DNA Methylation Pattern of Gene Promoters of MB-COMT, DRD2, and NR3C1 in Turkish Patients Diagnosed with Schizophrenia

Hasan Mervan Aytac1, Yasemin Oyaci2, Mustafa Pehlivan3, Sacide Pehlivan2
1Department of Psychiatry, Basaksehir Cam and Sakura City Hospital, 2Department of Medical Biology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, 3Department of Hematology, Gaziantep University, Faculty of Medicine, Gaziantep, Turkey

Objective: We aim to evaluate the methylation status of membrane-bound catechol-O-methyltransferase (MB-COMT) promotor, dopamine receptor D2 (DRD2), and nuclear receptor subfamily 3 group C member 1 (NR3C1) gene in patients with SCZ by comparing healthy controls.

Methods: A sample of 110 patients with SCZ and 100 age- and sex-matched healthy volunteers was included in the study. The interview was started by filling out data forms that included sociodemographic and clinical information. The Structured Clinical Interview for DSM-IV Axis I Disorders was used to confirming the diagnosis according to DSM-IV-TR criteria. Then the patients were evaluated with the Positive and Negative Symptoms Scale in terms of symptom severity. Methylation-specific polymerase chain reaction was used to determine the methylation status of MB-COMT promotor, DRD2, and NR3C1 gene from DNA material.

Results: When we compared the percentages of MB-COMT promotor, DRD2, and NR3C1 gene methylation status in SCZ patients with the healthy control group, the percentages of MB-COMT promotor (OR: 0.466; 95% CI: 0.268 – 0.809; p = 0.006), DRD2 (OR: 0.439; 95% CI: 0.375 – 0.514; p < 0.001), and NR3C1 (OR: 0.003; 95% CI: 0.001 – 0.011; p < 0.001) gene methylation status of SCZ was found to be significantly different from the control group. Whereas unmethylation of MB-COMT promotor and NR3C1 genes were associated with SCZ, the partial methylation of the DRD2 gene was related to the SCZ.

Conclusion: The MB-COMT promotor, DRD2, and NR3C1 gene methylation status may be associated with the SCZ in the Turkish population.

KEY WORDS: Schizophrenia; COMT; DRD2; NR3C1; Epigenetics; DNA methylation.

INTRODUCTION

Schizophrenia (SCZ) is characterized by the heterogenous presentation of positive, negative, and cognitive symptoms that cause significant impairments in psychosocial function, have a chronic course, and occur in nearly 1% of the world population [1]. The estimated heritability of SCZ and other psychotic disorders is as high as 60–80% [2]. However, genetics alone cannot explain its incidence. Because it is well-known that environmental exposures can modify DNA methylation patterns, long-lasting alterations in gene expression patterns after environmental exposures imply that epigenetic mechanisms might play a critical role in psychiatric disorders [3]. Therefore, environmental risk factors exposed during early development and young adulthood also contribute to SCZ in susceptible individuals via modifications of DNA and DNA-associated histone proteins by methylation, acetylation, and phosphorylation [4].

Catechol-O-methyltransferase (COMT) is the critical enzyme responsible for dopamine metabolism in the brain’s cortical regions [5]. The COMT gene is placed on chromosome 22q11.21, has eight exons, and produces 271 amino acids that metabolize catecholamines [6]. COMT gene polymorphisms are associated with the enzyme activity: higher activity is related to the COMT Val allele, and lower activity is associated with the COMT Met allele [7,8]. Growing evidence has proposed the as-
sociation of COMT genotypes in the pathophysiology of SCZ [9]. The relationship between COMT methylation and SCZ has concentrated on the soluble isoform (S-COMT) and the membrane-bound isoform (MB-COMT) [10]. Since the MB-COMT enzyme is included in dopamine and noradrenergic neurotransmission and is widely distributed in the peripheral blood and brain [11], therefore in our study, we studied the relationship between MB-COMT gene methylation and SCZ. Previous studies have published hypomethylation of MB-COMT in the brains of SCZ and bipolar disorder (BD) patients [12], and a similar hypo-methylated (~50%) MB-COMT promoter was in DNA derived from the saliva in SCZ and BD patients [13]. Recent research also reported hypomethylation in the peripheral blood leukocytes of Malaysian SCZ patients compared to the control group [10].

Again, the dopamine receptor D2 (DRD2) gene localized on chromosome 11q23.2 can have Val96Ala, Leu141Leu, Val154Ile, Pro310Ser, Ser311Cys, Taq1A, A-241G, and -141C Ins/Del polymorphisms [14]. The D2 receptor is involved in affect regulation, learning, motivation, reward processing, and decision-making [15], all of which are crucial processes involved in neuropsychiatric disorders such as mood disorders [16], cognitive sequelae of SCZ [17], and attention deficit hyperactivity disorder [18]. DRD2 -141C insertion/deletion and Taq1A have been investigated as treatment response markers [19,20]. While -141C insertion/deletion Del allele carriers were significantly associated with a poorer antipsychotic treatment response than the Ins/Ins genotype, there were no significant differences in the treatment response frequencies among Taq1A A1 allele carriers relative to SCZ patients with the A2/A2 genotype or A2 allele carriers relative to SCZ patients with the A1/A1 genotype [21]. Based on this dopamine hypothesis, several antipsychotics have been developed, and several studies have been conducted to identify biomarkers for SCZ diagnosis and the treatment response concerning the gene for DRD2. Yoshino et al. showed hypomethylation of DRD2 in Japanese SCZ patients [22]. Therefore, DNA methylation alterations in DRD2 may influence gene expression and may be associated with SCZ.

The genes like nuclear receptor subfamily 3 group C member 1 (NR3C1) play a critical role in the hypothalamic-pituitary-adrenal (HPA)-axis regulation, consisting of 8 introns and 9 exons on chromosome 5q31-32, encodes the glucocorticoid receptor [23]. Altered NR3C1 methylation is related to early stress exposure. It thus may cause the occurrence of psychopathologies including major depressive disorder [24], SCZ [25], post-traumatic stress disorder [26], suicidal behavior [27], bulimia nervosa [28], borderline personality disorder [29], alcohol-tobacco consumption [30], and cocaine use disorder [31]. Recently, Misiak et al. [32] published that the patients with SCZ-spectrum disorders show altered levels of NR3C1 methylation that are significantly lower in first-episode psychosis patients and significantly higher in acutely relapsed SCZ patients. No earlier researches have shown the methylation status of MB-COMT promoter, DRD2, and NR3C1 gene on SCZ in the Turkish population. We hypothesized that alteration of the methylation status of MB-COMT promoter, DRD2, and NR3C1 gene might be related to the SCZ. Therefore, we aimed to evaluate the relationship between SCZ and methylation status of MB-COMT promoter, DRD2, and NR3C1 gene by comparing healthy controls.

METHODS

This case-control study included 110 SCZ patients and 100 age- and sex-matched healthy controls and utilized a consecutive sampling design. SCZ patients were consecutively gathered from State Hospital Psychiatry Outpatient Clinic for three months. The study was approved by the Clinical Research Ethics Committee of Istanbul Faculty of Medicine, under the ethical standard for human experimentation established by the Declaration of Helsinki (03/22.01.2021) [33]. We informed the patients in detail about the study’s purpose, method, and procedures and obtained all the participants’ written consent. The interview was started by filling out data forms that included sociodemographic and clinical information. Afterward, the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-I) was used to exclude the psychiatric diagnosis and any psychiatric symptoms or non-specific psychological distress from the healthy control group. We recruited a healthy control group from the same geographical areas as the patients, and they were well-matched with the patients’ group in terms of similar age, ethnicity, and sex. The Positive and Negative Symptoms Scale (PANSS) was used to evaluate positive symptoms, negative symptoms, and general psychopathology in psy-
Methylation Status of MB-COMT, DRD2, and NR3C1 in SCZ

Table 1. Primer pairs and annealing temperatures for MB-COMT, DRD2, and NR3C1 genes

| Gene     | Primer set | Forward (5'-3')                        | Reverse (5'-3')                          | Annealing temp. (°C)/Cycle count |
|----------|------------|----------------------------------------|------------------------------------------|----------------------------------|
| DRD2     | MSP M      | CATTGTGATTGGATCGTCG                    | GACGCCCGAAGCGGAAAGCGG                    | 67/40                            |
|          | MSP U      | TGTTATTGGATGGATCGTCG                   | AACGCGGCAAGCGGAAAGCG                    | 56/40                            |
| MB-COMT  | MSP M      | TATTTTGTTAGGGGATTTGGTG               | AAGAACGCGAAGCGGAAAGCG                   | 56/40                            |
|          | MSP U      | TATTTTGTATTGGTGTGAGTGTG              | AAAGAACGCGAAGCGGAAAGCGG                 | 56/40                            |
| NR3C1    | MSP M      | TGTTTATTGGGATTGGATGGTG                | CGGCCGATCCGACTTTGCTAAG                  | 69/40                            |
|          | MSP U      | TTGTTTATTGGGATTGGATGGTG              | CCATCACAATACTCGTTAACTAAG                | 61/40                            |

MB-COMT, membrane-bound catechol-O-methyltransferase; DRD2, dopamine receptor D2; NR3C1, nuclear receptor subfamily 3 group C member 1; PCR, polymerase chain reaction; MSP, methylation-specific PCR; M, methylated; U, unmethylated.

RESULTS

MB-COMT Promotor, DRD2, and NR3C1 Gene Methylation Percentages of SCZ Patients

One hundred and ten patients diagnosed with SCZ were evaluated due to their clinical parameters, scale scores, MB-COMT promotor, DRD2, and NR3C1 gene methylation percentages, as shown in Table 2. In the MB-COMT gene methylation (partial methylation, unmethylation), 38.2% (n = 42) of the patients had partial methylated, 61.8% (n = 68) had unmethylated MB-COMT gene, in the DRD2 gene methylation (partial methylation, unmethylation), 100% (n = 110) of the healthy participants had partial methylated, 0% (n = 0) had unmethylated DRD2 gene, again in the NR3C1 gene methylation (partial methylation, unmethylation), 2.7% (n = 3) of the healthy participants had partial methylated, 97.3% (n = 107) had unmethylated NR3C1 gene.

Statistical Analyses

Statistical analysis was performed using IBM SPSS version 21.0 (IBM Corp. released 2012, Armonk, NY, USA). Quantitative data (clinical parameters, MB-COMT promotor, DRD2, and NR3C1 gene methylation) represented as descriptive statistics included the minimum, maximum, mean, standard deviation, frequency, and percentage. The Pearson chi-square or Fisher’s exact test analyzed comparisons of MB-COMT promotor, DRD2, and NR3C1 gene methylation of SCZ patients. We accepted statistical significance as p < 0.05 for the results of all analyses. The power analysis was performed with the “G*power” software (version 3.0.5, http://www.psychou.uni-duesseldorf.de/abteilungen/aap/gpower3/).

DNA Analyses

Blood samples and DNA extraction

The intravenous blood samples were collected from patients with SCZ and the control groups in 4 ml ethylenediaminetetraacetic acid tubes. Genomic DNA was obtained from the blood samples by using the GeneMark isolation kit (Plus Blood Genomic Purification Kit; Genemark, Atlanta, GA, USA).

Methylation-specific polymerase chain reaction

Bisulfite modification is accepted as the gold standard to determine the methylation status of DNA. To analyze, after isolation of DNA samples, we used the EZ-96 DNA Methylation-Gold kit according to manufacturer recommendations (Zymo Research): 10 minutes at 95°C, 40 cycles (30 seconds at 95°C, 40 seconds at annealing temperature for each primer, and 45 seconds at 72°C), and 72°C for 7 minutes. For methylation analysis of MB-COMT promotor [13], DRD2 [36], and NR3C1 [37] genes, two pairs of primers, one pair of methylated, and one pair of unmethylated, were used for each region. We showed primer sequences and annealing temperatures in Table 1.
Comparison of Percentages of MB-COMT Promotor, DRD2, and NR3C1 Gene Methylation Status in Patients with SCZ to the Control Group

When comparing the scale score (PANSS pos., PANSS neg., PANSS psycho., PANSS total), duration of the disorder, age of onset, and the number of hospitalizations according to MB-COMT promotor, DRD2, and NR3C1 gene methylation status in patients with SCZ, there was no statistically significant difference found between the groups (data not shown). When the percentages of MB-COMT promotor gene methylation in SCZ patients were compared with the control group, the percentages of MB-COMT promotor methylation of SCZ were found to be significantly different from the control group (odds ratio [OR]: 0.466; 95% confidence interval [CI]: 0.268–0.809; p = 0.006). Comparing the percentages of DRD2 gene methylation in SCZ patients with the control group, the percentages of DRD2 gene methylation of SCZ was found to be significantly different from the control group (OR: 0.439; 95% CI: 0.375–0.514; p < 0.001). Again, when the percentages of NR3C1 gene methylation in SCZ patients were compared with the control group, the percentages of NR3C1 gene methylation of SCZ was found to be significantly different from the control group (OR: 0.003; 95% CI: 0.001–0.011; p < 0.001) (Table 3).

DISCUSSION

This study demonstrated that the percentages of MB-COMT promotor, DRD2, and NR3C1 gene methylation status of SCZ patients were significantly different from the control group. Whereas unmethylation of MB-COMT promotor and NR3C1 genes were associated with SCZ, the partial methylation of the DRD2 gene was related to the SCZ. Growing evidence proposes that epigenetic alterations, such as DNA methylation and histone modifications, may contribute an additional explanation for the pathophysiology of SCZ [38]. Therefore, biological conditions like prenatal infections during development in the uterus [39], as well as social conditions like childhood trauma [40], the migrant status [41] might change gene expression leading to SCZ in genetically susceptible individuals [42]. There is abundant evidence for alterations of DNA methylation in SCZ, both at the site-specific and

Table 2. The clinical characteristics and scale scores of SCZ patients

| Variable                  | Schizophrenia (n = 110) |
|---------------------------|-------------------------|
| Sex                       | Male 80 (72.7)          |
|                           | Female 30 (27.3)        |
| MB-COMT                  | Partial 42 (38.2)       |
| Methylation               | Unmethylated 68 (61.8)  |
| DRD2                     | Partial 110 (100)       |
| Methylation               | Unmethylated 0 (0)      |
| NR3C1                    | Partial 3 (2.7)         |
| Methylation               | Unmethylated 107 (97.3) |
| Age                       | 39.2 ± 8.9              |
| Age of onset (yr)         | 24 ± 7.6                |
| Number of hosp.           | 3.4 ± 4.5               |
| Last hosp. (years ago)    | 5.5 ± 5.3               |
| PANSS total               | 57.8 ± 12.9             |
| PANSS psycho.             | 30.1 ± 7.2              |
| PANSS neg.                | 16.2 ± 5.3              |
| PANSS pos.                | 11.7 ± 3.7              |

Values are presented as number (%) or mean ± standard deviation. SCZ, schizophrenia; MB-COMT, membrane-bound catechol-O-methyltransferase; DRD2, dopamine receptor D2; NR3C1, nuclear receptor subfamily 3 group C member 1.

Table 3. Comparison of frequencies of the MB-COMT, DRD2, and NR3C1 methylation between SCZ patients and controls

| Methylation | Genotype   | SCZ (n = 110) | Healthy control (n = 100) | Odds ratio | 95% confidence interval | p value* |
|-------------|------------|--------------|---------------------------|------------|-------------------------|----------|
| MB-COMT     | Unmethylated | 68 (61.8)    | 43 (43.0)                 | 0.466      | 0.268–0.809              | 0.006*   |
| DRD2        | Partial methylation | 42 (38.2) | 57 (57.0) | 0.003 | 0.001–0.011 | 0.000* |
| NR3C1       | Unmethylated | 110 (100.0)  | 86 (86)                   | 0.439      | 0.375–0.514              | 0.000*   |
|             | Partial methylation | 107 (97.3) | 9 (9) |          |                        |          |

Values are presented as number (%).

SCZ, schizophrenia; MB-COMT, membrane-bound catechol-O-methyltransferase; DRD2, dopamine receptor D2; NR3C1, nuclear receptor subfamily 3 group C member 1.

*Pearson chi-square.
Methylation Status of MB-COMT, DRD2, and NR3C1 in SCZ

Genome-wide levels [43]. DNA methylation alterations in the promoters of COMT [13], glutamate decarboxylase 1 (GAD1) [44], reelin (RELN) [45], DNA methyltransferase 1 (DNMT1) [46], DNA methyltransferase 3a (DNMT3a) [46], serotonin receptor type-1 (HTR1A) [47], serotonin receptor type-2 (HTR2A) [48], and other genes have been shown [38]. Recent studies have investigated genome-wide methylation levels in the blood and brain tissue in SCZ with the development of the more advanced array- and sequencing-based techniques [43]. A few of these epigenome-wide association studies (EWAS) have validated the significance of GABA-associated genes in SCZ regarding aberrant methylation of Helt BHLH Transcription Factor (HELT) [49] and genes within the GAD1 regulatory network in SCZ brain tissue [50]. Moreover, Wockner et al. [51] recognized significant differential methylation in genes previously related to SCZ, including DNMT1, Nitric Oxide Synthase 1 (NOS1), and SRY-related HMG-box 10 (SOX10).

COMT has also been related to positive symptoms [52], negative symptoms [53], and cognitive deficits [54]. Besides, a recent meta-analysis showed that COMT was related to the severity of symptoms and cognitive parameters as well as treatment resistance in SCZ [55]. Previous researches in SCZ patients have reported hypomethylation of MB-COMT in the peripheral blood [10,56], saliva [13], and brain [12]. Contrastly, the DNA methylation of S-COMT seems to be increased in the peripheral blood and the brain tissues of SCZ patients [57]. Our results also showed that the MB-COMT gene overexpression was statistically significant. Theoretically, the overexpression of MB-COMT is related to increasing dopamine degradation, which is consistent with the critical role in dopamine neurotransmission and the dopamine hypothesis of SCZ. Therefore, we speculate that the epigenetic alteration of MB-COMT in the peripheral blood could be a potential peripheral biomarker of SCZ. When we compared the PANSS scale scores, duration of disease, age of onset, and the number of hospitalizations due to MB-COMT promoter methylation status in patients with SCZ, there was no statistically significant difference. Similarly, Nour El Huda et al. [10] did not find significant associations between the COMT methylation percentages and age at onset, duration of illness, BPRS, or DIPSS; even lower methylation rates were reported at other CpG sites of DRD2 in the SCZ group [22]. Again, Zhang et al. [61] did not find methylated CpG sites at the same CpG sites (CpG1 to 7) in a sib-pair study. These DRD2 genes’ methylation results have highlighted that the DRD gene network, overall, is actively involved in the increased risk of SCZ.

The HPA axis is thought the primary system involved in the regulation of stress. The glucocorticoid receptor (GR) is encoded by the NR3C1 gene, binds the HPA axis stress hormone cortisol [62]. Numerous researches have identified abnormalities of GR in the brains of patients with SCZ and BD. Besides, the transcript encoding GR’s overall expression levels are reduced in areas of the amygdala, hippocampus, and temporal cortex in SCZ and BD [63]. Earlier studies have mainly concentrated on identifying the relationship between SCZ-related genetic variants and clinical parameters [64,65]. Park et al. [66] published that two single nucleotide polymorphisms of NR3C1 (rs7701443 and rs2963155) may be associated with sus-
ceptibility to SCZ in the Korean population. In the present study, we reported a statistically significant difference between the methylation status of the NR3C1 gene of SCZ patients and control groups. The participants carrying the unmethylated NR3C1 gene had a higher risk of developing SCZ in the Turkish population. Our results are in line with the Misiak et al. [32] study. They reported significantly lower methylation of 4 CpG sites (CpG2, CpG4, CpG7, and CpG8) at the NR3C1 gene in first-episode psychosis patients than the high familial risk of psychosis individuals and healthy controls. Again, in a study examining the methylation state of the NR3C1 gene promoter region (exons 1D, 1B, 1F, and 1H) and its role in Chinese SCZ patients, have been reported that NR3C1 methylation at specific CpG sites in exon 1D, 1B, 1H, and 1F regions was related to SCZ, usually with sex specificity [67]. These results develop our experience concerning the HPA system’s function in developing SCZ and fill the gap in correlative research on NR3C1 gene methylation in SCZ.

Our study’s strength is that the first study showing the methylation status of MB-COMT promoter, DRD2, and NR3C1 gene on SCZ in the Turkish population. Secondly, since SCZ patients and healthy participants were collected from the same location, our study’s findings more valuable. Besides the strengths of the present research, there are also several limitations. The first limitation was the small sample size, which can limit the statistical power. Secondly, our study found that only DNA methylation was analyzed, while other epigenetic mechanisms such as histone deacetylation and hyperacetylation or miRNA were not studied. Also, the effect of psychotropic drugs on the epigenome can not be ruled out. Therefore, the dosage and duration of treatment were not accounted for in the statistical analysis due to difficulty collecting consistent data. Future studies are needed to elucidate the effects of psychotropic drugs on the epigenome. Lastly, confounding environmental factors such as diet and lifestyle are difficult to assess and were not evaluated in this study. In conclusion, whereas unmethylation of MB-COMT promoter and NR3C1 genes were associated with SCZ, the partial methylation of the DRD2 gene was related to the SCZ. Considering the advantage of blood samples in terms of accessibility, the DNA methylation status of the MB-COMT promoter, DRD2, and NR3C1 gene could potentially be helpful for developing biomarkers for this psychiatric disorder. Confirming these findings with different ethnicities will better investigate the association between these epigenetic alterations and SCZ.

**Funding**

None.

**Acknowledgments**

We want to thank all patients and healthy controls for their willingness to participate in the present study.

**Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

**Author Contributions**

Formulation of overarching research goals and aims: Hasan Mervan Aytac and Sacide Pehlivan. Conceived and designed the study: Hasan Mervan Aytac, Sacide Pehlivan, and Yasemin Oyaci. Provisions of study materials and laboratory samples: Sacide Pehlivan and Yasemin Oyaci. Acquired, analyzed, and interpreted all data: Hasan Mervan Aytac and Mustafa Pehlivan. Drafted the manuscript: Hasan Mervan Aytac. Supervised the study: Sacide Pehlivan and Mustafa Pehlivan.

**ORCID**

Hasan Mervan Aytac https://orcid.org/0000-0002-1053-6808
Yasemin Oyaci https://orcid.org/0000-0002-1338-0087
Mustafa Pehlivan https://orcid.org/0000-0002-6692-085X
Sacide Pehlivan https://orcid.org/0000-0003-1272-5845

**REFERENCES**

1. McGrath J, Saha S, Chant D, Welham J. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev 2008;30:67-76.
2. Pepper E, Cardno AG. Genetics of schizophrenia and other psychotic disorders. Curr Psychiatry Rev 2014;10:133-142.
3. Aleli-Paz R, Camorna FJ, Sanchez-Mut IV, Canega-Martinez A, Gonzalez-Corpes A, Ashour N, et al. Epigenetics in schizophrenia: a pilot study of global DNA methylation in different brain regions associated with higher cognitive functions. Front Psychol 2016;7:1496.
4. Aytac HM, Ozdilli K, Tuncel FC, Pehlivan M, Pehlivan S. Tumor necrosis factor-alpha (TNF-α) –238 G/A polymorphism is associated with the treatment resistance and attempted suicide in schizophrenia. Immunol Invest 2020:1-13.
5. Pehlivan S, Aydin PC, Aytac HM, Uysal MA, Sever Ü, Pehlivan M. Investigation of Catechol-O-Methyltransferase
and Cannabinoid Receptor 2 gene variants in tobacco use disorder or tobacco use disorder and schizophrenia comorbidity. Alpha Psychiatry 2020;21:572-578.
6. Jiménez-Jiménez FJ, Alonso-Navarro H, García-Martín E, Agüendé JA. COMT gene and risk for Parkinson’s disease: a systematic review and meta-analysis. Pharmacogenet Genomics 2014;24:331-339.
7. Aksoy S, Klenner J, Weinshilboum RM. Catechol O-methyltransferase pharmacogenetics: photoaffinity labelling and western blot analysis of human liver samples. Pharmacogenetics 1993;3:116-122.
8. Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melén K, Julkunen I, et al. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry 1995;34:4202-4210.
9. Craddock N, Owen MJ, O’Donovan MC. The catechol-O-methyltransferase (COMT) gene as a candidate for psychiatric phenotypes: evidence and lessons. Mol Psychiatry 2006;11:446-458.
10. Nour El Huda AR, Norsidah KZ, Nabil Fikri MR, Hanisah MN, Kartini A, Norlelawati AT. DNA methylation of membrane-bound catechol-O-methyltransferase in Malaysian schizophrenia patients. Psychiatry Clin Neurosci 2020;74:1-7.
11. Myöhänen TT, Schendzielorz N, Männistö PT, Pitelová R, Staif R, Znojil V, et al. Polymorphism of DRD2 gene and ADHD. Neuro Endocrinol Lett 2006;27:236-240.
12. Abdolmaleky HM, Cheng KH, Faraone SV, Wilcox M, Glatt SJ, et al. Association of DRD2 gene polymorphisms with mood disorders: a meta-analysis. J Affect Disord 2012;136:229-237.
13. Nohesara S, Ghadirivasfi M, Mostafavi S, Eskandari MR, Mostafavi M, et al. Epigenetic modification of the glucocorticoid receptor gene is linked to traumatic memory and post-traumatic stress disorder risk in genocide survivors. J Neurosci 2014;34:10274-10284.
14. Zahari Z, Teh LK, Ismail R, Razali SM. Influence of DRD2 polymorphisms on the clinical outcomes of patients with schizophrenia. Psychiatr Genet 2011;21:183-189.
15. Zou YF, Wang F, Feng XL, Li WF, Tian YH, Tao JH, et al. Association between methylation of the glucocorticoid receptor gene-specific (NR3C1) DNA methylation analysis in patients with cannabinoid or synthetic cannabinoid use disorder. Psychiatry Res 2021;298:113774.
16. Humphreys KL, Moore SR, Davis EG, Machaca JL, Lin DTS, Korb M, et al. DNA methylation of HPA-axis genes and the onset of major depressive disorder in adolescent girls: a prospective analysis. Transl Psychiatry 2019;9:245.
17. Vukojevic V, Kolassa IT, Fastenrath M, Spalek K, Chipchase J, et al. DNA hypomethylation of MB-COMT promoter in the DNA derived from saliva in schizophrenia and bipolar disorder. Hum Mol Genet 2006;15:3132-3143.
18. Vukojevic V, Kolassa IT, Fastenrath M, Spalek K, Chipchase J, et al. DNA hypomethylation of MB-COMT promoter in the DNA derived from saliva in schizophrenia and bipolar disorder. J Psychiatr Res 2011;45:1432-1438.
19. Zahari Z, Salleh MR, Zahri Johari MK, Musa N, Ismail R. A nested allele-specific multiplex polymerase chain reaction method for the detection of DRD2 polymorphisms. Malays J Med Sci 2011;18:44-57.
20. Collins AG, Frank MJ. Opponent actor learning (OpAL): modeling interactive effects of striatal dopamine on reinforcement learning and choice incentive. Psychol Rev 2014;121:337-366.
21. Conn KA, Burne THJ, Kelsey JP. Subcortical dopamine and cognition in schizophrenia: looking beyond psychosis in preclinical models. Front Neurosci 2020;14:542.
KH, et al. Glucocorticoid receptor gene variants and lower expression of NR3C1 are associated with cocaine use. Addict Biol 2019;24:730-742.

32. Misiak B, Samochowiec J, Konopka A, Gawrońska-Szklarz B, Beszlej JA, Szmida E, et al. Clinical correlates of the NR3C1 gene methylation at various stages of psychosis. Int J Neuropsychopharmacol 2021;24:322-332.

33. World Medical Association. World Medical Association’s Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013;310:2191-2194.

34. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987;13:261-276.

35. Kostakoglu E, Batur S, Tiryaki A, Gogus A. Reliability and validity of the Turkish version of the Positive and Negative Syndrome Scale (PANSS). Turk Psikiyri Dergisi 1999;14:23-34. Turkish.

36. Nohesara S, Ghadirivasfi M, Barati M, Chasemzadeh MR, Namirani S, Mousavi-Behbahani Z, et al. Methamphetamine-induced psychosis is associated with DNA hypomethylation and increased expression of AKT1 and key dopaminergic genes. Am J Med Genet B Neuropsychiatr Genet 2016;171:1180-1189.

37. Lind GE, Kleivi K, Meling GI, Teixeira MR, Thiss-Evensen E, Rognum TO, et al. ADAMTS1, CRABP1, and NR3C1 identified as epigenetically deregulated genes in colorectal tumorigenesis. Cell Oncol 2006;28:259-272.

38. 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;58:91-97.

39. Brown AS, Derkits EJ. Prenatal infection and schizophrenia: a meta-analysis. Int J Neuropsychopharmacol 2016;19:pyv132.

40. Arseneault L, Cannon M, Fisher HL, Polanczyk G, Moffitt TE, et al. Genetic and environmental factors in the development of schizophrenia. Acta Psychiatr Scand 2010;122:71-81.

41. Selten JP, Cantor-Graae E, Kahn RS. Migration and schizophrenia. Curr Opin Psychiatry 2006;19:111-115.

42. Bellette B, et al. DNA methylation in schizophrenia in different patient-derived cell types. NPJ Schizophr 2017;3:6.

43. Vonselvink R, Moreau V, Salloum E, et al. DNA methylation in schizophrenia and bipolar disorder: potential role in brain development and neurodevelopmental disorders. Transl Psychiatry 2021;11:1-9.

44. Huang HS, Akbarian S, GAD1 mRNA expression and DNA methylation in prefrontal cortex of subjects with schizophrenia. PLoS One 2007;2:e809.

45. Abdolmaleky HM, Cheng KH, Russo A, Smith CL, Farano SV, Wilcox M, et al. Hypermethylation of the reelin (RELN) promoter in the brain of schizophrenic patients: a preliminary report. Am J Med Genet B Neuropsychiatr Genet 2005;134B:60-66.

46. Zhuhi A, Veldic M, Puri NV, Kadiu B, Caruncho H, Loza I, et al. An upregulation of DNA-methyltransferase 1 and 3a expressed in telencephalic GABAergic neurons of schizophrenia patients is also detected in peripheral blood lymphocytes. Schizophr Res 2009;111:115-122.

47. Carrard A, Salzmann A, Malafosse A, Karege F. Increased DNA methylation status of the serotonin receptor 5HT1A gene promoter in schizophrenia and bipolar disorder. J Affect Disord 2011;132:450-453.

48. Abdolmaleky HM, Yaqubi S, Papageorgis P, Lambert AW, Ortizurk S, Sivaraman V, et al. Epigenetic dysregulation of HTR2A in the brain of patients with schizophrenia and bipolar disorder. Schizophr Res 2011;129:183-190.

49. Mill J, Tang T, Kaminsky Z, Khare T, Yazdanpanah S, Bouchard L, et al. Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. Am J Hum Genet 2008;82:696-711.

50. Ruzicka WB, Subharuraj B, Benes FM. Circuit-and diagnosis-specific DNA methylation changes at y-amino butyric acid-related genes in postmortem human hippocampus in schizophrenia and bipolar disorder. JAMA Psychiatry 2015;72:541-551.

51. Wockner LF, Noble EP, Lawford BR, Young RM, Morris CP, Whitehall VL, et al. Genome-wide DNA methylation analysis of human brain tissue from schizophrenia patients. Transl Psychiatry 2014;4:e339.

52. Goghri VM, Sponheim SR. Differential association of the COMT Val158Met polymorphism with clinical phenotypes in schizophrenia and bipolar disorder. Schizophr Res 2008;103:186-191.

53. Clelland CL, Drouet V, Rilett KC, Smeed JA, Nadrich RH, Rajparia A, et al. Evidence that COMT genotype and proline interact on negative-symptom outcomes in schizophrenia and bipolar disorder. Transl Psychiatry 2016;6:e689.

54. Loch AA, van de Bilt MT, Bio DS, Prado CM, de Sousa RT, Valiengo LL, et al. Epistasis between COMT Val158Met and DRD3 Ser9Gly polymorphisms and cognitive function in schizophrenia: genetic influence on dopamine transmission. Braz J Psychiatry 2015;37:235-241.

55. Huang E, Zai CC, Lisoway A, Maciukiewicz M, Felsky D, Tiwari AK, et al. Catechol-o-methyltransferase Val158Met polymorphism and clinical response to antipsychotic treatment in schizophrenia and schizoaffective disorder patients: a meta-analysis. Int J Neuropsychopharmacol 2016;19:pyw132.

56. Walton E, Liu J, Hass J, White T, Scholz M, Roessner V, et al. MB-COMT promoter DNA methylation is associated with working-memory processing in schizophrenia patients and healthy controls. Epigenetics 2014;9:1101-1107.

57. Murphy BC, O’Reilly RL, Singh SM. Site-specific cytosine methylation in S-COMT promoter in 31 brain regions with implications for studies involving schizophrenia. Am J Med Genet B Neuropsychiatr Genet 2005;133B:37-42.
58. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature 2014;511:421-427.

59. Dempster EL, Pidsley R, Schalkwyk LC, Owens S, Georgiades A, Kane F, et al. Disease-associated epigenetic changes in monozygotic twins discordant for schizophrenia and bipolar disorder. Hum Mol Genet 2011;20:4786-4796.

60. Kordi-Tamandani DM, Sahranavard R, Torkamanzehi A. Analysis of association between dopamine receptor genes’ methylation and their expression profile with the risk of schizophrenia. Psychiatr Genet 2013;23:183-187.

61. Zhang AP, Yu J, Liu JX, Zhang HY, Du YY, Zhu JD, et al. The DNA methylation profile within the 5’-regulatory region of DRD2 in discordant sib pairs with schizophrenia. Schizophr Res 2007;90:97-103.

62. Sinclair D, Tsai SY, Woon HG, Weickert CS. Abnormal glucocorticoid receptor mRNA and protein isoform expression in the prefrontal cortex in psychiatric illness. Neuropsychopharmacology 2011;36:2698-2709.

63. Sinclair D, Fullerton JM, Webster MJ, Shannon Weickert C. Glucocorticoid receptor 1B and 1C mRNA transcript alterations in schizophrenia and bipolar disorder, and their possible regulation by GR gene variants. PLoS One 2012;7:e31720.

64. Ripke S, O’Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, et al. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 2013;45:1150-1159.

65. Stein JL, Medland SE, Vasquez AA, Hilsar DP, Senstad RE, Winkler AM, et al. Identification of common variants associated with human hippocampal and intracranial volumes. Nat Genet 2012;44:552-561.

66. Park JS, Lee SM, Kim JY, Kang WS. NR3C1 polymorphisms for genetic susceptibility to schizophrenia. Korean J Biol Psychiatry 2019;26:88-93.

67. Liu L, Wu J, Qing L, Li J, Yang H, Ji A, et al. DNA methylation analysis of the NR3C1 gene in patients with schizophrenia. J Mol Neurosci 2020;70:1177-1185.