Faraji *et al.*
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RESEARCH

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rs3761548 (C/A) and *rs5902434 (del/ATT)* polymorphisms of *Foxp3* gene in Iranian patients with migraine

Fardin Faraji[1], Ghasem Mosayebi[2], Maryam Bahrami[2] and Mana Shojapour[2*]

Abstract

Background Migraine is a neurovascular disorder; several studies have demonstrated the immune system plays a key role in migraine pathogenesis**.** The aim of this study was to investigate the association between *FOXP3* gene polymorphism and susceptibility to migraine.

Methods In a case–control study, 55 whole blood samples of patients with migraine and 80 healthy samples were collected. After DNA extraction, genotyping of the *rs5902434* (*del/ATT*) and *rs3761548 (C/A) FOXP3* was performed using sequence-specific primers method (PCR-SSP).

Results The results of this study showed that there were statistically significant differences between patient and control group in genotype frequencies of *rs3761548*. In addition, the frequency of heterozygous genotype AC at *rs3761548* in patients was found to be significantly higher than controls. We also found no significant differences between cases and controls were found in the allelic and genotype distribution of the *rs5902434 (del/ATT)* polymorphism. None of the *rs5902434 (del/ATT)* genotypes showed any significant association with the migraine.

Conclusions According to finding of our study, polymorphism *rs3761548* in *FOXP3* gene were associated with susceptibility to migraine. Further studies with larger sample sizes and different populations in other parts of the world are needed to investigate relationship between this polymorphism on migraine susceptibility.

Keywords *FOXP3*, Migraine, *rs5902434*, *rs3761548*, Single nucleotide polymorphism

Introduction

Migraine is a neurovascular disorder that affects 10–20% of general population during periods of their lives [1], Based on the Global Burden of Disease (GBD) 2019 study, the global mean prevalence rate of migraine increased from 721.9 million (95% UI: 624.9–833.4) in 1990 to 1.1 billion (95% UI: 0.98–1.3) in 2019 [2]. A migraine attack can have a wide variety of symptoms may include throbbing pain, nausea and vomiting, phonophobia (sound sensitivity), photophobia (light sensitivity), stiffness of the neck and shoulders, diarrhea. Migraine attacks usually last from 4 to 72 h and migraine symptoms are often provoked by physical activity [3].

Although the underlying cause of migraines is unknown, a variety of factors, such as genetics and environmental factors can play a role in the development of migraine. Individual's susceptibility to migraine may be determined by genetic factor, while environmental factors are related to its development [4].

Various studies have indicated that an immune system plays a key role in the pathophysiology of migraine. Elevated blood levels of pro- and anti-inflammatory cytokines such as TNF and interleukin 10 (IL-10) were

*Correspondence:
Mana Shojapour
mana_shojapuor@yahoo.com
[1] Department of Neurology, School of Medicine, Arak University of Medical Sciences, Arāk, Iran
[2] Molecular and Medicine Research Center, Arak University of Medical Sciences, Arāk, Iran

observed during a migraine attack [5–7]. Furthermore, studies were performed on the relationship between the number of regulatory T cells (T_{reg} cells) and the pathophysiology of migraine [8, 9]. Clinical study showed that a significant increase in $CD4^+$ and a decrease in $CD8^+$ levels was observed in migraine patients [10]. Also studies on the relationship between T_{reg} population and migraine pathophysiology were done. Their results showed that the percentage of T_{reg} cells in migraine patients was significantly lower than the control group. The results of these studies suggest that deregulated immunity may be involved in the pathophysiology of migraine [9].

T_{reg} cells play an important role in prevents the development of autoimmune disease [11–13]. Forkhead box P3 (*FOXP3*) gene is located on the short arm of the human sex chromosome (*X* on XY model) (*Xp11.23*; 21 kb) [14]. *FOXP3* gene provides instructions for producing the forkhead box P3 (FOXP3) protein. The protein encoded by this gene is a member of the forkhead/winged-helix family of transcriptional regulators that plays a vital role in the development and function of regulatory T cells [15, 16].

FOXP3 gene mutations can disrupt immune regulation and lead severe autoimmune diseases [17–20]. The polymorphisms in the *FOXP3* promoter gene may change activity and production of *FOXP3* [19, 21, 22]. Numerous research studies have been conducted to try to understand the relationship between *FOXP3* gene polymorphisms and autoimmune diseases. The results of some of this research showed a positive association between *FOXP3* gene polymorphisms and susceptibility to type1 diabetes [23] or rejection in kidney transplant recipients [24], systemic lupus erythematosus [20], vitiligo [25], autoimmune thyroid disease [26], recurrent permanent abortion [27] and multiple sclerosis [28]. Given that, there is debate about the role of autoimmunity in the pathophysiology of migraine [29]. The aim of this study was to investigate the association between *rs3761548 (C/A)* and *rs5902434 (del/ATT) FOXP3* gene polymorphism and migraine susceptibility.

Materials and methods

Study population (patients and healthy controls)

In a case–control study 55 patients with headache complaints referred to neurology clinics and health centers in Arak University of Medical Sciences were evaluated by a neurologist according to the criteria from the International Classification of Headache Disorders were included [30]. For this study 80 age and sex matched healthy controls were enrolled, without headache history and immunological diseases. Written informed consent was obtained from all participants. The present study was approved by the Institutional Human Ethics Committees (IR.ARAKMU.REC.1397.238), Arak University of Medical Sciences, Iran. Degree of disability caused by migraine have been measured with Migraine Disability Assessment Scale (MIDAS).

Genotyping of *rs3761548 (C/A)* and *rs5902434* (*del/ATT*)

2 ml whole blood samples were collected; genomic DNA was extracted from whole blood using DNA extraction kit (YektatajhizAzma, Tehran, Iran) according to manufacturer's instructions, and stored at − 20 °C until subsequent analysis. *rs5902434* and *rs3761548* genotype were analysed through sequence-specific primers method (PCR-SSP). PCR-SSP was performed in a total volume of 25 ml using 1 ml of genomic DNA (1 mg/ml), 12.5 ml Taq DNA polymerase MasterMix (Yektatajhiz co, Tehran, Iran), 1 ml of each primer (5 mM) and sterile distilled H2O. The PCR temperature cycling conditions were as follows: initial denaturation at 95 °C for 4 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 1 min and extension at 72 °C for 1 min and the final extension of 72 °C for 5 min. Primers used in this study are listed in Table 1. PCR products analysed by electrophoresis on 2% agarose gel then visualized by transilluminator device.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences version 10.0 (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as mean ± standard error of the mean and qualitative data were presented as number (percentage). A P-value less than 0.05 were considered statistically significant. The chi-squared tests were used for compare to frequency of allele, genotype distribution and categorical variable. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by Logistic regression to estimate association between this SNP and disease susceptibility.

Table 1 Primers sets used for SNP genotyping analyses

SNP	Primer sequences	References
rs3761548	F1 5′-CTGGCTCTC TCCCCAACTGA-3′	[37]
	F2 5′-CTGGCTCTCTCCCCAACTGC -3′	
	R 5′-ACAGAGCC CATCATCAGACTCTCTA-3′	
rs5902434	F1 5′-ACCTTTAAGTCTTCTGCCATTATTCTATTA TTT- 3′	[37]
	F2 5′-CCTTTAAGTCTTCTGCCATTTATTCCTATT AT TA-3′	
	R 5′-TGATTATCAGCGCACACACTCAT-3′	

Results

In this study, a total of 135 individuals, including 55 with migraines and 80 healthy as a control group were examined. The demographic characteristics are presented in Table 2. No significant differences of age and gender were detected between cases and controls ($p > 0.05$).

Association of *FOXP3* polymorphisms with genetic susceptibility to migraine

Both polymorphisms were in Hardy–Weinberg equilibrium (HWE), ($p > 0.05$). Genotype and allele distributions of the *rs5902434* and *rs3761548* in migraine subjects and controls are shown in Table 3, $p < 0.05$ is considered statistically significant. For *rs5902434*, genotype frequency of TT, TA, AA in migraine patients were 29.1%, 65.5%, 5.5% and for healthy control were 21.3%, 75% and 3.8% respectively (Fig. 1). There was no statistically significant difference in genotype and allele frequency of *rs5902434* polymorphisms between the patient and control group. None of the genotypes showed any significant association with the migraine ($p = 0.48$ and $p = 0.61$ respectively). However, there were statistically significant differences between patient and control group in genotype frequencies of *rs3761548*. The frequency of heterozygous genotype AC (n = 16, 29.1%) in patients was found to be significantly higher than in controls ($p = 0.02$, OR = 2.57, 95% CI 1.08–1.09). In addition, the A allele was significantly more frequent in patients (36.4%) than in healthy controls (14.4%), and associated with increased susceptibility to migraine development ($p = 0.00$, OR = 1.40, 95% CI 0.890–1.12) (Fig. 2).

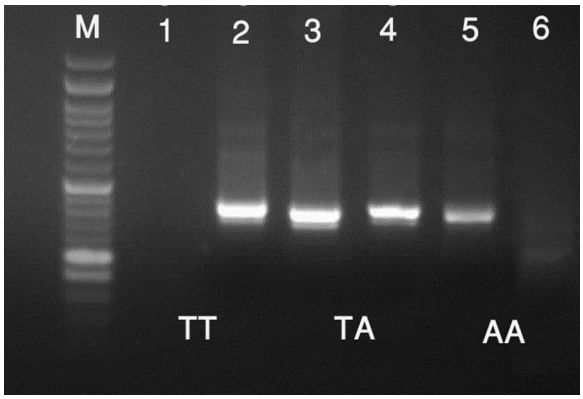

Fig. 1 Agarose gel electrophoresis for detection of *rs5902434*polymorphism. Lane (M): molecular weight marker, lanes 1,2 TT genotype; lanes 3, 4: TA genotype and lanes 5,6: AA genotype

Table 2 Demographical characteristics of migraine patients and healthy controls

Characteristics	Number of cases (%)	Number of controls (%)
Sample size	55	80
Age (years) mean ± SD	35.43 ± 0.99	33.69 ± 1.50
Gender		
Male	13 (23.6%)	19 (23.7%)
Female	42 (76.4%)	61 (76.2%)

Association of *FOXP3* polymorphisms with clinical characteristics of patients with migraine

The results of the MIDAS questionnaire showed that the grade of disability due to headache was 15%patients (grade 1), 32.5%patients (grade 2), 30% patients (grade 3) and 22.5%patients (grade 4). Stratified analysis was performed to evaluate the associations between

Table 3 Genotype and allele frequencies of FOXP3 *rs3761548* and *rs5902434* patients and control subjects in Iranian population

SNP	Model	Allele/genotype	Migraine (%)	Controls (%)	Odd ratio	*p* value
rs3761548		C	70 (63.6%)	137 (85.6%)	1.40 (0.89- 1.12)	0.00
		A	40 (36.4%)	23 (14.4%)		
	Dominant	C/C	27 (49.1%)	63 (78.8%)	0.26 (0.12–0.55)	0.00
		A/C-A/A	28 (50.9%)	17 (21.2%)		
	Recessive	C/C-A/C	43 (78.2%)	74 (92.5%)	2.44 (0.20–1.83)	0.01
		A/A	12 (21.8%)	6 (7.5%)		
rs5902434		ATT (A)	66 (41.2%)	45 (39.8%)	0.94 (0.57–1.54)	0.90
		Deletion (T)	94 (58.8%)	68 (60.2%)		
	Dominant	Deletion/Deletion	16 (29.1%)	17 (21.2%)	1.52 (0.68–1.35)	0.29
		Deletion/ATT-ATT/ATT	39 (70.9%)	63 (78.8%)		
	Recessive	Deletion/Deletion-Deletion/ATT	55 (94.8%)	77 (96.2%)	1.40 (0.27–1.19)	0.68
		ATT/ATT	3 (5.5%)	3 (3.8%)		

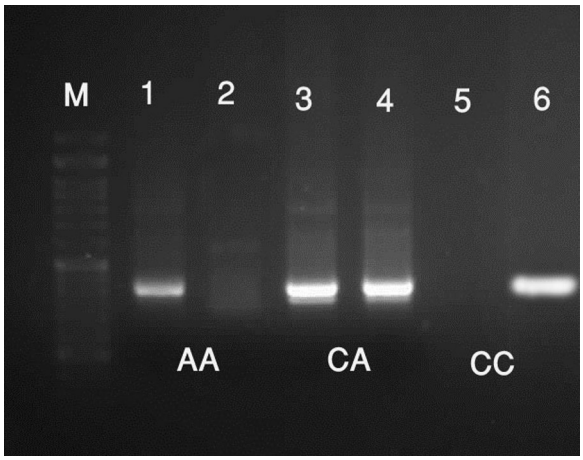

Fig. 2 Agarose gel electrophoresis for detection of *rs3761548* polymorphism. Lane (M): molecular weight marker, lanes 1,2 AA genotype; lanes 3, 4: AC genotype and lanes 5,6: CC genotype

each genetic polymorphism and clinical characteristics. There were no significant associations between *rs5902434* and *rs3761548* with clinical features.

Discussion

Migraine is a complex neurobiological disorder and pathophysiological mechanisms of migraine are not completely understood [31]. Several studies have proved the role of immune system in the pathogenesis of migraine [9, 32]. According to finding of our study, polymorphism *rs3761548* in *FOXP3* gene were associated with susceptibility to migraine. Furthermore, in our study, showed that there is no correlation between polymorphism *rs5902434* in of *FOXP3* gene with migraine.

Associations between *FOXP3* gene polymorphisms and several autoimmune diseases have been investigated in various studies. Wang et al. reported that of *rs3761548C/A* and *rs2294021C/T* polymorphisms were associated with the susceptibility to diabetes and diabetic nephropathy in the Han Chinese population [33]. In a case–control study by Hassannia et al., in 2011, the *FOXP3* gene polymorphism at positions -3279 A>C and -924 A>G were evaluated in patients with allergic rhinitis (AR). Researchers report that there is a significant association between *FOXP3* gene polymorphism and allergic rhinitis. *FOXP3*-3279 A alleles were associated with an increased risk of AR in an Iranian population [18]. In study by El-maadawy in 2022, showed that *FOXP3 rs3761548CC* and *AA* genotypes could be associated with an increased acute lymphoblastic leukemia (ALL) risk [34]. A 2013 study by Jahan et al. On patients with vitiligo showed that the polymorphism in *rs3761548* of the *FOXP3* gene may be associated with vitiligo susceptibility due to changes in expression. The CC genotype appears to be protective and the AC genotype appears to be approximately three times more likely to develop vitiligo in women group. Also, in another study of Iranian patient with multiple sclerosis (MS) revealed that the frequencies of AA and AC genotypes at *rs3761548* in the *FOXP3* gene were significantly higher in MS group as compared with healthy subjects [28]. In another investigation, Lan et al. reported that *FOXP3* gene -2383C/T polymorphism is associated with susceptibility to systematic lupus erythematosus (SLE) in Chinese Zhuang population [20].

Studies on the association of this polymorphism with migraine are limited. To our knowledge, our research is the first study to investigate the relationship between *rs5902434* in *FOXP3* polymorphisms and the risk of developing migraine in Iranian population. Only in one study association between *FOXP3* gene polymorphism and migraine was investigated, the results of this study showed that there was no significant difference between the allelic and genotypic distribution of *FOXP3 rs3761548* polymorphism in women and men with migraine compared to the control group [35].

The promoter region located upstream of the gene initiation site plays an important role in regulating gene expression. A polymorphism in the *FOXP3*, as rs3761548, may modify the transcription factor binding sites and change gene expression. In our study, we found A allele and AC genotype were significantly higher in migraine patients. To date, the exact mechanism of *FOXP3* regulation has not been determined, but a study by Shen et al. in 2010, indicated that A allele in patients may cause to reduction of *FOXP3* expression and leading to defective transcription of *FOXP3* gene [36].

In conclusion, the results of the present study showed SNP *rs3761548* in *FOXP3* gene was associated with the susceptibility to migraine disease. Also, we did not find a statistically significant association of rs5902434 with migraine. Further studies with larger sample sizes and different populations in other parts of the world are needed to validate the relationship between this polymorphism on migraine susceptibility.

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Authors' contributions

FF was responsible for diagnosis and confirmation of the disease, MS participated in design, data collection, and preparing the first draft of the manuscript. MB performed the experiments. GM participated in design the study and drafting the article. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this manuscript. In addition, more detail data are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The present study was approved by the Ethics Committee of Arak University of Medical Science, Arak, Iran (IR.ARAKMU.REC.1397.237). Informed consent was obtained from all participants. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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