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ARTICLE

Phenotype–genotype correlation in familial Mediterranean fever: evidence for an association between Met694Val and amyloidosis

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Familial Mediterranean fever (FMF) is an autosomal recessive disease characterised by recurrent attacks of inflammation of serosal membranes. Amyloidosis is the most severe complication of the disease. The aim of this study was to investigate the genotype–phenotype correlation and specifically the association between amyloidosis and the four common mutations in exon 10 of the gene causing FMF (MEFV) in a total of 83 FMF families from three ethnic groups: North African Jews, Armenians and Turks. A significant association was found between amyloidosis and the specific mutation at the *MEFV* gene: Met694Val (RR = 1.41, *P* = 0.02). Amyloidosis was present in 18 out of 87 homozygous FMF patients (20.7%) and in only two out of the 41 compound heterozygous FMF patients (4.9%). No patients carrying other mutations had amyloidosis. There was no significant association between the various mutations and the type or severity of the FMF symptoms. This finding underscores the importance of performing molecular studies on all suspect FMF patients. In addition to providing accurate diagnosis, these tests allow identification of presymptomatic genetically affected individuals, detection of carriers and assessment of the risk for amyloidosis in later life.

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Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disease affecting mainly non-Ashkenazi Jews (NAJ), Armenians, Turks and Arabs.[1] The carrier rate in these populations is very high, with estimates based on family studies as high as 1:5–1:7 among some non-Ashkenazi Jewish populations,[2] and 1:7 among Armenians in California.[3] The disease is characterised by recurrent short episodes of inflammation and serositis including fever, peritonitis, pleuritis, synovitis[1,3,4] and rarely pericarditis.[5]

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. 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.[6] 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 1–2% of the Armenians in the United States.[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] Therefore, identification of an amyloidosis-associated mutation will allow for directing prophylactic colchicine therapy to presymptomatic 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] Locus homogeneity was demonstrated for all ethnic groups studied.[13] Recently, the gene causing FMF has been cloned,[14,15] and four common missense mutations identified in exon 10. More recently, additional mutations have been found in exons 2, 5 and 10, bringing the current total of known mutations to twelve.[16] The protein encoded by the gene, named pyrin[14] or marenostrin,[15] is a member of a family of nuclear factors homologous to the Ro52 autoantigen.

Some recent studies have reported conflicting and inconclusive results with regard to the association between homozygosity for Met694Val and amyloidosis.[17–19] The aim of our study was to characterise the mutations in the *MEFV* gene in different ethnic groups, and to examine whether there is a correlation between the different mutations and the clinical symptoms in affected individuals. Specifically, we examined whether there is an association between FMF genotype and amyloidosis.

Subjects and Methods

In order to establish the frequency of the mutations in different ethnic groups, we studied 83 FMF families from three ethnic groups: 41 North African Jewish families (from Morocco, Algeria, Tunisia and Libya) (140 individuals, 65 affected), 16 Armenian families living in the USA (82 individuals, 31 affected), and 26 Turkish families (76 individuals, 42 affected). Most families were multiplex with two or more affected members, and in ten families a parent, an aunt or an uncle of the proband was also affected. Initially 134 affected individuals were identified in these families based on accepted clinical criteria[7] (Table 1). Following determination of the mutations at the MEFV locus, ten asymptomatic individuals were found to carry mutations in both alleles and were therefore considered genetically affected. Six individuals, who were clinically diagnosed as having FMF and treated with colchicine, were found not to carry the same mutations as their affected siblings or any of the other known mutations. They were, therefore, considered unaffected and excluded from the statistical analysis. Thus there were 138 affected and 160 unaffected individuals in the study population.

A detailed family history and a pedigree were obtained by interviewing each patient, and a comprehensive medical history was taken from each family member.

Of the patients studied, 20 had amyloidosis diagnosed by rectal and/or renal biopsy: ten non-Ashkenazi Jews, one Armenian and nine Turks. In all cases amyloidosis was diagnosed in previously untreated or inadequately treated patients.

Table 1 The FMF study population, first based on clinical criteria alone and then in combination with DNA testing

	North African Jews	*Armenians*	*Turks*
Families, no.	41	16	26
Patients based on clinical criteria alone, no.	62	31	41
Asymptomatic FMF based on DNA testing, no.	5	2	3
Clinically diagnosed FMF disproved by DNA testing, no.	2	2	2
Total FMF patients	65	31	42

Affected individuals were assigned according to the FMF mutation to one of the following groups: homozygotes for the mutation Met694Val, heterozygotes for this and another mutation, and homozygotes or heterozygotes for any two mutations other than Met694Val.

Variables related to the FMF phenotype such as: age of onset of the inflammatory attacks, number of attacks in a year, age of commencement of colchicine treatment, the presence of fever during attacks, the organs involved in the inflammatory process, and the presence of amyloidosis were compared between the different genotype groups. We also compared the clinical characteristics of the FMF patients with and without amyloidosis within each genotype (statistical analysis was performed when the number of patients was large enough to allow meaningful comparisons).

Statistical analysis was performed using Student's *t* test for two-group comparisons of continuous variables and analysis of variance for multiple groups comparisons. The χ^2 analysis was used to test for the significance of the association between the clinical characteristics of FMF and the presence of amyloidosis and the different genotypes. Fisher's exact test was performed to test for significance of the association when the sample size was small (expected counts in each cell less than 5).

Since awareness of the diagnosis of FMF may vary in different ethnic groups, especially in Israel where the disease is very common among the NAJ, we analysed the group of NAJ patients separately. Since only two NAJ patients were not homozygotes for the Met694Val mutation, we excluded them from the genotype–phenotype correlation analysis, and thus analysis was performed on four groups of patients: NAJ homozygotes for the Met694Val mutation, homozygotes for this mutation from other ethnic groups, compound heterozygotes from all ethnic groups where one allele carried this mutation, and patients not carrying this mutation at all.

Mutation Identification

The region that harbours the four common mutations in Exon 10 was amplified with PCR and specific primers: 10F1, 5'-ccagaagaactaccctgtccc-3' and 10R1, 5'-cagagcagctggcgaatg-tat-3'. PCR conditions were denatured at 95°C for 10 min; 30 cycles of 95°C for 15 s, 55 for 30 s and 72 for 3 min, with a final extension at 72 for 10 min. PCR products were purified with Sephadex P100 chromatography and sequenced directly, using specific primers and AmpliTaq FS Dye Termination cycle sequencing kit.

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

Results

Table 2 depicts the distribution of the four most common mutations in exon 10 of the *MEFV* gene in independent FMF chromosomes in each ethnic group. Mutation Met694Val was the most common mutation in each ethnic group, being significantly more frequent in NAJ compared with other ethnic groups ($P < 0.001$). The second most common mutation, Val726Ala, was found in 11% of Armenian and 12% of Turkish affected chromosomes. Two other mutations were found: Met680Ile, which was present in 1% of NAJ, 24% of Armenian and 11% of Turkish carrier chromosomes, and Met694Ile, which was present in 2% of Turkish carrier chromosomes. In 16 carrier chromosomes of clearly affected individuals no known mutation was identified (Table 2).

Table 3 summarises the clinical characteristics of the patients according to the different genotype groups. The NAJ homozygotes for the mutation Met694Val had a significantly earlier age of onset of FMF inflammatory attacks in comparison with the group of patients homozygous for this mutation in other ethnic groups (Turks and Armenians) (4.7 ± 3.4 *vs* 9.7 ± 8.1, $P < 0.0003$). They also commenced colchicine treatment at an earlier age (8.8 ± 8.1 *vs* 24.5 ± 16.5, $P < 0.0001$). There was no significant difference in the mean number of attacks in a year between these groups. Pleuritis was significantly more common among the Turks and Armenians than among the NAJ ($P < 0.001$). Amyloidosis was less prevalent among the NAJ patients, although the difference did not reach statistical significance (16% *vs* 33%, $P = 0.08$) (Table 4).

In the group of patients of Turkish and Armenian origin there were no significant differences between the different genotypes in parameters related to the severity of the FMF disease such as age of onset of

Table 2 Distribution of the four most common *MEFV* gene mutations in exon 10 among independent FMF chromosomes according to the different ethnic groups

	North African Jews (n=83)[a]	*Armenians* (n=37)	*Turks* (n=56)
Met694Val	80 (97%)	18 (49%)	34 (61%)
Val726Ala	—	4 (11%)	7 (12%)
Met680Ile	1 (1%)	9 (24%)	6 (11%)
Met694Ile	—	—	1 (2%)
Others	2 (2%)	6 (16%)	8 (14%)

[a] *n* = number of independent chromosomes. Only chromosomes from unrelated individuals were used for this analysis. Note: The number of independent chromosomes per family may be greater than 2. This is the case if an affected individual marries a carrier, resulting in affected parents producing affected offspring, and also in a situation where a carrier married to another carrier has affected siblings.

symptoms, age at diagnosis, number of attacks in a year and the organs involved in the attacks.

A statistically significant association was found between the mutation Met694Val and amyloidosis (P = 0.02) (Table 4). This was confirmed when analysis was done separately on the group of Turkish and Armenian patients. Whilst eight out of 24 FMF patients homozygous for the Met694Val mutation were diagnosed with amyloidosis, only two heterozygotes had amyloidosis (P = 0.004).

Since FMF amyloidosis can be prevented by colchicine treatment, we tested whether the association between the Met694Val mutation and amyloidosis held in patients who had never received colchicine and developed amyloidosis prior to age 20. The prevalence of amyloidosis developing between the ages of 13 and 20 in untreated homozygotes for this mutation was 67% (10 out of 15) in NAJ and 57% (8 out of 14) in Turks and Armenians. These rates were significantly higher than those in compound heterozygotes (10%, P = 0.001 and P = 0.007 respectively, Table 4).

Discussion

We have demonstrated in this study that the four common exon 10 mutations of the *MEFV* gene account for 98% of the affected chromosomes in the North African Jews, 84% in the Armenians and 86% in the Turks. Thus, a relatively simple molecular test can provide an accurate diagnosis in a large proportion of FMF patients.

Table 3 Characteristics of symptoms in FMF patients according to the different *MEFV* gene mutations

	NAJ	*Turks and Armenians*		
	Homozygote Met694Val (n=63)[a]	*Homozygote Met694Val* (n=24)	*Heterozygote Met694Val/other* (n=41)	*Non-Met694Val* (n=8)
Male/female	29/34	14/10	23/18	4/4
Age of onset, yrs	4.7±3.4[c]	9.7±8.1	10.6±8.1	14.9±8.2
Attacks/yr, no.	10.1±7.1	8.0±5.7	9.9±10.6	5.3±5.9
Treatment age, yrs	8.8±8.1[d]	24.5±16.5	23.5±10.3	20.6±11.8
Age diagnosis of amyloidosis, yrs	24.2±9.1	23.5±3.4	21.0±8.0	—
Asymptomatic, no.	5	2	3	—
Fever[b]	53 (91%)	22 (100%)	37 (92%)	7 (89%)
Peritonitis[b]	44 (76%)	21 (95%)	34 (85%)	8 (100%)
Pleuritis[b]	12 (21%)	12 (54%)	19 (48%)	3 (37.5%)
Arthritis[b]	26 (45%)	10 (45%)	14 (35%)	2 (25%)
Age at ascertainment, yrs	19.0±11.4[d]	32.6±17.0	36.0±14.8	26.7±10.0

[a] n = number of FMF patients; [b] in parenthesis: percentage of patients with a specific symptom out of all symptomatic patients; [c] NAJ *vs* homozygotes Met694Val Turks and Armenians, P<0.001; [d] NAJ *vs* homozygotes Met694Val Turks and Armenians, P<0.0001.

Table 4 Amyloidosis in FMF patients according to the various *MEFV* gene mutations

	NAJ	*Turks and Armenians*		
	Homozygote Met694Val	*Homozygote Met694Val*	*Heterozygote Met694Val/other*	*Non-Met694Val*
Total patients with amyloidosis	10/63 (16%)	8/24 (33%)	2/41 (5%)	0/8[a]
Patients (of the total with amyloidosis) not treated and developed amyloidosis prior to age 20, no.	10/15 (67%)	8/14 (57%)	2/19 (10%)	0/7[a]

[a] comparison between the three genotypes in Turks and Armenians (homozygotes *vs* heterozygotes *vs* non-carriers of the Met694Val mutation), P=0.001.

In addition to its value in considering whether to give colchicine or not, detection of the mutation allows identification of FMF patients at the presymptomatic stage, some of whom may present with amyloidosis as the first symptom (FMF type 2).

In our study population there were no significant differences in the clinical presentation of FMF patients carrying different mutations, although both Pras *et al*[20] and Dewalle *et al*[17] found that homozygosity for the Met694Val mutation was significantly associated with a more severe form of the disease. A possible explanation could be that the NAJ patients have a phenotype which is different from that of other ethnic groups, even when they have the same genotype. We did, in fact, find such differences between the NAJ and the Turks/Armenian homozygous for Met694Val, but no differences between genotypes within the same ethnic group. The previous studies did not control for ethnicity.

Within each genotype, we found no differences in parameters related to the severity of the disease between patients with and without amyloidosis, but a significant association was found between the mutation Met694Val and the development of amyloidosis. The finding that amyloidosis is associated with a certain genotype which within our study population was not correlated with a more severe clinical disease suggests that amyloidosis is not related to the severity of FMF, but rather is inherent in the specific MEFV mutation. This is also supported by earlier reports that showed no correlation between the severity of the disease and amyloidosis, and the fact that some patients developed amyloidosis prior to FMF episodes.[7,11] In addition, some patients with FMF do not benefit from colchicine in terms of prevention of attacks, yet do not develop amyloidosis while on this treatment.

Even though in our study population no cases of amyloidosis were found in patients who did not carry at least one Met694Val mutation, there have been three recent reports of such an association. Yalçinkaya *et al*[18] reported two patients with amyloidosis who were both compound heterozygotes Val726Ala/Met680Ile, and Dewalle *et al*[17] reported an Arab kindred where all the affected individuals were homozygotes for Met694Ile. The third case, reported by Pras,[19] was one patient with systemic amyloidosis who was homozygous for Val726Ala. Our study allows for the first time statistical analysis of the genotype–phenotype correlation with regard to amyloidosis and demonstrates a significant association between Met694Val and this complication.

Of interest are the findings of earlier age of onset and earlier age at commencement of colchicine treatment in Jews of North African origin compared with Turks and Armenians, all homozygotes for Met694Val. These differences may be explained by a greater awareness of FMF among North African Jews in Israel leading to earlier diagnosis and treatment. Earlier age of treatment may account also for the relatively lower prevalence of amyloidosis observed in this study in North African Jewish homozygotes for Met694Val compared with the Armenians and Turks. Supporting this is the finding that the prevalence of amyloidosis was similar in both groups when patients younger than age 20 and those who had been treated with colchicine prior to age 20 were excluded. Pleuritis was significantly less frequent in NAJ patients. This finding is at variance with that of Dewalle *et al*,[17] who found that both pleuritis and arthritis were significantly more frequent in homozygotes for the Met694Val mutation. However, since FMF is diagnosed and colchicine treatment commenced at an earlier age in the NAJ group compared to in other groups, this could be due to underdiagnosis of pleuritis in these young patients.

Our findings may have important clinical implications for the treatment of FMF families. Since colchicine has been shown to prevent the development of amyloidosis,[9,10] lifelong treatment is recommended for all FMF patients. Based on our findings, it appears that homozygotes for Met694Val are at the highest risk, and should be given colchicine treatment for life. If further studies confirm these findings, the question of treating asymptomatic individuals homozygotes for the Met694Val mutation with colchicine and withholding it from mildly affected patients who do not carry this mutation will need to be addressed.

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