Relationship of Tel Hashomer criteria and Mediterranean fever gene mutations in a cohort of Turkish familial Mediterranean fever patients

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ABSTRACT

Objectives: To evaluate the frequency of 5 mutations and their relationship with the Tel Hashomer criteria in 85 FMF patients.

Methods: We looked for mutations in the Mediterranean fever (MEFV) gene in 84 consecutive patients who admitted to the Department of Medical Genetics of Afyon Kocatepe University, with a variable (from high to low) clinical suspicion of FMF. By using polymerase chain reaction and Hybridization-ELISA methods, 5 mutations (M694V, M694I, V726A, M680I and E148Q) have been studied between December 2002 and January 2005.

Results: We detected homozygote mutations in 12 patients (25.3%) and heterozygote mutations in 23 patients (48.9%) out of 47 patients with high clinical suspicion of FMF using Tel Hashomer criteria. In 12 patients (25.3%), no mutation was detected despite the clinical diagnosis of FMF was likely according to the Tel Hashomer clinical criteria. On the other hand, we detected homozygote mutations in 2 patients (5.4%) and heterozygote mutations in 17 patients (45.9%) out of 37 patients with low clinical suspicion of FMF using Tel Hashomer criteria. In 18 out of 37 patients (48.6%) in this group no mutation was detected.

Conclusion: In patients with high or low clinical suspicion of diagnosis of FMF according to Tel Hashomer criteria, the frequency of homozygote patients was significantly higher than the frequency of patients with no mutation, but it was not higher than the frequency of heterozygote patients.

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Familial Mediterranean fever (FMF MIM 249100) is an autosomic recessive disease of the inflammatory pathway. Familial Mediterranean fever is the most frequent of the hereditary fevers, which include the hyperimmunoglobulinemia D with periodic fever syndrome (HIDS) (MIM 260920),[1,2] and autosomal dominant recurrent fevers such as Tumour-Necrosis-Factor-Receptor-1-associated Periodic Syndrome (TRAPS) (MIM 142680 and 134610).[3,4] Familial Mediterranean fever characterized by recurrent fever attacks, accompanied by peritonitis, arthritis pleuritis and neuropathic amyloidosis.[5,6] Episodic abdominal pain and fever, which were the typical findings of FMF, have been firstly reported by Janeway and Mosenthal in Jewish girls in 1908.[7] The typical feature of disease is acute onset fever attacks and accompanying abdominal, chest pain and arthralgia.[6] Although the most common site of inflammation is peritoneum, pleura or joints, any part of the body may be involved from skin to scrotum.[8] No agent has been found to be responsible for triggering of inflammation and, most patients

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could not define a specific cause triggering an attack. However, menstruation, emotional stress, and heavy physical activity have been reported to be associated with attacks in some patients.[9] No gender-difference has been reported in children whereas in adults the disease is more common in men (approximately 60% of cases).[10] The diagnostic criteria of the Tel Hashomer are one of commonly used criteria in clinical diagnosis of FMF since 1997. According to these criteria, for "high" clinical suspicion or definite diagnosis, 2 major criteria or one major criterion plus 2 minor criteria are needed and for "low" clinical suspicion or "possible" diagnosis, one major criterion plus one minor criterion are needed.[11] The major Tel Hashomer Criteria's are recurrent febrile episodes accompanied by peritonitis, synovitis or pleuritis, amyloidosis of the AA type (secondary amyloidosis) without predisposing disease and favorable response to continuous colchicine treatment. The minor Tel Hashomer Criteria's are FMF in a first-degree relative, Erysipelas-like erythema and Recurrent febrile episodes.[11] There is a positive family history in most of the FMF patients.[12,13] However, some generations may be skipped due to the autosomal recessive pattern of the disease. In 1992, the gene responsible for FMF was found to be localized on short arm of chromosome 16 (16p13.3); the likely order have been reported to be as follows telomer-D16S246-MEFV-D16S138-centromere (Mediterranean fever = MEFV) (OMIM 249100).[14] Following the cloning of the MEFV gene, 50 mutations have been identified on the 1st, 2nd, 3rd, 5th, 9th exons, and most commonly on the 10th exon.[15] In this study we evaluated the frequency of 5 mutations and their relationship with the Tel Hashomer criteria in 85 FMF patients.

Methods. Routine molecular diagnosis of FMF in our laboratory began in December 2002, since MEFV genotypes of 84 Turkish patients have been determined. Patients were referred by physicians from pediatrics. Main clinical data were registered on a standard form: age, gender, origin of both parents, consanguinity, familial history of FMF, age of onset of the inflammatory attacks, duration of attacks, organ involvement, frequency of the attacks, splenomegaly, amyloidosis and efficacy of colchicine. In order to standardize the symptoms and diagnosis, we requested the physician to use a specific set of clinical criteria (Tel Hashomer criteria).

Mutation analysis. Two milliliters of blood had been collected in EDTA containing tubes and the DNA of collected blood samples was isolated by using Puregene DNA Isolation Kit (Gentra Systems Inc, Minneapolis, MN, USA). The isolated DNA samples were studied for 4 mutations (M680I, M694V, M694I, V726A) in exon 10 and one mutation in exon 2 (E148Q) of MEFV gene, localized at 16p13.3. Polymerase chain reaction and hybridization-ELISA methods have been studied for this purpose. We used PRONTO® FMF kits in this research (Pronto Diagnostics Ltd., Rehovot, Israel).[16,17] The control of amplification products have been carried out in 2% agarose gel. The results have been evaluated according to the change in color following ELISA process which was described elsewhere.[16,17]

Clinical criteria tested versus genotype analysis results. We compared the results of the molecular analysis to the probability of the clinical diagnosis of FMF according to set of criteria. We chose to use the Tel Hashomer criteria as they were easily obtained from the referral. Major criteria were (i) recurrent febrile episodes accompanied by peritonitis, synovitis or pleuritis; (ii) amyloidosis of the (sekonder amiloidoz: AA type amiloidoz) AA type without predisposing disease; and (iii) the favorable response to continuous colchicine treatment. Minor criteria were (i) recurrent febrile episodes; (ii) erysipelas-like erythema; and (iii) FMF in a first-degree relative. Definite diagnosis requires 2 major, or one major and 2 minor criteria; 'probable' diagnosis requires one major and one minor.

Results. Mutations were detected in 54 of 84 (64.3%) patients. Fourteen (26%) patients had homozygote mutations, 16 (30%) had compound heterozygote mutations, 24 (44.4%) had heterozygosity for single mutations, and 30 (35,7%) patients had none of studied mutations. M694V was the most frequent mutation (38 [70%]). In total of 108 alleles from 54 patients; M694V mutation was detected in 49 (45.4%), E148Q in 15 (13.9%), M680I in 10 (9.3%), V726A in 7 (6.5%) and M694I in 4 (3.7%) alleles. In sixteen (28.3%) patients, ≥2 different mutations have been detected (**Table 1**).

The results of the genotypes according to the clinical probability of FMF using clinical criteria. Among the 47 patients with a definite clinical diagnosis of FMF, homozygote mutations were found in 12 patients (25.3%), heterozygote mutations were found in 23 patients (48.9%) and no mutation was identified in 12 patients (25.3%). Among the 37 patients in whom the clinical diagnosis of FMF was 'possible', homozygote mutations were found in 2 patients (5.4%), heterozygote mutations were found in 17 patients (45.9%) and no mutation was identified in 18 patients (48.6%)

Table 2 shows the clinical data for the patients homozygous or heterozygous for mutations in the

MEFV gene and patients with diagnosis of FMF without molecular evidence, according to the Tel Hashomer criteria. The mean age of the patients was 10.4 ± 4.4 years and onset varied from 1-16 years. In the major criteria group, recurrent febrile episodes with peritonitis was the most common symptom criterion presenting in 100% of the patients with homozygote mutations, 93.6% of the patients with compound heterozygote mutations, 100% of the patients with single mutation and 93.4% of the patients without mutations. A family history of FMF was found in 28 of the patients (33.3%). The frequencies of the other criteria were also reported in **Table 2**.

Discussion. Familial Mediterranean fever is most prevalent in people of Mediterranean basin and North Africa especially in Armenians, Sephardic-Jews, Arabs, and Turks.[18] The frequency of carrier state in Turks is 20%.[19] Recent studies showed that the most frequent mutations detected in Turks were M694V (40-52%), M680I (9-12%), E148Q (3.5%), M694I (0,4-3%) and, V726A (3-11%).[19,20] In this study, the most frequent mutations in Turkish revealed positive results in 54 of 84 (64.3%) patients. In our study, which was closely similar to that of Akar et al,[20] reported 19.1% of homozygote M694V mutation in their study and Yalcinkaya et al,[13] reported 24.6%, which was similar to our results (20.3%). Twenty-seven patients (50%) had heterozygosity of the same mutation. A number of studies reported the heterozygote mutation of M694V between 40-53% in Turkish.[13,19,20] The overall incidence of M694V mutation throughout the world was 30%, likewise North Africa had the highest ratio (90-97%).[21] Homozygosity of M694V mutation has been related with higher ratios of amyloidosis compared with other mutations.[21,22] In Turkish, the association of progression of amyloidosis and inflammatory diseases has been reported to be similar in patients with homozygous, heterozygous or compound mutations of M694V mutation, and with other mutations.[13,23] In our study, only one 18-year-old patient carrying M694V / M694V genotype has had amyloidosis with renal involvement. The V726A mutation prevalence throughout the world was approximately 17-20%.[24-26] The prevalence of this mutation in Turkey had been reported to be 3-14%.[13,19,20] In our study group, V726A mutation was in line with the literature and present in 6.5% of the patients. The frequency of E148Q mutation has been reported as 3.5% in Turkish.[19,20] E148Q was the most frequent mutation in Italian population (25-50%).[18] In our study group, we detected 10 (13.9%) mutations (7 heterozygote and 3 homozygote mutation). The M680I was the most frequent mutation in Armenians

Table 1 - Homozygous or compound heterozygous for mutations in the Mediterranean fever gene in the molecular analysis of the patients (n=54).

No.	Mutation	Number of cases (%)
1	M694V	13 (24)
2	E148Q	7 (13)
3	M680I	2 (3.7)
4	V726A	1 (1.9)
5	M694I	1 (1.9)
6	M694V / M694V	11 (20)
7	M694V / M680I	7 (13)
8	M694V / V726A	3 (5.6)
9	E148Q / E148Q	3 (5.6)
10	M694V / E148Q	2 (3.7)
11	M680I / V726A	1 (1.9)
12	M694V / M694I	1 (1.9)
13	M694I /V726A	1 (1.9)
14	M694V / M694I / E148Q	1 (1.9)

Table 2 - Clinical data of patients homozygous or heterozygous for mutations in the Mediterranean fever gene and patients with diagnosis of Familial Mediterranean fever (FMF) without molecular evidence, according to the Tel Hashomer criteria.

Tel Hashomer criteria	Homozygote (%)	Heterozygote (%)		No mutation (%)
		Compound	Single mutation	
Number of cases	14	16	24	30
Recurrent febrile episodes accompanied by peritonitis, synovitis or pleuritis	14 (100)	15 (93.6)	24 (100)	28 (93.4)
Amyloidosis of the AA type without predisposing disease	1 (7.1)	-	-	-
Favorable response to continuous colchine treatment	12 (85.7)	9 (56.3)	9 (37.5)	11 (36.7)
Major criterion per case	1.9	1.5	1.4	1.3
FMF in a first-degree relative	4 (28.6)	6 (35.7)	9 (37.5)	9 (30)
Erysipelas-like erythema	-	3 (18.8)	2 (8.4)	2 (6.7)
Recurrent febrile episodes	12 (85.7)	13 (81.3)	18 (75)	22 (73.4)
Minor criterion per case	1.1	1.4	1.2	1.1

(17-30%), and reported as frequent as 9-12% for Turkish.[9,19,20,26] The frequency in our study group was 9.3%. The M694I was the most frequent mutation in Arabs (18-54%).[26,27] In Turks, M694I mutation was reported as 0.4-3%.[19] The frequency in our study group was 3.7%. Dodé et al, found 10 (47.6%) different genotypes in 21 Armenian patients and 7 (29.2%) different genotypes in 24 Turkish patients. The most common variable genotypes were reported to be in Armenians and Turks.[26] In our group of patients, 14 out of 54 (26%) cases found to have different genotypes; of these, 7 cases were compound heterozygote (**Table 1**). One of our patients had three mutations (M694V/M694I/E148Q) (**Table 1**). Family screening was planned for this patient. M694V mutation was detected in his mother's blood sample. The mutations could not be evaluated for his father because he was deceased. We thought that M694I and E148Q mutations have been transmitted from his father in cis form. 'Recurrent febrile episodes accompanied by peritonitis, synovitis or pleuritis', 'favorable response to continuous colchicine treatment', and 'recurrent febrile episodes' were the most common criteria in all genotypes. On the other hand, 'amyloidosis of the AA type without predisposing disease' and 'erysipelas-like erythema' were the least common criteria in our patients (**Table 3**). The specificity of the tested criteria is also limited, as 30 patients with positive criteria had no identified mutations (**Table 3**). The following explanations could account for this observation. First, some of these patients are not of Mediterranean extraction, and some so-far-unknown mutations may exist in unexplored regions, though our mutation screening was near-exhaustive. Secondly, another as-yet-unidentified FMF locus may exist, as suggested in the Turkish population.[28] Thirdly, there is the question of the true diagnosis in these patients. Many diseases can present as fever of recurrent origin. The diagnosis of FMF is based on clinical criteria due to absence of pathognomic clinical findings or specific laboratory tests; the diagnosis was carried out by retrospective evaluation.[11,28] Familial Mediterranean fever may mimic acute peritonitis and appendicitis like conditions which may lead to inappropriate surgical interventions. Another important point is the necessity for lifelong colchicine medication, by which effective management of disease can be achieved. The frequency and severity of attack can be decreased and amyloidosis and renal failure may be prevented.[29] The molecular approach to FMF diagnosis now enables confirmation of FMF in most typical cases of the disease.[30]

In conclusion, we suggest that tests including more mutations should be done as screening tests for patients with clinical findings of FMF disease in order to make early diagnosis and prevent the progression of amyloidosis and inappropriate surgical interventions.

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