IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 19, Issue 3 Ser.8 (March. 2020), PP 23-27
www.iosrjournals.org

Evaluation of E148Q phenotype in patients with familial Mediterranean fever: a Moroccan case series

Mohamed Ahakoud[1], Hanane Sayel[1], Oussama Kettani[1], Tarik Sqalli Houssaini[2,4], MouniaLakhdar Idrissi[3,4], Fatima Zohra Souilmi[3,4], Laila Bouguenouch[1,2], Karim Ouldim[1,2]

Central Medical Genetics Unit, Hassan II University Hospital, Fez, Morocco[1]
nephrology department, Hassan II University Hospital, Fez, Morocco[2]
pediatrics department, Hassan II University Hospital, Fez, Morocco[3]
Faculty of Medicine and Pharmacy of Fez, Sidi Mohamed Ben Abdellah University, Fez, Morocco[4]

Abstract:
Background:*Mediterranean fever (FMF) is an autosomal recessive disease, characterized by recurrent short attacks of fever accompanied by abdominal pain, arthritis and pleuritis. It frequently occurs within Mediterranean populations, especially Armenians, Turks, Sephardic Jews and Arabs. The gene responsible for the FMF (MEFV), is located on 16p13.3 and it encodes a protein called pyrine.*
Objective: *To evaluate the phenotypic features of the patients with E148Q mutation.*
Subjects: *The MEFV gene of 5 patients is analyzed. 4 patients were found to be heterozygous for E148Q, and one compound heterozygous for E148Q.*
Results: *All of the five patients were symptomatic at the time of evaluation, fever was seen in 100% of patients, abdominal pain in 60% of the patients, arthralgia in 40%, arthritis in 20%. None of our patients had amyloidosis or a family history of amyloidosis. All the patients have a colchicine response.*
Conclusions: *Patients for E148Q have a heterogeneous clinical presentation. all are symptomatic and colchicine treatment is required in these patients.*
Key Word*: Familial Mediterranean fever, MEFV gene, renal amyloidosis Familial, E148Q*

Date of Submission: 01-03-2020 Date of Acceptance: 16-03-2020

I. Introduction

FMF (Familial Mediterranean Fever) is a hereditary disease transmitted as an autosomal recessive manifested by recurrent attacks of fever associated with inflammatory events affecting serous and articulation causing abdominal pain, chest and joints. Its major complication lies in the risk of occurrence of renal amyloidosis[1].

It is part of the disease called hereditary auto-inflammatory. FMF is the most common cause of hereditary recurrent fevers prevalent in individuals living in the Mediterranean region. The most affected are ethnic Armenian, Turkish, Sephardic Jews and Arabs, in which the frequency of the mutation in question is high[2].

The MEFV gene is the gene causing FMF disease, is located on chromosome 16 (16p13.3), and codes for a protein called pyrine[3] (figure 1), which is involved in the inflammatory response and apoptosis[4]. Over 300 MEFV sequence variations have been identified to date[5]. Five main mutations: M680I, M694V, M694I and V726A in exon 10 and E148Q in exon 2, are responsible for more than 85% of FMF cases[6].

The pre-symptomatic diagnosis of FMF is based on a bundle of clinical arguments and anamnestic elements, supported by the molecular study which consists in searching for mutations in the MEFV gene which helps to prevent complications of the disease.

Colchicine is the gold standard treatment aimed at controlling inflammatory attacks of FMF and preventing renal amyloidosis[7].

In Morocco this disease remains little known and under-diagnosed, sometimes to the point of kidney complications. In this work we propose to contribute to a better knowledge of the molecular pathology of the MEFV gene within the Central Medical Genetics Unit, Hassan II University Hospital of Fez, in order to evaluate the E148Q phenotype in our population.

Our objectives are thus defined in the continuous development of the genetic test for an adequate genetic counseling which is highly necessary for a better treatment of hereditary inflammatory pathologies in Morocco.

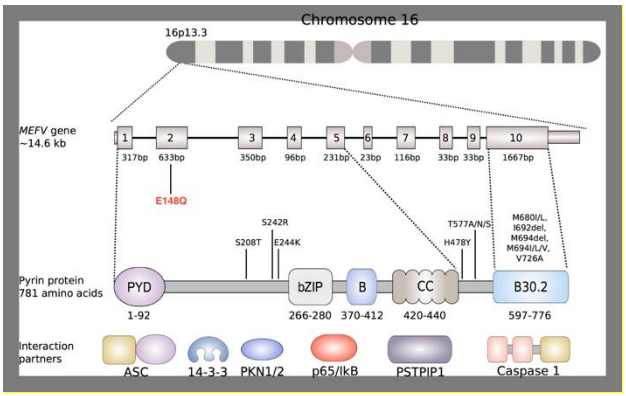

Fig 1: Schematic representation of the *MEFV* gene and the encoded pyrin protein. Of the more than 300 nucleotide variants described in the *MEFV* gene, only shown are the ones that are clearly associated to disease phenotypes. The most common FMF associated mutations reside in exon 10, that encodes the B30.2 domain (B30.2).[3]

II. Material And Methods

- **Patient population:**

This was a retrospective case series of five patients in who FMF was suspected on a clinical basis according to the Tel-Hashomer criteria and referred to the unit of medical genetics and onco-genetic.Informed consent becomes given by way of all patients. All of the patients had definite FMF according to the Tel Hashomer criteria[8].

Briefly, the aggregate of recurrent attacks of fever observed by using peritonitis, pleuritis, or arthritis that responded to colchicine is considered precise disease. In order to qualify as assembly the criteria, the fever and the abdominal pain needed to ultimate for as a minimum 12 hours (usually, 12–72 hours), and the abdominal pain needed to be severe enough that the patient turned into unable to get out of bed without assistance or that peritoneal symptoms had been elicited on physical examination.

Both parents of the patients were North-African Arabs originating from Morocco. Clinical details have been taken from the patients' clinical records.

- **Mutation search in MEFV :**

From each study participant, 5 ml of blood was drawn into tubes containing EDTA, and DNA was extracted using a commercial kit (The PureLink® Genomic DNA Mini Kit).

DNA amplifications were performed in a 25µl reaction volume containing 100 ng of DNA using the After a preliminary denaturation at 95°C for three minutes, 35 cycles have been performed (95°C for 30 seconds, 62°C for the Exon 2 for 30 seconds, and 72°C for 60 seconds), followed by a final extension at 72°C for 9 minutes.

Primers used are : Exon 2 was analyzed using primers F: 5′-ATC TTG GGC CCT AAA CGT GG-3′ and R: 5′-TCC TTC AGG TCC GCA GAT GC-3′.

Sequencing was performed using Applied Biosystems® 3500Dx Genetic Analyzer.

III. Result

- **Demographic Characteristics :**

The age of the studied population ranged from 5 to 28 years, with a mean of 15.1 years. The sex-ratio was 0,6. A family history of FMF wasn't present in all patientsand two patients (40%) admitted parental consanguinity.Consanguineous marriages were present in about 21% of the patients. As expected, for recessive disorders, this rate is higher than the consanguinity rate in the Moroccan general population reported by Jaouad et al.[9].

- **Clinical Manifestations and Treatment :**

The most prominent features were fever which were present in all of the patients. Abdominal pain present in 60% of patients. Arthritis and erysipelas-like erythema were rare. Disease symptoms were mild in most of the patients, and amyloidosis wasn't found. A favorable response to colchicine treatment was noted in all patients (100%).

Clinical details of the mutated patients are provided in Table 1.

Table 1 : Characteristics of the patients.

Patients	**Age**	Sex	Consanguinity	**Fever**	Abdominal Pain	Joint Pain	Chest Pain	Response to Colchicine	**Family** History	Mutation
1	10	F	1er degrees	+	-	-	-	Favorable	-	E148Q / M694V
2	13	F	1er degrees	+	+	-	-	Favorable	-	E148Q / -
3	5	M	-	+	-	-	-	Favorable	-	E148Q / -
4	28	M	-	+	+	+	-	Favorable	-	E148Q / -
5	10	F	-	+	+	+	-	Favorable	-	E148Q / -

- **Genetic Features :**

The Exon 2 of the MEFV gene in 5 patients is analyzed. four patients were found to be heterozygous for E148Q, and one compound heterozygous for E148Q.

Status of mutations	*Genotype*	*Number*
heterozygous	***E148Q/-***	***4***
Compound heterozygous	***E148Q / M694V***	***1***
	Total patients with mutations	***5***

Table 2 : Genotypes distribution of FMF gene in 5 Moroccans patients.

IV. Discussionand Conclusion

As a Mediterranean population, Maghrebians are particularly vulnerable to develop FMF. The carrier frequency of the disease in North Africa was estimated as 1%[10].

The clinical features of FMF in the studied group differ somewhat from those of other populations of the Mediterranean basin. Fever is the most frequent symptom, within which arthritis present at a similar frequency in other ethnic groups[2]. The low rate of amyloidosis in Arabs is similar to that we reported [11].

Since the MEFV gene was cloned and four missense mutations were identified in exon 10, several other mutations have been identified in exons 1, 3, 5, and 9 of the gene [1,12,13].

One of those mutations, we have the E148Q mutation, which results in the replacement of the amino-acid glutamine for glutamic acid in exon 2 at codon 148 [14,15]. It is more frequent inside the general population than in FMF patients inside the regions wherein FMF is prevalent, and it also occurs in geographically and ethnically various populations [13].

Because of the discrepancy between the prevalence of this mutation in FMF patients and in the healthy population, it is believed by far to be a polymorphism in place of a pathogenic mutation and its miles have claimed that homozygosity for E148Q is not sufficient to develop clinical disease [16–20].

Further, its miles stated that homozygosity for E148Q can also affect susceptibility to polygenic conditions consisting of AA amyloidosis and Behçet's disorder among patients with non-FMF periodic fever [21,22].

In our series from Morocco, the E148Q mutation has a low frequency among patients with FMF, with 6.5% allele. While this may suggest a low penetrance of the mutation, we observed symptomatic patients not only among those compound heterozygotes but also in cases heterozygous for E148Q. We had four patients who were heterozygous for E148Q. They all had a good response to colchicine. Clinical heterogeneity is observed in all patients, ranging from isolate fever to severe symptoms [23].

It could be suggested that we saw only patients with symptoms and that a large portion of the asymptomatic homozygotes do not present to a medical center.

On the other hand, other genetic modifiers that may link to E148Q and which are as yet unknown may play a role in enhancing the expression of the disease in the symptomatic homozygotes. The heterogeneity of the clinical picture is not limited to the E148Q mutation; it may be seen in homozygotes for other mutations, including M694V [24]. Furthermore, individuals with two MEFV mutations may present with three different clinical pictures [25]:

- Phenotype I: overt FMF, which includes patients with a wide range of manifestations and mutations;
- Phenotype II: isolated amyloidosis which includes patients with this as the sole or first manifestation of the disease;
- Phenotype III: subclinical or preclinical FMF which includes all patients clinically unaffected with two MEFV mutations.

In terms of phenotypic features there were no differences between the heterozygous and the compound heterozygous cases.

None of our five patients had amyloidosis at the time of evaluation, but FMF associated amyloidosis in patients who are heterozygous for E148Q and in a patient homozygous for both E148Q and V726A (E148Q V726A/E148Q V726A) has been reported [26,27].

Although most of the previous research showed that M694V changed into the leading mutation for the hazard of growing amyloidosis, the patients with mutations aside from M694V are also prone to this complication, and some other determinants inclusive of environmental factors and modifier genes can also have additional effects [28,29]. (Table 3)

Furthermore, the severity of the disease route does not continuously parallel the development of amyloidosis. On the basis of this information, one can't exclude the possibility that patients with E148Q may be at risk of growing FMF associated amyloidosis. Thus, our results do no longer completely rule out the opportunity that the E148Q mutation has an upregulating effect on infection in each FMF and other continual inflammatory processes.

We accept as true with that symptomatic those who are heterozygous for E148Q should no longer be ignored. As we discovered clinical heterogeneity and an excessive frequency of symptoms, our view is that symptomatic patients require colchicine treatment until as but undetermined modifier genes or environmental elements are found, especially in the regions wherein the disease is prevalent.

Ethnic groups (number of patients)	Mutations(%)					Refs.
	M694V	M694I	A744S	M680L	E148Q	
Algeria(85)	5	80	3	0	9	[10]
Tunisians(139)	27	13	3	0	18	[30]
Syrians(242)	45.8	4.8	1.2	-	6	[31]
Turks(1090)	52	4	-	-	4	[32]
Armenians(163)	45	-	-	-	2	[33]
Moroccans(120)	47	32	6.5	4	6.5	[34]

Table 3 : Frequency of Familial Mediterranean Fever gene mutations in Moroccan patients and comparisonwithfinding in otherethnic groups.

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Mohamed Ahakoud, etal. "Evaluation of E148Q phenotype in patients with familial Mediterranean fever: a Moroccan case series."*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 19(3), 2020, pp. 23-27.