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Common MEFV Mutations (M694V, V726A, M680I, M694I, and E148Q) in Patients with Behcet's Disease in Ardabil

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Abstract

Background and objectives: Behcet's Disease (BD) is a rare severe recurrent inflammatory disorder affecting several body organs. Since Familial Mediterranean Fever (FMF) and BD affect almost a specific population, both diseases can mimic the other clinically, and these two diseases sometimes occur in the same family and the same patient, also due to the high prevalence of BD in Iran and performing a small number of studies about MEFV gene mutations in patients with BD, this study aimed to determine the frequency of MEFV gene mutations in Ardabil patients with BD.

Material and Methods: Fifty patients with BD were diagnosed according to the International Study Group criteria for BD (for oral pests, genital pests, and ocular lesions, 2 points each, skin symptoms, vascular symptoms, positive pathogenesis test, and neurological symptoms, 1 point each). A score of 4 or higher indicated BD. All patients were analyzed for five common MEFV mutations (M694V, V726A, M680I, M694I, and E148Q) using amplification refractory mutation system and Polymerase Chain Reaction (PCR) restriction-digestion testing methods. A cohort of 224 healthy people who had been previously genotyped regarding the five common MEFV mutations served as the control group.

Results: The mean age of patients was 38.68±11 years. Most BD patients (56%) and 50.4% of the control group were women. Of all patients, 66% (33) mentioned that their parents had no family relationship. Among 50 patients with BD, 12 (24%) had mutations in the MEFV gene, of which seven patients (58.3%) had E148Q mutation. Among healthy individuals, 57 patients (25.4%) had mutations, of which 39 (68.4%) had E148Q mutation. The difference between the two groups was not significant.

Conclusion: Results showed that most patients with BD had mutations in the MEFV gene and the most common case was E148Q mutation which was similar to the healthy population in terms of BD agent.

Keywords: Behcet Syndrome [MeSH]; Mutation [MeSH]; Familial Mediterranean Fever [MeSH]

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Highlights

Behcet's Disease is prevalent in most areas of Iran, and most of them have mutations in MEFV. Similar to those without BD, the prevalent gene mutation in these patients was related to E148Q.

Introduction

Behcet's disease is a chronic multisystem autoinflammatory disease with pronounced recurrent clinical manifestations. Its common symptoms are oral plagues, genital sores, eye inflammation, and skin lesions that are thought to be due to vasculitis (1- 4). The average onset age of BD is 30 years old, and, its severity and prevalence in both sexes vary and depended to the race (5). Although the cause of BD is not fully understood, but due to high prevalence in populations of Asian and Mediterranean descent, HLA-B51 has been confirmed as the strongest genetic risk factor for BD in different racial populations. About 48% of BD patients and about 31.9% of the normal population have HLA-B51 (6- 8). Frequencies of p.M694V, p.E148Q, p.V726A, p.M680I, and p.M694I in Alibakhshi et al., study population were closer to those observed in the Mediterranean countries, especially in the Middle Eastern Arab populations (9).

A study showed that FMF and BD may have common etiopathogenetic mechanisms which may result in sacroiliitis. So, they recommended some further investigations to demonstrate whether this coexistence is due to chance or common etiopathogenetic characteristics (10).

According to a few statistics on the prevalence of BD in northwestern Iran and due to the location of Iran on the Silk Road with a high prevalence of BD (80 per 100000) after Turkey (420 per 100,000), despite the existence of previous studies in Turkish areas and on the other hand, assuming a change in genetic pattern and achieving better and more accurate scientific evidence in this field doing more studies in this regard can be essential (4, 11). The MEFV gene was identified on chromosome 16p.13.3 in 1997, and more than 20 FMF mutations have been detected in this gene (12). Given that FMF and BD affect specific populations, both diseases can mimic the other clinically and these two diseases sometimes occur in the same family and patient (13). This study aimed to determine the frequency of MEFV gene mutations in Ardabil patients with BD.

Materials and Methods

This case-control study was performed on 50 patients with BD from March 2020 to March 2021. The diagnosis was based on the ICBD criteria, giving two points each for oral pests, genital pests, and ocular symptoms and one point each for skin, vascular, positive pathogenesis, and neurological symptoms. A score of 4 or higher confirmed the disease. The study included 224 people from the normal population (without BD) as a control group. First, information was extracted from patients' files, including age, gender, contact number, location, and tests of patients, and then patients were contacted to obtain a complete history and possible history of the disease and also invited to attend the genetic laboratory. The patients were met again when visiting the laboratory, the necessary history and examinations were performed, and all information was entered into the designed checklist. Genomic DNA was extracted from peripheral blood leukocytes during genetic testing using standard protocols. Each sample was examined for 12 conventional MEFV mutations using the mutation Polymerase Chain Reaction (PCR) system and the PCR restriction fragment length polymorphism method. In various steps of the PCR method, four fragments of the MEFV gene were amplified using the Multiplex-PCR strategy using solutions (by using Taq DNA polymerase) and primers from the FMF strip Assay kit from Vienna Lab Diagnostics GmbH (Vienna Lab Diagnostics GmbH, Austria). Using 1% agarose gel electrophoresis, fragments with lengths of 206, 295, 236, and 318 bp were identified and confirmed. Reverse-hybridization and enzymatic color detection were carried out either manually, using thin-walled plastic incubation trays (Bio-

Rad Laboratories Inc., California, USA) and a shaking water bath (GFL) set to 45 °C, or in a fully automated device (profiBlot IIT; TECAN AG, Fornak Technologies, Austria). Patients with a score of 4 or more based on the ICBD criteria and no positive family history of FMF were included in the study with consent. The study was registered in the university's ethics committee with the code of IR.ARUMS.REC.1399.428. Data collected in SPSS 25 statistical analysis software were analyzed using chi-square, t-test, and ANOVA.

Results

In the present study, 50 patients with BS and 224 healthy individuals were included. The average age of patients was 38.68 years, with a standard deviation of 11.004. 56% of patients and 49.6% of healthy individuals were women, and there was no significant difference between the two groups regarding gender. 66% (33 patients) mentioned that their parents had no family relationship. Among 50 patients with BD, 12 (24%) had mutations in the MEFV gene. Among healthy people, out of 224, 57 (25.4%) had mutations. There was no significant difference between the two groups regarding mutation (Table 1).

Among those with mutations, seven patients (58.3%) had the E148Q mutation, which was the most common. Only three people had the V726A mutation, of which 2 were heterozygous and 1 was homozygous. The rest of the mutations were heterozygous in all subjects. From the healthy group, four mutants were homozygous and 53 heterozygous. The highest mutation among healthy individuals, such as the patient group, was related to the E148Q mutation with 39 patients (68.4%). The two groups did not differ significantly in the type of mutation (Table 2).

There was no significant relationship between gender and age of patients in the two groups and the presence or absence of mutations and the type of MEFV gene mutations. Because only one of the M694V and A744S mutations was seen among the patients, it was not possible to evaluate the average age for the mutations in question. No significant relationship was found between any of the studied symptoms of BD and the type of MEFV gene mutations in these patients (Table 3). No significant correlation was found between the laboratory tests performed for BD and the type of MEFV gene mutation in these patients (Table 4). There was no significant relationship between any of the studied symptoms of BD and the presence or absence of mutations in the MEFV gene in the sepatients.

Table 1. Frequency of MEFV mutations in two groups

Mutations		BD		control		*P*-value
		n	%	n	%	
-		38	76	167	74.6	0.97
+	Mutation of an allele (heterozygous)	11	22	53	23.7	
	Mutation of both alleles (homozygous)	1	2	4	1.7	

Table 2. Frequency of type of MEFV mutations in two groups

Mutations		BD		control		*P*-value
		n	%	n	%	
Without mutations		38	76	167	74.6	
Type of mutations	E148Q	7	14	39	17.4	0.48
	A744S	1	2	2	0.9	
	P364S	0	0	6	2.7	
	F479L	0	0	1	0.5	
	M694V	1	2	2	0.9	
	R761H	0	0	2	0.9	
	V726A	3	6	4	1.8	
	V625R	0	0	1	0.5	

Table 3. Frequency of MEFV mutations, based on patients by clinical manifestations

Mutations Clinical Manifestations		without	V726A	E148Q	M694V	A744S	*P*-value
Ocular manifestations	+	23	2	6	0	1	0.41
	-	15	1	1	1	0	
Neurological manifestations	+	22	1	4	0	0	0.54
	-	16	2	3	1	1	
Skin manifestations	+	23	0	4	1	1	0.22
	-	15	3	3	0	0	
Vascular manifestations	+	8	2	1	0	0	0.37
	-	30	1	6	1	1	
Oral plague	+	38	3	7	1	1	-
	-	0	0	0	0	0	
Genital plague	+	31	3	3	1	1	0.15
	-	7	0	4	0	0	
Joint manifestations	+	27	2	4	0	1	0.54
	-	11	1	3	1	0	
pathergy	+	12	1	2	1	0	0.49
	-	14	1	0	0	0	

Table 4. Frequency of MEFV mutationbased on patients by laboratory manifestations

Mutations		without	V726A	E148Q	M694V	A744S	*P*-value
HLAB5	+	16	1	3	0	1	0.77
	-	9	0	2	0	0	
HLAB51	+	16	1	3	0	1	0.77
	-	9	0	2	0	0	

No significant correlation was found between any of the BD tests and the presence or absence of mutations in the MEFV gene in these patients. Mutations V726A, E148Q, and A744SM694V were not significantly different in the two groups in terms of whether or not there was a mutation and its single allele and dual allele. In this study, 100 alleles of the patient group and 448 alleles of the healthy group were included. 13 cases (13%) of the patients and 62 cases (13.8%) of the healthy group had mutations that the distribution of mutations between the two groups was not significant.

We evaluated the specificity of the FMF Strip Assay by analyzing a series of amplification products obtained from mutant plasmid clones, as well as wild-type, heterozygous, or homozygous mutant genomic DNA samples (Fig 1, Table 5, Fig 2).

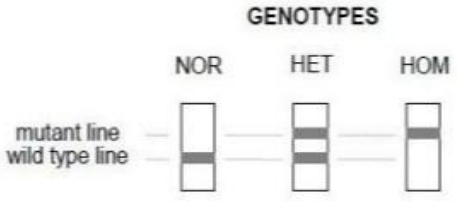

Figure 1. Identification process of MEFV mutations

Table 5. The identification process of MEFV mutations

	Wild Type Line	Mutant Line	Genotype
NOR	Positive	Negative	Normal
HET	Positive	Positive	Heterozygous
HOM	Negative	Positive	Homozygous Mutant

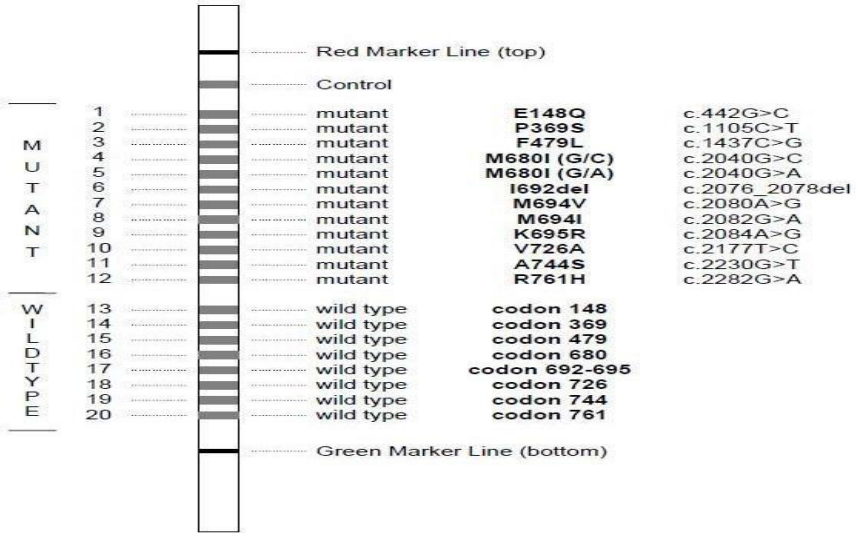

Figure 2. Identification process of MEFV mutations

Discussion

In the BD group, there was no significant relationship between MEFV mutations and different clinical manifestations, age, sex, and laboratory results of patients (HLA-B5 and HLA-B51). In our study, we concluded that most mutations in BD patients with 14% were related to the E148Q mutation, which was lower than the normal people with 14%. The second rank of the relevant gene mutation in the normal population with 2.7% was belonged to the P369S mutation, which was not seen in any of the BD group. This comparison shows that the risk of P369S mutation in patients with BD is much lower than the normal population. In total, 25.4% of the normal population of our study had mutations in the MEFV gene, which was 24% among patients with BD and therefore, it can be said that the probability of MEFV gene mutations in patients with BD is equal to the normal population. In the healthy population, 49.1% of mutations were in men and 50.9% in women, which was slightly different in BD patients because, 7 out of 12 mutations (58.33%) were related to men, which was more than the healthy population. It should be noted that in both BD and control groups there was no significant difference between gender and frequency of gene mutations.

In a study by Imirzalioglu et al., forty-two BD patients with no family history of FMF and 66 healthy controls for common MEFV gene mutations (E148Q, M680I, M694V, and V726A) were screened. Fifteen patients had MEFV mutations (not M694V, five E148Q, and one M680I), and the mutation rate significantly differed from the control group. Also, in this study, the E148Q mutation was one of the highest frequencies among patients, similar to our study patients. Also, in our study, the M694V mutation was the most common among patients, accounting for only 2%. This difference between our study and Imirzalioglu et al. can be considered as one of the genetic differences of this disease in these two important geographical regions in BD (13).

In a study by Esmaeili et al., entitled common MEFV mutations in Iranian Azeri patients with BD performed in 2011 at Tabriz, 53 BD patients were included. In this study, as in our study, the highest frequency was related to E148Q mutation. In the mentioned study, a total of 19 mutations out of 106 alleles studied (17.92%) were obtained, and in our research, this number was 13 out of 100 alleles studied (13%), which is a lower percentage than the neighboring province it shows (11).

A study by Çakır et al., entitled MEFV mutation frequencies in a Turkish cohort with a low prevalence of familial Mediterranean fever, was

performed. This study analyzed 263 unrelated healthy adults for the M694V, V726A, M680I, and E148Q mutations in the MEFV gene. 25 of the 263 individuals carried MEFV mutations (9.5%). The frequencies of the M694V, M680I, and E148Q mutations were not significantly different from allele frequencies (approximately 20%) determined for other regions of Turkey where FMF is more prevalent. In our study, among the BD group, M694V and V726A mutations (2% and 6%, respectively) were more than in the control group (0.9% and 1.8%). But the E148Q mutation with 14% in the BD group and 17.4% in the control group in our study was not in line with the findings of Çakır et al. (14). Further studies in this area are necessary to achieve the differences and similarities of the findings. Finally, according to the results of our research, there was no significant difference between the frequency of MEFV gene mutations in the BD patient and the healthy population of Ardabil province. Also, no significant differences were found in the type of MEFV gene mutations and the clinical manifestations of the BD patients.

Yazici et al. showed no significant relation between the mutations in the BD group and clinical findings, which was similar to our results. Also, the rate of E148Q and A744S mutations in this study were 3% and 1% which was lower than our study results with 14% and 2% (5).

Tasliyurt et al. found that the frequency of the MEFV mutation was significantly higher in patients with BD compared to the healthy control group. Based on our results, MEFV mutations appear to have a role in the pathogenesis of BD. Comparing our results with Tasliyurt study, we did not find any significant difference in the frequency of the MEFV mutation in patients with BD compared to the healthy control group, so we could not confirm the role of MEFV in the pathogenesis of BD (15).

Salehzadeh et al. found that Twenty-five percent of the normal population of the northwest of Iran carry a heterozygous variant of the MEFV gene, E148Q (18.3%) as the most common mutation, which can be considered as a normal variant in the healthy population. Similarly, in our study on BD patients, the rate of E148Q mutation of 14% was more than other mutations (16).

In a study by Bonyadi et al., five MEFV mutations (M694V, V726A, M680I, M694I, and E148Q) were examined in 200 healthy individuals. They showed 25% mutation in the normal Azeri population. The most common mutation was E148Q with 11.5% and V726A with 1.75 %. Our study examined common mutations and showed the same results with a higher percentage, E148Q mutation with 17.4%, and V726A mutation with 1.8% (8).

In a study by Ebadi et al., twelve common MEFV gene mutations were examined in 390 FMF patients from all areas of Iran. Two hundred thirty-four patients (60%) had at least one mutation, and 156 patients (40%) were without any common mutations. The most common variants were M694V (13.6%), followed by E148Q (10.4%), M694I (6.5%), V726A (4.1%), and M680I (3.8%), respectively (16). In our study, the most common mutation was E148Q (17.4%), followed by P364S with 2.7%, which was higher and different from Ebadi et al.(17).

In a study by Coskun et al., 220 patients with FMF were compared with a group of 228 healthy people as a control group. Five mutations of M694V, M694I, M680I, V726A, R761H, and E148Q were determined. The E148Q mutation, similar to our study, was significantly higher in the control group (17.4%) than in the BD group (18).

Conclusion

This study showed that the highest frequency of MEFV gene mutations was related to the E148Q mutation in BD patients and healthy individuals. It is suggested that the present study be conducted at the national level and with a larger statistical population and compare the necessary results. It is also suggested that other diseases be associated with mutations in this gene, especially in the geographical belt associated with FMF disease.

Limitations of this study:

Due to the high cost of genetic testing, we used the previously registered information of Salehzadeh et al. to study MEFV gene mutations in the normal population of Ardabil province. A few patients had no information about the results of the HLA-B5, HLA-B51, and Pathergy tests. Due to the COVID-19 pandemic status, access to patients was difficult and time-consuming.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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