The carrier rate and spectrum of *MEFV* gene mutations in central and southeastern European populations

M. Debeljak[1], N. Toplak[2,3], N. Abazi[4], B. Szabados[5], V. Mulaosmanović[6], J. Radović[7], D. Perko[2], J. Vojinović[7], T. Constantin[5], D. Kuzmanovska[4], T. Avčin[2,3]

[1]*Unit for Special Laboratory Diagnostics, and* [2]*Department of Allergology, Rheumatology and Clinical Immunology, University Children's Hospital, University Medical Centre, Ljubljana, Slovenia;*
[3]*Faculty of Medicine, University of Ljubljana, Slovenia;*
[4]*University Children Hospital, Medical Faculty, Ss. Cyril and Methodius University, Skopje, Macedonia, Republic Of Macedonia;*
[5]*Unit of Paediatric Rheumatology, Department of Pediatrics, Semmelweis University Budapest, Budapest, Hungary;*
[6]*Children's Hospital University, Clinical Centre, Sarajevo, Bosnia and Herzegovina;*
[7]*Faculty of Medicine, University of Niš, Serbia.*

Maruša Debeljak, PhD
Nataša Toplak, MD, PhD
Nora Abazi, MD
Beatrix Szabados, MD
Velma Mulaosmanović, MD
Jelena Radović, MD, PhD
Daša Perko, BSc
Jelena Vojinović, MD, PhD
Tamas Constantin, MD, PhD
Dafina Kuzmanovska, MD, PhD
Tadej Avčin, MD, PhD

Please address correspondence to:
Prof. Tadej Avčin,
University Children's Hospital, UMC,
Department of Allergology,
Rheumatology and Clinical Immunology,
Bohoričeva 20,
1000 Ljubljana, Slovenia.
E-mail: tadej.avcin@kclj.si

Received on September 14, 2014; accepted in revised form on October 15, 2014.

Clin Exp Rheumatol 2015; 33 (Suppl. 94): S19-S23.

Key words: *MEFV* mutations, carrier rate, central-southeastern Europe

Funding: this work was partially supported by the Slovenian Research Agency grants L3-4150 and P3-0343.

Competing interests: none declared.

ABSTRACT

Objective. *Familial Mediterranean fever (FMF) is an autosomal-recessive disorder caused by mutations in MEFV gene. Eastern Mediterranean populations have the highest number of carriers, whereas western Mediterranean populations are less frequently affected. The aim of this study was to determine the carrier rate and spectrum of MEFV gene mutations in apparently healthy populations and in suspected FMF patients from central and southeastern European (CSEE) countries.*

Methods. *We screened 507 apparently healthy persons from 5 CSEE countries. Exons 2 and 10 of the MEFV gene were PCR amplified and subsequently sequenced with ABI prism310 genetic analyser. Six most common mutations in the MEFV gene were tested: V726A, K695R, M694V, M694I, M680I in exon 10, and E148Q in exon 2. In suspected FMF patients we screened all MEFV exons in selected cases.*

Results. *The overall carrier frequency of all MEFV mutations was higher than expected (9.3%). In the whole cohort we did not find any apparently healthy persons with two mutations. Heterozygous mutations were found in apparently healthy subjects from different CSEE countries as follows: Macedonia 16%, Serbia 11%, Bosnia and Herzegovina 8%, Slovenia 6% and Hungary 5%. The most common mutation in healthy controls was K695R, appearing in 40% of mutated alleles. The most common mutation in suspected FMF patients was M694V, followed by K695R.*

Conclusion. *We found a higher than expected carrier rate of MEFV gene mutations in populations from CSEE countries. It is interesting to note that 40% of detected carriers carry the K695R mutation.*

Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disease (MIM 249100) caused by mutations in *MEFV* gene localised on chromosome 16. *MEFV* gene is composed of 10 exons encoding a 781 amino acid protein known as pyrin, which is expressed mainly in innate immune cells and has a distinct role in interactions with different proteins related to inflammation (1). Mutations in *MEFV* gene are responsible for the clinical presentation of FMF, characterised by recurrent fever accompanied by arthritis, serositis, myalgias and rash (2, 3). So far, 298 sequence variations and 169 disease associated mutations have been described in *Infevers* database (4). Since identification of the *MEFV* gene, the spectrum of mutations has been established in populations commonly affected by FMF, such as Sephardic Jews, Armenians, Arabs and Turks (5, 6), pointing to the obvious mutational "hot spot" in exon 10: about 90% of mutated alleles. The most common mutations in *MEFV* gene are E148Q, M694I, M694V, V726A and M680I. The distribution pattern of the *MEFV* mutation along the Mediterranean Sea is not uniform; eastern populations have the highest number of carriers (20–39%), whereas western Mediterranean populations are much less frequently affected. A haplotype analysis has demonstrated that most FMF chromosomes descend from a common ancestor, in keeping with a founder effect (5, 6). Each of the mutations other than those described above is found in 1% of FMF chromosomes; some of them are isolated mutations and are found especially in individuals who do not belong to commonly affected populations. However, in the absence of any functional test, the del-

eterious character of those mutations is difficult to assess. The FMF founder haplotypes were not established in populations other than the 4 classically affected populations (5-7). MEFV mutations have also been reported in Greeks and Italians (8, 9); however, those studies reported allele frequencies and/or geographic origins of the patients, and did not always describe their genotypes and ethnic origins. Moreover, the frequency of mutated alleles may be overestimated in those populations because of the high frequency of the E148Q variation (10-12).

The frequency of *MEFV* mutations in healthy populations from central and southeastern European (CSEE) countries has not previously been reported, therefore, the aim of our study was to evaluate carrier rate and spectrum of *MEFV* mutations in populations from several CSEE countries where FMF is apparently a very rare disease.

Methods

We performed a multicentre, prevalence study of *MEFV* gene mutations in five CSEE countries with different history of migration including Bosnia and Herzegovina, Hungary, Republic of Macedonia, Serbia and Slovenia. According to the 2014 Eurostat data for each included country, it is estimated that 25 million people live in these countries. DNA was collected from 507 apparently healthy adult subjects who donated their DNA for research purposes. An informed consent for participation in the study, drawing of blood for DNA isolation and genetic testing of *MEFV* mutations was obtained from the subjects. The study was approved by the Ethics Committees of the involved countries and was conducted according to the principles of the Helsinki Declaration.

Molecular analysis

DNA isolation was performed from peripheral blood using FlexiGene isolation kit (Qiagen, Germany), following the manufacturer's instructions. Polimerase chain reaction of part of exon 2 and exon 10 was performed using the AmpliTaq polimerase (Applied Biosystems, USA) and corresponding reagents. Six most common mutations in *MEFV* gene were tested: V726A, K695R, M694V, M694I, M680I in exon 10, and E148Q in exon 2. Primers used for the amplification were FMFe2f 5'AAAACGGCACAGATGATTCC3'/FMFe2r 5'CCTTCTCTCTGCGTTTGCTC3' and FMFe10f 5'TTGGAGACAAGACAGCATGG3'/FMFe10r 5'AGCAGGAAGAGAGATGCAGTG3' (Invitrogen, USA). The whole gene was PCR amplified for suspected patients (primers available upon request). The standard protocol mixture consisted of 4 ng/μL double stranded DNA, 0,2 μM of each deoxynucleoside triphosphate (dNTP), 0,4 μM primers, 1 mM $MgCl_2$, 1/10 of corresponding reaction buffer and 0,08 U/ μL Ampli*Taq* DNA polimerase (Applied Biosystems, USA).

PCR products were purified using ExoSAP-IT (USB, Cleveland, OH, USA) and subjected to direct nucleotide sequencing using the Big Dye Terminator cycle sequencing kit and ABI PRISM 310 automated sequencer (Applied Biosystems, Foster City, CA, USA). All identified mutations were validated in an additional independent round of PCR and sequenced. Data from the Sequence Analysis Software were aligned with the native *MEFV* sequence (NG_007871.1) (*http://www3.ncbi.nlm.nih.gov*).

The cDNA numbering system used is compliant with the HGVS website (*www.hgvs.org/mutnomen*). The amino acid numbering matches the one published previously and is based upon the first amino acid of mature protein to be +1. All genetic analyses were performed in the genetic laboratory of the University Children's Hospital Ljubljana, Slovenia.

Results

The carrier rate of MEFV mutations in apparently healthy populations

A total of 507 apparently healthy Caucasian controls from 5 CSEE countries were screened for different mutations in exons 2 and 10 of the *MEFV* gene. Heterozygous mutations were found in 47 out of 507 (9.3%) apparently healthy subjects. We did not find any sample with two mutations in the whole cohort. The highest carrier rate was found in Macedonia 16%, followed by Serbia 11%, Bosnia and Herzegovina 8%, Slovenia 6%, and Hungary 5%. (Table I, Fig. 1).

Spectrum of MEFV mutations in apparently healthy populations

Altogether, only five different mutations were identified in the study populations: V726A, K695R, E148Q, M694V and F756C. Not all mutations, for which the samples were screened, were detected. We found a heterozygous mutation in 47 out of 507 apparently healthy subjects. Of these, 19/47 were carriers of K695R, followed by 17/47 with E148Q and 9/47 with V726A, 1/47 with M694V, and 1/47 with F756C mutation (Fig. 2A). Mutations found in different populations are presented in Table I.

Spectrum of MEFV mutations in patients with undefined periodic fever syndrome in CSEE countries

In the period 2009-2014 we analysed overall 226 samples of patients with suspected periodic fever syndrome (mostly patients with suspected PFAPA syndrome and patients with unusual phenotypes) from 9 CSEE countries. In total cohort, we found 48 patients with *MEFV* mutations. Clinical data were not available for all patients. From the 226 patients only 56 (25%) patients had clinical presentation of FMF. They were referred to our Genetic laboratory from Croatia (3), Czech Republic (15), Hungary (2), Macedonia (3), Serbia (2), Slovakia (6), and Slovenia (25).We found mutations in 24/56 (43%) of patients. Only four patients were found with homozygous/combined heterozygous mutation in the whole cohort. The rest of the patients had only one heterozygous mutation. Patients from Slovakia and Czech Republic were previously reported (14, 15). The spectrum of mutations in the cohort of 56 suspected FMF patients from CSEE countries consisted of: M694V (10/28), K695R (9/28), V726A (3/28), E148Q (2/28), I591T (2/28), S730F (1/28) and A744S (1/28) (Fig. 2B). The nucleotide change I591T was tested for and hence detected only in patients, where the whole coding region was screened. The genotype-phenotype correlation in patients was not evaluated.

Table I. Carrier frequency data of the study population in %.

%	all	E148Q/wt	M694V/wt	K695R/wt	V726A/wt	F756C/wt
Total	9.3	3.4	0.2	3.8	1.8	0.2
Hungary	5	1	-	3	1	-
Slovenia	6	1	-	2	3	-
B&H	8	1	-	2	4	1
Serbia	11	6	-	5	-	-
Macedonia	16	8	1	7	-	-

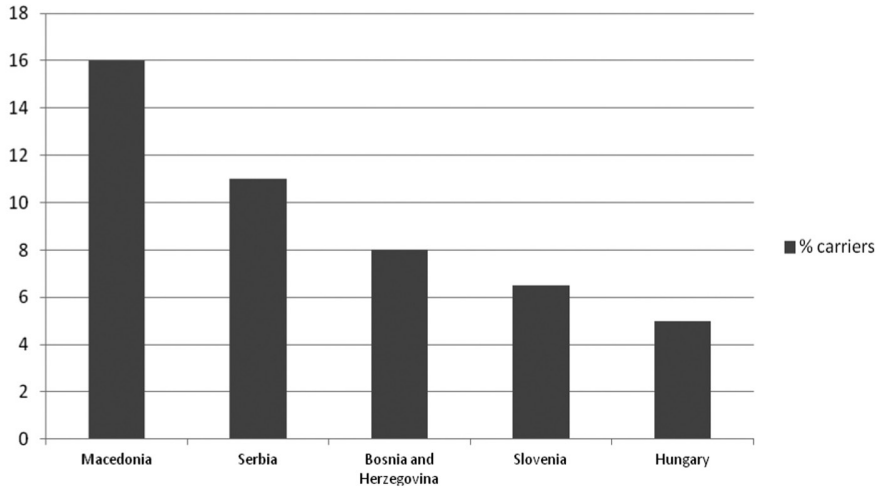

Fig. 1. Heterozygous mutations were found in 5% of Hungarians (1: 20), 6 % of Slovenians (1:15), 8% of Bosnians (1:13), 11% of Serbians (1:9) and in 16% of Macedonians (1:6), showing a decrease with the distance from the South-East Mediterranean basin, where FMF is a common disease.

Discussion

Relatively high frequency of MEFV mutations in the region

We present the first data about the carrier rate of *MEFV* mutations in CSEE countries. It was assumed that the carrier rate in CSEE countries would be significantly lower than in the neighbouring Mediterranean countries and similar to western European countries. However, we found a higher than expected carrier rate of mutations in populations with a presumably low number of patients with FMF. In the study population apparently healthy subjects from CSEE countries which are not directly neighboring to countries most affected with FMF, were included. Nevertheless, our study demonstrated surprisingly high prevalence of mutations (9.3%) for the whole region. The overall carrier frequency of all *MEFV* mutations was higher than expected from previous studies (5, 6).

The carrier rate in populations around the East Mediterranean basin is estimated to 1:3 to 1:5 and is rarer in other populations (6, 16). It was thought to be rare also in Italy, but this assumption was disproved in a study of recruited patients of Italian origin among those referred to Italian-French medical centres for fever of unknown origin or surgical emergencies (17). However, it appears that in western European Caucasian patients *MEFV* analysis is of particularly weak diagnostic value for recurrent fevers (18). First data about the number of FMF patients in CSEE countries was published in 2010 and the number of genetically confirmed FMF patients was extremely low. Only 11 genetically confirmed patients were found in a population of 121.5 million from 14 CSEE countries (19). It was suspected that FMF could be unrecognised in this region and might be more common than currently known. This assumption is supported by the results of the present study, demonstrating that the carrier rate is higher than expected from the low number of patients. On the other hand, it is possible that genotype-phenotype presentation is different than in Mediterranean countries. It was shown that the environment influences the clinical picture of FMF (20, 21, 22) resulting in an even more difficult recognition of this rare disease in CSEE countries. Heterozygous mutations were found in apparently healthy subjects from different CSEE countries as follows: Macedonia 16%, Serbia 11%, Bosnia and Herzegovina 8%, Slovenia 6%, and Hungary 5%. The frequency gradually decreases with the geographical distance from the South-East Mediterranean basin, where FMF is a common disease.

Limited heterogeneity of MEFV mutations in the region

Only five different mutations (K695R, E148Q, V726A, M694V, F756C) were found in healthy control subjects, whereas we found 7 different alteration (K695R, E148Q, V726A, M694V, I591T, S730F and A744S) in patients from CSEE. Only 4 different mutations (K695R, E148Q, V726A, M694V) occur more than once, showing a limited heterogeneity of *MEFV* mutations in the region. The most common mutation in healthy controls was K695R, appearing in 40% of subjects. This mutation was previously reported as a rare variant and was entered into the *Infevers* database in 2001 as a non founder mutation in Ashkenazi Jews (23). In the patient population the most common disease associated mutation was M694V (36%), mostly in heterozygous state. It is of interest that K695R represents 32% of all mutations found in patients, further supporting that this mutation is common in the CSEE region, with a similar prevalence in the healthy and patient populations.

New findings in the pathophysiology of FMF are suggesting that mutations in the *MEFV* gene are actually a gain of function mutations and not a loss of function as previously considered. A gene dosage effect can explain that as many as 30% of the patients diagnosed with FMF have only a single mutation in the *MEFV* genomic region (3, 22). In our patient populations reported from CSEE countries, this percentage was significantly higher (84%). The high carrier rate of *MEFV* mutations in the CSEE populations (9.3%) seem to suggest that the disease may be under-di-

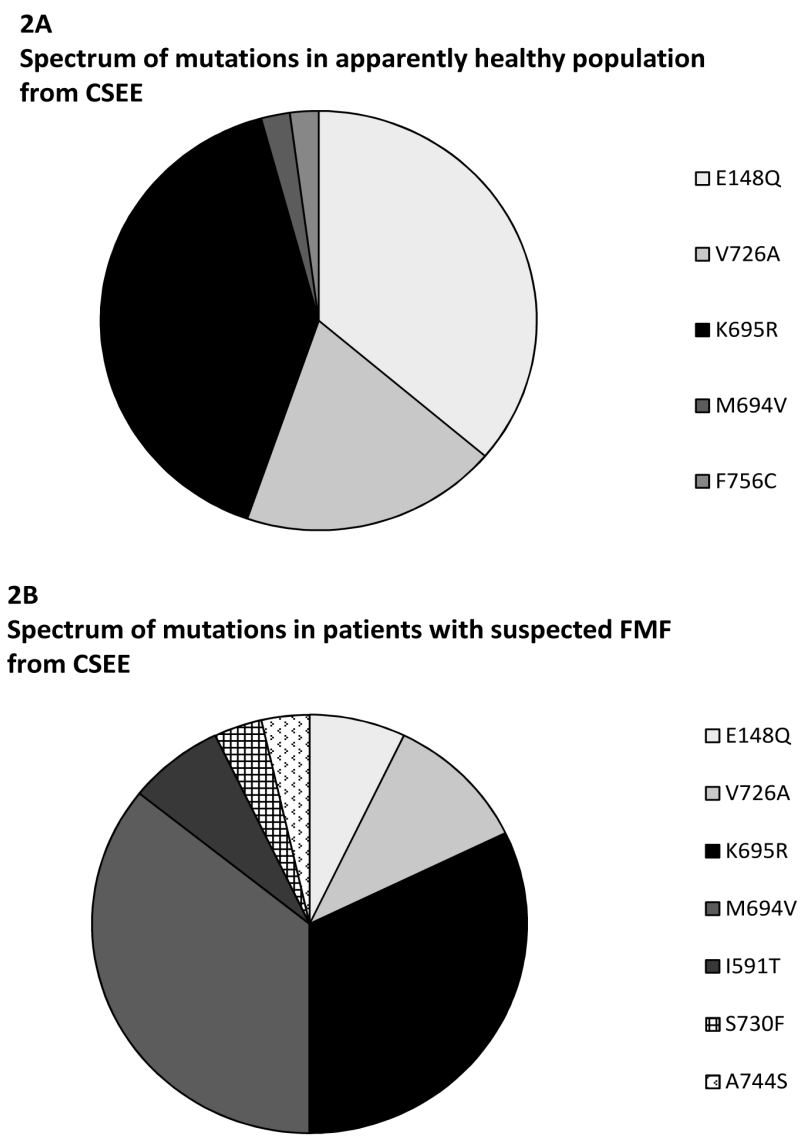

Fig. 2. Spectrum of mutations found in (**A**) healthy controls and (**B**) suspected FMF patients in CSEE countries.

agnosed in the region, but in fact mutations V726A, K695R and E148Q were reported to have a reduced penetrance (21, 22, 24). The V726A mutation commonly occurs in the Ashkenazi Jew population (25, 26), which may be the reason for occurrence only in the Slovenian, Bosnian and Hungarian populations, where Jewish communities live for centuries. The high prevalence of variation K695R which we observed in the whole CSEE region (3.8%) was not previously reported in any other population. It appears that there was a founder effect in the region, or that it was transferred from another population in the past, for example during expansion of the Ottoman Empire northwards into the Southeastern parts of Europe. We hypothesise that the origin of the majority of identified *MEFV* mutations in CSEE region were historical migrations within and from the Ottoman Empire which is supported also by the fact that the rate of carriers was higher in countries closer to Turkey.

Although all included subject were apparently healthy, which was the main inclusion criteria, it would be interesting to investigate, if subjects with mutations have any clinical symptoms which are not perceived as disease signs by themselves. Due to ethical concerns, this was not possible to investigate as a part of this study. It was already published that FMF could develop in persons with only one mutation (20, 21). Genotype-phenotype relationship in FMF patients in CSEE countries remain at the present time still unknown. We hope that results of our study might alert physicians to consider FMF in cases of unknown periodic inflammation also in CSEE countries, where it is still believed that FMF is a rare disease.

References

1. CHAE JJ, CHO YH, LEE GS *et al*.: Gain-of-function Pyrin mutations induce NLRP3 protein-independent interleukin-1β activation and severe autoinflammation in mice. *Immunity* 2011; 34: 755-68.
2. TUNCA M, AKAR S, ONEN F *et al*.: Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. *Medicine* (Baltimore) 2005; 84: 1-11.
3. KONÉ PAUT I, DUBUC M, SPORTOUCH J, MINODIER P, GARNIER JM, TOUITOU I: Phenotype-genotype correlation in 91 patients with familial Mediterranean fever reveals a high frequency of cutaneo mucous features. *Rheumatology* 2000; 39: 1275-9.
4. http://fmf.igh.cnrs.fr/ISSAID/infevers/
5. THE INTERNATIONAL FMF CONSORTIUM: Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell* 1997; 90: 797-807.
6. THE FRENCH FMF CONSORTIUM: A candidate gene for familial Mediterranean fever. *Nat Genet* 1997; 17: 25-31.
7. ALDEA A, CALAFELL F, ARÓSTEGUI JI *et al*.: The west side story: MEFV haplotype in Spanish FMF patients and controls, and evidence of high LD and a recombination "hot-spot" at the MEFV locus. *Hum Mutat* 2004; 4: 399.
8. KONSTANTOPOULOS K, KANTA A, DELTAS C *et al*.: Familial Mediterranean fever associated pyrin mutations in Greece. *Ann Rheum Dis* 2003; 62: 479-81.
9. LA REGINA M, NUCERA G, DIACO M *et al*.: Familial Mediterranean fever is no longer arare disease in Italy. *Eur J Hum Genet* 2003; 11: 550.
10. MIMOUNI A, MAGAL N, STOFFMAN N *et al*.: Familial Mediterranean fever: effects of geno-type and ethnicity on inflammatory attacks and amyloidosis. *Pediatrics* 2000; 105: E70.
11. BEN-CHETRIT E, LERER I, MALAMUD E, DOMINGO C, ABELIOVICH D: The E148Q mutation in the MEFV gene: is it a disease-causing mutation or a sequence variant? *Hum Mutat* 2000; 15: 385-6.
12. TCHERNITCHKO D, LEGENDRE M, CAZENEUVE C, DELAHAYE A, NIEL F, AMSELEM S: The E148Q MEFV allele is not implicated in the development of familial Mediterranean fever. *Hum Mutat* 2003; 22: 339-40.
13. http://www3.ncbi.nlm.nih.gov
14. DALLOS T, LUKAČIKOVA GALOVA L, MACEJKOVA E *et al*.: [Familial Mediterranean fever-first experiences in Slovakia.] *VnitrLek* 2014; 60: 80-5.
15. SEDIVA A, HORVATH R, MANASEK V *et al*.:

Cluster of patients with Familial Mediterranean fever and heterozygous carriers of mutations in MEFV gene in the Czech Republic. *Clin Genet* 2013; 84: 1-6.

16. PAPADOPOULOS VP, GIAGLIS S, MITROULIS I, RITIS K: The population genetics of familial Mediterranean fever: a meta-analysis study. *Ann Hum Genet* 2008; 72: 752-61.
17. LA REGINA M, NUCERA G, DIACO M *et al*.: Familial Mediterranean fever is no longer a rare disease in Italy. *Eur J Hum Genet* 2003; 11: 50-6.
18. TCHERNITCHKO D, MOUTEREAU S, LEGENDRE M *et al*.: MEFV analysis is of particularly weak diagnostic value for recurrent fevers in Western European Caucasian patients. *Arthritis Rheum* 2005; 52: 3603-5.
19. TOPLAK N, DOLEZALOVÀ P, CONSTANTIN T *et al*.: Periodic fever syndromes in Eastern and Central European countries: results of a pediatric multinational survey. *Pediatr Rheumatol Online J* 2010; 8: 29.
20. OZEN S, DEMIRKAYA E, AMARYAN G *et al*.: Results from a multicentre international registry of familial Mediterranean fever: impact of environment on the expression of a monogenic disease in children. *Ann Rheum Dis* 2014; 73: 662-7.
21. GUL A: Familial Mediterranean fever phenotype and MEFV variations. *Clin Exp Rheumatol* 2014; 32 (Suppl 87): S-12-13.
22. EISENSTEIN EM, BERKUN Y, BEN-CHETRIT E: Familial Mediterranean fever: a critical digest of the 2012-2013 literature. *Clin Exp Rheumatol* 2013; 31 (Suppl. 77): 103-7.
23. BERNOT A, DA SILVA C, PETIT JL *et al*.: Non-founder mutations in the MEFV gene establish this gene as the cause of familial Mediterranean fever (FMF). *Hum Mol Genet* 1998; 7: 317-25.
24. AKSENTIJEVICH I, TOROSYAN Y, SAMUELS J *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.
25. STOFFMAN N, MAGAL N, SHOHAT T *et al*.: Higher than expected carrier rates for familial Mediterranean fever in various Jewish ethnic groups. *Eur J Hum Genet* 2000; 8: 307-10.
26. MIKULA M, BULLER A, SUN W, STROM CM: Prevalence of known mutations in the familial Mediterranean fever gene (MEFV) in various carrier screening populations. *Genet Med* 2008; 10: 349-52.