Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations


Objective. To prospectively monitor inflammatory activity over a prolonged period in a cohort of Turkish patients with FMF, their healthy relatives and controls and to relate this to their MEFV genotypes.

Methods. 43 patients with FMF and 75 of their asymptomatic relatives underwent fortnightly assessments and venesection for measurement of CRP and SAA over 5 months. 50 unrelated healthy population matched controls were also studied. MEFV genotyping was performed on all participants and comparisons were made between the different groups.

Results. Paired MEFV mutations were detected in 84% of FMF patients and single mutations in 12%. Substantial acute phase reactivity was seen among the patients with FMF during attacks (median SAA 693 mg/l, CRP 115 mg/l). Between attacks there was also some inflammatory activity (median SAA 6 mg/l, CRP 4 mg/l). Among healthy controls 16% were heterozygotes for MEFV mutations and 4% had two mutations. As expected there was a substantial carrier rate among healthy relatives with mutations detected in almost 92%. Asymptomatic MEFV heterozygotes had elevated acute phase proteins compared to wild type subjects.

Conclusion. Substantial sub-clinical inflammation occurs widely and over prolonged periods in patients with FMF, indicating that the relatively infrequent clinically overt attacks represent the ‘tip of the iceberg’ in this disorder. Both basal and peak acute phase protein concentrations were greater in MEFV heterozygotes than in wild-type controls, regardless of mutation demonstrating a ‘pro-inflammatory’ phenotype among FMF carriers. Uprogulation of the acute phase response among carriers of FMF may augment their innate host response and contribute to better resistance to infection.

Key words: Familial Mediterranean fever, MEFV, Heterozygote, Carrier state, Acute phase response, CRP, SAA, Turkey.

Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disease characterized by recurrent, self-limiting attacks of fever, and serositis including, in some individuals, arthritis or rash [1, 2]. Most patients present in childhood or as young adults with attacks that commonly last for less than 3 days and typically occur every few weeks or months [3]. FMF occurs worldwide and predominantly affects the populations arising from the Eastern Mediterranean basin, particularly the non-Ashkenazi Jews, Armenians, Turks, Cypriots and Levantine Arabs [4–8]. FMF is caused by various mutations in the gene MEFV [9, 10], which encodes a protein named pyrin/marenostrin and is chiefly expressed in neutrophils [11]. Variant forms of pyrin/marenostrin are thought to allow inappropriate triggering of neutrophil activation, giving rise to the apparently unprovoked short-lived bursts of systemic inflammation that are evident clinically in FMF [12, 13]. MEFV genotyping has contributed greatly to knowledge of FMF, but the diagnosis remains predominantly clinical [2, 14] since mutations are not always penetrant and cannot always be identified on both alleles [7, 15].

Clinical attacks of FMF are accompanied by a number of laboratory abnormalities, including a low-grade neutrophilia and an acute-phase plasma protein response [16], but the most responsive and dynamic acute-phase markers, serum amyloid A protein (SAA) and C-reactive protein (CRP), have not previously been investigated in depth. SAA is the circulating precursor of AA amyloid fibrils [17, 18], and sustained long-term elevation of SAA concentration is the only known prerequisite for the development of AA amyloidosis [19]. The behaviour of SAA over time is of interest in patients with FMF because the natural history of this disease includes a remarkably high incidence of AA amyloidosis. Prior to the discovery in 1972 that colchicine prophylaxis decreases clinical attacks and the risk of amyloid in most patients, up to 60% of patients with FMF died of amyloidosis [20–22], but even recently it has been reported in 12.9% of patients in a large series from Turkey [23]. Individuals can present with amyloidosis before developing symptoms of FMF, described as phenotype II [24], which, along with the observation that most patients with FMF are symptomatic for less than 40 days per year [25], had led to suggestions that the genesis of amyloid in this disorder may differ from that in other chronic inflammatory diseases [20, 26, 27].

In order to further characterize the relationship between specific mutations and the inflammatory activity that occurs in FMF, we performed a prospective study in which the acute-phase proteins SAA and CRP were monitored fortnightly over a prolonged period.
Table 1. *MEFV* genotyping in patients with familial Mediterranean fever, their asymptomatic first-degree relatives and healthy Turkish controls

<table>
<thead>
<tr>
<th><em>MEFV</em> mutation</th>
<th>Clinical FMF (n = 43)</th>
<th>Asymptomatic relatives (n = 73)</th>
<th>Healthy controls (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 10</td>
<td>Exon 2 No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>V726A</td>
<td>E148Q</td>
<td>2</td>
<td>4.7%</td>
</tr>
<tr>
<td>V726A</td>
<td>E148V</td>
<td>4</td>
<td>9.3%</td>
</tr>
<tr>
<td>M680I</td>
<td>E148Q</td>
<td>4</td>
<td>9.3%</td>
</tr>
<tr>
<td>M694V</td>
<td>E148Q</td>
<td>2</td>
<td>4.7%</td>
</tr>
<tr>
<td>M694V/M694V</td>
<td>E148Q</td>
<td>6</td>
<td>14%</td>
</tr>
<tr>
<td>M694V/V726A</td>
<td>E148Q</td>
<td>2</td>
<td>4.7%</td>
</tr>
<tr>
<td>M694V/R761H</td>
<td>E148Q</td>
<td>6</td>
<td>14%</td>
</tr>
<tr>
<td>M694V</td>
<td>E148Q</td>
<td>6</td>
<td>14%</td>
</tr>
<tr>
<td>M694V/R761H</td>
<td>E148Q</td>
<td>2</td>
<td>4.7%</td>
</tr>
<tr>
<td>K695R</td>
<td>V726A</td>
<td>1</td>
<td>2.3%</td>
</tr>
<tr>
<td>V726A/V726A</td>
<td>E148Q</td>
<td>2</td>
<td>4.7%</td>
</tr>
<tr>
<td>V726A/R761H</td>
<td>E148Q</td>
<td>2</td>
<td>4.7%</td>
</tr>
<tr>
<td>No mutation identified</td>
<td>2</td>
<td>4.7%</td>
<td>6</td>
</tr>
<tr>
<td>Single mutation</td>
<td>5</td>
<td>11.6%</td>
<td>58</td>
</tr>
<tr>
<td>Two or more mutations</td>
<td>36</td>
<td>83.7%</td>
<td>9</td>
</tr>
<tr>
<td>Two mutations in exon 10</td>
<td>30</td>
<td>69.8%</td>
<td>4</td>
</tr>
</tbody>
</table>
of significant inflammatory activity and no clinical attacks during the study period (hs-CRP, median 1.1 mg/l, maximum 2.6 mg/l); the other had levels of inflammation compatible with FMF patients with confirmed mutations (hs-CRP, median 16.1 mg/l, maximum 100.2 mg/l).

The allele frequency of MEFV mutations among the 73 asymptomatic parents or siblings of patients with FMF was 0.58. Fifty-eight (80%) of these subjects were heterozygotes, and, despite their lack of symptoms, nine (12%) individuals had paired mutations (Table 1).

Serial measurements of the acute-phase reactants SAA and hs-CRP in the 38 asymptomatic individuals who were MEFV wild-type gave values that were within the range reported in healthy control populations, in which 90% values of are less than 3 mg/l. The median SAA value was 2.2 mg/l and the median hs-CRP value was 1.3 mg/l. Occasional spikes of acute-phase activity were recorded in these individuals but only in the presence of reported intercurrent illness, such as upper respiratory tract infections. The maximum recorded values for SAA (277 mg/l) and hs-CRP (24.4 mg/l) occurred during a self-reported attack of ‘influenza’.

By contrast, substantial acute-phase activity was evident among the patients with FMF. Of the 43 patients, 14 (33%) had two or three FMF attacks during the study period, a further 14 had a single attack and 15 patients reported no FMF symptoms, although seven of these individuals reported episodes of mild ‘flu’-like symptoms that they attributed to viral illnesses. Both SAA and hs-CRP were massively elevated during all reported clinical attacks of FMF in all patients, with median values of 693 (range 140–1330) mg/l and 115 (range 26–296) mg/l, respectively. SAA and hs-CRP were also both elevated compared with the healthy control group even when these patients were free of FMF symptoms [median SAA 6.0 (range 0.7–1230) mg/l; median hs-CRP 4.0 (range 2.7–262) mg/l]. Even when the patients were asymptomatic, only 29% of SAA measurements in the FMF patients were less than 3 mg/l, i.e. within the normal range; 65% were less than 10 mg/l and 13% of SAA values exceeded 50 mg/l. Markedly elevated SAA values in eight individuals who reported no FMF or other symptoms throughout the study period (hs-CRP, median 1.1 mg/l, maximum 2.6 mg/l); the other had levels of inflammation compatible with FMF patients with confirmed mutations (hs-CRP, median 16.1 mg/l, maximum 100.2 mg/l).

The up-regulation of SAA and CRP production during health and intercurrent illness observed here over a prolonged time course in characterized MEFV heterozygotes confirms and extends previous observations on the phenotype of FMF carriers [33].

The degree and periodic pattern with which SAA was elevated in patients with FMF is significant for several reasons. Firstly, it may help to point towards this diagnosis, since very few disorders are associated with repeatedly high SAA values of more than 1000 mg/l [34]. Secondly, the SAA concentration was always very significantly elevated during symptomatic attacks of FMF, and therefore a lack of intense acute-phase response during symptoms of abdominal pain, pleurisy and fever, etc., in an individual known to have FMF should suggest an alternative aetiology. Thirdly, the amount of acute-phase SAA production in asymptomatic patients with FMF is sufficient to account for their susceptibility to the development of AA amyloidosis, solving the enigma of why such an apparently intermittent disorder or, in the case of phenotype II FMF, an entirely subclinical process, should lead to this life-threatening complication.

Substantial clinical experience has shown that regular prophylactic treatment with daily colchicine, at doses between 500 μg and 2.5 mg a day, inhibits attacks of FMF in two-thirds of patients [35], and prevents the development of AA amyloidosis in the vast majority of cases [36]. This treatment had been prescribed to all patients studied here, but the remarkable degree and intensity of acute-phase activity led us to question the participants’ compliance with the drug. Our suspicion was supported by experience that we have acquired through monitoring disease activity with monthly SAA measurements among FMF patients who have been under long-term follow-up in our centre in London, in whom we have observed SAA values of less than 10 mg/l on most occasions in most patients who assert that they are compliant with colchicine. On subsequent questioning after the present study had been completed, about half of the patients reported here admitted using colchicine either erratically or otherwise inappropriately. Since there is no risk of AA amyloidosis developing in individuals who do not have abnormal overproduction of SAA, frequent SAA measurements in patients with FMF may help to reinforce drug compliance, as well as providing objective evaluation of response and reassurance to the attending physician.

Discussion

This study has shown that substantial subclinical inflammation occurs widely and over prolonged periods in patients with FMF, indicating that the relatively infrequent clinically overt attacks represent the tip of the iceberg in this disorder. Although measurements of hs-CRP and SAA are well established to be the most sensitive and dynamic indicators of the acute-phase response, the magnitude of their elevation in active FMF, especially SAA, far exceeds values seen in most other chronic inflammatory diseases. The up-regulation of SAA and CRP production during health and intercurrent illness observed here over a prolonged time course in characterized MEFV heterozygotes confirms and extends previous observations on the phenotype of FMF carriers [33].

FIG. 1. Fortnightly SAA measurements, plotted on a logarithmic scale, in the eight FMF patients who remained completely without symptoms throughout the study period, showing substantial subclinical inflammatory activity. Thirteen per cent of all SAA values were greater than 50 mg/l. The median SAA value is indicated for each patient. Three patients were M694V/E148Q, including patient 7, who had very little evidence of inflammation. The other five patients all had two exon 10 mutations.
Other notable findings in this study included the results of MEFV genotyping. The sequencing method used was relatively comprehensive but would not detect intronic mutations or indeed regulatory proteins. We identified pairs of mutations in more than 80% of patients with FMF, and single mutations in all but 5% of the remainder. The frequency of mutated MEFV alleles in the healthy control group was 22%, and pairs of mutations known to be associated with FMF were present in two of these individuals and in 12% of the patients' apparently healthy relatives. In contrast, no mutations were identified in two FMF patients, emphasizing the limitations of genotyping in the diagnosis of this disorder and the need to use clinical criteria to make the diagnosis.

We have previously demonstrated that obligate carriers of FMF had increased levels of acute-phase reactants [37], and the combination of MEFV genotyping coupled with high-sensitivity acute-phase response measurements over several months reported here enabled us to confirm and further elucidate the pro-inflammatory phenotype among characterized heterozygous FMF carriers. Both basal and peak acute-phase protein concentrations were greater in heterozygous FMF carriers than in wild-type heterozygotes, performed on the acute-phase parameters of each individual.

<table>
<thead>
<tr>
<th>MEFV mutation</th>
<th>Median hs-CRP (mg/l)</th>
<th>Median SAA (mg/l)</th>
<th>Maximum hs-CRP (mg/l)</th>
<th>Maximum SAA (mg/l)</th>
<th>Median number of attacks per patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single exon 10 and an exon 2 mutation</td>
<td>2.7 (1.1–11.3)</td>
<td>10.5 (4.1–270)</td>
<td>7.9 (2.0–644)</td>
<td>1 (0–5)</td>
<td></td>
</tr>
<tr>
<td>Two exon 10 mutations (n = 30)</td>
<td>4.9 (1.2–45)</td>
<td>7.0 (1.0–192)</td>
<td>4.8 (1.8–129.7)</td>
<td>3 (0–2)</td>
<td></td>
</tr>
<tr>
<td>M694V and another exon 10 mutation (n = 13)</td>
<td>3.6 (1.2–11.3)</td>
<td>4.6 (1.0–23.2)</td>
<td>48.9 (1.8–129.7)</td>
<td>91.2 (1.7–1230)</td>
<td>1 (0–2)</td>
</tr>
<tr>
<td>M694V homozygotes (n = 6)</td>
<td>16.6 (4.4–137)</td>
<td>31.3 (15.1–162)</td>
<td>51.0 (26.6–152)</td>
<td>279 (136–1030)</td>
<td>1 (0–3)</td>
</tr>
</tbody>
</table>

The Mann-Whitney U-test was performed on the acute-phase parameters of each individual. Comparisons were made between groups: *P < 0.05, **P < 0.01 compared with wild-type controls; P < 0.05, **P < 0.01 compared with asymptomatic MEFV heterozygotes; *P < 0.05 comparing the all other FMF patient genotypes with M694V homozygotes.

The authors have declared no conflicts of interest.

References


