Phospholipase C beta2 (PLCB2) is differentially expressed in the dorsolateral prefrontal cortex of patients with psychotic disorders.

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We mined two microarray datasets (published or public) (1, 2) to identify the most significant changes in gene expression in the brains of patients with psychotic disorders. We found that isoform 2 of phospholipase C beta (PLCB2) was among the genes most differentially expressed in the dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia and psychotic bipolar disorder (1). In a separate dataset (2), PLCB2 was again among the genes whose expression changed most significantly when comparing parvalbumin-positive layer 3 neurons from the DLPFC between patients with schizophrenia and schizoaffective disorder and control subjects. Multiple studies have documented dysregulated PLCB1 expression in the brains of patients with schizophrenia, including in the dorsolateral prefrontal cortex (3-6). These data suggest that PLCB2 expression may also be perturbed in the brains of patients with psychotic disorders.

Keywords: Schizophrenia, psychotic disorders, psychosis, systems biology of schizophrenia.

Schizophrenia is a serious psychiatric disorder characterized by psychosis, or a disconnection with reality and inability to reality test (7). Patients with schizophrenia, schizoaffective disorder, and psychotic bipolar disorder (bipolar disorder with psychotic features) can experience hallucinations, in the form of auditory hallucinations or visual hallucinations - seeing objects or hearing sounds that others do not (8). Understanding how gene expression is different in the affected organ of diseased patients as compared to non-affected subjects can reveal insights into molecular mechanisms of disease initiation, disease maintenance or disease progression. In this study compared global gene expression in the dorsolateral prefrontal cortex between patients with psychotic disorders and non-affected control subjects to identify differentially expressed genes in the brains of patients with psychotic disorders, including schizophrenia, schizoaffective disorder, and psychotic bipolar disorder, using published (1) or public (2) microarray data. In both datasets, isoform 2 of phospholipase C-beta (PLCB2) was identified as among the genes whose expression changed most significantly when comparing the DLPFC of patients with psychotic disorders with that of control subjects. Differential expression of PLCB1 has been documented in the left prefrontal cortex (4), superior temporal cortex (4), temporal lobe (5), and dorsolateral prefrontal cortex of patients with schizophrenia (6). These data changes in gene expression of beta phospholipase C isoforms may be a common feature of patients with psychotic disorders.

Methods

Differential Gene Expression Analyses

We utilized datasets GSE120340 (1) and GSE93577 (2) for this differential gene expression analysis in conjunction with GEO2R. GSE120340 (1) was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with post-mortem tissues from Brodmann's area 46 from control subjects (n=10 samples utilized in this analysis) and from

patients with psychotic disorders (schizophrenia and psychotic bipolar disorder) (n=16 samples utilized in this analysis). GSE93577 (2) was generated using Affymetrix Human Genome U219 Array technology from layer 3 (L3) parvalbumin (PV) positive neurons of the dorsolateral prefrontal cortex, and we used n=34 control subjects each with paired replicates (n=68 total samples) and n=35 patients with schizophrenia or schizoaffective disorder with paired replicates (n=70 total samples) from this dataset. These patient diagnoses in this dataset included schizoaffective disorder, paranoid schizophrenia, undifferentiated schizophrenia, chronic paranoid schizophrenia, chronic disorganized schizophrenia, chronic undifferentiated schizophrenia, and disorganized schizophrenia. The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used.

Statistical Analyses

A statistical test was performed to evaluate whether PLCB2 expression was significantly different between the dorsolateral prefrontal cortex of control subjects and the dorsolateral prefrontal cortex of patients with schizophrenia or psychotic bipolar disorder using a two-tailed, unpaired t-test with Welch's correction. A statistical test was performed to evaluate whether PLCB2 expression was significantly different between the PV+ L3 neurons of the DLPFC of control subjects and the PV+ L3 neurons of the DLPFC of patients with schizophrenia or schizoaffective disorder using a two-tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published microarray data to compare global transcription patients with psychotic disorders and control, non-affected subjects in the dorsolateral prefrontal cortex and in a specific cell-type of the DLPFC, parvalbumin-positive (PV+) layer 3 (L3) neurons.

PLCB2 is differentially expressed in the dorsolateral prefrontal cortex of patients with schizophrenia and psychotic bipolar disorder

We identified PLCB2 as among the genes whose expression was most significantly different in the dorsolateral prefrontal cortex of patients with schizophrenia and patients with psychotic bipolar disorder when compared to the DLPFC of control, non-affected subjects. When ranking all of the genes whose expression was measured by microarray based on change in expression between diseased and control subjects, PLCB2 ranked 129 out of 19070 transcripts (Table 1). Differential expression of PLCB2 in the DLPFC of patients with schizophrenia and patients with psychotic bipolar disorder was statistically significant (Table 1; $p=0.0013386$).

PLCB2 is differentially expressed in the PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia and psychotic bipolar disorder

Analysis of a separate dataset revealed that PLCB2 was again among the genes whose expression was most significantly different when comparing a region of the brain between patients with psychotic disorders - in this case, patients with schizophrenia and patients with schizoaffective disorder - and control, non-affected subjects. When ranking all of the genes whose expression was measured by microarray based on change in expression between diseased and control subjects, PLCB2 ranked 102 out of 49386 transcripts (Table 2). Differential expression of PLCB2 in the DLPFC of patients with schizophrenia and patients with schizoaffective disorder was statistically significant (Table 2; $p=6.09E-05$).

PLCB2 is expressed at significantly lower levels in the dorsolateral prefrontal cortex of patients with schizophrenia and psychotic bipolar disorder

Next, we obtained exact mRNA expression levels for PLCB2 from each patient tissue sample of the DLPFC, from patients with schizophrenia and patients with psychotic bipolar disorder and from control, non-affected subjects. PLCB2 was expressed at significantly lower levels in the DLPFC of patients with schizophrenia and patients with psychotic bipolar disorder when compared to that of control subjects (Figure 1). The difference in PLCB2 expression in the DLPFC of patients with schizophrenia or psychotic bipolar disorder and the DLPFC of control subjects was statistically significant (Figure 1; $p=0.0012$). We calculated a fold change of 0.9740 ± 0.0167 in PLCB2 expression when comparing the DLPFC of diseased patients to control subjects (Table 1).

PLCB2 is expressed at significantly higher levels in the PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia and psychotic bipolar disorder

We also obtained exact mRNA expression levels for PLCB2 from each patient tissue sample, from patients with schizophrenia and patients with schizoaffective disorder and from control, non-affected subjects, here in the PV+ L3 neurons of the DLPFC. PLCB2 was expressed at significantly higher levels in PV+ L3 neurons of the DLPFC in patients with schizophrenia and patients with schizoaffective disorder when compared to that of control subjects (Figure 2). The difference in PLCB2 expression in the PV+ L3 neurons of the DLPFC in patients with schizophrenia or schizoaffective disorder and that of control subjects was statistically significant (Figure 2; $p<0.0001$). We calculated a fold change of 1.1030 ± 0.1739 in PLCB2 expression when comparing the PV+ L3 neurons of the DLPFC of diseased patients to that of control subjects (Table 2).

Thus, we found that phospholipase C beta 2, PLCB2, was among the genes whose expression changed most significantly, globally, in the dorsolateral prefrontal cortex of patients with psychotic disorders when compared to that of control subjects. PLCB2 was expressed at

significantly higher levels in the parvalbumin-positive layer 3 neurons of the DLPFC of patients with schizophrenia and patients with schizoaffective disorder, while PLCB2 was expressed at significantly lower levels in the total DLPFC of patients with schizophrenia and patients with psychotic bipolar disorder.

Discussion

We used published (1) and public (2) microarray data to identify, in an unbiased fashion and at the systems-level, the most significant changes in gene expression in the brains of patients with a spectrum of psychotic disorders, including schizophrenia, schizoaffective disorder, and psychotic bipolar disorder. We found that changes in expression of isoform 2 of phospholipase C beta, PLCB2, were among the most significant changes in gene expression when comparing the dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia, psychotic bipolar disorder, and schizoaffective disorder with that of control subjects.

Three studies have identified changes in expression of isoform 1 of phospholipase beta C, PLCB1, in the brains of patients with schizophrenia, in the left prefrontal cortex (4), superior temporal cortex (4), temporal lobe (5), and dorsolateral prefrontal cortex of patients with schizophrenia (6). Together with the differential expression of PLCB2 identified here, these data suggest that changes in expression of phospholipase C beta may potentially be a common feature of patients with schizophrenia.

A genome-wide association study identifying single-nucleotide polymorphisms (SNPs) in patients with schizophrenia that correlated with an increased risk of development of the disease by comparing genetic information between diseased patients and control subjects found that among the 108 genetic loci that contained sequences with significant association with the risk for development of schizophrenia included one region on chromosome 15 that was proximal to

PLCB2 (9). Genome-wide expression data coupled with paired SNP data from brain tissue of deceased patients with psychotic disorders can reveal whether this region functions in any manner as a enhancer sequence for PLCB2 in the brain, through comparison of PLCB2 expression based on genotype (9, 10).

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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
129	5330_at	0.0013386	-3.56851724	-0.95951	0.9740 ± 0.0167	PLCB2	phospholipase C, beta 2

Table 1: PLCB2 is differentially expressed in the dorsolateral prefrontal cortex of patients with schizophrenia and psychotic bipolar disorder.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of PLCB2 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Rank	probe / transcript ID	p-value	t	B	Fold change	Gene	Gene name
102	11722131_a_at / NM_004573	6.09E-05	4.14	1.593177	1.1030 ± 0.1739	PLCB2	phospholipase C, beta 2

Table 2: PLCB2 is differentially expressed in the PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia and psychotic bipolar disorder

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of PLCB2 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: PLCB2 is expressed at significantly lower levels in the dorsolateral prefrontal cortex of patients with schizophrenia and psychotic bipolar disorder

The mRNA expression of PLCB2 in the dorsolateral prefrontal cortex of control subjects (left) and in the dorsolateral prefrontal cortex of patients with schizophrenia or psychotic bipolar disorder (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in PLCB2 expression between the dorsolateral prefrontal cortex of control subjects and patients with schizophrenia or psychotic bipolar disorder, a p-value, listed above.

Figure 2: PLCB2 is expressed at significantly higher levels in the PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia and psychotic bipolar disorder

The mRNA expression of PLCB2 in the PV+ L3 neurons of the dorsolateral prefrontal cortex of control subjects (left) and in the PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia or schizoaffective disorder (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in PLCB2 expression between the PV+ L3 neurons of the dorsolateral prefrontal cortex of control subjects and patients with schizophrenia or schizoaffective disorder, a p-value, listed above.