Amyloidosis induced, end stage renal disease in patients with familial Mediterranean fever is highly associated with point mutations in the MEFV gene

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Abstract

***Background*—Familial Mediterranean fever (FMF) is an autosomal recessive disease characterised by recurrent attacks of fever and serositis. Amyloidosis is the most significant complication of FMF, leading to end stage renal disease (ESRD). Recently the gene (MEFV) causing this disease was cloned and more than 18 mutations have been identified. The hypothesis that the development of amyloidosis is associated with one of these mutations was tested.**

***Methods*—23 patients with FMF and ESRD were analysed for their MEFV mutations and correlated with their corresponding rectal and renal biopsies. As case controls 23 patients with FMF free of renal disease, but with similar origin, sex, age, and age at onset of FMF, were chosen.**

***Results*—All the patients with ESRD induced by amyloidosis were homozygous for the M694V or M694I mutations. This finding was significantly different from that seen in the control group.**

***Conclusions*—Amyloidosis is highly associated with the 694 substitution in the MEFV gene causing FMF. It seems that genetic predisposition plays a part in the development of this complication of FMF.**

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Familial Mediterranean fever (FMF) is an autosomal recessive disease characterised by recurrent attacks of fever, peritonitis, pleuritis, synovitis, or erysipelas-like skin lesions.[1] The disease affects certain ethnic groups, mainly Sephardic Jews, Armenians, Turks, and Arabs.

One of the most significant complications of FMF is amyloidosis, usually affecting the kidneys, resulting in nephrotic syndrome and renal insufficiency progressing to end stage renal disease (ESRD). Amyloidosis may also affect the gastrointestinal tract, liver, spleen and, at a later stage, the heart and testes.[2] The amyloid is of the AA type, typical of secondary amyloidosis. Its prevalence differs among the various ethnic groups and usually depends on whether patients are treated with colchicine, which has significantly decreased its incidence. Some patients present with renal amyloidosis and no history of FMF attacks; however, questioning often shows that other family members have characteristic FMF manifestations. This presentation of amyloidosis without the attacks of serositis has been called "phenotype II".[3]

Two years ago the international and the French familial Mediterranean fever consortia independently cloned the gene responsible for FMF, found on the short arm of chromosome 16 (MEFV).[4 5] Four ancient missense mutations were identified on chromosomes of familial Mediterranean fever carriers in multiple ethnic groups. In these studies it was suggested that some of the phenotypic variations of the disease may be caused by different mutations—for example, M694V and V726A. Furthermore, the international consortium suggested that the milder V726A mutation may be protective against amyloidosis, whereas the patients homozygous for M694V would be prone to amyloidosis.[5] Recently, Yalcinkaya *et al* described four Turkish children with familial Mediterranean fever and renal amyloidosis, all of whom were heterozygous for V726A.[6] Pras has reported on a single patient with amyloidosis who was homozygous for the V726A mutation.[7] These results show that renal amyloidosis can accompany mutations other than the M694V. In two recent papers the role of the M694V mutation in amyloidosis was again discussed. Shohat *et al* and Livneh *et al* showed that this mutation significantly correlated with the presence of amyloidosis compared with other mutations.[8 9] Thus the question of whether amyloidosis of FMF has a specific predisposing mutation remains unsettled.

In our study we analysed the MEFV mutations of 23 patients with FMF and ESRD. The results were later correlated with the corresponding rectal or renal biopsies performed previously, in the evaluation of their renal disease. The MEFV mutations were also compared with those of a control FMF group without clinical renal disease.

Patients and methods

PATIENTS

All the available patients with FMF and ESRD in our medical centre were enrolled in the study. There were 23 such patients—13 were Jews of North African origin (mainly Moroccan) and 10 were Palestinian Arabs. All were from unrelated families. Half the patients were receiving dialysis, whereas the rest had undergone renal transplantation. All the patients were diagnosed as having FMF based on the following criteria: recurrent attacks of fever with peritonitis, pleuritis or synovitis, and favourable response to colchicine or a family

Table 1 Demographic and clinical features of the group with end stage remal disease (ESRD)

Patient/ sex	*Origin*	*Year of birth*	*Age of onset*	*Age at Dx**	*Age at Rx**	*Fever*	*Clinical peritonitis*	*Manifestation pleuritis*	*Arthritis*	*Erysipelas -like*	*Age at ESRD*	*Family history*
North African group												
1/M	Morocco	1938	5	19	36	+	+	+	−	−	50	Yes
2/M	Morocco	1944	18	20	28	+	+	+	−	−	50	No
3/M	Morocco	1947	2	7	36	+	+	+	+	−	20	Yes
4/M	Morocco	1954	4	5	44	+	+	−	+	−	45	Yes
5/M	Morocco	1954	3	12	22	+	+	−	+	−	35	No
6/F	Morocco	1955	5	5	20	+	+	−	+	−	25	Yes
7/F	Morocco	1957	9	18	18	+	+	+	+	−	34	No
8/M	Morocco	1959	6	13	18	+	+	+	+	+	29	No
9/F	Tunis	1960	3	20	20	+	+	+	+	+	24	Yes
10/M	Tunis	1960	4	5	17	+	+	−	+	−	35	No
11/F	Algier	1964	7	18	18	+	+	−	+	−	25	No
12/F	Morocco	1967	7	14	14	+	+	−	+	+	32	No
13/F	Morocco	1970	6	9	9	+	+	−	−	−	29	Yes
Mean (SD)			6.0 (4.1)	12.7 (5.7)	23 (10)						33.3 (9.3)	
Palestinian-Arab group												
14/M	Moslem	1938	10	40	40	+	+	−	−	−	40	Yes
15/F	Moslem	1945	22	54	54	+	+	+	−	−	54	No
16/M	Moslem	1949	20	22	22	+	+	−	+	−	41	No
17/M	Moslem	1957	20	34	34	+	+	−	−	−	34	No
18/M	Moslem	1960	7	17	−	+	+	+	−	−	33	No
19/M	Moslem	1962	5	29	29	+	+	+	−	−	30	Yes
20/F	Moslem	1965	2	13		+	−	−	+	−	17	Yes
21/M	Moslem	1967	15	23	23	+	+	−	−	−	26	Yes
22/M	Moslem	1969	6	9	9	+	+	−	−	−	20	Yes
23/F	Moslem	1977	12	15	15	+	+	−	−	−	17	Yes
Mean (SD)			11.7 (7.1)	25.6 (13.9)	28 (14.4)						31.2 (11.8)	

*Dx = diagnosis; Rx = treatment with colchicine; M = male; F = female.

history of FMF in a first degree relative.[10] All the patients underwent rectal or renal biopsies to look for amyloidosis. Clinical and demographic data were collected by direct interviews. The information included FMF symptomatology (fever, peritonitis, pleuritis, synovitis, skin lesions), disease duration, and frequency of attacks before the development of renal insufficiency. Mutation analysis was performed without knowledge of the rectal or kidney biopsy results.

From our outpatient clinic of more than 300 patients with FMF we chose 23 case controls—13 patients of North African origin and 10 Palestinian Arabs who were all free of renal disease. Controls who matched the patients with ESRD for their ethnic origin, age, sex, age at onset of the disease, and for the cumulative amount of colchicine treatment (approximately) were chosen from the FMF clinic data, consecutively. The diagnosis of FMF in the control group was strict, based on clinical grounds and a favourable response to colchicine in accordance with published criteria.[10] The kidney function of the controls was assessed by plasma or serum creatinine and urine analysis. All the patients in the control group had normal levels of serum creatinine and none had proteinuria. Nevertheless, because there was no medical indication, none of the controls had a rectal or renal biopsy or subcutaneous abdominal fat aspiration. Mutation analysis was also performed in the control group. The patients and the controls gave informed consent to participate in the study.

MUTATION ANALYSIS

A recently established restriction analysis polymerase chain reaction system was used for identification of the FMF mutations in the patients and in their controls.[5] We searched for the five mutations accounting for most of the FMF chromosomes in our FMF group: M694V, M694I, M680I, V726A, and E148Q. Screening for these mutations was blind, a code number being assigned to each patient. Correlation with the results of their corresponding rectal or renal biopsies was performed later.

STATISTICAL ANALYSIS

Results are reported as means (SD). χ^2 Analysis with Yates's correction was used for comparison of proportions, and a non-paired *t* test for comparison of means.

Results

Twenty three patients with FMF and ESRD and 23 matched control patients were studied. Table 1 summarises some of the demographic and clinical data of the patients with ESRD. Only seven of the patients in the North African group were female. The ages in this group ranged from 29 to 61 years (mean 45.7 (7.6)). Their ages at onset of symptomatic disease ranged from 5 to 18 years (mean 6.0 (4.1)) and disease duration until the development of ESRD and dialysis was 27.2 (8.2) years. In the Palestinian group (three patients female) the age of the patients ranged from 22 to 61 (mean 40.1 (11.3)), the age of onset of FMF ranged from 2 to 22 years (mean 11.7 (7.1)), and disease duration until the development of ESRD was 19.3 (8.9) years.

The clinical manifestations of the patients and controls included fever, peritonitis, pleuritis, arthritis, and erysipelas-like lesions.

Twelve of the ESRD group had a family history of FMF. Some of the patients received colchicine a few years after diagnosis of FMF as this drug was introduced to treat this disease only in 1972. Other patients did not take colchicine regularly or were non-compliant.

Comparison of the North African group with the Palestinian group showed several statistically significant differences. Age of onset of symptoms (6.0 (4.1) *v* 11.7 (7.1) years, $p<0.05$) and the diagnosis of FMF (12.7 (5.7)

Table 2 Mutation distribution among patients with familial Mediterranean fever (FMF) and end stage renal disease (ESRD), and controls

Allele 1	694	694	694	694	694	726	726	726	694*
Allele 2	694	?	726	148	694*	726	694	680	694*
North Africans									
FMF control	6	4	–	3	–	–	–	–	–
ESRD group	13	–	–	–	–	–	–	–	–
Palestinians									
FMF control	3	2	1	–	1	1	1	1	–
ESRD group	7	–	–	–	–	1†	–	–	2

694 = M694V; 694* = M694I; ? = none of the above mutations were found.
†Had no amyloid in rectal and renal biopsy specimens.

v 25.6 (13.9) years, p<0.02) occurred earlier in the North Africans, and they had a higher prevalence of joint involvement (9/13 *v* 2/10, p<0.05). Age at ESRD, however, was similar in both groups (33.3 (9.3) *v* 31.2 (11.8)) (table 1).

Analysis of FMF mutations disclosed that all the North African Jewish patients with ESRD were homozygous for M694V (table 2). Among the Palestinian patients with ESRD seven were homozygous for the M694V, two were homozygous for M694I, and one patient was homozygous for the V726A mutation. Review of the rectal and renal biopsies showed that 22 of the patients had evidence for amyloidosis, and one patient had only focal segmental glomerulosclerosis with no amyloidosis on rectal, renal, or transplanted kidney specimens. Opening the identifying code for the genotype of this patient disclosed that he was the one homozygous for the V726A mutation (table 2).

The clinical and demographic data of the control group were similar to those of the ESRD group (table 3). The frequency of FMF attacks and the cumulative dose of colchicine were similar in both groups. Genetic analysis of the control group showed that among the 13 patients with FMF of North African origin the mutation distribution was: six homozygous for the M694V mutation, seven heterozygous for this mutation, of whom three also bear the E148Q mutation (table 2). Analysis of the 10 Palestinian patients with FMF in the control group disclosed a higher variability in mutation combinations. Only three were homozygous for the M694V mutation and four were heterozygous for this mutation (table 2). In contrast with the North African control group, the Palestinian patients had a higher rate of V726A and none had the E148Q substitution.

Comparison of the frequency of homozygous 694 substitution in the ESRD and control groups showed a significantly greater frequency in the amyloid group (p<0.001). In fact, all the amyloid patients had a 694 substitution, most (21/23 (91%)) had the M694V and only 2/23 (9%), both Palestinians, had the M694I substitution.

Table 3 Comparison of clinical parameters between the group with end stage renal disease and the controls

	North African		*Palestinian Arabs*	
	Amyloid	*Control*	*Amyloid*	*Control*
Age* (mean (SD))	45.7 (7.6)	44.7 (7.3)	40.1 (11.3)	38.9 (11.1)
Age at onset of FMF	6.0 (4.1)	6.6 (3.7)	11.7 (7.1)	10.4 (5.4)
Disease duration†	27.2 (8.2)	27.9 (5.9)	19.3 (8.9)	24.7 (8.2)

*Age at the time of the study.
†In patient with amyloid: duration until renal failure. In controls: duration of the disease until the time of this study.

Discussion

Amyloidosis is the most serious complication of FMF, leading to ESRD. According to older publications amyloidosis occurred in 60% of Turkish patients, in 27% of the non-Ashkenazi Jews, and in only 1–2% of Armenians living in the United States.[11 12] The frequent occurrence of amyloidosis and its dramatic variation among different ethnic groups raises a question as to the inherent relation with FMF and whether it is transmitted by the gene for FMF or as a separate genetic trait. The fact that some subjects develop amyloidosis without febrile episodes (phenotype II) may suggest that the predilection for amyloid is a part of the underlying genetic background of FMF. Thus the search for a specific amyloid associated mutation may be a conceivable approach in order to test this hypothesis.

Colchicine has been shown to be effective in controlling attacks of FMF as well as preventing the development of amyloidosis.[13] Because most of the patients in Israel are treated with colchicine, it is quite difficult today to find patients with FMF with this complication, unless they are non-compliant. For that reason we chose to study the patients with FMF receiving dialysis or post-renal transplantation in order to analyse the distribution of mutations in this group and to compare it with a matched control group of patients with FMF without evidence of renal disease, reasoning that the distribution of amyloid associated mutations would be greater in the ESRD group. Of course, the absence of clinical renal disease does not exclude subclinical amyloidosis in the controls. However, the groups were matched for their age, length of disease activity, and colchicine treatment and would have been expected to behave similarly.

In the present study all the patients with FMF and amyloid induced ESRD were homozygous for a sequence alteration located to the 694 amino acid. The only patient who had renal failure owing to focal glomerulosclerosis was homozygous for the A726V mutation. Of special note is that the substitution in the 694 locus could be either V or I, suggesting that the location of the mutation rather than the type of the substitution is crucial for the association with amyloidosis. The fact that these mutations are in exon-10, an area encoding the functional portion of the protein, marenostrin/pyrin, may afford speculation upon its relation with the mechanism of amyloid production.

Because the prevalence of the M694V mutation is high among North African patients with FMF one may argue that the high association of this mutation with amyloidosis in our group with ESRD is expected. However, several points contradicting this explanation should be mentioned. Firstly, the prevalence of homozygosity in patients with FMF of North African origin who have no amyloidosis is far less than 100%. Many of these patients are heterozygotes or bear different mutations (table 2). In

a study by Dewalle *et al*, only 60 of 99 (61%) patients with FMF of North African origin were homozygous for the M694V mutation.[14] In another study Gershoni-Baruch *et al* found that only 118 of 160 (74%) chromosomes from North African patients with FMF bear this mutation.[15] Secondly, among the group of Palestinian patients with FMF, the prevalence of the M694V mutation was much less than that of the North African group and yet 100% of those who developed amyloidosis were homozygous for this 694 substitution (table 2). In the study of Gershoni-Baruch *et al* the prevalences of V726A, M680I, and M694V mutations in Palestinian patients with FMF were 22%, 20%, and 12.5% respectively.[15] Our results show that the prevalence of the M694V mutation among patients with FMF with amyloidosis is significantly higher than among North African and Palestinian patients with FMF without amyloidosis. These observations—although in a relatively small group—suggest that the M694V mutation either leads directly to the development of amyloidosis or is associated with other factors or processes leading to amyloid production. Our results are in agreement with other recent studies.[8 9 16]

The rate of amyloid deposition and thus the clinical expression of amyloid disease will theoretically depend on at least two factors: (*a*) the genetic predisposition to convert serum amyloid A (SAA) into AA amyloid fibrils, and (*b*) a sufficient supply of SAA. Conceivably, the supply of SSA differs between ESRD FMF M694 homozygotes and control FMF M694 homozygotes. This may be related to a number of factors, including differences in the punctuality of colchicine treatment, the occurrence of intercurrent other diseases (for example, infections, non-infectious inflammation, major surgery), or ethnic differences in disease expression. Support for these possibilities can be found in the observation that Armenians living in the United States have a very low prevalence of amyloidosis, whereas patients with FMF who live in Armenia have a higher prevalence of this complication.[12]

The mechanism by which a single mutation in MEFV gene can cause the production of amyloidosis is still obscure. Polymerisation of SAA into amyloid fibrils requires removal of the C-terminal third of the AA protein. Presumably, domains in this segment are incompatible with fibril formation. Because during FMF attacks there is an increase in the SAA serum levels it is possible that the defective protein, marenostrin/pyrin, loses its direct or indirect anti-amyloidotic effect, leading to a deposition of amyloid fibrils.

Several other points were found in the study. Firstly, the ages of FMF symptoms and diagnosis were significantly earlier in the North African group than those in the Palestinian group. It seems that greater awareness among the treating physicians in the North African group and a difference in medical care may explain this finding. Secondly, the North African patients with FMF had a significantly higher prevalence of joint disease than the Palestinian group, though both groups were homozygous for the M694V mutation. This observation suggests that the arthritis manifestation of FMF may be associated with ethnic factors rather than with the 694 mutation.

In summary, this study reports for the first time, the genetic analysis of a Palestinian Arab group of patients with FMF and amyloidosis. We found that amyloidosis is associated with homozygosity for the 694 substitution in North African Jews as well as in Palestinian Arab patients with FMF. Although the groups studied were relatively small, the observations are valid and in accord with findings of other recent publications. More studies are needed to elucidate the connection between the point mutations and amyloid production.

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