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Familial Mediterranean fever patients homozygous for E148Q variant

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Abstract

Aim: Familial Mediterranean fever (FMF) results from *MEFV* gene mutations. E148Q is a variant of unknown significance in *MEFV*. We aimed to define characteristics of FMF patients homozygous for E148Q, check for other *MEFV* variants in a subgroup, and compare the characteristics with FMF patients carrying other mutations.

Methods: Thirty FMF patients homozygous for E148Q were reviewed. *MEFV* variant analysis was performed with strip assay. All *MEFV* exons were screened by direct DNA sequencing in 14 randomly selected E148Q/E148Q patients. E148Q was also checked in 100 healthy adolescents. We compared the characteristics of FMF patients between three groups: E148Q/E148Q (n = 30), M694V/E148Q (n = 19) and exon 10/exon 10 *MEFV* mutations (n = 48).

Results: Among 30 FMF patients (E148Q/E148Q), the median age at disease onset and diagnosis were 60 (12–168) and 94 (41–196) months, respectively. Fifteen (50%) patients had mild, 14 (46.7%) moderate and one (3.3%) had severe disease. Twenty-two (73.3%) patients had complete, seven (23.3%) had incomplete response to colchicine, while only one was unresponsive. The detected *MEFV* variants in 14 E148Q/E148Q FMF patients were as follows: R314R (n = 9; 64.3%), E474E (n = 13; 92.9%), Q476Q (n = 13; 92.9%), D510D (n = 13; 92.9%), and P588P (n = 8; 57.1%). The E148Q allele frequency was 6.5% in healthy adolescents. When compared to FMF patients with other *MEFV* mutations, disease onset was later, disease was less severe and the ratio of patients responding completely to colchicine was higher in E148Q/E148Q patients.

Conclusion: Patients homozygous for E148Q and negative for other pathogenic *MEFV* variants may display FMF phenotype and may experience moderate/severe disease activity, although the disease may be milder when compared to FMF patients with other mutations.

Key words: familial Mediterranean fever, E148Q, *MEFV* gene, variant of unknown significance.

INTRODUCTION

Familial Mediterranean fever (FMF) is the most common monogenic auto-inflammatory disease (AID) characterized by recurrent, self-limited fever attacks associated with polyserositis.[1] The prevalence of FMF is very high among certain ethnic groups such as Jewish, Turkish, Armenian and Arabs, reaching figures as high as 1/500;[2,3] however, it can be seen all around the world.[4] FMF results from mutations in the *MEFV* gene on chromosome 16p.[5,6] Mutations in the *MEFV* gene encoding pyrin, causes exaggerated inflammatory response as a result of uncontrolled production of interleukin-1 (IL-1).[7] The diagnosis of FMF relies mainly on clinical findings, and molecular analysis of the *MEFV* gene provides genetic confirmation.[8]

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Colchicine is still the main form of treatment for patients with FMF.[9] It improves the quality of life by decreasing the frequency of fever flares and also prevents development of amyloidosis which is the scariest complication of FMF.[10,11]

There are about 300 known sequence variants of *MEFV* and all reported mutations and the associated phenotypes are now recorded in the INFEVERS database (http://fmf.igh.cnrs.fr/ISSAID/infevers/).[12] E148Q is the most frequent variant among carriers, while the most common disease-associated pathogenic variants are M694V, V726A, M680I and M694I.[13,14]

In 2012, Shinar *et al.*[15] proposed recommendations for interpretation of genetic testing in acquired immune deficiency syndrome and for FMF; the consensus was to test for 14 variants in the *MEFV* gene. Nine of the suggested variants (M680I, M694V, M694I, V726A, A744S, R761H, I692del, E167D, T267I) were clearly pathogenic, whereas five (E148Q, P369S, F479L, K695R, I591T) were defined as variants of uncertain significance. In fact, the jury is still out for these variants of uncertain significance, especially E148Q. This variant is present in > 1% of the healthy population and has a low penetrance, suggesting that it might be a benign polymorphism.[14] However, there are articles reporting patients homozygous for E148Q variant displaying FMF phenotype.[16,17] It is an issue of debate whether these patients have other associated genetic markers of inflammation.

The aim of our study was to evaluate the characteristics of symptomatic FMF patients homozygous for E148Q and look for other variants in the *MEFV* gene in a subgroup of these patients. We also compared the characteristics of E148Q/E148Q FMF patients with patients homozygous/compound heterozygous for exon 10 *MEFV* mutations or compound heterozygous for E148Q and exon 10 *MEFV* mutation (e.g., M694V/E148Q).

PATIENTS AND METHODS

We have reviewed the charts of 30 FMF patients who had a genetic analysis of the *MEFV* gene in the Department of Medical Biology at Hacettepe University, Ankara, and resulted to be homozygous for E148Q variant. All these patients were followed up in the Department of Pediatric Rheumatology and Nephrology at Hacettepe University, Ankara. To form comparison groups, we have reviewed FMF patients who were admitted to our clinic at least once during the last 6 months (November 2015 to April 2016) and who were being followed-up in our clinic at least for 2 years. We have included the patients either carrying two exon 10 *MEFV* mutations (n = 48; 23 had M694V/M694V, 18 M680I/M680I, five V726A/V726A, two M694V/M680I) or carrying E148Q and one exon 10 *MEFV* mutation (n = 19; all had M694V/E148Q).

All of these patients fulfilled both Tel Hashomer[18] and the Turkish pediatric FMF criteria.[19] Demographic data, family history, clinical manifestations, laboratory data (white blood cell count, erythrocyte sedimentation rate [ESR], C-reactive protein [CRP]), comorbidities, treatment, treatment response and complications were documented. Disease severity before colchicine treatment was assessed with the severity score system first suggested by Pras[20] and included the following features: age at onset (2 points for 11–20 years; 3 points for 6–10 years; 4 points for < 6 years), number of attacks per month (1 point for < 1; 2 points for 1–2; 3 points for > 2), acute or protracted arthritis (2 and 3 points, respectively), presence of erysipelas-like erythema (2 points), less than appropriate (0 point), appropriate (1 point), or more than appropriate dose (2 points) for colchicine, and development of amyloidosis (3 points). Patients with a score of 3–5 had mild, 6–9 moderate and > 9 severe disease. Complete response to colchicine treatment was defined as being attack-free with treatment. If the frequency of attacks decreases by ≥ 50%, this meant incomplete response and the patient was defined as unresponsive if the attack frequency decreased < 50%.[21]

In addition to the FMF patients, the presence of E148Q variant was checked in 100 healthy adolescents who attended the Department of Adolescent Medicine of our hospital for routine examination.

MEFV gene variant analysis was performed with strip assay. Twelve most common variants (E148Q, P369S, F479I, M680I (G-C), M680I (G-A), I692del, M694V, M694I, K695R, V726A, A744S, R761H) were investigated in the *MEFV* gene in Hacettepe University Department of Medical Biology. Fourteen FMF patients from our E148Q/E148Q cohort (n = 30) were randomly selected for further genetic analysis. In these patients, all protein encoding exons of the *MEFV* gene were screened by Sanger sequencing in Hacettepe University Nephrogenetics Laboratory. Sanger sequencing was performed using Big-Dye terminator chemistry 3.1 on the 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

The subjects' written consents were obtained according to the Declaration of Helsinki and the study was approved by the ethics committee of Hacettepe University.

Statistical analysis

Statistical analyses were performed using the SPSS software version 15 (SPSS Inc., Chicago, IL, USA). Descriptive analyses were presented using proportions, medians, minimum and maximum values as appropriate. The Chi-square test or Fischer's exact test, where appropriate, was used to compare the proportions in different groups. Kruskal-Wallis test was conducted to compare non-normally distributed numeric variables between independent groups. The Mann-Whitney *U*-test was performed to test the significance of pairwise differences using Bonferroni correction to adjust for multiple comparisons. A *P*-value of less than 0.05 was considered to show a statistically significant result.

RESULTS

The characteristics of FMF patients are summarized in Table 1. Among 30 patients who were homozygous for E148Q, the male-to-female ratio was 1.14. The median age of disease onset was 60 (12–168) months while the median age at diagnosis was 94 (41–196) months, and colchicine treatment had been started at the time of diagnosis. Fifteen (50%) patients had mild, 14 (46.7%) had moderate and one (3.3%) had severe disease according to the disease severity score. Twenty-two (73.3%) patients had complete and seven (23.3%) had incomplete response to colchicine treatment, while only one patient was unresponsive, thus anti-IL-1 treatment was commenced. This patient also had systemic juvenile idiopathic arthritis (SJIA).

The median duration of fever attacks was 3 (1–5.5) days, while the median attack number per year was 12 (4–22) before colchicine treatment. All patients had fever during attacks. Besides fever, the most common associated symptom was abdominal pain (80%). Other symptoms included arthralgia (50%), chest pain (16.7%), nausea/vomiting (13.3%), arthritis (6.7%),

Table 1 Characteristics of familial Mediterranenan fever (FMF) patients in three groups (patients with E148Q/E148Q; patients with M694V/E148Q; patients with two exon 10 *MEFV* mutations)

Characteristics	E148Q/E148Q (n = 30)	M694V/E148Q (n = 19)	Exon 10 mutation/Exon 10 mutation† (n = 48)	*P*-value
Male/female (n)	16/14	11/8	22/26	0.62
Age at symptom onset (months), median (min-max)	60 (12–168)	36 (12–132)	27 (2–192)	0.024
Age at diagnosis (months), median (min-max)	94 (41–196)	54 (24–168)	56.5 (1194)	0.008
Fever, n (%)	30 (100)	19 (100)	48 (100)	–
Abdominal pain, n (%)	24 (80)	16 (84.2)	43 (89.6)	0.49
Chest pain, n (%)	5 (16.7)	0 (0)	12 (25)	0.06
Arthralgia, n (%)	15 (50)	9 (47.4)	21 (43.8)	0.86
Arthritis, n (%)	2 (6.7)	4 (21.1)	2 (4.2)	0.07
Myalgia, n (%)	1 (3.3)	0 (0)	0 (0)	0.32
Headache, n (%)	1 (3.3)	0 (0)	0 (0)	0.32
Pharyngitis, n (%)	2 (6.7)	0 (0)	0 (0)	0.10
Nausea/vomiting, n (%)	4 (13.3)	1 (5.3)	3 (6.3)	0.47
Diarrhea, n (%)	2 (6.7)	0 (0)	0 (0)	0.10
Family history of FMF, n (%)	8 (26.7)	3 (15.8)	11 (22.9)	0.63
Pras severity score, median (min-max)	5.5 (3–11)	6 (5–9)	7 (4–11)	<0.001
Pras severity category, mild, n (%)	15 (50)	4 (21.1)	6 (12.5)	0.001
Pras severity category, moderate, n (%)	14 (46.7)	15 (78.9)	29 (60.4)	0.10
Pras severity category, severe, n (%)	1 (3.3)	0 (0)	13 (27.1)	0.002
Complete response to colchicine treatment, n (%)	22 (73.3)	5 (26.3)	10 (20.8)	<0.001
Partial response to colchicine treatment, n (%)	7 (23.4)	14 (73.7)	33 (68.7)	<0.001
No response to colchicine treatment, n (%)	1 (3.3)	0 (0)	5 (10.5)	0.49

†Twenty-three patients had M694V/M694V, 18 had M680I/M680I, five had V726A/V726A, two had M694V/M680I.

pharyngitis (6.7%), diarrhea (6.7%), myalgia (3.3%) and headache (3.3%). Family history of FMF was positive in eight patients (26.7%). Eleven (36.6%) patients had comorbidities as follows; SJIA ($n = 3$), immunoglobulin A vasculitis/Henoch-Schönlein purpura (IgAV/HSP) ($n = 2$), asthma ($n = 2$), allergic rhinitis ($n = 1$), Wilm's tumor ($n = 1$), Crohn disease ($n = 1$), celiac disease ($n = 1$) and right atrophic kidney ($n = 1$).

During fever flares, median values for white blood cell (WBC) count was 12.4 (8.8–15.3) $\times$ $10^3/mm^3$, ESR was 38.5 (14–56) mm/h (normal range 0–20) and CRP was 2.5 (1.4–16.8) mg/dL (normal range 0–0.5). During attacks CRP values were significantly higher than the values in attack-free intervals ($P = 0.012$).

The frequencies of detected *MEFV* variants in randomly selected 14 FMF patients (E148Q/E148Q) were as follows: R314R (exon 3) in nine patients (64.3%), E474E (exon 5) in 13 (92.9%), Q476Q (exon 5) in 13 (92.9%), D510D in 13 (92.9%) and P588P (exon 9) in eight patients (57.1%). Both parents of 10 children from this subgroup were heterozygous for E148Q. The fathers of two patients and the mothers of one other patient were homozygous for E148Q while the other pairs of parents were heterozygous for E148Q. The father of one patient did not carry E148Q while the mother was homozygous for E148Q. Uniparental disomy was excluded with 250K chip analysis; however, the probability of microdeletion still exists for this patient. Four parents who are homozygous for E148Q were asymptomatic.

The allele frequency for E148Q was 6.5% in 100 healthy adolescents.

When we compare three groups of FMF patients (E148Q/E148Q *vs*. M649V/E148Q *vs*. exon 10 mutation/exon 10 mutation), there was no significant difference with regard to the attack symptoms and acute phase reactant levels during attacks. However, the disease onset was later, the disease was less severe, and the ratio of patients responding to colchicine completely was higher in patients homozygous for E148Q (Table 1).

The median follow-up was 96 (12–192) months. None of our FMF patients included in this study had proteinuria and/or amyloidosis.

DISCUSSION

In our study, we have demonstrated that patients homozygous for E148Q displayed typical FMF phenotype and half of these patients had moderate/severe disease before colchicine treatment. On the other hand, the allele frequency for E148Q (6.5%) was high in 100 healthy individuals and four parents who were homozygous for E148Q were asymptomatic. We did not detect any other pathogenic variants or variants of unknown significance in the *MEFV* gene in a subgroup of symptomatic E148Q/E148Q FMF patients. When we compared E148Q/E148Q patients with FMF patients carrying exon 10 *MEFV* mutations, we demonstrated that the disease symptoms started at an older age, and the disease was less severe with more patients responding completely to colchicine treatment in the E148Q/E148Q group.

We searched the other *MEFV* variants detected in our patients (R314R, E474E, Q476Q, D510D and P588P) from the INFEVERS database (http://fmf.igh.cnrs.fr/ISSAID/infevers/).[12] These variants were defined as benign single nucleotide polymorphisms and they have never been shown to be associated with a clinical phenotype.[12]

E148Q variant results in the substitution of glutamine for glutamic acid at codon 148 in exon 2 of the *MEFV* gene.[22,23] It is one of the most frequent variants of the *MEFV* gene either as the only variant or in association with pathogenic variants.[22,24] Ben-Chetrit *et al.*[24] reported a similar E148Q variant frequency between patients and healthy controls and between patients and their asymptomatic relatives. They concluded that E148Q is a non-disease causing benign alteration of *MEFV* gene. In the same lines, Tchernitchko *et al.*[25] demonstrated that E148Q allele frequency was comparable among patients and asymptomatic relatives. In another study, it was demonstrated that patients carrying a variant of unknown significance such as E148Q with a clearly pathogenic mutation on the other allele often displayed the FMF phenotype.[26] In our previous study we showed that patients homozygous for E148Q had a heterogeneous presentation, while most were symptomatic and required colchicine treatment.[17] In this study, the age at onset and the male-to-female ratio were similar in the homozygous (E148Q/E148Q) and compound/complex (e.g., M694V/E148Q or E148Q/E148Q/M694V) cases.[17] Although the frequencies of fever, abdominal pain, chest pain, arthralgia, arthritis and myalgia were greater in compound heterozygous and complex cases, none of the symptoms attained statistical significance compared with patients who were homozygous for E148Q.[17] In the same lines, in our current study, there were no significant differences with regard to the attack symptoms; however, the disease

onset was later and the disease was less severe in patients homozygous for E148Q when compared to the patients with exon 10 *MEFV* mutations. In a recent study, Uluca *et al.*[27] reported later disease onset and milder disease in FMF patients with E148Q variants. Of note, 19 out of 507 FMF patients (3.7%) were homozygous for E148Q in this study. Altunoglu *et al.*[28] demonstrated that the most prevalent allelic variant was M694V followed by E148Q in patients with FMF phenotype II (in phenotype II, isolated amyloidosis is the sole and first manifestation of FMF). In their study group consisting of 22 FMF patients with phenotype II, four were heterozygous for E148Q. Therefore, the pathogenic role of E148Q still remains debatable. In the literature, symptomatic FMF patients homozygous for E148Q were mostly reported from Turkey. There may be an environmental or epigenetic factor affecting the phenotype in these patients with the same ethnic origin.

Most recently, the SHARE (Single Hub and Access point for pediatric Rheumatology in Europe) initiative has developed evidence-based recommendations for genetic diagnosis of FMF.[12] According to these recommendations, the E148Q variant is common, of unknown pathogenic significance and, as the only *MEFV* variant, does not support the diagnosis of FMF.

Mutations of *MEFV* may predispose to other rheumatic diseases and inflammatory conditions. E148Q was also shown to be associated with other rheumatic diseases.[29] Several reports demonstrated the frequent association of FMF with some vasculitides, especially IgAV/HSP and polyarteritis nodosa.[30–32] And another study by Ayaz *et al.*[33] showed that SJIA patients had a significantly higher frequency of *MEFV* mutations than the healthy population. In our study group, SJIA and IgAV/HSP were present in five E148Q/E148Q FMF patients (16.6%).

Our study is limited by the small number of patients.

In conclusion, we have evaluated our patients who were homozygous for E148Q and displayed typical FMF phenotype. The sequencing of all exons of the *MEFV* gene did not reveal new pathogenic variants or variants with unknown significance in these patients. Although the disease onset was later and the disease was less severe in E148Q/E148Q patients when compared to the FMF patients carrying exon 10 *MEFV* mutations, the disease severity was moderate/severe in half of E148Q/E148Q patients. Therefore, FMF patients homozygous for E148Q should be followed up as closely as patients with other *MEFV* variants.

AUTHOR CONTRIBUTIONS

Conception and design of the study: Çigdem Yıldız, Emine Korkmaz, Nesrin Beşbaş, Fatih Özaltın, Rezan Topaloglu. Collection of data: Rezan Topaloglu, Ezgi Deniz Batu, Çigdem Yıldız, Seza Özen. Analysis of data: Rezan Topaloglu, Ezgi Deniz Batu, Çigdem Yıldız, Emine Korkmaz, Nesrin Beşbaş, Fatih Özaltın. Preparation of the first draft of manuscript: Rezan Topaloglu, Ezgi Deniz Batu, Çigdem Yıldız, Seza Özen. Critical revision of the manuscript: Rezan Topaloglu, Ezgi Deniz Batu, Emine Korkmaz, Nesrin Beşbaş, Fatih Özaltın.

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