ABSTRACT

The MEFV (familial Mediterranean fever gene) researches were performed in the population of the Republic of Azerbaijan in 2016-2021. Seven mutations of the MEFV gene were identified in heterozygous, homozygous and compound homozygous conditions: R761H, M694I, M694V, V726A, R202Q, M680I and E148Q. The E148Q and R202Q mutations were discovered in exon 2 and R761H M694I, M694V, V726A, M680I were found in exon 10 in the population of the Republic of Azerbaijan. The highest gene frequency of the MEFV gene examined in 42 patients was 42.85% in the M694V mutations. The second highest frequency was the R761H and the third most frequent mutation was V726A. According to world literature, five mutations, M694V, V726A, M694I, R202Q, M680I and E148Q, constitute 75.0% of all mutations found today. In our studies, these five mutations belong to the same group, and make up 57.6% of the total mutations found. In order to prevent hereditary disease such as the familial Mediterranean fever (FMF) in the population of the Republic of Azerbaijan, it is planned to carry out prenatal diagnosis (PND) of the at-risk families.

Keywords: Exon; Familial Mediterranean fever (FMF); Gene; Inherited diseases; Protein.

INTRODUCTION

The MEFV gene (familial Mediterranean fever gene) is located on chromosome 16 (16.13.3), and it is composed of 3,242,028-3,256,776 nucleotides. It is specified as having an autosome-recessive hereditary type. Autosome-dominant hereditary species were also recorded [1].

The MEFV RoRet genes family contains exon 10, consisting of 10,000 nucleotide sequences. The length of the transcript consists of 3.7 thousand nucleotide sequences comprizing 761 synthesized pyridine protein amino acid bases. The pyrin (pyrin is a Greek word for “flame,” or marenostin meaning “our sea” in Latin, which stands mainly for Mediterranean Sea) is expressed in myeloid cells. The MEFV gene is located between the genes responsible for the kidney polycystosis and Rubinstein-Teybi syndrome [2].

The molecular-genetic analysis of these mutant-carrying haplotypes revealed that they belonged to the same ancestor haplotype. In the process of evolution, the ancestor haplotype has been subjected to divergence [3].

The majority of discovered mutations occur at the last exon 10. Approximately 70.0% of patients living in the Mediterranean Sea region have one of five mutations (M694V, V726A, M694I, M680I and E148Q) [4-6].

Familial Mediterranean fever could be encountered in medical literature as Armenian disease, non-European hereditary family amyloidosis, Daniel-Mozental paroxysmal syndrome, periodic peritonitis, Rayman syndrome, Seagull-Mamu disease. If there is a disease, the symptoms usually manifest themselves by the age of 30. It is a rare frequency hereditary disease [3,7-8].

The disease occurs mostly on the Mediterranean coast area and in the Asia Minor communities: mostly in Armenians, Turks, Sephardic and Ashkenazi Jews, Arabs, and less in Greeks, Spaniards and Italians. The heterogeneity of the disease among the people living in the Mediterranean Sea region is 20.0%, and the rate of births of homozygous children is 1:1000 to 1:2500. There is sporadic frequency in other ethnic groups [9-12].

1 Department of Medical Biology and Genetics, Azerbaijan Medical University, Baku City, Azerbaijan
2 Department of Genetics, Baku State University, Baku City, Azerbaijan
MATERIALS AND METHODS

All molecular genetic methods for detecting mutations are based on differences in the DNA sequence. Material used was venous blood with anticoagulant of 42 patients in 2016-2021. The age of the studied patients ranged from 2 to 29 years old. Genome DNA was obtained by automatic isolation from 200 µL of venous blood. The DNA concentration was measured by the Digital spectrometer. The integrity of the isolated genomic DNA was detected in a 2% agarose gel. The venous blood for research was drawn into a tube containing EDTA or heparin. Genomic DNA and RNA kits made by Qiagen GmbH (Hilden, Germany) were used for analysis. Integrity and quantity of genomic DNA and polymerase chain reaction (PCR) products were identified by electrophoresis on 2% agarose gel (PowerPacBasicGelDoc™ EZ; Bio-Rad Laboratories, Hercules, CA, USA).

The genome DNA underwent the PCR procedure for every protein-encoding exon of the MEFV gene. Positive PCR samples that were checked by electrophoresis in agarose gel were purified by an enzymatic method. Purified product was dyed with fluorescent dye by BiqDye Terminator V.3.1. (Applied Biosystems, Foster City, CA, USA) and processed by Cycle Sequencing PCR. Positive Cycle Sequencing PCR samples, controlled by electrophoresis in agarose gel, were extracted from the BiqDye XT (Applied Biosystems with dye-purifying agent. (Figure 1 and Figure 2).

Polymerase chain reaction was carried out in a following conditions: denaturation at 96 °C for 30 seconds; annealing at 55 °C for 30 seconds; extension at 75 °C for 1 min. This cycle was repeated 25 times, 72 °C for 10 min. and 4 °C pause. The PCR was carried out on a Professional Thermocycler Biometra system (Biometra Biomedizinische Analytik GmbH, Göttingen, Germany). A pair of for-ward and reverse primers was used for each genomic fragment. For the purification of DNA fragments after the first stage of PCR, a set of magnets was used: Agencourt AMPure XP PCR purification and SPROIPlate 96 Super Magnet Plate (Beckman Coulter Inc., Beverly, CA, USA). The second amplification of the purified DNA fragments was carried out in the following condition: denaturation at 95 °C for 30 seconds; annealing at 55 °C for 30 seconds; extension at 77 for 2 min. This cycle was repeated 25 times, and 72 °C for 10 min. and 4 °C pause. The nucleotide sequence of purified fragments was studied in GENOME Lab GeXP™ Sequencing (SCIEX, Brea, CA, USA).

The obtained nucleotide chains were identified through SeqScape® version 2.7 software program (Applied Biosystems, Foster City, CA, USA; http://tools.thermofisher.com/content/sfs/manuals/4401740.pdf), then compared by means of the National Center for Biotechnology Information (NCBI) Blast Ce, to normal MEFV nucleotide chains, and only then were the substitutions and mutations identified. Two DNA fragments were amplified: in exon 2, 360 nucleotide bases long and in exon 10, 400 nucleotide bases long. We used primers for exon 2 (forward): 5’-AAA ACG GCA CAG ATG ATT CC-3’ and (reverse): 5’-AAG GGC CTG CAC TCC TTC-3’; and for exon 10 (forward): 5’-AGC AGG AAG AGA GAT GCA GTG-3’ and (reverse): 5’-TTG GAG ACA AGA CAG CAT GG-3’.

RESULTS

For the first time in the population of the Republic of Azerbaijan, we aimed at studying the molecular-genetic characteristics of the MEFV gene in the FMF disease in ethnic Azerbaijanis. The molecular-genetic study of the MEFV gene isolated from the genome DNA suspected of carrying FMF has identified seven mutations: R761H, M694I, M694V, V726A, R202Q, M680I and E148Q. All of these seven mutations have been previously identified in the Turkish population [13].

21 of 42 examined patients were heterozygotes, 14 homozygotes, and 7 compound heterozygotes. Two mutations, R202Q and E148Q, were found in exon 2 (28.57%)...
of the *MEFV* gene, but the remaining five mutations, M860I, R761H, M694I, M694V and V726A, were located on exon 10 of the gene (71.43%). The R202Q polymorphism was found in two heterozygous patients, mutation E148Q was heterozygous in one patient, and as compound heterozygotes in two patients (R202Q/E148Q). The homozygous form of the R761H mutation was registered in four cases, and the M694I mutation in two persons in compound state (R761H/M694I). The M694I mutation was found in compound state separately with two other mutations as M694V and R202Q (M694I/M694V and M694I/R202Q).

M680I mutation was identified to be homozygous in two patients (M680I/M680I). The mutation of the V726A was identified as homozygous in three cases. The mutation of the V726A was identified as homozygous in 18 cases (Table 1). It should be noted, that patients with homozygous mutations had parents in consanguineous marriages.

Table 1. Gene frequencies of the studied mutations of the *MEFV* gene in the population of Azerbaijan in 2016-2021

| №  | Mutation | Location | Patients | Gene frequency (%) |
|----|----------|----------|----------|--------------------|
| 11 | E148Q    | Exon 2   | 3        | 7.14%              |
| 22 | M680I    | Exon 10  | 2        | 4.78%              |
| 33 | R761H    | Exon 10  | 13       | 30.95%             |
| 44 | M694I    | Exon 10  | 3        | 7.14%              |
| 55 | M694V    | Exon 10  | 18       | 42.85%             |
| 66 | V726A    | Exon 10  | 3        | 7.14%              |

According to the world literature, five mutations, M694V, V726A, M694I, R202Q, M680I and E148Q, constitute 75.0% of all mutations found today [14]. In our studies, those five of seven mutations belong to the same group, and makes up 57.6% of total mutations found. The results of molecular genetic studies of the *MEFV* gene in patients with a diagnosis of periodic disease (FMF) are described.

**DISCUSSION**

To prevent the hereditary disease of FMF, parents of 42 patients were invited to the consultation of physician-genetics in 2016-2021. Parents received information about a healthy child prognosis for the next pregnancy. When the inheritance type is autosomal-recessive, it has been reported that the risk of an affected child in the next pregnancy is 25.0%. As the majority of families are of reproductive age, they are prepared to consent undergoing prenatal diagnosis (PND) in future pregnancies.

The following mutations of the *MEFV* gene have been identified in Turkey: E148Q, R202Q, P369S, F479L, M680GA, M680GC, M694V, M694I, K695R, V726A, A744S and R761H [11]. In the diagnostics of the disease, great significance is given to who are the ancestors of the patient and to which ethnic group they belong [10].

Fragouli et al. [15] studied FMF in native Cretans, analyzing the 12 most frequent MEFV mutations in 71 patients and 158 healthy controls, and found that 59 (83.1%) of 71 FMF patients had at least one MEFV mutation, with five homozygotes and 54 heterozygotes; no mutations were detected in 16.9% of patients. Population genetic analysis showed an FMF carrier frequency in the healthy Cretan population of approximately 1:17 or about 6.0%. They noted that this placed the Cretan population in the ‘high risk’ category in terms of FMF prevalence [15].

By mutational analysis of 376 Lebanese patients with FMF, Jalkh et al. [16] found that the most common mutations were: M694V (28.98%), M694I (12.10%), V726A (19.28%), M680I (5.72%) and E148Q (10.10%), respectively. These mutations were estimated to be 7000, 8500, 15,000, 23,000, and 30,000 years old, respectively. Varying the mutation rate at one of the haplotype markers led to younger age estimates ranging from 3625 to 18,650 years. A total of 333 different haplotypes were found, 31 of which had a frequency greater than 5.0% in the whole sample. A comparison of haplotype distributions among religious groups showed that Muslim sub populations including Shiites and Sunnites, as well as Christians and Armenians [who were formerly settled in the southeastern part of Asia Minor (Cilicia)], were all descendants of an ancient common ancestral population, in which most of the MEFV mutations were already present with their respective associated haplotypes [16].

Bonyadi et al. [17] tested for five common mutations on the *MEFV* gene (E148Q, M680I, M694V, M694I, and V726A) in 524 unrelated Iranian patients of Azeri Turkish origin with FMF, and found their overall frequency to be 52.0%. Further analysis of 10 less common mutations enabled detection of approximately 9.0% of the unidentified alleles. The R761H mutation was the most frequently found of the rare alleles (4.7%), and the authors suggested that R761H should be included in routine molecular diagnosis of FMF patients from this ethnic group. Five different complex alleles were identified in 14 patients, including homozygosity for E167D/F479L in two patients. They noted that 43.0% of presumably mutated alleles remained elusive [17].

Otsuka et al. [18] reported a 32-year-old Japanese man with adult-onset fever, tonsillitis, and skin rash associated with leukocytosis, increased C-reactive protein, increased ferritin, and activation of monocytes. His rash consisted of painless papules and plaques. Skin biopsy showed neutrophilic dermatosis, and he was diagnosed clinically with adult-onset Sweet disease. He showed a favorable response to colchicine and low-dose corticos-
teroids. Genetic analysis identified a heterozygous E148Q variant in the MEFV gene, which the authors noted is found in about 20.0% of healthy individuals in Japan. Functional studies of the variant were not performed, but they suggested that the variant may have contributed to the development of the disorder [18].

Berg et al. [19] reclassified the R408Q mutation as ‘considered to imply carrier status’ for a recessive disorder. They noted that the R408Q and P369S mutations had been reported in cis as a single allele resulting in a highly variable clinical phenotype [19].

Masters et al. [20] noted that the molecular mechanism resulting from the S242R mutation differed from that of FMF-associated mutations M694V, M680I, and V726A, which had no appreciable effect on 14-3-3 binding. Of note, the carrier’s mother in Family C did not have neutrophilic dermatosis, but she did have some features of the disorder, including recurrent fevers and elevated acute-phase reactants. These findings suggested incomplete penetrance or clinical variability [20].

Kiyota et al. [21] reported a 45-year-old Japanese man with a systemic autoinflammatory disorder with intermittent fever and amyloidosis, consistent with FMF, as well as pustular dermatosis with neutrophilic aggregates since childhood. The authors noted that he had abdominal symptoms, rather than classic serositis, and that the skin problems had been the main symptom since childhood, suggesting a phenotype that overlapped with FMF. Genetic analysis identified compound heterozygous missense variants in the MEFV gene (S242R and E148Q). His unaffected mother was heterozygous for the S242R mutation, indicating incomplete penetrance of acute febrile neutrophilic dermatosis (AFND). Functional studies of the variants were not performed, but the authors suggested that the E148Q polymorphism may act as a disease modifier [21].

In three affected members of a Spanish family with acute febrile neutrophilic dermatosis, Moghaddas et al. [22] identified a heterozygous c.730G>A transition in exon 2 of the MEFV gene, which the authors noted is considered to imply carrier status for a recessive disorder. The mutation, which was found in about 20.0% of healthy individuals, was not identified in the 1000 Genomes Project, ExAC, or Exome Variant Server databases (https://research.monash.edu/en/publications/a-novel-pyrin-associated-autoinflammatory-mutation-with-neutrophilic-derma) or in 250 healthy Spanish controls [22].

Grossman et al. [23] reported a comprehensive characterization of the phenotypes of 57 patients with FMF who were homozygous for the M694V mutation on the MEFV gene compared to the phenotypes of a cohort of 56 patients with FMF and other MEFV genotypes. They found that disease severity and average frequency of attacks per year, both before and after treatment, were higher in the M694V homozygous group compared to the cohort of patients with other MEFV genotypes, although there was no difference in the length of attacks or in the proportion of patients with abdominal, erysipelas-like erythema and fever-alone attacks. They also found that the colchicine dose was higher and the response to colchicine was lower in the homozygous M694V cohort. Additionally, the homozygous cohort had a higher overall rate of diseases associated with FMF, including Crohn’s disease, Behçet disease, ankylosing spondylitis and Henoch Schönlein purpura, but not fibromyalgia [23].

Several reports have shown that the M694V mutation is associated with severe disease featuring early onset, high frequency of attacks, the need for the high doses of colchicine and high frequency of amyloidosis in untreated patients [24-26].

The high frequency of MEFV mutations in the four classically affected populations, ranging from 37.0 to 39.0% in Armenians and Iraqi Jews, to 20.0% in Turks, North Africans and Ashkenazi Jews and Arabs [11]. All molecular genetic methods for detecting mutations are based on differences in the DNA sequence.

We identified seven previously known mutations of the MEFV gene: R761H, M694I, M694V, V726A, R202Q, M680I and E148Q for the population of Azerbaijan. Two mutations, E148Q and R202Q, are located in the exon 2, the remaining five mutations, R761H, M694I, M694V, V726A, and M680I, in the exon 10 of the gene. In order to prevent periodic illness in families with a genetic risk of having a sick child, PND of the fetus in the first trimester of pregnancy is planned, using a molecular genetic method of research. (Table 1).

**CONCLUSIONS**

These MEFV gene researches were performed in the population of the Republic of Azerbaijan. Seven mutations of the MEFV gene were identified in heterozygous, homozygous and compound heterozygous and compound homozygous conditions: R761H M694I, M694V, V726A, R202Q, M680I and E148Q. The mutations E148Q and R202Q were discovered in exon 2 and R761H M694I, M694V, V726A, M680I were found in exon 10 in the population of the Republic of Azerbaijan.

The highest gene frequency of the MEFV gene examined in 42 patients was 42.85% for the M694V mutation, followed by R726H (30.95%) and M694I (7.14%) mutations, respectively (Table 1). According to the world literature, five mutations, M694V, V726A, M694I, R202Q, M680I and E148Q, constitute 75.0% of all mutations...
found today. In our studies, those five of seven mutations belong to the same group, and makes up 57.6% of total mutations found.

Acknowledgments. We express our deep gratitude to the Turkey GENOMED laboratory for its support. Part of the research we are looking for was carried out in the Laboratory of Human Genetics at the Institute of Genetic Resources of the Azerbaijan National Academy of Sciences. Special thanks to the management of the laboratory.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

1. Habahbeh LA, Hiary MA, Zaben SF, Al-Momanu A, Khawawneh R, Mallouh MA, et al. Genetic profile of patients with Familial Mediterranean Fever (FMF): Single center experience at King Hussein Medical Center KHMC. Med Arch. 2015; 69(6): 417-420.

2. Ozen S, Batu ED. The myths we believed in familial Mediterranean fever: What have we learned in the past years. Semin Immunopathol. 2015; 37(4): 363-639.

3. Rigante D, Frediani B, Cantarini L. A comprehensive overview of the hereditary periodic fever syndromes. Clin Rev Allergy Immunol. 2018; 54(3): 446-453.

4. Anwar GM, Fouad HM, Abd El-Hamid A, Mahmoud F, Musa N, Lotfi H, et al. A study of familial Mediterranean Fever (MEVF) gene mutations in Egyptian children with type 1 diabetes mellitus. Eur J Med Genet. 2015; 58(1): 31-34.

5. Beheshtian M, Izadi N, Kriegshauser G, Kahrizi K, Mehr EP, Rostami M, et al. Prevalence of common MEFV mutations and carrier frequencies in a large cohort of Iranian populations. J Genet. 2015; 58(1): 31-34.

6. Debeljak M, Toplak N, Abazi N, Szabados B, Mulaosmanović V, Radović J, et al. The carrier rate and spectrum of MEVF gene mutations in central and southeastern European populations. Clin Exp Rheumatol. 2015; 33(6 Suppl 94): S19-S23.

7. Wu B, Xu T, Li Y, Yin X. Interventions for reducing inflammation in familial Mediterranean fever. Cochrane Database Syst Rev. 2018; 10(10): CD010893.

8. Yates AD, Achuthan P, Akanni W, Allen J, Allen J, Alvaaraz-Jarreta J, et al. Ensembl 2020. Nucleic Acids Res. 2020; 48(D1): D682-D688.

9. Milenković J, Vojinović J, Debeljak M, Toplak N, Lazarević D, Avčin T, et al. Distribution of MEVF gene mutations and R202Q polymorphism in the Serbian population and their influence on oxidative stress and clinical manifestations of inflammation. Pediatr Rheumatol Online J. 2016; 14(1):39.

10. Yaşar Bilge Ş, Sari İ, Solmaz D, Şenel S, Emmungil H, Kılıç L, et al. The distribution of MEVF mutations in Turkish FMF patients: Multicenter study representing results of Anatolia. J Med Sci. 2019; 49(2): 472-477.

11. Yılmaz G, Senes M, Kayalp D, Yucel D. Is Turkish MEVF mutations spectrum different among regions?. J Clin Lab Anal. 2016; 30(5):641-644.

12. Zerkaoui M, Laarabi FZ, Ajhoun Y, Chkirate B, Sefiani A. A novel single variant in the MEVF gene causing Mediterranean fever and Behçet’s disease: A case report. J Med Case Rep. 2018; 12(1): 53.

13. Huseynova LS, Aiyeva KA, Najafzada GB, Yusufova KhJ, Hashimova AR. Molecular-genetic research of MEVF gene in population of Azerbaijan Republic. Poland Sylwan. 2018; 162(5):31-36.

14. Rigante D. A developing portrait of hereditary periodic fevers in childhood. Expert Opin Orphan Drugs. 2018; 6(1): 47-55.

15. Fragouli E, Eliopoulos E, Petraíki E, Sidiriropoulos P, Aksentijejívich I, Galanakis E, et al. Familial Mediterranean fever in Crete: A genetic and structural biological approach in a population of ‘intermediate risk.’ Clin Genet. 2008; 73(2): 152-159.

16. Jalkh N, Genin E, Chouery E, Delague V, Medlej-Hashim M, Idrac C-A, et al. Familial Mediterranean fever in Lebanon: founder effects for different MEVF mutations. Ann Hum Genet. 2008; 72(10): 41-47.

17. Bonyadi M, Esmaeili M, Jalali H, Somi MH, Ghaffari A, Rafeey M, et al. MEVF mutations in Iranian Azeri Turkish patients with familial Mediterranean fever. Clin Genet. 2009; 76(11): 477-480.

18. Otsuka M, Koga T, Sumiyoshi R, Koike Y, Furukawa K, Okamoto M, et al. A case of neutrophilic dermatosis with MEVF gene variant and abnormal activation of peripheral blood monocytes: A case report. Immun Med. 2019; 42(6): 45-49.

19. Berg JS, Adams M, Nassar N, Bizon C, Lee K, Schmitt CP, et al. An informatics approach to analyzing the incidentalome. Genet Med. 2013; 15(1): 36-44.

20. Masters SL, Lagou V, Jeru I, Baker PJ, Van Eyck L, Parry DA, et al. Familial autoinflammation with neutrophilic dermatosis reveals a regulatory mechanism of pyrin activation. Sci Transl Med. 2016; 8: 332-345.
21. Kiyota M, Oya M, Ayano M, Niiro H, Iwasaki T, Fujiwara M, et al. First case of pyrin-associated autoinflammation with neutrophilic dermatosis complicated by amyloidosis. Rheumatology (Oxford). 2020; 59(9): e.41-e43.

22. Moghaddas F, Llamas R, De Nardo D, Martinez-Banaclocha H, Martinez-Garcia JJ, Mesa-del-Castillo P, et al. A novel pyrin-associated autoinflammation with neutrophilic dermatosis mutation further defines 14-3-3 binding of pyrin and distinction to familial Mediterranean fever. Ann Rheum Dis. 2017; 76(12): 2085-2094.

23. Grossman C, Kassel Y, Livneh A, Ben-Zvi I. Familial Mediterranean fever (FMF) phenotype in patients homozygous to the MEFV M694V mutation. Eur J Med Genet. 2019; 62(6): 103532.

24. Mattit H, Joma M, Al-Cheikh S, El-Khateeb M, Medlej-Hashin M, Salem N, et al. Familial Mediterranean fever in the Syrian population: Gene mutation frequencies, carrier rates and phenotype-genotype correlation. Eur J Med Genet. 2006; 49(6): 481-486.

25. Jarjour RA. Familial Mediterranean fever in Syrian patients: MEFV gene mutations and genotype-phenotype correlation. Mol Biol Rep. 2010; 37(1): 1-5.

26. Jarjour RA, Dodaki R. Arthritis patterns in familial Mediterranean fever patients and association with M694V mutation. Mol Biol Rep. 2011; 38(3): 2033-2036.