1 The regulator of G-protein signaling 7 binding protein, RGS7BP, is differentially expressed in
2 the brains of patients with schizophrenia.

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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 the regulator of G-protein signaling 7 binding protein, RGS7BP, in neurons of the
10 dorsolateral pre-frontal cortex from patients with schizophrenia and schizoaffective disorder.

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27 Keywords: schizophrenia, schizoaffective disorder, psychotic disorders, psychosis, systems
28 biology of schizophrenia, RGS7BP, regulator of G-protein signaling 7 binding protein.

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. RGS7BP 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 RGS7BP 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 RGS7BP is differentially expressed in layer 3 parvalbumin-positive neurons of the dorsolateral
27 pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.
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We observed differential expression of RGS7BP 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, RGS7BP ranked 10 out of 49386 total transcripts. Differential expression of RGS7BP was statistically significant (Table 1; $p=1.38E-06$).

RGS7BP is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral prefrontal cortex in patients with schizophrenia.

We also observed differential expression of RGS7BP 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, RGS7BP ranked 3209 out of 49386 total transcripts (Table 3). Differential expression of RGS7BP in L3 and L5 pyramidal neurons of the DLPFC in patients with schizophrenia was statistically significant (Table 2; $p=1.37E-02$).

RGS7BP expression is significantly lower 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 RGS7BP transcript, from control subjects and from patients with schizophrenia and schizoaffective disorder, to directly compare RGS7BP expression levels in L3 PV+ DLPFC neurons. We also performed a statistical test to determine if this difference in RGS7BP expression was statistically significant. RGS7BP 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 0.9 ± 0.1 in RGS7BP 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 RGS7BP in two types of neuronal cell types in patients with schizophrenia; RGS7BP expression was significantly lower in L3 PV+ neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder.

Discussion

Regulators of G-protein signaling, or RGSs, consist of more than 20 family members, with the ability to modulate G-protein signaling in neuronal cells; the R7 family of RGSs, to which RGS7BP belongs, is expressed in both the central and peripheral nervous system⁶⁻⁹. Published information describing roles and functions of RGS7BP is somewhat limited. RGS7BP, also known as R7BP, shuttles between the nucleus and cytoplasm and is subject to

1 palmitoylation at its carboxy-terminus¹⁰. Palmitoylation modulates the ability of RGS7B to
2 inhibit inward rectifying potassium channel activation mediated by G-protein coupled
3 receptors¹⁰. RGS7, G α_{12} , and R7BP can form complexes in neuronal cells, and R7BP is
4 necessary to observe interactions between G α_{13} and R7-RGS proteins. Neurite retraction
5 induced by lysophosphatidic acid receptors dependent on G $\alpha_{12/13}$ was enhanced by expression of
6 RGS7BP, suggesting that RGS7BP can modulate neuritis morphogenesis¹¹. RGS7BP is a
7 differentially expressed gene in the caudate nucleus of patients with obsessive-compulsive
8 disorder¹². A haplotype containing 3 rare alleles of RGS7BP correlates with the risk of
9 developing aspirin-exacerbated respiratory disease¹³. Importantly, array comparative genomic
10 hybridization (aCGH) analysis revealed that three patients with juvenile myoclonic epilepsy
11 contained rare deletions at chromosome 5q12.3 partially encompassing the RGS7BP locus¹⁴.
12 Thus, RGS7BP is important for G-protein signaling in neurons, is post-translationally modified
13 by palmitoylation, and its expression or locus is implicated in a number of human diseases,
14 including diseases of the brain.

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16 We found differential expression of RGS7BP in neurons from patients with schizophrenia
17 in two published or public microarray datasets. Differential expression of RGS7BP should be
18 validated in separate and larger schizophrenia patient datasets. RGS7BP may be of relevance to
19 the biology of schizophrenia.

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Rank	ID	p-value	t	B	FC	Gene
10	11730316_a_at	1.38E-06	-5.05	4.944251	0.9 ± 0.1	RGS7BP

Table 1: RGS7BP 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 RGS7BP 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
3209	11730318_a_at	1.37E-02	2.4968277	-3.11481	RGS7BP

Table 2: RGS7BP 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: RGS7BP 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 RGS7BP 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 RGS7BP expression between control L3 PV+ DLPFC neurons and L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder, a *p*-value, listed above.