Familial Mediterranean Fever: Effects of Genotype and Ethnicity on Inflammatory Attacks and Amyloidosis

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ABSTRACT. Objective. The gene causing familial Mediterranean fever (FMF)—an autosomal recessive disease characterized by recurrent short episodes of fever associated most commonly with peritonitis, pleuritis, and arthritis—has recently been found and several mutations identified. The most severe complication of the disease is amyloidosis, which can lead to renal failure. The aim of this study was to investigate the role of genetic versus nongenetic factors on the phenotype as well as on the development of amyloidosis in FMF in a large and heterogeneous group of patients.

Methodology. We studied 382 patients from 4 ethnic origins living in different environments: North African Jews, other Jews, Turks, Armenians living in the United States, and Armenians from Yerevan, Armenia. Information regarding amyloidosis was available for 371 patients. We examined the association between the mutation M694V and the development of amyloidosis, and we also compared the clinical characteristics of the inflammatory attacks in patients from different ethnic origins, while controlling for the type of mutation.

Results. A significant association was found between amyloidosis and the most common mutation in exon 10 of the FMF gene (MEFV), M694V (for M694V homozygotes, relative risk = 1.77; 95% CI = 1.16–2.71). Amyloidosis was present in 44 of 171 homozygous FMF patients (25.7%), in 22 of 143 compound heterozygous FMF patients (15.4%), and in 7 of 57 patients carrying other mutations (12.5%). In homozygotes for M694V who had not been treated with colchicine before 20 years of age, the risk of amyloidosis developing before age was 61.0%. In our series, there were no cases of amyloidosis in 16 patients carrying the common mutation E148Q. We found that the type and severity of the FMF inflammatory symptoms were associated with both the genotype and the country of residence of the patient.

Conclusions. In the light of the high frequency of amyloidosis in homozygotes for the mutation M694V, colchicine treatment should be given to this group irrespective of the severity of the inflammatory attacks to prevent the development of amyloidosis. Our findings also suggest that factors other than genotype, such as environment or genes other than MEFV, play a role in the determination of the severity of the inflammatory attacks in FMF.

ABBREVIATIONS. FMF, familial Mediterranean fever; MEFV, Mediterranean fever (the FMF gene); PCR, polymerase chain reaction; CI, confidence interval.

Familial Mediterranean fever (FMF) is an autosomal recessive disease affecting primarily non-Ashkenazi Jews, Armenians, Turks, and Arabs.1 The carrier rate in these populations is very high, with estimates based on family studies as high as 1:5 to 1:7 among some non-Ashkenazi Jewish populations,2 and 1:7 among Armenians in California.3 The disease is characterized by recurrent short episodes of inflammation and serositis including fever, peritonitis, pleuritis, synovitis,1,3,4 and, rarely, pericarditis.3 The clinical symptoms can vary in different patients, sometimes even in the same family. Amyloidosis, similar to that seen in other chronic inflammatory diseases such as rheumatoid arthritis, is the most severe complication of FMF and leads to renal failure.

There is ethnic variability in the prevalence of amyloidosis, which occurs in 60% of the Turks, in 27% of the non-Ashkenazi Jews, and in 1% to 2% of the Armenians in the United States.6 In untreated Jewish patients of North African origin, the frequency of amyloidosis increases progressively with age, occurring in up to 75% of those over 43 years.7 Colchicine has been shown to be effective in preventing the attacks of FMF as well as the development of amyloidosis.8–10 Some individuals develop amyloidosis without having recurrent inflammatory episodes (FMF type 2).11 and therefore, identification of an amyloidosis-associated mutation will allow for directing prophylactic colchicine therapy to pre-symptomatic individuals who can greatly benefit from it.

Linkage between the gene responsible for FMF (MEFV) and the short arm of chromosome 16 was first shown in 1992,12 and locus homogeneity was first shown in 1992,12 and locus homogeneity was demonstrated for all ethnic groups studied.13 Recently, the gene causing FMF has been cloned,14,15 and 4 common missense mutations identified in exon...
10. More recently, additional mutations have been found in exons 2, 3, 5, and 10.16–19 The protein encoded by the gene, named pyrin14 or marenostrin,15 is a member of a family of nuclear factors homologous to the Ro52 autoantigen.

Previous phenotype/genotype correlation studies have shown conflicting results regarding the correlation between the severity of FMF and the mutation M694V.20–22 Two of these studies20,21 found that homozygosity for the mutation M694V was significantly associated with a more severe form of the disease, but they were both based on a population where it was not possible to differentiate between the effect of the mutation and the effect of the environment. The same studies have also reported conflicting and inconclusive results with regard to the association between homozygosity for M694V and amyloidosis21–24; however, they dealt with populations with insufficient variability of mutations or where most of the affected individuals had been treated.23,24

A previous study by us22 based on 138 affected individuals showed no significant phenotype-genotype correlation with regard to the inflammatory attacks, but did demonstrate a correlation between amyloidosis and the mutation M694V. Twenty out of the 138 affected individuals developed amyloidosis, but because of the small numbers involved, especially of those with amyloidosis, we could not make recommendations regarding treatment.

The aims of this study were to investigate in a large and heterogeneous group of patients the association between amyloidosis and the common mutations in MEFV, to compare the genotype to the phenotype in FMF patients before their commencing colchicine treatment, and to correlate the effect of ethnicity and environment (ie, country of residence) on the FMF attacks to determine clinical guidelines for molecular studies and treatment with colchicine.

METHODS

Subjects

We included in this study all 382 patients (314 probands and 68 siblings) diagnosed by us as having FMF. They were from 5 distinct groups living in 4 countries: 1) 131 North African Jews (from Morocco, Algeria, Tunisia, and Libya) living in Israel; 2) 51 Israeli Jewish patients of other ethnic origins (Ashkenazi, mixed Ashkenazi/other, and other); 3) 49 Turkish patients living in Turkey; 4) 29 Armenian patients living in California; and 5) 122 Israeli Jewish patients of other ethnic origins (Ashkenazi, mixed). The patients were ascertained in FMF clinics in the various countries (University of California, Los Angeles; FMF clinic in California; Rabin Medical Center in Israel; the Gata Medical Center in Ankara, Turkey; and the Emergency Medical Scientific Center in Yerevan, Armenia). For uniformity all the patients underwent clinical evaluation by the Israeli researchers (M.S., T.S., and M.K.) who examined the patients, reviewed the history, clinical symptoms and course of the disease of each patient, and completed the family history and pedigree.

Criteria for Diagnosis of FMF

The diagnosis of FMF in this study was based on the following criteria: 1) probands and/or sibs with 2 FMF-causing mutations in the MEFV gene (homozygotes or compound heterozygotes); and 2) probands found to carry only 1 known mutation but who demonstrated characteristic FMF symptoms that met the diagnostic criteria and who had no family history of FMF that could suggest a carrier state by descent. We believe that when more FMF mutations are discovered, these patients will be found to have 1 of these as yet undiscovered mutations.

The diagnosis of FMF in siblings when the proband was found to have 2 FMF mutations was based on the presence of these same 2 mutations in the sibling, and when the proband had only 1 known mutation, it was based on the demonstration of the sharing of 2 alleles with the proband, by studying polymorphic markers adjacent to the MEFV gene (D16S3070, D16S3370, D16S2617, and D16S5275).12 Asymptomatic individuals who were found to carry mutations in both alleles were considered affected.

Phenotype Parameters

The phenotype variables that were determined in all the affected individuals were age of onset of the inflammatory attacks, organs involved during the attacks (resulting in peritonitis, pleuritis, arthritis, or the presence of fever only), number of attacks before the commencement of colchicine treatment, age of diagnosis, and the presence and age of onset of amyloidosis. Age of commencement of colchicine treatment was also noted.

For several patients, information on some of the parameters of phenotype variables was missing. The number of attacks per year was considered missing when treatment with colchicine was initiated less than a year after the onset of the FMF symptoms (99 of 382), and the age of onset was considered missing when there was a problem with recall, usually in older patients (70 of 382). We excluded these patients when these parameters were analyzed.

Subjects With Amyloidosis

Information regarding amyloidosis was available in 371 patients. Amyloidosis was diagnosed in 73 of 371 patients and was the presenting symptom in 7 (FMF type 2). Of these 73 patients, 10 were North African Jews, 2 were other Jews (both of Iraqi origin), 3 were Armenians living in the United States, 47 were Armenians from Yerevan, and 11 were Turks.

Amyloidosis in the Armenians from Yerevan was diagnosed by rectal biopsy in 3 patients and by the presence of persistent proteinuria (>300 mg/dL) in 44 FMF patients—all whom were on dialysis at the time of the study. In the other ethnic groups, amyloidosis was diagnosed by rectal and/or renal biopsy. In all these cases, amyloidosis was diagnosed in previously untreated or inadequately treated patients.

Patient Genotype Groups

Affected individuals were assigned according to the FMF mutation to 1 of the following groups: group 1, homozygotes for the mutation M694V; group 2, compound heterozygotes for M694V and another mutation but not E148Q; group 3, homozygotes or compound heterozygotes for any 2 mutations other than M694V and E148Q; and group 4, patients with mutation E148Q.

Mutation Analysis

Mutation analysis was performed by genomic sequencing. Eleven mutations in exon 10 were tested—these were M680V, M680I, T681I, ΔI692, M694I, M694V, ΔI694, K695R, V726A, A744S, and R761H. We tested for these by polymerase chain reaction (PCR) amplification using the forward oligonucleotide 10F1: 5'-cagaagcaattcagtcgctc-3' and the reverse oligonucleotide 10R1: 5'-cagagctgtcgagtgatt-3'. The PCR amplification was performed under the following conditions: 95°C for 10 minutes followed by 30 cycles of 95°C for 15 seconds, 55°C for 30 seconds and 72°C for 30 seconds, and final extension at 72°C for 10 minutes. PCR products were purified with the High Pure PCR Product Purification kit (Boehringer, Mannheim, Germany) and sequenced directly, using specific primers and Thermo Sequenase kit (Amersham, Buckinghamshire, England).

Mutation E148Q in exon 2 was analyzed by restriction of PCR products from genomic DNA. The region harboring mutation E148Q was amplified using the forward oligonucleotide: 5'-gagcctgaagctttcaccggc-3' and the reverse oligonucleotide 5'-agggcttgagctctcttaccttc-3'. The amplified products were digested for 3 hours with 3 international units of BstNI. We did not test for mutations E167D, T267I, F479L, P369S, and R408Q.
Statistical Analysis
We compared the presence of amyloidosis among the 4 different genotype groups and the clinical characteristics of the patients with and without amyloidosis within each group.

All computations were performed using SAS statistical software (SAS, Cary, NC). Statistical analysis was performed using Student's t-test for 2-group comparisons of continuous variables and analysis of variance for comparisons of multiple groups. The $\chi^2$ test was used to test for the significance of the association between the clinical characteristics of FMF and the various mutations and ethnic origins. Fisher's exact test was performed to test for significance of the association when the sample size was small (expected counts in each cell $<5$). Fisher's exact test and the Monte Carlo test were used to test for the significance of the association between amyloidosis and the different genotypes. The effects of ethnicity and genotype on the characteristics of the FMF attacks (fever, abdominal pains, pleuritis, and arthritis) were studied by multiple regression analysis and odds ratio and 95% confidence intervals (CIs) were computed. The effects of ethnicity and genotype on the number of attacks, age of onset, and age at diagnosis of FMF were tested by a multiple linear regression model.

Because the inflammatory type of attack may change with age, it is possible that those groups with an earlier age of onset, diagnosis, and treatment may have a different pattern of symptoms. Therefore, we also tested for these variables, while controlling for the number of years since commencement of colchicine treatment.

This study was approved by the human subjects committees at the Rabin Medical Center, Beilinson Campus, Petah Tikva, Israel, and Cedars-Sinai Medical Center, Los Angeles, California, and informed consent was obtained from each participant.

RESULTS
For the analysis of the frequency of the mutations among the different ethnic groups we excluded the siblings. Table 1 depicts the distribution of the MEFV mutation groups among the 314 probands according to ethnic group. The frequency of homozygotes for the mutation M694V was highest among North African Jews (73.7%) compared with other ethnic groups. Interestingly, other Jews had a high frequency of the mutation E148Q.

Regarding individuals who were clinically affected but in whom only 1 known mutation had been identified, the number of unknown second mutations varied among the different ethnic groups. In North African Jews, the number was 8 of 160 alleles (5.0%); in other Jews, 18 of 92 alleles (19.6%); in Turks, 10 of 98 alleles (10.2%); in Armenians living in the United States, 3 of 38 alleles (7.9%); and in Armenians living in Yerevan, 41 of 240 alleles (17.1%).

<table>
<thead>
<tr>
<th>Mutation Group</th>
<th>North African Jews (n = 80)</th>
<th>Other (n = 46)</th>
<th>Turks (n = 49)</th>
<th>US Armenians (n = 19)</th>
<th>Yerevan Armenians (n = 120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M694V/M694V</td>
<td>73.7%</td>
<td>26.1%</td>
<td>42.3%</td>
<td>21.0%</td>
<td>29.6%</td>
</tr>
<tr>
<td>M694V/Other*</td>
<td>20%</td>
<td>41.3%</td>
<td>50%</td>
<td>52.6%</td>
<td>53.1%</td>
</tr>
<tr>
<td>Other*/Other*</td>
<td>0</td>
<td>17.4%</td>
<td>58.8%</td>
<td>26.4%</td>
<td>16.5%</td>
</tr>
<tr>
<td>Other*/Other*</td>
<td>6.3%</td>
<td>15.2%</td>
<td>1.9%</td>
<td>0</td>
<td>.8%</td>
</tr>
</tbody>
</table>

$\chi^2$ test was used to test for the significance of the association between the genotype M694V/M694V and amyloidosis (relative risk = 1.77; $P = .008$; 95% CI = 1.16–2.71). Amyloidosis was diagnosed in 44 (25.7%) of 171 homozygotes, and in only 7 (12.3%) of 57 patients who carried a mutation other than M694V. None of the 16 patients with mutation E148Q had amyloidosis. The association of amyloidosis with the M694V mutation was confirmed when analysis was performed separately on the group of Turkish patients ($P < .004$), other Jews ($P < .03$), and Armenian patients from Yerevan ($P < .009$) and from the United States ($P < .01$; Table 2).

Because FMF amyloidosis can be prevented by colchicine treatment, we tested whether the association between the mutation M694V and amyloidosis held in patients who had never received colchicine and developed amyloidosis before 20 years of age. The prevalence of amyloidosis developing before the 20 years of age in untreated homozygotes according to the mutation groups is shown in Table 3. Although 25 (61.0%) of 41 patients who were homozygous for the mutation M694V developed amyloidosis, the frequency of this complication in compound heterozygotes for M694V/other was 4.7% (2 of 43), and in individuals carrying the genotypes E148Q/other and other/other it was 21.0% (4 of 19; $P < .001$; Table 3). Amyloidosis was not diagnosed in any patients carrying the E148Q allele.

In addition, homozygotes for the mutation M694V were significantly more likely to develop amyloidosis at a younger age than patients with other genotypes. Thus, 25 (56.8%) of 44 patients who were homozygous for M694V and who developed amyloidosis were diagnosed by 20 years of age or earlier, compared with 2 of 20 compound heterozygotes for M694V/other and 4 of 7 individuals with the genotype other/other ($P = .001$).

Effect of the Genotype on the Inflammatory Attacks
Table 4 depicts the clinical characteristics of the FMF patients according to the ethnic groups and the genotype groups. After controlling for ethnic origin by linear regression analysis, we found that those patients who were homozygous for M694V (genotype group 1), compared with genotype groups 2 and 3, had a significantly younger age of onset (5.4 ± 5.7 years vs 9.1 ± 8.6 years and 9.5 ± 8.9 years, respectively; $P < .0001$), age at diagnosis (11.2 ± 9.9 years vs 18.5 ± 12.6 years and 17.7 ± 11.5 years, respectively; $P < .008$), and age at commencement of colchicine treatment (12.7 ± 9.8 years vs 23.1 ± 15.3 years and 19.3 ± 11.4 years, respectively; $P < .001$).

No significant differences were found among the genotype groups with regard to the number of attacks before commencement of colchicine treatment.

Table 5 depicts the type of inflammatory attack in FMF patients according to ethnic group and genotype group, and the results of the logistic analysis are shown in Table 6. The type of inflammation was

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TABLE 2. Association Between the Four Genotype Groups and Amyloidosis According to Ethnic Groups and Countries of Residence

<table>
<thead>
<tr>
<th>Mutation Group</th>
<th>M694V/M694V</th>
<th>M694V/Other</th>
<th>Other*/Other</th>
<th>E148Q/Other</th>
<th>Statistical Significance (P&lt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>North African Jews (n = 121)</td>
<td>9/96</td>
<td>1/20</td>
<td>0/0</td>
<td>0/5</td>
<td>.68</td>
</tr>
<tr>
<td>Other Jews (n = 51)</td>
<td>2/10</td>
<td>0/21</td>
<td>0/11</td>
<td>0/9</td>
<td>.03</td>
</tr>
<tr>
<td>Turks (n = 49)</td>
<td>9/21</td>
<td>2/24</td>
<td>0/3</td>
<td>0/1</td>
<td>.004</td>
</tr>
<tr>
<td>Armenians in United States (n = 29)</td>
<td>3/7</td>
<td>0/14</td>
<td>0/8</td>
<td>0/8</td>
<td>.01</td>
</tr>
<tr>
<td>Armenians in Yerevan (n = 121)</td>
<td>21/37</td>
<td>19/64</td>
<td>7/19</td>
<td>0/1</td>
<td>.009</td>
</tr>
<tr>
<td>Total (n = 371)</td>
<td>44/171</td>
<td>22/143</td>
<td>7/41</td>
<td>0/16</td>
<td>.008</td>
</tr>
</tbody>
</table>

* Other tested: M680V; M680I; V726A; M694I; ΔI692; K695R; A744S; R761H; T681I; and ΔM694.
Percentage of unknown second mutations: North African Jews (5.0%); other Jews (19.6%); Turks (10.2%); Armenians living in the United States (7.9%); and Armenians living in Yerevan (17.1%).
† Statistical comparison was performed with homozygotes for M694V and pooled data from the other 3 mutation groups.

TABLE 3. Association of Genotype and Ethnic Group With Onset of Amyloidosis Before 20 Years of Age in Untreated Patients

<table>
<thead>
<tr>
<th>Mutation Group</th>
<th>M694V/M694V</th>
<th>M694V/Other</th>
<th>Other*/Other</th>
<th>E148Q/Other</th>
<th>Statistical Significance (P&lt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>North African Jews</td>
<td>8/14</td>
<td>0/2</td>
<td>0/0</td>
<td>0/0</td>
<td>.47</td>
</tr>
<tr>
<td>Other Jews</td>
<td>2/2</td>
<td>0/3</td>
<td>0/3</td>
<td>0/1</td>
<td>.03</td>
</tr>
<tr>
<td>Turks</td>
<td>4/7</td>
<td>1/7</td>
<td>0/1</td>
<td>0/1</td>
<td>.10</td>
</tr>
<tr>
<td>Armenians in United States</td>
<td>3/6</td>
<td>0/9</td>
<td>0/3</td>
<td>0/0</td>
<td>.02</td>
</tr>
<tr>
<td>Armenians in Yerevan</td>
<td>8/12</td>
<td>1/22</td>
<td>3/9</td>
<td>0/1</td>
<td>.002</td>
</tr>
<tr>
<td>Total</td>
<td>25/41</td>
<td>2/43</td>
<td>3/16</td>
<td>0/3</td>
<td>.0001</td>
</tr>
</tbody>
</table>

* Other tested: M680V; M680I; V726A; M694I; ΔI692; K695R; A744S; R761H; T681I; and ΔM694.
Percentage of unknown second mutations: North African Jews (5.0%); other Jews (19.6%); Turks (10.2%); Armenians living in the United States (7.9%); and Armenians living in Yerevan (17.1%).
† Statistical significance between homozygotes for M694V and the other 3 mutation groups (M694V/other, E148Q/other, and other/other).
‡ These 4 were individuals with genotypes V726A/M680I (2 individuals), M680I/M680I (1 individual), and M680I/M694I (1 individual).

TABLE 4. Clinical Characteristics of FMF Patients According to Ethnicity, Country of Residence, and Genotype Groups

<table>
<thead>
<tr>
<th>Age of Onset</th>
<th>Age at Diagnosis</th>
<th>Age at Initiation of Colchicine</th>
<th>Number of Attacks per Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>North African Jews (n = 131)</td>
<td>4.7 ± 5.9</td>
<td>7.9 ± 8.7</td>
<td>8.8 ± 8.8</td>
</tr>
<tr>
<td>Other Jews (n = 51)</td>
<td>6.9 ± 8.5</td>
<td>8.3 ± 8.9</td>
<td>9.3 ± 10.5</td>
</tr>
<tr>
<td>Turks (n = 49)</td>
<td>12.3 ± 9.9</td>
<td>18.8 ± 10.3</td>
<td>18.2 ± 11.1</td>
</tr>
<tr>
<td>US Armenians (n = 29)</td>
<td>9.8 ± 7.2</td>
<td>24.1 ± 17.2</td>
<td>24.8 ± 17.5</td>
</tr>
<tr>
<td>Yerevan Armenians (n = 122)</td>
<td>8.0 ± 7.5</td>
<td>16.7 ± 8.9</td>
<td>23.3 ± 10.8</td>
</tr>
<tr>
<td>Linear regression (P&lt;)</td>
<td>.001</td>
<td>.0001</td>
<td>.0001</td>
</tr>
<tr>
<td>By genotype group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M694V/M694V (n = 179)</td>
<td>5.4 ± 5.7</td>
<td>11.2 ± 9.9</td>
<td>12.7 ± 9.8</td>
</tr>
<tr>
<td>M694V/other (n = 150)</td>
<td>9.1 ± 8.6</td>
<td>18.5 ± 12.6</td>
<td>23.1 ± 15.3</td>
</tr>
<tr>
<td>Other/other (n = 53)</td>
<td>9.5 ± 8.9</td>
<td>17.7 ± 11.5</td>
<td>19.3 ± 11.4</td>
</tr>
<tr>
<td>Linear regression (P&lt;)</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
</tr>
</tbody>
</table>

NS indicates not significant.
* The linear regression comparisons were made with the North African Jews ethnic group.
** The linear regression comparisons were made with the M694V/M694V group.

similar in all genotype groups except for arthritis, which was more common in homozygotes for M694V (39%) compared with group 2 (28%; P < .002) and group 3 (25%; P < .002). This difference was also found to be statistically significant, controlling for the number of years since commencement of colchicine treatment (in addition to controlling for ethnicity). Colchicine treatment was given to only 42% of the Armenian patients from Yerevan, which is a significantly lower percentage than that for the other ethnic groups (84%–94.2%). The percentage of patients treated with colchicine was significantly more common in homozygotes for mutation M694V (75.4%) compared with group 2 (62.7%) and group 3 (54.7%; P = .02).

Effect of Ethnicity on the Inflammatory Attacks

As depicted in Table 4, a significant association was found between the number of attacks (before commencement of colchicine treatment), country of residence, and ethnicity. Armenians living in Yerevan had a markedly higher number of attacks per year (30.8 ± 25.9) than all other ethnic groups (13.6 ± 12.2, 12.9 ± 11.3, 12.6 ± 27.3, and 5.2 ± 8.7; P < .0001). The North African Jews had a significantly earlier age of onset, age at diagnosis, and age at commencement of colchicine compared with all the other ethnic groups.

The inflammatory type of FMF attack was different in Armenians compared with in North African Jews (Tables 5 and 6). Armenians from Yerevan had...
a higher frequency of abdominal pain (89% vs 78%; 
P < .01), pleuritis (79% vs 18%; P < .002), and arthritis (44% vs 24%; P < .002). Armenians in California and Turks had a significantly higher prevalence of pleuritis (52%, P < .02 and 45%, P < .002), and arthritis (34%, P < .01 and 45%, P < .002), compared with North African Jews. No significant differences were found between North African Jews and other Jews living in Israel in any of these parameters. When this analysis was performed after controlling for the number of years since commencement of colchicine treatment (in addition to controlling for the mutation group), similar significant findings were demonstrated, except that the frequency of abdominal pain in the Armenians from Yerevan was no longer significantly different. Compared with North African Jews, Armenians from Yerevan were less commonly treated with colchicine (42% vs 94.2%; P < .001).

**DISCUSSION**

**Association of Mutation M694V and Amyloidosis**

The results of this study revealed a significant association between the presence of the M694V mutation in both the homozygous and heterozygous state and the development of amyloidosis. In addition, the findings suggest that the development of amyloidosis does not correlate with disease severity as assessed by the number of inflammatory attacks. This finding is consistent with previous reports that showed no correlation between the severity of the disease and amyloidosis, and the fact that some patients developed amyloidosis before FMF episodes, although there appear to be several patients with mutations other than M694V who also developed this complication.

In addition, there have been 3 previous reports of amyloidosis in patients not carrying the mutation M694V: 2 patients with amyloidosis who were both compound heterozygotes V726A/M680I22; an Arab kindred where all the affected individuals were homozygotes for M694I21; and a patient with systemic amyloidosis who was homozygous for V726A.24 Thus, the potential for development of amyloidosis in affected patients must be considered, even when the M694V mutation is not detected.

**Effect of Genotype Versus Environment/Ethnicity on FMF Inflammatory Attacks**

We also found an association between ethnicity and country of residence with disease severity. Because suspicion of the diagnosis of FMF is based on the clinical picture, it is influenced by various parameters, such as awareness of the physician and family, prevalence of the disease, and accessibility to and use of health services by the public. Therefore, we considered the number of attacks before commencement of treatment with colchicine to be the most objective characteristic for severity and the one the least influenced by the above mentioned parameters. We

**TABLE 5.** Clinical Characteristics of the FMF Inflammatory Attacks in Patients According to Ethnicity, Country of Residence, and Genotype Groups

<table>
<thead>
<tr>
<th></th>
<th>Fever</th>
<th>Abdominal Pain</th>
<th>Pleuritis</th>
<th>Arthritis</th>
<th>Percentage Treated with Colchicine*</th>
</tr>
</thead>
<tbody>
<tr>
<td>North African Jews (n = 131)</td>
<td>81.9%</td>
<td>78.4%</td>
<td>18.2%</td>
<td>24.3%</td>
<td>94.2%</td>
</tr>
<tr>
<td>Other Jews (n = 51)</td>
<td>84.8%</td>
<td>80.8%</td>
<td>13.3%</td>
<td>11.1%</td>
<td>93.5%</td>
</tr>
<tr>
<td>Turks (n = 49)</td>
<td>90.6%</td>
<td>87.5%</td>
<td>44.8%</td>
<td>45.2%</td>
<td>84.0%</td>
</tr>
<tr>
<td>US Armenians (n = 29)</td>
<td>93.1%</td>
<td>86.2%</td>
<td>51.7%</td>
<td>34.5%</td>
<td>93.3%</td>
</tr>
<tr>
<td>Yerevan Armenians (n = 122)</td>
<td>100%</td>
<td>89.3%</td>
<td>78.7%</td>
<td>44.3%</td>
<td>42.0%</td>
</tr>
<tr>
<td>By mutation group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M694V/M694V (n = 179)</td>
<td>86.9%</td>
<td>84.7%</td>
<td>38.6%</td>
<td>39.0%</td>
<td>75.4%</td>
</tr>
<tr>
<td>M694V/other (n = 150)</td>
<td>92.7%</td>
<td>83.0%</td>
<td>50.8%</td>
<td>27.8%</td>
<td>62.7%</td>
</tr>
<tr>
<td>Other/other (n = 53)</td>
<td>88.9%</td>
<td>81.2%</td>
<td>41.2%</td>
<td>25.4%</td>
<td>54.7%</td>
</tr>
</tbody>
</table>

† Multiple regression analysis (95% CI) compared with the M694V/M694V group.
‡ Multiple regression analysis (95% CI) compared with the North African Jews ethnic group.

* NS indicates not significant.
* Among known patients but excluding those on colchicine after the diagnosis of amyloidosis.
† Multiple regression analysis (95% CI) compared with the North African Jews ethnic group.
‡ Multiple regression analysis (95% CI) compared with the M694V/M694V group.
found that the number of attacks per year in Armenians living in Yerevan was significantly higher than in Armenians living in the United States (although both groups have the same genotype distribution) and than in other ethnic groups. In Israel, the number of attacks per year as well as the other parameters were found to be similar in both North African Jews and other Jews, even though the mutation/genotype distribution was different. Therefore, environment (country of residence) seems to exert the greater influence on the severity and type of FMF inflammation.

It is well known that the FMF inflammatory attacks can be triggered by stress and extreme physical exercise. Therefore, the effect of environment on the inflammatory attacks in cyclic diseases like FMF is expected and is also seen in other cyclic conditions, such as sickle cell anemia, etc. However, in contrast to sickle cell anemia, where only 1 mutation exists, in FMF the predisposition to the effect of environment is dependent on which mutations are present, demonstrated by the fact that this is greater in homozygotes for M694V. Recently, based on the screening of the Israeli normal population for MEFV mutations and comparison with the distribution of the mutations in the patients, it has been found that E148Q is frequent in the normal population but is markedly less frequently represented in the patients than is expected. This suggests a reduced penetrance of E148Q. As recent studies, including one by us, show that carriers of E148Q are more frequent than carriers of M694V in Jews, it seems that patients with E148Q are frequently undiagnosed and, therefore, untreated with colchicine.

Although it is possible that E148Q represents a polymorphism rather than a mutation, based on haplotype and family studies performed up to now, it is regarded as a mutation. In our current study, we found only 16 patients with the mutation E148Q, possibly because most such patients are phenotypically normal and none developed amyloidosis. These findings concur with those of other researchers. There have been no documented cases of amyloidosis in homozygotes for E148Q and only 1 in a patient who was a compound heterozygote M694V/E148Q, which suggests that this mutation carries a relatively low risk for FMF related amyloidosis. So far, it would seem that amyloidosis may develop rarely only when this mutation is combined with M694V (ie, M694V/E148Q).

Because it has also been found that individuals who are homozygous for M694V may be only mildly or not at all affected in terms of the inflammatory attacks, now that molecular testing is available, it is important to test for the common MEFV mutations in every patient suffering from unexplained recurrent abdominal pain with fever, arthritis, or recurrent fever. Such testing is also indicated and recommended for all siblings of known FMF patients.

CONCLUSION

Because colchicine has been shown to be effective in preventing the development of amyloidosis, lifelong treatment has been recommended for all FMF patients. Based on our findings, however, it seems that children who are homozygotes for M694V are at the highest risk, and this group should be given colchicine treatment for life. This requires a high level of awareness of all pediatricians working in communities with a high prevalence of FMF. In mildly affected patients (those with infrequent inflammatory attacks) who do not carry this mutation, we recommend that the patients should be either treated or tested every 6 months for the presence of proteinuria. The need for continuous treatment seems to be less indicated in the case of those patients where 1 or both mutations are E148Q, and in this group, colchicine treatment should be given to those patients who develop severe inflammatory episodes and/or proteinuria caused by amyloidosis.

ACKNOWLEDGMENTS

This project was partially supported by a research award from Mifal Hapayis, by the Israeli Ministry of Health Chief Scientist Grant 4209, and by Grant 32/97-18.0 from the Israeli Academy of Science.

We thank the FMF families for participating in the study.

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