Assessment of Proteomic Measures Across Serious Psychiatric Illness

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Abstract

The diagnoses of serious psychiatric illnesses, such as schizophrenia, schizoaffective disorder, and bipolar disorder, rely on the subjective recall and interpretation of often overlapping symptoms, and are not based on the objective pathophysiology of the illnesses. The subjectivity of symptom reporting and interpretation contributes to the delay of accurate diagnoses and limits effective treatment of these illnesses. Proteomics, the study of the types and quantities of proteins an organism produces, may offer an objective biological approach to psychiatric diagnosis. For this pilot study, we used the Myriad RBM Discovery Map 250+ platform to quantify 205 serum proteins in subjects with schizophrenia (n=26), schizoaffective disorder (n=20), bipolar disorder (n=16), and healthy controls with no psychiatric illness (n=23). Fifty-seven analytes that differed significantly between groups were used for multivariate modeling with linear discriminant analysis (LDA). Diagnoses generated from these models were compared to SCID-generated clinical diagnoses to determine whether the proteomic markers: 1) distinguished the three disorders from controls, and 2) distinguished between the three disorders. We found that a series of binary classification models including 8–12 analytes produced separation between all subjects and controls, and between each diagnostic group and controls. There was a high degree of accuracy in the separations, with training areas-under-the-curve (AUC) of 0.94–1.0, and cross-validation AUC of 0.94–0.95. Models with 7–14 analytes produced separation between the diagnostic groups, though less robustly, with training AUC of 0.72–1.0 and validation AUC of 0.69–0.89. While based on a small sample size, not adjusted for medication state, these preliminary results support the potential of proteomics as a diagnostic aid in psychiatry. The separation of schizophrenia, schizoaffective disorder, and bipolar disorder suggests that further work in this area is warranted.

Key Words: Schizophrenia, Schizoaffective Disorder, Bipolar Disorder, Diagnosis, Biomarkers, Proteomics

Introduction

Issues of the Symptomatic Diagnosis of Psychiatric Illness

In the treatment and research of serious psychiatric illnesses—schizophrenia, schizoaffective disorder, and bipolar disorder—there have been substantial, constructive efforts in the field to refine symptomatic diagnosis. The *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5)* (1) and the Research Domain Criteria (RDoC) (2, 3) represent two systems that are leading the effort to refine psychiatric diagnosis grounded in advances in research. Areas of study have developed structured interviews to ascertain and quantify specific patient symptoms to assist in diagnosis (e.g., SCID) (4) and to quantify the severity of the

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Clinical Implications

The results of this study demonstrate that high-quality samples for proteomic analysis provide a sound platform for discovery work, even given small sample sizes. We found that models based on 8–12 analytes were sufficient to separate serious psychiatric illnesses, combined or individually, from healthy subjects. Further, larger studies have confirmed differences between subjects with schizophrenia and controls. While based on a small sample size, not adjusted for medication state, these preliminary results support the potential of proteomics as a diagnostic aid in psychiatry. A step forward in this study has demonstrated not only differences between three serious psychiatric illnesses and the control group, but an initial exploration revealing proteomic measures that can separate schizophrenia, schizoaffective disorder, and bipolar illness.

illness, including the Brief Psychiatric Rating Scale (BPRS) (5), the Positive and Negative Syndrome Scale (PANSS) (6), the Young Mania Rating Scale (YMRS) (7), and the Montgomery-Asberg Depression Rating Scale (MADRS) (8). While these advances in symptom identification and quantification continue to benefit both research and its subsequent translation into clinical practice, contemporary clinical work maintains a dependence on traditional clinical interview, which probes a patient's subjective recall and a clinician's interpretation of that patient's recall. That is, psychiatric diagnosis is not centered on the objective measures of pathophysiology. A lack of scientific objectivity in reasoning yields uncertainty in the authority and credibility of conclusions. The lack of evidence-based statistics in current psychiatric diagnoses, particularly those with overlapping features (e.g., schizophrenia and schizoaffective disorder), limits the reliability and validity, and perhaps most importantly offers little predictive power in selecting efficacious personalized treatment. Furthermore, structured clinical interviews and rating scales, which truly strive to achieve objectivity, are laborious and time-consuming, and are often impractical in routine clinical settings.

It's important to note, however, that in response to these limitations, the field has persistently sought to investigate the physiological underpinnings of psychiatric diagnosis in an effort to move forward in this area. Robust recent examples include the examination of inflammatory markers and their potential relationship to diagnosis and treatment (9-11), meta-analyses examining oxidative stress and its relationship to psychiatric illnesses (12, 13), and the employment of immune factors and cytokine measures in schizophrenia subtype classification and clinical status (14, 15). In addition to these measures, Tamminga and colleagues (2013) have utilized the B-SNIP (Bipolar-Schizophrenia Network on Intermediate Phenotypes) for differential of psychotic illnesses (16). Further study of such blood tests, aimed to learn more about schizophrenia and other serious psychiatric illnesses, to explore potential subtypes, and to predict treatment response, would be a major advance in the medical model of research and clinical management.

Proteomics in the Diagnosis and Treatment of Schizophrenia and Related Illnesses

Protein markers change with time or state, representing the complex interaction of genes versus the environment. Proteomics refers to the large-scale study of the types and quantities of proteins an organism produces, their structures and functions. In this framework, proteomics provides a precise and unbiased functional profile of an organism's current physiological state, providing a biochemical indicator of disease progression. The study of biomarkers seeks to evaluate the potential of an analyte to aid in diagnosis and treatment selection in psychiatric illnesses (17). An ideal biomarker: 1) provides rapid and accurate diagnosis, 2) is easily obtainable, 3) is cost-effective, and 4) is acceptable by both patient and clinician. Proteomics may provide such an approach. Indeed, the use of biomarkers to inform diagnosis and treatment has revolutionized many fields of medicine (e.g., oncology), and proteomics has recently engaged the study of psychiatric illness.

Proteomics is currently being used to study the physiological underpinnings of schizophrenia, and the history leading up to this work and its present state have recently been reported in detail (18). In one of the first studies of proteomic measures as a diagnostic marker, antipsychotic-naive, early-stage schizophrenia patients (n=22) were compared to controls (n=33). In this early study, a statistical difference between the two groups was determined using 10 proteomic measures. Reflecting the complexity of the illness, the results were not in a single biological pathway—rather, they represented many functional pathways (19). In a successive larger study, the group collaborated with Rules-Based Medicine in the trial of serum-based tests of 250 subjects with a schizophrenia diagnosis and 230 control participants, which included a second study stage using an independent cohort of 577 individuals with a schizophrenia diagnosis whose results were compared to 229 controls. The outcome of this work was the conception of a 51-analyte proteomic panel which achieved 83% sensitivity and 83% specificity in diagnosis (20).

Table 1 Descriptive Summary of Subject Demographics

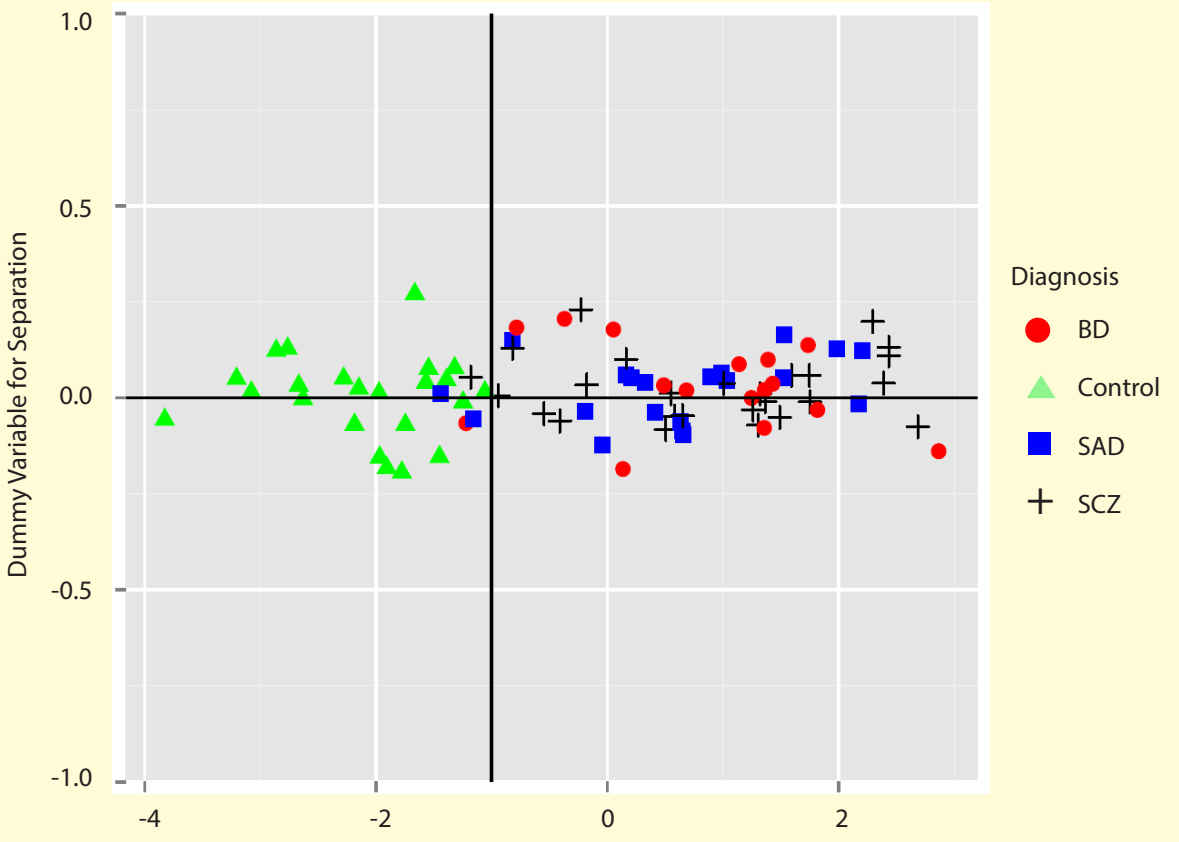
	Overall (n=85)	SCZ (n=26)	SAD (n=20)	BD (n=16)	Control (n=23)
Gender					
Female	30 (35.3%)	10 (38.5%)	5 (25.0%)	6 (37.5%)	9 (39.1%)
Male	55 (64.7%)	16 (61.5%)	15 (75.0%)	10 (62.5%)	14 (60.9%)
Race					
Asian	3 (3.5%)	0 (0.0%)	1 (5.0%)	1 (6.3%)	1 (4.4%)
African American	15 (17.7%)	7 (26.9%)	2 (10.0%)	3 (18.7%)	3 (13.0%)
Caucasian	50 (58.8%)	16 (61.5%)	14 (70.0%)	5 (31.2%)	15 (65.2%)
Asian/White	2 (2.4%)	1 (3.9%)	0 (0.0%)	1 (6.3%)	0 (0.0%)
African American/White	1 (1.2%)	0 (0.0%)	1 (5.0%)	0 (0.0%)	0 (0.0%)
Unreported	14 (16.4%)	2 (7.7%)	2 (10.0%)	6 (37.5%)	4 (17.4%)
Age at Baseline (yrs)					
Mean	36.8	39.7	40.7	32.6	33.1
StdDev	12.92	13.56	12.49	11.76	12.21
Median	37.1	42.0	43.9	29.3	27.0
Range	18.4–64.8	18.4–63.3	21.5–64.8	20.1–61.4	19.1–52.5
BMI at Baseline (kg/m^2)					
N	60*	22	14	8	16
Mean	28.8	29.9	30.8	27.8	26.2
StdDev	6.01	5.61	6.23	4.05	6.60
Median	27.9	29.6	29.1	27.7	25.3
Range	19.0–40.2	22.0–39.6	20.1–40.2	22.5–35.2	19.0–39.2

* Either height and/or weight measurements are missing for 25 subjects.

Next steps in utilizing proteomic measures were implemented by a multi-center trial, comparing individuals with a diagnosis of schizophrenia, major depressive disorder, bipolar disorder, or Asperger's syndrome, to control participants. Utilizing 181 proteins or small molecules, the authors noted a significant difference between schizophrenia participants and controls, further presenting a correlation of 0.81 between the analytes and clinically based diagnoses. The classification of schizophrenia participants and controls ranged between 60–75%. Consequently, the separation of other disorders was not as robust. That is, classification reached 50% for major depressive disorder vs. controls, and 10% for individuals with bipolar disorder and Asperger's syndrome (21).

Another study of proteomics quantification focused on postsynaptic density (PSD) in the anterior cingulate cortex, a brain area associated with dysfunction of excitatory activity in schizophrenia, utilizing the Stanley Medical Research Foundation post mortem brain samples (22). The authors noted differences between the schizophrenia samples and the controls in clathrin-mediated endocytosis and N-methyl-D-aspartate (NMDA) interacting proteins. The North American Prodrome Longitudinal Study (NAPLS) has also published the study of proteomics in their prodrome group (n=32) and their controls (n=35), who were prodromal patients and who had not converted to frank psychosis. Utilizing 15 analytes for classification, the model performed at Area-under-the-Receiver Operating Characteristic Curve AUC of 0.91 for the differentiation of individuals with clinical high-risk symptoms who developed

Figure 1 A Single Axis Classifies Controls from Illness (BD, SAD, and SCZ)



psychosis vs. unaffected comparison subjects. The same model performed at an AUC of 0.88 for the differentiation of persons with clinical high-risk symptoms who developed psychosis vs. persons with clinical high-risk symptoms who did not develop psychosis (23).

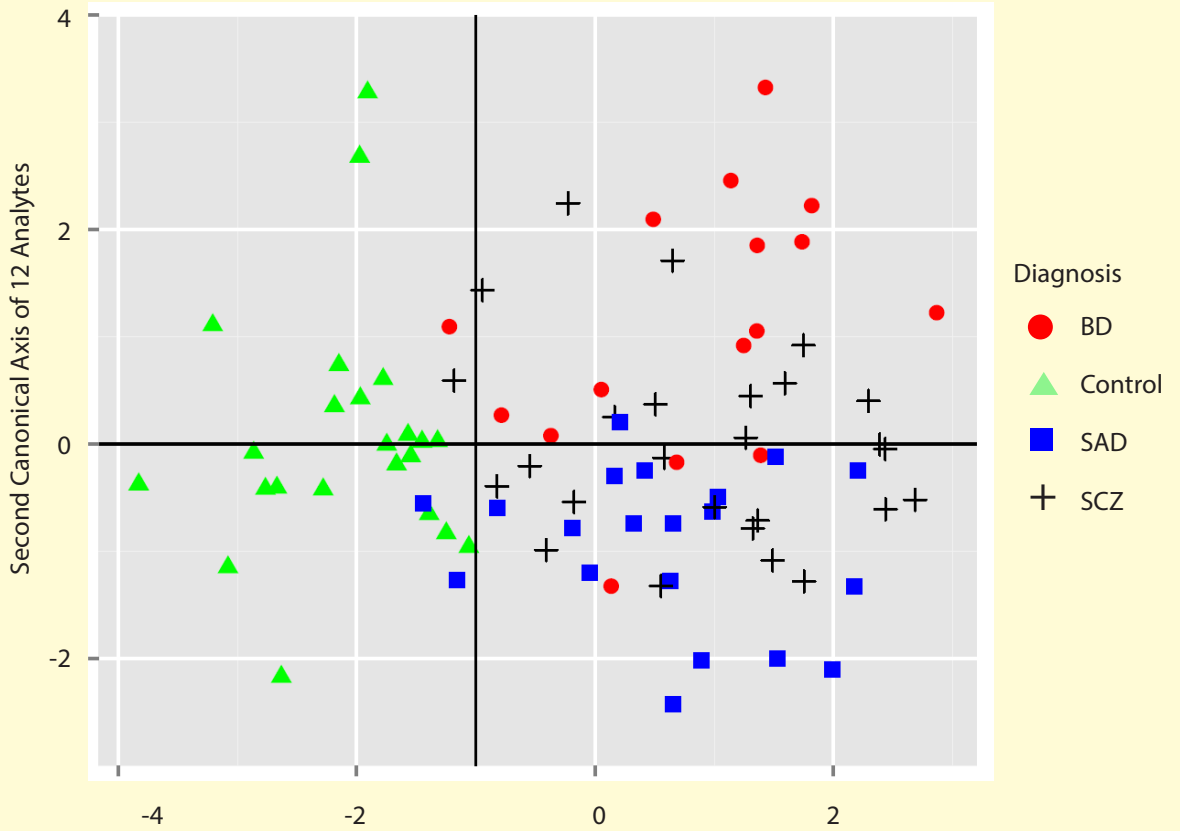
To extend that which has been presented, the goal of the present study was to measure a comprehensive panel of serum proteins in participants with schizophrenia, bipolar disorder, and schizoaffective disorder, and a control group with no history of psychiatric illness, to determine whether the proteomic markers could 1) distinguish these three disorders from the controls, and 2) distinguish between the 3 disorders. This work could have a major impact on facilitating rapid and accurate diagnosis, selecting and monitoring optimal, personalized treatments, and creating biologically homogeneous groups for research projects.

Methods

The study was approved by the IRB at the University of Minnesota. Recruitment fliers with the research center contact information were posted throughout the community. All potential subjects were screened by project coordinators to ensure criteria were met for participation. Study exclusion criteria ruled out any individuals unable to understand the consent process or who had a learning disability, a positive drug screen, any presence or history of acute infection, significant metabolic, cardiovascular, neurological, rheumatologic or autoimmune disorders, treatment using nonsteroidal anti-inflammatory or opioid agents, exposure to stimulators or shunts, chemotherapy or radiation within the past year, or treatment with monoclonal antibody therapy within the past three months. In addition to these general criteria, individuals with any psychiatric diagnosis, with exception of past substance abuse, were excluded from the healthy control group.

Subjects and controls were interviewed using the Structured Clinical Interview for DSM-IV (SCID) by trained raters. Following the SCID interview, each interaction summary, which included all assessments, was presented to and reviewed by a panel of clinical faculty for consensus of diagnosis. These assessments led to the enrollment of 62 subjects with a diagnosis of a psychotic spectrum disorder: schizophrenia (SCZ, n=26); schizoaffective disorder (SAD, n=20); bipolar disorder (BPD, n=16); healthy controls (n=23).

Figure 2 Addition of a Second Axis Further Classifies BD from SAD



In addition to the SCID, subjects underwent additional assessments to measure level of symptomatic severity: Calgary Depression Rating Scale (CDSS), Fagerstrom Test for Nicotine Dependence (FTND), Montgomery-Asberg Depression Rating Scale (MADRS), Positive and Negative Syndrome Scale (PANSS), and Young Mania Rating Scale (YMRS).

All individual participant data were collected within one week of the first visit, and for the convenience of participants restrictions for fasting and time of blood draw were not implemented. Each participant provided a 20cc blood sample at the laboratory, saved to clot for 30 minutes at room temperature, and then centrifuged at 1300xG for 10 minutes to obtain serum, following a standardized protocol instantiated by Myriad RBM (24). The samples were frozen at -80 degrees Celsius until the batch analysis.

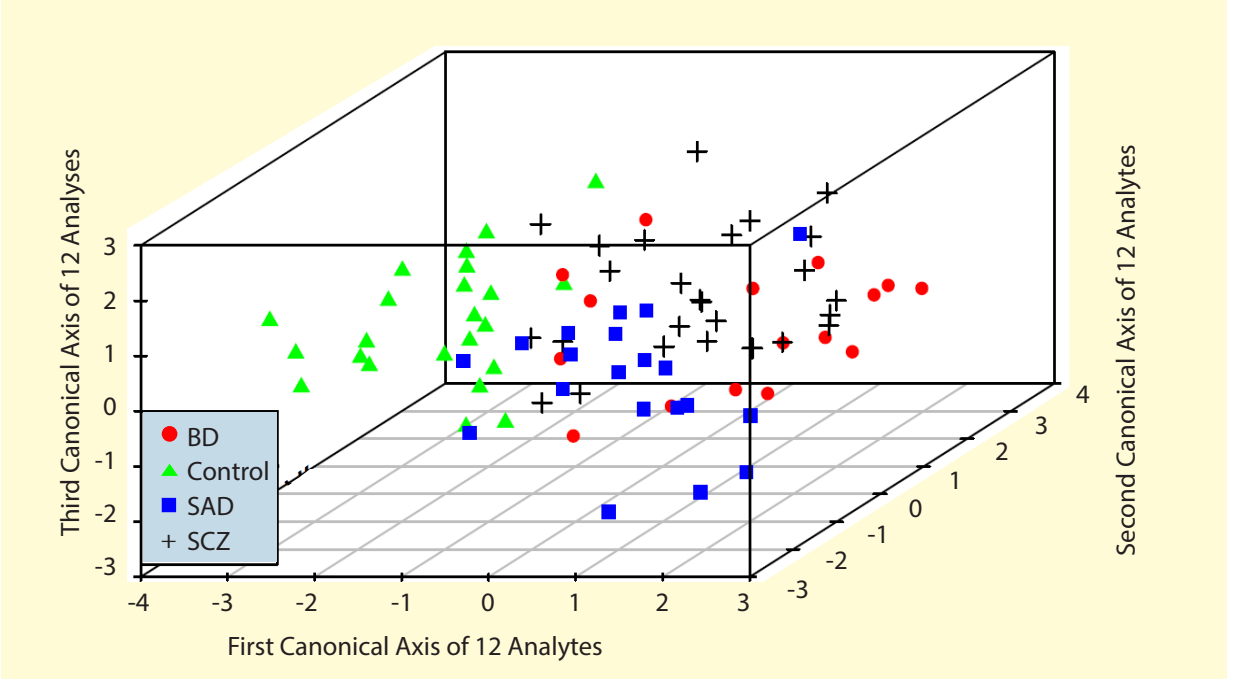
The assays utilized the Discovery Map 250+ platform for proteomic measures, and were carried out by blinded study staff (Myriad RBM). Of the 243 proteomic measures, 38 were excluded from analysis on the basis of being below the lower limit of quantification (LLOQ); thus, 205 analytes were used in the statistical analyses.

Multivariate exploratory techniques were used to explore dimensionality (27 to 50 dimensions), identify outliers (very few were detected), check effectiveness of the Log10 transformation used, and graph disease distributions in multivariate space. Univariate analyses (ANOVAs with 4 classifications based on clinical diagnosis) were conducted to analyze the differences between disease classifications over the 205 analytes and demographic variables. The p values obtained in the univariate analyses were adjusted for multiplicity to control the false discovery rate. The resulting set of 57 statistically significant analytes was used for multivariate modeling with Linear Discriminant Analyses (LDA). Diagnoses generated from these models were compared to the SCID diagnoses and model performance was measured in terms of AUC for both training and cross-validation (CxV).

Results

A total of 85 subjects and controls were included in the analyses. Their sociodemographic characteristics are listed in Table 1. There was no significant difference between psychiatric subjects and controls in gender, age, or BMI. These variables were included in preliminary modeling, but were

Figure 3 Three Axes Achieve a Decent 4-Way Classification Between Controls, BD, SAD, and SCZ



later removed due to non-significance and small effect sizes.

The first step of the analysis utilized an LDA model including 12 proteomic analytes. The first canonical axis revealed almost a complete separation of the control group from the serious psychiatric illness subjects. This is displayed in Figure 1. Note: although for visual clarity the model is displayed as 2-dimensional, it actually separated subjects along only a single dimension (i.e., X-axis). However, there was little separation between the three diagnostic groups.

In order to pursue potential differences between the psychiatric subjects with the 3 diagnoses, the next statistical step was the addition of a second model (axis), displayed in Figure 2. This figure is broader in distribution and interestingly shows pronounced separation between the bipolar disorder and schizoaffective disorder subjects, while schizophrenia subjects were less clearly separated from the other two diagnoses.

The third step was the application of 3 canonical models (axes), shown in Figure 3, which produced the best separation between the 3 psychiatric illnesses and controls.

Due to the multi-dimensional distribution of the illnesses, the final step in the analysis was to utilize an LDA approach to build various binary classification models. The results for the LDA models separating subjects from controls, and the analytes included in the models, are shown in Table 2. In these analyses, panels of 8–12 analytes produced separation between all subjects and controls, and between each diagnostic group and controls. There was a high degree of accuracy in the separations, with training AUC of 0.94–1.0, and cross-validation AUC of 0.94–0.95. The results for the LDA models distinguishing between the three subject groups are shown in Table 3.

Panels of 7–14 analytes produced separation between the diagnostic groups. The separations were not as robust as in the models separating subjects from controls, with training AUC of 0.72–1.0 and validation AUC of 0.69–0.89. The clearest separation was between schizophrenia and schizoaffective disorder, and the least clear between bipolar disorder and schizoaffective disorder, which may illustrate the closeness of the illnesses. This may seem to be in contrast to Figure 2. In Figure 2, however, a set of 12 analytes is being used to achieve a 4-way classification; whereas in Table 3 individual binary classification models are fitted.

Discussion

The results of this study demonstrate that high-quality samples for proteomic analysis provide a sound platform for discovery work, even given small sample sizes. We found that models based on 8–12 analytes were sufficient to separate serious psychiatric illnesses, combined or individually, from healthy subjects. In the separations, models ranged with training areas-under-the-curve (AUC) of 0.94–1.0, and cross-validation AUC of 0.94–0.95. Models based on 7–14 analytes produced separation between the diagnostic groups, though less robustly, with training AUC of 0.72–1.0 and validation AUC of 0.69–0.89. In a finding that may have relevance of psychiatric nosology, disease classifications did not lie along a single disease axis, and multiple models were therefore needed for optimal discrimination.

Table 2 LDA Models for Binary Classifications from Controls

	Illness vs. Control	BD vs. Control	SAD vs. Control	SCZ vs. Control
Samples (N)	85	39	43	49
Analytes (p)	12	8	10	12
Training AUC	0.94	1	1	0.98
CxV AUC	0.94	0.95	0.95	0.94
	IGFBP_1	IGFBP_1	IGFBP_1	IGFBP_1
	LH	LH	MMP_1	LH
	IL_8	IL_8	TTR	IL_8
	vWF	vWF	Apo_D	vWF
	MMP_1	MMP_1	MMP_7	TTR
	TTR	IL_2_r_a	IGFBP6	tPA
	IL_2_r_a	AFP	uPAR	MDC
	AFP	CD40	Apo_H	PARC
	MMP_7		CRP	IL_16
	uPAR		Apo_C_I	TNFR2
	CK_MB			A1Micro
	PARC			SAP

See full protein names below.

Table 3 LDA Models for Binary Classifications Between Illnesses

	BD vs. SAD	BD vs. SCZ	SAD vs. SCZ
Samples (N)	36	42	46
Analytes (p)	7	9	14
Training AUC	0.72	0.9	1
CxV AUC	0.69	0.79	0.89
	tPA	IL_2_r_a	IGFBP_1
	Apo_D	Apo_D	IL_8
	IL_13	IL_13	Apo_H
	Gelsolin	Gelsolin	MIP_1_beta
	IL_23	IGFBP6	Apo_E
	IGFBP6	MIP_1_beta	Fib_1C
	CRP	Apo_E	Apo_A_I
		Fib_1C	Sortilin
		HER_2	HB_EGF
			C3
			FAS
			ACE
			Ckine
			IGFBP4

See full protein names below.

The research in recent years of utilizing proteomic measures available in a blood test began with measures of young, never-medicated subjects with schizophrenia. As a statistical difference was noted after a selection of a group of proteomics, this then led to further measures of subjects with schizophrenia as well as autism and mood disorders. To date, these subjects were not as substantially different from controls.

The goals and design of this pilot study were to explore the domain of psychotic illnesses—schizophrenia, schizoaffective disorder, and bipolar illness. The first step in the analysis showed a very substantial difference between the subject group and the controls, with 94% accuracy. Largely, the proteins identified have prior evidence of alteration in drug-free schizophrenia (25). Predominating in the differentiation of disease vs. control were biomarkers implicated in inflammatory and immune response. Notably, all of these inflammatory markers appeared in at least one model differentiating specific disease type and control (see Table 2). The most highly ranked coefficient in the overall disease vs. control model—Insulin-like Growth Factor-Binding Protein 1 (IGFBP-1)—was also the chief classifier in all three of the individual disease vs. control models. Insulin-like Growth Factor 1 (IGF-1) is considered essential in the development and function of myelination and has recently gained focused attention in schizophrenia, given building evidence of dysfunctional oligodendrocytes in disrupted synaptic connectivity (26). IGFBP-1 alters the IGF interaction with cellular surface receptors, thereby inhibiting or stimulating the growth-promoting effects of IGFs on cell culture (27). This alteration of the IGF-1 is thought to contribute to the pathogenesis of schizophrenia and this signaling pathway has been highlighted in the exploration of new treatment options (28).

The finding that numerous immune/inflammatory proteins were important components of the models is in keeping with studies showing that they are relevant to the pathophysiology of mood and psychotic illnesses (10, 29-31), independent of treatment effects (29, 32). Several of these—including IL8, IL2 receptor antagonist, and matrix metaloproteinases 1 and 7—were components of the overall disease vs. control model, and the individual disease vs. control models. To a somewhat lesser extent, they were also constituents of the between-disease models: for example, IL13 was in the models distinguishing bipolar disorder from the other two illnesses. A number of cytokines cross the blood-brain barrier (BBB), and may also affect brain function by interactions with receptors on the BBB and the vagus nerve (33). Moreover, they have been shown in animal models and human studies to be strongly asso-

ciated with psychiatric syndromes such as depression (34, 35). These data are further supported by initial studies of proteomics in schizophrenia research, which have identified decreased apolipoproteins in the serum of schizophrenia patients (19). Thus, the proteins making up the models are largely in concordance with accepted theories of the pathophysiology of psychiatric illnesses.

After finding that the subject group of all three diagnoses was different from controls, the diagnoses groups were then compared to one another. To proceed in these comparisons, different proteomic measures were explored and separations between the 3 diagnoses were statistically determined, producing models ranging from 69–89% performance accuracy. The top performer, SCZ vs. SAD with AUC of 0.89, consisted of a 14-analyte model. Again, the proteins making up the models were largely in concordance with accepted theories of the pathophysiology of psychiatric illnesses. Through this analyte model were derived the following observations: Interleukin 8 (IL8) otherwise appeared only in the SCZ vs. Control model, and apolipoprotein H (ApoH) otherwise appeared only in the SAD vs. Control model. Notably, IL-8 levels have been reported as significantly higher in mothers of offspring with schizophrenia spectrum disorders than those of the mothers without (36), and ApoH has been identified as a candidate gene that could modulate the risk of schizophrenia (37). Additionally, three analytes from this SCZ vs. SAD model also appeared in the SCZ vs. BPD model: macrophage inflammatory protein-1Beta (MIP-1β), and apolipoprotein E (ApoE). Notably, significantly higher levels of MIP-1β have been identified in schizophrenia subjects when compared to controls, the literature pointing to evidence that schizophrenia may be accompanied by an activation of the monocyte-macrophage arm of cell-mediated immunity (38). Increased levels of ApoE have been associated with the pathology of schizophrenia (39), and ApoE has also been identified as a possible risk factor for schizophrenia (40), although this evidence has been debated (41).

Four analytes overlapped in BPD vs. SCZ and BPD vs. SAD models and were absent in the model differentiating SCZ from SAD: Apolipoprotein D (ApoD), Interleukin-13 (IL-13), Gelsolin, and Insulin-like growth factor binding protein 6 (Igfbp6). In a recent meta analysis, the main findings for the differential expression of proteins implicated in schizophrenia and bipolar disorder were 14-3-3 mediated signaling in schizophrenia and mitochondrial dysfunction in bipolar disorder (42). ApoD is elevated in many pathological situations involving mitochondrial damage (43), including bipolar disorder (44). The separation between schizophrenia and bipolar disorder has previously been demonstrated by significantly lower concentration levels of ApoD identified in the serum of schizophrenia subjects as compared to bipolar subjects (44) and, in another study, elevated levels of ApoD present in the amygdala and thalamus in subjects with bipolar disorder but not schizophrenia (45). Elevated levels of IL-13 have been identified in the orbitofrontal cortex of victims of suicide (46). Gelsolin is proposed to have a role in myelinogenesis (47), and hippocampal neurons lacking gelsolin may be vulnerable to glutamate toxicity (48). The Igfbp6 gene has previously been identified as a blood biomarker for mood disorders (49). As the pharmacological treatments between these three diagnoses are different from one another, there is potential for further study of this test in appropriate treatment. Currently being debated is the potential treatment of mental diseases using anti-inflammatory medication. It is important to note that the immune system is dynamic and is in fact sensitive to medication status, including antipsychotic drugs (31).

One limitation of this study is the lack of sufficient data to conduct a statistical analysis on medication use and smoking status, particularly across diagnostic groups. While investigations have probed at the potential effects of treatment on proteomic profiles and associated pathways (50, 51), to our knowledge none of the specific proteins identified in this work are known contributors to pharmacodynamically mediated therapeutic drug response. Recent work using a similar analyte panel has reported that the expressions of proteins under study were not primarily driven by medication use (52).

In conclusion, proteomics is an emerging test in this area of psychiatry as differences between young and never-medicated subjects have been demonstrated. Further, larger studies have confirmed differences between subjects with schizophrenia and controls. While based on a small sample size, not adjusted for medication state, these preliminary results support the potential of proteomics as a diagnostic aid in psychiatry. A step forward in this study has demonstrated not only differences between 3 serious psychiatric illnesses and the control group, but an initial exploration revealing proteomic measures that can separate schizophrenia, schizoaffective disorder, and bipolar illness.

Protein Abbreviations

IGFBP_1=Insulin-like Growth Factor Binding Protein 1; LH=Luteinizing Hormone; IL_8=Interleukin 8; vWF=von Willebrand factor; MMP_1=Matrix Metalloproteinase-1; TTR=Transthyretin; IL_2_r_a=Interleukin 2 Receptor Antagonist; AFP=Alpha-Fetoprotein; MMP_7=Matrix Metalloproteinase-7; uPAR=Urokinase-Type Plasminogen Activator Receptor; CK_MB=Creatine Kinase Muscle/Brain Type; PARC=Pulmonary and Activation-Regulated Chemokine; CD40=CD40 Ligand; Apo_D=Apolipoprotein D; IGFBP6=Insulin Like Growth Factor Binding Protein

6; Apo_H=Apolipoprotein H; CRP=C-reactive Protein; Apo_C_I=Apolipoprotein C-1; tPA=Tissue Type Plasminogen Activator; MDC=Macrophage-Derived Chemokine; IL_16=Interleukin 16; TNFR2=Tumor Necrosis Factor Receptor 2; A1Micro=Alpha-1 Microglobulin; SAP=Serum Amyloid P-Component; IL_13=Interleukin 13; Gelsolin=Gelsolin; IL_23=Interleukin 23; MIP_1_beta=Macrophage Inflammatory Protein-1 Beta; Apo_E=Apolipoprotein E; Fib_1C=Fibulin-1C; HER_2=Human Epidermal Growth Factor Receptor 2; Apo_A_1=Apolipoprotein A-1; Sortilin=Sortilin-1; HB_EGF=Heparin-Binding EGF-Like Growth Factor; C3=Complement C3; FAS=FAS Ligand Receptor; ACE=Angiotensin-Converting Enzyme; Ckine=Chemokine (C-C Motif) ligand 21; IGFBP4=Insulin Like Growth Factor Binding Protein 4.

References

1. American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). Washington, DC, American Psychiatric Association, 2013.
2. Insel TR. The NIMH Research Domain Criteria (RDoC) Project: precision medicine for psychiatry. Am J Psychiatry 2014;171(4):395-397. doi: 10.1176/appi.ajp.2014.14020138. PubMed PMID: 24687194.
3. Insel T, Cuthbert B, Garvey M, Heinssen R, Pine DS, Quinn K, et al. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. Am J Psychiatry 2010;167(7):748-751. doi: 10.1176/appi.ajp.2010.09091379. PubMed PMID: 20595427.
4. First M, Williams J, Karg R, Spitzer R. Structured Clinical Interview For DSM-5, Research Version (SCID-5 for DSM-5, Research Version; SCID-5-RV). Arlington, VA: American Psychiatric Association; 2015.
5. Overall JE, Gorham DR. The brief psychiatric rating scale. Psychol Rep 1962;10:799-812.
6. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987;13(2):261-276. PubMed PMID: 3616518.
7. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry 1978;133:429-435. PubMed PMID: 728692.
8. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry 1979;134:382-389. PubMed PMID: 444788.
9. Anderson G, Berk M, Dodd S, Bechter K, Altamura AC, Dell'osso B, et al. Immuno-inflammatory, oxidative and nitrosative stress, and neuroprogressive pathways in the etiology, course and treatment of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2013;42:1-4. doi: 10.1016/j.pnpbp.2012.10.008. PubMed PMID: 23085074.
10. Munkholm K, Brauner JV, Kessing LV, Vinberg M. Cytokines in bipolar disorder vs. healthy control subjects: a systematic review and meta-analysis. J Psychiatr Res 2013;47(9):1119-1133. doi: 10.1016/j.jpsychires.2013.05.018. PubMed PMID: 23768870.
11. Kirkpatrick B, Miller BJ. Inflammation and schizophrenia. Schizophr Bull 2013;39(6):1174-1179. doi: 10.093/schbul/sbt141.
12. Brown NC, Andreazza AC, Young LT. An updated meta-analysis of oxidative stress markers in bipolar disorder. Psychiatry Res 2014;218(1-2):61-68. doi: 10.1016/j.psychres.2014.04.005. PubMed PMID: 24794031.
13. Flatow J, Buckley P, Miller BJ. Meta-analysis of oxidative stress in schizophrenia. Biol Psychiatry 2013;74(6):400-409. doi: 10.1016/j.biopsych.2013.03.018. PubMed PMID: 23683390; PubMed Central PMCID: PMC4018767.
14. Schwarz E, van Beveren NJ, Ramsey J, Leweke FM, Rothermundt M, Bogerts B, et al. Identification of subgroups of schizophrenia patients with changes in either immune or growth factor and hormonal pathways. Schizophr Bull 2014;40(4):787-795. doi: 10.1093/schbul/sbt105.
15. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol Psychiatry 2011;70(7):663-671. doi: 10.1016/j.biopsych.2011.04.013.
16. Tamminga CA, Ivleva EI, Keshavan MS, Pearlson GD, Clementz BA, Witte B, et al. Clinical phenotypes of psychosis in the Bipolar-Schizophrenia Network on Intermediate Phenotypes (B-SNIP). Am J Psychiatry 2013;170(11):1263-1274. doi: 10.1176/appi.ajp.2013.12101339. PubMed PMID: 23846857.
17. Frey BN, Andreazza AC, Houenou J, Jamain S, Goldstein BI, Frye MA, et al. Biomarkers in bipolar disorder: a positional paper from the International Society for Bipolar Disorders Biomarkers Task Force. Aust N Z J Psychiatry 2013;47(4):321-332. doi: 10.1177/0004867413478217. PubMed PMID: 23411094.
18. Nascimento JM, Martins-de-Souza D. The proteome of schizophrenia. NPJ Schizophr 2015;1:14003. doi: 10.1038/npjschz.2014.3. PubMed PMID: 27336025; PubMed Central PMCID: PMCPMC4849438.
19. Levin Y, Wang L, Schwarz E, Koethe D, Leweke FM, Bahn S. Global proteomic profiling reveals altered proteomic signature in schizophrenia serum. Mol Psychiatry 2010;15(11):1088-1100. doi: 10.1038/mp.2009.54. PubMed PMID: 19546861.
20. Schwarz E, Izmailov R, Spain M, Barnes A, Mapes JP, Guest PC, et al. Validation of a blood-based laboratory test to aid in the confirmation of a diagnosis of schizophrenia. Biomark Insights 2010;5:39-47. PubMed PMID: 20520744; PubMed Central PMCID: PMC2879227.
21. Schwarz E, Guest PC, Steiner J, Bogerts B, Bahn S. Identification of blood-based molecular signatures for prediction of response and relapse in schizophrenia patients. Transl Psychiatry 2012;2:e82. doi: 10.1038/tp.2012.3. PubMed PMID: 22832819; PubMed Central PMCID: PMC3309553.
22. Zavitsanou K, Ward PB, Huang XF. Selective alterations in ionotropic glutamate receptors in the anterior cingulate cortex in schizophrenia. Neuropsychopharmacology 2002;27(5):826-833. doi: 10.1016/s0893-133x(02)00347-0. PubMed PMID: 12431856.
23. Perkins DO, Jeffries CD, Addington J, Bearden CE, Cadenhead KS, Cannon TD, et al. Towards a psychosis risk blood diagnostic for persons experiencing high-risk symptoms: preliminary results from the NAPLS project. Schizophr Bull 2015;41(2):419-428. doi: 10.1093/schbul/sbu099. PubMed PMID: 25103207; PubMed Central PMCID: PMC4332942.
24. Welsh B, Mapes J. An Overview of Assay Quality Systems at Myriad RBM, Inc. 2013. Available from: https://rbm.myriad.com/scientific-literature/white-papers/quality-control-white-paper/.
25. Sabherwal S, English JA, Focking M, Cagney G, Cotter DR. Blood biomarker discovery in drug-free schizophrenia: the contributionof proteomics and multiplex immunoassays. Expert Rev Proteomics 2016;13(12):1141-1155. doi: 10.1080/14789450.2016.1252262. PubMed PMID: 27771981.
26. Cassoli JS, Guest PC, Malchow B, Schmitt A, Falkai P, Martins-de-Souza D. Disturbed macro-connectivity in schizophrenia linked to oligodendrocyte dysfunction: from structural findings to molecules. NPJ Schizophr 2015;1:15034. doi: 10.1038/npjschz.2015.34. PubMed PMID: 27336040; PubMed Central PMCID: PMCPMC4849457.
27. O'Kusky J, Ye P. Neurodevelopmental effects of insulin-like growth factor signaling. Front Neuroendocrinol 2012;33(3):230-251. doi: 10.1016/j.yfrne.2012.06.002. PubMed PMID: 22710100; PubMed Central PMCID: PMC3677055.
28. Mitew S, Hay CM, Peckham H, Xiao J, Koenning M, Emery B. Mechanisms regulating the development of oligodendrocytes and central nervous system myelin. Neuroscience 2014;276:29-47. doi: 10.1016/j.neuroscience.2013.11.029. PubMed PMID: 24275321.
29. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol Psychiatry 2011;70(7):663-671. doi: 10.1016/j.biopsych.2011.04.013. PubMed PMID: 21641581; PubMed Central PMCID: PMCPMC4071300.

30. English JA, Pennington K, Dunn MJ, Cotter DR. The neuroproteomics of schizophrenia. Biol Psychiatry 2011;69(2):163-172. doi: 10.1016/j.biopsych.2010.06.031. PubMed PMID: 20887976.

31. Lai CY, Scarr E, Udawela M, Everall I, Chen WJ, Dean B. Biomarkers in schizophrenia: a focus on blood based diagnostics and theranostics. World J Psychiatry 2016;6(1):102-117. doi: 10.5498/wjp.v6.i1.102. PubMed PMID: 27014601; PubMed Central PMCID: PMCPMC4804259.

32. Zhang XY, Zhou DF, Zhang PY, Wu GY, Cao LY, Shen YC. Elevated interleukin-2, interleukin-6 and interleukin-8 serum levels in neuroleptic-free schizophrenia: association with psychopathology. Schizophr Res 2002;57(2-3):247-258. PubMed PMID: 12223256.

33. Capuron L, Miller AH. Immune system to brain signaling: neuropsychopharmacological implications. Pharmacol Ther 2011;130(2):226-238. doi: 10.1016/j.pharmthera.2011.01.014. PubMed PMID: 21334376; PubMed Central PMCID: PMC3072299.

34. Dunn AJ, Swiergiel AH, de Beaurepaire R. Cytokines as mediators of depression: what can we learn from animal studies? Neurosci Biobehav Rev 2005;29(4-5):891-909. doi: 10.1016/j.neubiorev.2005.03.023. PubMed PMID: 15885777.

35. Udina M, Hidalgo D, Navines R, Forns X, Sola R, Farre M, et al. Prophylactic antidepressant treatment of interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. J Clin Psychiatry 2014;75(10):e1113-1121. doi: 10.4088/JCP.13r08800. PubMed PMID: 25373120.

36. Brown AS, Hooton J, Schaefer CA, Zhang H, Petkova E, Babulas V, et al. Elevated maternal interleukin-8 levels and risk of schizophrenia in adult offspring. Am J Psychiatry 2004;161(5):889-895. doi: 10.1176/appi.ajp.161.5.889. PubMed PMID: 15121655.

37. Balan S, Iwayama Y, Toyota T, Toyoshima M, Maekawa M, Yoshikawa T. 22q11.2 deletion carriers and schizophrenia-associated novel variants. Br J Psychiatry 2014;204:398-399. doi: 10.1192/bjp.bp.113.138420. PubMed PMID: 24482440.

38. Reale M, Patruno A, De Lutiis MA, Pesce M, Felaco M, Di Giannantonio M, et al. Dysregulation of chemo-cytokine production in schizophrenic patients versus healthy controls. BMC Neurosci 2011;12:13. doi: 10.1186/1471-2202-12-13. PubMed PMID: 21266029; PubMed Central PMCID: PMCPMC3038147.

39. Dean B, Laws SM, Hone E, Taddei K, Scarr E, Thomas EA, et al. Increased levels of apolipoprotein E in the frontal cortex of subjects with schizophrenia. Biol Psychiatry 2003;54(6):616-622. PubMed PMID: 13129656.

40. Liu W, Breen G, Zhang J, Li S, Gu N, Feng G, et al. Association of APOE gene with schizophrenia in Chinese: a possible risk factor in times of malnutrition. Schizophr Res 2003;62(3):225-230. PubMed PMID: 12837518.

41. Gonzalez-Castro TB, Tovilla-Zarate CA, Hernandez-Diaz Y, Fresan A, Juarez-Rojop IE, Ble-Castillo JL, et al. No association between ApoE and schizophrenia: evidence of systematic review and updated meta-analysis. Schizophr Res 2015;169(1-3):355-368. doi: 10.1016/j.schres.2015.08.031. PubMed PMID: 26372448.

42. Saia-Cereda VM, Cassoli JS, Martins-de-Souza D, Nascimento JM. Psychiatric disorders biochemical pathways unraveled by human brain proteomics. Eur Arch Psychiatry Clin Neurosci 2017;267(1):3-17. doi: 10.1007/s00406-016-0709-2. PubMed PMID: 27377417.

43. Muffat J, Walker DW, Benzer S. Human ApoD, an apolipoprotein up-regulated in neurodegenerative diseases, extends lifespan and increases stress resistance in Drosophila. Proc Natl Acad Sci U S A 2008;105(19):7088-7093. doi: 10.1073/pnas.0800896105. PubMed PMID: 18458334; PubMed Central PMCID: PMCPMC2374552.

44. Thomas EA, Dean B, Pavey G, Sutcliffe JG. Increased CNS levels of apolipoprotein D in schizophrenic and bipolar subjects: implications for the pathophysiology of psychiatric disorders. Proc Natl Acad Sci U S A 2001;98(7):4066-4071. doi: 10.1073/pnas.071056198. PubMed PMID: 11274430; PubMed Central PMCID: PMCPMC31180.

45. Thomas EA, Dean B, Scarr E, Copolov D, Sutcliffe JG. Differences in neuroanatomical sites of apoD elevation discriminate between schizophrenia and bipolar disorder. Mol Psychiatry 2003;8(2):167-175. doi: 10.1038/sj.mp.4001223. PubMed PMID: 12610649.

46. Tonelli LH, Stiller J, Rujescu D, Giegling I, Schneider B, Maurer K, et al. Elevated cytokine expression in the orbitofrontal cortex of victims of suicide. Acta Psychiatr Scand 2008;117(3):198-206. doi: 10.1111/j.1600-0447.2007.01128.x. PubMed PMID: 18081924; PubMed Central PMCID: PMCPMC2612100.

47. Vouyiouklis DA, Brophy PJ. A novel gelsolin isoform expressed by oligodendrocytes in the central nervous system. J Neurochem 1997;69(3):995-1005. PubMed PMID: 9282921.

48. Furukawa K, Fu W, Li Y, Witke W, Kwiatkowski DJ, Mattson MP. The actin-severing protein gelsolin modulates calcium channel and NMDA receptor activities and vulnerability to excitotoxicity in hippocampal neurons. J Neurosci 1997;17(21):8178-8186. PubMed PMID: 9334393.

49. Le-Niculescu H, Kurian SM, Yehyawi N, Dike C, Patel SD, Edenberg HJ, et al. Identifying blood biomarkers for mood disorders using convergent functional genomics. Mol Psychiatry 2009;14(2):156-174. doi: 10.1038/mp.2008.11. PubMed PMID: 18301394.

50. Carboni L, Domenici E. Proteome effects of antipsychotic drugs: learning from preclinical models. Proteomics Clin Appl 2016;10(4):430-441. doi: 10.1002/prca.201500087. PubMed PMID: 26548651.

51. Schubert KO, Focking M, Wynne K, Cotter DR. Proteome and pathway effects of chronic haloperidol treatment in mouse hippocampus. Proteomics 2016;16(3):532-538. doi: 10.1002/pmic.201500242. PubMed PMID: 26607048.

52. Frye MA, Nassan M, Jenkins GD, Kung S, Veldic M, Palmer BA, et al. Feasibility of investigating differential proteomic expression in depression: implications for biomarker development in mood disorders. Transl Psychiatry 2015;5:e689. doi: 10.1038/tp.2015.185. PubMed PMID: 26645624; PubMed Central PMCID: PMCPMC5068585.