1 Pyridoxal kinase, encoded by the PDXK gene, is differentially expressed in the brains of patients
2 with schizophrenia.

3 Shahan Mamoor, MS¹

4 ¹shahanmamoor@gmail.com

5 East Islip, NY USA

6 Visual and auditory hallucinations are a cardinal feature of psychotic disorders¹. We mined
7 published and public microarray datasets^{2,3} to discover differentially expressed genes in
8 schizophrenia and schizoaffective disorder. We found significant differential expression of the
9 gene encoding pyridoxal kinase, PDXK in neurons of the dorsolateral pre-frontal cortex from
10 patients with schizophrenia and schizoaffective disorder.

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27 Keywords: schizophrenia, schizoaffective disorder, psychotic disorders, psychosis, systems
28 biology of schizophrenia, PDXK.

Psychiatric diseases schizophrenia and schizoaffective disorder have in common the existence of psychotic symptoms^{1,4,5}, like visual and auditory hallucinations, that can plague affected patients. Understanding the transcriptional profiles of cells and tissues from the brain of patients diagnosed with schizophrenia or schizoaffective disorder has the potential to provide insights into molecular mechanisms underlying disease initiation, progression and associated symptoms like psychosis. We mined published and public microarray data from layer 3 and layer 5 pyramidal neurons of the dorsolateral pre-frontal cortex from patients with schizophrenia and schizoaffective disorder and from the layer 3 parvalbumin-positive neurons of the dorsolateral prefrontal cortex of patients with schizoaffective disorder to discover genes whose expression was most significantly different in the brains of patients with psychosis at the systems-level. PDXK was among the most differentially expressed genes in patients with psychotic disorders in two cell types of the brain.

Methods

We used microarray datasets GSE93577² and GSE87610³ for this differential gene expression analysis in conjunction with GEO2R. GSE93577 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

1 included schizoaffective disorder, paranoid schizophrenia, undifferentiated schizophrenia,
2 chronic paranoid schizophrenia, chronic disorganized schizophrenia, chronic undifferentiated
3 schizophrenia, and disorganized schizophrenia. GSE87610 was generated using Affymetrix
4 Human Genome U219 with $n=72$ for control subjects and $n=65$ for patients with schizophrenia,
5 using L3 and L5 pyramidal neurons from the dorsolateral prefrontal cortex.
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8 The Benjamini and Hochberg method of p-value adjustment was used for ranking of
9 differential expression but raw p-values were used for assessment of statistical significance of
10 global differential expression. Log-transformation of data was auto-detected, and the NCBI
11 generated category of platform annotation was used. A statistical test was performed to evaluate
12 whether PDXK expression was significantly different when comparing neurons of the DLPFC
13 from control subjects and from patients with schizophrenia and schizoaffective disorder using a
14 two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses
15 (Version 8.4.0)(455).
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20 **Results**

21 We mined published and public microarray data^{2,3} to identify in an unbiased fashion and
22 at the global level genes most differentially expressed in the brains of patients with schizophrenia
23 and schizoaffective disorder.
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26 PDXK is differentially expressed in layer 3 parvalbumin-positive neurons of the dorsolateral pre-
27 frontal cortex in patients with schizophrenia and schizoaffective disorder.
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1 We observed differential expression of the gene encoding the pyridoxal kinase, PDXK, in
2 the layer 3 (L3) PV+ neurons of the dorsolateral prefrontal cortex (DLPFC) in patients with
3 schizophrenia and schizoaffective disorder². When sorting each of the transcripts whose
4 expression was measured by microarray based on significance of change in expression between
5 diseased and control patients, in L3 PV+ DLPFC neurons from patients with schizophrenia,
6 PDXK ranked 197 out of 49386 total transcripts, equating to 99.6% differential expression.
7 Differential expression of PDXK was statistically significant (Table 1; $p=1.36E-04$).
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12 PDXK is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal
13 cortex in patients with schizophrenia.
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15 We also observed differential expression of PDXK in the layer 3 and layer 5 (L5)
16 pyramidal neurons of the DLPFC in patients with schizophrenia. When sorting each of the
17 transcripts whose expression was measured by microarray based on significance of change in
18 expression between patients and control subjects, in L3 and L5 pyramidal neurons of the DLPFC
19 from patients with schizophrenia, PDXK ranked 4271 out of 49386 total transcripts (Table 2),
20 equating to 91.4% differential expression. Differential expression of PDXK in L3 and L5
21 pyramidal neurons of the DLPFC in patients with schizophrenia was statistically significant
22 (Table 2; $p=2.24E-02$).
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PDXK expression is significantly higher in layer 3 parvalbumin-positive neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.

We obtained exact mRNA expression levels for the differentially expressed PDXK transcript, from control subjects and from patients with schizophrenia and schizoaffective disorder, to directly compare PDXK expression levels in L3 PV+ DLPFC neurons. We also performed a statistical test to determine if this difference in PDXK expression was statistically significant. PDXK was expressed at higher levels in the L3 PV+ DLPFC neurons in patients with schizophrenia and schizoaffective disorder, and this difference was statistically significant (Figure 1; $p < 0.0001$). We calculated a mean fold change of 1.2 ± 0.3 in PDXK expression when comparing L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder to that of control subjects (Table 1).

Thus, we found significant differential expression of PDXK in two types of neuronal cell types in patients with schizophrenia; PDXK expression was significantly higher in L3 PV+ neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder.

Discussion

In the vitamin B6 salvage pathway, conversion of inactive forms of vitamin B6, including pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM) to the active form of vitamin B6, pyridoxal 5' phosphate (PLP), is catalyzed by two enzymes^{5,6}. One of these enzymes is pyridoxal kinase, encoded by the PDXK gene, which we have identified here as among the most

1 significant transcriptional differences in the brains of patients with schizophrenia; the other is
2 PNPO, or pyridoxine-5'-phosphate oxidase⁵. Pyridoxal kinase is expressed at relatively high
3 levels in the brain as evidenced by studies demonstrating its biochemical purification from the
4 bovine, sheep and human brain^{7,8,9}.

6 Multiple studies describe potential functions for B6 vitamers in the brain or in diseases of
7 the brain. Administration of a single high dose of pyridoxal to mice results in death due to
8 convulsions, and further investigation suggested that the cause of these convulsions was due to
9 decreased γ -aminobutyric acid (GABA) levels as a result of glutamate decarboxylase (GAD)
10 inhibition subsequent to decreased synthesis of pyridoxal phosphate (PLP)¹⁰. Importantly, in a
11 patient with schizophrenia who developed tardive dyskinesia (TD) as a result of treatment with
12 anti-psychotic medications, administration of pyridoxine resulted in amelioration of psychotic
13 symptoms as well as improvement in TD¹¹. In accordance, two studies describe efficacy, in
14 randomized clinical trials, of vitamin B6 for the treatment of tardive dyskinesia¹²⁻¹³. Moreover, a
15 study measuring plasma levels of pyridoxine in patients with schizophrenia demonstrated that in
16 the patients examined, male patients with schizophrenia or schizoaffective disorder who suffered
17 from TD had lower levels of pyridoxal 5' phosphate (PLP) than did male patients who suffered
18 from schizophrenia or schizoaffective disorder but without TD¹⁴. Finally, and perhaps most
19 significantly, in the Japanese population, single-nucleotide polymorphisms in PNPO, the second
20 enzyme involved in salvage of vitamin B6 (along with PDXK/pyridoxal kinase) were
21 significantly associated with risk of developing schizophrenia¹⁵.

1 We found that pyridoxal kinase, encoded by PDXK, was among the genes most
2 differentially expressed, transcriptome-wide, in two neuronal cell types of the dorsolateral
3 prefrontal cortex in patients with schizophrenia: the layer 3 PV+ neurons and the layer 3 and
4 layer 5 pyramidal neurons. Differential expression of PDXK should be validated in separate and
5 larger schizophrenia patient datasets. PDXK may be of relevance to the cell biology of the brain
6 in schizophrenia and associated psychoses: visual and auditory hallucinations.
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Rank	ID	p-value	t	B	FC	Gene
197	11759721_a_at	1.36E-04	3.92	0.887088	1.2 ± 0.3	PDXK

Table 1: PDXK is differentially expressed in the layer 3 PV+ neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.

The rank of differential expression, the probe 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 PDXK in L3 PV+ DLPFC neurons of patients with schizophrenia and schizoaffective disorder when compared to deep pyramidal neurons from control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
4271	11718138_at	2.24E-02	-2.3089481	-3.52352	PDXK

Table 2: PDXK is differentially expressed in the layer 3 and layer 5 pyramidal neurons of the dorsolateral pre-frontal cortex of patients with schizophrenia.

The rank of differential expression, the probe 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 gene and gene name are listed in this chart.

Figure 1: PDXK is expressed at significantly higher levels in the layer 3 PV+ neurons of the dorsolateral pre-frontal cortex of patients with schizophrenia and schizoaffective disorder.

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