

RESEARCH ARTICLE

GAD1 Gene Expression in Blood of Patients with First-Episode Psychosis

Jie Yin Yee^{1*}, Milawaty Nurjono², Stephanie Ruth Teo³, Tih-Shih Lee³, Jimmy Lee^{1,4}

1 Research Division, Institute of Mental Health, Singapore, Singapore, **2** Saw Swee Hock School of Public Health, National University of Singapore, Singapore, Singapore, **3** Neuroscience & Behavioural Disorders, Duke-NUS Medical School, Singapore, Singapore, **4** Department of General Psychiatry 1, Institute of Mental Health, Singapore, Singapore

* jie_yin_yee@imh.com.sg



Abstract

γ-Aminobutyric acid (GABA), the primary inhibitory neurotransmitter, has often been studied in relation to its role in the pathophysiology of schizophrenia. GABA is synthesized from glutamate by glutamic acid decarboxylase (GAD), derived from two genes, GAD1 and GAD2. GAD1 is expressed as both GAD67 and GAD25 mRNA transcripts with the former reported to have a lower expression level in schizophrenia compared to healthy controls and latter was reported to be predominantly expressed fetally, suggesting a role in developmental process. In this study, GAD67 and GAD25 mRNA levels were measured by quantitative PCR (qPCR) in peripheral blood of subjects with first-episode psychosis (FEP) and from healthy controls. We observed low GAD25 and GAD67 gene expression levels in human peripheral blood. There was no difference in GAD25 and GAD67 gene expression level, and GAD25/GAD67 ratio between patients with FEP and healthy controls. PANSS negative symptoms were associated with levels of GAD25 mRNA transcripts in patients with FEP. While the current study provides information on GAD25 and GAD67 mRNA transcript levels in whole blood of FEP patients, further correlation and validation work between brain regions, cerebrospinal fluid and peripheral blood expression profiling are required to provide a better understanding of GAD25 and GAD67.

OPEN ACCESS

Citation: Yee JY, Nurjono M, Teo SR, Lee T-S, Lee J (2017) GAD1 Gene Expression in Blood of Patients with First-Episode Psychosis. PLoS ONE 12(1): e0170805. doi:10.1371/journal.pone.0170805

Editor: Xiang Yang Zhang, University of Texas Health Science Center at San Antonio Cancer Therapy and Research Center at Houston, UNITED STATES

Received: November 29, 2016

Accepted: January 11, 2017

Published: January 25, 2017

Copyright: © 2017 Yee et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the manuscript.

Funding: This study was funded by the Singapore Ministry of Health's National Medical Research Council under the Centre Grant Programme (Grant No.: NMRC/CG/004/2013). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Introduction

Alterations in GABAergic inhibitory neurotransmission have been reported in schizophrenia [1]. *GAD1* is the gene which encodes for a 67kDa isoform of glutamic acid decarboxylase, a key enzyme involved in GABA synthesis, and has been intensively studied [1]. GAD67 was found to be localized in several brain regions, in particular, the prefrontal cortex and hippocampus; its expression gradually increased from 14 weeks of gestational age until approximately 10 years of age and plateauing throughout the rest of lifespan [2,3]. Changes in molecular and cellular mechanisms affecting GAD67 were found to interfere with neuronal activity, connectivity formation during development, glutamatergic and dopaminergic neurotransmission and neurotrophin or glycoprotein signaling [1]. Studies comparing GABA levels in subjects in schizophrenia suggested

Competing Interests: The authors have no potential conflict of interest to report.

that a GABA synthesis deficit maybe the cause of cognitive dysfunction in schizophrenia and not unlikely the consequence of the disorder itself [4]. This hypothesis was supported by reports of reduction in GAD67 expression levels in brain regions obtained from postmortem collections in patients with schizophrenia [1].

GAD25, another *GAD1* transcript, lacks an enzymatic domain and is predominantly expressed in human fetal prefrontal cortex and hippocampus, as well as in the olfactory bulb in adult mouse model [3,5]. Its localization suggests possible roles in cell proliferation, migration, maturation of neuroblasts, synaptogenesis and synaptic plasticity [3,5]. As both GAD25 and GAD67 leads to the synthesis of GABA, therefore GAD25:GAD67 ratio may be an indicator of the maturation state of GABA function in human prefrontal cortex and hippocampus [3]. In normal individuals, Hyde et al reported a period of rapid decline in GAD25:GAD67 ratio beginning from fetal development to the first decade of life before it remains stable across the lifespan [3]. However, GAD25:GAD67 ratio was increased in the hippocampus of adult schizophrenics, reflecting an immature state of GABA system [3].

There has been a continuous effort to identify suitable biomarkers for the diagnosis, prognosis and personalization of treatments in people with psychosis [6,7]. However, obtaining brain samples in living individuals for research is a challenge, and it has been done under opportunistic circumstances. However, serial sampling of human brain tissues in living individuals to examine the development of the GABA system is probably near impossible and there had been a continuous effort to explore the possibility of using blood as a peripheral surrogate of brain regions [8]. The present study is the first attempt to explore levels of GAD25 and GAD67 mRNA transcripts and their relative expression in human peripheral blood. We hypothesized that FEP patients would have a higher GAD25/GAD67 ratio than matched controls, suggesting a potentially immature GABA system. We also hypothesized that there will be an association between GAD25 and GAD67 gene expression levels and symptom severity in the FEP patient group.

Materials and Methods

Study Participants

The current case control study was conducted at the institute of Mental Health, Singapore. Cases were a mixture of males and females diagnosed with FEP who have had less than 4 weeks of antipsychotic treatment. Controls were healthy individuals with no history of mental illness. Controls were matched for age, ethnicity and gender. Socio-demographic data such as age, gender, ethnicity and smoking status were obtained from all study participants. Participants with a history of substance use, mental retardation and neurological disorders were excluded from this study. Ethics approval for the study was provided by the Domain Specific Review Board of the National Healthcare Group, Singapore. Written informed consent was obtained from all study participants.

Assessments

All FEP participants were assessed using the Structured Clinical Interview for DSM-IV-TR (SCID-I). Clinical symptoms were assessed on the Positive and Negative Syndrome Scale (PANSS) by trained raters with established inter-rater reliability at > 0.8 . Controls were assessed on the SCID-I to determine history of mental illness at recruitment.

Quantitative Polymerase Chain Reaction

RNA isolation. 3ml of venous blood was collected from all study participants into TempusTM Blood RNA tube (Applied Biosystems, Foster City, CA) and stored in -80°C until RNA

extraction. Total RNA was extracted using TempusTM Spin RNA Isolation Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The concentration of extracted total RNA and purity ratio (260/280 and 260/230) were measured using NanoDrop ND-1000 spectrophotometer (Fisher Scientific, Oslo, Norway). The 260/280 ratio of all samples were > 2.1 . The quality of total RNA was assessed on ethidium bromide (EtBr)-stained agarose gel. Only samples with clearly defined ribosomal peaks were used in the study. Total RNA was stored at -80°C for later use.

1 μg of RNA obtained from the RNA extraction procedure was reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories Inc., Hercules, CA, USA) according to the manufacturer's protocol on an iCycler analyzer (Bio-Rad Laboratories Inc., Hercules, CA, USA).

qPCR. Fast real-time PCR was carried out using fluorescent labeled TaqMan[®] gene expression assays. Three FAM labeled probes were used: (1) β -Actin as endogenous control (ABI assay ID: HS99999903_m1), (2) GAD25 as target (ABI assay ID: HS00247564_m1), (3) GAD67 as target (ABI assay ID HS01065886_m1). The assays were conducted on an ABI7900HT analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. cDNA sample was added to a master mix containing 2X TaqMan[®] Fast Advanced Master Mix, 20X TaqMan[®] Gene Expression Assay and DEPC water. Relative quantification using the $\Delta\Delta\text{Ct}$ method was selected as the assay type. All three genes were run in parallel and in triplicates on the same 96-well plate for each sample. The PCR amplification was carried out under the following conditions: 2 minutes at 50°C (UNG incubation), 20 seconds at 95°C (polymerase activation), and 40 cycles with 1 second at 95°C and 20 seconds at 60°C . Results were analyzed on the SDS and RQ Manager Version 1.2 software (Applied Biosystems, Foster City, CA) which reported the comparative CT values according to the $2^{-\Delta\Delta\text{Ct}}$ formula.

Statistical Analysis

Data was analyzed on SPSS Statistics version 23 (IBM Co., Armonk, NY, USA). Descriptive statistics were tabulated for case and control groups. Statistical significance was set at $p < 0.05$. Categorical variables were examined using chi-squared test. Continuous variables were analyzed via independent Student's t-test or Mann-Whitney U test and data were reported as mean and standard deviation (SD). Univariate linear regression was used to identify variables [age, gender, smoking status and total daily chlorpromazine (CPZ) equivalent] associated with the expression of GAD25 and GAD67. Predictors with p-values 0.2 or less will be included in the final multivariate linear regression model for examining the association of GAD25 and GAD67 expression levels with symptom severity using a PANSS 5-factor model that was previously validated in our local datasets [9]. Only Ct values less than 40 were used for comparison between controls and patients, and linear regression analysis as Ct values above 40 would suggest no amplification according to manufacturer's protocol.

Results

57 pairs of FEP patients and healthy controls, matched for age, gender and ethnicity, were recruited for current study. As shown in [Table 1](#), there was no significant difference in age, gender, ethnicity and smoking status between cases and controls.

To explore the feasibility of using blood as a suitable surrogate for *GAD1* gene expression in the central nervous system, qPCR was employed to assess the gene expression profiles of GAD25 and GAD67 in blood of healthy controls and patients with FEP. The lowest Ct values of GAD25 for controls and FEP patients were 31.04 and 31.71, respectively. The lowest Ct values for GAD67 for controls and FEP patients were 33.00 and 33.41, respectively. More than

Table 1. Study participants' demographics.

	Patients	Controls	p-value
N	57	57	
	n (%)	n (%)	
Gender			0.851
Male	30 (52.6%)	29 (50.9%)	
Female	27 (47.4%)	28 (49.1%)	
Ethnicity			1.000
Chinese	36 (63.2%)	36 (63.2%)	
Malay	17 (29.8%)	17 (29.8%)	
Indian	4 (7.0%)	4 (7.0%)	
Smoking	16 (28.1%)	13 (22.8%)	0.519
Age, years	29.26 (7.94)	28.96 (7.91)	0.841
Duration of illness, years	1.80 (4.01)		
PANSS Total	64.63 (15.11)		

doi:10.1371/journal.pone.0170805.t001

50% of both patients and controls had Ct values above 35 for both *GAD1* transcripts, suggesting low expression levels in human peripheral blood. On the other hand, β -actin, the reference gene, showed low Ct values, indicating sufficient cDNA template for qPCR assay.

We noted a slight increase in *GAD25* (Mann-Whitney $U = 968$, $p = 0.722$) and *GAD67* (Mann-Whitney $U = 640$, $p = 0.783$) fold changes, and *GAD25*/*GAD67* ratio (Mann-Whitney = 460.5, $p = 0.212$) in patients with FEP but these differences were not statistically significant when compared to healthy controls (Fig 1) (Table 2). Factors known to affect *GAD1* expression levels were determined using univariate linear regression with *GAD25* and *GAD67* expression levels as dependent variables and age, gender, smoking status and total daily CPZ equivalents as independent variables. Significant predictors for *GAD25* were age ($F = 3.196$, $df = 1,88$, $p = 0.077$, $R^2 = 0.035$) and smoking ($F = 2.297$, $df = 1,88$, $p = 0.133$, $R^2 = 0.025$) (Table 3). Significant predictors for *GAD67* were age ($F = 3.863$, $df = 1,70$, $p = 0.053$, $R^2 = 0.052$), gender ($F = 2.896$, $df = 1,70$, $p = 0.093$, $R^2 = 0.040$) and CPZ equivalents ($F = 7.513$, $df = 1,35$, $p = 0.010$, $R^2 = 0.177$) (Table 3).

Associations between expression levels of *GAD25* and *GAD67* with symptom severity were examined via multivariate linear regression. Significant predictors mentioned above were included in the models (Tables 4 and 5). After adjusting for the predictors of *GAD25* and

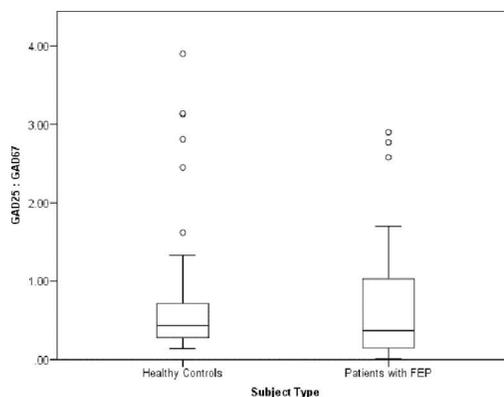


Fig 1. Boxplot of *GAD25*/*GAD67* gene expression levels of patients and controls.

doi:10.1371/journal.pone.0170805.g001

Table 2. Expression levels of GAD25 and GAD67 mRNA.

	Patients	Controls	p-value
GAD25 Fold Change			
Mean (SD)	9.49 (12.13)	7.83 (7.21)	0.722
< Ct35	26 (46.4%)	26 (48.1%)	
Ct35—Ct39	18 (31.2%)	20 (37.0%)	
≥ Ct40	12 (21.4%)	8 (14.8%)	
GAD67 Fold Change			
Mean (SD)	25.43 (34.35)	24.63 (31.29)	0.783
< Ct35	21 (37.5%)	13 (24.5%)	
Ct35—Ct39	17 (30.4%)	21 (39.6%)	
≥ Ct40	18 (32.1%)	18 (35.8%)	
GAD25:GAD67	0.70 (0.78)	0.88 (1.03)	0.212

doi:10.1371/journal.pone.0170805.t002

GAD67 expression levels, there was a significant association between GAD25 expression level and negative symptom factor score ($F = 2.790$, $df = 5,38$, $p = 0.002$, $R^2 = 0.269$) (Table 4).

Discussion

While studies in the literature on *GAD1* in psychosis were conducted primarily on postmortem brain samples, the current study is the first to examine *GAD1* expression levels in human blood using qPCR. We found that both transcripts, GAD25 and GAD67, were lowly expressed in human peripheral blood.

There had been varying findings from published reports; while majority observed a reduction in GAD67 expression levels in patients with schizophrenia and mood disorders, two reported that the reduced GAD67 levels were due to subset of GABA neurons and two other studies noted an increase in mRNA level in elderly with schizophrenia [10–17]. It has been reported that progressive switches in expression from GAD25 to GAD67 leads to GABA synthesis and higher GAD25/GAD67 ratios in the hippocampus of individuals with schizophrenia were observed, suggesting an immature GABA physiology [3]. The present study observed no differences in expression levels of GAD25, GAD67 and in GAD25/GAD67 ratio between FEP patients and controls. Some of the possible reasons for the differing findings could be due to different cell or tissue types, subsets of patients analyzed and illness duration. A potential reason might be the different duration of illness and treatment. The mentioned studies had patient groups with chronic schizophrenia with at least 15 years of illness compared to the current study which recruited FEP patients. Given differences in GAD67 mRNA levels noted between subgroups of GABA neurons and low GABA levels in the cerebrospinal fluid in subset of patients diagnosed with schizophrenia, whole blood which is made up of a composite group of cells may exhibit different expression profiles [17–19]. The low gene expression level of *GAD1* in peripheral blood may not accurately reflect the *GAD1* profiles in FEP and healthy controls. A report on the comparability of gene expression in blood and brain suggested

Table 3. The association between GAD25 and GAD67 gene expression levels and its predictors.

	Age		Gender		BMI		Smoking		CPZ Equivalent	
	β	P-value	β	P-value	β	0050	β	P-value	β	P-value
GAD25	0.187	0.077	0.114	0.284	0.089	0.405	-0.159	0.133	0.126	0.414
GAD67	0.229	0.053	0.199	0.093	-0.001	0.995	-0.014	0.905	0.420	0.01
GAD25/67	-0.123	0.325	-0.093	0.458	0.047	0.709	-0.087	0.468	-0.381	0.026

doi:10.1371/journal.pone.0170805.t003

Table 4. Association between GAD25 and psychopathology.

GAD25	Unadjusted			Adjusted for age, gender, smoking and CPZ equivalent		
	β	P-value	95% CI	β	P-value	95% CI
Positive	0.035	0.821	-0.583 to 0.731	0.088	0.633	-0.570 to 0.942
Negative	0.437	0.003	0.476 to 2.179	0.479	0.002	0.555 to 2.358
Excitement	-0.155	0.315	-2.379 to 0.786	-0.134	0.421	-2400 to 1.024
Depression	-0.175	0.256	-1.692 to 0.462	-0.102	0.561	-1.594 to 0.878
Cognitive	0.264	0.084	-0.199 to 3.043	0.270	0.101	-0.298 to 3.211

doi:10.1371/journal.pone.0170805.t004

GAD1 to be expressed in the prefrontal cortex only [6]. It was then suggested that peripheral blood levels may better correlate with tissues with direct access to blood such as pituitary and hypothalamus [6]. Expression of *GAD1* gene was noted to be highest in the cerebral cortex, hippocampus and cerebellum and lowly expressed in the esophagus, kidney, urinary bladder and testis [19]. The lack of difference in *GAD1* gene expression levels between patients and controls may be due to the effects of antipsychotics as patients had underwent 4 weeks of anti-psychotic treatment prior to participation in the study. The present study also lacks genetic information of *GAD1* to further examine its expression profile in blood. Three single nucleotide polymorphisms at 5' regulatory region of *GAD1* have been associated with decreased expression levels, increased risk for childhood-onset schizophrenia and cortical gray volume loss [20]. An increase in *GAD25/GAD67* expression ratio was associated with a *GAD1* single nucleotide polymorphism (rs3749034), with variant alleles predicting the immature GABA signaling pattern (HYDE 2011) [3]. Moreover, decreased expression of *GAD1* was reported in mutant mice with interneuron-specific ablation of NMDA glutamate receptor subunits [21]. A reduction in *GAD1* expression at prefrontal cortex of people with schizophrenia was observed to be associated with three DNA methylation sites at *GAD1* [22].

Our study found that higher levels of *GAD25* mRNA expression in the blood were associated with greater negative symptom burden. This suggests that negative symptoms such as emotional and social withdrawal, poor rapport, and motor retardation may be related to immature GABA development.

The strengths of our study include a consistent sampling time for all participants to minimize possible diurnal variation [18]. We also focused on FEP patients, with minimal exposure to antipsychotics which may interfere with *GAD67* expression levels [23–25]. Whole blood was collected directly into Tempus™ blood collection tubes which stabilize the transcriptome with proprietary agents coated within the tubes [26].

To the authors' knowledge, this is the first attempt to measure *GAD1* transcript levels in whole blood of FEP patients. We did not find any significant difference between *GAD25* and *GAD67* levels between patient and control groups, probably due to low expression levels. Future attempts might pursue prospective blood samples collected from FEP patients to

Table 5. Association between GAD67 and psychopathology.

GAD67	Unadjusted			Adjusted for age, gender, smoking and CPZ equivalent		
	β	P-value	95% CI	β	P-value	95% CI
Positive	-0.392	0.016	-4.164 to -0.449	-1.465	0.186	-3.672 to 0.742
Negative	0.266	0.112	-0.567 to 5.202	1.768	0.226	-1.159 to 4.685
Excitement	-0.276	0.098	-8.134 to 0.716	-3.576	0.130	-8.363 to 1.112
Depression	-0.088	0.604	-4.317 to 2.548	-0.923	0.583	-4.312 to 2.467
Cognitive	0.382	0.020	1.003 to 10.895	4.571	0.093	-0.801 to 9.943

doi:10.1371/journal.pone.0170805.t005

examine changes in expression levels in relation to chronicity of illness, treatment exposure and clinical outcomes. Studies may also be carried out on antipsychotic-naïve schizophrenia patients to assess the treatment effects on *GAD1* gene expression profile. This information may provide in-depth understanding for the development of psychosis.

Acknowledgments

We thank the study participants for their time and effort in this study.

Author Contributions

Conceptualization: MN JL.

Data curation: JYY SRT.

Formal analysis: JYY.

Methodology: MN JL.

Project administration: MN JL.

Validation: JYY.

Writing – original draft: JYY.

Writing – review & editing: JYY MN SRT TSL JL.

References

1. Akbarian S, Huang H-S (2006) Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. *Brain research reviews* 52: 293–304. doi: [10.1016/j.brainresrev.2006.04.001](https://doi.org/10.1016/j.brainresrev.2006.04.001) PMID: [16759710](https://pubmed.ncbi.nlm.nih.gov/16759710/)
2. Mitchell AC, Jiang Y, Peter C, Akbarian S (2015) Transcriptional regulation of GAD1 GABA synthesis gene in the prefrontal cortex of subjects with schizophrenia. *Schizophrenia research* 167: 28–34. doi: [10.1016/j.schres.2014.10.020](https://doi.org/10.1016/j.schres.2014.10.020) PMID: [25458568](https://pubmed.ncbi.nlm.nih.gov/25458568/)
3. Hyde TM, Lipska BK, Ali T, Mathew SV, Law AJ, et al. (2011) Expression of GABA signaling molecules KCC2, NKCC1, and GAD1 in cortical development and schizophrenia. *The Journal of Neuroscience* 31: 11088–11095. doi: [10.1523/JNEUROSCI.1234-11.2011](https://doi.org/10.1523/JNEUROSCI.1234-11.2011) PMID: [21795557](https://pubmed.ncbi.nlm.nih.gov/21795557/)
4. Curley AA, Arion D, Volk DW, Asafu-Adjei JK, Sampson AR, et al. (2011) Cortical deficits of glutamic acid decarboxylase 67 expression in schizophrenia: clinical, protein, and cell type-specific features. *American Journal of Psychiatry*.
5. Trifonov S, Yamashita Y, Kase M, Maruyama M, Sugimoto T (2014) Glutamic acid decarboxylase 1 alternative splicing isoforms: characterization, expression and quantification in the mouse brain. *BMC Neurosci* 15: 114. doi: [10.1186/1471-2202-15-114](https://doi.org/10.1186/1471-2202-15-114) PMID: [25322942](https://pubmed.ncbi.nlm.nih.gov/25322942/)
6. Prata D, Mechelli A, Kapur S (2014) Clinically meaningful biomarkers for psychosis: a systematic and quantitative review. *Neuroscience & Biobehavioral Reviews* 45: 134–141.
7. Mirzakhani H, Singh F, Cadenhead KS (2014) Biomarkers in psychosis: an approach to early identification and individualized treatment. *Biomarkers in medicine* 8: 51–57. doi: [10.2217/bmm.13.134](https://doi.org/10.2217/bmm.13.134) PMID: [24325224](https://pubmed.ncbi.nlm.nih.gov/24325224/)
8. Sullivan PF, Fan C, Perou CM (2006) Evaluating the comparability of gene expression in blood and brain. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 141: 261–268.
9. Jiang J, Sim K, Lee J (2013) Validated five-factor model of positive and negative syndrome scale for schizophrenia in Chinese population. *Schizophr Res* 143: 38–43. doi: [10.1016/j.schres.2012.10.019](https://doi.org/10.1016/j.schres.2012.10.019) PMID: [23148897](https://pubmed.ncbi.nlm.nih.gov/23148897/)
10. Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, et al. (1995) Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. *Archives of general psychiatry* 52: 258–266. PMID: [7702443](https://pubmed.ncbi.nlm.nih.gov/7702443/)

11. Dracheva S, Elhakem SL, McGurk SR, Davis KL, Haroutunian V (2004) GAD67 and GAD65 mRNA and protein expression in cerebrocortical regions of elderly patients with schizophrenia. *J Neurosci Res* 76: 581–592. doi: [10.1002/jnr.20122](https://doi.org/10.1002/jnr.20122) PMID: [15114630](https://pubmed.ncbi.nlm.nih.gov/15114630/)
12. Guidotti A, Auta J, Davis JM, Di-Giorgi-Gerevini V, Dwivedi Y, et al. (2000) Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Arch Gen Psychiatry* 57: 1061–1069. PMID: [11074872](https://pubmed.ncbi.nlm.nih.gov/11074872/)
13. Hakak Y, Walker JR, Li C, Wong WH, Davis KL, et al. (2001) Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proceedings of the National Academy of Sciences* 98: 4746–4751.
14. Hashimoto T, Bergen SE, Nguyen QL, Xu B, Monteggia LM, et al. (2005) Relationship of brain-derived neurotrophic factor and its receptor TrkB to altered inhibitory prefrontal circuitry in schizophrenia. *The Journal of neuroscience* 25: 372–383. doi: [10.1523/JNEUROSCI.4035-04.2005](https://doi.org/10.1523/JNEUROSCI.4035-04.2005) PMID: [15647480](https://pubmed.ncbi.nlm.nih.gov/15647480/)
15. Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, et al. (2003) Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *J Neurosci* 23: 6315–6326. PMID: [12867516](https://pubmed.ncbi.nlm.nih.gov/12867516/)
16. Impagnatiello F, Guidotti AR, Pesold C, Dwivedi Y, Caruncho H, et al. (1998) A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *Proc Natl Acad Sci U S A* 95: 15718–15723. PMID: [9861036](https://pubmed.ncbi.nlm.nih.gov/9861036/)
17. Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA (2000) Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. *Arch Gen Psychiatry* 57: 237–245. PMID: [10711910](https://pubmed.ncbi.nlm.nih.gov/10711910/)
18. Huhman KL, Hennessey AC, Albers HE (1996) Rhythms of glutamic acid decarboxylase mRNA in the suprachiasmatic nucleus. *Journal of biological rhythms* 11: 311–316. PMID: [8946258](https://pubmed.ncbi.nlm.nih.gov/8946258/)
19. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, et al. (2015) Proteomics. Tissue-based map of the human proteome. *Science* 347: 1260419. doi: [10.1126/science.1260419](https://doi.org/10.1126/science.1260419) PMID: [25613900](https://pubmed.ncbi.nlm.nih.gov/25613900/)
20. Addington A, Gornick M, Duckworth J, Sporn A, Gogtay N, et al. (2005) GAD1 (2q31.1), which encodes glutamic acid decarboxylase (GAD67), is associated with childhood-onset schizophrenia and cortical gray matter volume loss. *Molecular psychiatry* 10: 581–588. doi: [10.1038/sj.mp.4001599](https://doi.org/10.1038/sj.mp.4001599) PMID: [15505639](https://pubmed.ncbi.nlm.nih.gov/15505639/)
21. Belforte JE, Zsiros V, Sklar ER, Jiang Z, Yu G, et al. (2010) Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nature neuroscience* 13: 76–83. doi: [10.1038/nn.2447](https://doi.org/10.1038/nn.2447) PMID: [19915563](https://pubmed.ncbi.nlm.nih.gov/19915563/)
22. Huang H-S, Akbarian S (2007) GAD1 mRNA expression and DNA methylation in prefrontal cortex of subjects with schizophrenia. *PLoS one* 2: e809. doi: [10.1371/journal.pone.0000809](https://doi.org/10.1371/journal.pone.0000809) PMID: [17726539](https://pubmed.ncbi.nlm.nih.gov/17726539/)
23. Chertkow Y, Weinreb O, Youdim MB, Silver H (2006) The effect of chronic co-administration of fluvoxamine and haloperidol compared to clozapine on the GABA system in the rat frontal cortex. *Int J Neuropsychopharmacol* 9: 287–296. doi: [10.1017/S1461145705005626](https://doi.org/10.1017/S1461145705005626) PMID: [15967061](https://pubmed.ncbi.nlm.nih.gov/15967061/)
24. Lipska BK, Lerman DN, Khaing ZZ, Weinberger DR (2003) The neonatal ventral hippocampal lesion model of schizophrenia: effects on dopamine and GABA mRNA markers in the rat midbrain. *Eur J Neurosci* 18: 3097–3104. PMID: [14656305](https://pubmed.ncbi.nlm.nih.gov/14656305/)
25. Rupniak NM, Prestwich SA, Horton RW, Jenner P, Marsden CD (1987) Alterations in cerebral glutamic acid decarboxylase and 3H-flunitrazepam binding during continuous treatment of rats for up to 1 year with haloperidol, sulpiride or clozapine. *J Neural Transm* 68: 113–125. PMID: [3806083](https://pubmed.ncbi.nlm.nih.gov/3806083/)
26. Lee J, Goh L-K, Chen G, Verma S, Tan C-H, et al. (2012) Analysis of blood-based gene expression signature in first-episode psychosis. *Psychiatry research* 200: 52–54. doi: [10.1016/j.psychres.2012.03.021](https://doi.org/10.1016/j.psychres.2012.03.021) PMID: [22503327](https://pubmed.ncbi.nlm.nih.gov/22503327/)