Cdx2 is differentially expressed in the brains of patients with schizophrenia.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Visual and auditory hallucinations are a cardinal feature of psychotic disorders¹. We mined published and public microarray datasets^{2,3} to discover differentially expressed genes in schizophrenia and schizoaffective disorder. We found significant differential expression of Cdx2 in neurons of the dorsolateral pre-frontal cortex from patients with schizophrenia and schizoaffective disorder.

Keywords: schizophrenia, schizoaffective disorder, psychotic disorders, psychosis, systems biology of schizophrenia, Cdx2.

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^{2,3} 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. *Cdx2* was among the genes most differentially expressed in two cell types of the brain in patients with psychotic disorders.

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 Cdx2 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 Cdx2 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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We observed differential expression of Cdx2 in the layer 3 (L3) PV+ neurons of the dorsolateral prefrontal cortex (DLPFC) in patients with schizophrenia and schizoaffective disorder². When sorting each of the transcripts whose expression was measured by microarray based on significance of change in expression between diseased and control patients, in L3 PV+ DLPFC neurons from patients with schizophrenia, Cdx2 ranked 158 out of 49386 total transcripts. Differential expression of Cdx2 was statistically significant (Table 1; $p=1.04E-04$).

Cdx2 is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia.

We also observed differential expression of Cdx2 in the layer 3 and layer 5 (L5) pyramidal neurons of the DLPFC in patients with schizophrenia. When sorting each of the transcripts whose expression was measured by microarray based on significance of change in expression between patients and control subjects, in L3 and L5 pyramidal neurons of the DLPFC from patients with schizophrenia, Cdx2 ranked 6095 out of 49386 total transcripts (Table 3), equating to differential expression greater than 87.7% of all transcripts whose expression was detected and measured. Differential expression of Cdx2 in L3 and L5 pyramidal neurons of the DLPFC in patients with schizophrenia was statistically significant (Table 2; $p=4.03E-02$).

Cdx2 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 Cdx2

transcript, from control subjects and from patients with schizophrenia and schizoaffective disorder, to directly compare Cdx2 expression levels in L3 PV+ DLPFC neurons. We also performed a statistical test to determine if this difference in Cdx2 expression was statistically significant. Cdx2 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 1.063 ± 0.090 in Cdx2 expression when comparing L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder to that of control subjects (Table 1).

Thus, we found significantly differential expression of Cdx2 in two types of neuronal cell types in patients with schizophrenia; Cdx2 expression was significantly higher in L3 PV+ neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder.

Discussion

The homeobox gene Cdx2 is one of three mammalian homologues of *Drosophila caudal*⁶. Cdx2 null homozygote mice die between day 3.5 and 5.5 post-coitum, while 90% of Cdx2 heterozygotes develop intestinal adenomatous polyps, some of which contain frank metaplasia⁷. Changes in the vertebrae affecting cervical and thoracic vertebral elements indicated homeosis, and Cdx2 heterozygotes also possessed tail defects, stunting of growth and malformations of the ribs⁷. Cdx2 is among the earliest trophectoderm-specific transcription

factors whose expression is induced in the 8-cell embryo⁸, and in embryonic stem (ES) cells, expression of Cdx2 results in morphological differentiation to trophectoderm (TE). Moreover, cloned blastocysts resulting from nuclear transfer-derived mouse blastocysts from donor fibroblasts depleted of Cdx2 by short hairpin RNA were lacking in trophoblast and were defective in their ability to implant into the uterus but were competent in generation of pluripotent embryonic stem cells in culture⁹. However, Cdx2 was dispensable for TE differentiation of ES cells induced by Oct3/4 repression, but required for ES cell self-renewal¹⁰. Together, the data indicate a role for the transcription factor Cdx2 in specification of the trophectoderm, the first cell type which is specified during mammalian development and from which the placenta is derived.

In the mouse embryo, Cdx2 is first expressed at embryonic day 3.5 (E3.5), absent from the inner cell mass but expressed in the TE¹¹. Starting at E8.5, Cdx2 expression can be observed in the neural tube, the neural plate, and the notochord caudal to somite 6, and continues in the posterior neural plate from E12.5¹¹. Cdx2 is important for elongation of the posterior axis, with axial truncation observed in Cdx2 conditional null mutant embryos; at E8.5 shortening of the anteroposterior axis is observed¹². Cdx2 can drive the expression of Wnt3a, brachyury (T), and Cyp26A1¹². The neural tube of Cdx1/2 double knockouts mouse embryos fails to close, a neural tube defect known as craniorachischisis marked by a failure of early neurulation¹³. Ptk7, a gene important for neural tube closure, was found to be a Cdx target gene. In embryos deficient for β -catenin in the dorsal neural folds, expression of Cdx2 is repressed in the dorsal posterior neuropore (PNP), and Pax3 is required but not sufficient for expression of Cdx2 in the PNP¹⁴.

1 We found, by mining independently published microarray data that the homeobox gene
2 Cdx2, a transcription factor important for the development of neural tube, was among the genes
3 most differentially expressed in two neuronal cell types from patients with schizophrenia. Cdx2
4 may be relevant to the biology of psychotic disorders including schizophrenia and
5 schizoaffective disorder.
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Rank	ID	p-value	t	B	FC	Gene
158	11731754_at	1.04E-04	4.00	1.121768	1.063 ± 0.090	CDX2

Table 1: Cdx2 is differentially expressed in the layer 3 PV+ neurons of the dorsolateral prefrontal 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 Cdx2 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
6095	11731754_at	4.03E-02	2.0698867	-4.00082	CDX2

Table 2: Cdx2 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: Cdx2 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 Cdx2 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 Cdx2 expression between control L3 PV+ DLPFC neurons and L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder, a *p*-value, listed above.