

Dr. Frye is a consultant (for Mayo Clinic) to Janssen, Mitsubishi Tanabe Pharma Corporation, Myriad, Sunovion, and Teva Pharmaceuticals; Dr. Choi is a scientific advisory board member to Peptron Inc.; none of these funding sources contributed to any work carried out in this study. Other authors have none to declare. All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jad.2020.03.105.

Results: Eleven protein groups differed significantly between groups, including the reduction of tenascin C ($q = 0.005$, $Log_2FC = -1.78$), the elevations of synaptoporin ($q = 0.235$, $Log_2FC = 1.17$) and brain-specific angiogenesis inhibitor 1-associated protein 3 ($q = 0.241$, $Log_2FC = 2.10$) in BD with psychosis. The between-group differences of these proteins were confirmed by Western blots. The top enriched pathways ($p < 0.05$ with ≥ 3 hits) were the outgrowth of neurons, neuronal cell proliferation, growth of neurites, and outgrowth of neurites, which were all predicted to be upregulated in BD with psychosis.

Limitations: Small sample size and uncertain relationships of the observed proteomic differences with illness stage and acute psychosis.

Conclusions: These results suggested BD with psychosis history may be associated with abnormalities in neurodevelopment, neuroplasticity, neurotransmission, and neuromodulation in the DLPFC.

1. Introduction

Psychosis can occur during manic or depressive mood episodes of bipolar disorder (BD). More than 50% of BD patients develop psychosis during the course of their illness (Dunayevich and Keck, 2000). Compared to BD with affective disturbance only, BD patients with psychosis have more inpatient admissions and younger illness onset (Bora et al., 2010), and demonstrate more severe cognitive impairments in planning, reasoning, working memory, and processing speed (Bora et al., 2010; Bowie et al., 2018; Glahn et al., 2007). These disparities in the course of illness and cognition, albeit small, reflect differences in the neurobiology of affective psychosis from affective-only BD (Bora et al., 2010). Compared to schizophrenia patients, patients with affective psychoses (BD or major depressive disorder [MDD] with psychosis) show a similar cognitive profile but milder degrees of impairments, suggesting that common mechanisms may cause the cognitive dys-function across psychotic disorders (Barch and Sheffield, 2014). Genetic studies also found a higher genetic loading for schizophrenia in BD patients with psychosis compared to those without psychosis, implying some degree of common genetic liability between schizophrenia and BD psychosis (Allardyce et al., 2018; Markota et al., 2018). Despite the clinical similarities with schizophrenia, the neurobiological characteristics of BD with psychosis remain poorly understood.

Working memory is one of the cognitive domains that are compromised in both BD with psychosis and schizophrenia (e.g., Glahn et al., 2006; Jenkins et al., 2018; Jimenez-Lopez et al., 2019). The dorsolateral prefrontal cortex (DLPFC) and its interaction with other brain regions play key roles in working memory (Barbey et al., 2013; Moser et al., 2018). Patients with psychotic disorders showed abnormalities in the cortical structures and networks that involve the DLPFC. For example, reduced gray matter volume in the frontal, insular, parietal, and cingulate cortices was observed in first-episode psychosis patients compared to healthy controls (de Castro-Manglano et al., 2011; Koo et al., 2008). Recent brain connectome studies in patients with psychotic illnesses found graded reduction in the frontoparietal control network connectivity, which encompasses the dorsolateral prefrontal, dorsomedial prefrontal, lateral parietal and posterior temporal cortices (Baker et al., 2019, 2014). Compared to patients with affective illnesses without psychosis, BD patients with

psychosis and patients with schizophrenia showed reduced within-network connectivity across various cortical regions that involved the default network, the dorsal and ventral attention networks, and motor and visual systems (Baker et al., 2019). Decreased frontoparietal network connectivity was found to correlate with increased symptoms of mania and positive psychotic symptoms (Baker et al., 2019). These findings support the DLPFC as a critical region that differentiates affective psychosis from affective-only BD.

Proteomics techniques have been increasingly used to investigate the potential diagnostic biomarkers for mood disorders (Frye et al., 2015) and to study the molecular foundation of affective psychosis in human brain tissues. Using label-free proteomics, Gottschalk et al. (2014) detected the associations of hypoglutamatergic state and hypofunction of energy metabolism with psychotic illnesses (schizophrenia and affective psychosis), in contrast to the associations of hyperglutamatergic state and hyperfunction of energy metabolism with affective disorders (MDD and BD). Martins-de-Souza et al. (2012) observed that the proteomic differences between MDD with and without psychosis in the DLPFC (Brodmann Area [BA] 9) markedly overlapped with the schizophrenia brain proteome. Using targeted proteomics, Wesseling et al. (2014) found that calcium signaling was affected in the anterior prefrontal cortex (BA10) in schizophrenia and affective psychosis. These studies demonstrate that proteomics is an efficient method for screening proteins and pathways that potentially contribute to affective psychosis. However, the DLPFC, which is important for decision making, emotion regulation, cognitive and executive functions (Clark and Sahakian, 2008), has not been explored in BD with psychosis. In this study, we performed label-free proteomics on *postmortem* human DLPFC gray matter to explore proteins that were associated with psychosis history in BD. We hypothesized that the proteomic profile of BD patients with psychosis history would differ from that of BD patients without psychosis history.

2. Methods

2.1. Subjects

All procedures were conducted according to the Declaration of Helsinki and approved by the Institutional Review Boards of the University of Mississippi Medical Center, Jackson, MS, and the University Hospitals Cleveland Medical Center, Cleveland, OH. Details of case recruitment and the procedures of diagnosis had been described in Ho et al. (2019). Briefly, subjects' psychopathology was determined in consensus by the review of comprehensive information obtained from interviews with next-of-kin, the medical examiner's report, and prior medical records by a board-certified clinical psychologist and a board-certified psychiatrist, in addition to obtaining the consensus of a Master-level social worker interviewed knowledgeable informants regarding the subjects. Subjects meeting DSM-IV criteria for BD were included and subjects with any known neuropathological or neurological disorders were excluded. We further selected BD subjects with and without psychosis history ($n = 5$ per group) and pair-matched them for sex (all males), age, and cause of death (natural causes or illnesses). Blood and/or urine toxicology was performed by the medical examiner to detect psychotropic medications and substances of abuse.

2.2. Sample preparation

The overall procedure of the untargeted proteomics method is depicted in Fig 1A. Approximately 20 mg of DLPFC (BA9) gray matter tissue was dissected and homogenized by Minilys ceramic bead homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) in lysis buffer (0.1% SDS, 50 mM Tris, 1 mM $MgCl_2$, pH 8.2), with the addition of Halt protease inhibitors (Thermo Fisher Scientific, Waltham, MA), Simple Step phosphatase inhibitors (GoldBio, St Louis, MO), and benzonase (Invitrogen, Carlsbad, MA). The lysate was incubated at 80 °C for 10 min while shaking. The cooled lysate was then centrifuged at 10 000 *g* for 10 min at 4 °C. The resulting supernatant was collected for SDS-PAGE. Protein concentration was measured by BCA protein assay (ThermoFisher) using bovine serum albumin (BSA) to derive standard curves.

For each sample, 15 μg protein was first mixed with reducing LDS sample buffer (with 5% β-mercaptoethanol) and then incubated at 85 °C for 5 min. Subsequently, the treated samples were subjected to electrophoresis on Criterion XT Bis-Tris gels in MES running buffer (BioRad, Hercules, CA). The gels were stained with BioSafe Coomassie stain (BioRad) to visualize lanes. Using protein markers as a guide, each lane was divided into six equal horizontal segments to fractionate proteins by size prior to mass spectrometry analysis.

Gel segments were destained with 40% acetonitrile (in 50 mM Tris, pH 8.1) until clear, reduced with 50 mM TCEP (in 50 mM Tris, pH 8.1) at 60 °C for 40 min, followed by alkylation with 25 mM iodoacetamide (in 50 mM Tris, pH 8.1) at room temperature for 1 hr in the dark. Proteins were digested in situ with 0.16 μg trypsin (Promega, Madison, WI) in 25 mM Tris (pH 8.1) and 0.0002% Zwittergent 3–16 (Millipore Sigma, Burlington, MA) at 37 °C overnight. Subsequently, peptides were extracted with 2% trifluoroacetic acid and acetonitrile and dried.

2.3. Mass spectrometry

The dried trypsin-digested peptide samples were suspended in 0.2% formic acid, 0.1% trifluoroacetic acid (TFA), and 0.002% Zwittergent 3–16. Nano-flow liquid chromatography electrospray tandem mass spectrometry (nanoLC-ESI-MS/MS) using a Thermo Scientific Q-Exactive Mass Spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled to a Thermo Ultimate 3000 RSLCnano HPLC system was used to analyze the samples. Briefly, the sample was loaded onto a 330 nL HALO 2.7 ES-C18 trap (Optimized Technologies, Oregon City, OR). Chromatography was performed using A solvent (98% water, 2% acetonitrile, and 0.2% formic acid) and B solvent (80% acetonitrile, 10% isopropanol, 10% water, and 0.2% formic acid) over a 2–45% B gradient for 90 min at 400 nL/min through a PicoFrit 100 μm × 33 cm column (New Objective, Woburn, MA) hand-packed with Poroshell 120 ECeC18 bond phase (Agilent, Santa Clara, CA). The Q-Exactive mass spectrometer was set to acquire an MS1 survey scan from 340–1600 *m/z* at a resolution of 70,000 (200 *m/z*) with an automatic gain control (AGC) target of 3e6 ions and a maximum ion inject time of 60 msec. Survey scans were followed by HCD MS/MS scans on the top 15 ions at a resolution of 17,500 with an AGC target of 2e5 ions and a maximum ion inject time of 60 ms. Dynamic exclusion placed selected ions on an exclusion list for 40 s.

2.4. Mass spectrometry data analysis

MaxQuant version 1.5.1.2 (Cox and Mann, 2008) embedded with Andromeda search engine (Max-Plank Institute; Cox et al., 2011) was used to search the mass spectrometry generated data against a human Uniprot database (release 2018_05), based on species using reviewed proteins. Precursor mass tolerance was set at 10.5 ppm and ms/ms mass tolerance was set at 20 ppm. Carbamidomethyl cysteine was set as a fixed modification, oxidation methionine and n-terminal acetylation were set as variable modifications, and trypsin/P was designated as the digestion enzyme with up to 3 missed cleavages allowed. A protein was identified when at least two peptides were matched and at least one peptide was unique to the protein. Peak features were aligned by time to allow peak identifications across samples.

An in-house script in the R programming language was applied to perform differential expression analysis using protein group intensities. Protein group intensities of each sample were first Log_2 transformed and normalized using a quantile method. For each protein, the normalized intensities observed in each experimental group were modeled using a Gaussian-linked generalized linear model. ANOVA was used to detect differentially expressed proteins between experimental groups, and resulted test p-values were corrected for multiple comparisons by the FDR-controlling Benjamini–Hochberg–Yekutieli procedure. Proteins with an FDR-adjusted p-value (q) < 0.4 and an absolute Log_2 fold change (FC) ≥ 1 (i.e., absolute FC ≥ 2) were considered significantly differentially expressed in order to include as many true positives as possible (i.e., not to exclude possible true positives) and to allow more accurate interpretations of the proteomics data due to the physiological interdependency between proteins (Martins-de-Souza et al., 2012).

2.5. Multivariate analyses

Principal component analysis (PCA) was performed on the list of proteins which were non-redundant, not related to keratin or trypsin contamination, and were detected in all samples using ClustVis (Metsalu and Vilo, 2015). Hierarchical clustering was performed on the top between-group proteins (with $p < 0.05$ in addition to the aforementioned criteria) using Euclidean distances as a measure of dissimilarity and $k = 2$ clusters for unsupervised clustering. Association between the clusters and clinical/sample factors (psychosis history, age, cause of death [suicide vs. illness], last recorded mood, postmortem interval, and tissue pH) were tested by Fisher's exact tests.

2.6. Western blot

Three proteins that were significantly different between groups (tenascin-C [TNC], synaptoporin [SYNPR], BAI1 associated protein 3 [BAIAP3]) were selected for Western blot analyses due to their involvement in synaptic functions. Total tissue lysate samples of all ten subjects were included in the same gel and the gel was repeated as a technical replicate. For each sample, 30 μg of total protein was loaded into each well of a 4–20% Mini-PROTEAN TGX precast gel (BioRad, Hercules, CA, USA), which was then electrophoresed at 100 V for 90 min, followed by wet transfer to an Immun-Blot PVDF membrane (BioRad). The membrane was blocked with 5% non-fat dry milk (BioRad) for 2 h at room temperature following by incubation with a primary antibody overnight at 4 °C: rabbit anti-TNC at 1:1000 (ab108930; Abcam, Cambridge, UK), rabbit anti-SYNPR at

1:500 (ab175224; Abcam), rabbit anti-BAIAP3 1:200 (24836–1-AP; Proteintech, Rosemont, IL, USA), or mouse anti-GAPDH at 1:4000 (MAB374; Millipore, Burlington, MA, USA). The membrane was washed with TBST for 10 min three times and was then incubated at room temperature for 1 h with an HRP-linked secondary antibody at 1:2000: goat anti-rabbit (#7074; Cell Signaling, Danvers, MA, USA) or goat anti-mouse (STAR117P; BioRad). After washing three times with TBST, the membrane was incubated in Clarity Western ECL Substrate (BioRad) for 5 min before imaging with a ChemiDoc MP Imaging System (BioRad). Band intensities were measured using Image Lab software v.3.0 (BioRad). For each sample, the band intensities of the proteins of interest were each adjusted for the GAPDH band intensity on the same blot, and the geometric mean between blots was used for between-group comparisons by Mann–Whitney U test.

2.7. Functional enrichment analysis

We performed Ingenuity Pathway Analysis (IPA; QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis) on protein groups differentially expressed between groups at unadjusted $p < 0.05$ with an absolute $Log_2FC \geq 1$. IPA applies Fisher's exact test for significant enrichment detection. The content was limited to human brain tissues, cerebral cortex and gray matter in particular, and related cell lines. Significant pathway enrichment was considered at Benjamini–Hochberg (B–H) corrected $p < 0.05$.

3. Results

3.1. Subject and tissue characteristics

Table 1 lists the characteristics of the subjects and tissues by groups. Since our subjects were pair-matched, no significant differences in sex (all males), age (overall median [range] = 37.5 [25–69] years) and cause of death (overall 80% suicide vs. 20% not suicide) were found between groups ($p > 0.05$). Neither *postmortem* interval (overall median [range] = 20 [16–26] h) nor tissue pH (overall median [range] = pH 6.8 [5.9–7.0]) were significantly different between groups ($p > 0.05$). Notably, *postmortem* toxicology examination detected antidepressant, antipsychotic and anxiolytic medications only in urine/blood samples of BD subjects with psychosis history, while the history of substance abuse/dependence was only reported in BD subjects without psychosis history.

3.2. Proteomics results

Ten *postmortem* DLPFC samples matched to 5 647 non-redundant proteins, of which 5 150 were detected in all samples. Another 20 proteins were excluded due to keratin and trypsin contamination, hence 5 130 proteins were considered for differential comparison. PCA on these proteins did not show obvious clustering effect by psychosis group despite the first two principal components (PCs) contributed to ~40% of variations (Fig. 1B). The hierarchical clustering of the top between-group proteins demonstrated a classification of subjects according to their psychosis group identity (Fig. 1C). No association was found between the two clusters (i.e., psychosis groups) and the clinical/sample factors of interest.

3.3. Differentially expressed proteins between BD subjects with and without psychosis

Comparing between BD subjects with and without psychosis history, 62 proteins differed at unadjusted $p < 0.05$ level with an absolute $Log_2FC \geq 1$ (Fig. 1D). Furthermore, 11 proteins differed between groups significantly at $q < 0.4$ level with an absolute $Log_2FC \geq 1$ (Fig. 1E and Table 2). In BD subjects with psychosis history, six of these protein groups were decreased (VASP, TNC, HIKESHI, CPNE1, AMPD3, and ENPP2) and five were increased (GNG3, FBXO3, LYSM1, SYNPR, and BAIAP3) compared to those without psychosis history. Upon further examination of the peptides matched to these proteins, three proteins (TNC, SYNPR, and BAIAP3) showed discernible and coherent between-group differences among their peptides (Fig. S1) and were involved in synaptic functions, hence they were selected for validation by Western blot analyses.

Western blot results confirmed the between-group differences detected by mass spectrometry. The psychosis group had a significantly lower level of TNC ($p = 0.032$) and significantly higher levels of SYNPR ($p = 0.016$) and BAIAP3 ($p = 0.008$; Fig. 2 and whole blots in Fig. S2–S5).

3.4. Functional enrichment analysis

We further performed functional enrichment analysis on the proteins that differed between BD subjects with and without psychosis history at unadjusted $p < 0.05$ an absolute $Log_2FC \geq 1$ (Table S1). No significant enrichments in canonical pathways related to neurotransmitters and other nervous system signaling (Table S2) or in functional annotations (Table S3) were detected (B–H $p > 0.05$). However, four functional annotations related to the nervous system were enriched at $p < 0.05$ level with more than three molecule hits: outgrowth of neurons (activation z-score = 1.631 based on 7 molecules; $p = 0.003$; B–H $p = 0.074$), proliferation of neuronal cells (activation z-score = 1.892 based on 8 molecules; $p = 0.006$; B–H $p = 0.084$), growth of neurites (activation z-score = 1.633 based on 7 molecules; $p = 0.009$; B-H $p = 0.098$), and outgrowth of neurites (activation z-score = 1.390 based on 6 molecules; $p = 0.012$; B-H $p = 0.108$), which were all predicted to be upregulated in the psychosis group (activation z-score > |2| is considered significant). A few proteins participated in multiple nervous system functions, such as TNC, ACAN, and NLGN1 (Fig. 3).

4. Discussion

In order to understand the neurobiological basis of BD psychosis, we employed untargeted proteomics to identify the proteomic differences in the DLPFC gray matter of BD subjects with and without psychosis history. We found significant differences in eleven proteins, in which three selected proteins (TNC, SYNPR, and BAIAP3) were validated by Western blot. Among proteins that showed potential between-group differences, we further detected enrichment in nervous system functions related to the growth of neuronal processes and neuronal proliferation. The between-group difference of the proteins mapped to these functions suggested that these functions were upregulated in BD subjects with psychosis history.

Tenascin-C (TNC) is an extracellular matrix glycoprotein with diverse functions. During central nervous system (CNS) development, TNC plays extensive and pivotal roles in regulating neuronal migration, neuronal assembly segregation, neuronal process extension, neuronal pattern formation and plasticity (Faissner et al., 1996), oligodendrocyte precursor cell maturation, proliferation and migration (Garcion et al., 2001; Garwood et al., 2004), and neovascularization (Zagzag and Capo, 2002). In the mature CNS, TNC expression is reported to be restricted to regions exhibiting neuroplasticity (e.g., subventricular zone, hippocampus) while it is virtually undetectable in most regions (Faissner et al., 1996), except in cases of brain tumors (Leins et al., 2003), inflammation (Jakovcevski et al., 2013), and injury (Fujimoto et al., 2016). Interestingly, we found a substantially high TNC protein level in the DLPFC gray matter of the non-psychotic BD subjects, in contrast to a drastically lower TNC protein level in BD subjects with psychosis history (Fig. 2A). These results also appear to contradict the elevated *TNC* gene expression in the prefrontal cortex of schizophrenia patients reported previously (Arion et al., 2007) and the associations of higher serum TNC protein levels with disease onset in schizophrenia patients (Chan et al., 2015). The observed TNC reduction in the BD-psychosis group could be due to the acceleration of TNC protein degradation. Although we did not ascertain the enzymatic activities of the proteins responsible for TNC degradation (matrix metalloproteases and gingipain cysteine proteinases; Midwood et al., 2016), we did not find significant between-group differences in these protein levels. Note that TNC degradation can release soluble fragments that exert functions unique to the intact form (Midwood et al., 2016). Observations in adult *Tnc* knockout mice suggest the potential impacts of TNC deficiency, including exceptionally high neuronal density (in particular non-GABAergic neurons) and astrogliosis, reduced oligodendrocytes-to-neurons and inhibitory-to-excitatory neurons ratios, aberrant dendrite tortuosity, and stubby spine distribution. These cytoarchitectural abnormalities imply a diminished inhibitory circuitry in *Tnc* knockout mice (Irintchev et al., 2005). Inhibitory output deficiency in the DLPFC is associated with impaired working memory function, a core feature of psychotic disorders (Lewis et al., 2005). However, whether similar effects could be observed in human brains of BD psychosis needs to be ascertained. Our results suggest that TNC protein could still be present in the adult DLPFC and its reduction may be associated with BD psychosis, but the temporal relationship between TNC reduction and the development of psychosis require further investigation.

Synaptoporin (SYNPR) and brain-specific angiogenesis inhibitor 1 associated protein 3 (BAIAP3) are both presynaptic proteins. SYNPR is a synaptic vesicle membrane protein and shares homology with synaptophysin (Knaus et al., 1990). Although not directly involved in exocytosis, SYNPR and synaptophysin bind to synaptobrevin to regulate synaptic vesicle maturation by reserving synaptobrevin for exocytosis during high synaptic activity (Becher et al., 1999). In the hippocampus, SYNPR was found to express in mossy fiber axon terminals and was necessary for the homeostatic upregulation of synapses in response to chronic inhibition (Lee et al., 2013), but whether it performs similar synaptoplastic function in the cerebral cortex remains unclear. SYNPR has been implicated in language impairments (Chen et al., 2017; de la Hoz et al., 2015), which may be relevant to our findings as communication disturbances are an important feature of psychopathology in patients with psychosis (Merrill et al., 2017). BAIAP3 is a member of the Munc13 protein family that

regulates neurotransmitter exocytosis and dense-core vesicle release (Shiratsuchi et al., 1998; van de Bospoort et al., 2012). Its function in the cerebral cortex is understudied. A recent study revealed BAIAP3 involvement in dense-core vesicle exocytosis (for the release of neuropeptides, monoamines, and neurotrophins) in neuroendocrine cells by regulating the recycling of dense-core vesicle protein (Zhang et al., 2017). In mouse hypothalamic neuronal punctate, Baiap3 overlapped with neither glutamatergic nor GABAergic vesicular transporters but located close to them instead (Wojcik et al., 2013), demonstrating that BAIAP3/Baiap3 may modulate neurotransmission. Moreover, the hypothalamic slices of *Baiap3* knockout mice showed higher action potential firing rates than those from wild-type mice, which might contribute to the increased seizure propensity and anxiety-like behaviors exhibited in the knockout mice (Cliteur et al., 2012; Wojcik et al., 2013). A genetic study reported the associations of *BAIAP3* gene polymorphisms with family history of psychotic illness (Bigdeli et al., 2016), further associating BAIAP3 with psychosis. Our findings of increased SYNPR and BAIAP3 protein levels in the psychotic group imply that psychotic BD patients may have an enhanced exocytosis of synaptic vesicles and dense-core vesicles and a disturbed regulation between the release of neurotransmitters and neuromodulators in the DLPFC.

We found marginal enrichment in pathways related to neuronal proliferation and neuronal outgrowth and the between-group differences in the proteins matched to this pathway suggested an upregulation of these processes in the BD with psychosis group. Abnormal neuronal proliferation and outgrowth has been reported in mouse and *in vitro* models for the study of schizophrenia and psychosis. Using a chronic low dose of MK-801, a N-methyl-D-aspartate [NMDA] receptor antagonist, in mice, Genius et al. (2012) observed a two-fold increase of proliferating cells in the germinal zone of the dentate gyrus. The group also found increased proliferation and neuronal differentiation in stem cells exposed to MK-801 (Genius et al., 2012). However, the exposure of another NMDA receptor antagonist, phencyclidine (PCP), suppressed neurite outgrowth in primary mouse prefrontal cortex culture and reduced dendritic spine synapses in rat prefrontal cortex (Hajszan et al., 2006; Zhang et al., 2016). In contrast, proliferation deficits were reported in postmortem hippocampal tissue of schizophrenia patients and in induced pluripotent stem cells (iPSCs) derived from schizophrenia and BD patients (Allen et al., 2016; Madison et al., 2015; Seshadri et al., 2017). Despite the inconsistencies in findings (which may be attributed to the methodological differences), our results are the first to suggest a potential increase in neuronal proliferation and neuronal outgrowth in postmortem human DLPFC of BD psychosis. Further studies in the histological features of the human DLPFC in BD with and without psychosis are needed to confirm this hypothesis.

4.1. Limitations

A few limitations need to be noted. Firstly, the sample size was small and only males were included. The top findings of this study must be replicated in larger samples and include both genders for the exploration of sex differences. Secondly, although subjects were pair-matched for age and cause of death, some clinical factors, such as medications and substance misuse, could not be matched between groups, hence they might have contributed to some of the proteomic differences. Thirdly, while comparing BD with and without

psychosis allowed us to detect the proteomic differences associated with psychotic feature in BD, we could not attribute the proteomic differences to BD or BD-psychosis specifically without comparing to a control group. Moreover, our findings might also apply to non-affective psychosis. For instance, Martin-de-Souza et al. (2012) reported that the proteins that were different in the MDD with and without psychosis comparison overlapped significantly with a previously reported schizophrenia proteome. Similarly, Gottschalk et al. (2014) reported that the biological pathways identified in the MDD psychosis vs. MDD without psychosis and the schizophrenia vs. control comparisons showed similar directional changes. Fourthly, since the postmortem tissues were collected in adults, we could not discern when the proteomic differences appeared (e.g., the stage of development, prior to first psychosis episode, after repeated psychotic episodes, after receiving medical treatment) nor could we identify whether such differences were the causes or the results of the affective psychotic episodes. Lastly, the findings in this study might not reflect the proteomic changes of acute psychosis due to limited information about the subjects' mental states shortly prior to death.

4.2. Conclusions

To conclude, we have identified DLPFC proteomic differences that are associated with psychosis in BD, including proteins responsible for cell adhesion, cell migration, neurodevelopment, neuroplasticity, synaptic and dense-core vesicle exocytosis. The protein differences between groups imply enhanced neuronal proliferation and neuronal outgrowth in BD with psychosis history compared to BD without psychosis history. These results suggest that psychosis in BD may be associated with abnormalities in neurodevelopment, neuroplasticity, neurotransmission, and neuromodulation in the DLPFC. Future studies are needed to validate these findings and to establish the causal relationships between these abnormalities and BD psychosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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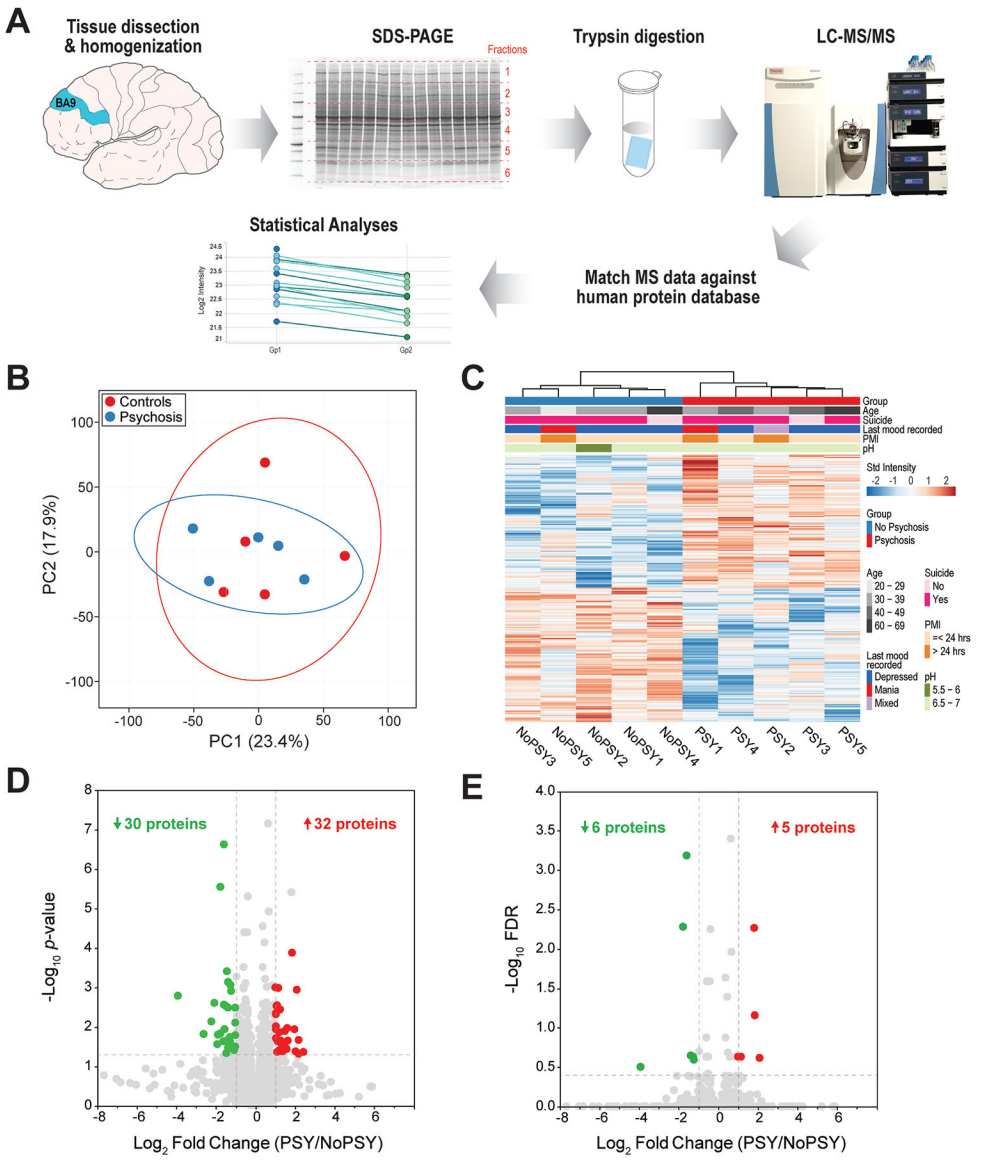

Fig. 1.

(A) Overview of the untargeted proteomics analysis procedures. Multivariate analysis of postmortem human brain proteomics by (B) principal component analysis and (C) hierarchical clustering of the top proteins differed between groups at $p < 0.05$ and Log fold change (FC) ≥ |1|. Volcano plots of proteins that differed between BD subjects with (PSY) and without psychosis (NoPSY). (D) at $p < 0.05$ level (list in Table S1) or (E) at $q < 0.4$ (listed in Table 2) with $Log_2FC \geq |1|$.

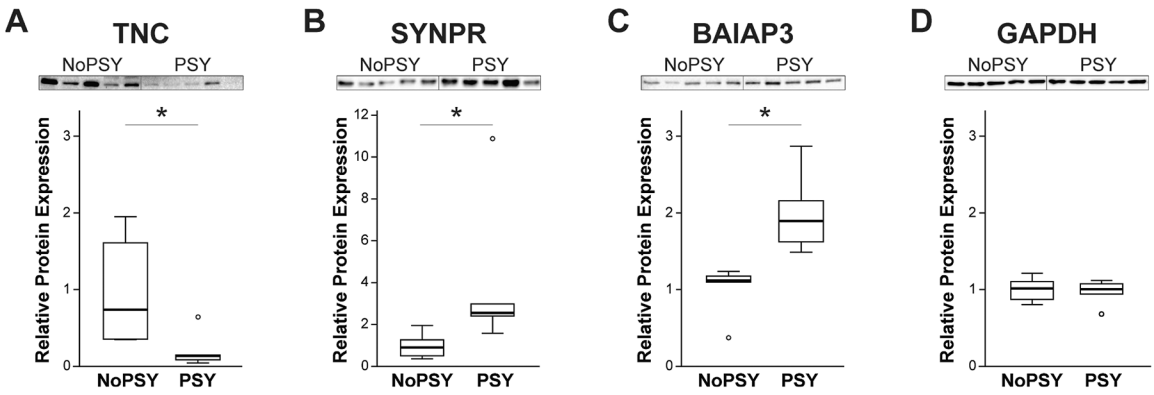

Fig. 2.

Western blot validation of (A) TNC, (B) SYNPR, and (C) BAIAP3 with (D) GAPDH as housekeeping gene reference. Significant differential protein levels (median with interquartile range) were detected between BD subjects with (PSY) and without psychosis (NoPSY). *Mann–Whitney U test $p < 0.05$.

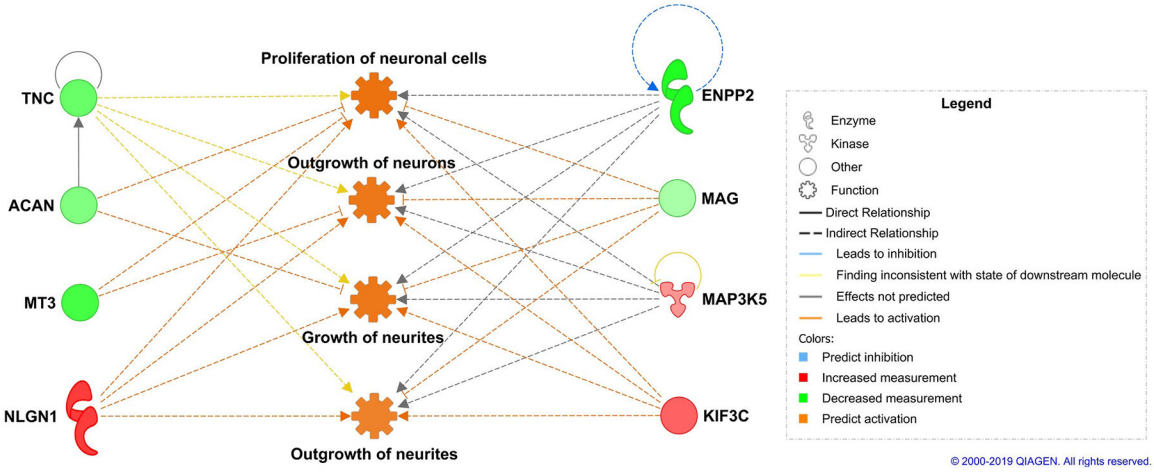

Fig. 3.

Top enriched Ingenuity Pathway Analysis functional annotations related to the nervous system and their predicted activities by the proteins differed between the dorsolateral prefrontal cortices of BD subjects with and without psychosis history ($p < 0.05$).

Table 1

Subject demographics.

	BD subjects without psychosis history	BD subjects with psychosis history	*p*-value
Male (n;%)	5 (100%)	5 (100%)	-
Age (years; median & range) †	36 (25–69)	41 (32–63)	0.548
Last recorded mood (*n*;%) †			0.565
Depression	4 (80%)	3 (60%)	
Mania	1 (20%)	1(20%)	
Mixed	0	1(20%)	
Cause of death (*n*;%) *			1.000
Suicide	4 (80%)	4 (80%)	
Not suicide	1 (20%)	1 (20%)	
Postmortem toxicology (*n*; %) *	0	1 (20%)	1.000
Antidepressant	0	2 (40%)	0.444
medication	0	0	
Antipsychotic	0	1 (20%)	1.000
medication	1 (20%)	0	1.000
Mood stabilizer	1 (20%)	0	1.000
medication	0	0	
Anxiolytic medication	0	0	
Alcohol	1 (20%)	0	1.000
Cocaine			
Cannabinoids			
Opiates			
Other drugs			
Post mortem interval (h; median & ranged) †	18 (10–26)	24 (16–26)	0.310
pH (median & ranged) †	6.9 (5.9–7.0)	6.8 (6.3–7.0)	1.000

* Fisher's exact test.

† Mann–Whitney *U* test.

Table 2

Differentially expressed protein groups ($q < 0.4$ and Log2 fold change [FC] ≥ |1|) in the DLPFC of BD patients with psychosis history (PSY) compared to BD without psychosis history (NoPSY).

Protein group name	Gene name	Log_2FC (PSY/ NoPSY)	*p*-value	*q*-value	Functions
Vasodilator-stimulating phosphoprotein	*VASP*	−1.585	2.34E-07	0.0007	Promotes actin polymerization; involves in cortical neuronal migration, neurovascular angiogenesis, modulate dendritic spine and synapse formation and maturation
Tenascin C	*TNC*	−1.779	2.80E-06	0.0053	Extracellular matrix glycoprotein; involves in cell adhesion and migration during development, neurite outgrowth, formation and stabilization of neuronal contacts
G protein subunit γ3	*GNG3*	1.833	3.88E-06	0.0055	A subunit of the heterotrimeric G protein complex for signal transduction
F-box only protein	*FBX03*	1.863	1.36E-04	0.0696	Protein-ubiquitin ligase
Protein Hikeshi	*HIKESHI*	−1.378	7.24E-04	0.2271	Nuclear transport receptor: mediates heat-shock nuclear import of Hsp70s
Copine-1	*CPNE1*	−1.262	8.69E-04	0.2352	Phospholipid binding protein on plasma membrane
LysM domain-containing protein 1	*LYSM1*	1.021	1.00E-03	0.2352	Unknown
Synaptoporin	*SYNPR*	1.165	1.05E-03	0.2352	Synaptic vesicle membrane protein belonging to synaptophysin family
Brain-specific angeiogenesis inhibitor 1-associated protein	*BAIAP3*	2.098	1.15E-03	0.2409	Synaptic regulator of neurotransmitter exocytosis
AMP deaminase 3	*AMPD3*	−1.219	1.27E-03	0.2562	Deamination of adenosine monophosphate to inosine monophosphate in adenylate catabolism
Ectonucleotide pyrophosphatase/ phosphodiesterase family member 2	*ENPP2*	−3.917	1.62E-03	0.3159	Phosphodiesterase: cleaves phosphodiester bonds at 5’ end of oligonucleotides Phospholipase: converts lysophosphatidylcholine to lysophosphatidic acid (LPA), whose receptors are involved in neurogenesis, neuronal migration, axon extension and myelination