Short Report

‘Silent’ carriage of two familial Mediterranean fever gene mutations in large families with only a single identified patient


The presence of two mutations in the familial Mediterranean fever gene, without overt familial Mediterranean fever (FMF), designated as phenotype III, predisposes to developing ‘silent’ AA amyloidosis, recognized as phenotype II, due to the absence of medical supervision and colchicine prophylaxis. We sought to determine the prevalence of phenotype III in large families with only one subject affected with FMF, in order to assess the population at risk for transformation to phenotype II. A total of seven large families were recruited for the study. Siblings were screened for \( \text{MEFV} \) mutations and underwent a clinical interview to assess for unrecognized FMF manifestations. Phenotype III, most commonly associated with a V726A/E148Q genotype, was detected in 10% of siblings of index cases from informative families, corresponding to a 10-fold increase in comparison to the expected rate in the general population (\( p < 0.01 \)). Unnoticed ‘FMF-like’ manifestations were detected among two siblings in the five families in which the index case was heterozygous, but in none of the siblings of the homozygous index cases. The enrichment for phenotype III and detection of occult FMF in large families, in which only a single member is afflicted with FMF, mandates routine clinical evaluation and genetic screening of siblings.

Conflict of interest

The authors declare no conflict of interest.

Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disorder, prevalent among populations of the Mediterranean basin, including Jews, Armenians, Turks and Arabs. The disease is characterized by recurrent, unprovoked attacks of fever, serositis and erysipelas-like skin rash, which abate with continuous colchicine therapy in the majority of afflicted individuals (1).

Reactive AA amyloidosis, caused by the extracellular deposition of amyloid fibrils, is considered the most severe complication of FMF, as it inevitably culminates in organ dysfunction, most prominent in the kidneys. Renal involvement due to AA amyloidosis initially manifests as proteinuria, and gradually progresses into nephrotic syndrome and renal dysfunction. Before the advent of colchicine therapy, amyloidosis was reported to occur in about 75% of FMF patients, over the age of 40 (2). Ethnicity, carriage of the M694V mutation, the SAA1 \( \alpha/\alpha \) genotype, male gender, joint attacks and a positive family history are all risk factors for amyloidosis (3, 4).

Uncommonly, amyloidosis may develop in individuals carrying two Familial Mediterranean fever gene (\( \text{MEFV} \)) mutations without overt clinical symptoms of FMF, a condition designated as phenotype II (5). Furthermore, two \( \text{MEFV} \) mutations may be harbored without signs or symptoms of FMF nor of reactive amyloidosis. This ‘silent’ homozygous or compound...
heterozygote state is termed phenotype III and is estimated to occur in 1:300 Ashkenazi and 1:25 Iraqi Jews (6). Putatively, these asymptomatic individuals, particularly those with a family history of FMF, are at risk of transforming into phenotype II due to lack of colchicine prophylaxis and of medical supervision. The aim of this study is to determine the presence and rate of phenotype III in large families in which only a single subject is identified as suffering from FMF.

**Patients and methods**

**Identification of large families**

A computerized search of the registry of the National Center for FMF, encompassing around 12,000 subjects, yielded 30 unrelated patients with at least 7 healthy siblings, aged 18 years or older. The size of the family was based on a recessive inheritance, by which one would expect a mean of two affected siblings per family. Successful contact was made with 20 of the 30 candidate index patients, 12 of whom were willing, together with all or most of their siblings, to participate in the study.

**Intervention**

After verification of the diagnosis of FMF in the index patients, according to the Tel Hashomer criteria (7), the patients and their siblings donated blood samples. These were screened for the three most common MEFV mutations in the Israeli-Jewish population (M694V, V726A and E148Q), using polymerase chain reaction amplification and restriction enzyme digestion, as previously described (8). In Arabic families, the five most common mutations in the population were studied (M694I and M680I, in addition to the three mentioned above), utilizing a similar technique. If only a single MEFV mutation was detected in the index patient, a complete exon 10 sequencing was performed, as previously described, in an attempt to reveal the second mutation (9). In addition, siblings were interviewed and the presence of obscure FMF manifestations was determined. The study was approved by the hospital’s institutional review board and all index patients and their siblings signed an informed consent for participation.

**Statistical analysis**

Differences between categorical variables were analyzed using the chi-squared test or Fisher’s exact test, according to the size of the cells examined. All tests of significance were two-tailed; p-values <0.05 were considered statistically significant.

**Results**

Twelve large families, including at least eight siblings, of whom only one is diagnosed with FMF, formed the study group. Of these 12 families, only 7 were informative for calculating the prevalence of phenotype III, as their index case was found to carry two MEFV mutations. In the other five index cases, only a single MEFV mutation was identified, even after exon 10 sequencing, rendering their families non-informative for phenotype III calculations. Fifty-two of 55 unaffected siblings, who were available for study from the informative families, 5 subjects (9.6%) were found to carry two MEFV mutations, without any clinical manifestations and were thus diagnosed as phenotype III of FMF (Table 1).

Out of a total of 87 clinically unaffected siblings in all 12 families, 5.7% (5/87) were found to express hitherto unnoticed periodic disease consistent with FMF. The genotypes and clinical manifestations of these newly diagnosed patients are detailed in Table 2.

The different genotypes associated with phenotype III are detailed in Table 3. The V726A/E148Q genotype appears to present mutations on two separate alleles (compound heterozygosity) (Fig. 1) and not on the same allele (complex allele), as might be found in the Druze population (10). Expectedly, the homozygous state of M694V was associated with full penetrance, that is to say, all who harbored this double mutation displayed

---

**Table 1. Prevalence of phenotype III**

<table>
<thead>
<tr>
<th>Family no.</th>
<th>Healthy siblings* (#)</th>
<th>Index case genotype</th>
<th>Siblings with two MEFV mutations (#)</th>
<th>Frequency of phenotype III in family (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>M694V/E148Q</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>V726A/E148Q</td>
<td>4</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>M694V/M694V</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>M694V/E148Q</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>M694V/M694V</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>E148Q/E148Q</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>V726A/V726A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td></td>
<td>5 Average (%): 9.6</td>
<td></td>
</tr>
</tbody>
</table>

*Included only siblings who agreed to be examined.

**Table 2. Genotype and clinical characteristics of hitherto occult ‘FMF-like’ disease**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Origin</th>
<th>Genotype</th>
<th>Clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>Iraq</td>
<td>M694V/0</td>
<td>Episodic arthritis without fever</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Iraq-Syria</td>
<td>M694V/0</td>
<td>Febrile abdominal attacks in childhood which spontaneously remitted in third decade</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Lybia</td>
<td>M694V/0</td>
<td>Afebrile abdominal attacks</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>Lybia</td>
<td>M694V/0</td>
<td>Afebrile abdominal attacks</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>Arab-Muslim</td>
<td>E148Q/0</td>
<td>Episodic arthritis without fever</td>
</tr>
</tbody>
</table>

F, female; M, male.

*Patients 3 and 4 are siblings.
Camus et al.

Table 3. Genotype associations of phenotype III

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of family members with Mediterranean fever (phenotype I)</th>
<th>Number of ‘healthy’ family members with two MEFV mutations (phenotype III)</th>
<th>Penetrance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M694V/E148Q</td>
<td>2</td>
<td>1</td>
<td>66</td>
</tr>
<tr>
<td>V726A/E148Q</td>
<td>1</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>M694V/V694V</td>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>V726A/V726A</td>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>E148Q/E148Q</td>
<td>1</td>
<td>1</td>
<td>50</td>
</tr>
</tbody>
</table>

A clinical phenotype. Also, the penetrance rate of the compound heterozygous state of M694V/E148Q was almost thrice that of V726A/E148Q. Unexpectedly, V726A homozygosity was also associated with 100% penetrance. However, this genotype was carried by only one family member.

Discussion

Our study shows that the frequency of phenotype III in families, in which only a single member has FMF is 10%, a significant enrichment compared to the general population, in which the rate is estimated at 1% (p < 0.01) (6). The enrichment in MEFV homozygosity or compound heterozygosity among large families with only a single recognized FMF patient validates the hypothesis underlying the current study, which states that screening for mutations should be actively undertaken in these families.

The importance of early diagnosis of individuals with phenotype III stems from their putative risk of transformation to phenotype II. Although the magnitude of such a transformation has not been formally evaluated, one may assume that it is negligible in the general population. However, as it has been shown that a family history of FMF increases one’s risk of developing amyloidosis (3, 4), it may be presumed that transformation to phenotype II will be more prevalent in phenotype III individuals who have a family history of the disease.

Furthermore, 5.7% of siblings evaluated, all of which were MEFV heterozygous mutation carriers, were found to suffer from not formerly suspected FMF or FMF-like disease, as they described symptoms compatible with FMF, although a rather mild, or incomplete form of the disease. Hence, one may suspect FMF in these siblings, initiate periodic follow-up and consider administering colchicine. Alternatively, especially in cases with only a single MEFV mutation, one may deduce that the clinical phenotype is not FMF but rather, a non-specific periodic clinical picture, associated with a variety of other inflammatory (e.g. incomplete form of Behçet’s disease or spondyloarthropathy) or autoinflammatory (e.g. cryopyrin-associated periodic syndrome; CAPS, Tumor-necrosis factor receptor type 1 (TNFR)-Associated Periodic Syndrome; TRAPS) diseases. However, based on the prevalence of these inflammatory diseases in our Jewish and Druze population and the family history of FMF, the option of incomplete FMF seems the most likely.

The concept of FMF symptom expression in patients heterozygous for an MEFV mutation, in a disease traditionally viewed as autosomal recessive, has gained attention over the past several years (11, 12). Leading to the realization that FMF may manifest in carriers of only one mutation, were exhaustive attempts, which failed to detect less common MEFV mutations as well as mutations in the non-coding regions and promoter region and other abnormal genetic mechanisms (12, 13). Why some heterozygous subjects display only sub-clinical inflammation, evident by elevated C-reactive protein and serum amyloid A levels, while others manifest clinical disease, and why some carriers experience non-specific and mild symptoms while others exhibit FMF manifestations in their entirety, still remains an enigma. It is assumed that the FMF genotype combined with other, as yet unrecognized, modifier genes and environmental factors determine MEFV mutation penetrance. The five ‘occult’ patients we had unveiled opted for clinical follow-up without colchicine therapy at present.

Our findings of genotype-dependent variable penetrance of MEFV mutations conform in general with previous observations (6), excluding that of V726A and E148Q. The role of the E148Q polymorphism as a disease-causing mutation has been the subject of debate (14, 15), suggesting that even if it has some effect, as claimed by several studies (8, 16), the 50% penetrance revealed in this study is higher than expected and could be incidental, as it is based on a single case.

Fig. 1. Family tree genotypes of family #2.
A similar reservation may apply to the 100% penetrance of the V726A/V726A genotype.

In summary, our study shows that phenotype III and unrecognized, mild FMF or ‘FMF-like’ disease are prevalent in siblings of identified FMF patients with respective rates of 10 and 5%. We suggest that individuals with phenotype III be followed in dedicated clinics for the development of FMF symptoms, asymptomatic proteinuria and/or elevation of inflammatory markers, all being indications for prompt initiation of colchicine therapy. Prospective follow-up will also enable us to determine the rate of transformation to phenotype II, which may serve as justification for publicly supported genetic screening of families.

Acknowledgements

This study was supported by a grant devoted to the memory of Johana Feler and her family members who perished in the Holocaust.

References