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E148Q IS a DISEASE CAUSING MEFV MUTATION: A PHENOTYPIC EVALUATION in PATIENTS with FAMILIAL MEDITERRANEAN FEVER

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ABSTRACT
FMF is one of the periodic fever syndromes, and is extremely common among Turks, Jews, Arabs and Armenians. After identifying the first four mutations several other mutations including E148Q have been identified in the MEFV gene as the disease causing mutation. The E148Q mutation has been suggested to be the mildest mutation and some reports questioned its disease association. This prompted us to evaluate the phenotypic features of the patients with E148Q mutation. Twenty six patients homozygous for E148Q, 10 compound heterozygous for E148Q and 8 complex cases were assessed. Although 4 of 26 patients with E148Q/E148Q were asymptomatic at the time of evaluation, abdominal pain was seen in 77% of the patients, fever in 66%, arthralgia in 50%, arthritis in 15.4% and vomiting in 23.8%. Compound heterozygous and complex cases for E148Q had a higher frequency of abdominal pain, fever, arthralgia, arthritis myalgia and chest pain than patients homozygous for E148Q, but none of them reached statistical significance. None of our patients had amyloidosis but 2 of the patients with E148Q/E148Q had a family history of amyloidosis and one had rapidly progressive glomerulonephritis secondary to vasculitis, which progressed to chronic renal failure. Thus patients homozygous for E148Q may have a heterogeneous clinical presentation, majority of them were symptomatic and colchicine treatment was required in these patients.

INTRODUCTION

Familial Mediterranean Fever (FMF) is an autosomal recessive disease affecting mainly Turks, Jews, Armenians and Arabs. The disease is characterized by recurrent short episodes of fever, sterile peritonitis, arthritis, and pleurisy. Attacks are accompanied by a
strong acute phase response and the most severe complication is the development of renal amyloidosis (1). The gene responsible for FMF, MEFV, was identified by positional cloning in 1997 (2,3). It is located on chromosome 16p13.3, comprises 10 exons and 781 codons. The product of MEFV gene, named pyrin/Marenostrin is expressed in polymorphonuclear cells and monocytes and it is proposed that it regulates inflammatory responses at the level of leukocyte cytoskeleton organization (4). Thirty-six mutations located in the MEFV gene have been identified so far, mostly in exon 10 followed by exons 2, 3, 5, 1 and 9. Four of five founder mutations, M694V, V726A, M680I and M694I are located in exon 10 and E148Q in exon 2 (5). These founder mutations account for 74% of FMF chromosomes in typical patients from the Mediterranean basin (5). M694V is found to be the most common mutation in North African Jews with more than 70% allele frequency; V726A is predominant in Ashkenazi and Iraqi Jews, Armenians and Arabs (6). Allele frequencies in Turkey have been reported from 2 independent centers (7,8). Yalcinkaya et al (7) reported the allele frequencies in patients as follows: M694V 43.5%, M680I 13%, V726A 11.1% and M694I 2.8% for the four founder mutations, and most recently our group reported the 5 most common founder mutations frequencies as follows: M694V 51.5%, M680I 9.2%, E148Q 3.6%, V726A 2.9% and M694I 0.4% (8).

Carrier frequency of E148Q mutation has been reported to be 12% in Turks, 10% in Ashkenazi Jews, 6.4% in Jews of Moroccan origin and as high as 53% in Jews from the various ethnic groups (8, 10, 11). Because carrier frequencies were far more higher among health carriers than among patients with FMF, it has been proposed that E148Q is a polymorphism, not a disease causing mutation, and has a low penetrance (9-12). Since E148Q is one of the common mutations in Turkish patients with FMF, we intended to evaluate the phenotypic features of Turkish FMF patients with E148Q mutation in homozygous, heterozygous and complex states in order to shed light on the clinical presentation of this mutation.

**PATIENTS and METHODS**

Molecular diagnosis of FMF at our institution began in 1999 by screening the four most common MEFV mutations, followed by screening 14 MEFV mutations in exon 10 along
with E148Q in exon 2 in 2000. The patients with FMF diagnosed at our department and in whom MEFV mutation analysis was available between the years 2000 and 2003, were retrospectively screened. Forty-four of 2723 patients had E148Q mutation either homozygous or heterozygous and complex state and these 44 patients were enrolled in the evaluation. While twenty-six patients were homozygous for E148Q, 10 were compound heterozygous and 8 were complex. Four of 26 patients homozygous for E148Q, detected through family studies, were asymptomatic at the time of evaluation and remained asymptomatic during the follow-up for 2-4 years. None of them had colchicine prior to testing.

The diagnosis of FMF was established according to previously described criteria (1). These patients were seen again and the patient file was reviewed and updated by the same physician. Clinical information included age of onset, frequency of attacks prior to colchicine treatment, the presence of fever, abdominal pain, pleurisy, arthralgia, arthritis, response to colchicine, the family history of amyloidosis and the development of amyloidosis.

**Mutational analysis**

Molecular diagnosis of FMF in the patients was performed at our Medical Biology Department. The strategy for mutation analysis includes two steps. Exon 10 is first analyzed by denaturing gradient gel electrophoresis (DGGE). According to the band pattern, subsequent analysis is done either by restriction endonuclease enzyme digestion or genomic sequencing. E148Q in exon 2 is analyzed by restriction endonuclease enzyme digestion of PCR products from genomic DNA. The region harboring the mutation was amplified and the amplified products were digested with enzyme BstN1.

**Statistical analysis**

Results are given as median (min-max) for age and as percentage for the phenotypic features.

Comparisons between groups were carried out by using Mann-Whitney U test and $\chi^2$ test. p<0.05 was considered significant.

**RESULTS**

In the patients homozygous for E148Q the median age of onset was 6, male/female ratio was 1.6 (Table 1). Fever was seen in 64%, abdominal pain in 76.9%, arthralgia in 50%
and arthritis in 15.4% of these patients. None of them had erysipelas like lesions or scrotal pain but 23.8% had severe vomiting and 12.5% had diarrhea during the attack. None of them had amyloidosis but three had a family history of amyloidosis. One had rapidly progressive glomerulonephritis secondary to vasculitis and progressed to CRF. Currently she is on chronic hemodialysis program.

Of ten compound heterozygous, 6 were carrying E148Q/M694V, 3 E148Q/V726A and one E148Q/M680I. Of eight complex cases, 7 were carrying E148Q/E148Q/M694V and one E148Q/E148Q/M694I. The age of onset and male/female ratio were similar in the homozygous and compound/complex cases for E148Q (Table 1).

Table 1. Demographic Features of the Groups

<table>
<thead>
<tr>
<th>Phenotypic feature</th>
<th>E148Q/E148Q (n=26)</th>
<th>E148Q Compound &amp; Complex (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at onset (year)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>6 (3-15)</td>
<td>6.5 (2-16)</td>
</tr>
<tr>
<td><strong>Age at diagnosis (year)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>8.5 (3.5-16)</td>
<td>8.5 (3.5-19)</td>
</tr>
<tr>
<td><strong>Male/Female</strong></td>
<td>16/10</td>
<td>6/12</td>
</tr>
</tbody>
</table>

p>0.05 between the groups

Although the frequency of fever, abdominal pain, chest pain, arthralgia, arthritis and myalgia were higher in compound heterozygous and complex cases, none of the symptoms attained statistical significance compared to the patients homozygous for E148Q (Table 2 Fig.1). One of the complex cases had a history of acute rheumatic fever.

It was striking that severe vomiting was observed in around ¼ of the patients in both homozygous and compound/complex cases.

Table 2-Phenotypic features of patients

<table>
<thead>
<tr>
<th>Phenotypic feature</th>
<th>E148Q/E148Q (n=26) N(%)</th>
<th>E148Q/others E148Q/E148Q-others (n=18) N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>16 (64)</td>
<td>15 (83.3)</td>
</tr>
<tr>
<td>Condition</td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>20(80)</td>
<td>18(100)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>13(50)</td>
<td>7(38.9)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>4(15.4)</td>
<td>6(33.3)</td>
</tr>
<tr>
<td>Chest pain</td>
<td>6(23.1)</td>
<td>8(44.4)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>3(11.5)</td>
<td>4(22.2)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5(23.8)</td>
<td>4(22.2)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3(12.5)</td>
<td>2(11.1)</td>
</tr>
<tr>
<td>Family history of FMF</td>
<td>8(36.4)</td>
<td>7(38.9)</td>
</tr>
<tr>
<td>Family History of Amyloidosis</td>
<td>3(11.5)</td>
<td>3(16.6)</td>
</tr>
<tr>
<td>Appendectomy</td>
<td>3(11.5)</td>
<td>4(22.2)</td>
</tr>
</tbody>
</table>

### Frequency of attack (pre-Rx)

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4(15.4)</td>
<td>-</td>
</tr>
<tr>
<td>1-10/year</td>
<td>16(61.5)</td>
<td>9(52.9)</td>
</tr>
<tr>
<td>11-20/year</td>
<td>3(11.5)</td>
<td>3(17.6)</td>
</tr>
<tr>
<td>&gt;20/year</td>
<td>3(11.5)</td>
<td>5(29.4)</td>
</tr>
</tbody>
</table>

### Duration of attacks

<table>
<thead>
<tr>
<th>Duration</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4(15.4)</td>
<td>-</td>
</tr>
<tr>
<td>&lt;1 day</td>
<td>9(34.6)</td>
<td>3(17.6)</td>
</tr>
<tr>
<td>1-2 days</td>
<td>9(34.6)</td>
<td>10(58.8)</td>
</tr>
<tr>
<td>3-5 days</td>
<td>3(11.5)</td>
<td>3(17.6)</td>
</tr>
<tr>
<td>&gt;5 days</td>
<td>1(3.9)</td>
<td>1(5.9)</td>
</tr>
</tbody>
</table>

### Response to colchicine

<table>
<thead>
<tr>
<th>Response</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>16(72.8)</td>
<td>12(67)</td>
</tr>
<tr>
<td>Partial</td>
<td>3(13.6)</td>
<td>2(11)</td>
</tr>
<tr>
<td>No response</td>
<td>-</td>
<td>2(11)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3(13.6)</td>
<td>2(11)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Since the gene causing FMF (MEFV) was cloned and four missense mutations were identified in exon 10, several other mutations have been identified in exons 1,2,3,5,9 and 10 of the MEFV gene (2,3,5). One of them is the E148Q mutation, which results in the substitution of glutamine for glutamic acid at codon 148 in exon 2 (9,13). Thus, it is one...
of the most common mutations in patients with FMF; it is more frequent in the general population than in FMF patients in the regions where FMF is prevalent and it also occurs in geographically and ethnically diverse populations (5). As a result of the discrepancy between the prevalence of this mutation in FMF patients and in the healthy population it is thought to be a polymorphism rather than a disease causing mutation and it is claimed that homozygosity for E148Q is not sufficient for developing a clinical disease (10-12, 14,15). Further it is reported that it may influence susceptibility to polygenic conditions such as Behçet and AA amyloidosis among patients with non-FMF periodic fever (16,17).

In our series from Turkey, the E148Q mutation is the one of the common mutations among the patients with FMF with 3.5% allele frequency, the carrier frequency being found to be 12% in the healthy Turkish population. This may suggest the low penetrance of the mutation, we observed quite symptomatic patients not only in compound heterozygous or complex cases for E148Q but homozygous patients for E148Q as well.

In regard to the homozygous state for E148Q we had 26 patients. While 4 of them (%15) were as yet asymptomatic and detected through family studies, the remaining patients had typical FMF attacks. During the acute attacks their acute phase reactants were elevated and after an attack they returned to normal. They all gave good response to colchicine.

Clinical heterogeneity is observed in the patients homozygous for E148Q, ranging from absence of symptoms to severe symptoms (18). It may be suggested that we observed only the patients with symptoms and a large portion of the asymptomatic homozygotes do not present to a medical center. On the other hand other genetic modifiers which may link to E148Q and 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 only limited to the E148Q mutation, this may be seen in the homozygotes for other mutations including homozygotes for M694V (19). Furthermore individuals with two MEFV mutations may present in three different clinical pictures. 1) Phenotype I-overt FMF, which includes patients with a wide range of manifestations and mutations 2) phenotype II -isolated amyloidosis which includes patients with this as the sole or first manifestation of the disease 3) phenotype III- sub or pre clinical FMF which includes all
patients clinically unaffected with two MEFV mutations (6). Our 4 patients with no symptoms at the moment may be considered as Phenotype III. Moreover, in terms of phenotypic features there were no differences between the homozygous and compound heterozygous/complex cases for E148Q.

As to developing amyloidosis, none of our 44 patients had amyloidosis at the time of evaluation but FMF associated amyloidosis in patients heterozygous for E148Q and in a patient homozygous for both E148Q and V726A (E148Q V726A/ E148Q V726A) have been reported (16,17). Although most of the previous studies showed that M694V was the leading mutation of risk for developing amyloidosis, the patients with mutations other than M694V are also prone to this complication and some other determinants such as environmental factors and modifier genes may have additional effects (7,22).

Additionally the severity of disease course is not consistently parallel to development of amyloidosis. Based on this information, one can not exclude the possibility that the patients with E148Q may be at risk for developing FMF related amyloidosis.

Thus our results could not completely decline the propositions that E148Q mutation has an up regulating effect on inflammation both in FMF and in other chronic inflammatory processes. We think that the symptomatic individuals homozygous for E148Q should not be ignored.

Since we observed clinical heterogeneity but quite symptomatic patients, we think that symptomatic patients require colchicine treatment until as yet undetermined modifier genes or environmental factors are found especially in the areas where the disease is prevalent.

**Competing interests: None declared.**

**REFERENCES**


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Ann Rheum Dis published online September 30, 2004

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