

Analysis of the Most Common Three MEFV Mutations in 630 Patients with Familial Mediterranean Fever in Iranian Azeri Turkish Population

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ABSTRACT

Introduction: The aim of the present study was to determine the frequency of M694V, M680I and V726A mutations of the MEFV gene in 630 Azeri Turkish patients with family Mediterranean fever.

Material and Methods: The MEFV gene mutations were detected using allele-specific oligonucleotide polymerase chain reaction.

Outcomes: 630 cases with a mean age \pm SD of 28.54 \pm 16.54 ranging from 2.5 to 76 years old including 268 (42.54%) males and 362 (57.46%) females, were tested. Nineteen patients were homozygote for one mutation (3.02%), 127 were heterozygote for one mutation (20.2%) and 18 were compound heterozygote for two mutations (2.86%). Mutation analysis confirmed that the most common mutation was M694V 109 (8.65%). V726A and M680I mutations accounted for 4.44% of the alleles; V726A 32 (2.54%) and M680I 24 (1.9%). In this study, compound heterozygote for M694V and V726A, M694V and M680I, and V726A and M680I mutations were found in 1.43%, 0.79%, and 0.63% from West Azerbaijan province in exon 10. Mutation was found in 164 (26.03%) of cases regarding analysis of the three most common MEFV mutations, but in 466 (73.97%) of cases, no mutation was detected. Among our samples, the frequencies of mutant genotypes were 15 (2.38%), 1 (0.15%), 3 (0.47%), 9 (1.42%), 4 (0.63%) and 5 (0.79%), regarding M694V/M694V, M680I/M680I, V726A/V726A, M694V/ V726A, M680I/ V726A and M680I/M694V, respectively. In our samples, 79 (12.53%), 26 (4.12%), and 22 (3.49%) cases had M694V/normal, V726A/normal, and M680I/normal genotypes regarding M694V, V726A, and M680I mutations, respectively.

Conclusions: The M694V mutation is the most common risk factor for family Mediterranean fever in our group.

Keywords: MEFV, FMF, M694V, M680I, V726A.

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INTRODUCTION

Familial Mediterranean fever (FMF) is an autosomal recessive auto-inflammatory disease that mainly affects several populations such as Greeks, Arabs, Armenians, Sephardic Jews, and Turks in the Mediterranean region (1). It is recognized by periodic acute attacks of fever, abdominal pain, pleurisy, peritonitis, synovitis, rashes or arthritis, pericarditis, serositis, erysipelas-like erythema, systemic amyloidosis, and kidney failure (1, 2). Familial Mediterranean fever pathogenesis is clinically diagnosed according to Tel Hashomer criteria (3, 4). It can be prevented via daily or life-long colchicine consumption (5). The Mediterranean fever gene (*MEFV*; MIM# 249100) on chromosome 16p13.3 is responsible for FMF predisposition (6, 7). The human *MEFV* gene is constructed of 10 exons and contains approximately 15 kb of genomic DNA (6, 7). The *MEFV* gene encodes a 3.7 Kb transcript encoding a protein named pyrin (marennostin) that has an important role in the anti-inflammatory activity via caspase-1 and interleukin-1 β pathways (8). More than 180 mutations or polymorphisms have been found in the *MEFV* gene (9). Most of the mutations are located in exon 10 (M694V, M680I, V726A, M694I, A744S, K654R, R761H, T681I, I692del, M694del) (10). Results of several studies implied that the presence of four mutations within exon 10 (M694V, V726A, M680I, M694I) and a mutation within exon 2 (E148Q) correspond to 85% of FMF causing mutations in the Mediterranean ethnic groups (11). The *MEFV* gene mutations cause a decreased level of pyrin, impaired inflammation control, and prolonged inflammatory response (12). Most of the *MEFV* gene mutations are point mutations; but a few mutations are small deletions (10). The most common mutation, Met694Val or M694V, is related with an increased risk of amyloidosis that can lead to renal failure (13). A large body of studies suggest that variations within other genes, called serum amyloid A1 (SAA1) and A2 (SAA2), can persuade the risk of amyloidosis among individuals with M694V mutation (14, 15). The M694V mutation is frequent in Azeri Turks in the North-West of Iran (16). The prevalence of M694V mutation was about 47% in the carrier chromosomes among North African Jewish FMF patients. So, M694V/M694V is the most frequent

genotype among North African Jewish patients. M694V/M694V genotype has been associated to severe form of FMF disease (17). The M694V mutation is very rare among Ashkenazi FMF patients (17).

The aim of the present study was to determine the frequency of M694V, M680I and V726A mutations of the *MEFV* gene in 630 Azeri Turkish FMF patients. □

MATERIALS AND METHODS

This study was performed in Urmia University of Medical Sciences in the city of Urmia, West Azarbaijan, Iran. Between 2009 and 2014, a total number of 630 unrelated FMF patients entered the study. FMF diagnosis was carried out based on the Tel Hashomer criteria as well as on exclusion of other disorders (3, 4). All cases had Azeri Turkish origin and were analyzed for their familial and medical history, had clinical examinations by several specialists such as pediatricians, gastroenterologists, and rheumatologists, and referred for genetic testing to the department of genetics (Mottahari Hospital, Urmia, Iran). A 3-4 mL sample of peripheral blood in EDTA-containing tubes was obtained from every case. The "salting out" method was used for isolation of genomic DNA as previously described (18). In each sample, three mutations of M694V, M680I, and V726A in the *MEFV* gene were screened using allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) by primer pairs, as reported earlier (19-21). A PCR test was performed in a 25 μ L solution with 100 ng of DNA, 1x reaction buffer, 10 pmol of each primer, 0.2 unit of Taq DNA polymerase, 200 μ mol of each dNTPs, and 1.5 mmol MgCl₂. Following the PCR tests, PCR products were analyzed by electrophoresis on 2.5% agarose gel containing CinnaGen DNA safe Stain (CinnaGen Co. Tehran, Iran). The presence or absence of fragments was visualized in standard UV transilluminator. Frequencies of alleles and genotypes were counted directly. This study was approved by the ethics committee of the Institutional Review Board of Urmia University of Medical Sciences (permit number ir.umsu.rec.1395.157). □

RESULTS

Findings of this study are summarized in Table 1 and Figures 1 and 2. Six hundred thirty cases

Mutation	Frequency
M694V	8.65%
V726A	2.54%
M680I	1.9%
M694V/M694V	2.38%
M680I/M680I	0.15%
V726A/V726A	0.47%
M694V/ V726A	1.43%
M680I/ V726A	0.63%
M680I/M694V	0.79%

TABLE 1. Frequency of tested mutations in 630 cases

with a mean age \pm SD of 28.54 ± 16.54 ranging from 2.5 to 76 years old, including 268 (42.54%) males (mean \pm SD: 30.04 ± 17.93) and 362 (57.46%) females (mean \pm SD: 27.16 ± 15.36), were analyzed. Nineteen out of the 630 patients were homozygote for one mutation (3.02%), 127 out of 630 patients were heterozygote for one mutation (20.2%) and 18 out of 630 patients were compound heterozygote for two mutations (2.86%). Mutation analysis confirmed that the most common mutation was M694V 109 (8.65%). V726A and M680I mutations accounted for 4.44% of the alleles; V726A 32 (2.54%) and M680I 24 (1.9%).

In this study, compound heterozygote for M694V and V726A, M694V and M680I, and V726A and M680I mutations were found in 1.43% (9 out of 630 patients), 0.79% (5 out of 630 patients) and 0.63% (4 out of 630 patients) from West Azerbaijan province in exon 10. Mutation was found in 164 (26.03%) of cases regarding analysis of the three most common *MEFV* mutations; but in 466 (73.97%) of cases, no mutation was detected. Among our samples, the frequencies of mutant genotypes were 15 (2.38%), 1 (0.15%), 3 (0.47%), 9 (1.43%), 4 (0.63%) and 5 (0.79%), regarding M694V/M694V, M680I/M680I, V726A/V726A, M694V/ V726A, M680I/ V726A and M680I/M694V, respectively. In our samples, 79 (12.53%), 26 (4.12%), and 22 (3.49%) cases had M694V/normal, V726A/ normal, and M680I/normal genotypes regarding M694V, V726A, and M680I mutations, respectively. □

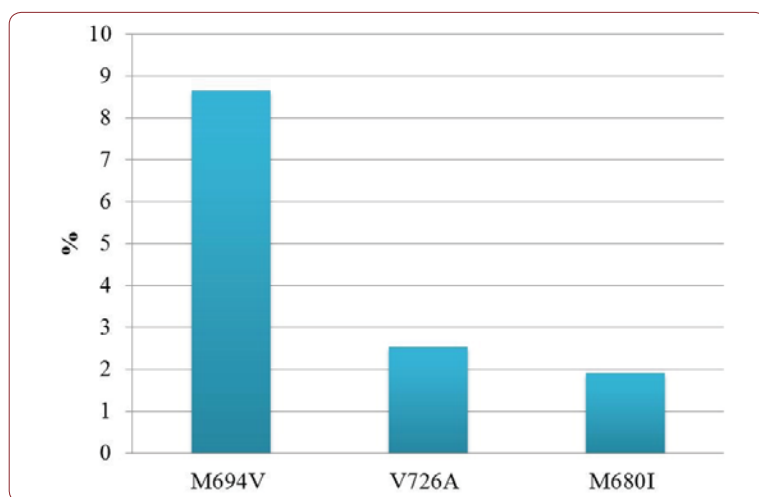


FIGURE 1. The frequencies of three mutations in this study were 8.65%, 2.54% and 1.9%, regarding M694V, V726A and M680I mutations, respectively

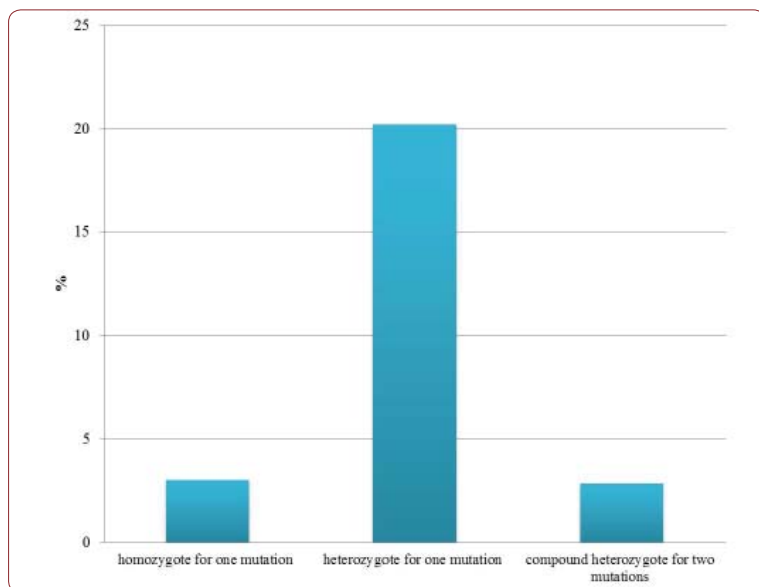


FIGURE 2. The frequencies (%) of homozygote for one mutation (3.02%), heterozygote for one mutation (20.2%) and compound heterozygote for two mutations (2.86%) in this study

DISCUSSION

Familial Mediterranean fever as a genetic disease is caused by *MEFV* gene (21, 22). The *MEFV* gene has 10 exons and 781 codons. There are three mutational hot spots in exons 2 and 10 of the *MEFV* gene. These hot spots are located at codons 680 and 694 (exon 10) as well as at codon 148 (exon 2) (21, 22). It has been shown that the genetics of FMF was more complicated due to the variable expressivity and incomplete (reduced) penetrance regarding clinical findings among patients heterozygous for FMF (22).

In this study, we analyzed the most common three *MEFV* mutations including M694V (Met694Val), V726A (Val726Ala) and M680I (Met680Ile) in cases who referred to Motahari

Hospital (Urmia, Iran). These disease-associated mutations are missense mutations. The carrier frequency has been estimated to reach up to 25.5% in Iranian Azeri Turks (23). Bonyadi *et al.* (2010) showed no carriers for M694V, M694I, and M680I mutations in *MEFV* gene in Azeri Turkish general population (23). This study was carried out on the largest group of FMF patients in West Azerbaijani and showed that M694V mutation, namely p.Met694Val, in exon 10 was a dominant mutation. FMF patients with M694V/M694V genotype had more severe disease compared to other genotypes (24, 25). The M694V mutation is frequent in the majority of different ethnic groups worldwide along the Mediterranean Sea area (26). The frequency of the M694V mutation was found to range from 0% (27) to 42.4% (28) in the different groups of Iranian FMF patients. The M694V mutation was more frequent in our samples (8.65%). Our findings were in agreement with Sabokbar *et al.* 2014 (29). The V726A and M680I mutations, which are recognized to be general in Armenians (10), were found to be the next most frequent mutations among our samples (2.54% and 1.9%).

The penetrance of FMF-causing mutations has a variable rate ranging from a high penetrance to a non-penetrance rate regarding M694V and E148Q mutations, respectively. Results of recent reports showed that cases who were carrier for E148Q mutation may lack clinical findings. This study had two main limitations: lack of registry systems and incomplete medical history of cases. □

CONCLUSION

Our results demonstrate that the M694V mutation is the most common risk factor for FMF in the studied population. □

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Conflicts of interest: none declared.

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