Familial Mediterranean Fever in Siblings

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ABSTRACT. ***Objective.*** Genetic and environmental factors have been implicated in disease severity and development of amyloidosis in familial Mediterranean fever (FMF). We investigated similarities in clinical characteristics, disease severity, and treatment response within siblings with FMF.

Methods. The study group consisted of 2 or more siblings who were followed in our center with the diagnosis of FMF. Siblings were evaluated for demographic data, clinical and laboratory disease features, genetic analysis of *MEFV* mutations, and disease severity score. The intraclass correlation coefficient (ICC), which can be interpreted as the expected correlation between 2 siblings, was used to reflect within-family similarity.

Results. The study included 67 pediatric patients from 31 different families. When we investigated the similarity of siblings after adjusting for genetic effects, we found very low ICC with $p > 0.05$ in the majority of clinical features, disease severity, and colchicine dosages. However, age at disease onset, age at onset of therapy, attack-free acute-phase reactant levels, and presence of amyloidosis were found to be similar within siblings (relatively high ICC with $p < 0.05$).

Conclusion. Siblings with FMF had different clinical findings and disease severity. They had similar amyloidogenic potential, proven by both similar presence of amyloid and increased levels of acute-phase reactants between attacks. Our findings strongly support that genetic factors may be more dominant in the development of amyloidosis. (J Rheumatol First Release Oct 1 2012; doi:10.3899/jrheum.120530)

Key Indexing Terms:
FAMILIAL MEDITERRANEAN FEVER PEDIATRIC SIBLINGS
DISEASE SEVERITY AMYLOIDOSIS

Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent self-limited attacks of fever and serosal inflammation accompanied by a marked acute-phase response[1]. FMF is an ancient disease and the most common inherited periodic syndrome; since the 1970s, its clinical features have been understood and pertinent treatment was discovered[2,3,4]. In 1997, 2 independent groups defined the gene *MEFV* responsible for this autoinflammatory disease, a major milestone for understanding it[5,6].

Genotyping has shown that the disease is associated with a wide variety of symptoms. Yet the genotype-phenotype relationship is not well established and the spectrum of clinical findings may differ considerably from one patient to another. Any patient with 2 mutations may be defined as having FMF on genetic grounds but may not always have the phenotype. Similarly, there are many patients who have FMF phenotype but no *MEFV* mutations[7,8]. While some patients with severe disease do not develop the fatal complication of amyloidosis, others acquire amyloidosis within only a few years after disease onset. In addition, there is no correlation between the frequency and severity of febrile attacks and amyloidosis, and the incidence varies among different ethnic groups. Both genetic and environmental factors have been implicated in disease severity and development of amyloidosis in patients with FMF[9,10,11,12].

The aim of our study was to investigate similarities in siblings with FMF in their clinical characteristics, disease severity, and treatment response. Our main hypothesis was that these siblings, with the same genetic background, living in the same environment, probably had similar clinical findings and disease severity.

MATERIALS AND METHODS

The study group consisted of 2 or more siblings that were followed in our center with the diagnosis of FMF. Parents of the patients were interviewed about the onset and clinical features of the disease. Patients' files were evaluated for demographic data, clinical and laboratory features of the disease, and genetic analysis of *MEFV* mutations. Diagnosis of FMF was based on the presence of clinical criteria[13,14]. At least 6 predominant mutations (p.M694V, p.M680I, p.M694I, p.V726A, p.K695R, p.E148Q) in the *MEFV* gene were studied. Exon 10 of *MEFV* gene was screened using direct sequencing of polymerase chain reaction (PCR) amplified fragments. The p.E148Q mutation was analyzed with a PCR restriction fragment-length polymorphism protocol[15,16]. Disease severity was determined by the use of scoring systems described by Pras, *et al*[9] (Table 1) and Mor, *et al*[17] (Table 2). We made some changes in these scoring systems according to children, in the age factor and also in the colchicine dosages. Informed consent was obtained from the parents of each patient and the study was approved by the institutional ethics committee.

Statistical analysis. The intraclass correlation coefficient (ICC), which can

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Table 1. Severity score by Pras, *et al*[9] modified for children. The severity score is the sum of the score of each variable. A score of 3–5 is accepted as mild, 6–9 is moderate, and > 9 is severe disease.

Variable	Score
Age at onset, yrs	
11–20	2
3–10	3
< 3	4
No. attacks per month	
< 1	1
1–2	2
> 2	3
Arthritis	
Acute	2
Protracted	3
Erysipelas-like erythema	2
Amyloidosis	3
Dose of colchicine, mg/m^2	
< 1	0
1	1
> 1	2
> 2 mg/day*	3

* Not responsive to 2 mg/day.

Table 2. Severity score by Mor, *et al*[17] modified for children. Severe disease ≥ 3 criteria; intermediate disease = 2 criteria; mild disease ≤ 1 criterion.

Score	Criteria
1	> 1 site in a single attack*
2	> 2 sites in the course of disease
3	> 1 mg/m^2/day colchicine to achieve remission
4	≥ 2 pleuritic attacks during course of the disease
5	≥ 2 Erysipelas-like erythema attacks during course of the disease
6	Age of onset ≤ 10 years.

* In at least 25% of attacks.

be interpreted as the expected correlation between 2 siblings, was used to reflect within-family similarity. In order to calculate ICC, standard errors and 95% CI multilevel models were fitted. The genetic effect was added to models as a covariate to adjust for differences within siblings in terms of genetics. With respect to the type of outcome variable, random intercept regression, random intercept binary logistic regression, or random intercept ordinal logistic regression models were fitted[18,19,20]. A p value < 0.05 was considered significant.

RESULTS

The study included 67 pediatric patients (32 male, 35 female) from 31 different families. There were 4 siblings from one family, 3 siblings from 3, and 2 siblings from 27 families. Demographic features and clinical findings of the study group are shown in Table 3. The most frequent mutations were homozygous pM694V (43%), pM694V/pM680I (21.5%), and heterozygous pM694V mutation (13.8%). The mutations were the same in 17 families, different in 12 families, and not determined in one of the 2 siblings in 2 families. Consanguinity was present in 5 families, family history of FMF was present in 16, and family history of renal disease was present in 14 families. Attacks completely disappeared in 64%, and frequency and duration decreased in 36% of the patients after colchicine therapy. Attack-free acute-phase reactant levels during the followup period were high in 17 patients (25.4%) and colchicine side effects were observed in 7 patients (10%).

Table 3. Demographic features and clinical findings of the study group (n = 67).

Characteristic	N (%), Mean ± SD
Sex	
Boys	32 (47.8)
Girls	35 (52.2)
Age at time of study, yrs	15.7 ± 5.8
Age at disease onset, yrs	4.5 ± 3.5
Age at onset of therapy, yrs	9.2 ± 4.8
Attack frequency before colchicine, attacks/year	21.8 ± 16.7
Attack duration before colchicine, hours	54.5 ± 27.7
Clinical findings	
Abdominal pain	55 (82.1)
Fever	61 (91)
Chest pain	29 (43.3)
Arthritis	22 (32.8)
Arthralgia	18 (26.8)
Erysipelas-like erythema	8 (11.9)
Leg pain	20 (29.9)
Heel pain	13 (19.4)
Vasculitis	8 (11.9)
Protracted arthritis	3 (4.5)
Protracted febrile myalgia	1 (1.5)
Fussy about trifles	13 (19.4)
Amyloidosis	6 (9)
Accompanying disease	18 (26.9)
Appendectomy history	5 (7.5)
Colchicine dosage	
mg/kg/day	0.03 ± 0.02
mg/m^2/day	0.96 ± 0.31
Disease severity (Pras[9])	
Mild	16 (23.9)
Moderate	33 (49.2)
Severe	18 (26.9)
Disease severity (Mor[17])	
Mild	25 (37.3)
Moderate	14 (20.9)
Severe	28 (41.8)

When we analyzed similarity of the siblings after adjusting for genetic effects (Appendix) in terms of the clinical features using the ICC, these factors were found to be similar within siblings ($p < 0.05$): age at disease onset, age at onset of therapy, attack duration, presence of abdominal pain and fever, attack-free acute-phase reactant levels, and presence of amyloidosis. The ICC for the majority of clinical features, disease severity, and colchicine dosages were found to be nonsignificant, indicating nonsimilarity within siblings ($p > 0.05$; Table 4).

Table 4. Intraclass correlation coefficients (ICC) of the siblings.

Characteristic	ICC ± SE	p	95% CI Lower–Upper
Age at disease onset	0.342 ± 0.151	0.031	0.032–0.652
Age at onset of therapy	0.523 ± 0.127	0.0003	0.263–0.783
Attack frequency before colchicine	0.120 ± 0.176	0.499	0.000–0.481
Attack duration before colchicine	0.411 ± 0.147	0.008	0.111–0.712
Clinical findings			
Abdominal pain	0.605 ± 0.258	0.026	0.077–1.133
Fever	0.650 ± 0.292	0.033	0.053–1.246
Chest pain	0.141 ± 0.215	0.516	0.000–0.581
Arthritis/Arthralgia	0.155 ± 0.265	0.562	0.000–0.697
Erysipelas-like erythema	0.285 ± 0.377	0.454	0.000–1.056
Leg pain	0.119 ± 0.253	0.640	0.000–0.636
Heel pain	0.043 ± 0.275	0.876	0.000–0.606
Vasculitis	0.000 ± 0.000	0.999	0.000–0.000
Protracted arthritis	0.000 ± 0.000	0.999	0.000–0.001
Protracted febrile myalgia	0.000 ± 0.002	0.999	0.000–0.004
Fussy about trifles	0.366 ± 0.341	0.291	0.000–1.063
Amyloidosis	0.675 ± 0.293	0.028	0.075–1.275
Colchicine dosage			
mg/kg/day			
mg/m^2/day	0.000 ± 0.002	0.999	0.000–0.004
Disease severity (Pras[9])	0.242 ± 0.184	0.198	0.000–0.619
Disease severity (Mor[17])	0.303 ± 0.191	0.122	0.000–0.693
Response to therapy	0.401 ± 0.234	0.097	0.000–0.879
Colchicine side effects	0.333 ± 0.410	0.423	0.000–1.172
Attack-free APR levels	0.837 ± 0.098	< 0.0001	0.636–1.038

APR: acute-phase reactant.

DISCUSSION

In this study we investigated the similarity in clinical features and disease severity within siblings with FMF. We found that siblings with FMF were dissimilar with regard to the majority of clinical features and disease severity, but age at disease onset, attack-free acute-phase reactant levels in the followup period, and presence of amyloidosis were similar within the same family.

Our first result was that clinical disease features in the same family seemed not to resemble each other. Although presence of fever and abdominal pain were significantly similar, these symptoms were present in the majority of patients (fever in 91% and abdominal pain in 82% of patients) and this was the reason for this similarity. However, less frequent symptoms such as chest pain, arthritis, and leg pain and uncommon findings such as erysipelas-like erythema, protracted arthritis, and vasculitis were not similar in the same family. Attack frequency before colchicine therapy, final colchicine dosages, response to therapy, and colchicine side effects were also dissimilar. These all suggest that siblings with FMF have different clinical findings. Ben-Zvi, *et al*[21] also showed that monozygotic and dizygotic twins with FMF show variable intrapair concordance of disease phenotype. The variability is greater in dizygotic twins compared to monozygotic twins.

The second finding was that disease severity according to the 2 scoring systems was dissimilar within the same family as well. In 1967, Sohar, *et al*[22] stated that the disease was characterized by a marked variability in clinical expression between and within families. Pras, *et al*[9] compared disease severity between North African and Iraqi Jews (living in Israel) and found that the former group had more severe disease. They also indicated that disease symptoms may differ in severity among affected siblings from the same family (unpublished data). Ozen, *et al*[12] compared disease severity in Turkish children living in Turkey and Germany, and found more severe disease in patients living in Turkey. They suggested that environment affects the phenotype of this monogenic disease. In contrast, in our study, siblings living in the same environment had different severity scores. All these results indicate that FMF is a private disease even in the same family.

Finally, the most important finding was that attack-free levels of acute-phase reactants in the followup period and the presence of amyloidosis were similar within the same family. Acute-phase reactants are almost always elevated during the attacks in patients with FMF. Some patients continue to have elevated levels of acute-phase reactants between the attacks as well[23] and this was seen in one-quarter of our patients.

Reactive AA amyloidosis is the most devastating complication of FMF and amyloidosis continues to occur in the colchicine era in untreated and noncompliant patients. Recently, its prevalence has been reported in 12.9% of patients in a large series from Turkey[24] and also in 11.4% of patients in the metaFMF database[11]. Secondary amyloidosis occurs only in patients with the most intense expression of inflammation. Delay in diagnosis of FMF, positive family history of amyloidosis, M694V homozygosity, and polymorphisms of serum amyloid A were suggested to be risk factors for the development of amyloidosis[24,25,26]. It was also shown that patients with mutations other than M694V would still be prone to this complication[27]. However, according to results from the metaFMF database, country of recruitment rather than *MEFV* genotype is the chief risk factor for renal amyloidosis. This risk, which parallels infant mortality rates, indicates a possible environmental origin of susceptibility to amyloidosis[11]. Similarly, it was shown that although development of amyloidosis is frequent in Armenia, this complication did not occur in a cohort of Armenian patients living in the United States[28]. These findings imply that not only genetic but also environmental factors are predictors for development of amyloidosis in FMF. On the other hand, it was well known that family history of amyloidosis was an important risk factor for development of amyloidosis (a 4.5- to 6-fold increased risk)[24,29]. The siblings in our study were from the same ethnic origin and were living in the same environment. They had dissimilar clinical findings and disease severity. However, they had similar amyloidogenic potential, proven by both similar presence of amyloid and increased acute-phase reactant levels between the attacks. Thus, our findings strongly support that genetic factors may be more dominant in the development of amyloidosis. Similarly, genetic factors such as polymorphisms in the gene coding for serum amyloid A have been implicated in development of secondary amyloidosis[26]. In other words, for the development of amyloidosis in a patient with FMF, some genetic factors other than *MEFV* play a role.

Variability in clinical expression of disease phenotype is a common feature of many genetic disorders. This phenomenon may result from allelic heterogeneity and/or from the influence of environmental and modifying genetic factors. We showed that siblings with FMF living in the same environment had different clinical findings and disease severity, but similar amyloidogenic potential. However, our study has the limitation of inclusion of a limited number of siblings from the same ethnic origin. Comparisons with larger number of patients, and more importantly genetic studies in patients with amyloidosis, are needed to shed light on the exact cause of amyloidosis in FMF.

APPENDIX

The random intercept model for continuous response variables can be written as:

$$y_{ij} = ß_0 + ß_1\, genetic + u_j + e_{ij}$$
$$u_j \sim N(0, \delta_u^2);\ e_{ij} \sim N(0, \delta_e^2)$$

where y_{ij} is the response for the i'th sibling in the j'th family, β_0 is the overall mean, u_j is a departure for the j'th family so that $\beta_0 + u_j$ gives the mean for the j'th family, e_{ij} is the departure of the i'th sibling from the j'th family's mean. u_j can be described as a "shared environmental" effect, between-family variation, or clustering of measurements at the family level; e_{ij} can be described as a "non-shared environmental" effect, within-family variation, or sibling-specific effect. Lastly, β_1 is added to the model to adjust the parameter estimates with respect to genetic effect[18].

The ICC then can be calculated as:

$$\frac{ICC = \delta_u^2}{\delta_u^2 + \delta_e^2}$$

The random intercept logistic regression model for binary response variables can be written as:

$$\ln\left(\frac{\Pr(Y_{ij} = 1)}{1 - \Pr(Y_{ij} = 1)}\right) = ß_0 + ß_1 genetic + u_j$$
$$u_j \sim N(0, \delta_u^2)$$

The random intercept logistic regression model for ordinal response variables can be written as:

$$\ln\left(\frac{\Pr(Y_{ij} \le c)}{1 - \Pr(Y_{ij} \le c)}\right) = ß_0 + ß_1 genetic + u_j$$
$$u_j \sim N(0, \delta_u^2)$$

where $c = 1, \ldots, C - 1$ for the C categories of the ordinal outcome.

The ICC for random-effects binary and ordinal regression models can be calculated as:

$$ICC = \frac{\delta_u^2}{\delta_u^2 + (\pi^2/3)}$$

As the $\delta_u^2 + (\pi^2/3)$ for the standard logistic distribution, which is assumed to be the underlying distribution of the binary and ordinal responses, it is not estimated from the model[19]. The standard error of the ICC was calculated using the delta method[20]. These standard errors were then used to compute corresponding p values from t statistics (with degrees of freedom = number of families − 1) and 95% CI.

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