

Original Research

Association of Dopamine D2 Receptor (DRD2) C939T and a Lack of Association of Tumor Necrosis Factor- β (TNF- β) +A252G Polymorphisms with Susceptibility to Migraine in A Northern Iranian Population

Negar Gorjizadeh ^{1,2}, Payam Saadat ³, Abbas Azadmehr ², Saeed Irian ^{1,*}

1. Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran; E-Mails: negar.gorjizadeh@gmail.com; Irian@khu.ac.ir; ORCID: 0000-0002-8276-9037; 0000-0002-4190-2380
2. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran; E-Mail: azadmehr2010@gmail.com
3. Mobility Impairment Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran; E-Mail: sepanta1968@yahoo.com

* **Correspondence:** Saeed Irian; E-Mail: Irian@khu.ac.ir; ORCID: 0000-0002-4190-2380

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Abstract

Genetic factors including TNF- β and DRD2 have been considered as essential components in the etiology of migraine. Several studies have investigated the association between TNF- β +A252G or DRD2 C939T polymorphisms and migraine risk, with debatable results. We aimed to examine whether TNF- β +A252G and DRD2 C939T polymorphisms confer genetic susceptibility to migraine in a Northern Iranian case-control sample. In total, 151 migraineurs (105 with MO 46 with MA) and 144 healthy control subjects were included in this study. Genotyping of the two SNPs, rs6275 DRD2 and rs909253 TNF- β , was determined by PCR-RFLP. The RNA secondary structure was predicted using the RNAfold web server online software. Considering the distribution of genotypes and allelic frequencies between the patient and control groups, no significant difference was detected in genotype and allele frequencies for



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the rs909253 nucleotide polymorphism. In contrast, the DRD2 C939T polymorphism in an over dominant model significantly ($P = 0.002$) contributed to increased risk of migraine susceptibility, with its dominant model ($P = 0.005$) decreasing the risk of migraine. Our case-control study supports a protective effect of the variant genotype CC and allele C of rs6275 DRD2 SNP against migraine, while demonstrating a lack of involvement of TNF- β +A252G in susceptibility to migraine.

Keywords

Migraine without aura; migraine with aura; tumor necrosis factor; dopamine receptor D2; genetic polymorphism

1. Background

Migraine as the most common disabling neurovascular disorder characterized by recurrent headache attacks and often moderate to severe in intensity [1] is a complex, polygenic, multifactorial disease caused by the interaction between genetic variants and environmental factors [2, 3]. According to the International Headache Society, migraine is clinically subdivided into two main types of migraine without aura (MO) and migraine with aura (MA) [4]. In MA, a third of patients experience transient focal neurological symptoms that usually take place before the onset of the headache [5]. Although the exact pathophysiology of migraine is not yet fully understood, much evidence suggests the role of both proinflammatory cytokines and the dopaminergic system in migraine and its clinical subtypes.

The tumor necrosis factor (TNF- α and TNF- β) superfamily, the proinflammatory cytokines involved in various pathophysiological processes such as systemic inflammatory reactions and immune responses, appears to play a role in migraine [6-8]. The TNF- β gene as a member of the TNF family is expressed mainly by activated lymphocytes (T, B cells), natural killer (NK), and lymphoid tissue inducer (LTi) cells under physiological conditions [9-11]. The gene is located on the short arm of human chromosome 6 (6p21.3) within the MHC class III region, spans 2 kb, and consists of four exons which by undergoing alternative splicing produce at least 7 isoforms. TNF- β 252A>G polymorphism within the first intron of the TNF- β gene (rs909253) is a variant with a silent point mutation that influences the expression of the gene and has been investigated in migraine risk studies in various populations [12, 13].

The dopamine receptors, on the other hand, are involved in the repair of the nervous system in the human brain [14, 15], act as key elements of the dopaminergic system, and play roles in multiple psychiatric and neuropsychiatric disorders [16, 17]. Indeed, DRD2 antagonists have been used as effective agents in the acute treatment of migraine [18]. The dopamine receptor D2 is a G protein-coupled receptor consisting of two alternatively spliced isoforms, D2S (short isoform) and D2L (long isoform) [17, 19, 20], encoded by the eight-exon DRD2 gene, located on chromosome 11q22-23 [21]. The rs6275 (DRD2 939C>T) is a synonymous polymorphism (a silent C/T transition) (His313His) located in exon 7 of the DRD2 gene [3], initially implicated by Peroutka and colleagues (1997) as being associated with clinical susceptibility to migraine with aura (MA) [18].

Considering a lack of knowledge on the molecular pathophysiology of migraine, and the strong genetic component of the disorder [22], and given the association between the two polymorphisms rs6275 and rs909253 and susceptibility to migraine, we undertook the present research investigation to separately test the hypothesis that the DRD2 939C>T (rs6275) exonic and TNF- β 252A>G intronic (rs909253) polymorphisms are associated with the susceptibility to migraine in a Northern Iranian population.

2. Methods

2.1 Patients and Controls

The present case-control study evaluated 151 migraine patients, including 105 patients with MO (12 males and 93 females with a mean age $\pm$ SD years of 40.09 ± 10.93) and 46 patients with MA (3 males and 43 females with a mean age $\pm$ SD years of 36.72 ± 10.63), in the clinic of Ayatollah Rouhani Hospital in Babol-Iran (Participant recruitment and sample collection were conducted between June 2018 and March 2019). Migraine was diagnosed according to the International Classification of Headache Society criteria [23]. The sociodemographic data and headache characteristics, including age, sex, headache frequency, family history, and the influence of menstruation on headache severity, were collected from all participants. Patients and controls were matched for age and sex, and no significant differences were observed between the two groups in these variables. The control group consisted of individuals without any symptoms or family history of headache. Participants with other primary headaches, such as common types of tension-type and cluster headaches, as well as secondary headaches due to conditions like sinusitis, allergy, hypertension, hormonal disorders, or sudden caffeine withdrawal, which usually appear as symptoms of other underlying diseases, were excluded from the study.

The control group included 144 healthy controls (12 males and 132 females with a mean age $\pm$ SD of 36.72 ± 10.63 years), selected from the same demographic area, and matched for age and sex with patients. Individuals with a genetic relationship to migraine patients were excluded from the study. Ethical approval for the present study was obtained from the Research Ethics Committee at Kharazmi University, Tehran, Iran (IR.KHU.REC.1398.010).

2.2 DNA Extraction and Genotyping

Patients' and controls' whole blood was drawn into tubes containing K3EDTA, and genomic DNA was extracted from peripheral blood leukocytes according to the salting-out method and stored at -20°C [24]. Two single-nucleotide polymorphisms (SNPs) in the TNF and DRD2 genes (TNF- β +A252G and DRD2 C939T) were genotyped by PCR restriction fragment length polymorphism (PCR-RFLP) (Table 1). The PCR products were digested with NcoI restriction enzyme (SinaClon BioScience, Tehran, Iran) according to the manufacturer's instructions. The forward and reverse primer sequences for the two SNPs are listed in Table 1. Finally, to ensure the reliability of the PCR-RFLP results, the PCR products were sequenced by the Sanger sequencing method, and sequences of the PCR products were analyzed by the Codon Code Aligner software version 6.0 (CodonCode Corporation, Centerville, MA, USA). The software was used to align the obtained sequences with reference sequences, and to confirm the accuracy of the identified nucleotide variations. Nucleotide variations were confirmed to ensure accurate genotyping.

Table 1 Primer sets used for the amplification of TNF- β and DRD2 to detect polymorphism.

Polymorphism	Primer Sequences	Restriction enzyme	Product size (bp)
TNF-β A252G (rs909253)	5'GCCGCTTCCTCTATAAAGGGAC3' 5'GGAGAACCTGCAGAGAAAGAGAG3'	NcoI	A = 494 G = 298 and 205
DRD2 C939T (rs6275)	5'CCATGCCTCAGTGACATCT3' 5' AATGGCGAGCATCTGAGTGG 3'	NcoI	C = 474 T = 279 and 195

2.3 Polymerase Chain Reaction (PCR)

2.3.1 Thermal Cycling

The PCR program consisted of an initial denaturation-activation step at 95°C for 10 min, followed by a 35-cycle program including: denaturation at 95°C for 30 seconds, annealing conditions 60°C TNF- β +A252G and 59°C DRD2 C939T for 35 seconds, Extension at 72°C for 40 seconds, and final Extension at 72°C for 5 min.

2.4 RNA Structural Analysis

The RNA secondary structure was predicted based on the minimum free energy (MFE) folding of an RNA sequence using RNAfold web server online software (<http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>).

2.5 Statistical Analysis

The relationship between polymorphism and the risk of migraine was calculated using the odds ratio (OR) and 95% confidence interval (CI). Genotype distribution in both control and migraine populations was in Hardy-Weinberg equilibrium (HWE). In this study, genotypic distributions: dominant (AG + GG vs. AA for TNF- β A252G; CC + TC vs. TT for DRD2 C939T), recessive (AA + AG vs. GG for TNF- β A252G; TT + TC vs. CC for DRD2 C939T), and over-dominant (AA + GG vs. AG TNF- β A252G; CC + TT vs. CT for DRD2 C939T) were calculated using the chi-square test (<https://statpages.info/ctab2x2.html>). P values < 0.05 were considered statistically significant. Statistical analyses were performed using SPSS software (version 23, IBM Corp., Armonk, NY, USA) to ensure reproducibility of the results.

Post-hoc power analysis was performed to assess whether the sample size (151 patients and 144 controls) was sufficient to detect moderate differences in genotype frequencies. Assuming Cohen's $w = 0.3$ and $\alpha = 0.05$, the study achieved a statistical power of approximately 82%. Power calculations were conducted using G*Power software (Universität Düsseldorf, Germany).

2.6 Ethics Statement

Ethical approval for the present study was obtained from the Research Ethics Committee at Kharazmi University, Tehran, Iran (IR.KHU.REC.1398.010). Written informed consent was obtained

from all participants. The study was conducted according to the guidelines of the Declaration of Helsinki.

3. Results

Clinical characteristics of patients with migraine and the healthy control group.

The demographic and clinical characteristics of patients and the healthy control group are summarized in Table 2.

Table 2 Summary of clinical characteristics of patients with migraine and controls.

Characteristics	Patients (n = 151) Frequency (%)	Control (n = 144)
Age (years $\pm$ SD)	40.09 $\pm$ 10.93	36.72 $\pm$ 10.63
Gender		
Female	136 (90.1)	132 (91.7)
Male	15 (9.9)	15 (8.3)
Family history of migraine		
Positive	57 (37.7)	-
Negative	94 (62.3)	-
Classification of migraine		
Migraine with aura	46 (30.5)	-
Migraine without aura	105 (69.5)	-
Frequency of attacks		
≤ 1 /month	51 (33.8)	-
2-4/month	64 (42.4)	-
≥ 5 /month	36 (23.8)	-
Character of headache		
Throbbing/pulsating	111 (73.5)	-
Pressure/tightening	40 (26.5)	-
Pain severity		
Low	0	-
Moderate	36 (23.8)	-
Severe	115 (76.2)	-
Accompanying symptoms		
Nausea	114 (75.5)	-
Vomiting	54 (35.8)	-
Photophobia	130 (86.1)	-
Phonophobia	133 (88.1)	-
Aggravation of headache		
Emotional stress	120 (79.5)	-
Physical activity	56 (37.1)	-
Menstruation	87 (64.0)	-
Sleep changes	121 (80.1)	-

3.1 Genotype Analysis

For the TNF- β A252G polymorphism, the uncut fragment was 494 bps (A allele: no NcoI restriction site) and the digestion products were 298 and 205 bps (G allele: presence of NcoI restriction site). The NcoI restricted products of DRD2 C939T allele C (CC) and allele T (TT) genotypes were 474 bps and 279 + 195 bps long, respectively. Restriction enzymes and the expected fragment sizes for the two polymorphisms are shown in Figure 1 and Figure 2. Genotype distribution and allele frequencies of the TNF- β A252G and DRD2 C939T polymorphisms in patients and control subjects are shown in Table 3 and Table 4. The genotype distributions in migraine and control groups were in accordance with the Hardy-Weinberg equilibrium.

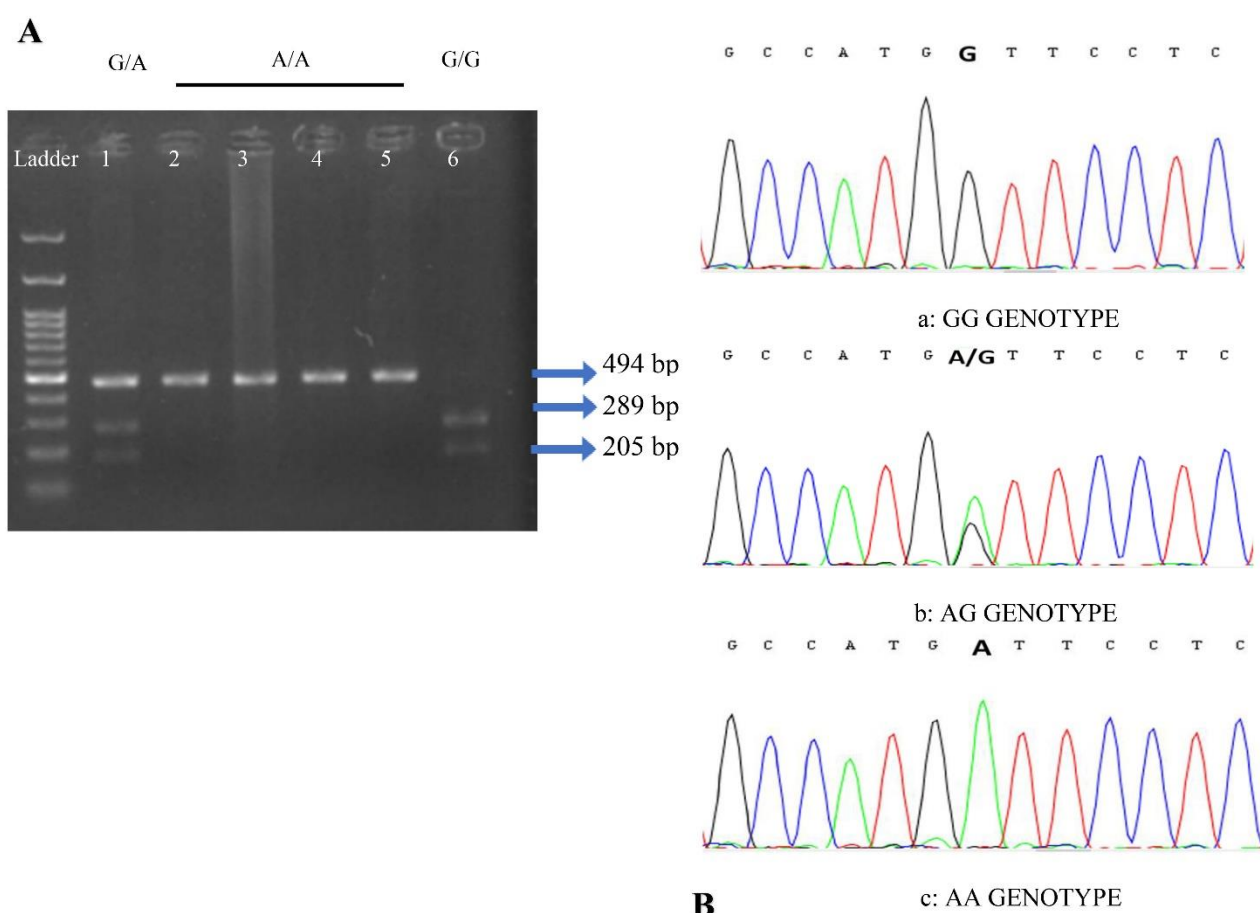


Figure 1 (A) 1.5% agarose gel showing the expected bands for the three TNF- β genotypes (G/A, A/A and G/G) following digestion of DNA samples with NcoI restriction enzyme (Lanes 1-6) and the 100 bp molecular marker (Ladder). (B) Electropherograms of Sanger sequencing analysis of the three genotypes at +A252G site of the TNF- β gene: a: GG genotype, b: AG genotype, and c: AA genotype.

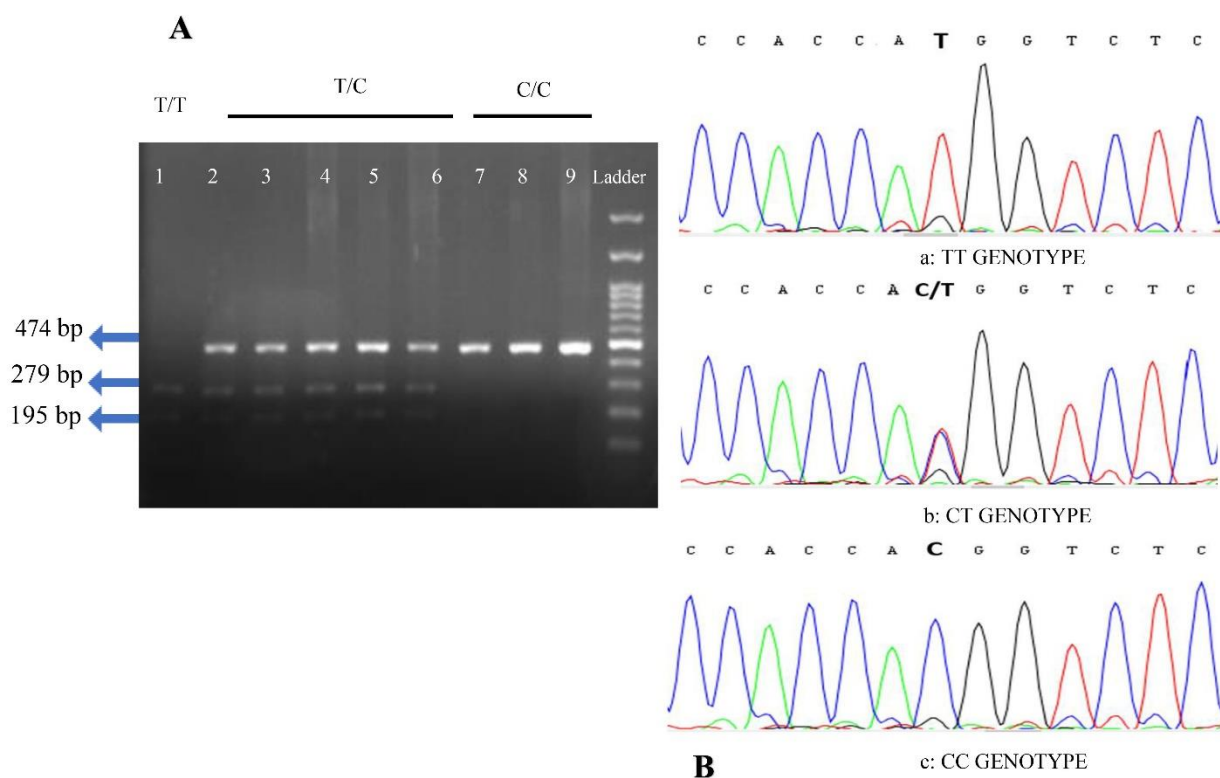


Figure 2 (A) 1.5% agarose gel showing the expected bands for the three DRD2 genotypes (T/T, T/C and C/C) following digestion of DNA samples with NcoI restriction enzyme (Lanes 1-6) and the 100 bp molecular marker (Ladder). (B) Electrocadiograms of Sanger sequencing analysis of the three genotypes at the C939T site of the DRD2 gene: a: TT genotype, b: CT genotype, and c: CC genotype.

Table 3 Frequencies of TNF- β +A252G genotype distributions in patients and the controls.

dbSNP	Genotype/alleles	Control (n = 144) n (%)	All Patients (n = 151) n (%)	MO (n = 105) n (%)	MA (n = 46) n (%)	P1	P2	P3
Codominant	AA	81 (56.3)	83 (55.0)	56 (53.3)	27 (58.7)	-	-	-
	GG	5 (3.5)	9 (6.0)	7 (6.7)	2 (4.3)	0.326	0.240	0.830
	AG	58 (40.3)	59 (39.1)	42 (40.0)	17 (37.0)	0.976	0.862	0.716
Dominant	AA	81 (56.3)	83 (55.0)	56 (53.3)	27 (58.7)	-	-	-
	GG + AG	63 (43.8)	68 (45.5)	49 (46.7)	19 (41.3)	0.825	0.648	0.771
Recessive	GG	5 (3.5)	9 (6.0)	7 (6.7)	2 (4.3)	0.315	0.245	0.784
	AA + AG	139 (96.5)	142 (94.0)	98 (93.3)	44 (95.7)	-	-	-
	AG	58 (40.3)	59 (39.1)	42 (40.0)	17 (37.0)	0.833	0.995	0.688
Over dominant	AA + GG	86 (59.7)	92 (60.9)	63 (60.0)	29 (63.0)	-	-	-
	A	220 (76.39)	225 (74.50)	154 (77.17)	71 (77.17)	-	-	-
ALELES	G	68 (23.61)	77 (25.50)	56 (22.83)	21 (22.83)	0.595	0.436	0.877

TNF- β : Tumor necrosis factor β ; OR: Odds ratios; CL = confidence interval; MO: Migraine without aura; MA: Migraine with aura. P1: P value of all patients vs. control. P2: P value of migraine without aura vs. control. P3: P value of migraine with aura vs. control.

A p-value of less than 0.05 ($P < 0.05$) is statistically significant.

Table 4 Frequencies of DRD2 C939T genotype distributions in patients and the controls.

dbSNP	Genotype/alleles	Control (n = 144) n (%)	All Patients (n = 151) n (%)	MO (n = 105) n (%)	MA (n = 46) n (%)	P1	P2	P3
Codominant	CC	77 (53.6)	56 (37.1)	40 (38.1)	16 (34.2)	-	-	-
	TT	15 (10.4)	14 (9.3)	7 (6.7)	7 (15.2)	0.543	0.829	0.123
	TC	52 (36.5)	81 (53.6)	58 (55.2)	23 (50.0)	0.002	0.005	0.040
Dominant	CC	77 (53.5)	56 (37.1)	40 (38.1)	16 (34.2)	-	-	-
	TC + TT	67 (46.5)	95 (62.9)	65 (61.9)	30 (65.2)	0.005	0.016	0.027
Recessive	TT	15 (10.4)	14 (9.3)	7 (6.7)	7 (15.2)	0.741	0.303	0.376
	CC + TC	129 (89.6)	68 (90.7)	98 (93.3)	39 (84.8)	-	-	-
	TC	52 (36.6)	81 (53.6)	58 (55.2)	23 (50.0)	0.002	0.003	0.093
Over dominant	TT + CC	92 (63.6)	70 (46.4)	47 (44.2)	23 (50.0)	-	-	-
	C	206 (71.53)	193 (63.91)	138 (65.71)	55 (59.8)	-	-	-
ALELES	T	82 (28.47)	109 (36.09)	72 (34.29)	37 (40.2)	0.048	0.166	0.034

DRD2: Dopamine receptor D2; OR: Odds ratios; CL = confidence interval; MO: Migraine without aura; MA: Migraine with aura. P1: P value of all patients vs. control. P2: P-value of migraine without aura vs. control. P3: P-value of migraine with aura.

A p-value of less than 0.05 ($P < 0.05$) is statistically significant.

3.2 TNF- β +A252G Polymorphism and Susceptibility to Migraine

Genotype distributions AA, AG, and GG for migraine and control groups were 81 (56.3), 58 (40.3), and 5 (3.5), and 83 (55.0), 9 (6.0), 59 (39.1), respectively. In the migraine group, the frequency of the A allele was 76.39 % and of the G allele was 23.61%. In the control group, the frequencies of the A and G alleles were 74.50% 25.50%, respectively. Allele frequency of TNF- β +A252G allele was approximately the same between the case and control subjects. There was no significant difference in the genotypic distribution and allele frequency between patients and controls ($P > 0.05$). There was no association of polymorphism with the risk of each subtype of migraine.

3.3 DRD2 C939T Polymorphism and Susceptibility to Migraine

Genotype distributions TT, TC, and CC for migraine and control groups were 14 (9.3), 81 (53.6), and 56 (37.1), and 15 (10.4), 52 (36.5), 77 (53.5), respectively, while allele frequencies were 28.47% and 36.09% (T) and 71.53% and 63.91% (G), respectively.

There were no significant differences in TT genotypes between the controls and patients; however, the latter group had a significantly higher frequency of the CT heterozygote ($P = 0.002$).

Carriers of the CC genotype vs. the TT + TC genotype in controls were higher than in all patients ($P = 0.005$). A significant association in genotype distribution, including CC and TT + TC genotypes, was observed between the patient and control groups, whereas no association was detected in the recessive model (TT vs. CC and TC; $P = 0.741$). Carriers of TC vs. TT + CC genotypes in patients, however, were higher than in the controls ($P = 0.002$). Allele frequency distribution in patients (36.09% for T and 28.47% for C) and the control group (63.01% for T and 71, 53% for C) differed significantly ($P = 0.048$). With respect to the distribution frequencies of DRD2 C939T genotypes in patients with MO and MA, carriers of CC vs. TT + TC genotype in controls were higher than in MO patients ($P = 0.016$). In comparison, carriers of TC vs. TT + CC genotype in MO patients were higher than in controls ($P = 0.003$). Regarding the allele frequency in MO patients (34.29% for T and 28.47% for C) and the control (65.71% for T and 71.53% for C), the differences were not significant ($P = 0.166$). Carriers of CC vs. TT + TC genotype in the controls, however, were higher than in the MA patients ($P = 0.027$), with no significant difference observed in TC vs. TT + CC genotype between MA patients and the controls ($P = 0.093$). Finally, a substantial difference in the frequency of C and T alleles was observed between the patient and the control groups ($P = 0.034$).

3.4 RNA Structural Analysis

The RNAfold analysis revealed that the C939T polymorphism alters the minimum free energy (MFE), with predicted minimum free energy values of -89.20 and -90.50 kcal/mol for T and C alleles, respectively. In addition, a minor change in the size of the terminal loop was detected (Figure 3).

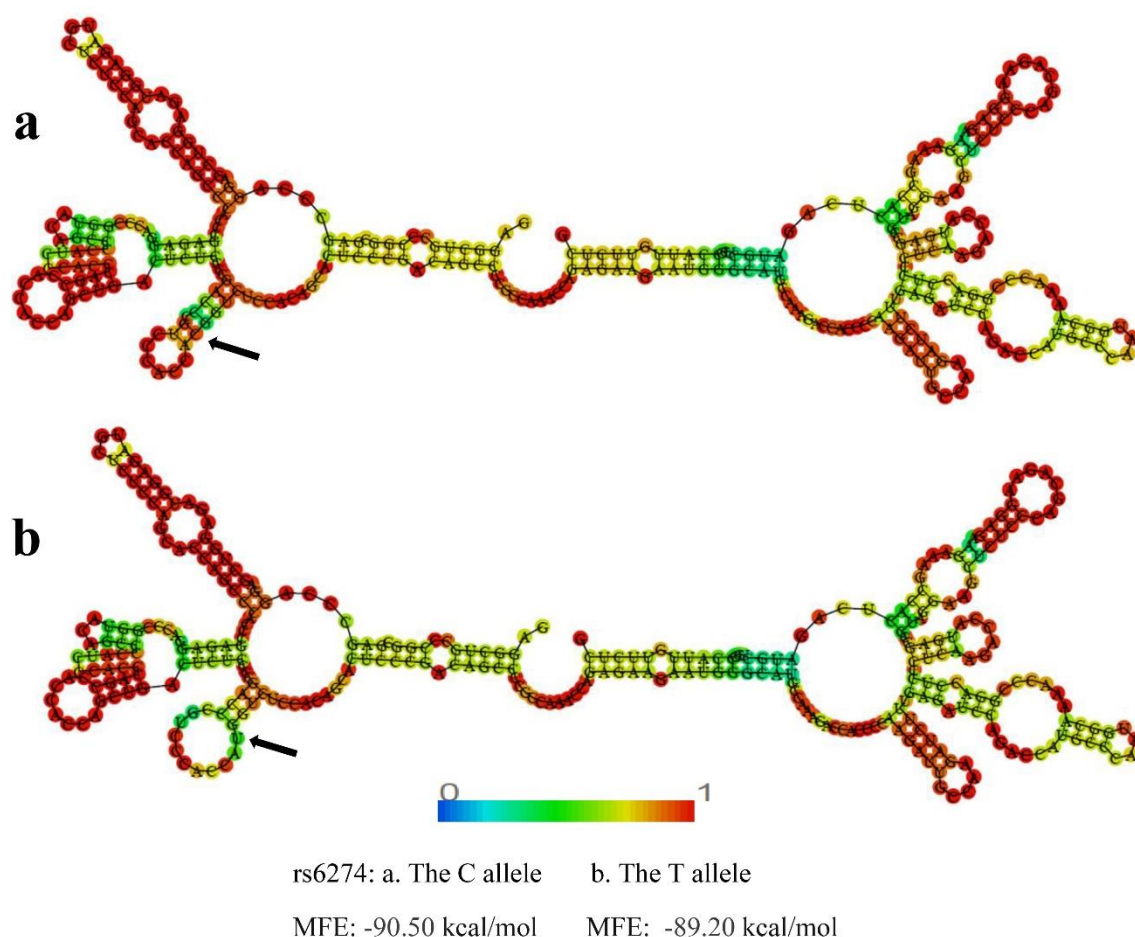


Figure 3 The SNP rs6275 leads to a change in terminal loop size of hairpin structures. A and b indicate the structures corresponding to the wild-type C939 and mutant T939 alleles, respectively. Arrows mark the alleles' position in the structures.

4. Discussion

Migraine is a multifactorial disorder where both susceptibility genes and environmental factors contribute to the manifestation of the disorder [25]. In recent years a great deal of attention has been directed to the field of migraine genetics in search of factors potentially contributing to migraine. Evidence from twin studies indicates that 34% to 57% of migraine cases can be linked to genetics [26]. According to systematic studies conducted in Iran, the prevalence of migraine is estimated at 14%, similar to or even higher than reported worldwide. Migraines can affect the health of people in any country, so it is essential to educate individuals on early detection and the discovery of effective migraine treatments [27]. Although the pathophysiology of migraine is still not wholly understood, existing evidence points to the involvement of neurotransmitters and inflammatory pathways in the pathogenesis of the disease [25, 28]. Indeed, association studies of close to 200 polymorphisms in ~ 100 genes have been published for migraine [29]. In this study, we assessed the impact of genetic polymorphisms in the TNF- β and DRD2 genes on susceptibility to migraine in a Northern Iranian population.

4.1 Association of TNF- β A252G with Migraine

TNF- β is a proinflammatory cytokine that plays a vital role in the mediation of inflammatory reactions and autoimmune diseases [12]. Evidence suggests that the TNF- β gene is involved in susceptibility to migraine, with the A252G polymorphism being reported by at least three different groups [2, 30-32]. Trabas et al., showed for the first time a genetic association of the G allele for TNF- β G252A polymorphism with migraine, particularly MA. Trabace et al. also reported that carriage of the A allele for the TNF- β A252G polymorphism was associated with increased risk for MO, compared with G homozygosity, but not MA. Asuni et al. also state that in homozygous (G/G) and heterozygous (G/A) patients with MO, the observed increase of the G allele suggests that this allele could be implicated in predisposing towards the development of the disease [31]. During a meta-analysis on a total of 5,557 migraineurs and 20,543 unrelated healthy controls from seven publications, TNF- β 252A.G polymorphism was associated with a risk of migraine in Asian subjects, but not in Caucasians [12]. In a study by Stuart et al., nine SNPs (rs1800683, rs2229094, rs2009658, rs2071590, rs2239704, rs909253, rs1800630, rs1800629, and rs3093664) in the TNF gene cluster were examined in relation to migraine susceptibility in an Australian Caucasian population. The results did not show a significant association of the SNPs with migraine, nor between migraine subtype and gender for disease risk. They therefore concluded that prospective studies that examine several family gene variants concerning HLA class I-related haplotypes would be helpful to [33]. In the present study, our results revealed no significant association of the A252G polymorphism of the TNF- β gene with migraine susceptibility in a Northern Iranian population, with subgroup analysis being based on MO and MA. These results are in line with those of Ghose et al and Pappa et al, who were also unable to confirm the association between A252G polymorphism and migraine susceptibility (Table 5) [34, 35]. Such discrepancies have generally been associated with ethnic differences [2].

Table 5 General characteristics of the studies in different populations.

Study	SNP	Racial Descent/Origin	Results
Tumor necrosis factor (TNF)-β			
Martelletti et al. (2000)	TNF- β	Caucasian/Italian (77/1010)	Significant association $P \leq 0.05$ for MO
Trabace et al. (2002)	TNF- β	Caucasian/Italian (79/101)	Significant association $P \leq 0.05$ for MO
Asuni et al. (2009)	TNF- β	Caucasian/Sardinian (219/278)	Significant association $P \leq 0.05$
Gosh et al. (2010)	TNF- β	Asian/India (216/216)	No association $P \geq 0.05$
Pappa et al. (2010)	TNF- β	Caucasian/Greece (103/178)	No association $P \geq 0.05$
Ishii et al. (2012)	TNF- β	Asian/Japan (91/119)	Significant association $P \leq 0.05$
Our Study	TNF- β	Asian/Iran (151/144)	No association $P \geq 0.05$

Dopamine receptor D2 (DRD2)			
Peroutka et al. (1997)	DRD2	United States (129/121)	Significant association $P \leq 0.05$ for MA
Lea et al. (2000)	DRD2	Caucasian/Australia (177/182)	No association $P \geq 0.05$
Stochino et al. (2003)	DRD2	Caucasian/Sardinian (100)	No association $P \geq 0.05$ for migraine-panic phenotype
Rebaudengo et al. (2004)	DRD2	Caucasian/Italian (118)	No association $P \geq 0.05$ for the clinical features of migraine
Asuni et al. (2007)	DRD2	Caucasian/Sardinian (50)	Significant association $P \leq 0.05$ for clinical response to rizatriptan
Ghosh et al. (2012)	DRD2	Asian/India (335/200)	Significant association $P \leq 0.05$
Our Study	DRD2	Asian/Iran (151/144)	Significant association $P \leq 0.05$

4.2 Association of DRD2 C939T with Migraine

The involvement of the dopaminergic system in the pathogenesis and dysfunction of migraine has been confirmed by various authors through clinical, pharmacological, and genetic studies [18, 36]. The dopamine D2 receptor (DRD2) C939T mutation is a change from an optimal to a non-optimal codon. The results of the present study revealed an increased migraine risk for individuals carrying the TC genotype of DRD2 C939T polymorphism compared to homozygotes, a protective role for the CC genotype vs. TC + TT in a dominant model ($P = 0.005$), and a contribution to increased risk of migraine susceptibility in the over-dominant model ($P = 0.002$). In addition, the DRD2 NcoI C allele could reduce the risk of migraine ($P = 0.048$).

Furthermore, with respect to migraine group, the heterozygote TC genotype vs. CC + TT in the over dominant model contributes to increased risk of migraine susceptibility with MO compared to individuals with MA, suggesting a protective role for the CC genotype vs. TC + TT in a dominant model in the development of MA ($P = 0.027$) and MO ($P = 0.016$). Finally, the association of the DRD2 NcoI C allele was only detected with MA, not with MO.

The idea of DRD2 NcoI C allele modifying the clinical susceptibility to migraine aura was initially coined by Peroutka et al. in 1997 [18], who later reported that DRD2 NcoI C/C genotype was higher in individuals with migraine with aura, major depression, generalized anxiety disorder, panic attacks, and phobia than in individuals with DRD2 NcoI T allele (Table 5) [36]. These findings were later followed up by others revealing a significant association between the DRD2 NcoI C allele and the clinical response to rizatriptan, a 5-HT_{1B} receptor agonist used for treating migraine attacks, and a lack of association between dopaminergic genes and the migraine-panic phenotype [3, 37]. Still, a lack of interaction between this polymorphism and the clinical features of migraine was reported by other independent studies [19, 38]. Finally, the most recent case-control study led by Gosh and co-workers (2012) revealed a protective effect for the variant genotype TT and allele T [21]. Our findings, though in accordance with those reporting on the association of DRD2 C939T polymorphism with migraine susceptibility, however, the protective effect was attributed to the variant genotype CC and allele C. Such variations have mainly been attributed to stratification.

Multiple possibilities may result in the differential relation, but the two most likely explanations are ethnicity and the difference in frequency of the C allele in different populations. This may imply that the genetic background is a potential contributing factor to the genetic predisposition to migraine [12].

There are hypotheses suggesting the contribution of common genetic variations to the structural and functional diversity of the mRNA, which could account for a significant fraction of human phenotypic variations [39]. Finally, despite a minor variation in C939T DRD2 mRNA conformation revealed by our RNA fold analysis, other factors may potentially affect DRD2 gene regulation when considering the effects that synonymous codons might have on mRNA stability [40-42], protein synthesis rate (translation elongation, translation efficiency), and protein folding [43]. Future experiments aimed at investigating these effects, along with information on tRNAs abundance and regulation, may shed light on how exactly this codon-mediated gene regulation operates [44].

5. Conclusion

Despite multiple studies in human models, the complex polygenic nature of migraine, along with a lack of knowledge on the number of migraine susceptibility genes, has made unravelling the migraine molecular mechanisms challenging. Future investigations aimed at identifying novel migraine susceptibility genes will undoubtedly improve our understanding and treatment of the disorder.

Abbreviations

TNF- β	Tumor necrosis factor- β
DRD2	Dopamine receptor D2
MO	Migraine without aura
MA	Migraine with aura

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Author Contributions

SI and NG designed and performed the experiments, derived the models, analyzed the data, and writing the main manuscript text and tables, and review the manuscript. PS conceived of the presented idea and contributed to data acquisition. AA developed the theory and contributed to data acquisition. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no conflict of interest.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available in the [Association of dopamine D2 receptor (DRD2) C939T and a lack of association of Tumor Factor- β (TNF- β) +A252G polymorphisms with susceptibility to Migraine in A Northern Iranian population] repository, [<https://figshare.com/s/5ba2f6b5ff387790576b>], [<http://dx.doi.org/10.6084/m9.figshare.14774709>].

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