A new aspect on the correlation of ten SNPs in MIR and their target genes in dopaminergic pathways in schizophrenia

Ali Molaei1†, Mohadeseh Agahi1†, Mahtash Malekian1, Bahareh Moradhasel1, Ardalan Tajrezaee1, Ava Lajevardi1, Iman Salahshourifar1, Niloufar Mahdavi Hezaveh2, Gholamreza Javadi1 and Zahra Noormohammadi1*

Abstract
Background: Schizophrenia (SCZ) is a severe mental disorder in which people interpret reality abnormally. Different studies indicated a complex polygenic control over SCZ. In the present study, we investigated the potential correlation between ten SNPs among MicroRNA (MIR) and their target genes; rs369770942, rs143525573, rs200982455, rs530404895, rs753764536, rs374732351, rs4680, rs165599, rs340597269, and rs10759, and schizophrenia in the Iranian population.

Results: The results revealed that the T allele in rs200982455 increased the risk factor by 3.19 times. We obtained a significant association between rs165599 and schizophrenia in codominant, dominant, and overdominant inheritance models (P = 0.016, P = 0.01, P = 0.004, respectively). Moreover, the risk of schizophrenia increased in the presence of the G allele in rs165599 up to 2.12, 2.35, and 2.28 times, respectively. The A allele in rs10759 increased the risk factor up to 1.05 times.

Conclusion: Our finding showed that some of the studied SNPs within the genes and MIRs involved in the dopaminergic pathway may consider as a biomarker in the diagnostic patterns in Schizophrenia.

Keywords: Dopamine, MicroRNA, Polymorphism, Schizophrenia, SNPs

Highlights
• The association between SNPs in COMT gene and schizophrenia
• The correlation of SNPs in target genes and MIRs in the dopaminergic pathway

*Correspondence: marjannm@yahoo.com; z-nouri@srbiau.ac.ir
†Ali Molaei and Mohadeseh Agahi contributed equally to this work.
1 Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
Full list of author information is available at the end of the article

Background
Schizophrenia (SCZ) is a mental disorder with 1% lifetime prevalence. Different genes and processes have been considered as potential causes of schizophrenia. For instance, malfunctioning dopamine receptors, which play an essential role in dopaminergic pathways, are one of the causes of developing schizophrenia (Kuncara 2019; Liu et al. 2014). The level of dopamine content is significantly higher in patients with schizophrenia than that of healthy individuals (Grace 2016; Kesby et al. 2018). The genetics of SCZ is considered a complex disease without a specified inheritance model. A multi-locus model has been proposed to indicate the pattern of heritability in this complicated disorder. The model illuminates that a composition of various genetic facets is involved in schizophrenia (Risch 1990). Thereby, the development of SCZ
might be affected by more than one locus and consists of a complex polygenic interaction (Estrada et al. 2011; Raznahan et al. 2011; Singh et al. 2012). Dopamine receptors, such as the Dopamine D1 family (DRD1 and DRD5 receptors) as well as the Dopamine D2 family (including DRD2, -DRD4 receptors), act as G-protein coupled receptors (Funahashi et al. 2019; Kuncara 2019; Liu et al. 2014; Poorshekar et al. 2019). The regulator of G-protein signaling 4 (RGS4) is another candidate gene for schizophrenia, which is known to have a significant role in brain development stages, such as neuronal differentiation and the formation of new axons (Schwarz 2018; Chowdari et al. 2008; Ding et al. 2016; Paspalas et al. 2009).

The Neuregulin-1 gene (NRG-1), located on chromosome 8p13, is also considered a potential gene for schizophrenia (Zhang et al. 2008). Alternations in the NRG1 function expressed in dopaminergic neurons are associated with schizophrenia (Ledonne et al. 2015). Catechol-O-methyltransferase (COMT) is an enzyme that has a significant role in dopamine catabolism and has been reported to be associated with schizophrenia (Gozukara Bag 2018; Morozova et al. 2019). The rs4680 (Val158Met polymorphism) and rs165599 in the COMT gene are two polymorphism and rs10759 in the MIRs snps, and schizophrenia. Shifman claimed that the C-GG haplotype for the SNPs rs737865-rs4680-rs165599 was correlated with schizophrenia in Ashkenazi Jews (Shifman et al. 2002). Additionally, rs10759 (RGS4) raised the risk of schizophrenia by miRNA altering the binding of miRNAs to their targets, influencing susceptibility to schizophrenia (Gong et al. 2013), although Williams and Nunokawa reported that rs4680 and rs165599 might not be associated with schizophrenia (Williams et al. 2005; Nunokawa et al. 2007). Therefore, in the current research, we aimed to study (1) Correlation between ten SNPs: rs369770942, rs143525573, rs200982455, rs530404895, rs753764536, rs374732351, and rs340597269 needed to be studied, not only because of their remarkable genomic locations but were also explored neither Iran nor across the world.

Despite their probable effect on dopaminergic pathways and brain development stages, which are supposed to play a crucial role in SCZ, their correlation with the disorder was entirely neglected to be explored up to the current study. We faced either inadequate or inconsistent results due to the correlation studies between rs4680, rs10759, rs165599, and schizophrenia. Shifman claimed that the C-GG haplotype for the SNPs rs737865-rs4680-rs165599 was correlated with schizophrenia in Ashkenazi Jews (Shifman et al. 2002). Additionally, rs10759 (RGS4) raised the risk of schizophrenia by miRNA altering the binding of miRNAs to their targets, influencing susceptibility to schizophrenia (Gong et al. 2013), although Williams and Nunokawa reported that rs4680 and rs165599 might not be associated with schizophrenia (Williams et al. 2005; Nunokawa et al. 2007). Therefore, in the current research, we aimed to study (1) Correlation between ten SNPs: rs369770942, rs143525573, rs200982455, rs530404895, rs753764536, rs374732351, and rs340597269, and rs10759 in MIRS and their target genes, and (2) Investigating the potential association of these SNPs with schizophrenia in the Iranian population.

We opted for five SNPs situated in 3’UTR of NRG1, DRD2, COMT, and RGS4 genes for this study. They are presumed to be associated with schizophrenia (Cui and Jiang 2012; Luykx et al. 2017; Shariati et al. 2011; Shifman et al. 2002). Also, the other five SNPs which are located in MIR-125-a, MIR-326, and MIR-124 genes, are supposed to be related to either adjustment of NRG1, DRD2, COMT, and RGS4 genes or schizophrenia (Camkurt et al. 2016; Gong et al. 2013; Shi et al. 2014).

Methods

SNP data retrieval

NCBI dbSNP is the most extensive SNP database; thus, the ten SNPs were chosen and retrieved from this database. The criteria for choosing these SNPs were their susceptibility to being digested with restriction enzymes in desired locations in the 3’UTR region of the target genes and the 5’UTR region of the MicroRNA genes. The workflow of choosing SNPs is shown in Fig. 1. PANSS (Positive and negative syndrome scale): a 30-item rating scale with three subscales which evaluates positive and negative symptoms and global psychopathology. Patients can rate each item from 1 to 7, so the minimum score is 30 and the maximum is 210. The reliability and validity of the scale have been approved and showed good psychometric properties (Kay et al. 1988), and it has been used widely in patients with schizophrenia in Iran (Chaychi et al. 2015; Ghanbari Jolfaei et al. 2012; Hatami et al. 2017).
In the present study, 102 patients with schizophrenia (67 males and 35 females) referred to the psychiatric ward of Imam Hossein Educational Hospital and 506 Artesh hospital, Tehran, Iran, were selected. For this purpose, the subjects’ medical records were first studied, and their details were compared to this research’s conditions. According to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, Text Revision (DSM-IV-TR), patient entry requirements for this study were verified using research tools, including demographic questionnaire (age, gender, and treatment response), the positive and negative syndrome scale test (PANSS), and clinical interview. Finally, the disorder was confirmed by a psychiatrist. The criteria for excluding patients from the study included schizoaffective or any other psychiatric disorder, mental retardation, drug, and stimuli abuse. In order to observe ethical considerations, each patient and their supervisor received written consent. On the other hand, 100 control subjects (62 males and 38 females) were selected from healthy individuals whose mental health was confirmed by clinical interview. The conditions for selecting control subjects included lack of schizophrenia, severe psychiatric disorder or other physical illness, lack of family history of severe mental disorders, and non-use of drugs and stimuli. The PANSS also was done for controls.

This research has an ethical code IR.IAU.SRB.REC.1396.43. The demographic characters were questioned, including sex, age, education, ethnics, job, alcohol and drug usage, relative responses to treatment, and family history.

**Genomic DNA extraction**

The peripheral blood sample was collected and transferred to the laboratory and stored at −70 °C for further study. Genomic DNA was extracted by using an optimized salting-out method and then stored at −20 °C. The quantity and quality of genomic DNA were examined by a 0.8% agarose gel electrophoresis and spectrophotometer.

**Genotyping**

In order to determine the genotypes, polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and PCR-sequencing were performed. Eight pairs of specific primers (which were designed by Primer3 and Gene Runner software) were used to amplify MIRs and their 3'UTR region target genes (Table 1).

PCR reactions for studied regions of the genome were performed in 25 μl containing 50 ng template DNA, 1X PCR buffer, 200 μM dNTP, 1.5 mM MgCl2, 0.4 μM of each of the forward and reverse primers, 1U Taq DNA polymerase (Cinnagen, Tehran, Iran).

PCR thermal program (Bio-Rad, USA) was as follows: initial denaturation at 94 °C for 5 min, followed by 40 cycles in three steps: 30 s at 94 °C, the annealing step for each pair of primers based on Table 1, 40 s at 72 °C, and final extension at 72 °C for 7 min. The PCR products were loaded on a %1.5 agarose gel and visualized by GelRed™ Nucleic Acid Gel Staining. Fragment size was measured by using a 100-bp molecular size ladder (Vivantis, Malaysia).

For digestion PCR products, restriction enzymes were used for each SNP (Table 1). The digested products were run on 2% agarose gel electrophoresis. For MIR326 SNPs, RGS1 (rs10759), as well as COMT (rs165599) (Table 1), the PCR products were sequenced. For confirming
genotypes in PCR–RFLP, PCR products were sequenced (Pishgam Company, Tehran, Iran).

**Data analysis**

The crude odds ratio (COR) and 95% confidence interval (CI) indicate a potential association between genotypes and the disease. Multiple comparisons were performed to avoid false-positive results using principal component analysis (PCA) using the PAST program. Hardy–Weinberg equilibrium was estimated by using the chi-square test. We considered \( p \)-value < 0.05 to be statistically significant. Hardy–Weinberg equilibrium was estimated by using the chi-square test. We considered \( p \)-value < 0.05 to be statistically significant. Haplotype frequency of SNPs’ genotypes and haplotype association between control and case samples were estimated using SNPstats software (https://www.snpstats.net/start.htm). The Holm–Bonferroni Method was used to correct the \( p \)-value LD test. For the prediction of single-nucleotide polymorphism effects on the miRNAs’ second structure, we used the RNAfold MFE tool. RNA fold (http://rna.tbi.univie.ac.at/cgibin/RNAWebSuite/RNAfold.cgi) is a web-based software developed to analyze the noncoding RNAs’ second structures (Lorenz et al. 2011).

**Results**

The demographic characters were obtained, including sex, age, education, ethnics, job, alcohol and drug usage, relative responses to treatment, and family history are provided in Additional file 1: Tables S1, S2 and S3.

The enzymatic digestion of rs369770942 (NC-000008.11) by DdeI produced two fragments with 148 bp and 652 bp size length in the presence of A allele (ancestral allele). There were three fragments with 441 bp, 211 bp, and 148 bp size length, which would be observed in the presence of the G allele (Table 2). The enzymatic digestion of rs143525573 (NC-000019.10) by DdeI gave rise to four fragments with 10 bp, 75 bp, 90 bp, and 359 bp size length in the presence of the G allele (ancestral allele). Conversely, we did not observe these five fragments in the presence of the A allele (Table 2).

Table 1 The primers, their sequences, Tm, product size used for PCR

| Region/SNP Primer sequence | Tm (°C) | Product size (bp) |
|----------------------------|---------|------------------|
| 3′UTR NRG1/rs369770942 Forward GGAGTATGAAAGCACCAAGAG 58/4 800 DdeI | | |
| miR-125a-3p/rs143525573 Reverse GAGGCCCTAGAGTGTG 58/3 800 DdeI | | |
| 3′UTR DRD2 Forward TTCTAGGCTCTGCCCCCTCC 56 534 DdeI | | |
| MIR326 Forward CTTGAGCACATGGACACATT 51 239 – | | |
| rs374732351 Reverse GCAAGAGGAAGAGAGACGACAGA 54 – | | |
| 3′UTR COMT rs4680 Forward CTCATCACCATGAGATCAACC 61 383 NLaII | | |
| rs166599 Reverse GCCATCTTTACACCCCATACA 59 – | | |
| 3′UTR COMT Forward CTCATCACCATGAGATCAACC 59 160 – | | |
| rs10759 Reverse TGCAGGTTCCTTCTAAATGTACAC 58 – | | |
| MIR124 Forward CCCCCTGGCTGTTACACAG 62 202 Asel | | |
| rs340597269 Reverse GCCATGTCGGCCGATTG 62 – | | |
Genotyping of the other SNPs, including rs530404895 (NC-000011.10), rs753764536 (NC-000011.10), rs374732351 (NC-000022.11), and rs10759 (NC-000022.11) genotypes, was done by using PCR sequencing (Table 2). The results obtained for the genotype frequencies and the inheritance models are presented in Table 2. Due to lack of heterogeneity, the assessment of p-value, OR (95% CI), and Linkage disequilibrium analysis were not performed for rs369770942, rs143525573, rs530404895, rs753764536, rs374732351, and rs4680.

A significant difference did not occur between case and control groups for rs200982455 (P = 0.28). Logistic regression indicated that the T/T genotype increased the risk factor 3.19 times.

A significant difference was detected between the case and control groups in codominant, dominant, and overdominant models for the rs165599 (P = 0.01, P = 0.01, and P = 0.004, respectively).

Logistic regression revealed that in the codominant model, the A/G genotype increased the risk factor by 2.35 times, while the G/G genotype increased the risk factor by 1.17 times.

In the dominant model, the risk factor was increased up to 2.14 times in the A/G-G/G genotypes presence. Similarly, in the recessive model, the risk factor was increased up to 0.71 times in the G/G genotype presence.

In the overdominant model, the A/G genotype increases the risk factor by 2.28 times.

A significant difference did not occur between the case and control groups for rs10759 (P = 0.89). Logistic regression revealed that the A/A genotype raised the risk factor by 1.05 times.

The PCA ordination based on 10 SNPs data showed partially differentiation between case and control samples while some samples showed admixture genotypes between two groups (Fig. 2).

The haplotype analysis of ten SNPs indicated that the highest frequency is belonged to AGC CCG AATC haplotype (0.495, rs369770942, rs143525573, rs200982455, rs530404895, rs753764536, rs374732351, and rs4680, rs165599, rs340597269, and rs10759, respectively). By considering the haplotype’s OR = 1.00, the approximate risk in 95% CI = 1.33 (0.80–2.23) P = 0.28, (OR 95% CI) = 0.88 (0.52–1.50) P = 0.64, (OR 95% CI) = 2.03 (0.88–4.70), P = 0.1 and (OR 95% CI) = 1.15 (0.28–4.72) P = 0.84, (Table 3).

### Table 2  Association of the ten studied polymorphisms with Schizophrenia assuming different genetic models

| Polymorphism                  | Model                  | Genotype | Case, n (%) | Control, n (%) | OR (95% CI) | P-value |
|------------------------------|------------------------|----------|-------------|----------------|-------------|---------|
| NRG1 /rs369770942            | –                      | AA       | 102 (100)   | 98 (100)       | –           | –       |
| miR-125a-3P/rs143525573      | –                      | GG       | 102 (100)   | 98 (100)       | –           | –       |
| 3′ UTR DRD2 rs200982455      | –                      | CC       | 102 (100)   | 98 (100)       | –           | –       |
| MIR326 rs330404895           | –                      | CC       | 102 (100)   | 98 (100)       | –           | –       |
| MIR326 rs753764536           | –                      | GG       | 102 (100)   | 98 (100)       | –           | –       |
| MIR326 rs374732351           | –                      | GG       | 102 (100)   | 98 (100)       | –           | –       |
| 3′ UTR COMT rs4680           | Codominant             | AA       | 47 (46.1%)  | 28 (28.6%)     | 1.00        | 0.016   |
|                              | AG                     | 45 (44.1%) | 63 (64.3%) | 2.35 (1.28–4.30) | 0.016 |
|                              | GG                     | 10 (9.8%)  | 7 (7.1%)    | 1.17 (0.40–3.44)| 0.016 |
| 3′ UTR COMT rs165599         | Dominant               | AA       | 47 (46.1%)  | 28 (28.6%)     | 1.00        | 0.016   |
|                              | AG/GG                  | 55 (53.9%) | 70 (71.4%) | 2.14 (1.19–3.84)| 0.016 |
|                              | Recessive              | AA/AG    | 92 (90.2%)  | 91 (92.0%)     | 1.00        | 0.5     |
|                              | GG                     | 10 (9.8%)  | 7 (7.1%)    | 0.71 (0.26–1.94)| 0.004 |
| 3′ UTR COMT rs4680           | Overdominant           | AA/GG    | 57 (55.9%)  | 35 (35.7%)     | 1.00        | 0.5     |
|                              | AG                     | 45 (44.1%) | 63 (64.3%) | 2.28 (1.29–4.03)| 0.004 |
| MIR124 rs340597269           |                        | TT       | 102 (100)   | 98 (100)       | –           | –       |
| 3′ UTR RGS4 rs10759          | –                      | CC       | 82 (80.4%)  | 78 (79.6%)     | 1.00        | 0.89    |
|                              | AA                     | 20 (19.6%) | 20 (20.4%) | 1.05 (0.53–2.10)| 0.89    |
Linkage disequilibrium (LD) was surveyed between three SNPs: rs200982455, rs165599, and rs10759. The analysis was based on the $D$ and $D'$ value, $r$ and associated $p$-value. These three SNPs of 3′UTR COMT, 3′UTR RGS4, and 3′UTR DRD2 genes showed no link together [(rs200982455- rs4680 ($D$ value = $-0.0021$, $D'$ value = 0.296, $R$ = $-0.0314$, $P$ = 0.5304), rs200982455-rs10759 ($D$ value = $-0.0039$, $D'$ value = 0.9851, $R$ = $-0.0704$, $P$ = 0.1594) and rs4680- rs10759 ($D$ value = $-0.006$, $D'$ value = 0.0843, $R$ = $-0.0313$, $P$ = 0.5317)].

The recessive inheritance model of rs165599 and rs10759, which have been examined in various populations, the association was not observed in the Iranian population, although logistic regression revealed that T/T genotype in rs200982455 increased the risk of schizophrenia up to 3.19 times.

The rs4680 is supposed as a significant SNP for conceiving the genetic etiology of psychiatric disorders (Taylor 2018). González-Castro declared that rs4680 is remarkably associated with schizophrenia in the allelic model in the Caucasian population (Gonzalez-Castro et al. 2016). On the other hand, the association was not observed in the Asian population in genetic models after eliminating heterogeneity. Our result showed homozygous genotype AA in all samples studied, minor allele frequency (MAF)
in this SNP. We have also found no association between rs4680 and schizophrenia in the Iranian population.

The rs165599 is considered a dubious SNP for schizophrenia (Okochi et al. 2009). Reported evidence is not consistent, with regard to the correlation between rs165599 and schizophrenia.

According to a meta-analysis, which contained both case–control and family-based studies, there was no association between rs165599 and schizophrenia (Okochi et al. 2009).

Acar stated that no association was detected between rs165599 and schizophrenia (Acar et al. 2015). Meanwhile, our result showed the association in codominant, dominant, and overdominant inheritance models between rs165599 and schizophrenia in the population studied ($P = 0.016$, $P = 0.01$, $P = 0.004$ respectively).

Funke declared that the presence of a G allele in rs165599 led to an increase in the risk of psychiatric disorders, such as schizophrenia (Funke et al. 2005). We have also noticed that the presence of a G allele in codominant, dominant, and overdominant inheritance models has increased the risk factor up to 2.12, 2.35, and 2.28 times, respectively.

Polymorphisms might influence the miRNA binding to specified mRNAs and the risk of diseases in 3′ UTRs of genes. In miRNA maturation, primary miRNAs, which are transcribed from DNA sequence processed to precursor miRNA by RNA binding protein named DGCR8 and Drosha, a ribonuclease III enzyme. In the next stage, RNase III endonuclease, Dicer produces mature miRNA from the pre-miRNA, interacting with the target mRNAs to change their expression (O’Brien et al. 2018). Regulatory functions of miRNAs are dependent upon their secondary structure (Yu et al. 2018). For the formation of RNA secondary structure, the complementary base pairing of the single-stranded RNA is needed. The development of optimal two-dimensional secondary structures occurs in the minimum thermodynamic free energy known as the minimum free energy structure (MFE) (George and Thomas 2016). As shown in Table 4, the single-nucleotide polymorphisms can affect the MFE structure of desired miRNAs at a low level, but there are no shreds of evidence in desired miRNAs dysfunction since none of the variants were included in mature miRNA structures. For instance, rs10759 is situated in 3′ UTR of the RGS4 gene. The SNP is supposed to interfere with miR-124 binding to RGS4 and augments the risk of schizophrenia (Gong et al. 2013). Cui and Jiang indicated rs10769 is associated with schizophrenia by studying rs10759 in 662 persons (including 315 patients and 347 controls) (Cui and Jiang 2012). We did not find an association between rs10759 and schizophrenia in the Iranian population ($P = 0.89$). C/C genotype increased the risk factor up to 1.05 times in the presence of the A/A genotype as reference.

Achieved results indicated that rs165599 genotypes in codominant, dominant, and overdominant inheritance models are associated with schizophrenia in the Iranian population. The T allele in rs200982455, G allele in rs165599, and the A allele in rs10759 must be considered the risk factor.

The present study reveals neither association between haplotypes and schizophrenia nor linkage between the three SNPs; rs200982455, rs165599, and rs10759. Although there is exclusively no report on the association between these three SNPs in other populations, we anticipate getting conflicting findings in other case–control studies. In addition to the influence of structure and stratification of different populations, the interaction between miRNAs and mRNAs should be considered a remarkable agent, significantly affecting the findings.

Convincing the patients, especially those with paranoia for completing written consent, was the limitation of this study. The expensive price of the next-generation sequencing tools for studying SNPs in the great scale, the small number of cases was the other study limitations. Also, comorbid psychiatric conditions could not be identified using the PANSS test.

| SNP        | miRNA   | Inclusion in mature miRNA | MFE in the presence of ancestral allele (kcal/mol) | MFE in the presence of variant allele (kcal/mol) |
|------------|---------|---------------------------|---------------------------------------------------|-------------------------------------------------|
| rs530404895| miRNA-326| –                         | −54.70                                            | −42.60                                          |
| rs374732351| miRNA-326| –                         | −54.70                                            | −38.40                                          |
| rs753764536| miRNA-326| –                         | −54 to 70                                         | −39.00                                          |
| rs143525573| miRNA-125A| –                        | −44.74                                            | −45.50                                          |
| rs34059726 | miRNA-124-3| –                        | −41.93                                            | −41.93                                          |
Conclusions
These results are a preliminary report in the Iranian population in these SNPs. Our finding showed the significant correlation of some of the studied SNPs in genes and miRNAs in the dopaminergic pathway. It may consider as a biomarker in the diagnostic pattern in schizophrenia.

However, the difference in the results of researchers’ reports stems from the influence of the structure and stratification of different populations.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran. 2 Department of Psychiatry, School of Medicine, Imam Hossein Educational Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Received: 14 January 2022 Accepted: 21 February 2022 Published online: 04 March 2022

Abbreviations
SCZ: Schizophrenia; SNP: Single-nucleotide polymorphism; MIR: MicroRNA; DRD: Dopamine receptors D; GPCR: G-protein-coupled receptor; NRG-1: Neuregulin-1; COMT: Catechol-O-methyltransferase; PANS: Positive and negative syndrome scale; PCR-RFLP: Polymerase chain reaction–restriction fragment length polymorphism; CI: Crude odds ratio; CI: Confidence interval; LD: Linkage disequilibrium; MAF: Minor allele frequency; MFE: The minimum free energy structure.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s42269-022-00744-w.

Additional file 1: Table S1. Demographic characters: sex, job and age. Table S2. Demographic characters: drug usage, family history and education. Table S3. Demographic characters: alcohol usage, response to treatment and Ethics.

Acknowledgements
The authors gratefully acknowledge Science and Research Branch, Islamic Azad University, for providing laboratory.

Authors’ contributions
AM, MA, MM, AL, BM, and AT collected the samples and performed the PCR tests. ZN conceptualization of the project and data analyzed and interpreted. IS and GHU data analyzed and interpreted. NMH was co-advisor and psychiatrist regarding to diagnose patients. All authors were contributors in writing the manuscript and read and approved the final manuscript.

Funding
No funding was obtained for this study.

Availability of data and materials
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to ethical or privacy restrictions. PANSS test and clinical interview according to DSM-IV-TR are not public because of ethical restrictions.

Declarations
Ethics approval and consent to participate
The written informed consent was obtained from members of the case and control groups or their supervisors. This study was approved by the Ethical Committee of Islamic Azad University, Science and Research Branch, Tehran (approval No. IR.IAU.SRB.REC.1396.43). Informed consent was obtained from patients before commencement of the research. The authors confirm that the present manuscript has not been published or submitted for publication elsewhere.

Consent for publication
The statement of consent to publish from the patient is not applicable. All authors agree to publish research findings. They guarantee that the research findings have not been previously published.

References
Acar C, Sozen MM, Gozukara H, Orman K, Kartalci Ş. (2015) Lack of association between catechol-O-methyltransferase and schizophrenia in a Turkish population. Turk J Biochem 40:205–209
Camkurt MA, Karababa F, Erdal ME, Bayazit H, Kandemir SB, Ay ME, Kandemir H, Ay ÖL, Çiçek E, Selek S, Taşdelen B (2016) Investigation of dysregulation of several miRNAs in peripheral blood of schizophrenia patients. Clin Psychopharmacol Neurosci 14:256
Chaychi I, Foroughipour M, Haghir H, Talaei A, Chiachi A (2015) Electroencephalographic characteristics of Iranian schizophrenia patients. Acta Neurol Belg 115:665–670
Chowdhuri KV, Bame M, Wood J, Ťalkowski ME, Mirnics K, Levitt P, Lewis DA, Nimchangoan KL. (2008) Linkage disequilibrium patterns and functional analysis of RGS4 polymorphisms in relation to schizophrenia. Schizophr Bull 34:118–126
Cui D, Jiang K (2012) Research in China on the molecular genetics of schizophrenia. Shanghai Arch Psychiatry 24:187
Ding L, Styblo M, Drobna Z, Hegde AN (2016) Expression of the longest RGS4 splice variant in the prefrontal cortex is associated with single nucleotide polymorphisms in schizophrenia patients. Front Psychiatry 7:26
Dwivedi Y (2017) microRNA-124: a putative therapeutic target and biomarker for major depression. Taylor & Francis, Routledge
Estrada G, Fatjo-Vilas M, Munoz MJ, Pulido G, Minano MJ, Toledo E, Ilia JM, Martin M, Miralles ML, Miret S, Campanera S, Bernabeu C, Navarro ME, Fananas L. (2011) Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism. Acta Psychiatry Scand 123:485–492
Funahashi Y, Yoshino Y, Yamazaki R, Ozaki Y, Mori Y, Mori T, Ochi S, Iga JI, Ueno SI (2019) Analysis of methylation and C141C Ins/Del polymorphisms of the dopamine receptor D2 gene in patients with schizophrenia. Psychiatry Res 278:135–140
Funke B, Malhotra AK, Finn CT, Plocki AM, Lake SL, Lenzc T, DeRosse P, Kane JM, Kucherlapati R (2005) COMT genetic variation confers risk for psychotic and affective disorders: a case control study. Behav Brain Funct 1:19
George TP, Thomas T (2016) Novel approach to analyzing MFE of noncoding RNA sequences. Genomics Insights 9:41–49
Ghanbari Jolfaei A, Moshtikh A, Ashgharpour M, Moshtik H. (2012) The Relationship between attention/vigilance and symptom severity in schizophrenic patients. Iran J Psychiatr 7:22–25
Gong Y, Wu CN, Xu J, Feng G, Xing QH, Fu W, Li C, He L, Zhao XZ (2013) Polymorphisms in microRNA target sites influence susceptibility to schizophrenia by altering the binding of miRNAs to their targets. Eur Neuropsychopharmacol 23:1182–1189
Gonzalez-Castro TB, Hernandez-Diaz Y, Juarez-Rejop IE, Lopez-Narvaez ML, Toivilla-Zarate CA, Genis-Mendoza A, Alpuin-Reyes M. (2016) The role of C957T, TaqI and Ser311Cys polymorphisms of the DRD2 gene in schizophrenia: systematic review and meta-analysis. Behav Brain Funct 12:29
Gozukara Bag HG (2018) Association between COMT gene rs165599 SNP and schizophrenia: a meta-analysis of case-control studies. Mol Genet Genom Med 6:845–854
Grace AA (2016) Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. Nat Rev Neurosci 17:524
Hatami M, Karamghadri N, Mohaghegh H, Yoosefie S, Karimipoor M, Hadjighasem M, Ananloo ES (2017) Neuregulin-1 gene and schizophrenia, and its negative symptoms in an iranian population. Iran J Psychiatry Behav Sci 11:4484
Schwarz E (2018) A gene-based review of RGS4 as a putative risk gene. Psychiatr Res 239:99–110
Kesby JP, Eyles DW, McGrath JJ, Scott JG (2018) Dopamine, psychosis and schizophrenia: the widening gap between basic and clinical neuroscience. Transl Psychiatry 8:1–12
Kuncara DB (2019) Polymorphism of Dopamine D2–141C Ins/Del receptor gene in paranoid schizophrenia and non schizophrenia patients of batak ethnicity in sumatera utara. Bull Farmatera 4:18–28
Ledonne A, Nobili A, Latagliata EC, Cavallucci V, Guatiero E, Puglisi-Allegra S, D’Amello M, Mercun NB (2015) Neuregulin 1 signalling modules mGluR1 function in mesencephalic dopaminergic neurons. Mol Psychiatry 20:959–973
Liu L, Fan D, Ding N, Hu Y, Cai G, Wang L, Xin L, Xia Q, Li X, Xu S, Xu J (2014) The relationship between DRD2 gene polymorphisms (C957T and C939T) and schizophrenia: a meta-analysis. Neurosci Lett 583:43–48
Lorenz R, Bernhart SH, Hörner zu Siederdissen C, Tafer H, Flamm C, Stadler PF, Hofacker IL (2011) ViennaRNA Package 2.0. Algorithms Mol Biol 6:26
Luykx JJ, Broersen JL, de Leeuw M (2017) The DRD2 rs1076560 polymorphism and schizophrenia-related intermediate phenotypes: a systematic review and meta-analysis. Neurosci Biobeh Rev 74:214–224
Morozova A, Zorkina Y, Pavlov K, Pavlova O, Storozheva Z, Zubkov E, Zakharova N, Karpenko O, Reznik A, Chekhonin V, Kostyuk G (2019) Association of rs4680 COMT, rs6280 DRD3 and rs7322347 5HT2A with clinical features of youth-onset schizophrenia. Front Psychiatry 10:830
Nunokawa A, Watanabe Y, Muratake T, Kaneko N, Koizumi M, Someya T (2007) Relationship between SNP8NRG241930 in the 5′ End of Neuroglin 1 gene with schizophrenia in a Japanese population. Neurosci Res 58:291–296
O’Brien J, Hayder H, Zayed Y, Peng C (2018) Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol (Lausanne) 9:402
Okochi T, Ikeda M, Kishi T, Kawashima K, Kinoshita Y, Kitajima T, Yanamouchi Y, Tomita M, Inada T, Ozaki N, Iwata N (2009) Meta-analysis of association between genetic variants in COMT and schizophrenia: an update. Schizophr Res 110:140–148
Paspalas CD, Selenkon LD, Arminst AFT (2009) Mapping the regulator of G protein signaling 4 (RGS4): presynaptic and postsynaptic substrates for neuroregulation in prefrontal cortex. Cereb Cortex 19:2145–2155
Paul P, Chakraborty A, Sarkar D, Langthasa M, Rahman M, Bari M, Singha RS, Malakar AK, Chakraborty S (2018) Interplay between miRNAs and human diseases. J Cell Physiol 233:2007–2018
Paul S, Reyes PR, Garza RS, Sharma A (2019) MicroRNAs and child neuropsychiatric disorders: a brief review. Neurochem Res 45:232–240
Poorshekar S, Firoozfar A, Yassini Ardekani SM, Kalantar SM (2019) Study of the association between DRD2 Gene Ser311Cys and GSTM1 gene polymorphism in schizophrenia. SSU J 27:1602–1611
Qi J, David M (2012) MicroRNAs and lung cancers: from pathogenesis to clinical implications. Front Med 6:134–155
Raznahan A, Greenstein D, Lee Y, Long R, Clasen L, Geschm P, Addington A, Gredd JN, Rapoport JL, Gogtay N (2011) Catechol-o-methyl transferase (COMT) val158met polymorphism and adolescent cortical development in patients with childhood-onset schizophrenia, their non-psychotic siblings, and healthy controls. Neuroimage 57:1517–1523
Risch N (1990) Linkage strategies for genetically complex traits. III. The effect of linkage disequilibrium and population structure on power. Am J Hum Genet 46:242–253
Singh J, Kour K, Jayaram MB (2012) Acetylcholinesterase inhibitors for schizophrenia. Cochrane Database Syst Rev 1:CD007967
Taylor S (2018) Association between COMT Val158Met and psychiatric disorders: a comprehensive meta-analysis. Am J Med Genet B Neuropsychiatr Genet 177:199–210
Williams HJ, Glaser B, Williams NM, Norton N, Zammit S, MacGregor S, Kirov GK, Owen MJ, O’Donovan MC (2005) No association between schizophrenia and polymorphisms in COMT in two large samples. Am J Psychiatry 162:1736–1738
Yu H, Yan H, Wang L, Li J, Tan L, Deng W, Chen Q, Yang G, Zhang F, Lu T, Yang J, Li K, Lv L, Tan Q, Zhang H, Xiao X, Li M, Ma X, Yang F, Li L, Wang C, Li T, Zhang D, Yue W, Consortium Chinese Antipsychotics Pharmacogenomics (2018) Five novel loci associated with antipsychotic treatment response in patients with schizophrenia: a genome-wide association study. Lancet Psychiatry 5:327–338
Zhang Y, Li L, Xu W, Xu L, Jiang Y, Lu Y, Zhang X, Zhu X (2015) Dopamine receptor D2 and associated microRNAs are involved in stress susceptibility and resistance to escitalopram treatment. Int J Neuropsychopharmacol 18:pyv025

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.