ERp29 is differentially expressed in the brains of patients with schizophrenia.

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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 the gene encoding ERp29 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, ERp29.

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. A 29 kilodalton protein of the endoplasmic reticulum, ERp29 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 ERp29 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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19 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 ERp29 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 29 kilodalton endoplasmic reticulum protein
2 ERp29, in the layer 3 (L3) PV+ neurons of the dorsolateral prefrontal cortex (DLPFC) in patients
3 with 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 ERp29 ranked 9 out of 49386 total transcripts. Differential expression of ERp29 was statistically
7 significant (Table 1; $p=9.17E-07$).

8 ERp29 is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal
9 cortex in patients with schizophrenia.

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12 We also observed differential expression of ERp29 in the layer 3 and layer 5 (L5)
13 pyramidal neurons of the DLPFC in patients with schizophrenia. When sorting each of the
14 transcripts whose expression was measured by microarray based on significance of change in
15 expression between patients and control subjects, in L3 and L5 pyramidal neurons of the DLPFC
16 from patients with schizophrenia, ERp29 ranked 6377 out of 49386 total transcripts (Table 3),
17 equating to differential expression greater than 87.1% of all transcripts whose expression was
18 detected and measured. Differential expression of ERp29 in L3 and L5 pyramidal neurons of the
19 DLPFC in patients with schizophrenia was statistically significant (Table 2; $p=4.38E-02$).

20 ERp29 expression is significantly lower in layer 3 parvalbumin-positive neurons of the
21 dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.

We obtained exact mRNA expression levels for the differentially expressed ERp29 transcript, from control subjects and from patients with schizophrenia and schizoaffective disorder, to directly compare ERp29 expression levels in L3 PV+ DLPFC neurons. We also performed a statistical test to determine if this difference in ERp29 expression was statistically significant. ERp29 was expressed at lower 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 0.930 ± 0.103 in ERp29 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 ERp29 in two types of neuronal cell types in patients with schizophrenia; ERp29 expression was significantly lower in L3 PV+ neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder.

Discussion

Complementary DNA (cDNA) for ERp29 was cloned from rat enamel cells and characterized as a protein 259 amino acids in length and containing a variant of the KDEL endoplasmic reticulum (ER) retention signal, KEEL⁶. ERp29 possesses some level of homology with the protein disulfide isomerase (PDI) and is expressed ubiquitously in the rat⁶. Whether ERp29 expression could be induced by exposure to agents causing ER stress responses such as

1 tunicamycin, like is characteristic of reticuloplasmins, is debated^{6,7}. ERp29 constitutes a
2 significant portion of microsomal protein, representing 0.1% of rat microsomal proteins in the
3 liver. One report indicated that ERp29 could associate with the molecular chaperone BiP⁷, while
4 a separate study reported that size-exclusion chromatography indicated that ERp29 was not in
5 stable association with any other proteins⁸. In dental enamel cells, the expression of ERp29 was
6 down-regulated during mineralization, and because expression of ERp29 was highest in
7 secretory tissues like the testis, lung and spleen, and because the expression of ERp29 in rat
8 dental tissues was highest during the secretory phase as opposed to the differentiation and
9 maturation phases, it was suggested that ERp29 was associated with secretory protein synthesis⁸.
10 At the three-dimensional structural level, ERp29 shares similarity with Wind, an escort
11 chaperone protein with an amino-terminal thioredoxin domain which binds a client protein
12 named Pipe⁹, and indeed a report presenting the crystal structure of human ERp29 described
13 ERp29 as a homolog of *Drosophila* Wind¹⁰. The amino-terminus of ERp29 contains a
14 thioredoxin-like domain and the carboxy-terminus is likely responsible for solubilization of the
15 protein⁹. In FRTL-5 thyroid culture cells, expression of ERp29 can be induced following
16 stimulation with the thyroid-stimulating hormone (TSH)¹¹ and it has been reported that ERp29
17 can associate with thyroglobulin and is involved in folding and maturation of thyroglobulin in
18 the ER¹². ERp29 can associate with Connexin43 (Cx43), function in the transport of
19 Connexin43 to the plasma membrane, and regulate oligomerization of Cx43 in the Golgi
20 apparatus by stabilizing the monomeric form of Cx43 in the ER¹³. Since Cx43 functions at gap
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1 junctions, depletion of ERp29 affects intracellular communication at gap junctions¹³. ERp29 has
2 also been reported to interact with TNFAIP2, also known as MSec, a component of tunneling
3 nanotubes (TNT), another structure that can mediate intracellular communication¹⁴.

5 In the brain, ERp29 has ubiquitous expression in both neuronal and non-neuronal cell
6 types¹⁵. Two reports describe a role for ERp29 in protection of neurons from apoptotic cell
7 death^{16,17}; after spinal cord transection, expression of ERp29 was decreased in the motor cortex 3
8 days post-transection, and it was suggested that ERp29 could support neurite outgrowth and
9 regeneration of cortical neurons through pathways involving up-regulation of Erk1 and PI3-
10 kinase and down-regulation of Caspase-3¹⁶. Thus, ERp29 is a protein of the ER which may be
11 involved in the ER stress response, may have functions in the secretory pathway, in stabilization
12 and folding of some proteins, and through interactions with other proteins, at intracellular
13 junctions.

17 We found differential expression of ERp29 in neurons from patients with schizophrenia
18 in two published or public microarray datasets. Differential expression of ERp29 should be
19 validated in separate and larger schizophrenia patient datasets. ERp29 may be of relevance to
20 the biology of schizophrenia.
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Rank	ID	p-value	t	B	FC	Gene
9	11742681_s_at	9.17E-07	-5.14	5.307568	0.930 ± 0.103	ERP29

Table 1: ERp29 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 Erp29 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
6377	11742681_s_at	4.38E-02	-2.034725	-4.06693	ERP29

Table 2: ERp29 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: ERp29 is expressed at significantly lower levels in the layer 3 PV+ neurons of the dorsolateral pre-frontal cortex of patients with schizophrenia and schizoaffective disorder.

The mRNA expression of ERp29 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 ERp29 expression between control L3 PV+ DLPFC neurons and L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder, a *p*-value, listed above.