Rheumatol Int (2007) 27:517–522
DOI 10.1007/s00296-006-0265-6

ORIGINAL ARTICLE

The value of the levels of acute phase reactants for the prediction of familial Mediterranean fever associated amyloidosis: a case control study

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Received: 7 January 2006 / Accepted: 18 October 2006 / Published online: 14 November 2006

Abstract In order to determine the role of levels of acute phase proteins (APPs) for the development of amyloidosis in familial Mediterranean fever (FMF) patients, the levels of serum amyloid A (SAA), C reactive protein (CRP), fibrinogen and erythrocyte sedimentation rate were measured in paired sera of 36 FMF patients during and in between acute attacks, 39 of their healthy parents (obligate heterozgotes), and 15 patients with FMF associated amyloidosis. To compare the levels of APPs, 39 patients with chronic infections or inflammatory diseases who may develop secondary amyloidosis, 20 patients with acute infections who are known to have elevated acute phase response but will never develop amyloidosis and 19 healthy controls were included. The median levels of all APPs are increased in the patients with FMF during attacks and a significant decrease was observed after the attack was over. The level of SAA was above reference range in all FMF patients during the attack free period and the level of at least one other APP was also above normal in 64% of the patients. Both CRP and SAA levels were found to be higher in obligate heterozygotes compared to controls. The levels of SAA in patients with FMF during the attack-free period, obligate heterozygotes and patients with FMF-amyloidosis were found to be similar. The levels in each group were found to be higher than SAA levels found in healthy controls yet lower than the levels measured in the patients with acute infections and patients with chronic inflammation or chronic infections. In conclusion, our results show that SAA level reflects subclinical inflammation with high sensitivity but its value for the prediction of amyloid formation process seems to be low.

Keywords Familial Mediterranean fever · Amyloidosis · Serum amyloid A · Acute phase proteins

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Introduction

Familial Mediterranean fever (FMF) is an autoinflammatory disease characterized by recurrent episodes of fever and serosal inflammation along with a very intense acute phase response (APR). AA amyloidosis,

which in the absence of treatment may develop over several years in a large proportion of the patients, is the most important complication of the disease. While some patients with severe disease do not develop this fatal complication, others acquire amyloidosis within only a few years after the onset of the disease. The clinical variability of FMF is wide, ranging from mild symptoms to severe manifestations and there is no correlation between the frequency and severity of febrile attacks and amyloidosis. In addition, there are variations in the incidence of amyloidosis within and among different ethnic groups [1, 2]. Although early studies indicated that certain mutations in the FMF gene (*MEFV*) were associated with the development of amyloidosis [3, 4] it was shown that alterations in *MEFV* are not sole determinants to renal amyloidosis susceptibility [2, 5–7]. More recently, the α/α genotype in the *SAA1* gene has been found to be associated with renal amyloidosis in patients with biallelic *MEFV* mutations. Yet, the mechanism of the accumulation of amyloidosis in FMF is not fully understood [8–11].

Secondary amyloidosis also occurs in some patients with chronic inflammatory conditions and chronic infections. The AA amyloid fibrils in these patients are derived from the circulating acute phase reactant serum amyloid A (SAA) protein. The activation pattern of SAA protein in the presence of inflammation is similar to that of C reactive protein (CRP). Its sensitivity was shown to be equal or more than that of CRP. Both are acute phase proteins (APPs), high levels were reported to show the disease activity, rapid progression of the disease and poor outcome in patients with rheumatic diseases. The level of SAA increases during acute and chronic infections and chronic inflammation [12, 13].

Increase in APPs during attacks returning to normal levels in between is well known in FMF. However, recent data have suggested that continuous subclinical inflammation might be present in at least some patients without symptoms. It was also noticed that levels of APPs were elevated in close relatives of patients with FMF [14, 15]. However, the levels of different APPs and their relative sensitivities in FMF have not yet been determined.

The aims of this study are to determine the levels of SAA and other APPs in FMF and to investigate a hypothetical role of levels of APPs for the development of amyloidosis. We therefore included patients with FMF but no amyloidosis (during and between acute attacks) and patients with amyloidosis due to FMF. In order to determine if there is a critical level of APR for the development of amyloidosis we also included patients with chronic inflammatory or infectious diseases who may develop secondary amyloidosis. In addition we included individuals with acute infections and obligate carriers (heterozygotes) of FMF, who are known to have elevated APR but will never develop amyloidosis and healthy controls to compare the levels of APPs.

Patients and methods

This is a case-control study that compares FMF patients with and without amyloidosis. The levels of acute phase reactans [SAA, erythrocyte sedimentation rate (ESR), CRP and fibrinogen levels] were measured in paired sera of 36 FMF patients during acute attacks (within the first 72 h after the onset), and in remission phase of the disease (at least 2 weeks after the end of the attack), 15 patients with FMF associated amyloidosis and 39 of their healthy parents (obligate heterozgotes). A correlation of SAA levels was assessed with ESR, CRP and fibrinogen levels. A questionnaire that includes age, sex, consanguinity, family history of FMF and amyloidosis, the presence of fever, abdominal pain, chest pain, arthritis and erysipelas like erythema was developed. The diagnosis of FMF was established according to previously described criteria [16]. The deposition of amyloidosis was histologically established by using Congo red staining and $KMnO_4$ in renal or rectal biopsy specimens of all the patients with amyloidosis. Mutation analysis was available in 21 patients with FMF (The M694V, M680I, M694I, V726A and E148Q mutations accounted for 61.1, 16.7, 11.1, 8.3 and 2.8% of the alleles, respectively) and ten patients with amyloidosis (The M694V, V726A, M694I and M680I mutations accounted for 53.3, 33.3, 6.7 and 6.7% of the alleles, respectively). Thirteen patients with FMF and six patients with amyloidosis had two mutations. Six FMF patients and two patients with amyloidosis were found to have only one mutation. No mutations could be detected in two patients of each group, and no difference for the presence of different mutations was noted between the two groups all of the patients with FMF and amyloidosis were on regular colchicine treatment (0.02–0.05 mg/kg/days) at the time of the study. Thirty-nine patients who had chronic inflammatory diseases (24 JRA, three Behçet disease, one Takayasu arthritis and three Crohn disease) or chronic infectious diseases (six tuberculosis and two osteomyelitis), 20 patients with various acute infectious diseases (ten pneumonia, nine bacteraemia and one cellulites) and 19 healthy subjects (ten females, nine males; mean age 15 years; age range 5–37 years) who did not have a family history of FMF were studied as controls.

Fibrinogen levels could not be studied in the patients with acute infections. Patients with other inflammatory conditions were treated based on their particular diagnoses. Analyses of the ESR (normal <20 mm/h), CRP (normal 0–0.8 mg/dl) and fibrinogen levels (normal 140–430 mg/dl) were performed immediately at the hospital laboratory, by standard methods. Serum samples for SAA were liquated, stored at −70°C and measured at the end of the study. All serum samples were labelled in a blinded fashion. SAA was measured by an ELISA method in accordance with the manufacturer's instructions (Cytoscreen Human SAA ELISA kit, Biosource International, Camarillo, CA, USA). The normal SAA level was <10 mg/l.

Informed consent was obtained from all of the patients and/or their parents. The study was approved by the Ethics Committee of Ankara University School of Medicine, Ankara, Turkey.

Statistical analysis

The sample size required for the study was calculated based on the primary outcome variable, that is, SAA. Group sample sizes of 36 and 15 achieve 81% power to detect a difference of 90 between the null hypothesis that both group means are 200 and the alternative hypothesis that the mean of group 2 is 110 with known group standard deviations of 100 and 100 and with a significance level of 0.05 using a two-sided Mann–Whitney *U*-test assuming that the actual distribution is normal [17]. According to our findings, we found that logit was not linear in the variables (CRP, ESR, fibrinogen and SAA). So we dichotomized continuous variables by using optimal cut-off points. On the other hand, contingency tables for these dichotomized variables concluded with some zero cells. Inclusion of such a variable into any logistic regression analysis will cause one of a number of undesirable numerical outcomes to occur, i.e., high-standard error estimate. As a result, because of small sample size, we could not perform logistic regression analysis [18].

Nominal variables were evaluated by chi-square test or Fisher's Exact test, where appropriate. Degree of association between variables was calculated by Spearman's correlation coefficient. Comparison of APPs for FMF patients during attack period and attack free period were assessed by Wilcoxon Signed Ranks test. Mann–Whitney *U*-test was used to test if there is any significant difference between two groups. Differences among five groups for CRP, ESR, fibrinogen, SAA were evaluated by Kruskal–Wallis variance analysis. When the *P*-value from the Kruskal–Wallis test statistics is statistically significant, multiple comparison test was used to know which groups differ from which others [19].

Results

Demographic and clinical features of the patients with FMF and amyloidosis are shown in Table 1. Table 2 summarizes the median values of ESR, fibrinogen, CRP and SAA levels in the study population and control groups.

The median levels of all APPs are increased in the patients with FMF during attacks and a significant decrease was observed after the attack was over. However, the level of SAA was above reference range in all FMF patients during the attack free period. In addition, the level of at least one other APP was still above normal in 23 (64%) of the patients 3 weeks after the end of attacks. While the increased levels for CRP were detected in 25% of the patients, raised fibrinogen levels and ESR were found in 47 and 33% of the patients, respectively.

Pearson correlation analysis showed that the positive correlation between SAA and CRP during the attack free period; $r = 0.557$ ($P < 0.001$). The coefficient was less than 0.5 for other parameters during attack and attack free periods. Both CRP and SAA levels were found to be higher in obligate heterozygotes compared to controls ($P < 0.05$).

The levels of SAA in patients with FMF during the attack-free period, obligate heterozygotes and patients with FMF-amyloidosis were found to be similar. These levels were found to be higher in each of the three groups compared to those measured in healthy controls

Table 1 Demographic and clinical features of the patients with FMF and FMF-amyloidosis

	FMF	FMF-amyloidosis
n	36	15
M/F	23/13	7/8
Age (years)	12 (3.5–24)	19 (9–40)
Age at onset (years)	3 (1–20)	5 (1–10)
Age at diagnosis (years)	7.5 (2–23)	11 (3–30)
Family history of FMF (%)	38.9	53.3
Family history of amyloidosis* (%)	2.8	40
Consanguinity* (%)	33.3	80
Fever (%)	91.7	93.3
Abdominal pain (%)	97.2	80
Chest pain (%)	33.3	20
Arthritis (%)	61.1	53.3
Protracted arthritis (%)	2.8	6.7
Erysipelas like erytheme (%)	27.8	26.7

*$P < 0.01$

Table 2 Levels of acute phase response in the patients with FMF and FMF-amyloidosis in comparison with those in the control groups

	ESR (<20 mm/h)	Fibrinojen (150–320 mg/dl)	CRP (0–0.8 mg/dl)	SAA (<10 mg/l)
FMF (during attack) ($n = 36$)	41.5 (18–112)	474 (355–640)	4.55 (1–23)	720.94 (19.48–1,003)
FMF (attack free) ($n = 36$)	20 (1–53)	320.5 (179–551)	0.58 (0.1–3.2)	40.51 (16–1,043)
FMF-amyloidosis ($n = 15$)	70 (20–104)	552 (331–911)	1.41 (0.1–6.59)	40 (9–448.46)
Healthy parents (Obligate heterozygotes) ($n = 39$)	10 (4–37)	257 (186–534)	0.48 (0.1–5.79)	40.38 (6–733)
Chronic inflammation/chronic infection ($n = 39$)	33.5 (2–136)	307.25 (168–870)	1.76 (0.1–18.8)	199.22 (7–1,350)
Acute infection ($n = 20$)	74 (20–125)	–	8.15 (0.5–29.7)	862.99 (56.43–1,250)
Healthy controls ($n = 19$)	8 (2–16)	225 (137–272)	0.24 (0.01–0.31)	16 (6–120.45)

Data are shown as minimum–maximum (median)

($P < 0.01$). They were found to be lower compared to those SAA levels obtained in patients with acute infections and patients with chronic inflammation or chronic infections ($P < 0.001$ and <0.01, respectively). In addition, CRP levels were similar in patients with FMF during the attack-free period and in obligate heterozygotes. Both were higher than CRP levels found in healthy controls ($P < 0.01$ and <0.05, respectively) yet lower than the levels measured in patients with FMF-amyloidosis ($P < 0.05$ and <0.01, respectively), in patients with acute infections ($P < 0.001$ and <0.01, respectively) and in patients with chronic inflammation or chronic infections ($P < 0.01$ and < 0.001, respectively).

Patients with FMF-amyloidosis had also increased levels of all studied APPs. Fibrinogen and ESR were the most evident parameters ($P < 0.001$ and <0.001, respectively).

Discussion

How and why amyloidosis develops during the course of FMF is largely unknown. In this study, we tried to find some markers that could establish the activity of the disease and predict the risk for amyloidosis. Although the patients with amyloidosis have a longer disease duration and longer delay for the diagnosis, the clinical and demographic features of the patients with and without amyloidosis did not have statistically significant differences. Besides it cannot be excluded that those patients without amyloidosis will develop amyloidosis to a later time point as all were on colchium therapy. We are aware of the limitation of our study such small sample size and case control design. Interestingly, although the patients in both groups had similar frequencies of the family history of FMF, consistent with the results of other studies from Turkey [20, 21] family history of amyloidosis and consanguinity were higher in the patients with amyloidosis.

As expected, the levels of all APPs were increased in our patients with FMF during attacks and a significant decrease was observed after the attacks were over. However, during the attack-free period, increased levels of fibrinogen, ESR and CRP were found in nearly half, one third and a quarter of the patients, respectively. Moreover, the levels of SAA were measured above normal in all of our patients 3 weeks after the attacks. These results confirm the presence of continuing subclinical inflammation during the attack free-periods in patients with FMF receiving regular colchicine therapy. Similar observations have been made more recently by other groups [14, 22].

Reactive amyloidosis can occur in some inflammatory diseases, chronic infections as well as FMF. The disease develops with the conversion of normally soluble proteins into insoluble aggregates that disrupt tissue structure and cause amyloid deposition. SAA protein is a circulating APR that is known to be the principal component of secondary amyloidosis. The level of SAA can increase more than 1,000-fold from healthy reference of 10 mg/l during acute and chronic infections and can persist indefinitely [23]. It was suggested that SAA is a more sensitive marker than CRP in infections with low-inflammatory activity including many viral infections [24]. Serum concentrations of SAA were shown to reflect the activity of rheumatic disease and rapid progression of secondary amyloidosis. It was also reported that a persistently high concentration of SAA is a prerequisite for the development of AA amyloidosis. Moreover, Gillmore et al. have shown that reduction of SAA to less than 10 mg/l is associated with regression of amyloid deposits [25]. As expected, in our study plasma levels of SAA were increased not only in patients with FMF but also in patients with acute infections and in the patients with chronic infections or chronic inflammatory conditions. Increased levels of SAA during asymptomatic periods were reported to contribute to the risk of AA amyloidosis in patients with FMF and

some authors recommended frequent estimation of SAA concentration and regulation of colchicine dose according to its level [22]. However, we have found increased levels of SAA in all of our patients in the attack-free period. These results show that the level of SAA reflects the inflammatory process with high sensitivity but its predictive value for the prediction of amyloid formation process is low. To make it more complicated, the levels of SAA were found to be very close in patients with FMF during the attack-free period, in obligate heterozygotes and in patients with FMF-amyloidosis. Interpreting this data is very difficult; for the known fact that using regular colchicine prevents the development of amyloidosis in all FMF patients, probably regardless the levels of SAA. Although it has been demonstrated that more severe inflammation is associated with higher levels of SAA, our results in this study, show that high concentration of SAA is not a specific predictor for the development of amyloidosis in patients with FMF. It should be noted here that the presence of SAA1α/α genotype was found not to have an effect on SAA levels [11, 26], even though it was demonstrated to be associated with the development of amyloidosis.

In conclusion, the increased risk for the development of amyloidosis seems not to be related to the increased levels of SAA. Further studies should elucidate the clinical consequences of subclinical inflammation in patients with FMF and in obligate heterozygotes.

References

1. Tunca M, Akar S, Önen F, Özdoğan H, Kasapçapur Ö, Yalçınkaya F, Tutar E, Özen S, Topaloğlu R, Yılmaz E, Arıcı M, Bakkaloğlu A, Beşbaş N, Akpolat T, Dinç A, Erken E (2005) Turkish FMF study group. Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. Medicine 84:1–11
2. Samuels J, Aksentijevich I, Torosyan Y, Centola M, Deng Z, Sood R, Kastner DL (1998) Familial Mediterranean fever at the millenium. Clinical spectrum, ancient mutations and a survey of 100 American referrals to the National Institutes of Health. Medicine 77:268–297
3. Cazeneuve C, Sarkisian T, Pêcheux C, Dervichian M, Nédelec B, Reinert P, Ayvazyan A, Kouyoumdjian J-C, Ajrapetyan H, Delpech M, Goossens M, Dodé C, Grateau G, Amselem A (1999) MEFV gene analysis in Armenian patients with familial Mediterranean fever: prognostic value and unfavourable renal prognosis of the M694V homozygous genotype—genetic and therapeutic implications. Am J Hum Genet 65:88–97
4. Dewalle M, Domingo C, Rozebaum M, Ben-Chetrit E, Cattan D, Bernot A, Dross C, Dupont M, Notarnicola C, Levy M, Rosner I, Demaille J, Touitou I (1998) Phenotype-genotype correlation in Jewish patients suffering from familial Mediterranean fever (FMF). Eur J Hum Genet 6:95–97
5. Yalçınkaya F, Akar N, Mısırlıoğlu M (1998) Familial Mediterranean fever—amyloidosis and the Val726Ala mutation. N Eng J Med 338:993–994
6. Booth DR, Gillmore JD, Booth SE, Pepys MB, Hawkins PN (1998a) Pyrin/Marenostrin mutations in familial Mediterranean fever. Q J Med 91:603–606
7. Yalçınkaya F, Çakar N, Mısırlıoğlu M, Tümer N, Akar N, Tekin M, Taştan H, Koçak H, Özkaya N, Elhan AH (2000a) Genotype-phenotype correlation in a large group of Turkish patients with familial Mediterranean fever: evidence for mutation-independent amyloidosis. Rheumatology 39:67–72
8. Booth DR, Booth SE, Gillmore JD, Hawkins PN, Pepys MB (1998b) SAA1 alleles as risk factors in reactive systemic amyloidosis. Amyloid 5:262–265
9. Cazaneuve C, Ajrapetyaan H, Papin S, Roudot-Thoraval F, Geneviève D, Mndjoyan E, Papazian M, Sarkisian, Babloyan A, Boissier B, Duquesnoy P, Kouyoumdjian C, Girodon-Boulandet E, Grateau G, Sarkisian T, Amselem S (2000) Identification of MEFV-Independent modifying genetic factors for familial Mediterranean fever. Am J Hum Genet 67:1136–1143
10. Akar N, Hasipek M, Akar E, Ekim M, Yalçınkaya F, Çakar N (2003) Serum amyloid A and tumor necrosis factor-alpha alleles in Turkish familial Mediterranean fever patients with and without amyloidosis. Amyloid 10:12–16
11. Bakkaloğlu A, Düzova A, Özen S, Balcı B, Beşbaş N, Topaloğlu R, Özaltın F, Yılmaz E (2004) Influence of serum amyloid A (SAA1) and SAA2 gene polymorphisms on renal amyloidosis and on SAA/C-recative protein values in patients with familial Mediterranean fever in the Turkish population. J Rheumatol 31:1139–1142
12. De Beer FC, Mallya RK, Fagan EA, Lanham JG, Hughes GR, Pepys MB (1982) Serum amyloid-A protein concentration in inflammatory diseases and its realtionship to the incidence of reactive systemic AA-amyloidosis. Lancet 2:231–234
13. Benson MB, Cohen AS (1979) Serum amyloid A protein in amyloidosis, rheumatic and neoplastic diseases. Arthritis Rheum 22:36–42
14. Tunca M, Kırıkali G, Soytürk M, Akar S, Pepys MB, Hawkins Pn (1999) Acute phase response and evolution of familial Mediterranean fever. Lancet 353:1415
15. Korkmaz C, Özdoğan H, Kasapçapur Ö, Yazıcı H (2002) Acute phase response in familial Mediterranean fever. Ann Rheum Dis 61:79–81
16. Livneh A, Langevitz P, Zemer D, Zaks N, Kees S, Lidar T, Migdal A, Padeh S, Pras M (1997) Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum 40:1879–1885
17. Hintze J (2001) NCSS and PASS. Number Cruncher Statistical Systems. UTAH, Kaysville
18. Hosmer DW, Lemeshow S (1989) Applied Logistic Regression. Wiley, New York, p 84
19. Conover WJ (1980) Practical nonparamertric statistics. Some methods based on ranks. Wiley, New York, pp 229–239
20. Saatçi Ü, Özen S, Özdemir S, Bakkaloğlu A, Beşbaş N, Topaloğlu R, Arslan S (1997) Familial Mediterranean fever in children: report of a large series and discussion of the risk and prognostic factors of amyloidosis. Eur J Pediatr 156:619–623
21. Yalçınkaya F, Tekin M, Çakar N, Akar N, Tümer N (2000b) Familial Mediterranean fever and systemic amyloidosis in untreated Turkish patients. Q J Med 93:681–684
22. Düzova A, Bakkaloğlu A, Beşbaş N, Topaloğlu R, Özen S, Özaltın F, Başsoy Y, Yılmaz E (2003) Role of A-SAA in monitoring subclinical inflammation and in colchicines dosage in familial Mediterranean fever. Clin Exp Rheum 21:509–514

23. Uhlar CM, Whitehead AS (1999) Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem 265:501–523
24. Lannergård A, Larsson A, Kragsbjerg P, Friman G (2003) Correlations between serum amyloid A protein and C-reactive protein in infectious diseases. Scand J Clin Lab Invest 63:267–272
25. Gillmore JD, Lovat LB, Persey MR, Pepys MB, Hawkins PN (2001) Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein. Lancet 358:24–29
26. Yamada T, Wada A, Itoh Y, Itoh K (1999) Serum amyloid A1 alleles and plasma concentrations of serum amyloid A. Amyloid 6:199–204