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Concise report

Amyloidosis and its related factors in Turkish patients with familial Mediterranean fever: a multicentre study

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Abstract

Objective. The primary aim of this study was to investigate the prevalence of amyloidosis and its related factors in a large number of FMF patients.

Methods. Fifteen centres from the different geographical regions of Turkey were included in the study. Detailed demographic and medical data based on a structured questionnaire and medical records were collected. The diagnosis of amyloidosis was based on histological proof of congophilic fibrillar deposits in tissue biopsy specimens.

Results. There were 2246 FMF patients. The male/female ratio was 0.87 (1049/1197). The mean age of the patients was 34.5 years (S.D. 11.9). Peritonitis was the most frequent clinical finding and it was present in 94.6% of patients. Genetic testing was available in 1719 patients (76.5%). The most frequently observed genotype was homozygous M694V mutation, which was present in 413 (24%) patients. Amyloidosis was present in 193 patients (8.6%). Male sex, arthritis, delay in diagnosis, M694V genotype, patients with end-stage renal disease (ESRD) and family history of amyloidosis and ESRD were significantly more prevalent in patients with amyloidosis compared with the amyloidosis-negative subjects. Patients with homozygous M694V mutations had a 6-fold higher risk of amyloidosis compared with the other genotypes (95% CI 4.29, 8.7, $P < 0.001$).

Conclusion. In this nationwide study we found that 8.6% of our FMF patients had amyloidosis and homozygosity for M694V was the most common mutation in these patients. The latter finding confirms the association of homozygous M694V mutation with amyloidosis in Turkish FMF patients.

Key words: familial Mediterranean fever, amyloidosis, prevalence.

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CLINICAL SCIENCE

Introduction

FMF is an autosomal recessive autoinflammatory disease that occurs worldwide and predominantly affects populations of Mediterranean origin [1]. Clinically, FMF is characterized by self-limiting febrile attacks of polyserositis, lasting on average 24–72 h [1]. Acute attacks are followed by attack-free intervals, but subclinical inflammation continues during these periods [1, 2]. The *MEFV* (Mediterranean fever) gene is associated with FMF and is located on the short arm of chromosome 16 [3–5]. It is suggested that mutated pyrin, a protein that is encoded by the *MEFV* gene, may cause uncontrolled inflammation [6]. Secondary (AA) amyloidosis is the most devastating complication of FMF, especially in untreated and non-compliant patients. It has been reported that, before colchicine therapy, renal amyloidosis was the primary cause of death before the age of 40 years [7, 8]. More recent reports have indicated that 11–13% of FMF patients had amyloidosis in the colchicine era [7–9]. However, the pathogenesis and risk factors of this complication still remain only partially understood in FMF. The primary aim of this study was to investigate the prevalence of amyloidosis and its related factors in a large number of FMF patients from different centres.

Methods

In the present multicentre, retrospective study, we included 2246 patients with a diagnosis of FMF according to the Tel-Hashomer or Sheba Medical Center criteria [10, 11]. Fifteen centres dealing with FMF patients from the different geographical regions of Turkey were included in the study between January and December 2012. A structured questionnaire including the following variables was sent to the centres: age, sex, age at onset of attacks, age at diagnosis, family history of FMF, clinical features, colchicine use, *MEFV* mutations (if present), presence of concomitant diseases (AS, HScP and polyarteritis nodosa), chronic renal failure and presence of amyloidosis.

MEFV mutations were studied with PCR restriction fragment length polymorphism (PCR-RFLP) or the reverse hybridization assay (FMF StripAssay), depending on the laboratory. The following mutations were tested: M694V, M680I, V726A and E148Q. The diagnosis of amyloidosis was based on histological proof of congophilic fibrillar deposits. Histological samples were obtained from the following tissues: renal (54%), rectal (30%), spleen (10%), gastric or duodenum (4%), bone marrow (1%) and abdominal s.c. fat (1%). This study was approved by the Institutional Ethics Committee of the Eskisehir Osmangazi University School of Medicine. Due to the retrospective nature of this study, patient consent was not required.

Statistical analysis

The Shapiro–Wilk normality test was used to determine the distribution pattern of the variables. Continuous data are presented as mean (S.D.) and median with minimum–maximum values. The Mann–Whitney *U* test was used for comparisons between two groups of continuous variables. Chi-square testing was performed for the comparison of categorical variables. A double-tailed *P*-value <0.05 was considered statistically significant. The statistical analysis was carried out using Statistical Package of Social Science (SPSS) software, version 15.0 (SPSS, Chicago, IL, USA).

Results

There were 2246 FMF patients. The male/female ratio was 0.87 (1049/1197). The mean age of patients was 34.5 years (S.D. 11.9) [median 33 (6–84)] and the mean disease duration was 18.8 years (S.D. 12.4) [median 17 (0–77)]. The delay in diagnosis was 10.1 years (S.D. 9.9) [median 7 (0–73)]. The mean colchicine dosage and treatment durations were 1.4 g/day (S.D. 0.12) and 4.08 years (S.D. 0.11), respectively. The demographic characteristics of the patients are summarized in Table 1. Peritonitis was the most frequent clinical finding and was present in 94.6% of the patients. The other clinical features (in decreasing order of frequency) were fever (91.9%), pleuritis (47.9%), arthritis (39.8%), erysipelas-like erythema (ELE, 23.7%), and myalgia (13%). Vasculitis (HSP or PAN) was present in 2.7% of patients. The prevalence of AS in this population was 6.5%. Genetic testing was available in 1719 patients (76.5%). The most frequently observed genotype was a homozygous M694V mutation, which was present in 413 patients (24%). Other mutations (in decreasing order of frequency) were as follows: M694V heterozygous (17%), M694V/M680I (8.4%), M694V/V726A (7.3%), M694V/E148Q (5.3%), V726A/M680I (4.2%), E148Q heterozygous (4.1%), M680I heterozygous (3.7%) and M680I homozygous (3%). There were 154 (9%) patients without mutations (wild type). The frequency of end-stage renal disease (ESRD) was 4.94% ($n=111$). Amyloidosis was the responsible aetiology for the ESRD in 101 cases (96%).

Table 1 Demographic and clinical characteristics of the study group ($n=2246$)

Age, mean (S.D.), years	34.5 (11.9)
Male/female	1049/1197
Age at symptom onset, mean (S.D.), years	15.7 (9.6)
Age at diagnosis, mean (S.D.), years	25.8 (11.6)
Delay in diagnosis, mean (S.D.), years	10.1 (9.9)
Family history of FMF, %	57.3
Family history of amyloidosis, %	21.5
Family history of ESRD, %	5
Fever, %	91.9
Peritonitis, %	94.6
Pleuritis, %	47.9
Arthritis, %	39.8
ELE, %	23.7
Amyloidosis, %	8.6
ESRD, %	4.9
Myalgia, %	13
AS, %	6.5
Vasculitis, %	2.1

ESRD: end stage renal disease; ELE: erysipelas-like erythema

TABLE 2 Comparison of patients with and without amyloidosis

	Amyloidosis positive ($n = 193$)	Amyloidosis negative ($n = 2053$)	P-value
Male sex, *n* (%)	105 (54.4)	944 (46)	0.026
Peritonitis, *n* (%)	170 (88.1)	1956 (95.3)	<0.001
Pleuritis, *n* (%)	73 (37.8)	1002 (48.8)	<0.001
Arthritis, *n* (%)	99 (51.3)	796 (38.8)	0.001
Patients with ESRD, *n* (%)	101 (52.3)	10 (0.5)	<0.001
M694V homozygous, *n* (%)	90 (45.6)	323 (15.7)	<0.001
Delay in diagnosis, mean (S.D.), years	12.3 (11.8)	9.9 (9.7)	0.006
Family history of amyloidosis, *n* (%)	54 (28)	428 (20.8)	0.027
Family history of ESRD, *n* (%)	32 (16.6)	81 (3.9)	<0.001

ESRD: end stage renal disease

Comparison of patients with and without amyloidosis

Amyloidosis was present in 193 patients (8.6%). Male sex, arthritis, M694V genotype, patients with ESRD and a family history of amyloidosis and ESRD were significantly more prevalent in patients with amyloidosis compared with the amyloidosis-negative subjects. In contrast, the frequencies of peritonitis and pleuritis were significantly higher in the patients without amyloidosis (Table 2). The delay in the diagnosis was significantly longer in the amyloidosis group [mean 12.3 years (S.D. 11.8) *vs* 9.9 (S.D. 9.7), median 9 years (0–51) *vs* 7 (0–73)]. The distribution of genotypes in patients with and without amyloidosis revealed that M694V homozygous (61.2% *vs* 20.5%, $P < 0.001$, respectively) frequencies were significantly higher in the group of patients with amyloidosis (Table 2).

Comparison of patients with M694V homozygous mutations and other genotypes

Patients with homozygous M694V mutations had a significantly increased frequency of arthritis ($P < 0.001$), ELE ($P < 0.001$), ESRD ($P < 0.001$), family history of FMF ($P = 0.009$) and family history of ESRD ($P = 0.002$) compared with the other genotypes. The amyloidosis rate was significantly increased in patients with M694V homozygous mutations compared with non-M694V patients [odds ratio (OR) = 6.1, 95% CI 4.29, 8.7, $P < 0.001$).

Other comparisons

In 1633 patients FMF symptoms started before 18 years of age. These patients had an increased frequency of arthritis and M694V homozygous mutations compared with the patients whose symptoms started after the age of 18 years ($P < 0.001$, 44% *vs* 31% and $P < 0.001$, 29% *vs* 15%, respectively). On the other hand, no significant difference was determined regarding amyloidosis between the two groups ($P = 0.223$, 9% *vs* 8% respectively).

Discussion

In this study we found that 8.6% of our FMF patients had amyloidosis, and homozygosity for M694V was the most common mutation in these patients. Amyloidosis is the most important complication of FMF and continues to be a major problem, especially in countries where FMF is prevalent, even in the colchicine era. In 2005 the Turkish FMF Study Group reported that amyloidosis was present in 316 of 2838 FMF patients with a frequency of 12.9% [12]. In the current study we analysed 2246 FMF patients and there were 193 (8.6%) biopsy-proven cases of amyloidosis. Because of its lethal consequences, determination of high-risk patient groups for amyloidosis is crucial for FMF. For that reason, several studies have focused on identifying FMF patients who are at risk of amyloidosis. The development of amyloidosis has been shown to be associated with various factors including certain *MEFV* mutations, a family history of amyloidosis, male sex, environmental factors and country of residence [9, 13]. It has been reported that the *MEFV* M694V mutation has been associated with renal amyloidosis in FMF patients of Armenian, Jewish and Arabic heritage [9, 14–17]. Although there are plenty of studies indicating a relationship between the presence of homozygous M694V mutation and the development of amyloidosis in certain ethnic groups, there are contradictory reports regarding the association of M694V mutation and amyloidosis in Turkish FMF patients [9, 12]. An earlier Turkish study ($n = 2838$) did not show any association between the homozygous M694V genotype and amyloidosis [12]. In that study there were 316 patients with secondary amyloidosis, but *MEFV* mutations were available in only 126 of them. Thus it is possible that they may have missed some substantive changes (type II error). In another study, Touitou *et al.* [9] investigated the susceptibility for amyloidosis among 2482 FMF patients (260 patients with amyloidosis) from 14 countries, including Turkey. These authors reported that the association of M694V with renal amyloidosis was borderline and just barely missed significance in Turkish patients ($P = 0.055$) [9]. In another study, Yalcinkaya *et al.* [18] did not show any association with severe disease, amyloidosis and M694V and suggested that Turkish FMF patients had a broad spectrum of mutation combinations, which might reflect the intercultural interactions of ancient ethnic groups that lived in Anatolia and might explain this condition. However, in a recent report, Akpolat *et al.* [13] reviewed 27 studies from 20 centres, including 3505 Turkish

subjects, and found that the homozygous M694V genotype was significantly more common in FMF patients with amyloidosis. In our study of different geographic regions of Turkey, we found that the frequency of the homozygous M694V genotype was significantly increased in patients with amyloidosis compared with patients without amyloidosis. Furthermore, we found that presence of the homozygous M694V mutation in FMF patients confers a 6-fold higher risk of amyloidosis than other mutations. In the current study we confirmed the results of the previously mentioned Turkish studies, which suggest a link between M694V and amyloidosis. In our subgroup analysis, amyloidosis patients had a significantly higher frequency of male sex, peritonitis, pleuritis, arthritis and a family history of amyloidosis. Amyloidosis patients were also significantly younger than the non-amyloidosis FMF patients and there was a significant diagnosis delay in the amyloidosis group.

In the current study, genotype analysis was present in 76.5% of the patients. According to our results, M694V was the most frequently observed mutation (allelic frequency 43.05%), followed by M680I (11.2%), V726A (5.79%) and E148Q (4.17%). In the literature there are several studies concerning genotype–phenotype correlations. These showed that homozygosity for M694V is associated with more severe disease, including early onset, more frequent attacks, significantly more joint disease and a higher rate of amyloidosis [19]. Similarly, in our study, comparison of M694V homozygous patients with other genotypes showed that the frequencies of amyloidosis and arthritis were significantly higher in patients carrying the M694V homozygous genotype. In addition, the rates of ELE and ESRD were also increased in these patients.

In our multicentre study we also collected the clinical and demographic characteristics of the FMF patients. The ratio of male to female patients in the current study was 0.88. The frequency of clinical features such as fever, peritonitis, pleuritis, arthritis and ELE were quite similar with earlier Turkish nationwide study data [12].

It has been reported that nearly 90% of FMF patients experience their first attack before

20 years of age. Some studies suggest that late-onset FMF (>18 years) has a more benign disease course [20]. In our study the comparison of early *vs* late onset of disease yielded an increased frequency of arthritis and M694V homozygous mutations in late-onset FMF patients. On the other hand, the frequency of amyloidosis was similar between the groups.

Our study did have some limitations. First, the study centres provided data for six of the seven geographical regions of Turkey. However, the maternal and paternal ethnic origins of the patients were not included in the study. Second, we did not assess data regarding acute phase proteins and biomarkers such as serum amyloid A. Including these data may provide additional information to the study.

In conclusion, in this nationwide study, our results confirm the association of the homozygous M694V mutation with amyloidosis in Turkish FMF patients. However, amyloidosis is a complex disease and other factors in addition to certain mutations should be considered in every patient individually. Identification of other genetic and environmental factors on the development of amyloidosis in patients with FMF may be possible in the future. Based on the available data, for prevention of amyloidosis, early diagnosis and colchicum therapy at effective dosages must be the golden rule for all FMF patients, especially patients carrying the homozygous M694V mutation. Future studies are needed to clarify responsible aetiologies of amyloidosis in FMF.

Rheumatology key messages

- Amyloidosis is still a serious complication and a challenging issue in FMF.
- Homozygosity for M694V confers a 6-fold higher risk of amyloidosis than other mutations.

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