Clinical Psychopharmacology and Neuroscience - Manuscript Submission

- **Manuscript ID**: CPN-21-902
- **Title**: DNA Methylation Pattern of Gene Promoters of MB-COMT, DRD2, and NR3C1 in Turkish Patients Diagnosed with Schizophrenia
- **Running Title**: Methylation Status of MB-COMT, DRD2, and NR3C1 in SCZ
- **Article Type**: Original Article
- **KeyWords**: Schizophrenia, COMT, DRD2, NR3C1, DNA methylation, epigenetics, methylation-specific polymerase chain reaction

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

ABSTRACT

Objective: Genetics alone can not explain the etiopathology of schizophrenia (SCZ). It is well-known that environmental risk factors exposed during early development and young adulthood also contribute to SCZ in susceptible individuals. 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 ~~schizophrenia (~~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 (SCID-I) was used to confirming the diagnosis according to DSM-IV-TR criteria. Then the patients were evaluated with the Positive and Negative Symptoms Scale (PANSS) in terms of symptom severity. Methylation-specific polymerase chain reaction (MSP-PCR) 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% Cl: 0.268–0.809; p=~~0~~.006), *DRD2* (OR: 0.439; 95% Cl: 0.375–0.514; p<~~0~~.001), and *NR3C1* (OR: 0.003; 95% Cl: 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.

Keywords: Schizophrenia; ~~MB~~-COMT; DRD2; NR3C1; epigenetics; DNA methylation; methylation-specific polymerase chain reaction

서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴
서식 지정함: 글꼴: 기울임꼴

Introduction:

Schizophrenia (SCZ) is characterized by the heterogonous 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 can not 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 association 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, TaqI A, 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 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 (23). Altered *NR3C1* methylation is related to early stress exposure. It thus may cause the occurrence of psychopathologies including major depressive disorder (24), BD (25), SCZ (26), post-traumatic stress disorder (27), suicidal behavior (28), bulimia nervosa (29), borderline personality disorder, alcohol-tobacco consumption (30), and cocaine use disorder (31). Recently, Misiak et al. 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 (32). No earlier researches have shown the methylation status of *MB-COMT* promotor, *DRD2*, and *NR3C1* gene on SCZ in the Turkish population. We hypothesized that alteration of the methylation status of *MB-COMT* promotor, *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* promotor, *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 The study was approved by the Clinical Research Ethics Committee of Istanbul Faculty of

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 gender. The Positive and Negative Symptoms Scale (PANSS) was used to evaluate positive symptoms, negative symptoms, and general psychopathology in psychotic patients and measure the severity of these symptoms (34, 35). Subjects of 18 to 65 years of age, of either gender, literate, agreed on the participation in the study, diagnosed with SCZ according to the SCID-I interview, had no other systemic/neurological disorder that may affect cognitive functions included in the study. We had excluded subjects who refused participation or had mental retardation, neurodevelopmental disorders, a diagnosis of axis-1 disorder other than SCZ, psychosis secondary to a general medical condition.

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 (EDTA) tubes. Genomic DNA was obtained

from the blood samples by using the GeneMark isolation kit. (Genemark, Plus Blood Genomic Purification Kit, USA).

Methylation-specific polymerase chain reaction (MSP PCR):

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 95^0 C, 40 cycles (30 seconds at 95^0 C, 40 seconds at annealing temperature for each primer, and 45 seconds at 72^0 C), and 72^0 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.

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.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/).

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.

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* promoter gene methylation in SCZ patients were compared with the control group, the percentages of *MB-COMT* promoter methylation of SCZ were found to be significantly different from the control group (OR: 0.466; 95% Cl: 0.268–0.809; p=.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% Cl: 0.375–0.514; p<.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% Cl: 0.001–0.011; p<.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 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. recognized significant differential methylation in genes previously related to SCZ, including *DNMT1*, Nitric Oxide Synthase 1 (*NOS1*), and SRY-related HMG-box 10 (*SOX10*) (51).

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. did not find significant associations between the *COMT* methylation percentages and age at onset, duration of illness, PANSS total score except for the psycho-pathological symptoms (10). We assume that the lower *COMT* methylation rates are specific to SCZ pathogenesis and independent of scale scores and other clinical parameters.

Recently, large-scale genome-wide association research identified a significant relationship between *DRD2* and SCZ (58). However, no trait biomarkers regarding *DRD2* are

available. Again, methylation of DNA is a critical epigenetic mechanism in the regulation of gene expression. The genetic and environmental factor's impact on specific changes in DNA methylation is mainly unknown. From genome-wide methylation research comparing peripheral blood from SCZ discordant monozygotic twins, and healthy controls, differences in DNA methylation in several genes were identified as diagnostic biomarkers. However, *DRD2* was not detected in those studies (38, 59). One notable finding in our study is that the methylation percentages of the *DRD2* gene were significantly higher in SCZ patients compared to control subjects. When the literature was reviewed, it was seen that only several types of research about DNA methylation of *DRD2* by using peripheral leukocytes had been reported. Increased promoter methylation of dopamine receptor genes, including *DRD4*, *DRD5*, and *DRD2*, was shown in SCZ patients, similar to our study (60). Conversely, in a previous study, Yoshiro did not find significant associations between the methylation percentages at individual CpG sites and age at onset, duration of illness, BPRS, or DIEPSS; even lower methylation rates were reported at other CpG sites of *DRD2* in the SCZ group (22). Again, Zhang et al. did not find methylated CpG sites at the same CpG sites (CpG1 to 7) in a sib-pair study (61). These *DRD2* genes' methylation results have highlighted that the *DRD* gene network, overall, is actively involved in the increased risk of SCZ.

The hypothalamic-pituitary-adrenal (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. published that two single nucleotide polymorphisms of *NR3C1* (rs7701443 and rs2963155) may be associated with susceptibility to SCZ in the Korean population (66). 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. study. They reported significantly lower Our results are in line with the Misiak et al. study. They reported significantly lower Our results are in line with the Misiak et al. 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 (32). 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* promotor, *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* promotor 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* promotor, *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.

Acknowledgments

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

Statement of interest

All authors declare not to have any conflicts of interest that might be interpreted as influencing the manuscript's content.

Funding

The authors received no specific funding for this work.

Contributions of authors

HMA and SP are responsible for the formulation of overarching research goals and aims. HMA, SP, and YO conceived and designed the study. SP and YO are the responsible provisions of study materials and laboratory samples. HMA, MP acquired, analyzed, and interpreted all data. HMA drafted the manuscript. SP and MP supervised the study.

References:

1. McGrath J, Saha S, Chant D, Welham J. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiologic reviews. 2008;30(1):67-76.

2. Pepper E, G Cardno A. Genetics of schizophrenia and other psychotic disorders. Current Psychiatry Reviews. 2014;10(2):133-42.

3. Alelú-Paz R, Carmona FJ, Sanchez-Mut JV, Cariaga-Martínez A, González-Corpas A, Ashour N, et al. Epigenetics in schizophrenia: a pilot study of global DNA methylation in different brain regions associated with higher cognitive functions. Frontiers in psychology. 2016;7:1496.

4. Vitale AM, Matigian NA, Cristino AS, Nones K, Ravishankar S, Bellette B, et al. DNA methylation in schizophrenia in different patient-derived cell types. npj Schizophrenia. 2017;3(1):1-11.

변경된 필드 코드

5. Pehlivan S, AYDIN PÇ, Aytaç 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. Anatolian Journal of Psychiatry. 2020;21:0-.

6. Jiménez-Jiménez FJ, Alonso-Navarro H, García-Martín E, Agúndez JA. COMT gene and risk for Parkinson's disease: a systematic review and meta-analysis. Pharmacogenetics and Genomics. 2014;24(7):331-9.

7. Aksoy S, Klener J, Weinshilboum RM. Catechol O-methyltransferase pharmacogenetics: photoaffinity labelling and western blot analysis of human liver samples. Pharmacogenetics. 1993;3(2):116-22.

8. Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen 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(13):4202-10.

9. Craddock N, Owen MJ, O'Donovan MC. The catechol-O-methyl transferase (COMT) gene as a candidate for psychiatric phenotypes: evidence and lessons. Molecular psychiatry. 2006;11(5):446-58.

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 and clinical neurosciences. 2018;72(4):266-79.

11. Myöhänen TT, Schendzielorz N, Männistö PT. Distribution of catechol-O-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice. Journal of neurochemistry. 2010;113(6):1632-43.

12. Abdolmaleky HM, Cheng K-h, 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. Human molecular genetics. 2006;15(21):3132-45.

13. Nohesara S, Ghadirivasfi M, Mostafavi S, Eskandari M-R, Ahmadkhaniha H, Thiagalingam S, et al. DNA hypomethylation of MB-COMT promoter in the DNA derived from saliva in schizophrenia and bipolar disorder. Journal of psychiatric research. 2011;45(11):1432-8.

14. Zahari Z, Salleh MR, Zahri MK. A Nested Allele-Specific Multiplex Polymerase Chain Reaction Method for the Detection of DRD2 Polymorphisms. The Malaysian Journal of Medical Sciences: MJMS. 2011;18(4):44.

15. Collins AG, Frank MJ. Opponent actor learning (OpAL): modeling interactive effects of striatal dopamine on reinforcement learning and choice incentive. Psychol Rev. 2014;121(3):337-66.

16. Zou Y-F, Wang F, Feng X-L, Li W-F, Tian Y-H, Tao J-H, et al. Association of DRD2 gene polymorphisms with mood disorders: a meta-analysis. Journal of affective disorders. 2012;136(3):229-37.

17. Conn K-A, Burne TH, Kesby JP. Subcortical Dopamine and Cognition in Schizophrenia: Looking Beyond Psychosis in Preclinical Models. Frontiers in Neuroscience. 2020;14:542.

18. Serý O, Drtílková I, Theiner P, Pitelová R, Staif R, Znojil V, et al. Polymorphism of DRD2 gene and ADHD. Neuro Endocrinol Lett. 2006;27(1-2):236-40.

19. Schäfer M, Rujescu D, Giegling I, Guntermann A, Erfurth A, Bondy B, et al. Association of short-term response to haloperidol treatment with a polymorphism in the dopamine D2 receptor gene. American Journal of Psychiatry. 2001;158(5):802-4.

20. Zahari Z, Teh LK, Ismail R, Razali SM. Influence of DRD2 polymorphisms on the clinical outcomes of patients with schizophrenia. Psychiatric genetics. 2011;21(4):183-9.

21. Zhang J-P, Lencz T, Malhotra AK. D2 receptor genetic variation and clinical response to antipsychotic drug treatment: a meta-analysis. American Journal of Psychiatry. 2010;167(7):763-72.

22. Yoshino Y, Kawabe K, Mori T, Mori Y, Yamazaki K, Numata S, et al. Low methylation rates of dopamine receptor D2 gene promoter sites in Japanese schizophrenia subjects. The World Journal of Biological Psychiatry. 2016;17(6):449-56.

23. 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(8):1177-85.

24. Humphreys KL, Moore SR, Davis EG, MacIsaac JL, Lin DT, Kobor MS, et al. DNA methylation of HPA-axis genes and the onset of major depressive disorder in adolescent girls: a prospective analysis. Translational psychiatry. 2019;9(1):1-10.

25. Perroud N, Dayer A, Piguet C, Nallet A, Favre S, Malafosse A, et al. Childhood maltreatment and methylation of the glucocorticoid receptor gene NR3C1 in bipolar disorder. Br J Psychiatry. 2014;204(1):30-5.

26. 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. Journal of Molecular Neuroscience. 2020:1-9.

27. Vukojevic V, Kolassa IT, Fastenrath M, Gschwind L, Spalek K, Milnik A, 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(31):10274-84.

28. Park S, Hong JP, Lee J-K, Park Y-M, Park Y, Jeon J, et al. Associations between the neuron-specific glucocorticoid receptor (NR3C1) Bcl-1 polymorphisms and suicide in cancer patients within the first year of diagnosis. Behavioral and Brain Functions. 2016;12(1):22.

29. McDonald S. Understanding the genetics and epigenetics of bulimia nervosa/bulimia spectrum disorder and comorbid borderline personality disorder (BN/BSD-BPD): a systematic review. Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity. 2019:1-16.

30. Dogan MV, Lei MK, Beach SR, Brody GH, Philibert RA. Alcohol and tobacco consumption alter hypothalamic pituitary adrenal axis DNA methylation. Psychoneuroendocrinology. 2016;66:176-84.

31. Schote AB, Jäger K, Kroll SL, Vonmoos M, Hulka LM, Preller KH, et al. Glucocorticoid receptor gene variants and lower expression of NR3C1 are associated with cocaine use. Addict Biol. 2019;24(4):730-42.

32. Misiak B, Samochowiec J, Konopka A, Gawrońska-Szklarz B, Beszłej JA, Szmida E, et al. Clinical correlates of the NR3C1 gene methylation at various stages of psychosis. International Journal of Neuropsychopharmacology. 2021;24(4):322-32.

33. Association WM. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. Jama. 2013;310(20):2191-4.

34. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull. 1987;13(2):261-76.

35. Kostakoğlu A, Batur S, Tiryaki A, Göğüş A. Pozitif ve negatif sendrom ölçeğinin (PANSS) Türkçe uyarlamasının geçerlilik ve güvenilirliği. Türk Psikoloji Dergisi. 1999;14(44):23-32.

36. Nohesara S, Ghadirivasfi M, Barati M, Ghasemzadeh MR, Narimani S, Mousavi-Behbahani Z, et al. Methamphetamine-induced psychosis is associated with DNA hypomethylation and increased expression of AKT1 and key dopaminergic genes. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2016;171(8):1180-9.

37. Lind GE, Kleivi K, Meling GI, Teixeira MR, Thiis-Evensen E, Rognum TO, et al. ADAMTS1, CRABP1, and NR3C1 identified as epigenetically deregulated genes in colorectal tumorigenesis. Analytical Cellular Pathology. 2006;28(5, 6):259-72.

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. Journal of human genetics. 2013;58(2):91-7.

39. Brown AS, Derkits EJ. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. American Journal of Psychiatry. 2010;167(3):261-80.

40. Arseneault L, Cannon M, Fisher HL, Polanczyk G, Moffitt TE, Caspi A. Childhood trauma and children's emerging psychotic symptoms: a genetically sensitive longitudinal cohort study. American journal of Psychiatry. 2011;168(1):65-72.

41. Selten J-P, Cantor-Graae E, Kahn RS. Migration and schizophrenia. Current opinion in psychiatry. 2007;20(2):111-5.

42. Vitale AM, Matigian NA, Cristino AS, Nones K, Ravishankar S, Bellette B, et al. DNA methylation in schizophrenia in different patient-derived cell types. NPJ Schizophr. 2017;3:6.

43. Ovenden ES, McGregor NW, Emsley RA, Warnich L. DNA methylation and antipsychotic treatment mechanisms in schizophrenia: Progress and future directions. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2018;81:38-49.

44. Huang H-S, Akbarian S. GAD1 mRNA expression and DNA methylation in prefrontal cortex of subjects with schizophrenia. PloS one. 2007;2(8):e809.

45. 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. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2005;134(1):60-6.

46. Zhubi A, Veldic M, Puri N, Kadriu 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. Schizophrenia research. 2009;111(1-3):115-22.

47. Carrard A, Salzmann A, Malafosse A, Karege F. Increased DNA methylation status of the serotonin receptor 5HTR1A gene promoter in schizophrenia and bipolar disorder. Journal of affective disorders. 2011;132(3):450-3.

48. 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. Schizophrenia research. 2011;129(2-3):183-90.

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. The American Journal of Human Genetics. 2008;82(3):696-711.

50. Ruzicka WB, Subburaju S, Benes FM. Circuit-and diagnosis-specific DNA methylation changes at γ-aminobutyric acid–related genes in postmortem human hippocampus in schizophrenia and bipolar disorder. JAMA psychiatry. 2015;72(6):541-51.

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. Translational psychiatry. 2014;4(1):e339-e.

52. Goghari VM, Sponheim SR. Differential association of the COMT Val158Met polymorphism with clinical phenotypes in schizophrenia and bipolar disorder. Schizophrenia research. 2008;103(1-3):186-91.

53. Clelland C, Drouet V, Rilett K, Smeed J, Nadrich R, Rajparia A, et al. Evidence that COMT genotype and proline interact on negative-symptom outcomes in schizophrenia and bipolar disorder. Translational psychiatry. 2016;6(9):e891-e.

54. Loch AA, van de Bilt MT, Bio DS, Prado CMd, Sousa RTd, Valiengo LL, et al. Epistasis between COMT Val158Met and DRD3 Ser9Gly polymorphisms and cognitive function in schizophrenia: genetic influence on dopamine transmission. Brazilian Journal of Psychiatry. 2015;37(3):235-41.

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 schizo-affective disorder patients: a meta-analysis. International Journal of Neuropsychopharmacology. 2016;19(5).

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(8):1101-7.

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. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2005;133(1):37-42.

58. Consortium SWGotPG. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511(7510):421-7.

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. Human molecular genetics. 2011;20(24):4786-96.

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. Psychiatric Genetics. 2013;23(5):183-7.

61. Zhang A-p, Yu J, Liu J-x, Zhang H-y, Du Y-y, He G, et al. The DNA methylation profile within the 5′-regulatory region of DRD2 in discordant sib pairs with schizophrenia. Schizophrenia research. 2007;90(1-3):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(13):2698-709.

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(3):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(10):1150-9.

65. Stein JL, Medland SE, Vasquez AA, Hibar DP, Senstad RE, Winkler AM, et al. Identification of common variants associated with human hippocampal and intracranial volumes. Nat Genet. 2012;44(5):552-61.

66. Park J-S, Lee S-M, Kim J-W, Kang W-S. NR3C1 Polymorphisms for Genetic Susceptibility to Schizophrenia. Korean Journal of Biological Psychiatry. 2019;26(2):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. Journal of Molecular Neuroscience. 2020;70(8):1177-85.

Tables:

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. (ºC)/Cycle count
DRD2	**MSP M**	CGTTTAGGTCGGGGATCGTCG	GACGCCCGAACGCGAAAAACGCG	67/40
	MSP U	TGTTTAGGTTGGGGATTGTTG	AACACCCAAACACAAAAAACACA	56/40
MB-COMT	**MSP M**	TATTTTGGTTATCGTCGCGC	AACGAACGCAAACCGTAACG	56/40
	MSP U	TATTTTGGTTATTGTTGTGT	AACAAACACAAACCATAACA	56/40
NR3C1	**MSP M**	TCGGTTTCGTTCGTTCGTTTAGGTC	CGTCCCGATCCCAACTACTTCGAC	69/40
	MSP U	TTGGTTTTGTTTGTTTGTTTAGGTT	CCATCCCAATCCCAACTACTTCAAC	61/40

Abbreviations: PCR, Polymerase chain reaction; MSP, Methylation-specific PCR; M, Methylated; U, Unmethylated

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

		Schizophrenia (N:110)
		n (%)
Gender	**Male**	80 (72.7)
	Female	30 (27.3)
MB-COMT Methylation	**Partial**	42 (38.2)
	Unmethylated	68 (61.8)
DRD2 Methylation	**Partial**	110 (100)
	Unmethylated	0 (0)
NR3C1 Methylation	**Partial**	3 (2.7)
	Unmethylated	107 (97.3)

	Mean±SD
Age	39.2±8.9
Age of onset (year)	24±7.6
Number of Hospt.	3.4±4.5
Last Hospt. (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

Methylation	Genotype	SCZ	Healthy Control	OR	95% CI	p*
		n= [a] (%)	n=100 (%)			
COMT	Unmethylation	68 (61.8)	43 (43.0)			
	Partial methylation	42 (38.2)	57 (57.0)	0.466	0.268-0.809	.006*
DRD2	Unmethylation	0 (0.0)	14 (14)			
	Partial methylation	110 (100.0)	86 (86)	0.439	0.375-0.514	.000*
NR3C1	Unmethylation	107 (97.3)	9 (9)			
	Partial methylation	3 (2.7)	91 (91)	0.003	0.001-0.011	.000*

[a]n= 110; *, Pearson chi-square.

Abbreviations: SD, standard deviation; hospt., hospitalization PANSS, positive and negative syndrome scale; pos., positive; neg., negative; psycho., psychopathology

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

~~Methylation~~	~~Genotype~~	~~SCZ~~	~~Healthy Control~~	~~OR~~	~~95% CI~~	~~p*~~
		~~n=* (%)~~	~~n=100 (%)~~			
~~COMT~~	~~Unmethylation~~	~~68 (61.8)~~	~~43 (43.0)~~			
	~~Partial methylation~~	~~42 (38.2)~~	~~57 (57.0)~~	~~0.466~~	~~0.268-0.809~~	~~0.006*~~
~~DRD2~~	~~Unmethylation~~	~~0 (0.0)~~	~~14 (14)~~			
	~~Partial methylation~~	~~110 (100.0)~~	~~86 (86)~~	~~0.439~~	~~0.375-0.514~~	~~0.000*~~
~~NR3C1~~	~~Unmethylation~~	~~107 (97.3)~~	~~9 (9)~~			
	~~Partial methylation~~	~~3 (2.7)~~	~~91 (91)~~	~~0.003~~	~~0.001-0.011~~	~~0.000*~~

~~*n= 110; *, Pearson chi-square.~~