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Amyloidosis in familial Mediterranean fever patients: correlation with *MEFV* genotype and *SAA1* and *MICA* polymorphisms effects

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Abstract

Background: Familial mediterranean fever (FMF) is a recessively inherited disease characterized by recurrent crises of fever, abdominal, articular and/or thoracic pain. The most severe complication is the development of renal amyloidosis. Over 35 mutations have been discovered so far in the gene responsible for the disease, *MEFV*. This article aims at determining a correlation between the *MEFV* genotype and the occurence of amyloidosis in FMF patients, in addition to the study of the modifying effects of the *SAA1* (type 1 serum amyloid A protein) and *MICA* (Major Histocompatibility Complex (MHC) class-I-chain-related gene A) genes on this severe complication.

Methods: Fourteen *MEFV* mutations were screened and the *SAA1* and *MICA* polymorphisms tested in 30 FMF patients with amyloidosis and 40 FMF patients without amyloidosis.

Results: The M694V and V726A allelic frequencies were, respectively, significantly higher and lower in the group with amyloidosis, compared to the control FMF group. The beta and gamma *SAA1* alleles were more frequently encountered in the group without amyloidosis, whereas the alpha allele was significantly more observed in FMF patients with amyloidosis ($p < 0.025$). All the *MICA* alleles were encountered in both patients' groups, but none of them was significantly associated with amyloidosis.

Conclusions: The results suggest a protective effect of the *SAA1* beta and gamma alleles on the development of amyloidosis and show the absence of a *MICA* modifying effect on amyloidosis development. Testing these polymorphisms on a larger sample will lead to more definite conclusions.

Background

Familial mediterranean fever (FMF, MIM 249100) is a recessively inherited disease mostly prevalent in Jews, Armenians, Arabs and Turks [1]. It is characterized by

recurrent bouts of fever, accompanied by serosal inflammation, leading to abdominal, articular and/or thoracic pain. The most severe complication of FMF is the development of secondary renal amyloidosis, and two disease phenotypes have been identified, wether amyloidosis appears years after the appearance of the other clinical signs (phenotype I), or wether it is the first or the only FMF presenting sign (phenotype II), which is less frequent [1,2]. Over 35 mutations [3] have been discovered so far, in the gene responsible for the disease, *MEFV* [4,5]. The 5 most frequent mutations are M694V, M694I, V726A, M680I and E148Q.

Correlation of FMF patients genotypes with the various phenotypes of the disease, especially amyloidosis, has been often studied. The latter complication has been often associated with a specific mutation in exon 10, M694V, especially at the homozygous state [6-11], while others found that there was no specific genotype correlated with the development of amyloidosis [12]. Moreover, 2 genes, namely *SAA1* (type 1 serum amyloid A protein) and *MICA* (Major Histocompatibility Complex (MHC) class-I-chain-related gene A) have been investigated in FMF patients and were found to have an effect on amyloidosis and on the course of the disease respectively. The genotype alpha/alpha of the *SAA1* gene was associated with a sevenfold increase in the incidence of renal amyloidosis, especially in patients homozygous for M694V [13,14], whereas the *MICA* alleles A4 and A9 were associated with a diminished attacks frequency and an earlier age of onset, respectively, notably in patients homozygous for M694V [15].

The purpose of the present article is to determine a correlation between the *MEFV* genotype and the occurence of amyloidosis in FMF patients, in addition to the study of the modifying effects of the *SAA1* and *MICA* genes on this severe complication.

Methods

The main study group was composed of thirty Lebanese and Jordanian amyloidosis affected FMF patients from 24 non-related families. Renal amyloidosis was detected either by biopsy or persistent proteinuria. Thirteen of these patients were either having regular renal dialysis or had a renal transplantation. A comparative group was composed of 40 FMF patients, either homozygous or compound heterozygous for *MEFV* mutations, having an age superior to 20 years old. These patients did not have amyloidosis, nor did they have any sign of proteinuria, and were not under any medication, including colchicine, before the time of the study. Patients of both groups willingly filled up a questionnaire form where clinical and social information were recorded.

Blood samples were withdrawn from all the patients on EDTA, and DNA was extracted from leucocytes by standard technique [16].

Fourteen mutations previously detected in the Lebanese and Jordanian populations were screened (M694V, M694I, V726A, M680I, E148Q, E167D, T267I, R761H, P369S, A744S, F479L, I692del, M694del, K695R) as previously described [11,17].

SAA1 and *MICA* genes polymorphisms were tested in the 2 FMF patients' groups. The DNA fragment including the *SAA1* gene polymorphisms was amplified using the Saa1F (5'-GCCAATTACATCGGCTCAG-3') and Saa1R (5'-TGGCCAAAGAATCTCTGGAT-3') primers. The 2 polymorphisms of the SAA1 gene were determined with 2 restriction enzymes, BanI and BclI, that allowed the identification of the amino acids at positions 52 and 57, respectively [18]. Valine/Alanine, Alanine/Valine and Alanine/Alanine amino acids at positions 52/57 defined respectively the alpha, beta and gamma *SAA1* alleles.

The MICA transmembrane region polymorphism, which is a polyalanine repeat, was tested by genotyping using fluorescent dUTP (2'-deoxyuridine 5'-triphosphate) on an ABI 310 genetic analyzer. Sequencing was performed whenever needed to determine the different *MICA* alleles. The primers used to amplify the exon 5 polymorphism containing DNA fragment were Mica-5F (5'-CCTTTTTT-TCAGGGAAAGTGC-3') and Mica-5R (5'-CCTTAC-CATCTCCAGAAACTGC-3').

Chi-squared test was performed to test the significance of differences in FMF crises frequency and in *SAA1* genotypes and *MICA* alleles distribution between the 2 groups of FMF patients, with and without amyloidosis. A *P* value of 0.05 or less meant that the difference was statistically significant.

Results

Fifty percent and 20 % of the 24 unrelated amyloidosis affected FMF patients and of the control FMF group, respectively, were consanguineous ($p < 0.02$). All amyloidosis affected FMF patients in this study were of the phenotype I. Twelve of the unrelated amyloidosis affected patients never took colchicine before the development of renal amyloidosis, one patient was not taking it regularly, 3 of them stopped taking it after having taken it for some years, in one case because of the inefficacy of the drug in relieving the patient from painful crises.

Ages at the moment of diagnosis of FMF varied from 3 to 50 years, and from 7 to 44 years, in the groups of patients with and without amyloidosis, respectively. Diagnosis was mainly made between 20 and 35 years of age in

Table 1: Genotypes of the FMF patients with and without amyloidosis

Genotypes	FMF patients with amyloidosis	FMF patients without amyloidosis
M694V/M694V	17	6
V726A/M680I	3	7
M694V/V726A	2	4
M694V/M694I	2	1
M694V/M680I	1	1
V726A/F479L	1	0
M694I/M694I	1	3
M694I/M680I	1	1
V726A/M694I	0	4
V726A/V726A	0	6
M680I/M680I	0	1
M694V/E148Q	0	1
M694V/E148Q/I692del	0	1
V726A/E148Q	0	1
V726A/E148Q/E148Q	0	1
V726A/R761H	0	1
V726A/K695R	0	1
M694V/?	1	-
V726A/?	1	-
Total	30	40

Table 2: Number of SAA1 genotypes observed in FMF patients with and without amyloidosis.

Genotypes	Alpha/alpha	Alpha/beta	Beta/beta	Beta/gamma*	Total
FMF patients with amyloidosis	11	16	3	0	30
FMF patients without amyloidosis	10	15	11	4	40
Total	21	31	14	4	70

*Once the genotypes beta/beta and beta/gamma are grouped, $X^2 = 6.789$ ($p < 0.05$)

patients without amyloidosis, whereas the disease manifested earlier, mostly between 10 and 20 years, in patients with amyloidosis ($p < 0.005$). Moreover, patients with amyloidosis had a significantly higher crises frequency, compared to the FMF control group ($p < 0.02$).

The genotypes of the FMF patients with and without amyloidosis are shown in table 1. The 6 siblings in the group with amyloidosis were homozygous for M694V. The M694V, V726A and M694I allelic frequencies were respectively 67%, 12%, and 8% in the group of patients with amyloidosis, and 25%, 39% and and 15% in patients without amyloidosis ($p < 0.001$).

The alpha, beta and gamma *SAA1* alleles were encountered in patients without amyloidosis, whereas only the alpha and beta alleles were detected in amyloidosis patients. Table 2 shows the allele combinations detected in the patients. Once the beta/beta and beta/gamma genotypes were grouped, the difference between the 2 groups was significant ($p < 0.05$). Comparison of the alpha and beta allelic frequencies in the 2 groups of patients was also significant ($p < 0.025$). However, the distribution of the *SAA1* alleles among the M694V homozygous FMF patients with amyloidosis was not different from the M694V homozygous patients without amyloidosis.

All the different *MICA* alleles were encountered in both patients' groups. Once the 2 groups were combined, no specific *MICA* allele was significantly associated neither with the age of onset of the disease, nor with the attacks frequency. Moreover, no specific *MICA* genotype or allele was significantly associated with the development of amyloidosis (Table 3), even in the M694V homozygotes subgroup.

Discussion

Familial mediterranean fever is a clinically heterogeneous disease. The quite variable phenotypes among FMF patients have suggested specific mutational effects as well

Table 3: Distribution of *MICA* alleles in the 2 groups of FMF patients, with and without amyloidosis (Non significant)

MICA alleles	A4	A5	A5.1	A6	A9	Total
FMF patients with amyloidosis	7	13	12	19	9	60
FMF patients without amyloidosis	15	12	13	30	10	80
Total	22	25	25	49	19	140

as the presence of some genes that would affect the appearance of the different clinical signs, especially amyloidosis. The significantly more important consanguinity in the group with amyloidosis ($p < 0.02$), compared with the control group, suggests a recessive modifying aspect of this phenotype. The present study associates amyloidosis with some aspects of disease severity, and with the specific mutation M694V, although it points out the involvment of other mutations in the occurence of this severe phenotype. Moreover, it associates *SAA1* alleles with the presence or absence of amyloidosis, whereas *MICA* was not found to have any association with amyloidosis.

The development of glomerular renal amyloidosis in FMF patients, unlike the secondary amyloidosis that occurs in other different diseases, was not previously associated with the frequency, nor the severity of the febrile crises [19]. The development of amyloidosis in patients having never experienced FMF attacks (phenotype II) is in favor of the lack of association between amyloidosis and FMF severity. However, in the present study, amyloidosis development in FMF patients has been significantly associated with a beginning of the disease prior to 20 years old ($p < 0.005$), and with a higher FMF crises frequency ($p < 0.02$), compared with the control FMF group, which allowed the association of amyloidosis with FMF severity in phenotype I patients.

Contradictory genotype-phenotype studies tried to associate amyloidosis with a specific mutation or genotype. The M694V mutation, that has often been correlated with a severe FMF phenotype, has also been correlated, especially at the homozygous state, with amyloidosis development [6,7,9,14], whereas a study on Turkish families presenting FMF patients with amyloidosis revealed that the M694V mutation was not the only mutation presenting a risk for amyloidosis development [12]. In the present study, a significant increase in the number of patients homozygous for the M694V mutation was noted in the group with amyloidosis ($p < 0.001$), compared to all the other genotypes. In parallel, the M694V allelic frequency was significantly higher and the V726A allelic frequency significantly lower in the group with amyloidosis, compared to the control FMF group ($p < 0.001$). However, the simultaneous occurence of V726A with another mutation such as M694V or M680I seems to modify the mild effect of V726A, and thus increase the severity of the disease. Indeed, 3 FMF patients with amyloidosis were compound heterozygous for V726A and M680I (Table 1). The latter genotype was also previously encountered in FMF patients with amyloidosis [7].

The serum amyloid protein (SAA) is an acute phase protein which serum level increases considerably during inflammation and leads, by proteolytic cleavage, to amyloid A proteins, the major fibrillar proteins in secondary amyloidosis [20]. Two isotypes, SAA1 and SAA2, with quite homologous sequences have been distinguished, and allelic forms of these isotypes have been identified [21]. The SAA1 allelic forms have been associated with the incidence of amyloidosis in different diseases and various populations. The SAA1 alpha/alpha genotype has already been associated with the occurence of amyloidosis in Armenian FMF patients [13], whereas both the alpha SAA1 allele and the alpha/alpha genotype were significantly associated with this phenotype in Caucasian juvenile chronic arthritis patients [22]. In the present study, the beta/beta and beta/gamma *SAA1* genotypes, as well as the alleles beta and gamma, were more frequently encountered in the group without amyloidosis than in the group with amyloidosis, whereas the alpha/alpha and the alpha/beta genotypes, and hence the alpha allele were much more observed in patients with amyloidosis (Table 2). This study shows therefore the association of the alpha allele with this severe phenotype, as well as the protective effect of the beta and gamma alleles on the development of amyloidosis in FMF patients, an effect that was not mentioned previously.

The *MICA* gene has been recently associated with different inflammatory diseases. The MICA transmembrane exon 5 polymorphism consisting of a (GCT/AGC)n triplet repetition has been particularly studied. Five alleles have been identified so far: A4, A5, A6, A9, corresponding to 4, 5, 6 and 9 repetitions and A5.1, corresponding to a 5 triplet repetition and the insertion of one nucleotide (G/C). MICA appears to be a susceptibility factor in many multifactorial diseases such as Behcet's disease [23], psoriatic arthritis [24] and diabetes [25,26]. In FMF, the first monogenic disease associated with *MICA*, the latter was found to have a modifying effect on the course of the disease, namely on the attacks frequency and the age of onset, but

not on its clinical manifestations [15]. As it was not, however, tested in FMF patients having developed renal amyloidosis, we tried to investigate any possible correlation between the *MICA* polymorphism and this specific phenotype. The *MICA* alleles and genotypes were found in a non-significant way in both FMF groups, with and without amyloidosis. Hence, no clear cut association was detected between any *MICA* allele or genotype and this severe phenotype (Table 3). Moreover, the lack of association between any *MICA* allele and the age of onset of the disease on one hand, and the attack frequency on the other hand, in the combined group of FMF patients, is in contradiction with previously published results, that correlated the A4 and the A9 *MICA* alleles with a reduced frequency of attacks and an early age of onset, respectively [15]. Such observation could suggest either a weak effect of MICA hidden by a stronger effect of *MEFV* or *SAA1* genotypes, or a possible varying effect of *MICA* on the course of the disease in different populations, which could be expected in the case of genes that have minor modifying effects on a disease phenotype.

Conclusions

With the availability of the genetic FMF test, since the cloning of the *MEFV* gene in 1997, and the automatic prescription of colchicine to the confirmed patients, amyloidosis is being far less encountered. But the reason why it develops in some patients, not in others, is still unknown. The effect of the *MEFV* genotypes, mainly the ones homozygous for the M694V mutation, seems evident, but there are definitely other modifying factors or genes that play a role in the manifestation of amyloidosis. In the present study, no association was found between the *MICA* gene exon 5 polymorphism and the development of renal amyloidosis in FMF patients, whereas the alpha allele and the alpha/alpha genotype of the *SAA1* gene were associated with this severe phenotype, and the beta and gamma SAA1 alleles showed a protective effect on its development. Testing these polymorphisms on a larger sample will allow more accurate results and will lead to more definite conclusions.

Authors' contributions

Myrna Medlej-Hashim looked for the patients, tested the *SAA1* and *MICA* alleles and wrote the article. Valérie Delague helped in the recruitment of Lebanese FMF patients and *MICA* alleles' analysis. Eliane Chouery and Nabiha Salem helped in DNA extraction from patients' blood leucocytes and testing of the *MEFV* genotype. Mohammed Rawashdeh helped in the recruitment of Jordanian FMF patients with amyloidosis. Gérard Lefranc participated in the study design and coordination, Jacques Loiselet helped in the results analysis and statistical calculations and André Mégarbané directed and supervised the study. All authors read and approved the final manuscript.

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