Research Article

İbrahim Fettahoğlu, Şükrü Kartalcı, Harika G. Gözükara Bağ and Ceren Acar*

Relationship between methylation pattern of the *SYN2* gene and schizophrenia

https://doi.org/10.1515/tjb-2023-0249
Received November 15, 2023; accepted March 20, 2024;
published online May 1, 2024

Abstract

Objectives: Schizophrenia is a neuropsychiatric disease, and its etiology is not exactly understood. DNA methylation is an important phenomenon that affects the rise of abnormal phenotypes in many cases. Investigation of the association between DNA methylation and schizophrenia is crucial for elucidating the basis of schizophrenia. Previous association studies confirm that the *SYN2* gene is a strong candidate gene for schizophrenia. In the current study, the relationship between the methylation status of the *SYN2* gene and schizophrenia was investigated. The aim is to obtain crucial results for illuminating the effects of the *SYN2* methylation changes in the etiology of schizophrenia.
Methods: In light of this scientific information, we investigated the methylation status of three different CpG regions in the promoter of the *SYN2* gene and compared them in healthy controls and schizophrenia patients. Thirty-three healthy controls and 36 schizophrenia patients were included in this study. Sequencing was performed using the pyrosequencing method to reveal the methylation pattern.
Results: As a result of the statistical analysis, it was confirmed that there is a significant relationship between the methylation pattern of the *SYN2* gene and schizophrenia. Schizophrenia patients showed more methylation in position 2 and position 3. Additionally, the average methylation ratio is increased in schizophrenia patients.
Conclusions: We find an association between the DNA methylation pattern of the *SYN2* gene and schizophrenia. These results can help to the understanding of the etiology of schizophrenia. Except for these, DNA methylation changes in the *SYN2* gene in people who live in urban and rural areas can be one of the reasons for the different incidences of schizophrenia in these regions.

Keywords: schizophrenia; *SYN2*; DNA methylation; epigenetics; synapse

Introduction

Schizophrenia is one of the serious psychiatric disorders that is characterized by the impairment of social relations, thought, emotion, and behavior. The high prevalence of schizophrenia and considerable cost to society are reasons that make schizophrenia a remarkable research subject. Schizophrenia affects approximately 1 % of the worldwide population [1]. Despite the high prevalence of the disorder, there are no acceptable biomarkers to use in the diagnosis of schizophrenia. Instead, clinicians use The Diagnostic and Statistical Manual of Mental Disorders – fifth edition (DSM-5) to diagnose the disorder. Treatment of schizophrenia generally occurs with antipsychotic drugs.

Schizophrenia has a complex and heterogeneous etiological background. Although scientific studies have been going on for a long time, its etiology is still unknown completely. Both biological and environmental factors play a crucial role together in the existence of the disorder [2]. While the genetic architecture is the most effective biological factor, there are a lot of different environmental factors such as obstetric complications, the season of birth, prenatal and postnatal infections, urbanity, maternal malnutrition, maternal stress, child abuse, abnormal life events, immigration, head injury, drug abuse, adverse childhood experiences, and extreme living conditions [3–6].

Thousands of different genes were associated with schizophrenia by genome-wide association studies (GWAS). Beyond the genetics studies of schizophrenia, more complicated studies than single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) were performed with

***Corresponding author: Ceren Acar**, PhD, Prof. Dr., Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Inonu University, Malatya, Türkiye, Phone: +90 422 377 4122, E-mail: ceren.acar@inonu.edu.tr. https://orcid.org/0000-0003-1842-9203
İbrahim Fettahoğlu, Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Inonu University, Malatya, Türkiye. https://orcid.org/0000-0001-8208-0611
Şükrü Kartalcı, Department of Psychiatry, Faculty of Medicine, Inonu University, Malatya, Türkiye. https://orcid.org/0000-0003-2560-0355
Harika G. Gözükara Bağ, Department of Biostatistics, Faculty of Medicine, Inonu University, Malatya, Türkiye. https://orcid.org/0000-0003-1208-4072

the development of epigenetics. Epigenetics refers to a field of science that is interested in the changes in gene expression without any changes in DNA sequence [7]. DNA methylation is one of the major processes that control gene expression epigenetically. The most frequent form of DNA methylation is seen as adding a methyl (CH3) group to the fifth carbon position of cytosine in CG dinucleotide and it can then be inherited by cell division [8]. CpG islands are special regions that include more CG dinucleotides than others, and these regions are generally the target of epigenetic studies that try to search DNA methylation patterns. DNA methylation patterns of specific genes play a critical role in the pathophysiology of schizophrenia. For instance, *RELN* [9, 10], *5HTR1A* [11], *COMT* [12], *DRD2,* and *NR3C1* [13] are important genes that show significant changes in methylation pattern in schizophrenia patients.

Synapsins are a family of synaptic vesicle-associated phosphoproteins, and they are involved in releasing synaptic vesicles to the synaptic cleft. They are the most abundant synaptic vesicle proteins and consist of 9 % of all proteins in synaptic vesicles. Except those, 1 % of total neuronal proteins are synapsins. There are three members of the synapsin protein family, Synapsin 1, Synapsin 2, and Synapsin 3, and they are all effective in neuronal plasticity, regulation of the kinetics of synaptic vesicle exocytosis, axon elongation and formation of the presynaptic terminal. Their most known function is the control of the synaptic vesicle exocytosis by attaching and releasing them. Synapsin 2 is a member of the Synapsin group and coded by the *SYN2* gene that localizes in the 3p25.2 position of the human genome [14].

All members of the Synapsin family are attractive candidate genes for the etiology and molecular pathology of schizophrenia and other psychiatric disorders because of their role in organisms [15, 16]. All scientific literature and the role of Synapsin 2 in the synapse are sufficient to raise suspicion to investigate the relationship between the DNA methylation status of *SYN2* and schizophrenia [17–19]. In the present study, we have performed a pyrosequencing approach to detect and compare the methylation status of three specific CpG regions that are found in the promoter region of the *SYN2* gene in schizophrenia patients and healthy controls. We mainly expect to find a significant difference in methylation status rate between the healthy control and schizophrenic groups.

Materials and methods

Participants

Peripheral blood samples from both schizophrenia patients (n=36) and healthy controls (n=33) were collected by professional medical staff.

Table 1: Sociodemographic characteristics and medication status of the case group.

Variables	Cases (n=36)
Age, year	45.4±12.9
Age at onset, year	25 (17–50)
Sex, n (%)	
Female	11 (31 %)
Male	25 (69 %)
Education level, n (%)	
Non-university graduate	32 (88.9 %)
University graduate	4 (11.1 %)
Marital status, n (%)	
Single	24 (66.7 %)
Married	9 (25.0 %)
Divorced	3 (8.3 %)
Region, n (%)	
Rural	14 (38.9 %)
Urban	22 (61.1 %)
Antipsychotic medication, n (%)	
Atypical	28 (77.8 %)
Atypical+typical	8 (22.2 %)

Age parameter is given as mean±standard deviation, age at onset as median (min to max).

Schizophrenia patients who applied to the Department of Psychiatry in Turgut Özal Medical Center (Affiliated with İnönü University) and agreed to participate as volunteers were included in the study. Thirty-nine blood samples were collected from patients, but three schizophrenia patients were discarded who did not meet the inclusion criteria, and 36 patients were included in the analysis. Informed consent forms were obtained from all patients. The case group consisted of 11 female (31 %) and 25 male (69 %) volunteers. The average age of the case group was 45.4±12.9. The age of onset of schizophrenia in the case group was about 25 (17–50 years). Most of them had lower education degrees (88.9 %). All sociodemographic characteristics and medication status of the case group were given in Table 1. All experimental steps have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All schizophrenia patients were diagnosed by professional psychiatrists according to the DSM-V criteria. The healthy controls were unrelated individuals with no current or past psychiatric or physical diagnoses and no first-degree relatives had schizophrenia. All of the participants (both patients and controls) were Turkish. Ethics approval for the project was obtained from the Malatya Clinical Research Ethics Committee of İnönü University.

DNA extraction and bisulfite conversion

10 mL of venous blood samples were collected in EDTA-coated tubes from each patient or healthy individual after an overnight fast (10 h) between 8:00 and 10:00 a.m. All blood samples were collected by professional medical staff. Whole blood samples were then stored at 4 °C until DNA extraction. DNA isolation was completed using a PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, United States, Cat. No: K182001) according to the manufacturer's instructions. DNA

quality was assessed using a DeNovix DS-11 FX+ spectrophotometer (DeNovix, United States) and gel electrophoresis. Bisulfite conversion was performed to convert unmethylated cytosines to the uracil and make a difference between methylated and unmethylated positions of the DNA before the PCR amplification and sequencing. Bisulfite conversion of the isolated genomic DNA was performed using EpiTect Bisulfite Kit (Qiagen, United States, Cat. No: 59104) following the manufacturer's instructions. Bisulfite-modified genomic DNA was stored at 4 °C to amplify and analyze the methylation pattern of the sequence region in the promoter of the *SYN2* gene.

PCR amplification

Bisulfite-converted genomic DNA was used as a template in a PCR reaction to amplify the sequence region. All of the PCR reactions were set up according to the PyroMark PCR Kit (Qiagen, United States, Cat. No: 978703) instruction. Summarily, we made a mixture by adding 12.5 µL of PyroMark PCR Master Mix, 2.5 µL of CoralLoad Concentrate, 2.5 µL of PyroMark assay primers A and B and 5.5 µL of RNase-free water, respectively per reaction. 2 µL of bisulfite-modified DNA as a template was added to the mix per reaction. 2 µL of RNase-free water was added extra to the NTC tube. The thermal cycler was set up to 95 °C for 15 min, 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, 45 cycles, and 72 °C for 10 min. The samples obtained from PCR were stored at −20 °C to perform pyrosequencing.

Pyrosequencing

Pyrosequencing was performed using the PyroMark Q24 ID system (Qiagen, United States) to analyze and compare the DNA methylation pattern of the *SYN2* gene both in schizophrenia patients and healthy controls. Considering that DNA methylation generally occurs in the promoter regions and controls gene expression by blocking the recruitment of transcription factors to the promoter, we decided to analyze three different CpG positions they found on the promoter of the *SYN2* gene. Hs_SYN2_01_PM PyroMark CpG assay (Qiagen, United States, Cat. No: 978746) was used to set up all the experiments according to our target region. 5′-**CG**CCCTCCC**CG**CATAGTCA**CG**T-3′ was analyzed by pyrosequencing using Hs_SYN2_01_PM PyroMark CpG assay. Three different CpG positions are shown in bold in sequence (Position 1 for the first, Position 2 for the second, and Position 3 for the third CG dinucleotide). According to the outputs from methylation analysis of schizophrenia patients and the healthy control group, statistical methods were applied to the results.

Statistical analysis

The distribution of the qualitative data was presented by count and percentage. The normality of the quantitative data was examined using the Shapiro-Wilk test. Normally distributed quantitative data were summarized by mean and standard deviation, the others by median, minimum, and maximum values. For comparison of two independent groups, the Mann-Whitney U test was used. The correlation between quantitative data was analyzed using Spearman's rank correlation coefficient. The two-sided significance level was accepted as 0.05 in all tests. Analyses were performed by IBM SPSS Statistics for Windows version 22.0 (Armonk, NY: IBM Corp.).

Results

Relationship between demographic and clinical characteristics and DNA methylation

There was a significant difference in methylation of position 1 and position 3 in schizophrenia patients who live in urban and rural areas. Schizophrenia patients who come from urban areas show a higher methylation ratio in the first and third CpG positions compared with patients from rural areas (p=0.027 for position 1 and p=0.038 for position 3) (Table 2). On the other hand, there was no significant difference between DNA methylation and age of onset, gender, educational status, age, and marital status of patients. Some clinical data such as the age of onset, type of antipsychotic medication, the scale for the assessment of negative symptoms (SANS) score, and the scale for the assessment of positive symptoms (SAPS) score were also analyzed. There was another significant relation between SANS scores and the type of antipsychotic medication. The patients who have been medicated with both typical and atypical antipsychotics have higher SANS scores than patients who have been medicated only with atypical antipsychotics (p=0.007) (Table 3). The correlations between SAPS/SANS and methylation status are given in Table 4. Except for those, there was not a significant relation between SANS and SAPS score and DNA methylation, age, age of onset, gender, marital status, educational status, and living in urban or rural areas.

Table 2: The relationship between DNA methylation and patients who come from urban and rural areas.

	Rural areas (n=14) Median (min.–max.)	Urban areas (n=22) Median (min.–max.)	p-Value
CpG position 1 Meth, %	32 (5–44)	40.5 (14–69)	**0.027**
CpG position 2 Meth, %	100 (76–100)	100 (99–100)	0.215
CpG position 3 Meth, %	73 (30–80)	76 (53–85)	**0.038**

Bold p-values indicate statistical significance (p<0.05).

Table 3: The linkage between SANS and SAPS scores and type of antipsychotic medication.

	Single medication (atypical, n=28) Median (min.–max.)	Combined medication (atypical+typical, n=8) Median (min.–max.)	p-Value
SAPS	43 (16–90)	51 (40–70)	0.284
SANS	56 (16–84)	76 (48–86)	**0.007**

Bold p-values indicate statistical significance (p<0.05). SANS, scale for the assessment of negative symptoms; SAPS, scale for the assessment of positive symptoms.

Table 4: Correlations between SAPS/SANS and methylation status.

	SAPS		SANS	
	r_s	p-Value	r_s	p-Value
Position 1 Meth, %	−0.217	0.204	0.072	0.678
Position 2 Meth, %	0.008	0.965	−0.164	0.338
Position 3 Meth, %	−0.201	0.240	0.061	0.725

r_s, Spearman's correlation coefficient; SANS, scale for the assessment of negative symptoms; SAPS, scale for the assessment of positive symptoms.

Methylation of the *SYN2* gene in schizophrenia patients and healthy controls

As stated earlier, our main hypothesis was that there is a significant difference in the DNA methylation pattern of the *SYN2* gene between schizophrenia patients and the healthy control group. Each CpG region was analyzed separately for its significant difference between schizophrenia and healthy controls. Statistical analysis showed that the DNA methylation of CpG islands in schizophrenia is higher than healthy controls for second and third CpG positions (p=0.006 for position 2 and p=0.003 for position 3). Beyond these, average CpG methylation was significantly higher in CpG regions of schizophrenia patients than in healthy controls (p=0.014) (Table 5).

Discussion

The idea of the heritability of schizophrenia arose from the adoption, twin, and family studies in the first years of schizophrenia research. Research to reveal the genetic basis of schizophrenia proves that thousands of different genes are responsible for the existence of the disorder. Scientists have more doubts about some of these genes because of their critical roles in synapse and neuronal plasticity. In addition to the early studies like SNPs and CNVs, recent studies like GWAS show crucial evidence about the heritability of schizophrenia [20, 21]. Beyond the DNA sequence, it is clear epigenetic modifications have a huge effect on the existence of abnormal phenotypes. In the present study, we investigate the difference in DNA methylation pattern of the *SYN2* gene between schizophrenia patients and healthy people. To our knowledge, this is the first study that investigates the association of methylation status of the *SYN2* gene that codes Synapsin 2 protein with schizophrenia. We succeeded in finding significant results about the association of DNA methylation in the *SYN2* gene with schizophrenia.

Synapsins were first reported synaptic vesicle proteins and were reported by Edward Johnson and colleagues in 1972 [22]. As we mentioned before, there are three different types of synapsins as Synapsin 1, Synapsin 2 and Synapsin 3. All types of Synapsins have different isoforms as "a" and "b". The number of isoforms can show variety from cell to cell [23]. Knockdown experiments of the *SYN2* show their huge role in the synapse. Studies performed by Ferreira et al. reveal the problems in synapse formation in the case of *SYN2* knockout. In addition to troubles with vesicular docking, presynaptic terminal problems and deficiency in axon elongation exist in the lack of *SYN2* [24, 25]. As mentioned before, they control the synaptic vesicles via binding or separating them by phosphorylation.

Table 5: CpG methylation ratios in schizophrenia and healthy controls.

	Control (n=33) Median (min.–max.)	Schizophrenia (n=36) Median (min.–max.)	p-Value
CpG position 1 Meth, %	28 (9–97)	37 (5–69)	0.176
CpG position 2 Meth, %	100 (54–100)	100 (76–100)	**0.006**
CpG position 3 Meth, %	60 (18–97)	75 (30–85)	**0.003**
Average, %	62 (32.3–97.7)	70.3 (38–83.7)	**0.014**

Bold p-values indicate statistical significance (p<0.05).

Previously, DNA methylation pattern analysis and alterations in DNA methylation in schizophrenia patients have been reported especially for candidate genes of schizophrenia such as *EGR3* [26], *COMT* [12], *RELN* [27], *GABRB2* [28], *MMP9* [29], *HTR2A* [30, 31], *HTR1A* [11], *BDNF* and *DAT1* [32]. Synapse and neuroplasticity-related genes have come into prominence as candidate genes for schizophrenia because of their role in organisms in recent years. Schizophrenia, bipolar disorder (BD), and major depression (MD) are primary psychiatric disorders linked with *SYN2* [17, 33]. Previous studies about the association of *SYN2* with schizophrenia were at the level of SNP or indel association studies [34–36]. These studies also found significant results and these results create a suggestion for our study. Different from previous studies, we investigated the relationship at the level of DNA methylation and epigenetically. A study performed by Cristiana Cruceanu et al. investigated the relationship between *SYN2* methylation pattern and BD and MD in 2016 [37]. Their results show hypomethylation in the *SYN2* gene in BD and MD patients compared with healthy controls. Unlike the BD and MD patients, our investigation says schizophrenia patients show more methylation in the *SYN2* gene compared with healthy controls. When considering BD, MD, and schizophrenia have common points in mechanisms and pathophysiology, these results may look inconsistent, but it should not be forgotten that DNA methylation patterns can show differences in different tissues. Their studies were performed using post-mortem brain tissue. Instead, we detected methylation patterns in DNA extracted from blood. On the other side, association studies were performed on different populations. While cases in their study were White Caucasian individuals with diagnoses of BD or MD and who died primarily by suicide, our study was performed by using cases of Turkish individuals who were diagnosed with schizophrenia. Different populations may show different methylation patterns. Except for different populations and tissues, there are many other factors that affect the DNA methylation pattern, like diet, hormones, stress, drugs, or exposure to environmental chemicals.

The effects of the methylation changes in the *SYN2* gene in the etiology of schizophrenia are open to interpretation. Depending on the multifactorial and polygenic characteristics of schizophrenia, it is hard to say that differential methylation of the *SYN2* gene has a huge effect on the pathogenesis of schizophrenia, but certainly, our results will help the understanding of the etiology of schizophrenia. On the other hand, synapse-related genes are one of the important gene groups that are candidates for schizophrenia. Our study shows the importance of epigenetic alterations in synapse-related genes, which can be one of the reasons for schizophrenia.

Except those, our other results confirm that the living area of patients has a relationship with the methylation status of the *SYN2* gene. It is known that the living area of individuals has an effect on DNA methylation in other species [38]. The incidence of schizophrenia is already showing a significant difference between people from urban and rural areas. The people who live in urban show more methylation in positions 1 and 3 in the *SYN2* gene. The reasons for these different methylation ratios are arguable. The people who live in urban areas are exposed to more chemicals. Soil components of rural and urban areas can show a variety in their minerals and toxic materials. These chemicals can affect the methylation patterns of people. On the other hand, stress factors are different between urban and rural areas. It is known that people who live in urban areas are exposed to more stress factors compared to those who live in rural areas. These results are also compatible with the higher schizophrenia incidence in people who live in urban areas. More investigations are required to understand whether these differences in incidence originate from DNA methylation or not. Another important point is that no significant pattern difference was found for the *SYN2* gene in previous blood methylation studies, unlike our study [39–41]. There could be many reasons for this. The use of different populations is among the main reasons. Different populations can show different methylation status. Previous methylome studies use Swedish and Japanese populations [39–41]. On the other hand, the environment to which populations were exposed is highly effective for changes in DNA methylation patterns. The climate and geography of Türkiye are wildly different from Japan and Sweden. Air pollution may be another important reason for this difference. Although there are studies showing that air pollution alters DNA methylation, it is also known that there is a significant difference between Japan, Türkiye, and Sweden in terms of air pollution [42].

In our study, some limitations prevent the investigation of the gene expression levels of *SYN2*. *SYN2* is only expressed in neuronal tissues, and it is not expressed in the blood [43]. The biggest problem was finding post-mortem tissues to investigate the gene expression level. DNA methylation studies can be carried forward by gene expression studies.

Previous transcriptome [44, 45] and proteome [46] studies showing the expression of the *SYN2* gene have been performed in brain tissues. Our study was completed using blood samples. Considering that DNA methylation shows different patterns between tissues, comparisons with previous transcriptome and proteome studies may not provide reliable results. Except from these, as mentioned before, different populations may show different results. Such a study on the Turkish population has not been conducted before. On the other hand, animal modelling is an important field in studying psychiatric disorders. In rats modelled for

schizophrenia using phencyclidine, Synapsin 2 protein levels were found to be significantly reduced in the medial prefrontal cortex [47]. Considering that methylation in the promoter region is generally inversely correlated with gene expression, the results of our study and the animal modelling study [47] support each other. On the other hand, methylation status can show differences from region to region, as mentioned before. Although the results support our study, it should be kept in mind that there are many points that remain to be clarified. However, it should be kept in mind that the validity of animal modelling and its power to reflect schizophrenia is still a matter of debate. Besides gene expression studies, miRNA studies can help to figure out the relationship between *SYN2* and schizophrenia. There is another potential research subject to understand the basics of DNA methylation in schizophrenia, which can be the effects of antipsychotic drugs on DNA methylation.

Conclusions

We find an association between the DNA methylation pattern of the *SYN2* gene and schizophrenia. These results can help to understand the etiology of schizophrenia. Except for these, DNA methylation changes in the *SYN2* gene in people who live in urban and rural areas can be one of the reasons for the different incidences of schizophrenia in these regions. To our knowledge, this is the first study that investigates the relationship between *SYN2* and schizophrenia epigenetically. Further studies are necessary to figure out the importance of *SYN2* (and other genes that are synapse-related) in the pathophysiology of schizophrenia.

Acknowledgments: Authors would like to thank Inonu University Scientific Research Coordination Unit for their support.

Research ethics: The research related to human use has complied with all the relevant national regulations, institutional policies, and in accordance with the tenets of the Helsinki Declaration and has been approved by the authors' Institutional Review Board (Malatya Clinic Research Ethical Board ref #: 2021/143).

Informed consent: Informed consent was obtained from all individuals included in this study.

Author contributions: İbrahim Fettahoğlu: experimental analysis, interpretation of the results, writing the manuscript. Şükrü Kartalcı: Sample collection, interpretation of the results, writing the manuscript. Harika G. Gözükara Bağ: Statistical analysis, interpretation of the results, writing the manuscript. Ceren Acar: Study design, interpretation of the results, writing the manuscript, principal investigator of the grant (#FYL-2021/2719). All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Research funding: This study was finacially supported by Inonu University Scientific Research Coordination Unit with project number #FYL-2021/2719.

Data availability: The raw data can be obtained on request from the corresponding author.

References

1. Marder SR, Cannon TD. Schizophrenia. N Engl J Med 2019;381:1753–61.
2. Walker E, Kestler L, Bollini A, Hochman KM. Schizophrenia: etiology and course. Annu Rev Psychol 2004;55:401–30.
3. Fearon P, Morgan C. Environmental factors in schizophrenia: the role of migrant studies. Schizophr Bull 2006;32:405–8.
4. Li Q, Cheung C, Wei R, Hui ES, Feldon J, Meyer U, et al. Prenatal immune challenge is an environmental risk factor for brain and behavior change relevant to schizophrenia: evidence from MRI in a mouse model. PLoS One 2009;4:9.
5. Misiak B, Stramecki F, Gaweda L, Prochwicz K, Sasiadek MM, Moustafa AA, et al. Interactions between variation in candidate genes and environmental factors in the etiology of schizophrenia and bipolar disorder: a systematic review. Mol Neurobiol 2018;55:5075–100.
6. Morgan C, Fisher H. Environment and schizophrenia: environmental factors in schizophrenia: childhood trauma – a critical review. Schizophr Bull 2007;33:3–10.
7. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21.
8. Wockner LF, Noble EP, Lawford BR, Young RM, Morris CP, Whitehall VLJ, et al. Genome-wide DNA methylation analysis of human brain tissue from schizophrenia patients. Transl Psychiatry 2014;4:e339.
9. Kho S-H, Yee JY, Puang SJ, Han L, Chiang C, Rapisarda A, et al. DNA methylation levels of RELN promoter region in ultra-high risk, first episode and chronic schizophrenia cohorts of schizophrenia. Schizophrenia 2022;8:81.
10. Fikri RMN, Norlelawati AT, El-Huda ARN, Hanisah MN, Kartini A, Norsidah K, et al. Reelin (RELN) DNA methylation in the peripheral blood of schizophrenia. J Psychiatr Res 2017;88:28–37.
11. Carrard A, Salzmann A, Malafosse A, Karege F. Increased DNA methylation status of the serotonin receptor 5HTR1A gene promoter in schizophrenia and bipolar disorder. J Affective Disord 2011;132:450–3.
12. Abdolmaleky HM, Cheng KH, Faraone SV, Wilcox M, Glatt SJ, Gao F, et al. Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. Hum Mol Genet 2006;15:3132–45.
13. Aytac HM, Oyaci Y, Pehlivan M, Pehlivan S. DNA methylation pattern of gene promoters of MB-COMT, DRD2, and NR3C1 in Turkish patients diagnosed with schizophrenia. Clin Psychopharmacol Neurosci 2022; 20:685–93.
14. Mirza FJ, Zahid S. The role of synapsins in neurological disorders. Neurosci Bull 2018;34:349–58.
15. Longhena F, Faustini G, Brembati V, Pizzi M, Benfenati F, Bellucci A. An updated reappraisal of synapsins: structure, function and role in neurological and psychiatric disorders. Neurosci Biobehav Rev 2021; 130:33–60.

16. Höltje M, Mertens R, Schou MB, Saether SG, Kochova E, Jarius S, et al. Synapsin-antibodies in psychiatric and neurological disorders: prevalence and clinical findings. Brain Behav Immun 2017;66:125–34.
17. Cruceanu C, Freemantle E, Alda M, Rouleau GA, Turecki G. Epigenetic regulation of synapsin genes in mood disorders. Neuropsychopharmacology 2013;38:239–41.
18. Kao HT, Porton B. Synaptic vesicle associated proteins and schizophrenia. In: Lajtha A, Javitt D, Kantrowitz J, editors. Handbook of neurochemistry and molecular neurobiology: schizophrenia. Boston, MA: Springer US; 2009:267–84 pp.
19. Browning MD, Dudek EM, Rapier JL, Leonard S, Freedman RJB. Significant reductions in synapsin but not synaptophysin specific activity in the brains of some schizophrenics. Biol Psychiatry 1993;34:529–35.
20. Gershon ES, Alliey-Rodriguez N, Liu CY. After GWAS: searching for genetic risk for schizophrenia and bipolar disorder. Am J Psychiatry 2011;168:253–6.
21. Duncan LE, Ratanatharathorn A, Aiello AE, Almli LM, Amstadter AB, Ashley-Koch AE, et al. Largest GWAS of PTSD (N=20070) yields genetic overlap with schizophrenia and sex differences in heritability. Mol Psychiatr 2018;23:666–73.
22. Johnson EM, Ueda T, Maeno H, Greengard P. Adenosine 3′,5-monophosphate dependent phosphorylation of a specific protein in synaptic membrane fractions from rat cerebrum. J Biol Chem 1972;247: 5650–2.
23. Sudhof TC, Czernik AJ, Kao HT, Takei K, Johnston PA, Horiuchi A, et al. Synapsins – mosaics of shared and individual domains in a family of synaptic vesicle phosphoproteins. Science 1989;245:1474–80.
24. Ferreira A, Kosik KS, Greengard P, Han HQ. Aberrant neurites and synaptic vesicle protein-deficiency in synapsin II-depleted neurons. Science 1994;264:977–9.
25. Ferreira A, Han HQ, Greengard P, Kosik KS. Suppression of synapsin II inhibits the formation and maintenance of synapses in hippocampal culture. Proc Natl Acad Sci U S A 1995;92:9225–9.
26. Hu TM, Hsu SH, Tsai SM, Cheng MC. DNA methylation analysis of the EGR3 gene in patients of schizophrenia. Psychiatry Res 2017;251:115–7.
27. Abdolmaleky HM, Cheng KH, Russo A, Smith CL, Faraone SV, Wilcox M, et al. Hypermethylation of the reelin (RELN) promoter in the brain of schizophrenic patients: a preliminary report. Am J Med Genet Part B 2005;134:60–6.
28. Zong L, Zhou L, Hou Y, Zhang LL, Jiang W, Zhang WW, et al. Genetic and epigenetic regulation on the transcription of GABRB2: genotype-dependent hydroxymethylation and methylation alterations in schizophrenia. J Psychiatr Res 2017;88:9–17.
29. Gao J, Yi HW, Tang XW, Feng XT, Yu M, Sha WW, et al. DNA methylation and gene expression of matrix metalloproteinase 9 gene in deficit and non-deficit schizophrenia. Front Genet 2018;9:10.
30. Abdolmaleky HM, Yaqubi S, Papageorgis P, Lambert AW, Ozturk S, Sivaraman V, et al. Epigenetic dysregulation of HTR2A in the brain of patients with schizophrenia and bipolar disorder. Schizophr Res 2011; 129:183–90.
31. Ghadirivasfi M, Nohesara S, Ahmadkhaniha HR, Eskandari MR, Mostafavi S, Thiagalingam S, et al. Hypomethylation of the serotonin receptor type-2a gene (HTR2A) at T102C polymorphic site in DNA derived from the saliva of patients with schizophrenia and bipolar disorder. Am J Med Genet Part B 2011;156:536–45.
32. Kordi-Tamandani DM, Sahranavard R, Torkamanzehi A. DNA methylation and expression profiles of the brain-derived neurotrophic factor (BDNF) and dopamine transporter (DAT1) genes in patients with schizophrenia. Mol Biol Rep 2012;39:10889–93.
33. Cesca F, Baldelli P, Valtorta F, Benfenati F. The synapsins: key actors of synapse function and plasticity. Prog Neurobiol 2010;91:313–48.
34. Chen Q, He G, Wang XY, Chen QY, Liu XM, Gu ZZ, et al. Positive association between synapsin II and schizophrenia. Biol Psychiatry 2004;56:177–81.
35. Chen Q, He G, Qin W, Chen QY, Zhao XZ, Duan SW, et al. Family-based association study of synapsin II and schizophrenia. Am J Hum Genet 2004;75:873–7.
36. Saviouk V, Moreau MP, Tereshchenko IV, Brzustowicz LM. Association of synapsin 2 with schizophrenia in families of Northern European ancestry. Schizophr Res 2007;96:100–11.
37. Cruceanu C, Kutsarova E, Chen ES, Checknita DR, Nagy C, Lopez JP, et al. DNA hypomethylation of synapsin II CpG islands associates with increased gene expression in bipolar disorder and major depression. BMC Psychiatry 2016;16:286.
38. McNew SM, Beck D, Sadler-Riggleman I, Knutie SA, Koop JAH, Clayton DH, et al. Epigenetic variation between urban and rural populations of Darwin's finches. BMC Evol Biol 2017;17:183.
39. Nishioka M, Bundo M, Koike S, Takizawa R, Kakiuchi C, Araki T, et al. Comprehensive DNA methylation analysis of peripheral blood cells derived from patients with first-episode schizophrenia. J Hum Genet 2013;58:91–7.
40. Kinoshita M, Numata S, Tajima A, Shimodera S, Ono S, Imamura A, et al. DNA methylation signatures of peripheral leukocytes in schizophrenia. NeuroMol Med 2013;15:95–101.
41. Aberg KA, McClay JL, Nerella S, Clark S, Kumar G, Chen W, et al. Methylome-wide association study of schizophrenia: identifying blood biomarker signatures of environmental insults. JAMA Psychiatry 2014; 71:255–64.
42. Rider CF, Carlsten C. Air pollution and DNA methylation: effects of exposure in humans. Clin Epigenet 2019;11:131.
43. Corradi A, Fadda M, Piton A, Patry L, Marte A, Rossi P, et al. SYN2 is an autism predisposing gene: loss-of-function mutations alter synaptic vesicle cycling and axon outgrowth. Hum Mol Genet 2014;23:90–103.
44. Olde Loohuis LM, Mangul S, Ori APS, Jospin G, Koslicki D, Yang HT, et al. Transcriptome analysis in whole blood reveals increased microbial diversity in schizophrenia. Transl Psychiatry 2018;8:96.
45. Song X, Liu Y, Pu J, Gui S, Zhong X, Chen X, et al. Transcriptomics analysis reveals shared pathways in peripheral blood mononuclear cells and brain tissues of patients with schizophrenia. Front Psychiatry 2021;12: 716722.
46. Smirnova L, Seregin A, Boksha I, Dmitrieva E, Simutkin G, Kornetova E, et al. The difference in serum proteomes in schizophrenia and bipolar disorder. BMC Genom 2019;20:535.
47. Thomson S, Dyck B, Daya R, Ho J, Bernardo A, Tian Y, et al. Reduced expression of synapsin II in a chronic phencyclidine preclinical rat model of schizophrenia. Synapse 2019;73:e22084.