ORIGINAL ARTICLE

E148Q as a Familial Mediterranean Fever-causing Mutation: A Clinical-based Study

RA Jarjour

ABSTRACT

Objective: *To evaluate the clinical implications of E148Q mutation in familial Mediterranean fever (FMF) patients and compare it with other FMF-causing mutations.*

Methods: *The clinical features of 137 FMF patients with E148Q have been evaluated. Moreover, the clinical features of those patients have been compared with the clinical symptoms of FMF patients with other mutations.*

Results: *The clinical features of FMF in the patients with E148Q in this study are not different from those we previously reported in FMF patients with different* MEFV *mutations. However, there is a clear difference in terms of severity between the E148Q patients and those with other mutations. The data from this study show that E148Q causes mild symptoms of FMF, while other* MEFV *mutations are associated with the severe form of FMF. There were no significant statistical differences between patients with homozygote E148Q mutation, compound heterozygote or heterozygote.*

Conclusion: *This study showed that E148Q variant is associated with FMF. Patients who are heterozygous or homozygous for E148Q should not be ignored and should be followed up and treated liked other FMF patients. The main aim of this study was to evaluate the clinical implications of E148Q mutation in FMF patients and compare it with other FMF-causing mutations.*

Keywords: E148Q mutation, familial Mediterranean fever.

INTRODUCTION

Familial Mediterranean fever (FMF) (OMIM #249100) is a genetic disease with autosomal-recessive inheritance. The disease most commonly occurs in Arabs, Jews, Armenians and Turks (1). Familial Mediterranean fever is clinically characterized by recurrent and self-limited attacks of fever accompanied by peritonitis, pleuritis, synovitis or erysipelas-like erythema and may be complicated by AA amyloidosis. Colchicine was found to be very effective in preventing attacks of FMF and in the development of amyloidosis (2).

The responsible gene, *MEFV*, has been mapped to chromosome 16p13.3 and consists of 10 exons and encodes a 781 amino acid protein called Pyrin which is expressed in granulocytes and is thought to be a negative regulator of inflammation. Since the cloning of the *MEFV* gene (3, 4), about 244 sequence variants for *MEFV* have been described (5). Five founding mutations M694V, V726A, M680I, M694I (in exon 10) and E148Q (in exon 2) are the most frequently encountered mutations and account for 74% of FMF mutations in typical patients (6). In Syrian FMF patients, the most frequent mutation is M694V (36.5%), followed by V726A (15.2%), E148Q (14.5%), M680I (G/C) (13.2%) and M694I (10.2%) mutations (7).

The clinical implications of different *MEFV* mutations (*eg* M694V, M694I and M680I (G>C)) in FMF have been clearly implicated (3, 4, 7). However, the clinical implication of E148Q mutation is still controversial. Initially, E148Q was described as a disease causing mutation with low penetrance and mild symptoms (8, 9), but in more recent studies some investigators found a

From: Clinical Genetics Unit, Molecular Biology and Biotechnology Department, Atomic Energy Commission of Syria (AECS), Damascus, Syria.

Correspondence: Dr R Jarjour, Clinical Genetics Unit, Molecular Biology and Biotechnology Department, Atomic Energy Commission of Syria (AECS), PO Box 6091, Damascus, Syria. Email: ascientific@aec.org.sy

DOI: 10.7727/wimj.2016.162

similar frequency of E148Q among patients and controls and therefore suggested that it is no more than a benign polymorphism (10, 11). The carrier frequency of E148Q is 7.93% in Syrians (Rami Jarjour and Rami Abou Jamra, unpublished data), 5% in Lebanese (12), 12% in Turks (13) and 10% in Ashkenazi Jews (14).

The main aim of this study was to evaluate the clinical implications of E148Q mutation in FMF patients and compare it with other FMF-causing mutations.

SUBJECTS AND METHODS

A total of 787 Syrian FMF patients were referred to the Clinical Genetics Unit, Atomic Energy Commission of Syria (AECS), Damascus, Syria, for FMF mutation detection and genetic counseling between April 2005 and December 2011. All the patients were of Syrian Arab origin. The following *MEFV* mutations were investigated: E148Q in exon 2, P369S in exon 3, F479L in exon 5, and M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, and R761H in exon 10. A clinical diagnosis of FMF was made by the author according to the international FMF criteria (15).

Out of the 787 FMF patients, records of 137 unrelated FMF patients with E148Q mutation were retrospectively reviewed. The disease severity was evaluated according to the previously described criteria (16) and assigned to 1 of 3 severity levels (mild, intermediate or severe). Every patient was informed about the study, and a written consent was signed either by the patient or his/her parent for blood sampling. This study has been approved by the Institutional Review Board of the AECS.

For all patients, EDTA blood was sampled and DNA was isolated from frozen blood samples by the phenol-chlorophorm extraction method (17).

MEFV mutation analysis was performed for 12 mutations using a reverse-hybridization assay (FMF StripAssay™) according to the manufacturer's instructions (ViennaLab Labordiagnostika, Vienna, Austria).

Statistical analysis

Differences between groups were assessed using Z-test for two proportions and T-test for means. All p values were two-tailed, and confidence intervals were set at 95%. A p value < 0.05 was accepted as statistically significant.

RESULTS

Among the 137 FMF patients with E148Q mutation, 72 (52.6%) were males and 65 (47.4%) were females (female-to-male ratio was 0.9) (Table 1). A positive family history of FMF was observed in 54 (39.42%) of the patients. The mean age of onset of FMF in Syrian patients was 15.19 ± 10.63 years.

The main clinical characteristics of the patients were as follows (Table 1): peritonitis was observed in 95.62% of the patients, fever in 70.8%, arthritis in 8.03%, pleuritis in 36.5% and myalgia in 2.19%. None of the patients showed erysipelas-like erythema or pericarditis. None of the patients developed amyloidosis.

Table 1: Comparison of clinical features and FMF severity score between patients with E148Q and patients with other mutations*

	Group I E148Q (this study) (No. 137)	Group II Other mutations* (7) (No. 97)	Significance (Z test or T test)
Male/female	72/65	48/49	No
Age of onset (years)	15.19 ± 10.63	11.68 ± 10.11	Yes[a]
Abdominal pain	131 (95.62%)	88 (90.72%)	No
Nausea	72 (52.55%)	46 (47.42%)	No
Vomiting	64	47	No
Diarrhoea	40	34	No
Fever	97 (70.8%)	83 (85.57%)	Yes[b]
Chest pain	50 (36.5%)	16 (16.49%)	Yes[c]
Arthralgia	59	–	–
Arthritis	11 (8.03%)	31 (31.96%)	Yes[d]
Erysipelas-like erythema	0 (0%)	4 (4.12%)	No
Myalgia	3 (2.19%)	2 (2.06%)	No
Family history	54 (39.42%)	40 (41.24%)	No
Colchicine treatment	63	57	–
Response to colchicine	46 (73.02%)	46 (80.7%)	No
Attacks frequency/year	14.39 ± 11.77	17.42 ± 10.18	Yes[e]
Duration of attacks (days)	3.11 ± 2.23	3.42 ± 3.15	Yes[f]
Amyloidosis	0 (0%)	0 (0%)	No
Mean severity score			
Severe	11 (8.03%)	31 (31.96%)	Yes[g]
Intermediate	33 (24.09%)	32 (32.99%)	No
Mild	93 (67.88%)	31 (31.96%)	Yes[h]

[a]p value = 2.56; [b]Z value = 2.484; [c]Z value = 3.204; [d]Z value = 4.526; [e]Z value = 2.04; [f]Z value = 8.9; [g]Z value = 4.526; [h]Z value = 5.291.
* P369S, F479L, M680I (G/C), M680I (G/A), E148Q, I692del, M694V, M694I, K695R, V726A, A744S, and R761H (7).

Of the 137 patients, 63 (45.9%) were treated with colchicine and 46 (73.02%) responded with complete remission or significant improvement (reduction of number and severity of FMF episodes).

In a previous study, the author identified the frequency and distribution of 12 *MEFV* mutations in 97 Syrian patients and performed a genotype–phenotype correlation in the patients' cohort (7). The following *MEFV* mutations were investigated: E148Q in exon 2, P369S in exon 3, F479L in exon 5, and M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A,

Table 2: Clinical features of different subgroups according to the E148Q mutation

	Group (A) Homozygote E148Q/E148Q (No. 6)	Group (B) Compound heterozygote E148Q/+ (No. 49)	Group (C) Heterozygote E148Q/− (No. 82)	Significance		
				A and B	A and C	B and C
1—Age of onset (years)	11.33 ± 3.07	14.2 ± 11.02	16.08 ± 10.72	No	No	No
2—Abdominal pain	6	45	80	No	No	No
3—Nausea	5	22	45	No	No	No
4—Vomiting	5	23	36	No	No	No
5—Diarrhoea	1	17	22	No	No	No
6—Fever	6	39	52	No	No	No
7—Chest pain	3	17	30	No	No	No
8—Arthralgia	4	22	33	No	No	No
9—Arthritis	0	6	5	No	No	No
10—Erysipelas-like erythema	0	0	0	No	No	No
11—Myalgia	0	1	2	No	No	No
12—Family history	1	27	26	No	No	Yes[a]
13—Colchicine treatment	2	27	34	–	–	–
14—Response to colchicine	2	18	26	No	No	No
15—Attacks frequency/year	20.5 ± 12.53	16.43 ± 10.71	12.51 ± 12.23	No	No	No
16—Duration of attacks (days)	2.8 ± 1.16	3.02 ± 1.84	3.1 ± 2.51	No	No	No
17—Mean severity score						
Severe	1	4	6	No	No	No
Intermediate	2	16	7	No	No	Yes[b]
Mild	3	29	61	No	No	No

[a]Z value = 2.45; [b]Z value = 3.27.

A744S, and R761H in exon 10. The most frequent mutation was M694V (36.5%), followed by V726A (15.2%), E148Q (14.5%), M680I (G/C) (13.2%) and M694I (10.2%) mutations. Rare mutations (R761H, A744S, M680I (G/A), K695R, P369S, F479L, and I692del) were also detected in the patients. M694V was associated with the severe form of the disease.

In order to evaluate the clinical features of the patients in this study, the clinical features were compared to that in the previous study (Table 1). There were statistical significant differences between these two groups regarding age of onset, fever, arthritis, attacks frequency/year, and duration of attacks. However, there were no statistical significant differences between male-to-female ratio, abdominal pain, nausea, vomiting, diarrhoea, chest pain, erysipelas-like erythema, myalgia, family history, and response to colchicine.

The severity of the disease was assessed in all 137 patients (Table 1). There were statistically significance differences between the mean severity scores of this cohort and the previous cohort especially between the severely affected patients in both groups. Moreover, the mildly affected patients in both groups showed a statistically significant difference.

In order to compare the clinical features of the FMF patients with E148Q mutation, 137 patients were divided into three groups according to the presence of the E148Q mutation on both alleles (E148Q/E148Q) (homozygote) (group A), on one allele with other identified mutation (E148Q/+) (compound heterozygote) (group B) and on one allele without other identified mutation (E148Q/–) (heterozygote) (group C) (Table 2). There were no statistically significant differences between these three groups regarding age of onset, abdominal pain, nausea, vomiting, diarrhoea, fever, chest pain, arthralgia, arthritis, erysipelas-like erythema, myalgia, response to colchicines, attacks frequency/year, and duration of attacks. However, there were statistically significant differences between groups B and C regarding the family history and the severity score (intermediate).

DISCUSSION

E148Q mutation is one of the most common mutations in FMF patients. Initially, E148Q was described as a disease-causing *MEFV* mutation with low penetrance and mild symptoms (8, 9). Patients who are homozygous for E148Q have a heterogeneous clinical presentation and may be at risk of developing amyloidosis (18). However,

the role of E148Q in FMF is controversial. In more recent studies, some investigators found a similar frequency of E148Q among patients and controls and therefore suggested that it is no more than a benign polymorphism (10, 11). Others could not provide evidence that would support the notion that E148Q is a mutation or a polymorphism (19).

A recent study using a quantum chemistry model showed that the impact of the E148Q on the structure of pyrin is indeed low but not zero (20). This study concluded that the E148Q variation is associated with a mild effect on the structure and perhaps the function of the pyrin. Since there is no biochemical testing for FMF, the diagnosis of this genetic disease is still clinical. E148Q may have a potential role in other diseases. A recent study has shown that E148Q is a promising candidate risk factor for multiple sclerosis (21). E148Q is the most frequent mutation in patients with Behcet's disease (22, 23). In China, it has been shown that E148Q is associated with Henoch–Schönlein purpura (24). This study aimed to investigate the association between E148Q and FMF based on the clinical features of the patients carrying this mutation.

The clinical features of FMF in the patients with E148Q in this study (group I) are not different from those we previously reported in FMF patients with different *MEFV* mutations (group II) (7) (Table 1). Peritonitis was the most common symptom in this study (95.62%). Fever is the second most common symptom in this study (70.8%). However, there is a clear difference in terms of severity between the E148Q patients (group I) and those with other mutations (group II). The difference in severity score could be mainly explained by the presence of the M694V mutation in the other group (II) of FMF patients which is associated with severe form of the disease, early onset, high frequency of attacks, and higher frequencies of arthritis as we and others have previously shown (7, 25). The data from this study show that E148Q causes mild symptoms of FMF, while other *MEFV* mutations are associated with the severe form of FMF.

The rate of amyloidosis in the Syrian FMF patients is low (range 0%–5%) (7, 26). This might be due to the fact that these data were obtained after the introduction of colchicine as a recommended treatment for FMF. None of the 137 patients in this study had amyloidosis. However, FMF-associated amyloidosis in patients who are heterozygote for E148Q has been reported (27). It should be noted that there was no significant difference regarding response to colchicine treatment between groups I and II (Table 1). This notion is of great value as response to colchicine is one of the criteria of diagnosis of FMF (28). This suggests that E148Q might be a disease-causing mutation.

There were no significant statistical differences between patients with homozygote E148Q mutation (group A), compound heterozygote (group B) or heterozygote (group C) (Table 2). This shows that there were no differences between the homozygote patients (group A) and the other two groups (B and C) regarding the clinical features. Moreover, there was no difference between the heterozygote patients (group C) and the other two groups (A and B). Recently, it has been reported that a detection of a single *MEFV* mutation is sufficient in the presence of clinical symptoms for the diagnosis of FMF and the initiation of a trial of colchicines (29, 30). Moreover, a recent report has shown that the clinical picture of French heterozygote patients with recurrent fevers resembles that of homozygote patients. This report has also shown that E148Q accounts for 15% of those heterozygote patients (31). Our data are in agreement with what has been previously published. Therefore, the author suggests that patients who are heterozygous or homozygous for E148Q should be considered as FMF patients and treated with colchicine.

In conclusion, this study showed that E148Q variant is associated with FMF. Moreover, patients with E148Q should not be ignored and should be followed up and treated liked other FMF patients.

ACKNOWLEDGEMENTS

I would like to thank Mrs. Reem Dodaki and Mr. Rami Majdalawi for technical assistance. I would also like to thank the Director General of AECS, and Head of Department, for their support. This work was financially supported by Atomic Energy Commission of Syria (AECS).

AUTHORS' NOTE

The author has declared no conflicts of interest.

REFERENCES

1. Sohar E, Gafni J, Pras M, Heller H. Familial Mediterranean fever. A survey of 470 cases and review of the literature. Am J Med 1967; **43:** 227–53.
2. Lidar M, Livneh A. Familial Mediterranean fever: clinical, molecular and management advancements. Neth J Med 2007; **65:** 318–24.
3. Consortium TFF. A candidate gene for familial Mediterranean fever. Nat Genet 1997; **17:** 25–31.
4. Consortium TIF. Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. Cell 1997; **90:** 797–807.

5. Stenson PD, Ball EV, Howells K Phillips AD, Mort M, Cooper DN. The Human Gene Mutation Database: providing a comprehensive central mutation database for molecular diagnostics and personalized genomics. Hum Genomics 2009; **4:** 69–72.
6. Touitou I. Standardized testing for mutations in familial Mediterranean fever. Clin Chem 2003; **49:** 1781–2.
7. Jarjour RA. Familial Mediterranean fever in Syrian patients: MEFV gene mutations and genotype-phenotype correlation. Mol Biol Rep 2010; **37:** 1–5.
8. Aksentijevich I, Torosyan Y, Samuels J, Centola M, Pras E, Chae JJ et al. Mutation and haplotype studies of familial Mediterranean fever reveal new ancestral relationships and evidence for a high carrier frequency with reduced penetrance in the Ashkenazi Jewish population. Am J Hum Genet 1999; **64:** 949–62.
9. Bernot A, da Silva C, Petit JL, Cruaud C, Caloustian C, Castet V et al. Non-founder mutations in the MEFV gene establish this gene as the cause of familial Mediterranean fever (FMF). Hum Mol Genet 1998; **7:** 1317–25.
10. Ben-Chetrit E, Lerer I, Malamud E, Domingo C, Abeliovich D. The E148Q mutation in the MEFV gene: is it a disease-causing mutation or a sequence variant? Hum Mutat 2000; **15:** 385–6.
11. Tchernitchko D, Legendre M, Cazeneuve C, Delahaye A, Niel F, Amselem S. The E148Q MEFV allele is not implicated in the development of familial Mediterranean fever. Hum Mutat 2003; **22:** 339–40.
12. Medlej-Hashim M, Serre JL, Corbani S, Saab O, Jalkh N, Delague V et al. Familial Mediterranean fever (FMF) in Lebanon and Jordan: a population genetics study and report of three novel mutations. Eur J Med Genet 2005; **48:** 412–20.
13. Yilmaz E, Ozen S, Balci B, Duzova A, Topaloglu R, Besbas N et al. Mutation frequency of familial Mediterranean fever and evidence for a high carrier rate in the Turkish population. Eur J Hum Genet 2001; **9:** 553–5.
14. Stoffman N, Magal N, Shohat T, Lotan R, Koman S, Oron A et al. Higher than expected carrier rates for familial Mediterranean fever in various Jewish ethnic groups. Eur J Hum Genet 2000; **8:** 307–10.
15. Livneh A, Langevitz P, Zemer D, Zaks N, Kees S, Lidar T et al. Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum 1997; **40:** 1879–85.
16. Mor A, Shinar Y, Zaks N, Langevitz P, Chetrit A, Shtrasburg S et al. Evaluation of disease severity in familial Mediterranean fever. Semin Arthritis Rheum 2005; **35:** 57–64.
17. Sambrook J, Maniatis T, Fritsch EF. Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press; 1989.
18. Topaloglu R, Ozaltin F, Yilmaz E, Ozen S, Balci B, Besbas N et al. E148Q is a disease-causing MEFV mutation: a phenotypic evaluation in patients with familial Mediterranean fever. Ann Rheum Dis 2005; **64:** 750–2.
19. Marek-Yagel D, Bar-Joseph I, Pras E, Berkun Y. Is E148Q a benign polymorphism or a disease-causing mutation? J Rheumatol 2009; **36:** 2372.
20. Naimushin A, Lidar M, Ben Zvi I, Livneh A. The structural effect of the E148Q MEFV mutation on the pyrin protein: a study using a quantum chemistry model. Isr Med Assoc J 2011; **13:** 199–201.
21. Kuempfel T, Gerdes LA, Wacker T, Blaschek A, Havla J, Krumbholz M et al. Familial Mediterranean fever-associated mutation pyrin E148Q as a potential risk factor for multiple sclerosis. Mult Scler 2012; **18:** 1229–38.
22. Yazici A, Cefle A, Savli H. The frequency of MEFV gene mutations in Behcet's disease and their relation with clinical findings. Rheumatol Int 2012; **32:** 3025–30.
23. Ayesh S, Abu-Rmaileh H, Nassar S, Al-Shareef W, Abu-Libdeh B, Muhanna A et al. Molecular analysis of MEFV gene mutations among Palestinian patients with Behcet's disease. Scand J Rheumatol 2008; **37:** 370–4.
24. He X, Lu H, Kang S, Luan J, Liu Z, Yin W et al. MEFV E148Q polymorphism is associated with Henoch-Schonlein purpura in Chinese children. Pediatr Nephrol 2010; **25:** 2077–82.
25. Shohat M, Magal N, Shohat T, Chen X, Dagan T, Mimouni A et al. Phenotype-genotype correlation in familial Mediterranean fever: evidence for an association between Met694Val and amyloidosis. Eur J Hum Genet 1999; **7:** 287–92.
26. Mattit H, Joma M, Al-Cheikh S, El-Khateeb M, Medlej-Hashim M, Salem N et al. Familial Mediterranean fever in the Syrian population: gene mutation frequencies, carrier rates and phenotype-genotype correlation. Eur J Med Genet 2006; **49:** 481–6.
27. Akar N, Akar E, Yalcinkaya F. E148Q of the MEFV gene causes amyloidosis in familial Mediterranean fever patients. Pediatrics 2001; **108:** 215.
28. Ben-Chetrit E, Sagi M. Genetic counselling in familial Mediterranean fever: has the time come? Rheumatology 2001; **40:** 606–9.
29. Booty MG, Chae JJ, Masters SL, Remmers EF, Barham B, Le JM et al. Familial Mediterranean fever with a single MEFV mutation: where is the second hit? Arthritis Rheum 2009; **60:** 1851–61.
30. Marek-Yagel D, Berkun Y, Padeh S, Abu A, Reznik-Wolf H, Livneh A et al. Clinical disease among patients heterozygous for familial Mediterranean fever. Arthritis Rheum 2009; **60:** 1862–6.
31. Kone-Paut I, Hentgen V, Guillaume-Czitrom S, Compeyrot-Lacassagne S, Tran TA, Touitou I. The clinical spectrum of 94 patients carrying a single mutated MEFV allele. Rheumatology 2009; **48:** 840–2.