Male Sex Coupled with Articular Manifestations Cause a 4-fold Increase in Susceptibility to Amyloidosis in Patients with Familial Mediterranean Fever Homozygous for the M694V-MEFV Mutation

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ABSTRACT. ***Objective.*** Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by attacks of fever, serositis, and a predisposition to the development of amyloidosis. The wide clinical variability of the disease has been partly attributed to MEFV allelic heterogeneity and partly to the influence of additional genetic and/or environmental modifiers. Of these, male sex was found to influence disease penetrance and susceptibility to amyloidosis. We investigated the role of sex as an independent contributor to the phenotypic profile in FMF and further defined the factors affecting disease expression and severity.

Methods. A total of 124 patients with FMF who were all homozygous for the M694V mutation, including 47 patients with nephropathic amyloidosis, were identified. A detailed chart review and physical examination were undertaken to determine demographic characteristics, history, clinical manifestations, and treatment, and we calculated the disease severity score from the Tel-Hashomer key.

Results. A preponderance of male patients was documented (73:51; 1.4). The overall male:female ratio was significantly higher among patients with amyloidosis (32:15; 2.1) compared to patients without amyloidosis (41:36; 1.1). FMF severity scores, independently calculated for male and female patients, were equally high (9.5 ± 3.0 and 9.7 ± 2.8, respectively). The frequency of arthritic attacks, significantly higher in women than men ($p = 0.015$), remained notably higher in male FMF patients with amyloidosis compared to male FMF patients without amyloidosis ($p = 0.002$). Significant correlation between arthritis attacks and amyloidosis was found ($R > 0.285$, $p < 0.001$).

Conclusion. Susceptibility to renal amyloidosis is influenced both by sex and the occurrence of joint attacks, acting as 2 MEFV independent factors (OR 2.37, 95% CI 1.06-5.26 and OR 3.27, 95% CI 1.23-8.68, respectively). (J Rheumatol 2003;30:308–12)

Key Indexing Terms:
FAMILIAL MEDITERRANEAN FEVER AMYLOIDOSIS ARTHRITIS MEFV SEX

Familial Mediterranean fever (FMF) is an autosomal recessive disorder (MIM# 249100), occurring most commonly in populations of Mediterranean extraction[1]. It is characterized by recurrent acute self-limited episodes of fever and peritonitis, pleuritis, arthritis, and erysipelas-like skin disease[1]. In some patients, systemic amyloidosis develops, manifested most notably as nephropathy[2]. The clinical variability is wide, from mild symptomatology to severe and life threatening manifestations. The age of onset is before 10 years in 60% of the patients and before 20 in 90%[1].

Mutations in the pyrin/marenostrin (MEFV) gene have been identified in the majority of FMF patients[3-9]. These include 4 conservative missense mutations (M680I, M694V, M694I, V726A) clustered in exon 10 that together with mutation E148Q in exon 2 account for the vast majority of FMF chromosomes identified in our patients[10-12]. The phenotypic variability of the disease is partly due to allelic heterogeneity. Mutation M694V and the complex V726A-E148Q allele are associated with a severe phenotype and amyloidosis[13-19]. Mutation M680I is associated with a moderate form of disease. Mutations E148Q and V726A have reduced penetrance and many individuals, homozygotes or compound heterozygotes for these mutations, remain symptom-free[9-12]. Recent population based studies have shown that the prevalence of FMF chromosomes far exceeds those previously estimated based on disease prevalence, indicating that the majority of individuals who fulfil the genetic criteria for FMF remain unaffected[9-12]. This is mainly attributed to the high frequency of mutations E148Q and V726A, noted for their low penetrance[9-12].

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Submitted February 12, 2002; revision accepted June 6, 2002.

Considering both the high frequency of mutations and the fact that individuals who carry identical mutations still vary in their clinical manifestations, a role for additional genetic and/or environmental modifiers seems reasonable. Polymorphisms at the SAA1 gene and the major histocompatibility complex class I chain related gene A (MICA) gene have been shown to play a role as modifiers in FMF[20,21]. In addition, an unbalanced sex ratio has been observed in many population samples[22,23]. Cazenueve, *et al* clearly demonstrated that men with FMF are at higher risk of developing amyloidosis[21].

We investigated the role of sex as an independent contributor to the phenotypic profile in FMF and attempted to further define the factors affecting disease expression and severity.

MATERIALS AND METHODS

Patient selection. From a large cohort of patients with FMF who were offered a genetic test as part of their diagnostic investigation, only those homozygous for M694V were selected for further analysis. These included 49 pediatric cases referred to the pediatric rheumatology or FMF clinic, 28 adults referred to the genetic counseling clinic at Rambam Medical Center, and 47 patients with amyloidosis, recruited at the FMF clinic of the Sheba Medical Center. Recruitment of these patients was random and based on sequential arrival to the clinics for followup or treatment. The patients were examined and their charts reviewed.

Clinical features, prior to onset of colchicine therapy, were recorded through a standardized form featuring an established set of clinical criteria[24], such as fever, abdominal, thoracic and articular attacks, renal manifestations, and duration and frequency of the attacks. Colchicine dosage (used to control the attacks) at the time of the interview and disease severity calculated from the Tel-Hashomer key[25] were recorded. The study had the approval of the hospital's institutional review board. Patients were stratified according to ethnic descent: Ashkenazi and non-Ashkenazi Jews, and Arabs of Muslim, Druze, and Christian origin.

Genetic analysis. The predominant mutations in the MEFV gene (M694V, M680I, M694I, V726A, E148Q) were investigated by polymerase chain reaction (PCR) amplification followed by digestion with appropriate enzymes made to distinguish the wild-type allele from the mutant allele, as described[9,26].

Statistical analysis. The statistical significance of differences between groups was calculated by either the chi-square test for categorical data or the t test for quantitative data. All statistical tests were 2 sided. Logistic regression analysis was used to study the contribution of 2 independent variables (sex and arthritis) to the development of amyloidosis. Results are given as odds ratios (OR) and 95% confidence intervals (CI). Spearman partial correlation analysis was used to study the independent and combined contribution of sex and/or arthritis attacks to amyloidosis.

RESULTS

We identified 124 FMF patients (73 men, 51 women) homozygous for the M694V mutation. These included 105 of North African Jewish descent, 7 non-Ashkenazi Jews, and 7 of Muslim Arab descent (Table 1). Table 2 shows the clinical manifestations, mean age at disease onset, and severity scores separately for men and women. The mean severity scores were equally high for male and female patients (9.5 ± 3.0 and 9.7 ± 2.8, respectively). The mean age at disease onset, the mean number of attacks, and the mean dose of colchicine used to control these attacks did not differ between male and female patients. Women manifested arthritis attacks more frequently than men (p = 0.015). In men a higher trend for amyloidosis was noted (p = 0.103) (Table 2).

Table 1. Distribution of FMF patients according to sex and ethnic origin.

Ethnic Background	n	Men	Women
North African Jews	105	64	41
Non-Ashkenazi Jews	12	4	8
Muslim Arabs	7	5	2
Total	124	73	51

Amyloidosis. The factors governing amyloidosis were further analyzed (Table 3). The male:female ratio was higher in patients with renal amyloidosis (n = 47), than in patients without renal amyloidosis [32:15 (2.1) vs 41:36 (1.1)]. The group recruited at Tel-Hashomer, consisting of the patients with amyloidosis, had a higher male:female ratio compared to the sample recruited at the Rambam Medical Center. However, the ethnic distribution of patients with amyloidosis (38 North African Jews, 7 non-Ashkenazi Jews, and 2 Muslim Arabs) was similar to that of patients without amyloidosis. In patients with renal amyloidosis, in contrast to patients without renal amyloidosis, the mean severity scores were higher (12 ± 2.8 and 8.17 ± 2.2, respectively; p < 0.001), the frequency of FMF attacks lower (p < 0.001); and the mean dose of colchicine used to control these attacks higher (p < 0.001) (Table 3). However, when male and female patients with amyloidosis were compared to each other, and male and female patients without amyloidosis were compared to each other, there were no significantly different variables (Table 4).

Compared to patients without renal amyloidosis, a significantly higher proportion of patients with amyloidosis manifested arthritis (p = 0.042). Women, however, although less prone to amyloidosis (p = 0.103), manifested arthritis more frequently than men (p = 0.015). This effect was stronger when women without amyloidosis were compared to men without amyloidosis (p = 0.002). Altogether, the prevalence of amyloidosis was highest in men with arthritis (Table 5).

These data taken together imply that male sex and arthritis independently influence susceptibility to renal amyloidosis. A logistic regression analysis showed that, in our population sample, the risk for men with FMF to develop amyloidosis was twice that of women (OR 2.37, 95% CI 1.06–5.26). Patients with arthritis had a 3-fold increased risk for amyloidosis (OR 3.27, 95% CI 1.23–8.68), while men with arthritis had a 4-fold increased risk for amyloidosis (OR 4.44, 95% CI 0.55–36.2).

To study the relative contribution of sex and/or arthritis to amyloidosis, we utilized Spearman partial correlation analysis. A significant correlation was found between arthritis attacks and amyloidosis when sex was entered as a covariate (R > 0.285, p = 0.001). A weaker correlation was found between sex and amyloidosis when arthritis was entered as a

Table 2. Clinical characteristics of 124 M694V/M694V FMF patients (by sex) show that arthritis attacks are more common in women.

	n	Distribution Arthritis, n (%)	Amyloidosis, n (%)	Peritonitis, n (%)	Fever, n (%)	Age at Disease Onset, yrs ± SD	Attacks per Month*	Colchicine, mg/day*	Severity Score*
Men	73	49 (67.1)	32 (43.8)	66 (90.4)	66 (90.4)	6.2 ± 6.0	1.6 ± 1.5	1.7 ± 0.5	9.5 ± 3.0
Women	51	44 (86.3)	15 (31.9)	46 (90.2)	47 (92.2)	6.6 ± 7.2	1.8 ± 1.5	1.8 ± 0.6	9.7 ± 2.8
p		0.015	0.103	0.968	0.737	0.694	0.512	0.300	0.773
Total	124	93 (75)	47 (37.9)	112 (90.3)	113 (91.1)	6.4 ± 6.5	1.7 ± 1.5	1.7 ± 1.4	9.6 ± 2.9

*Mean ± SD

Table 3. Clinical characteristics of 124 M694V/M694V FMF patients (according to the presence or absence of amyloidosis) show that arthritis is more common in patients with amyloidosis.

	n	M/F (ratio)	Frequencies Arthritis %	Peritonitis %	Age at Disease Onset, yrs ± SD	Attacks per Month*	Colchicine, mg/day*	Severity Score*
Without amyloidosis	77	41/36 (1.1)	53 (68.8)	68 (88.3)	5.7 ± 7.4	2.1 ± 1.6	1.5 ± 0.5	8.1 ± 2.2
With amyloidosis	47	32/15 (2.1)	40 (85.1)	44 (93.6)	7.5 ± 4.4	1.1 ± 0.9	2.1 ± 0.4	12 ± 2.8
p			0.042	0.332	0.159	< 0.001	< 0.001	< 0.001
Total	124	73/51 (1.4)	93 (75.0)	112 (90)	6.4 ± 6.5	1.7 ± 1.5	1.7 ± 1.4	9.6 ± 2.9

*Mean ± SD

Table 4. Clinical characteristics of 124 M694V/M694V FMF patients by presence or absence of amyloidosis. Arthritis acts as an independent contributor to the development of amyloidosis.

	With Amyloidosis Men, n = 32	Women, n = 15	p	Without Amyloidosis Men, n = 41	Women, n = 36	p	Men vs Women With/Without Amyloidosis
Age of onset, yrs ± SD	7.6 ± 4.8	7.1 ± 3.5	NS	5.1 ± 6.5	6.5 ± 8.3	NS	0.416
Severity score ± SD	12.0 ± 2.0	12.1 ± 2.7	NS	7.6 ± 2.1	8.7 ± 2.1	NS	< 0.001
Attacks per month ± SD	1.0 ± 0.7	1.3 ± 1.2	NS	2.1 ± 1.8	2.1 ± 1.5	NS	0.004
Colchicine, mg/day ± SD	2.0 ± 0.4	2.2 ± 0.4	NS	1.4 ± 0.4	1.6 ± 0.6	NS	0.000
Peritonitis, n (%)	30 (90.3)	14 (93.3)	NS	36 (87.8)	32 (88.9)	NS	0.809
Fever, n (%)	32 (100)	14 (93.3)	NS	34 (82.9)	33 (91.7)	NS	0.085
Arthritis, n (%)	27 (84.4)	13 (86.7)	0.837	22 (53.7)	31 (86.1)	0.002	0.002

Table 5. Prevalence of renal amyloidosis among men with arthritis compared to men without arthritis and women with and without arthritis.

Patients	n* Total	%	p
Men with arthritis	27/45	60.0	
Men without arthritis	4/24	16.7	< 0.01
Women with arthritis	13/44	29.5	< 0.02
Women without arthritis	2/7	28.6	NS
Men and women without arthritis	7/31	22.6	< 0.01
All excluding men with arthritis	20/75	26.6	< 0.01

* Refers to patients with amyloidosis.

covariate (R = 0.12, p = 0.071). Significant correlation was found (R > 0.284, p = 0.001) when arthritis and sex were both considered in the analysis.

DISCUSSION

The wide clinical variability observed in FMF is partly attributed to allelic heterogeneity and partly to the influence of environmental and/or additional genetic modifiers. Of these, patients' sex seems to play an important role[21-23]. FMF is more common in men[22] and the risk of developing amyloidosis seems to be higher in male patients[21]. To preclude the con-

founding effect of allelic heterogeneity, we investigated a large number of FMF M694V homozygotes. Consistent with previous observations, our cohort of patients included more men than women (ratio 1.4). This effect was mainly due to the preponderance of male patients among those who developed amyloidosis (ratio 2.1). Among patients who did not develop amyloidosis and among our pediatric and adolescent cases (n = 49), this effect was much less pronounced (ratios 1.1 and 1.26, respectively). These numbers are in accord with those reported for both adult and pediatric FMF cases[21-23]. We can conclude that male sex somehow modifies the penetrance of the disease and predisposes to systemic amyloidosis (OR 2.37, 95% CI 1.06–5.26). The disease severity scores, the mean age at disease onset, the frequency of FMF attacks, and the dosage of colchicine required to control these attacks do not seem to be influenced by sex. Higher severity scores, calculated for patients with amyloidosis, could be partly attributed to the presence of amyloidosis *per se*, and partly to the increased dosage of colchicine regularly prescribed to these patients. Arthritis, on the other hand, frequently seen in women with FMF and in men with FMF who developed amyloidosis, acts as another MEFV-independent factor that influences susceptibility to amyloidosis (OR 3.27, 95% CI 1.23–8.68) (Table 4).

Amyloidosis, the major complication of FMF, occurs more readily in M694V/M694V homozygotes[10,15,17-19]. It was recently shown that, regardless of MEFV genotype, men with FMF are more susceptible to amyloidosis than women with FMF[21]. As we studied a large cohort of FMF M694V homozygotes, there was no need to adjust for MEFV allelic heterogeneity and our results support this observation. Since the development of amyloidosis is less frequently seen now due to early diagnosis and treatment with colchicine, the preponderance of men among those who developed amyloidosis could have been, theoretically, attributed to decreased compliance to colchicine in men compared to women. This argument can be refuted by Cazenueve, *et al*[21], who studied a cohort of Armenian patients with no access to colchicine. That study found a 4-fold higher risk of developing nephropathic amyloidosis for men compared to women.

Most importantly, we found that patients who manifest arthritis have a 3-fold increased risk of developing amyloidosis. It could be argued that arthritis *per se* reflects more severe disease, and as such is more frequently associated with amyloidosis. Yet among men and women with FMF with the same disease severity scores, arthritis was significantly more common in women, either with or without amyloidosis, while in men it occurred only among those who developed amyloidosis. Using the Spearman partial correlation, a significant correlation was found between arthritis attacks and amyloidosis when sex was entered as a covariate ($R > 0.285$, $p = 0.001$).

Serum amyloid A (SAA), a major acute phase reactant potentially involved in the pathogenesis of inflammatory diseases, is the precursor of the amyloid A deposited in amyloid A amyloidosis[27]. One can speculate that during arthritic attacks, the *in vivo* concentration of SAA exceeds that generated during attacks of peritonitis and thereby increases susceptibility to amyloidosis. In addition, it remains questionable whether sex steroids, also known to influence the concentration of several acute phase proteins[28], differentially influence the inflammatory process in men.

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