

Influence of Serum Amyloid A (SAA1) and SAA2 Gene Polymorphisms on Renal Amyloidosis, and on SAA/C-Reactive Protein Values in Patients with Familial Mediterranean Fever in the Turkish Population

AYSIN BAKKALOGLU, ALI DUZOVA, SEZA OZEN, BANU BALCI, NESRIN BESBAS, REZAN TOPALOGLU, FATIH OZALTIN, and ENGIN YILMAZ

ABSTRACT. Objective. To evaluate the effect of serum amyloid A (SAA) 1 and SAA2 gene polymorphisms on SAA levels and renal amyloidosis in Turkish patients with familial Mediterranean fever (FMF).

Methods. SAA1 and SAA2 gene polymorphisms and SAA levels were determined in 74 patients with FMF (39 female, 35 male; median age 11.5 yrs, range 1.0–23.0). All patients were on colchicine therapy. SAA1 and SAA2 gene polymorphisms were analyzed using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). SAA and C-reactive protein (CRP) values were measured and SAA/CRP values were calculated.

Results. The median SAA level was 75 ng/ml (range 10.2–1500). SAA1 gene polymorphisms were: α/α genotype in 23 patients (31.1%), α/β genotype in 30 patients (40.5%), α/γ genotype in one patient (1.4%), β/β genotype in 14 patients (18.9%), β/γ genotype in 5 patients (6.8%), and γ/γ genotype in one patient (1.4%). Of the 23 patients who had α/α genotype for the SAA1 polymorphism, 7 patients had developed renal amyloidosis (30.4%) compared to only one patient without this genotype (1/51; 2.0%); $p < 0.001$. SAA2 had no effect on renal amyloidosis. SAA1 and SAA2 genotypes had no significant effect on SAA levels. SAA/CRP values were significantly lower in patients with the SAA1 α/α genotype, compared to other SAA1 genotypes: 0.16 (0.025–1.96) versus 0.23 (0.012–28.20), $p < 0.05$.

Conclusion. SAA1 α/α genotype is one genetic factor that confers a significant risk for amyloidosis in the Turkish FMF population. Neither the SAA1 nor SAA2 genotypes had a significant effect on SAA level. (J Rheumatol 2004;31:1139–42)

Key Indexing Terms:

FAMILIAL MEDITERRANEAN FEVER
SERUM AMYLOID A

AMYLOIDOSIS
POLYMORPHISM

Familial Mediterranean fever (FMF) is a recessively inherited disorder. The diagnosis is based on the occurrence of recurrent episodes of fever and serous inflammation manifested by sterile peritonitis, arthritis, and pleurisy. The disease primarily affects Sephardic Jews, Armenians, Turks, and North African Arabs. Amyloidosis of AA type, mainly renal, is the major complication of FMF¹. It develops over years and progresses to renal failure.

From the Departments of Pediatrics, Pediatric Nephrology, and Medical Biology, and the Rheumatology Unit, Hacettepe University Faculty of Medicine, Ankara, Turkey.

A. Bakkaloglu, MD, Professor of Pediatrics; A. Duzova, MD, Assistant Professor of Pediatrics; S. Ozen, MD, Professor of Pediatrics; N. Besbas, MD, Professor of Pediatrics; R. Topaloglu, MD, Professor of Pediatrics; F. Ozaltin, MD, Fellow in Pediatric Nephrology, Department of Pediatrics, Pediatric Nephrology and the Rheumatology Unit; B. Balci, PhD; E. Yilmaz, PhD, Associate Professor of Medical Biology, Department of Medical Biology.

*Address reprint requests to Dr. A. Bakkaloglu, Department of Pediatrics, Hacettepe University Faculty of Medicine, 06100, Ankara, Turkey.
E-mail: aysin@hacettepe.edu.tr*

Submitted August 26, 2002; revision accepted January 6, 2004.

In 1997, 2 independent groups (French FMF Consortium and International FMF Consortium) identified the gene responsible for FMF, designated MEFV, on chromosome 16 (16p13.3)^{2,3}. The most common MEFV mutations are M694V, M680I, V726A, and M694I located on exon 10 and E148Q located on exon 2. Genotype-phenotype correlation is not well established. There are unexplained ethnic differences in the rates of amyloidosis^{1,4-6}. Although early data suggested that M694V mutation was a risk factor for amyloidosis, there are also patients with FMF who have renal amyloidosis and do not carry the M694V homozygous genotype⁷⁻¹⁰. Unidentified genetic and environmental factors might have a protective or exacerbating effect on renal amyloidosis.

Serum amyloid A (SAA) protein family is known to contain a number of differentially expressed apolipoproteins that are synthesized primarily by the liver. The concentration of acute phase SAA can increase to more than 1000-fold during inflammation¹¹. Its sensitivity was shown to be equal or more than that of C-reactive protein (CRP) in

several studies; persistently high SAA levels were correlated with activity of rheumatic disease, rapid progression of secondary amyloidosis, and poor outcome in patients undergoing certain surgical interventions^{12,13}.

Acute phase isotypes of human SAA are encoded at 2 different loci, SAA1 and SAA2. SAA3 is a pseudogene¹⁴. It has been suggested that AA-fibrils deposited in tissues were predominantly derived from SAA1 α ¹⁵. SAA1 and SAA2 are serum precursors of amyloid A1 (AA1) and A2 (AA2) proteins, the principal components of the secondary amyloid plaques. Although prolonged high plasma levels of SAA may lead to the deposition of its products, AA proteins, in tissues, high concentrations of SAA are not sufficient to promote the development of amyloidosis. Recent studies have focused on the polymorphisms of SAA as the genetic background for amyloidogenesis¹⁶⁻¹⁸.

Recently, Cazeneuve, *et al* showed in a group of Armenian patients with FMF that the SAA1 α / α genotype was associated with a 7-fold increased risk for renal amyloidosis, compared with other SAA1 genotypes¹⁹. No correlation with SAA levels was examined.

There is continuous subclinical inflammation in patients with FMF²⁰. Our group has previously shown that FMF patients homozygous and compound heterozygous for MEFV mutation had higher SAA levels compared to heterozygotes²¹. Elevated SAA levels in FMF patients can be secondary to either subclinical inflammation or to SAA1 genotype. It is well known that CRP correlates markedly with SAA in most inflammatory disorders, and that no study has reported the effect of a genetic factor in producing a variation in serum CRP levels. The SAA/CRP ratio can be a useful measure when evaluating the relation between SAA values and SAA1 genotypes in patients with continuous inflammation²².

We evaluated the influence of SAA1 and SAA2 polymorphisms on renal amyloidosis, SAA level, and SAA/CRP values in Turkish patients with FMF.

MATERIALS AND METHODS

Patients. SAA1 and SAA2 gene polymorphisms and SAA levels were determined in 74 FMF patients (39 female, 35 male; median age 11.5 yrs, range 1.0–23.0) who were homozygous or compound heterozygous for MEFV mutation (M694V, M694I, M680I, V726A, and E148Q). Homozygous or compound heterozygous patients were recruited in order to clarify the effect of MEFV genotype on SAA level²¹.

The clinical data were registered on a standardized form that included age, sex, familial consanguinity, history of the disease, age at onset of symptom(s), presence of fever, abdominal pain, pleurisy, joint pain, duration of FMF disease, duration of colchicine prophylaxis, and colchicine dosage. All patients were on colchicine therapy and drug compliance was estimated to be good. Informed consent was obtained from the patient or the parents.

Mutation analysis. Our strategy for mutation analysis included 2 steps. The hot spot, exon 10, which harbors 18 mutations, was first analyzed by denaturing gradient gel electrophoresis (DGGE). According to the band pattern, subsequent analysis was done either by restriction endonuclease enzyme digestion or sequencing. Further, E148Q in exon 2 was analyzed by restriction endonuclease enzyme digestion.

SAA measurement. SAA levels were measured by solid phase sandwich ELISA method (Cytoscreen, Human SAA immunoassay kit; Biosource International, Camarillo, CA, USA). The normal SAA level was < 10 ng/ml. Blood samples for SAA and CRP measurements were drawn at least 2 weeks after an FMF attack. This period was chosen since we aimed to measure subclinical inflammation in these patients and not levels during an attack. The SAA/CRP ratio was calculated.

Analysis of polymorphisms in SAA1 and SAA2 genes. The SAA1 α , β , and γ isoforms are encoded by the V52-A57, A52-V57, and A52-A57 SAA1 alleles, respectively. The SAA2 α and β isoforms are encoded by the H71 and R71 SAA2 alleles. These polymorphisms are located in the coding region of the gene. SAA1 and SAA2 gene polymorphisms were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as described²³. SAA2 gene polymorphism was available in 69 patients.

Statistical analysis. Results were expressed as median (minimum-maximum) for data not showing normal distribution and as mean \pm standard deviation (SD) for data showing normal distribution. Mann-Whitney U and chi-squared tests were used for comparison. P values < 0.05 were considered significant.

RESULTS

Clinical features, MEFV mutation analysis, and distribution of SAA1 and SAA2 gene polymorphisms are summarized in Table 1. Median age at onset of clinical symptoms was 3 (0.5–13) years, median age at diagnosis was 7 (1–16) years. The mean duration of FMF was 7.5 \pm 4.40 years (range 1–15) and duration of colchicine prophylaxis 3.50 \pm 3.64 years (range 1–13). Colchicine dosage ranged from 0.5 to

Table 1. Clinical characteristics, MEFV mutation analysis, and SAA1 and SAA2 gene polymorphisms of 74 patients with FMF.

Features	n (%)
M/F	35/39
Current age, yrs median, (minimum-maximum)	11.5 (1–23)
Amyloidosis	8/74 (10.8)
Consanguinity	20/74 (27.0)
Family history	34/74 (45.9)
History of appendectomy	7/74 (9.5)
Clinical features	
Fever	70/74 (94.6)
Abdominal pain	69/74 (93.2)
Pleurisy	34/74 (45.9)
Arthritis/arthralgia	59/74 (69.7)
MEFV mutations	
M694V/M694V	44/74 (59.5)
M694V/M680I	21/74 (28.4)
M694V/V726A	9/74 (12.2)
SAA1 genotypes	
α / α	23/74 (31.1)
α / β	30/74 (40.5)
α / γ	1/74 (1.4)
β / β	14/74 (18.9)
β / γ	5/74 (6.8)
γ / γ	1/74 (1.4)
SAA2 genotypes	
α / α	55/69 (79.7)
α / β	13/69 (18.8)
β / β	1/69 (1.4)

1.5 mg/day. Frequency and duration of attacks before colchicine treatment were 12 (0–52)/year and 2 (0–15) days, respectively. Eight patients (10.8%) presented with renal amyloidosis; their MEFV mutation analysis revealed M694V/M694V genotype in 6 patients, and M694V/M680I in 2 patients. They all had typical recurrent attacks before the diagnosis.

Of the 23 patients who had α/α genotype for SAA1 polymorphism, 7 patients had developed renal amyloidosis (30.4%), whereas only one patient without this genotype developed renal amyloidosis (1/51, 2.0%; $p < 0.001$) (Table 2).

Although all of the amyloidosis patients were in the SAA2 α/α genotype group, and none of the 14 patients with non- α/α genotype for SAA2 had developed amyloidosis, the difference was not statistically significant (7/55, 12.7% vs 0/14, 0%; $p > 0.05$) (Table 2).

The median SAA level was 75 ng/ml (range 10.2–1500). The difference between SAA levels of patients with α/α and non- α/α genotypes for both SAA1 and SAA2 genes was not statistically significant (Table 2). The groups were comparable for duration of FMF, duration of colchicine prophylaxis, and colchicine dosage. Of the 13 patients who were homozygous for M694V and SAA1 α alleles, only 5 patients had renal amyloidosis.

The median CRP levels were not different between SAA1 α/α and non- α/α genotypes. On the other hand SAA/CRP values were significantly lower in patients with SAA1 α/α genotype, compared to other SAA1 genotypes (Table 2).

SAA1 and SAA2 genotypes had no significant effect on the presence of fever, abdominal pain, pleurisy, arthritis, consanguinity, and family history. There was no correlation between the MEFV genotype and SAA1 and SAA2 gene polymorphisms.

DISCUSSION

Previous studies focused on SAA1 genotype effect on secondary amyloidosis. SAA1 α/α genotype was associated with an increased risk of renal amyloidosis in Caucasian patients with juvenile chronic arthritis¹⁷. SAA1 γ/γ genotype and γ -allele frequency were associated with an increased

risk of renal amyloidosis in Japanese patients with rheumatoid arthritis (RA)^{16,18}.

Cazeneuve, *et al* showed that the SAA1 α/α genotype was associated with a higher risk of renal amyloidosis in FMF¹⁹. They studied 137 Armenian FMF patients; 47 patients had renal amyloidosis. Their results showed a 7-fold increased risk of amyloidosis in patients with SAA1 α/α genotypes, compared to other genotypes. Another striking point was that all 11 patients who were homozygous for both M694V and SAA1 α alleles had developed renal amyloidosis¹⁹.

Mutation analysis may give different results in different ethnic groups. It is of particular importance to evaluate the effect of the SAA1 α/α genotype in different FMF populations. In our study, SAA1 α/α genotype was significantly associated with the development of renal amyloidosis: 7/23 (30.4%) versus 1/51 (2.0%); $p < 0.001$. In contrast to the observation by Cazeneuve, *et al*, 5 out of 13 patients who were homozygous for M694V and SAA1 α alleles had renal amyloidosis in our study. These data support the view that genetic factors other than MEFV and SAA1 genotype and/or environmental factors play a role in the pathogenesis of renal amyloidosis in patients with FMF. Early and regular use of colchicine in our study population may be another factor responsible for a lower rate of amyloidosis in this selected subgroup, compared to Armenian patients.

We propose 3 hypotheses to explain the increased risk of renal amyloidosis with SAA1 α/α genotype. First, a greater macrophage uptake of SAA1 α isoform: this hypothesis is supported by Meek, *et al*²⁴ and Kluge-Beckerman, *et al*²⁵, who showed that in mice the plasma clearance of α/α isoform was increased. Second, the α/α isoform may also have a high intrinsic potential to promote (RA) fibrillogenesis, compared with the β and γ isoforms. Lastly, the product of the α/α genotype may somehow be more prone to deposition¹⁹. Since the polymorphisms for which we screened are not in the promoter region, we cannot be sure that the levels of the protein are affected.

As described above, the SAA1 γ/γ genotype was associated with renal amyloidosis in Japanese patients with RA¹⁶, but SAA levels of healthy Japanese subjects carrying

Table 2. The effect of SAA1 and SAA2 genotypes on amyloidosis and SAA levels.

Genotypes	Amyloidosis n (%)	SAA ng/ml median (minimum–maximum)	CRP ng/ml median (minimum–maximum)	SAA/CRP
SAA1				
α/α	7/23 (30.4)*	56 (10.2–1500)	367.5 (100–8210)	0.16 (0.025–1.96)**
Non- α/α	1/51 (2.0)*	160 (16.4–1500)	312.5 (10–9710)	0.23 (0.012–28.20)**
SAA2				
α/α	7/55 (12.7)	196 (10.2–1500)	120 (100–351)	0.18 (0.160–0.31)
Non- α/α	0/14 (–)	125 (15.6–1400)	292 (100–1600)	0.17 (0.012–2.4)

* $p < 0.001$, ** $p < 0.05$.

SAA1 γ/γ genotype were lower than those of individuals with other SAA1 genotypes²².

In our study, SAA values did not depend on SAA1 genotype. Since they were receiving colchicine, our patients' SAA levels were affected by the drug as well. The SAA/CRP values were even lower in patients with the SAA1 α/α genotype, compared to other SAA1 genotypes. Observations by Yamada, *et al*²² and our results suggest that the increased risk for renal amyloidosis is not directly related to SAA level. The question of why the SAA1 γ/γ genotype is associated with renal amyloidosis in Japanese patients with RA and the SAA1 α/α genotype is associated with renal amyloidosis in FMF patients remains to be answered.

Our study confirms an association between the SAA1 α/α genotype and renal amyloidosis in patients with FMF. SAA/CRP value is a better measure for evaluating the influence of SAA1 α/α genotype on SAA level in patients with FMF. Whether greater macrophage uptake or increased (RA) fibrillogenesis of the α/α isoform plays a role in the pathogenesis of amyloidosis in FMF merits further study.

REFERENCES

1. Sohar E, Gafni J, Pras M, Heller H. Familial Mediterranean fever: A survey of 470 cases and review of the literature. *Am J Med* 1967;43:227-53.
2. The French Consortium. A candidate gene for FMF. *Nature Genet* 1997;17:25-31.
3. The International FMF Consortium. Ancient missense mutations in a new member of the RoRet gene family are likely to cause FMF. *Cell* 1997;90:797-807.
4. Saatci U, Ozen S, Ozdemir S, et al. Familial Mediterranean fever in children: report of a large series and discussion of the risk and prognostic factors of amyloidosis. *Eur J Pediatr* 1997;156:619-23.
5. Schwabe AD, Peters RS. Familial Mediterranean fever in Armenians: Analysis of 100 cases. *Medicine* 1974;53:453-62.
6. Barakat MH, Karnik AM, Majeed HW, El-Sobki NI, Fenech FF. Familial Mediterranean fever (recurrent hereditary polyserositis) in Arabs. A study of 175 patients and review of the literature. *Q J Med* 1986;60:837-47.
7. Dewalle M, Domingo C, Rozenbaum M, et al. Phenotype-genotype correlation in Jewish patients suffering from familial Mediterranean fever. *Eur J Hum Genet* 1998;6:95-7.
8. Booth DR, Gillmore JD, Booth SE, Pepys MB, Hawkins PN. Pylrin/marenostrin mutations in familial Mediterranean fever. *Q J Med* 1998;91:603-6.
9. Yalcinkaya F, Akar N, Misirlioglu M. Familial Mediterranean fever: amyloidosis and the Val726Ala mutation. *N Engl J Med* 1998;338:993-4.
10. Yalcinkaya F, Cakar N, Misirlioglu M, et al. Genotype-phenotype correlation in a large group of Turkish patients with familial Mediterranean fever: evidence for mutation independent amyloidosis. *Rheumatology* 2000;39:67-72.
11. Uhlar CM, Whitehead AS. Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem* 1999;265:501-23.
12. Benson MB, Cohen AS. Serum amyloid A protein in amyloidosis, rheumatic and neoplastic diseases. *Arthritis Rheum* 1979;22:36-42.
13. Berendes E, Möllhoff T, van Aken H, Erren M, Deng MC, Loick HM. Increased plasma concentration of serum amyloid A: an indicator of acute phase response after cardiopulmonary bypass. *Crit Care Med* 1997;25:1527-33.
14. Sipe JD. Nomenclature Committee of the International Society of Amyloidosis. Part 2. Revised nomenclature for serum amyloid A (SAA). *Amyloid* 1999;6:67-70.
15. Liepnieks JJ, Kluve-Beckerman B, Benson MD. Characterization of amyloid A protein in human secondary amyloidosis: the predominant deposition of serum amyloid A1. *Biochim Biophys Acta* 1995;1270:81-6.
16. Baba S, Masago SA, Takahashi T, et al. A novel allelic variant of serum amyloid A. SAA1g: genomic evidence, evolution, frequency, and implication as a risk factor for reactive systemic amyloidosis. *Hum Mol Genet* 1995;4:1083-7.
17. Booth DR, Booth SE, Gillmore JD, Hawkins PN, Pepys MB. SAA1 alleles as risk factors in reactive systemic amyloidosis. *Amyloid* 1998;5:262-5.
18. Okuda Y, Yamada T, Takasugi K, et al. Serum amyloid A (SAA)1, SAA2 and apolipoprotein E isotype frequencies in rheumatoid arthritis patients with AA amyloidosis. *Ryumachi* 1999;39:3-10.
19. Cazeneuve C, Ajrapetyan H, Papin S, et al. Identification of MEFV-independent modifying genetic factors for familial Mediterranean fever. *Am J Hum Genet* 2000;67:1136-43.
20. Tunca M, Kirkali G, Soyutürk M, Akar S, Pepys MB, Hawkins PN. Acute phase response and evolution of familial Mediterranean fever [letter]. *Lancet* 1999;353:1415.
21. Duzova A, Bakkaloglu A, Besbas N, et al. Role of A-SAA in monitoring subclinical inflammation and in colchicine dosage in familial Mediterranean fever. *Clin Exp Rheumatol* 2003;21:509-14.
22. Yamada T, Okuda Y, Takasugi K, Itoh K, Igari J. Relative serum amyloid A (SAA) values: the influence of SAA1 genotypes and corticosteroid treatment in Japanese patients with rheumatoid arthritis. *Ann Rheum Dis* 2001;60:124-7.
23. Moriguchi M, Terai C, Koseki Y, et al. Influence of genotypes at SAA1 and SAA2 loci on the development and the length of the latent period of secondary AA-amyloidosis in patients with rheumatoid arthritis. *Hum Genet* 1999;105:360-6.
24. Meek RL, Hoffman JS, Benditt EP. Amyloidogenesis: one serum amyloid A isotype is selectively removed from the circulation. *J Exp Med* 1986;163:499-510.
25. Kluve-Beckerman B, Yamada T, Hardwick J, Liepnieks JJ, Benson MD. Differential plasma clearance of murine acute phase serum amyloid A proteins SAA1 and SAA2. *Biochem J* 1997;322:663-9.