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Genetic Risk Factors of Amyloidogenesis in Familial Mediterranean Fever

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Key Words
Familial Mediterranean fever · Amyloidosis · Familial Mediterranean fever gene · Serum amyloid A1 gene · Childhood

Abstract
Background/Aims: Evaluation of the risk factors, and phenotype-genotype correlation of familial Mediterranean fever (FMF) gene (MEFV) and serum amyloid A1 (SAA1) gene polymorphisms in renal amyloidosis. ***Methods:*** We investigated MEFV and SAA1 genotypes (α, β, and γ isoforms) in 50 FMF patients and 50 healthy children. Tel-Hashomer criteria were used for the diagnosis and severity scoring of FMF. ***Results:*** The most common MEFV mutation and SAA1 genotype were M694V/M694V (n = 26/50) and SAA1 α/α (n = 26/50), respectively. Positive family history for amyloidosis was significantly higher ($p < 0.001$) with more severe clinical course ($p = 0.006$) in the amyloidosis group than the non-amyloid group. In M694V/M694V mutation, erysipelas-like skin erythema ($p = 0.029$), arthritis ($p = 0.004$), arthralgia ($p < 0.001$) were significantly more frequent with higher severity scores ($p = 0.008$) than the patients with other mutations. Comparison of the SAA1 α/α genotype with other genotypes revealed more frequent arthritis ($p = 0.003$) in the SAA1 α/α genotype. In amyloidosis group patients having both M694V/M694V and SAA1 α/α genotypes were the largest subgroup (n = 14, $p < 0.001$). Logistic regression analysis for amyloidosis corrected risk revealed a 1.2 times increase in M694V/M694V, a 2.4 times increase in SAA1 α/α genotypes and a 2.5 times increase when both are together. ***Conclusion:*** Positive family history for amyloidosis and presence of SAA1 α/α genotype in M694V/M694V mutation may predispose to amyloidosis by increasing the clinical severity. Therefore, in such children early colchicine treatment might be recommended even if they are asymptomatic.

Introduction

Familial Mediterranean fever (FMF) is an autosomal-recessive disease characterized by recurrent fever and inflammation of the serosal membranes, which is predominantly seen in various Mediterranean populations including Turks, Jews, Armenians, and Arabs. Clinical presentation may vary and the most serious complication is renal amyloidosis that carries the potential risk for end-stage renal disease. The incidence of amyloidosis is relatively higher in Turks compared to other Mediterranean populations [1–4]. It has been reported that FMF is the underlying disease in 64% of secondary renal amyloidosis cases in Turkey [5].

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In 1997, FMF gene (MEFV) was defined on chromosome 16 and was shown to encode the protein named as 'pyrin' or 'marenostrin', which is mainly involved in the regulation of neutrophil inflammation [6, 7]. Until now about 85 mutations of this gene have been reported [8, 9]. The mutated pyrin in FMF undergoes structural changes leading to exaggerated leukocyte migration to serosal surfaces and inflammation and certain mutations have been reported to be correlated with renal amyloidosis [6, 7]. For example, homozygous M694V, being the most frequent MEFV mutation in the Turkish population as well, is reported to be associated with a more severe clinical picture and more frequent amyloidosis, while some other mutations like V726A seem to have a somewhat protective effect against amyloidosis. There is insufficient data regarding the impact of other less prevalent MEFV mutations such as M680I on amyloidogenesis, that needs to be elucidated [6, 7, 10].

Amyloidosis seen in FMF is of the AA type. Amyloid A proteins (SAA1 and SAA2) are acute-phase proteins, the major source of which is liver. And their 7,500-dalton cleavage products comprise the amyloid fibrils. In secondary amyloidosis SAA1 comprises the majority of the amyloid deposition. There are three isoforms of serum amyloid A1 (SAA1 α, SAA1 β, and SAA1 γ) [11–15]. During the inflammation, as in FMF attacks, IL-6 induces hepatic transcription of SAA mRNA and production of SAA increases about 1,000-fold. Catabolism of overproduced SAA protein is disturbed and it is deposited in the form of protease-resistant fibrils mainly in phagocytic cells in tissues. In SAA1 gene polymorphism especially the SAA1 α/α genotype has been found to facilitate the development of amyloidosis in FMF patients [16–20].

In this study, we aimed to evaluate the risk factors for the development of renal amyloidosis in Turkish children with FMF and to determine the phenotype-genotype correlation of MEFV and SAA1 gene polymorphisms with amyloidosis.

Patients and Methods

In this study 50 children with FMF and 50 age- and sex-matched healthy children as a control group were investigated for MEFV and SAA1 gene mutations. Informed consent was obtained from the parents. Tel-Hashomer criteria were used for the diagnosis of FMF [21]. Clinical severity was defined as mild (severity scores 2–5), moderate (severity scores 6–10), or severe (severity scores >10) according to the 'Tel-Hashomer key to severity score' [22]. Renal amyloidosis was confirmed with renal biopsy in all patients.

The patients were divided into two groups according to the presence or absence of amyloidosis and both groups were compared with each other in terms of clinical features, laboratory findings, severity of the disease, MEFV and SAA1 gene polymorphisms. They were also grouped according to MEFV mutations as patients with M694V/M694V mutation and patients with other MEFV mutations; according to SAA1 gene polymorphism as patients with SAA1 α/α genotype and patients with other SAA1 mutations. These groups were also compared with each other according to clinical and laboratory findings, severity of the disease, and development of amyloidosis. Additionally clinical pictures of the patients were compared according to the number of MEFV gene alleles carrying the mutation (two alleles, one allele, or none).

SAA1 gene polymorphism was compared between patients and control group and between amyloid patients and control group. Furthermore, nonamyloid patients with M694V/M694V, M694V/M680I, and M694V/V726A mutations were compared with each other and with the control and amyloid group for SAA1 gene polymorphism.

Clinical picture, MEFV and SAA1 gene polymorphisms were analyzed as the risk factors for amyloidosis.

Methods

Detection of MEFV Gene Mutations: Polymerase Chain Reaction (PCR) and Restriction Endonuclease Digestion

Genomic DNA was isolated from peripheral leukocytes using standard procedures. In all patients the hot spot exon 10, which harbors 18 mutations of the MEFV gene, was first analyzed by denaturing gradient gel electrophoresis (DGGE) [23]. The amplification conditions were 94°C for 2 min followed by 35 cycles of 30 s at 94°C, 30 s at 61°C and 30 s at 72°C, followed by a 2-min incubation at 72°C. According to the band pattern, subsequent analysis was done by restriction endonuclease enzyme digestion. Furthermore, exon 2 was also analyzed by restriction endonuclease enzyme digestion of PCR products from genomic DNA. The PCR products were separated on 8% nondenaturating polyacrylamide gel, stained with ethidium bromide, and visualized under an ultraviolet lamp.

Analysis of Polymorphism in SAA1 Genes

The SAA1α, SAA1β and SAA1γ isoforms are encoded by the V52-A57, A52-V57 and A52-A57 SAA1 alleles, respectively [24]. The PCR products from the SAA1 gene were digested with *Bcl*I and *Ban*I restriction enzymes to detect α, β, and γ alleles (fig. 1).

Statistical Analysis

The Statistical Package for Social Science for Windows 10.0 (SPSS Inc., Chicago, Ill., USA) program was used. The statistical significance of differences between groups was calculated by either χ^2 test for categorical data or t test for quantitative data. All statistical tests were 2-sided. Kruskal-Wallis test was used for variance analysis. For survival analysis Kaplan-Meier and Cox regression analyses were used. Logistic regression analysis was used to study the contribution of independent variables to the development of amyloidosis. Results were given as the odds ratio (OR) with 95% confidence interval (95% CI) and corrected risk ratio was calculated according to the formula in the publication of Zhang et al. [25].

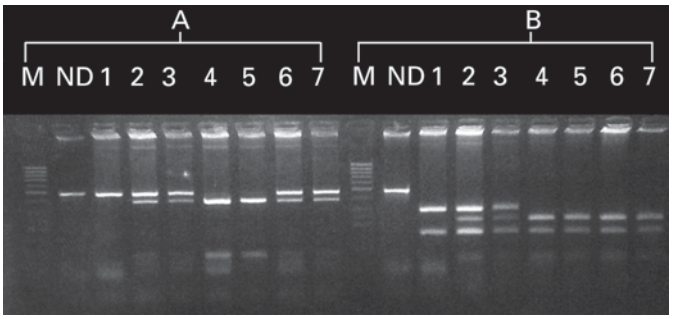

Fig. 1. Polymerase chain reaction-restriction fragment polymorphism (PCR-RFLP analysis of SAA1). **A** *Bcl*I digestion of SAA1 gene. **B** *Ban*I digestion of SAA1 gene. M = 50-bp DNA ladder; ND = non-digested PCR products. DNA samples: 1 = α/α, 2 = α/β, 3 = α/β, 4 = β/β, 5 = β/β, 6 = γ/β, 7 = γ/β.

Results

There were 50 patients (19 male, 31 female) with FMF with a mean age of 12.3 ± 0.6 years (range: 4–24 years) in the study. Twenty-seven of them had developed renal amyloidosis. According to severity scores 9 patients (18%) had mild, 20 (40%) moderate and 21 (42%) severe disease. Renal functions were normal in the nonamyloid and control groups. But in the amyloid group 4 patients were in the dialysis program and 7 patients had elevated blood urea nitrogen or creatinine levels. However, no statistically significant difference between renal functions of the patients according to their genotypes (MEFV and SAA1) was detected.

As shown in table 1, the majority of the patients had homozygous M694V MEFV mutation (52%) and α/α SAA1 genotype (52%). In the amyloidosis group homozygous M694V mutation was found in 16 patients, heterozygous M694V mutations in 5 and R726H/(–) and M680I/M680I mutations in 1 patient each (table 2). There were no mutations in 4 patients. In the non-amyloidosis FMF group 10 patients (20%) had M694V/M694V, 10 patients (20%) M694V/M680I and 3 patients (6%) M694V/V726A mutations. Homozygous M694V mutation was seen more frequently in amyloid group than the nonamyloid group. But the difference was not statistically significant (p = 0.255). SAA1 α/α genotype was detected significantly more frequent than the other SAA1 genotypes in amyloidosis group ($p < 0.001$). Similarly, the association of M694V/M694V and SAA1 α/α genotypes was significantly more frequent in amyloidosis group when compared with other mutations ($p < 0.001$).

Table 1. Distrubition of MEFV mutations and SAA1 gene polymorphisms in patients and control group

	FMF patients			Control group (n = 50)
	amyloid (–) (n = 23)	amyloid (+) (n = 27)	total (n = 50)	
MEFV mutations				
M694V/M694V	10	16	26	
M694V/M680I	10	1	11	
M694V/V726A	3	1	4	
M694V/(–)		3	3	
M680I/M680I		1	1	
R761H/(–)		1	1	
(–)/(–)		4	4	50
SAA1 gene polymorphism				
α/α	5	21	26	12
α/β	10	6	16	24
β/β	6		6	10
γ/β	2		2	1
α/γ				3

Table 2. Distrubition of MEFV mutations and SAA1 gene polymorphisms in patients and control group

MEFV mutations	SAA1 genotype	Amyloid (+) (n = 27)	Amyloid (–) (n = 23)	Total (n = 50)	Control (n = 50)
M694V/M694V	α/α	14	2	16	
	α/β	2	5	7	
	β/β		3	3	
M694V/M680I	α/α	1	3	4	
	α/β		4	4	
	β/β		1	1	
	γ/β		2	2	
M694V/V726A	α/α	1		1	
	α/β		1	1	
	β/β		2	2	
M694V/(–)	α/α	1		1	
	α/β	2		2	
M680I/M680I	α/α	1		1	
R761H/(–)	α/β	1		1	
(–)/(–)	α/α	3		3	12
	α/β	1		1	24
	α/γ				1
	β/β				10
	γ/β				3

Table 3. Clinical characteristics according to the MEFV and the SAA1 genotype

Clinical characteristics	Patients (n = 50)	MEFV genotype			SAA1 genotype		
		M694V/M694V (n = 27)	other/other (n = 23)	p	α/α (n = 26)	other/other (n = 24)	p
Fever	48	26	22	1	24	24	0.491
Peritonitis	47	26	21	0.588	23	24	0.236
Pleuritis	11	7	4	0.515	6	5	1.000
Arthritis	27	20	7	0.004	19	8	0.010
Erysipelas	15	12	3	0.029	7	8	0.760
Myalgia	22	15	7	0.093	11	11	1.000
Arthralgia	28	21	7	<0.001	16	12	0.569
Amyloidosis	27	16	11	0.255	21	6	<0.001
Severity score	9.26 ± 3.67	10.52 ± 3.67	7.78 ± 3.13	0.008	10.69 ± 3.07	7.71 ± 3.69	0.003

Table 4. Risk factors in development of amyloidosis

	Crude OR (95% CI)	Corrected RR (95% CI)
Male sex	0.947 (0.466–1.922)	0.971 (0.622–1.011)
Female sex	1.034 (0.668–1.601)	1.018 (0.785–1.260)
M694V/M694V mutation	1.448 (0.837–2.506)	1.242 (0.890–1.609)
SAA1 α/α gene polymorphism	3.578 (1.606–7.972)	2.401 (1.440–3.429)
M694V/M694V(–) SAA1 α/α	6.389 (1.629–25.057)	2.546 (1.385–3.239)
Arthritis attack	2.981 (1.456–6.106)	2.076 (1.323–2.875)

OR = Odds ratio; CI = confidence interval; RR = indicates risk ratio.

The between-group genotypic comparisons were done. Significant differences were detected in severity score and amyloid development rate between M694V/M694V and M694V/M680I in favor of latter (p = 0.034 and 0.003). Similar differences were detected between M694V/(–) and M694V/M680I genotypes (p = 0.022 and 0.011). Age of onset and amyloid development rate were also significantly different between M694V/M680I genotype and controls (p = 0.040 and 0.006). In the control group none of the studied MEFV mutations were detected.

When FMF patients were compared with the control group in terms of SAA1 genotype, the SAA1 α/α genotype was significantly more frequent in the patient group (p = 0.004). This difference was more significant in the amyloidosis group when compared with the control group (p < 0.001). However, there was no difference between the nonamyloid group and the control group (table 1).

When the amyloid and nonamyloid groups were compared with each other, family history of amyloidosis (p = 0.006), severity scores (p < 0.001), arthritis (p = 0.004) and arthralgia (p = 0.001) attacks were significantly higher in the amyloidosis group. In the group with M694V/M694V mutation arthritis, erysipelas-like erythema (p = 0.029), arthralgia (p < 0.001) and severity scores were significantly higher than in the patients with other mutations (table 3). In patients with SAA1 α/α genotype mean severity score (p = 0.003), arthritis (p = 0.010) and amyloidosis (p < 0.001) were significantly higher compared with other SAA1 groups. A logistic regression analysis for the risk of development of amyloidosis revealed a 1.2 times increase in risk in the M694V/M694V genotype (crude OR 1.448, 95% CI 0.837–2.506, corrected RR 1.242, 95% CI 0.890–1.609), a 2.4 times increase in SAA1 α/α genotype (crude OR 3.578, 95% CI 1.606–7.972, corrected RR 2.401, 95% CI 1.440–3.249), a 2.5 times increase in M694V/M694V and SAA1 α/α genotypes together (crude OR 6.389, 95% CI 1.629–25.057, corrected RR 2.546, 95% CI 1.385–3.239) and a 2 times increase in patients having arthritis attack (crude OR 2.981, 95% CI 1.456–6.106, corrected RR 2.076 95% CI 1.323–2.875) (table 4).

The median survival of the patients was 86 ± 27 months (95% CI 32–140). Cox regression analysis revealed significantly shorter survival times in patients with SAA1 α/α genotype (p = 0.029).

Discussion

The most important clinical presentation determining the prognosis of FMF is amyloidosis that might occur in up to 90% of untreated patients until 40 years of age [3]. The rate of amyloidosis varies because of genetic and environmental factors even with the same population such as the lower prevalence of amyloidosis in American Armenians than those living in Armenia [26]. Detection of the MEFV gene brought important explanations for the pathogenesis of FMF and genotype-phenotype analysis has been made in various ethnic groups. In many studies the presence of the M694V mutation was reported to increase the risk of renal amyloidosis [26–30]. The homozygous M694V mutation was also reported to be associated with a more severe clinical course and higher risk to develop renal amyloidosis when compared with patients carrying the V726A mutation [6, 7]. Cazeneuve et al. [10] found similar results for this genotype in terms of prevalence of amyloidosis and arthritis in 90 Armenian FMF patients. Gershoni-Baruch et al. [31] evaluated 124 FMF patients 47 of whom had amyloidosis, and they reported that all the patients had M694V homozygous mutation. They also reported a positive correlation between male sex, arthritis and amyloidosis. Similarly, we found the M694V/M694V genotype as the most common MEFV mutation and showed a positive correlation between amyloidosis and arthritis/arthralgia attacks with this mutation. Mutations such as complex E148Q-V726A other than M694V were also reported to be associated with a tendency for amyloidosis in recent studies [17, 27]. Yalçınkaya et al. [32] reported that a severe clinical picture and renal amyloidosis had developed in M680I, V726A mutations in untreated Turkish patients as well. The same investigators, in another study with 238 FMF patients, reported no association between M694V mutation and renal amyloidosis except for a negative correlation between homozygous M680I mutation and arthritis frequency [33]. Other studies in the Turkish population also reported an increased amyloidosis rate with the Ala138Gly mutation (second exon) or homozygous M694V genotype being the most common mutation in phenotype II FMF patients [34, 35]. Finally, the Turkish FMF Study Group reported earlier onset and significantly more frequent arthritis/arthralgia in patients with the M694V/M694V mutation (p = 0.001, p = 0.001 and p < 0.05) but without any genotype correlation with amyloidosis. Therefore, they proposed to investigate other genetic factors that might predispose to amyloidosis [36]. Association of more severe clinical picture with homozygous M694V mutation was encountered in our patients too. However, we also showed the correlation of this mutation with amyloidosis like Balcı et al. [35]. All these studies on FMF in the Turkish population signify the presence of other genetic and environmental factors involved in the development of amyloidosis.

Homozygous M694V mutations, early onset, arthritis, erysipelas-like rash episodes and requirement of higher doses of colchicine are the most commonly reported factors facilitating the development of amyloidosis [27, 30, 31]. However, controversial results do exist such as the occurrence of different phenotypes in the same family or even with the same genotype, suggesting a role of other environmental and genetic modifiers [26]. With this objective Touitou el al. [37] found an association of major histocompatibility complex chain-related gene A9 (MICA9) with the homozygous M694V mutation. On the other hand, the presence of MICA A4 was reported to decrease the attack rate of FMF. Baba et al. [13] showed the allelic variants of the SAA1 gene and their importance in the development of AA amyloidosis. Locus polymorphism of SAA1 or SAA1 α/α genotype in FMF patients have been found to facilitate the development of amyloidosis. But no relation between SAA1 and amyloidosis could be demonstrated. Cazeneuve et al. [16] reported a 7-fold increased risk for amyloidosis in the SAA1 α/α genotype by detecting the relation between the SAA1 and SAA2 genotypes in FMF patients. Yılmaz et al. [18] evaluated the SAA and TNFα gene polymorphisms in 173 Turkish patients with amyloidosis. The frequency of SAA1 α/α was significantly higher in patients with amyloidosis but no correlation between SAA2 and TNFα could be detected. Akar et al. [19] also found a higher frequency of the SAA1 α/α gene in amyloidosis patients and showed a statistically 2.5-fold higher risk of amyloidosis in FMF patients having this gene than in patients with amyloidosis due to diseases other than FMF. Medlej-Hashim et al. [20] studied SAA1 and MICA polymorphisms in FMF patients and suggested a protective effect for SAA1 β and γ alleles on the development of amyloidosis. They also reported that the absence of MICA had a modifying effect on amyloidosis development. In our study, we found that the SAA1 α/α genotype had a role in the development of amyloidosis and patients having

this genotype had shorter survival (p = 0.029), more frequent arthritis attacks and higher mean severity scores. When both M694V/M694V and SAA1 α/α genotypes were together, the amyloidosis risk increased much more.

The mechanism responsible for the SAA1 α/α genotype to predispose amyloidosis is not clear. Many hypotheses have been proposed. One of these is that SAA1 α/α gene product is more potent inflammatory protein than the other SAA products. Amyloid A protein composed of SAA1 is deposited more easily and metabolized minimally. In severe inflammation, the SAA level is elevated leading to amyloidosis. Association of amyloidosis with more frequent arthritis attacks may also be due to elevated SAA levels in FMF patients.

In conclusion, the factors affecting severity of disease and amyloidogenesis in FMF are complex and plenty as in many other genetic diseases. MEFV and SAA1 loci and presence of arthritis seem to be the most important ones. In FMF, positive family history for amyloidosis and presence of SAA1 α/α genotype in M694V/M694V mutation probably cause perdisposition to amyloidosis by increasing disease severity. Therefore, early colchicine treatment might be recommended in asymptomatic children with a positive family history of amyloidosis due to FMF, when they are detected to have M694V/M694V mutation and SAA1 α/α genotype. Of course, mostly mild but sometimes severe side effects of colchicine in children such as diarrhea, vomiting, bone marrow suppression, myopathy, neuropathy, oligo- or azoospermia and many others should be kept in mind by the clinician. But the probable risk of amyloidosis in defined population seems to overcome this concern [4, 38]. However, since our data set is small the analyses are subject to type 1 error. Therefore, further studies with a larger number of patients investigating the other genetic and environmental factors are also needed.

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