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

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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-
eterous 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 over-estimated 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 polymerase (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’AAAACGGCAGAGTGTCTCC3’/FMFe2r 5’CCCTTCTCTGGGTTCGCTC3’ and FMFe10f 5’TGGAGACAAAGCAGCAATGG3’/FMFe10r 5’AGCAGGAAGAGATGCGG3’ (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 MgCl₂, 1/10 of corresponding reaction buffer and 0.08 U/μL AmpliTaq DNA polymerase (Applied Biosystems, USA). PCR products were purified using Exo-SAP-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 (1/1).

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 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.
the distance from the South-East Mediterranean basin, where FMF is a common disease. It was thought to mated to 1:3 to 1:5 and is rarer in other populations (6, 16). It was assumed that the disease may be under-diagnosed 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 (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 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.

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

<table>
<thead>
<tr>
<th>%</th>
<th>all</th>
<th>E148Q/wt</th>
<th>M694V/wt</th>
<th>K695R/wt</th>
<th>V726A/wt</th>
<th>F756C/wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>9.3</td>
<td>3.4</td>
<td>0.2</td>
<td>3.8</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Hungary</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Slovenia</td>
<td>6</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>B&amp;H</td>
<td>8</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Serbia</td>
<td>11</td>
<td>6</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Macedonia</td>
<td>16</td>
<td>8</td>
<td>1</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**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 neighboring 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 alterations (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.

agonsed 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 Jewish 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
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