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

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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

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

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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 single-nucleotide polymorphisms, potentially candidates related to schizophrenia (Morozova et al. 2019; Gozukara Bag 2018).

The association between many human diseases and microRNAs have been shown (Paul et al. 2018, 2019). MicroRNAs are noncoding RNAs, which bind to 3′ regions (3′UTRs) of the target mRNAs to regulate their expression. Some variants in 3′ UTR of the genes may increase the risk of diseases due to altering microRNAs' binding to the target mRNAs. The miR-124 is one of the important miRNAs which act in the development of the brain. The *RGS4* gene is the target for this miR (Dwivedi 2017). Similarly, miR-326 positively regulates the expression of the *DRD2* gene, so it affects the dopamine system and causes the development of schizophrenia (Shi et al. 2014; Zhang et al. 2015). MiR-125a-3p, a member of the miR-125a family, is derived from the 3′-end of pre-miR-125a. Based on laboratory evidence and using Bioinformatics software, the *NRG1* gene is predicted as a target gene for miR-125a-3p, and it has been determined that the 3′UTR region of the *NRG1* gene has highly protected regions that may be considered as a Connection site for miR-125a-3p (Qi and David 2012).

Concerning the association of these genes with schizophrenia, no similar studies have investigated the correlation between SNPs located in these genes with schizophrenia in the Iranian population. 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, rs4680, rs165599, 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 we chose, 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.

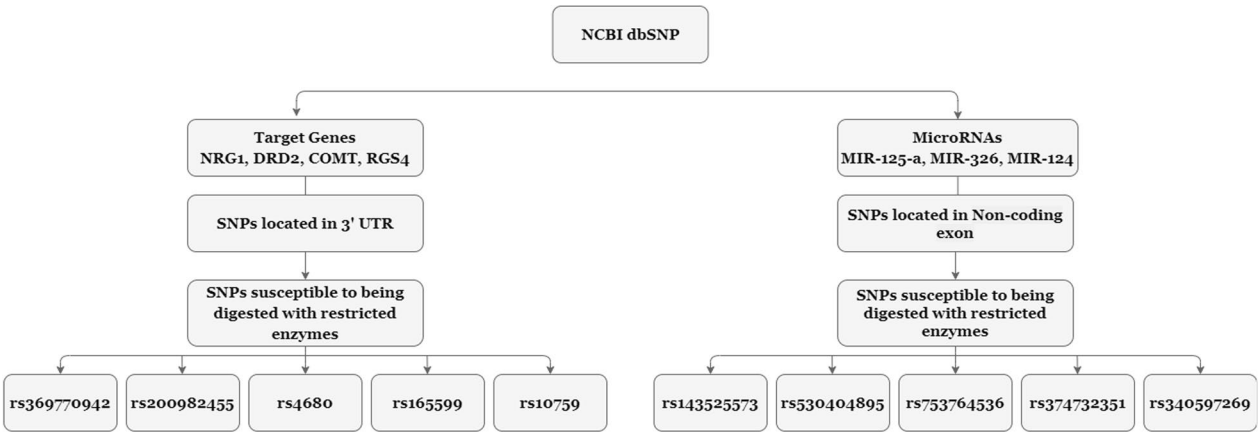

Fig. 1 The chart of choosing desired SNPs located in the 3′region of the target genes and the 5′UTR region of the MicroRNA genes

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 observance 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 $MgCl_2$, 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

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	GGAGTATGAAACGACCCAAGAG	58/4	800	DdeI
		GAGGCATATCTGGATGGATGTG	58/3		
miR-125a-3P/rs143525573	Forward	TTCTAGGTCTCTGCCCCTCC	56	534	DdeI
	Reverse	GAGGCGCTCAGAGTAGGTTG	58		
3′UTR DRD2	Forward	CTTGAATGCCAAGCACAGAA	49	249	DdeI
rs200982455	Reverse	CTCCTGTTTCCCTTCCCTTC	53		
MIR326 rs530404895 rs753764536	Forward	CCTGAGCACATGGACACATT	51	239	–
rs374732351	Reverse	GGCAAGAGAAAGACAGACAGA	54		
3′UTR COMT rs4680	Forward	CTCATCACCATCGAGATCAACC	61	383	NLaIII
	Reverse	GCCATCTTTACACACCCATACA	59		
3′UTR COMT	Forward	CTCATCACCATCGAGATCAACC	59	160	–
rs165599	Reverse	GCCATCTTTACACACCCATACA	59		
3′UTR RGS4	Forward	CTCAGAGTTCACTGGCACA	60	589	–
rs10759	Reverse	TGCAGGTTTTCTAATTGTACCC	58		
MIR124	Forward	CCCTCTGCGTGTTCACAG	62	202	AseI
rs340597269	Reverse	GCATTGTTCGCCGGATTTG	62		

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 *Dde*I 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 *Dde*I 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).

The enzymatic digestion of rs200982455 (NC-000011.10) using *Dde*I identified four fragments with 17 bp, 36 bp, 43 bp, and 153 bp size length in the presence of the C allele (ancestral allele), whereas five fragments with 17 bp, 36 bp, 40 bp, 43 bp, and 113 bp size length were obtained in the presence of the T allele (Table 2).

The enzymatic digestion of rs4680 (NC-000022.11) by *Nla*III produced three fragments with 242, 87, and 54 bp size length in the presence of G allele (ancestral allele), while four fragments with 242, 69, 54, and 18 bp were obtained in the existence of A allele (Table 2).

The enzymatic digestion of rs34059726 (NC-000020.11) using *Ase*I showed one fragment with a 202 bp size length in the presence of the G allele (ancestral allele). On the other hand, two fragments with 45 bp and 157 bp size length were detected in the presence of the T allele (Table 2).

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 TT	101 (99) 1 (0.01)	95 (97) 3 (0.03)	1 3.19 (0.33–31.20)	0.28
MIR326 rs530404895	–	CC	102 (100)	98 (100)	–	–
MIR326 rs753764536	–	GG	102 (100)	98 (100)	–	–
MIR326 rs374732351	–	GG	102 (100)	98 (100)	–	–
3′UTR COMT rs4680	–	AA	102 (100)	98 (100)	–	–
3′UTR COMT rs165599	Codominant	AA AG GG	47 (46.1%) 45 (44.1%) 10 (9.8%)	28 (28.6%) 63 (64.3%) 7 (7.1%)	1.00 2.35 (1.28–4.30) 1.17 (0.40–3.44)	0.016
	dominant	AA AG/ GG	47 (46.1%) 55 (53.9%)	28 (28.6%) 70 (71.4%)	1.00 2.14 (1.19–3.84)	0.01
	Recessive	AA/AG GG	92 (90.2%) 10 (9.8%)	91 (92.9%) 7 (7.1%)	1.00 0.71 (0.26–1.94)	0.5
	Overdominant	AA/GG AG	57 (55.9%) 45 (44.1%)	35 (35.7%) 63 (64.3%)	1.00 2.28 (1.29–4.03)	0.004
MIR124 rs340597269	–	TT	102 (100)	98 (100)	–	–
3′UTR RGS4 rs10759	–	CC AA	82 (80.4%) 20 (19.6%)	78 (79.6%) 20 (20.4%)	1.00 1.05 (0.53–2.10)	0.89

Genotyping of the other SNPs, including rs530404895 (NC-000011.10), rs753764536 (NC-000011.10), rs374732351 (NC-000011.10), rs165599 (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 ($P=0.016$, $P=0.01$, 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 AGCCGGAATC haplotype (0.495, rs369770942, rs143525573, rs200982455, rs530404895, rs753764536, rs374732351, rs4680, rs165599, rs34059726, and rs10759, respectively). By considering the haplotype's OR = 1.00, the approximate risk in 95%C for AGCCGGAGTC, AGCCGGAATA, AGCCGGAGTA, and AGTCGGAATC haplotypes none of them show considerable correlation with schizophrenia [(OR (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)].

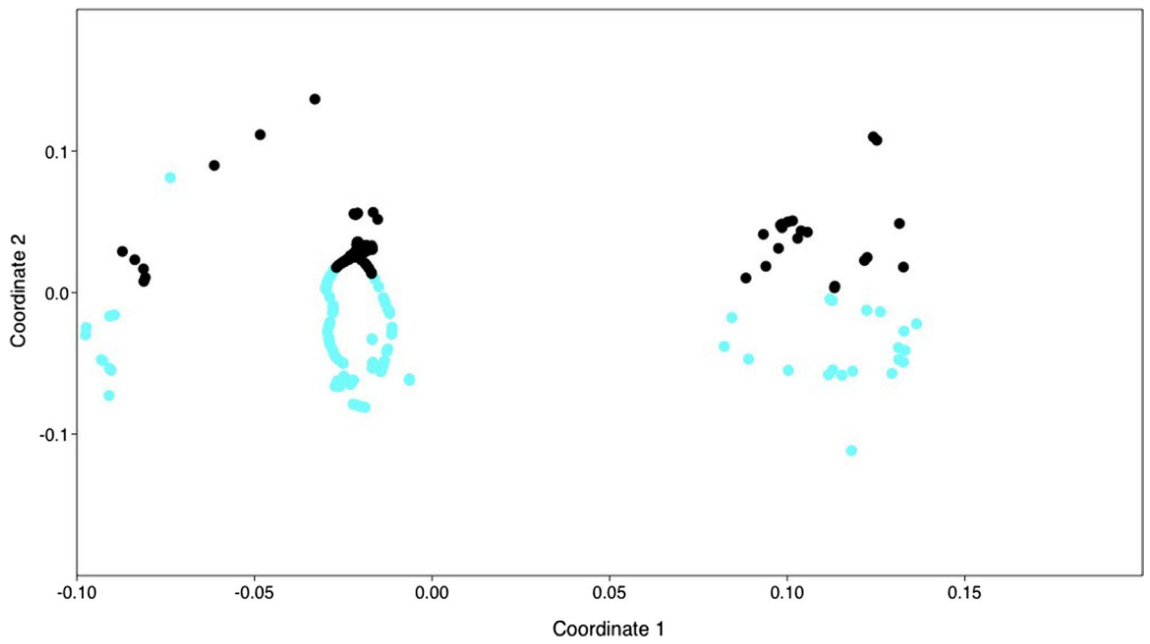

Fig. 2 PCA ordination based on ten SNP genotyping in two groups studied (blue dot: case and black dot: control samples)

Table 3 Haplotypes of ten SNPs studied

No	Haplotype	Frequency	OR (95% CI)	*P*-value
1	AGCCGGAATC	0.495	1.00	–
2	AGCCGGAGTC	0.285	1.33 (0.80–2.23)	0.28
3	AGCCGGAATA	0.135	0.88 (0.52–1.50)	0.64
4	AGCCGGAGTA	0.065	2.03 (0.88–4.70)	0.1
5	AGTCGGAATC	0.015	1.15 (0.28–4.72)	0.84

The ordination of each allele is based on SNPs ordination in Table 1

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)].

Discussion

Five of the ten studied SNPs are situated in 3′UTR of *NRG1*, *DRD2*, *COMT*, and *RGS4* genes. They are presumed to be associated with schizophrenia (Cui and Jiang 2012; Luykx et al. 2017; Shariati et al. 2011; Shifman et al. 2002). Another five SNPs have been chosen, which are located in *MIR-125-a*, *MIR-326*, and *MIR-124* genes. They are supposed to be related to either adjusting *NRG1*, *DRD2*, *COMT*, and *RGS4* genes or schizophrenia (Camkurt et al. 2016; Gong et al. 2013; Shi et al. 2014).

The rs369770942, rs143525573, rs200982455, rs530404895, rs753764536, rs374732351, and rs34059726 are capable to be nominated as critical SNPs in dopaminergic pathways, because of their genomic locations. Their correlations with schizophrenia have not been reported in an Iranian population as well as other populations. We found no association between these SNPs and schizophrenia 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 recessive inheritance model of rs165599 and rs10759, which have been examined in various populations, the association was not observed in the Iranian population.

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.

Table 4 Estimated minimum free energy (MFE) of miRNAs second structures using RNAfold

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 MIRs 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.

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; PANSS: Positive and negative syndrome scale; PCR–RFLP: Polymerase chain reaction–restriction fragment length polymorphism; COR: 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 Ethnics.

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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 GhJ data analyzed and interpreted. NMH was co-advisor and psychiatrist regards to diagnose patients. All authors were contributors in writing the manuscript and read and approved the final manuscript.

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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 privacy or ethical 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.

Competing interests

The authors declare that they have no competing interests.

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