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ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

The Effect of E148Q Variant on Disease Severity in Familial Mediterranean Fever Patients with Compound Heterozygous Mutation

Bileşik Heterozigot Mutasyonu Olan Ailevi Akdeniz Ateşi Hastalarında E148Q Varyantının Hastalık Ağırlığına Etkisi

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ABSTRACT

Aim: In this study, we aimed to evaluate the demographic and clinical findings of familial Mediterranean fever (FMF) patients carrying a compound heterozygous mutation and to investigate the effect of the E148Q variant on disease severity.

Material and Method: Patients between the ages of 0-18 years diagnosed with FMF and carrying a compound heterozygous mutation were included in the study. Disease severity was assessed using the international severity scoring for FMF (ISSF). Patients were divided into two groups according to mutation type: those with exon 10/E148Q compound heterozygous mutation (Group 1) and exon 10/exon 10 compound heterozygous mutation (Group 2).

Results: A total of 317 FMF patients with compound heterozygous mutations had a male/female ratio of: 1.07, median age at diagnosis was 6.7 (IQR:6.3) years. The most common symptom was abdominal pain (85.8%). The median age at diagnosis was 6 years in Group 2 (n=219) and 8.2 years in Group 1 (n=98) (p=0.005). Fever (75.8%) was more common in patients with two exon 10 mutations (75.8%) (p=0.001). The presence of more than two different attack types, more than two findings in an attack and an attacks lasting longer than 72 hours were more frequent in patients in Group 2 (p=0.021, p<0.001, p=0.043, respectively). The ISSF score of Group 2 was higher than the other group (p<0.001).

Conclusion: Patients with two exon 10 mutations have more severe disease than patients with other compound heterozygous mutations including the E148Q mutation. ISSF score is lower in patients with E148Q mutation in one allele.

Keywords: Familial Mediterranean fever, E148Q variant, ISSF score

ÖZ

Amaç: Bu çalışmada bileşik heterozigot mutasyon taşıyan ailevi Akdeniz ateşi (AAA) hastalarının demografik, klinik bulgularının değerlendirilmesi ve E148Q varyantının hastalık ağrılığı üzerine etkisinin araştırılması amaçlandı.

Gereç ve Yöntem: 0-18 yaşları arasında, Yalçınkaya-Özen kriterlerine göre AAA tanısı konulmuş olan ve bileşik heterozigot mutasyon saptanmış olan hastalar çalışmaya alındı. Klinik ve demografik verileri kaydedildi. Hastalık ağırlığı AAA için uluslararası şiddet skorlama sistemi (ISSF) ile değerlendirildi. Hastalar mutasyon tipine göre ekzon 10/E148Q bileşik heterozigot mutasyonu olanlar (Grup 1) ile ekzon 10/ekzon 10 bileşik heterozigot mutasyonu olanlar (Grup 2) olarak iki gruba ayrılarak değerlendirildi.

Bulgular: Toplam 317 bileşik heterozigot mutasyona sahip AAA hastalarında kadın/erkek oranı: 1.07, ortanca tanı yaşı 6.7 (IQR: 6.3) yıldı. En sık görülen semptom %85.8 oranında karın ağrısı idi. Grup 2'de (n=219) yer alan hastaların ortanca tanı yaşı 6 yıl, Grup 1'dekilerin (n=98) ise 8.2 yıldı (p=0.005). İki ekzon 10 mutasyonu olan hastalarda ateş (%75.8) diğer gruba göre daha sık görüldü (p=0.01). İkiden fazla farklı atak tipi, atakta ikiden fazla bulgu ve 72 saatten uzun süren atak varlığı Grup 2'deki hastalarda daha sıktı (sırasıyla p=0.021, p<0.001, p=0.043). Tüm hastalarda toplam ISSF skoru ortanca 2 (IQR:3) olarak bulunurken Grup 2'nin ISSF skoru diğer gruba göre daha yüksekti (p<0.001).

Sonuç: İki ekzon 10 mutasyonu olan hastalar, E148Q mutasyonunu içeren diğer bileşik heterozigot mutasyonu olan hastalara göre daha şiddetli hastalığa sahiptir. ISSF skoru bir alelinde E148Q mutasyonu olan hastalarda daha düşüktür.

Anahtar Kelimeler: Ailevi Akdeniz ateşi, E148Q varyant, ISSF skoru

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INTRODUCTION

Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disease that is characterized by recurrent episodes of fever and serosal inflammation (1,2). The predicted prevalence of FMF is 1/1000, the prevalence of carriage in the Turkish population is 20% (3).

A typical FMF attack lasts 12-36 hours and patients are mostly asymptomatic between attacks. The frequency of attacks ranges from once a week to several times a year (2). Approximately 96% of FMF attacks are characterized by fever. Following fever, the most common finding is abdominal pain in 90-95% of cases. Joint findings are observed in 20-75% and chest pain in 30-50% of cases (4,5).

The diagnosis is based on clinical findings, supported by family history and genetic testing. After the identification of the Mediterranean FeVer (MEFV) gene, genetic testing has been helpful in the diagnosis, especially in patients without typical clinical findings (1, 6). The MEFV gene is localized on chromosome 16p13.3 and consists of 10 exons (4). Most of the mutations associated with FMF are located in exons 2, 3, 5 and 10. The most common mutations are M694V, M680I, V726A, M694I in exon 10 and E148Q in exon 2 (5). Although studies have been reported on the genotype-phenotype relationship, the correlation has not been clearly clarified yet. Homozygous mutations in the exon 10 such as M694V, M680I, V726A are known to be associated with more severe clinical presentation. However, the potential pathologic role of the E148Q variant is still controversial (7).

The aim of this study was to evaluate the demographic and clinical findings of patients with compound heterozygous mutations and to investigate the effect of the E148Q variant on disease severity when combined with exon 10 mutations.

MATERIAL AND METHOD

This study was approved by the Ankara City Hospital No:2 Clinical Researches Ethics Committee (Date: 23/11/2022, Decision No: E2-22-2844). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study was conducted retrospectively in patients with a compound heterozygous mutation in the MEFV gene, aged younger than 18 years at diagnosis, who were evaluated in the Pediatric Rheumatology Department of our hospital between January 2019 and January 2023. FMF patients with MEFV mutation other than compound heterozygous mutation or FMF patients without genetic testing were excluded from the study.

Demographic data, clinical findings, attack frequency, attack duration, family history, presence of comorbidity, treatment data and MEFV mutation analysis were recorded.

The diagnosis of FMF was based on Yalçınkaya-Özen criteria with the presence of at least 3 episodes lasting 6-72 hours of fever, abdominal pain, chest pain, arthritis and the positive family history. (8). Disease severity was assessed by the international severity score for FMF (ISSF). Nine parameters were evaluated and classified as mild, moderate or severe disease with a total score of 10 (**Table 1**) (9).

Table 1: The international severity score for familial Mediterranean fever (ISSF)

1) Chronic sequela (including amyloidosis, growth retardation, anaemia, splenomegaly)	1 point
2) Organ dysfunction (nephrotic range proteinuria, FMF related)	1 point
3) Organ failure (heart, renal, etc, FMF related)	1 point
4-a) Frequency of attacks (average number of attacks between 1 and 2 per month)	1 point
4-b) Frequency of attacks (average number of attacks >2 per month)	2 points
5) Increased acute-phase reactants (any of C-reactive protein, serum amyloid A, erythrocyte sedimentation rate, fibrinogen) during the attack-free period, ≥2 weeks after the last attack (at least two times 1 months apart)	1 point
6) Involvement of more than two sites during an individual acute attack (pericarditis, pleuritis, peritonitis, synovitis, ELE, testis involvement, myalgia, and so on)	1 point
7) More than two different types of attack during the course of the disease (isolated fever, pericarditis, pleuritis, peritonitis, synovitis, ELE, testis involvement, myalgia, and so on)	1 point
8) Duration of attacks (more than 72 h in at least three attacks in a year)	1 point
9) Exertional leg pain (pain following prolonged standings and/or exercising, excluding other causes)	1 point

Severe disease ≥6, intermediate disease 3–5, mild disease ≤2.
*Criterion 4a/4b can give 0 or 1 or 2 points altogether according to the definition.
ELE, erysipelas-like erythema; FMF, familial Mediterranean fever.

At least five predominant mutations in the MEFV gene (p.M694V, p.M680I, p.M694I, p.V726A, p.E148Q) were analyzed. Patients carrying different mutations in two alleles were defined as compound heterozygotes. Patients were divided into two groups according to mutation type: Group 1 included patients with exon 10/E148Q compound heterozygous mutation and Group 2 included patients with exon 10/exon 10 compound heterozygous mutation. The two groups were compared according to clinical and demographic characteristics and ISSF score components.

Statistical Analyses

Statistical analyses were performed using the SPSS software version 25. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. Descriptive analyses were presented using means and standard deviations for normally distributed variables, medians and interquartile range for the non-normally

distributed and ordinal variables, and frequencies for the categorical variables. In the comparisons between groups, the Mann-Whitney U test for non-normally distributed variables and the Chi-square or Fisher tests for categorical variables were used. A p-value of less than 0.05 was considered to show a statistically significant result.

RESULTS

Three hundred and seventeen FMF patients with compound heterozygous mutations were included in the study. One hundred and sixty-four (51.7%) of the FMF patients were female (female/male ratio: 1.07).

The median age at onset of complaints was 4 years (IQR: 5.3), while the median age at diagnosis was 6.7 years (IQR: 6.3). During the attack, 85.8% (n=272) had abdominal pain, 70.3% (n=223) had fever, 50.5% (n=160) had arthralgia, 31.9% (n=101) had chest pain, 14.8% (n=47) had arthritis, 8.2% (n=26) had erysipelas-like erythema (ELE), and 12.6% (n=40) had diarrhea.

M694V/M680I mutation was detected in 91 (28.7%), M694V/V726A in 63 (19.9%), M694V/E148Q in 62 (19.6%), M680I/V726A in 25 (7.9%), M694V/R761H in 21 (6.6%), M680I/E148Q in 12 (3.8%) and V726A/E148Q in 11 (3.5%) patients. The most common allele was M694V in 246 (77.6%) patients.

Ninety-eight patients with exon 10/E148Q compound heterozygous mutation were classified as Group 1 and 219 patients with exon 10/exon 10 compound heterozygous mutation were classified as Group 2. Demographic, clinical, treatment characteristics and ISSF scores between the two groups are given in **Table 2**.

Table 2: Demographic, clinical, treatment characteristics and ISSF scores of patients according to the mutation type

	Group 1 Exon 10/ E148Q (n= 98)	Group 2 Exon 10/Exon 10 (n=219)	p value
Female/Male	56/42	108/111	0.20[a]
Age at onset of complaint† (n=261)	5 (6)	3.5 (5)	0.22[b]
Age at diagnosis†	8.2 (6.9)	6 (6.1)	0.005[b]
Diagnostic delay (month)	21.5 (32.4)	22.3 (28.2)	0.68[b]
Attack characteristics*			
Fever	57 (58.2)	166 (75.8)	0.001[a]
Abdominal pain	79 (80.6)	193 (88.1)	0.076[a]
Chest pain	28 (28.6)	73 (33.3)	0.40[a]
Arthralgia	44 (44.9)	116 (53)	0.18[a]
Arthritis	10 (10.2)	37 (16.9)	0.12[a]
Erysipeloid rash	7 (7.1)	19 (8.7)	0.65[a]
Exercise-related leg pain*	25 (25.5)	47 (21.5)	0.43[a]
Attack duration† (day) (n=265)	2 (2)	2 (1)	0.15[b]
Attack frequency (last 6 months) †	0 (1)	1 (2)	0.016[b]
Treatment characteristics*			0.92[a]
Colchicine	97 (99)	216 (98.6)	
Anakinra	0 (0)	1 (0,5)	
Canakinumab	1 (1)	2 (0.9)	
More than two different attack*	19 (19.4)	70 (32)	0.021[a]
More than two symptoms in an attack*	60 (61.2)	177 (80.8)	<0.001[a]
Three episodes lasting longer than 72 hours*	15 (15.3)	56 (25.6)	0.043[a]
Increased acute-phase reactants *	1 (1)	9 (4.1)	0.18[c]
Comorbidity*			0.84[a]
IgA vasculitis	2 (2)	10 (4.6)	
Sacroiliitis	2 (2)	4 (1.8)	
Juvenile idiopathic arthritis	4 (4.1)	6 (2.7)	
Inflammatory bowel disease	3 (3.1)	4 (1.8)	
Family history*, n=290	46 (49.5)	107 (54.3)	0.44[a]
ISSF score†	2 (2)	3 (2)	<0.001[b]
ISSF score, group*			<0.001[a]
Mild	67 (68.4)	94 (42.9)	
Intermediate	31 (31.6)	120 (54.8)	
Severe	0 (0)	5 (2.3)	

*n (%), †median (IQR)
aChi-Square, bMann-Whitney U, cFisher's Exact Test
ISSF: The international severity score for familial Mediterranean fever

The median age at diagnosis was 6 years in Group 2 and 8.2 years in Group 1 (p=0.005). Fever (75.8%) was more common in patients with two exon 10 mutations compared to the other group (p=0.001). The presence of more than two different attack types, more than two symptoms in an attack and an attack lasting longer than 72 hours were significantly more frequent in patients in Group 2 (p=0.021, p<0.001, p=0.043, respectively).

The median total ISSF score was 2 (IQR:3) in all patients, while the ISSF score of Group 2 was significantly higher than the other group (p<0.001). There was no organ dysfunction, organ failure, or amyloidosis in any patient in the study. Splenomegaly was detected in 16 patients (5%).

The disease was well-controlled with colchicine in 299 patients (94.3%). Four (1.3%) patients were accepted colchicine-resistant and received anakinra or canakinumab. There was no difference between the two groups in terms of treatment characteristics.

DISCUSSION

The effect of exon 10 mutation on disease severity in patients with FMF is known, however, the importance of E148Q mutation on disease severity is still controversial. In this study, compound heterozygous patients with two exon 10 mutations were found to have an earlier age at diagnosis, a longer duration of attacks and a higher rate of febrile attacks than compound heterozygous patients with the E148Q variant. In addition, ISSF score indicating disease severity was found to be higher in patients with two exon 10 mutations.

Familial Mediterranean fever begins before the age of 20 in 90% of patients (10). The average age of onset is between 3-9 years (5). In our study, the median age at onset of complaints was 4 years and the median age at diagnosis was 6.7 years (5). In 2010, Çağlayan et al. evaluated 66 patients with only compound heterozygous mutation and found that the age at diagnosis was 16 years. (11). Nowadays, increased awareness of FMF and easy availability of genetic analyses lead to earlier diagnosis. The mutation detected in the patient is the determinant of the age at diagnosis. It is known that patients with M694V homozygous mutation have an earlier age of onset (3). In this study, the number of exon 10 mutations was found to be effective on the age at diagnosis. Patients with exon 10 mutation in two alleles had a younger age at onset of symptoms and age at diagnosis than patients with exon 10 mutation in one allele and E148Q in the other allele. It suggests that patients who are compound heterozygous with the exon 10 mutation have a more severe phenotype than compound heterozygous with the E148Q variant on one allele.

The type and frequency of FMF attacks vary according to ethnic groups and the result of studies. According to studies conducted in the Turkish population, the frequency of clinical symptoms of FMF was 68.6-92.5% fever, 88.2-94.8% abdominal pain, 17.8-50.4% chest pain, 46.4-77.7% arthritis and 5.4-27.5% erysipelas-like erythema (12-14). The most common clinical findings in our patients were abdominal pain, fever and arthralgia. In addition, fever was more common in patients with exon 10 mutations in both alleles.

Several studies have reported that patients carrying the M694V/M680I and M694V/V726A compound heterozygous mutation have a relatively severe clinical course similar to the M694V homozygous mutation and pointing to non-M694V exon 10 mutations (7, 15, 16). However, suspicions about the potential pathogenic role of the E148Q variant continue to persist. Some authors consider the E148Q variant to be a benign polymorphism, while others consider it to be a mild disease-causing mutation with less penetrance and a cumulative worsening effect (7, 17, 18). In our study similarly reported that patients with two exon 10 mutations had a more severe clinical course. These patients had longer attack durations, more clinical findings and more than two attack types more frequently. This may be related to the fact that other exon 10 mutations such as M694V cause a similarly more severe clinical presentation.

Several scoring systems are used to assess disease severity in patients with FMF (19). In our study, ISSF score was higher in patients with two exon 10 mutations. In the study using the Pras disease severity score, it was found that patients whose age at diagnosis was older than 8 years had a lower disease activity score, lower frequency of attacks and lower rate of febrile attacks (10). Bilge et al. evaluated disease severity with the ISSF score and found that early-onset disease and more frequent pleuritis, ELE, arthritis and myalgia were associated with more severe disease (20). Studies comparing age at diagnosis and disease severity have shown that earlier disease onset is associated with a more severe disease outcome (21, 22).

The retrospective design of the study and the small number of patients are the mail limitations of the study. The fact that this study was conducted with FMF patients with a specific mutation allowed a more comprehensive evaluation of this group.

CONCLUSION

The clinical significance of the E148Q mutation in FMF is still controversial. In this study, patients with an exon 10 compound heterozygous mutation with E148Q in one allele were shown to have milder disease than patients carrying two exon 10 mutations. This result may be attributed to the fact that carrying two exon 10 mutations is associated with a severe phenotype rather than the

E148Q variant being associated with a mild phenotype. Detailed evaluation in FMF patient groups with different exon mutations is needed to better understand the genotype-phenotype relationship.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Ankara City Hospital No:2 Clinical Researches Ethics Committee (Date: 23/11/2022, Decision No: E2-22-2844).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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REFERENCES

1. Ayaz NA, Tanatar A, Karadağ ŞG, Çakan M, Keskindemirci G, Sönmez HE. Comorbidities and phenotype–genotype correlation in children with familial Mediterranean fever. Rheumatol Int. 2021;41(1):113-20.
2. Padeh S, Bilginer Y, Ozen S. Familial mediterranean fever. Textbook of Autoinflammation. 2019:293-313.
3. Coşku S, Kurtgöz S, Keskin E, Sönmez F, Bozkurt G. Frequency of mutations in Mediterranean fever gene, with gender and genotype–phenotype correlations in a Turkish population. J Genet. 2015;94:629-35.
4. Kunt SŞ, Aydın F, Çakar N, Özdel S, Yalçınkaya F, Özçakar ZB. The effect of genotype on musculoskeletal complaints in patients with familial Mediterranean fever. Postgrad Med. 2020;132(2):220-4.
5. Ozdogan H, Ugurlu S. Familial mediterranean fever. La Presse Médicale. 2019;48(1):e61-e76.
6. Barut K, Sahin S, Adrovic A, et al. Familial Mediterranean fever in childhood: a single-center experience. Rheumatol Int. 2018;38:67-74.
7. Gangemi S, Manti S, Procopio V, et al. Lack of clear and univocal genotype-phenotype correlation in familial Mediterranean fever patients: A systematic review. Clin Genet. 2018;94(1):81-94.
8. Yalçınkaya F, Özen S, Özçakar ZB, et al. A new set of criteria for the diagnosis of familial Mediterranean fever in childhood. Rheumatology. 2009;48(4):395-8.
9. Demirkaya E, Acikel C, Hashkes P, et al. Development and initial validation of international severity scoring system for familial Mediterranean fever (ISSF). Ann Rheum Dis. 2016;75(6):1051-6.
10. Özdel S, Özçakar ZB, Kunt SŞ, Elhan AH, Yalçınkaya F. Late-onset disease is associated with a mild phenotype in children with familial Mediterranean fever. Clin Rheumatol. 2016;35:1837-40.
11. Caglayan AO, Demiryilmaz F, Ozyazgan I, Gumus H. MEFV gene compound heterozygous mutations in familial Mediterranean fever phenotype: a retrospective clinical and molecular study. Nephrol Dial Transplant. 2010;25(8):2520-3.
12. Balta B, Erdogan M, Kiraz A, Akalın T, Baştug F, Bayram A. A comprehensive molecular analysis and genotype–phenotype correlation in patients with familial mediterranean fever. Mol Biol Rep. 2020;47:1835-43.
13. Tunca M, Ozdogan H, Kasapcopur O, et al. Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. Medicine (Baltimore). 2005;84(1):1-11.
14. Yaşar Bilge NŞ, Sari I, Solmaz D, et al. The distribution of MEFV mutations in Turkish FMF patients: multicenter study representing results of Anatolia. Turk J Med Sci. 2019; 49(2), 472-477..
15. Ece A, Çakmak E, Uluca Ü, et al. The MEFV mutations and their clinical correlations in children with familial Mediterranean fever in southeast Turkey. Rheumatol Int. 2014;34:207-12.
16. Jarjour RA. Familial Mediterranean fever in Syrian patients: MEFV gene mutations and genotype–phenotype correlation. Mol Biol Rep. 2010;37:1-5.
17. Giancane G, Ter Haar NM, Wulffraat N, et al. Evidence-based recommendations for genetic diagnosis of familial Mediterranean fever. Ann Rheum Dis. 2015;74(4):635-41.
18. Kilic A, Varkal MA, Durmus MS, et al. Relationship between clinical findings and genetic mutations in patients with familial Mediterranean fever. Pediatr Rheumatol. 2015;13(1):1-9.
19. Ozcan G, Cayci S, Celikel Acar B, et al. Is the performance of the international severity scoring system for familial mediterranean fever in children better than other scoring systems? Int J Clin Pract. 2021;75(11):e14678.
20. Bilge NŞY, Bodakçi E, Bilgin M, Kaşifoğlu T. Comparison of clinical features in FMF patients according to severity scores: An analysis with the ISSF scoring system. Eur J Rheumatol. 2020;7(2):68.
21. Sönmez HE, Esmeray P, Batu ED, et al. Is age associated with disease severity and compliance to treatment in children with familial Mediterranean fever? Rheumatol Int. 2019;39:83-7.
22. Tanatar A, Karadağ ŞG, Çakan M, Sönmez HE, Ayaz NA. Age of onset as an influencing factor for disease severity in children with familial Mediterranean fever. Mod Rheumatol. 2021;31(1):219-22.