Paediatric rheumatology

Familial Mediterranean fever with a single MEFV mutation: comparison of rare and common mutations in a Turkish paediatric cohort

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ABSTRACT

Objective. Presence of common MEFV gene mutations strengthened the diagnosis of FMF in addition to the typical clinical characteristics of FMF. However, there are also rare mutations. P369S, A744S, R761H, K695R, F479L are the main rare mutations in Turkish population. We aimed to evaluate FMF patients with a single allele MEFV mutation and to compare patients with common and rare mutations.

Methods. We retrospectively reviewed the medical records of FMF patients with a single allele mutation who were followed up between 2008 and 2013 in six centres. We compared the patients with rare and common mutations for disease severity score, frequent exacerbations (>1 attack per month), long attack period (>3 day), symptoms, age at the onset of symptoms, gender, consanguinity, and family history.

Results. Two hundred and seventeen patients (MF=101/116) with the diagnosis of FMF and single mutation were included. Heterozygote mutations were defined as common (M694V, V726A, M680I) and rare mutations (A744S, P369S, K695R, R761H, F479L). Sixty-seven patients (27 males, 40 females) had one single rare mutation and 150 (74 males, 76 females) had one single common mutation. No difference was found between the rare and common mutations with respect to the disease severity score. There was no significant difference between common and rare heterozygote form of mutations in terms of disease severity.

Conclusion. Patients with typical characteristics of FMF, with some rare mutations (A744S, P369S) should be treated in the same manner as patients with a common mutation.

Introduction

Familial Mediterranean fever (FMF) is a hereditary inflammatory disease characterised by recurrent fever that is accompanied by peritonitis, pleuritis, arthritis, and erysipelas-like rash in an autosomal recessive pattern. Disease is mostly seen in the Mediterranean region; particularly Turks, Arabs, Armenians, and Jews are affected (1, 2). Responsible gene for the disease, MEFV, is mapped on chromosome 16p13.3 (3). In 80% of typical cases, mutations are within exon 10. Other less common allele mutations have been shown in exons 2, 3, and 5 (4). A meta-analysis in FMF patients and normal individuals in 14 affected populations showed that MEFV mutations are distributed non-uniformly along the Mediterranean Basin. The most frequent mutations are M694V (39.6%), V726A (13.9%), M680I (11.4%), E148Q (3.4%), and M694I (2.9%), while 28.8% of chromosomes bear unidentified mutations or no mutations at all (5). Genetic analysis provides valuable information, supporting the clinical diagnosis and reassuring patients for the necessity of therapy. Currently, diagnostic criteria based only on clinical findings may not be sufficient in certain cases. New diagnostic criteria using a combination of clinical findings and molecular analysis may help clinicians make the diagnosis of FMF (6). Classically defined MFM phenotype is actually a heterogeneous group including typical autosomal recessively inherited FMF patients, multifactorial FMF patients with a single exon 10 variation, and patients with FMF-like disease without MEFV mutations. On the other hand, the MEFV gene variations may not necessarily be associated with the FMF-phenotype (7).

Competing interests: none declared.

Key words: familial Mediterranean fever, genotype, phenotype, rare mutations, mutation:
Phenotype and genotype correlation in FMF has not been conclusively explained, but several researchers have observed more severe disease expression, increased susceptibility to amyloidosis, and unresponsiveness to colchicine therapy in patients with M694V mutation (8-11). Clinical characteristics of patients carrying a single mutated ME芙 allele may be as severe as that of patients carrying two-mutant alleles (12).

Beside the common mutations there are also rare mutations. We do not know yet whether these rare mutations are as important as common mutations with respect to disease severity and phenotype. P369S, A744S, R761H, K695R, and F479L are the main rare mutations in the Turkish population (12-14). The genetic analysis method for FMF was the same performed in all 6 centres. There were more than 217 patients in the pool of FMF patients with single mutation, but the numbers were very small and we could not include to study for statistical reasons.

In this study, we aimed to investigate role of ME芙 mutations on the phenotype of patients with a single mutation and compare the patients with common and rare mutations in the Turkish paediatric population. We enrolled heterozygote persons, since FMF is a typically recessive disease.

Materials and methods
We retrospectively reviewed the medical records of FMF patients, who had a single allele mutation, followed in six paediatric nephrology-rheumatology centres in Turkey between 2008 and 2013. Main clinical data concerning the age, sex, consanguinity, age at the onset of the symptoms, duration of the attacks, frequency of the attacks, symptoms related with the attacks (fever, abdominal pain, arthralgia, arthritis, chest pain, headache, vomiting, diarrhoea, constipation, myalgia), erysipelas-like erythema, amyloidosis, family history of FMF or amyloidosis, history of FMF associated disease, especially Henoch Schönlein purpura (HSP), and efficacy of colchicine were registered on a standard form.

The genetic analysis method for FMF was the same performed in all 6 centres. Genomic DNA was isolated from blood using standard protocols. ME芙 exons and the flanking inonic sequences were PCR amplified from genomic DNA as described PCR products were sequenced bidirectionally by automated DNA-sequencing (Qiagen – FMF sequencing kit) that allows the detection of the 12 most frequent ME芙 mutations (E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H).

Table I. Demographic and clinical characteristic of Turkish FMF patients with regard to the common and rare mutations.

<table>
<thead>
<tr>
<th>Features</th>
<th>Rare mutations</th>
<th>Common mutations</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (m/n)</td>
<td>27 (40.3%)</td>
<td>74 (49.3%)</td>
<td>0.278</td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>6.76±3.94</td>
<td>6.75±4.27</td>
<td>0.831</td>
</tr>
<tr>
<td>Current age (years)</td>
<td>11.6±5.8</td>
<td>11.5±5.2</td>
<td>0.849</td>
</tr>
<tr>
<td>Early age onset (n/%)</td>
<td>36 (53.7%)</td>
<td>79 (52.7%)</td>
<td>0.885</td>
</tr>
<tr>
<td>Age of diagnosis (years)</td>
<td>8.62±3.85</td>
<td>8.63±4.21</td>
<td>0.902</td>
</tr>
<tr>
<td>Fever (n/%)</td>
<td>58 (86.6%)</td>
<td>127 (84.7%)</td>
<td>0.875</td>
</tr>
<tr>
<td>Abdomen pain (n/%)</td>
<td>61 (91%)</td>
<td>130 (86.7%)</td>
<td>0.489</td>
</tr>
<tr>
<td>Arthritis (n/%)</td>
<td>14 (20.9%)</td>
<td>23 (15.4%)</td>
<td>0.430</td>
</tr>
<tr>
<td>Chest pain (n/%)</td>
<td>5 (7.5%)</td>
<td>27 (18%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Attacks with long duration (n/%)</td>
<td>51 (76.1%)</td>
<td>125 (83.3%)</td>
<td>0.286</td>
</tr>
<tr>
<td>Frequency of attack (&gt;1/month) (n/%)</td>
<td>31 (46.3)</td>
<td>115 (76.7%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean severity score</td>
<td>7.53±1.49</td>
<td>7.24±1.37</td>
<td>0.412</td>
</tr>
<tr>
<td>High severity score rate</td>
<td>15 (22.4%)</td>
<td>27 (18%)</td>
<td>0.652</td>
</tr>
<tr>
<td>Mild severity score rate</td>
<td>51 (76.1%)</td>
<td>108 (72%)</td>
<td>0.640</td>
</tr>
<tr>
<td>Low severity score rate</td>
<td>1 (1.5%)</td>
<td>15 (10%)</td>
<td>0.025*</td>
</tr>
<tr>
<td>Consanguinity (n/%)</td>
<td>13(19.4%)</td>
<td>15 (10%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Family history (n/%)</td>
<td>13 (19.4%)</td>
<td>6 (42%)</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

*p<0.05.

Statistical analysis
Data were analysed with SPSS (Statistical Package for Social Science) 16.0 program. Male rate, frequent exacerbations, long attack period, early age onset of symptoms, frequency of attacks (fever, abdominal pain, chest pain), consanguinity and family history of FMF were assessed using Chi-square test. The mean severity score and age of onset of symptoms were assessed with Mann Whitney U-test. The level of significance was set at p<0.05.

Results
In order to evaluate the phenotype-genotype correlation, 217 (M/F=101/116) heterozygous FMF patients were divided into two groups that were formed by the presence of the common (M694V, M680I, V726A) and rare (A744S, P369S, K695R, R761H, F479L) mutations. Sixty-seven patients (27 males, 40 females) had one of the rare mutations, namely A744S, P369S, R761H, K695R, and F479L, while 150 patients (74 males, 76 females) had one of the common mutations; M694V, V726A, and M680I. The demographic and clinical characteristics of the study group with regard to the common and rare mutations were shown in Table I.

Phenotype genotype correlation
Comparison of the demographic and clinical findings of the patients with...
common and rare one-allele mutations showed that there was no difference in terms of gender, current age, arthritis/arthralgia, fever, abdominal pain, chest pain, consanguinity, age of onset, and early-onset symptoms. However, the frequency of attack rate (>1 attack/months) \( p < 0.001 \) and family history \( p = 0.002 \) were found significantly higher among the patients who had common mutations. There was no significant difference between the common and rare mutations in terms of disease severity score. Moreover, there was only one patient (1.5%) with a low severity score in the rare mutation group as compared to 15 patients (10%) in the common mutation group \( p = 0.025 \) (Table 1).

No difference was found for the comparison of disease severity score between P369S, A744S, and M694V, and V726A and M680I, respectively. Moreover, there was no significant difference between P369S and A744S mutated groups in terms of disease severity score. A significantly decreased attack frequency was found in patients with P369S compared to the frequent mutations \( p < 0.001 \) for P369S vs. M694V, and for P369S vs. V726A, \( p = 0.006 \) for P369S vs. M680I. A significantly decreased frequency of family history was found in FMF patients with A744S in comparison to the patients who bear M694V \( p = 0.013 \) and M680I \( p = 0.043 \) mutations. Arthritis was more common in patients with P369S as compared to the patients with M680I mutation \( p = 0.039 \). The ratio of male patients with M694V was significantly higher than the patients with A744S \( p = 0.011 \). No statistically significant difference was found between the patients with A744S and P369S with respect to age at disease onset, number of attacks before treatment, presentation of symptoms (fever, abdominal pain, arthritis, and chest pain), duration of attacks, family history of FMF, consanguinity, and gender. None of the patients developed proteinuria. HSP was recorded in 8 patients. Three of these patients had M694V, two had V726A, and the remaining three patients had M680I, P369S, and K695R single allele mutations.

### Discussion

Recent molecular genetic studies have revealed disease-causing mutations in FMF (4). Clinician’s experience and the clinical criteria are particularly important in decision-making. Before the discovery of FMF gene, the Tel Hashomer criteria (16) were the most widely used criteria for the diagnosis of FMF. Recently, Yağkınkaya et al. (17) proposed a new set of criteria for the diagnosis of FMF in a cohort of Turkish children with FMF. Due to the clinical heterogeneity of FMF and the lack of specific diagnostic biochemical tests, genetic analysis may help establish a definitive diagnosis.

The severity of the attacks may be associated with the MEFV genotype in FMF. Homozygous M694V and M680I, V726A-E148Q complex allele mutations are expected to occur in severe illness (18, 19). Moreover, clinical characteristics of FMF in patients with single mutation may be as severe as that of patients with two-mutant allele for M694V (20). However, the clinical spectrum of the heterozygote form of the rare mutations is not obvious. The most frequent rare mutations in single allele form previously reported in Turkish pediatric study groups are P369S (1.6–2.2%), A744S (1–2.2%), and R761 (1–1.8%) (13, 14, 21). The findings of our study (most frequent 3 rare mutations: A744S, P369S, R761H) confirm these results.

There are some rare MEFV mutations that tend to be linked with specific populations. Examples are T177I, S108R, and E474K identified in Lebanese (22), I591T found in Western Europeans (French/Spaniards) (23), and E225K identified in a Greek family (24). There is a need to conduct multinational studies investigating the genotype-phenotype correlation of FMF involving all these rare MEFV gene mutations.

It has been demonstrated that a some of the patients with clinical FMF (up to 30%, depending on the population) possess only 1 demonstrable mutation despite sequencing of the entire coding region (25-27). The lack of sensitivity in screening techniques is an explanation for his phenomenon. The majority of FMF patients are screened for a limited number of mutations, which account for a majority of carrier chromosomes in a given population. Another explanation is that the second disease-associated mutation may reside in the noncoding (intronic) or regulatory regions of MEFV, possibly affecting messenger RNA (mRNA) expression or splicing. The entire genomic region encompassing the MEFV transcript is 15 kb in size; therefore, it is not practical for diagnostic sequencing using standard techniques. Although most disease-associated mutations are missense nucleotide changes, the possibility of genomic rearrangements (e.g. deletions or copy number variations) cannot be excluded as another mechanism of disease (28).

Studies from Israel and Turkey (12-14, 19, 29) have investigated the phenotype-genotype correlation in FMF patients. However, these studies include only the most common 3 mutations (M694V, V726A, E148Q). To our knowledge no study yet has evaluated the role of the rare mutations in disease phenotype. Ozturk et al. (12) studied the phenotype among patients with a single mutation. In this study, records of 452 FMF children living in western Anatolia, Turkey, were retrospectively reviewed. The phenotype-genotype correlation in this study revealed that there was no significant difference between M694V/-, E148Q/- and the other heterozygous groups (M680I, V726A, A744S, P369S, K695R, R761H) with respect to clinical characteristics and the severity score, so that their analysis was not a comparison between the common and rare mutations.

Our study showed that there was no difference in the disease severity between patients with rare and common mutations in single allele form, also in detailed comparison between the patient group who bear P369S, A744S and M694V, M680I, V726A mutations. In our study the number of the patients with R761H, K695R, and F479L single allele mutations was not sufficient to perform a mutation-specific statistical analysis. To draw clear conclusions, therefore, new studies are needed, which will ideally include a large patient population having these rare mutations.

There was no difference between the common and rare mutations with re-
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spect to age of onset of the symptoms. Padéh et al. (29) showed that the severity score correlated with the number of mutations. Children with no mutations presented at an older age compared to children with one or 2 mutations. Children who were homozygous for the M694V mutation presented at a younger age, had a higher severity score, and had arthritis at an increased rate.

Several authors have suggested that a single heterozygous mutation, especially M694V, M680I, and V726A, is sufficient both to diagnose FMF and to start a therapeutic trial with colchicine in patients with typical characteristics of FMF (28). Although it is accepted that a patient with typical characteristics of FMF, deserves prophylactic colchicine treatment whatever the result of genetic analysis is, our results suggest that having a rare single allele mutation, particularly A744S and P369S would support the diagnosis.

We have some limitations in the study. The study has the disadvantages of being a retrospective study and the patient records might not be standard thus some data could be missing in some patients. But we tried to solve the problem by using a scoring system. Although we do not have a scoring system for paediatric patients we have used the Pras score system which was an another limitation of the study.

Our study was supported by 6 centres so that we accomplished to enroll a relatively high number of patients with A744S and P369S mutations. This increased the statistical power of our study. As far as we know, this is the largest ever study investigating the FMF patients with rare mutations A744S, P369S. Our study provided new aspects in clinical spectrum of rare mutations.

In clinical practice the diagnosis of FMF is usually based on clinical criteria, although molecular studies can also be used for detection of disease-causing mutations. Our results proved that there was no difference between heterozygote common and rare mutations in terms of disease severity. Patients with typical characteristics of FMF with some rare mutations (A744S, P369S) be treated in the same manner as patients with a common mutation. Our findings may help manage FMF patients better and improve counselling provided for their families.

References