GABARAPL1 is differentially expressed in the brains of patients with psychotic disorders.

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We used public and published microarray data^{1,2} to identify the most significant gene expression changes in the brains of patients with psychotic disorders. We identified the γ -aminobutyric acid receptor-associated protein-like 1 GABARAPL1³ as differentially expressed in the dorsolateral prefrontal cortex of patients with schizophrenia as well as in the parvalbumin-positive layer 3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder. The brain tissues of patients with psychotic disorders expressed significantly lower levels of GABARAPL1 than that of non-affected control subjects. GABARAPL1 may be relevant to the biology of schizophrenia and related psychotic disorders.

Keywords: schizophrenia, psychotic disorders, systems biology of schizophrenia, psychosis, GABARAPL1.

Psychotic disorders including schizophrenia and schizoaffective disorder are characterized by the presence of psychosis, or an inability to engage in reality testing^{4,5}. Patients with psychotic disorders can experience positive as well as negative symptoms⁶. Positive symptoms include hallucinations, visual and auditory, as well as delusions, or fixed false beliefs⁶. Negative symptoms include anhedonia, or an inability to experience pleasure, as well as avolition, or loss of motivation⁶. To understand how specific regions of the brain differ most significantly in terms of gene expression in patients with psychotic disorders, we used published and public microarray data to compare global gene expression profiles of brain tissues from patients with psychotic disorders with that of non-affected, control subjects^{1,2}. We found that the gene encoding γ -aminobutyric acid receptor-associated protein-like 1, GABARAPL1, was among the genes whose expression changed most significantly in patients with schizophrenia in the dorsolateral prefrontal cortex, as well as in the layer 3 parvalbumin-positive neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder. GABARAPL1 may be relevant for future study of genes pertinent to the biology of psychotic disorders.

Methods

We used microarray datasets GSE87610¹ and GSE93577² for this differential gene expression analysis in conjunction with GEO2R. GSE87610 was generated using Affymetrix Human Genome U219 with n=72 for control subjects and n=65 for patients with schizophrenia. GSE93577 was generated using Affymetrix Human Genome U219 Array technology with n=68 for control subjects and n=70 for patients with schizophrenia and schizoaffective disorders. 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. A statistical test was performed to

1 evaluate whether GABARAPL1 expression was significantly different when comparing normal
2 DLPFC tissue from control subjects and DLPFC or L3 PV+ neurons of the DLPFC from patients
3 with schizophrenia or schizophrenia and schizoaffective disorder, respectively, using a two-
4 tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered
5 statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).
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7 **Results**

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9 We mined microarray datasets, public¹ and published², to identify genes whose
10 expression was most different in the brains of patients with psychotic disorders when
11 compared to control subjects.

12 GABARAPL1 is differentially expressed in the dorsolateral prefrontal cortex of patients with schizophrenia.

13 We identified the gene encoding γ -aminobutyric acid receptor-associated protein-like 1,
14 GABARAPL1, as among the genes whose mRNA expression was most different in the
15 dorsolateral prefrontal cortex of patients with schizophrenia when compared to control
16 subjects¹. When sorting all of the genes expressed in the dorsolateral prefrontal cortex, as
17 measured by microarray, based on change in expression between psychotic patients and
18 control subjects, GABARAPL1 ranked 32 out of 12188 total transcripts. Differential expression
19 of GABARAPL1 in the dorsolateral prefrontal cortex of patients with schizophrenia was
20 statistically significant (Table 1; p=8.42E-07).
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23 GABARAPL1 is differentially expressed in the PV+ L3 neurons dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder.

24 In a separate dataset, we identified GABARAPL1 as differentially expressed in the
25 parvalbumin (PV)-positive L3 neurons of the dorsolateral prefrontal cortex of patients with
26 schizophrenia or schizoaffective disorder when compared to control subjects². When sorting
27 all of the genes expressed in PV+ L3 neurons of the dorsolateral prefrontal cortex, as measured
28 by microarray, based on change in expression between patients with schizophrenia or

1 schizoaffective disorder and control subjects, GABARAPL1 ranked 10 out of 49386 total
2 transcripts. Differential expression of GABARAPL1 in the PV+ L3 neurons of the DLPFC of
3 patients with schizophrenia or schizoaffective disorder was statistically significant (Table 2;
4 $p=1.61E-06$).

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6 GABARAPL1 expression is significantly lower in the dorsolateral prefrontal cortex of patients with schizophrenia.

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8 Next we obtained exact mRNA expression levels of GABARAPL1 from the dorsolateral
9 prefrontal cortex of patients with schizophrenia and from the dorsolateral prefrontal cortex of
10 control subjects to directly compare GABARAPL1 expression between these groups. We also
11 performed a statistical test to evaluate whether this difference in expression was statistically
12 significant. GABARAPL1 was expressed at significantly lower levels in the dorsolateral
13 prefrontal cortex of patients with schizophrenia when compared to control subjects (Figure 1;
14 $p<0.0001$). We calculated a 0.9789 ± 0.0278 fold change in GABARAPL1 expression in the
15 dorsolateral prefrontal cortex of patients with schizophrenia when compared to control
16 subjects (Table 1).

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18 GABARAPL1 expression is significantly lower in the PV+ L3 neurons dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder.

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20 We also obtained exact mRNA expression levels of GABARAPL1 from PV+ L3 neurons
21 of the dorsolateral prefrontal cortex from patients with schizophrenia or schizoaffective
22 disorder and from the dorsolateral prefrontal cortex of control subjects to directly compare
23 GABARAPL1 between these two groups. We also performed a statistical test to evaluate
24 whether this difference in expression was statistically significant. GABARAPL1 was expressed
25 at significantly lower levels in the PV+ L3 neurons of the dorsolateral prefrontal cortex of
26 patients with schizophrenia or schizoaffective disorder when compared to control subjects
27 (Figure 2; $p<0.0001$). We calculated a 0.970 ± 0.043 fold change in GABARAPL1 expression in
28 the PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia and
schizoaffective disorder when compared to control subjects (Table 2).

Thus, we found using public and published microarray datasets^{2,3} that GABARAPL1 was among the genes most differentially expressed in the brain tissues of patients with psychotic disorders, in the dorsolateral prefrontal cortex and in the PV+ L3 neurons of the dorsolateral prefrontal cortex.

Discussion

We compared transcriptome information from published¹ and public² microarray datasets generated using the brain tissues of patients with psychotic disorders, specifically, in the dorsolateral prefrontal cortex, to that of non-affected control subjects, to identify the most significant changes in gene expression in the brains of patients with psychotic disorders. Changes in GABARAPL1 gene expression were more significant than greater than 99% of transcriptome in both groups of patients.

GABARAPL1 is an ATG8 paralogue and the most abundantly expressed ATG8 protein in the central nervous system⁶. In mice, GABARAPL1 expression could be detected by embryonic day 11 (E11), in fiber tracts and immature neurons in the embryo, and with highest expression in adults, but restricted to neurons⁶. Spatially, GABARAPL1 expression could be observed in every structure of the brain examined except for the stratum lacunosum-moleculare, stratum radiatum, stratum oriens, and stratum lucid of the hippocampus⁶. In humans, GABARAPL1 is expressed at very high levels in the brain, heart, peripheral blood leukocytes, liver, kidney, placenta, and skeletal muscle⁷. GABARAPL1 is thought to associate with autophagic vesicles⁸. Like other ATG8 family members, GABARAPL1 is cleaved at a conserved glycine residue. GABARAPL1 was found conjugated to phospholipids in HEK293T cells and Atg4B could delipidate GABARAPL1. The majority of GABARAPL1 localizes to lysosomal structures, exhibits punctate staining and partially co-localizes with LC3. Investigators using Dlx5-Cre to selectively delete Atg7 from the forebrain GABAergic interneurons of mice performed quantitative proteomic analysis in conjunction with semi-

denaturing detergent gel electrophoresis to identify aggregated and insoluble proteins, finding that GABARAPL2 was among the proteins most significantly aggregated in high-molecular weight fractions⁹. These Atg7-deficient mice exhibited behavioral deficits including signs of reduced social behavior, as evidenced by three-chamber social interaction tests and disturbed nesting behavior⁹. In humans, investigators found that in the brains of patients with autistic-spectrum disorder (ASD), the detergent-insoluble fraction of both GABARAPL1 and GABARAPL2 were significantly increased, suggesting that the GABARAPL1 protein aggregates in the brains of patients with ASD⁹.

Autophagosome biogenesis is orchestrated by Atg4 proteins, which once activated can delipidate Atg8 proteins like GABARAPL1¹⁰. Caspase-3 was found to cleave Atg4D, and while full-length Atg4D has poor delipidating activity *in vitro*, amino-terminally truncated Atg4D (Δ N63 Atg4D) can stimulate the delipidation of GABARAPL1, and silencing Atg4D blocks autophagosome formation by GABARAPL1¹⁰. The LIR motifs of PIK3C3, BECN1 and ATG14 all have the capacity to interact with GABARAPL1¹¹. p62 interacts with both ubiquitinated cargoes and GABARAPL1 on autophagosomes¹². Interestingly, proteasome inhibition causes selective induction of p62 and GABARAPL1 but no other autophagy-related machinery, and depleting cells of p62 or GABARAPL1 reduced survival of cells following proteasome inhibition, suggesting that p62 induction following proteasome inhibition promoted cell survival through sequestration of ubiquitinated proteins¹². In Dlx-Cre-driven Atg7-deficient mice, both GABARAPL1 and GABARAPL2 were localized to p62+ aggregates, further supporting the notion that the function of the GABARAP proteins might be altered under conditions of autophagic dysfunction⁹.

Thus, by mining published and public microarray data generated using the brains of patients with psychotic disorders, we found that among the most striking transcriptional features of the brain in psychosis was a significant change in GABARAPL1 expression. GABARAPL1 is expressed at significantly lower levels in the dorsolateral prefrontal cortex of

1 patients with psychotic disorders when compared to the brain in health. GABARAPL1 function
2 may be perturbed in the brains of patients with psychotic disorders.
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Rank	ID	p-value	t	B	Fold change	Gene
32	11758023_x_at	8.42E-07	-5.1616696	5.45481	0.9789 ±0.0278	GABARAPL1

Table 1: GABARAPL1 is differentially expressed in the dorsolateral prefrontal cortex of patients with schizophrenia when compared to control subjects.

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 GABARAPL1 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

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Rank	ID	p-value	t	B	Fold change	Gene
10	11758023_x_at	1.61E-06	-5.0090524	4.793089	0.970 ± 0.043	GABARAPL1

Table 2: GABARAPL1 is differentially expressed in PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia or schizoaffective disorder when compared to control subjects.

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 GABARAPL1 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: GABARAPL1 is expressed at significantly lower levels in the dorsolateral prefrontal cortex of patients with schizophrenia when compared to control subjects.

The mRNA expression of GABARAPL1 in the dorsolateral prefrontal cortex of control subjects (left) and in the dorsolateral prefrontal cortex of patients with schizophrenia (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in GABARAPL1 expression between the dorsolateral prefrontal cortex of control subjects and patients with schizophrenia, a p-value, listed above.

Figure 2: GABARAPL1 is expressed at significantly lower levels in the PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia or schizoaffective disorder than in the PV+ L3 neurons of the DLPFC of control subjects.

The mRNA expression of GABARAPL1 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 GABARAPL1 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.