Common *MEFV* mutations in Iranian Azeri Turkish patients with Behçet's disease

M Esmaeili[1], M Bonyadi[1,2], A Khabbazi[3], AA Ebrahimi[3], S-K Sharif[3], M Hajialilo[3], S Kolahi[3], S Dastgiri[4]

[1]Liver and Gastrointestinal Disease Research Centre, Drug Applied Research Centre, Tabriz University of Medical Sciences, [2]Centre of Excellence for Biodiversity, Faculty of Natural Sciences, University of Tabriz, [3]Rheumatology Ward, Imam Reza Hospital, Faculty of Medicine, Tabriz University of Medical Sciences, and [4]Department of Community and Family Medicine, School of Medicine, National Public Health Management Centre, Tabriz University of Medical Sciences, Tabriz, Iran

Objective: Behçet's disease (BD) is an inflammatory disorder of unknown cause with higher prevalence along the ancient Silk Road. BD shares epidemiological and clinical features with familial Mediterranean fever (FMF). Moreover, association of BD and certain *MEFV* gene mutations has been described in recent decades. We studied the role of *MEFV* mutations in Iranian Azeri Turkish patients with BD.

Methods: Fifty-three BD patients who met the International Study Group criteria for BD were analysed 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 200 healthy Azeri Turkish individuals who had been previously genotyped regarding the five common *MEFV* mutations served as the control group.

Results: Eighteen patients were found to carry a single *MEFV* mutation and one additional patient was compound heterozygote. There was a statistically significant difference between the patient group and ethnically matched healthy individuals regarding M694V and M680I mutations ($p = 0.01$ and $p = 0.04$, respectively). Both BD groups (carriers and non-carriers of *MEFV* mutations) were similar in their clinical symptoms.

Conclusion: Definite *MEFV* mutations seem to be a susceptibility factor for BD in our cohort of Iranian Azeri Turkish patients.

Behçet's disease (BD) is a multisystemic inflammatory disorder of unknown cause, characterized by recurrent oral and genital ulcers, uveitis, and skin lesions. The disease may involve joints, the central nervous system, and the gastrointestinal tract (1, 2). BD is not a Mendelian disorder; however, considering its peculiar distribution, its occasional familial aggregation, and its close association with genes of major histocompatibility complexes, BD is under partial genetic control (3, 4).

The results of recent studies have suggested that some *MEFV* (the gene responsible for familial Mediterranean fever, FMF) mutations may act as additional susceptibility genetic factors in BD (4–9). FMF is an autosomal recessive autoinflammatory disorder characterized by self-limiting recurrent bouts of fever and painful episodes of sterile serositis that typically involve the peritoneum, pleura, and synovia and is sometimes associated with erysipelas-like erythema (10). Both FMF and BD have been highly prevalent along the ancient Silk Road, which extends from the Mediterranean Basin to eastern Asia (10, 11). These two diseases share some common symptoms, laboratory findings, and treatment with colchicine (5). *MEFV* encodes a protein called pyrin or marenostrin, expressed primarily in the myeloid cell lineage. Pyrin belongs to a class of proteins involved in the regulation of apoptosis and inflammation (10).

Iran is among the countries in which BD is fairly common (12) and this study was designed to determine the frequency of five common *MEFV* mutations among Iranian Azeri Turkish BD patients.

Materials and methods

A total of 53 BD patients who had no symptoms or family history of FMF was included in the study. All patients were of Azeri Turkish origin and were referred by rheumatologists according to the diagnostic criteria proposed by the International Study Group for BD (2). Informed consent was obtained from all patients after an explanation of the purpose of the study. Genomic DNA was extracted from peripheral blood leucocytes using standard protocols. Each sample was tested for the five

Mortaza Bonyadi, Centre of Excellence for Biodiversity, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.
E-mail: jabbarpour@tabrizu.ac.ir

Accepted 9 February 2011

DOI: 10.3109/03009742.2011.562532

Table 1. Distribution and p-values of five MEFV mutations in BD patients and the control group.

Mutation	Patient group (n=53)	Control group (n=200)	p-value*	OR (95% CI)*
E148Q	12	45†	1	1.01 (0.49–2.08)
M694V	3	0	0.01	
V726A	3	7	0.69	1.65 (0.41–6.63)
M680I	2	0	0.04	
Total	19‡	52	0.15	1.59 (0.83–3.03)
Total without E148Q	7	7	0.01	4.19 (1.40–12.55)

OR, Odds ratio; CI, confidence interval.
*Some figures were rounded up.
†One control was homozygous for E148Q.
‡One patient was compound heterozygous.

common *MEFV* mutations (M694V, M694I, M680I (G to C transversion), V726A, and E148Q) by using amplification refractory mutation system-polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism methods as described previously (13). A total of 200 unrelated ethnically matched healthy subjects who had been previously genotyped regarding the five common mutations served as the control group (14).

The χ^2 test and Fisher's exact test were used for comparison of frequencies of gene carriage. A p-value < 0.05 was regarded as statistically significant. All tests were two-tailed. A χ^2 test of goodness-of-fit was also applied to test for Hardy–Weinberg equilibrium. A p-value > 0.05 was considered as consistency with Hardy–Weinberg equilibrium.

Results

Of the 53 referred patients, 31 were males and 22 were females (male-to-female ratio of 1.4:1). The age range of the patients was 17 to 50 years (mean 34.25 years). The main clinical characteristics of the patients were the same as reported previously (15). Mutation analysis of the *MEFV* gene revealed that 19 (36%) patients carried one or two common mutations. Of those with mutations, 12 (22.6%) had an E148Q, two (3.8%) had an M694V, two (3.8%) had a V726A, and two (3.8%) had an M680I. In addition, one patient was compound heterozygous to the M694V/V726A mutations. This patient was a 38-year-old man who had experienced recurrent oral and genital aphthosis, anterior uveitis, and pyoderma gangrenosum-like lesions. His clinical manifestations did not conform to the criteria for the diagnosis of FMF (16). Mutation M694I was not found in any of the patients. No homozygous mutations were found in this cohort. Although the difference in *MEFV* mutation frequency between BD patients and the control individuals from the same ethnic group (14) was not statistically significant ($p = 0.15$) (Table 1), the mutation distribution differed significantly between the two groups for M694V ($p = 0.01$) and M680I ($p = 0.04$). The mutation frequencies of E148Q and V726A in the patient group were not statistically different from those of the control group ($p = 1$ and $p = 0.69$, respectively) (Table 1).

The genotype distributions of BD patients and healthy controls conformed to Hardy–Weinberg equilibrium ($p > 0.05$).

Table 2 shows the clinical features of two patient groups, categorized by their *MEFV* carrier status. There was no significant difference between the two groups regarding the disease manifestations compared.

Table 2. Clinical features of BD patients by their *MEFV* carrier status.

Clinical feature	BD with mutation (n=19)	BD without mutation (n=34)	p-value
Oral ulcer	19	33	1
Genital ulcer	12	23	1
Skin lesion*	10	16	0.92
Uveitis	10	20	0.88
Arthritis	1	3	1
Gastrointestinal	0	2	0.53
Neurological	1	2	1
Pathergy test	9	14	0.88
HLA-B51	9	11	0.43

*Erythema nodosum, pseudofolliculitis, and pyoderma gangrenosum-like lesion.

Discussion

BD is an inflammatory multisystemic disorder with unknown aetiology and it seems that both genetic and environmental factors trigger the disease. In recent years, the possible association between FMF and BD has been of interest. The present study is the first assessment of common *MEFV* mutations in Iranian Azeri Turkish BD patients. Thirty-six per cent of BD patients were found to carry *MEFV* mutations compared with 25.5% in the control group of the same ethnic background ($p = 0.15$). At first glance it seems that there is no significant difference in the frequency of *MEFV* mutations between the control group and the patients;

Table 3. Frequencies of the five most common *MEFV* mutations (%) among the BD alleles studied in different ethnic groups.

Study	Ethnic origin (n)	M694V	M680I	V726A	M694I	E148Q
Touitou et al (4)	Mixed (38)	2.6	0	2.6	0	5.2
Ben-Chetrit et al (5)	Mixed (53)	4.7	1.9	2.8	1.9	5.6
Atagunduz et al (6)	Turkish (57)	9.6	0.9	2.6	nd	nd
Im1rzalioglu et al (7)	Turkish (42)	14	1.2	0	nd	6
Rabinovich et al (8)	Jewish (54)	15.7	nd	1	nd	8.3
Ayesh et al (9)	Arab (42)	4.7	0	1.2	1.2	9.5
The present study	Azeri Turkish (53)	2.8	1.9	2.8	0	11.3

n, Number of patients; nd, not determined.

and therefore our results are not in line with previously published material (4–9). However, the M694V mutation, which was reported to be the most prevalent mutation in Azeri Turkish FMF patients (42.4% of the identifiable mutations) (17), was observed in 15% of BD mutated alleles and its putative effect in predisposing individuals to develop BD is significant (Table 1). Regarding the insignificant differences for E148Q and V726A mutations between patients and controls, it is noteworthy that these mutations are not fully penetrant. In the control group the prevalence of the E148Q mutation is relatively high (with a carrier rate of 22%) (14). It has also been suggested that E148Q is a mild mutation with reduced penetrance, and a considerable proportion of the genetically affected individuals who were homozygous for E148Q remain asymptomatic (18, 19). Although the role of pyrin is yet to be more clearly defined, there is a possibility that the E148Q-mutated pyrin protein could trigger the inflammation cascade in BD. Analysing the results when excluding this mutation shows that the frequency of mutated BD alleles is significantly higher than that determined among the normal population (p=0.01) (Table 1).

Our finding of 36% of patients with *MEFV* mutations lies between the values reported by Touitou et al (21%) (4), Atagunduz et al (26%) (6), Ben-Chetrit et al (30%) (5), Imirzalioglu et al (36%) (7), Ayesh et al (40.5%) (9), and Rabinovich et al (44%) (8).

The M694V frequency in the Azeri Turkish BD alleles studied is very different from those of almost all other studies (Table 3). The frequencies of M680I and V726A mutations in our cohort are similar to those of other studies, whereas the frequency of E148Q is much more than that of other populations (Table 3).

The possible modifying role of *MEFV* mutations on the clinical expression of BD is not supported by our observations. That is, the group of BD patients with *MEFV* mutations is almost identical to the BD group without them (Table 2). This finding is concordance with Ben-Chetrit et al (5) and Espinosa et al (20), but in contrast to others (6, 8).

No homozygous mutations were detected in the patients studied and no mutations were identified in 34 patients (64%), although we cannot exclude the possibility that some of the patients had unknown or rare mutations.

In conclusion, we found that some *MEFV* mutations (M694V and M680I) could be an additional genetic susceptibility factor in BD, although they are only found in a minority of patients with BD (five out of 53). This finding could be confirmed by sequencing coding regions of *MEFV* in a larger study.

Acknowledgements

We thank all of the participating patients. This project was supported financially by the Drug Applied Research Centre, Tabriz University of Medical Sciences.

References

1. Sakane T, Takeno M, Suzuki N, Inaba G. Behçet's disease. N Engl J Med 1999;341:1284–91.
2. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's disease. Lancet 1990;335:1078–80.
3. Chajek T, Fainaru M. Behçet's disease report of 41 cases and a review of the literature. Medicine 1975;54:179–95.
4. Touitou I, Magne X, Molinari N, Navarro A, Quellec AL, Picco P, et al. *MEFV* mutations in Behçet's disease. Hum Mutat 2000; 16:271–2.
5. Ben-Chetrit E, Cohen R, Chajek-Shaul T. Familial Mediterranean fever and Behçet's disease – are they associated? J Rheumatol 2002;29:530–4.
6. Atagunduz P, Ergun T, Direskeneli H. *MEFV* mutations are increased in Behçet's disease (BD) and are associated with vascular involvement. Clin Exp Rheumatol 2003;21:S35–7.
7. Imirzalioglu N, Dursun A, Tastan B, Soysal Y, Yakicier MC. *MEFV* gene is a probable susceptibility gene for Behçet's disease. Scand J Rheumatol 2005;34:56–8.
8. Rabinovich E, Shinar Y, Leiba M, Ehrenfeld M, Langevitz P, Livneh A. Common FMF alleles may predispose to development of Behçet's disease with increased risk for venous thrombosis. Scand J Rheumatol 2007;36:48–52.
9. Ayesh S, Abu-Rmaileh H, Nassar S, Al-Shareef W, Abu-Libdeh B, Muhanna A, et al. Molecular analysis of *MEFV* gene mutations among Palestinian patients with Behçet's disease. Scand J Rheumatol 2008;37:370–4.
10. Lidar M, Livneh A. Familial Mediterranean fever: clinical, molecular and management advancements. Neth J Med 2007:65:318–24.
11. Kaklamani VG, Variopoulos G, Kaklamanis PG. Behçet's disease. Semin Arthritis Rheum 1998;27:197–217.
12. Shahram F, Nadji A, Jamshidi AR, Chams H, Chams C, Shafaie N, et al. Behçet disease in Iran, analysis of 5059 cases. Arch Iran Med 2004;7:9–14.
13. Esmaeili M, Bonyadi M, Rafeey M, Sakha K, Somi MH. Common *MEFV* mutation analysis in Iranian Azeri Turkish patients with familial Mediterranean fever. Semin Arthritis Rheum 2008;37:334–8.

14. Bonyadi M, Esmaeili M, Karimi A, Dastgiri S. Common Mediterranean fever gene mutations in the Azeri Turkish population of Iran. Genet Test Mol Biomarkers 2010;14:149–51.
15. Bonyadi M, Jahanafrooz Z, Esmaeili M, Kolahi S, Khabazi A, Ebrahimi AA, et al. TNF-alpha gene polymorphisms in Iranian Azeri Turkish patients with Behçet's disease. Rheumatol Int 2009;30:285–9.
16. Livneh A, Langevitz P, Zemer D, Zaks N, Kees S, Lidar T, et al. Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum 1997;40:1879–85.
17. Bonyadi M, Esmaeili M, Jalali H, Somi MH, Ghaffari A, Rafeey M, et al. *MEFV* mutations in Iranian Azeri Turkish patients with familial Mediterranean fever. Clin Genet 2009;76:477–80.
18. Aksentijevich I, Torosyan Y, Samuels J, Centola M, Pras E, Chae JJ, et al. Mutation and haplotype studies of familial Mediterranean fever reveal new ancestral relationships and evidence for a high carrier frequency with reduced penetrance in the Ashkenazi Jewish population. Am J Hum Genet 1999; 64:949–62.
19. Stoffman N, Magal N, Shohat T, Lotan R, Koman S, Oron A, et al. Higher than expected carrier rates for familial Mediterranean fever in various Jewish ethnic groups. Eur J Hum Genet 2000;8:307–10.
20. Espinosa G, Arostegui JI, Plaza S, Rius J, Cervera R, Yague J, et al. Behçet's disease and hereditary periodic fever syndromes: casual association or causal relationship? Clin Exp Rheumatol 2005;23:S64–6.