1 The $\alpha 1$ subunit of the γ -aminobutyric acid receptor, Gabra1, is differentially expressed in the
2 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 $\alpha 1$ subunit of the γ -aminobutyric acid (GABA) receptor Gabra1 in neurons of the dorsolateral
10 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, Gabra1.

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. *Gabra1* 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 *Gabra1* 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 *Gabra1* 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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1 We observed differential expression of the $\alpha 1$ subunit of the γ -aminobutyric acid (GABA)
2 receptor, encoded by the Gabra1 gene, in the layer 3 (L3) PV+ neurons of the dorsolateral
3 prefrontal cortex (DLPFC) in patients with schizophrenia and schizoaffective disorder². When
4 sorting each of the transcripts whose expression was measured by microarray based on
5 significance of change in expression between diseased and control patients, in L3 PV+ DLPFC
6 neurons from patients with schizophrenia, Gabra1 ranked 31 out of 49386 total transcripts.
7 Differential expression of Gabra1 was statistically significant (Table 1; $p=1E-05$).
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11 Gabra1 is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal
12 cortex in patients with schizophrenia.
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14 We also observed differential expression of Gabra1 in the layer 3 and layer 5 (L5)
15 pyramidal neurons of the DLPFC in patients with schizophrenia. When sorting each of the
16 transcripts whose expression was measured by microarray based on significance of change in
17 expression between patients and control subjects, in L3 and L5 pyramidal neurons of the DLPFC
18 from patients with schizophrenia, Gabra1 ranked 187 out of 49386 total transcripts (Table 3),
19 equating to differential expression greater than 99.6% of all transcripts whose expression was
20 detected and measured. Differential expression of Gabra1 in L3 and L5 pyramidal neurons of the
21 DLPFC in patients with schizophrenia was statistically significant (Table 2; $p=4.1E-05$).
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24 Gabra1 expression is significantly lower in layer 3 parvalbumin-positive neurons of the
25 dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.
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We obtained exact mRNA expression levels for the differentially expressed Gabra1 transcript, from control subjects and from patients with schizophrenia and schizoaffective disorder, to directly compare Gabra1 expression levels in L3 PV+ DLPFC neurons. We also performed a statistical test to determine if this difference in Gabra1 expression was statistically significant. Gabra1 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 0.952 ± 0.071 in Gabra1 expression when comparing L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder to that of control subjects (Table 1).

Gabra1 expression is significantly lower in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia.

We also obtained exact mRNA expression levels for the differentially expressed Gabra1 transcript, from control subjects and from patients with schizophrenia, to directly compare Gabra1 expression levels in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex. We also performed a statistical test to determine if this difference in Gabra1 expression was statistically significant. Gabra1 was expressed at lower levels in the L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia, and this difference was statistically significant (Figure 2; $p < 0.0001$). We calculated a mean fold change of 0.984 ± 0.027 in Gabra1 expression when comparing L3 and L5 pyramidal neurons of the dorsolateral

pre-frontal cortex in patients with schizophrenia to that of control subjects (Table 2).

Thus, we found significant differential expression of Gabra1 in two types of neuronal cell types in patients with schizophrenia; Gabra1 expression was significantly lower in both L3 PV+ neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder and in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia.

Discussion

γ -aminobutyric acid, or GABA, is an inhibitory neurotransmitter that functions, in the context of the GABA_A receptor, to decrease neuronal action potential by generating inhibitory post-synaptic potentials through modulation of chlorine flux across a ligand-gated channel, increasing conductance of Cl⁻ across the cell membrane⁶. The α 1 subunit of the GABA_A receptor is expressed in the rat brain in the mitral cells of the olfactory bulb, in the Purkinje cells and in the granule cells of the cerebellum from embryonic day 19 to post-natal day 6⁷. Mice with conditional deletion of the GABA_A receptor α 1 subunit in corticotropin-releasing factor neurons display increased anxiety-like behavior and failure to properly extinguish conditioned fear⁸. Mice heterozygous for whole brain ablation of the α 1 subunit of the GABA_A receptor on a C57BL/6 background manifest absence-like seizures and spike-wave discharges on electroencephalogram⁹. GABA_A receptor α 1 subunit-deficient mice manifest loss of over half of all GABA_A receptors in the brain, lose the ability to bind zolpidem in the cerebral cortex¹⁰, and

1 display tremor¹⁰ that shares similarity with human essential tremor¹¹.

2 The importance of the $\alpha 1$ subunit of the GABA_A receptor in the human brain is
3 demonstrated by the fact that a mutation in the GABRA1 gene, causing a conversion of alanine
4 at residue 322 to aspartate is the cause of juvenile myoclonic epilepsy in a French Canadian
5 family; the current activated by GABA in these mutant GABA_A receptors is of decreased
6 amplitude, suggesting that epileptic seizures in this family may be a result of loss of inhibitory
7 function of the ligand-gated channel¹². Other disorders found in humans with GABRA1
8 mutation include unspecified epilepsy, juvenile myoclonic epilepsy, photosensitive idiopathic
9 generalized epilepsy, generalized epilepsy with febrile seizures, and severe epileptic
10 encephalopathies¹³. The major seizure type in 9 participants was of the tonic-clonic type, while
11 in 5 others, the predominant seizure type was myoclonic¹³. In a study of the Japanese
12 population, the 17-C haplotype of the IVS-712(GT)_n polymorphism was significantly associated
13 with bipolar I disorder and bipolar-type schizoaffective disorder; this association was confirmed
14 in a National Institutes of Mental Health (NIMH) pedigree and appeared to be protective¹⁴.

15 We identified four separate studies investigating relationships between psychotic
16 disorders and GABRA1. One study concluded that variants in the GABRA1 gene were not
17 significantly associated with mania, depression, delusion or disorganization, and that there was
18 no interaction between GABRA1 and the D4 dopamine receptor, for which a positive association
19 with delusional symptomology had previously been established¹⁵. However, a separate study
20 found that in a Portuguese patient population, haplotypes Hap9A and Hap10A were found in
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1 excess in patients as compared to controls; haplotype blocks 9 and 10 contain all schizophrenia-
2 associated single nucleotide polymorphisms (SNP)¹⁶. In a German population, a significant
3 association between SNP rs1037715 and schizophrenia was observed, and GABRA1 haplotype
4 Hap10A was found to be over-transmitted to affected probands, confirming association between
5 genetic variation in GABRA1 and schizophrenia in two ethnically distinct populations¹⁶. A third
6 study described decreased expression of GABRA1 mRNA in the pre-frontal cortex of patients
7 with schizophrenia¹⁷, with a fourth study describing decreased expression of GABRA1 mRNA in
8 the superior temporal gyrus in patients with schizophrenia. Thus, while association of genetic
9 variation in the GABRA1 gene and risk of development of or protection from schizophrenia is
10 debated, decreased expression of GABRA1 in the pre-frontal cortex and superior temporal gyrus
11 in patients with schizophrenia has been described, consistent with findings from our analysis.

12 We found by mining independently published microarray data that the gene encoding the
13 alpha subunit of the γ -aminobutyric acid receptor, Gabra1, was among the genes most
14 differentially expressed in two neuronal cell types of the dorsolateral pre-frontal cortex in
15 patients with schizophrenia. Gabra1 may be relevant to the biology of psychotic disorders
16 including schizophrenia and schizoaffective disorder.

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Rank	ID	p-value	t	B	FC	Gene
31	11741084_a_at	1E-05	-4.59	3.184884	0.952 ± 0.071	GABRA1

Table 1: Gabra1 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 Gabra1 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	FC	Gene
187	11741084_a_at	4.1E-05	-4.2389736	1.96044	0.984 ± 0.027	GABRA1

Table 2: Gabra1 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 and gene are listed in this chart.

Figure 1: Gabra1 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 Gabra1 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 Gabra1 expression between control L3 PV+ DLPFC neurons and L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder, a *p*-value, listed above.

Figure 2: Gabra1 is expressed at significantly lower levels in the layer 3 and layer 5 pyramidal neurons of the dorsolateral pre-frontal cortex of patients with schizophrenia.

The mRNA expression of Gabra1 in layer 3 and layer 5 pyramidal neurons of the DLPFC from control subjects (left) and from 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 Gabra1 expression between control layer 3 and layer 5 pyramidal neurons of the DLPFC and layer 3 and layer 5 pyramidal neurons from patients with schizophrenia, a *p*-value, listed above.