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Research paper

Genotype-phenotype correlation in FMF patients: A “non classic” recessive autosomal or “atypical” dominant autosomal inheritance?

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ABSTRACT

Background: Uncertainty remains on the pathogenetic mechanisms, model of inheritance as well as genotype-phenotype correlation of FMF disease.
Objective: To investigate the impact of genetic factors on the FMF phenotype and the disease inheritance model.
Methods: A total of 107 FMF patients were enrolled. Patients were diagnosed clinically. All patients underwent genetic analysis of the FMF locus on 16p13.3.
Results: 9 distinct mutations were detected. Specifically, the 85.98% of patients showed a heterozygous genotype. The most common genotypes were p.Met680Ile/wt and p.Met694Val/wt. The most frequent clinical findings were fever, abdominal pain, joint pain, thoracic pain, and erysipelas-like erythema. Analysis of clinical data did not detect any significant difference in clinical phenotype among heterozygous, homozygous as well as compound homozygous subjects, further supporting the evidence that, contrary to the recessive autosomal inheritance, heterozygous patients fulfilled the criteria of clinical FMF. Moreover, subjects with p.Met694Val/wt and p.Met680Ile/wt genotype reported the most severe clinical phenotype. p.Ala744Ser/wt, p.Glu148Gln/Met680Ile, p.Met680Ile/Met680Ile, p.Met680Ile/Met694Val, p.Pro369Ser/wt, p.Met694Ile/wt, p.Glu148Gln/Glu148Gln, p.Lys695Arg/wt resulted in 100% pathogenicity.
Conclusions: The existence of a “non classic” autosomal recessive inheritance as well as of an “atypical” dominant autosomal inheritance with incomplete penetrance and variable expressivity cannot be excluded in FMF.

1. Introduction

Familial Mediterranean fever (FMF) is the most common Mendelian autoinflammatory disease, characterized by uncontrolled activation of the innate immune system, resulting in recurrent brief fever and serositis, chest, abdominal, joint and muscle pain (Onen, 2006; Sohar et al., 1967).

Predominantly, FMF affects people from Mediterranean and Middle Eastern ethnic origins (1/200–1/1000) (Onen, 2006; Sohar et al., 1967; Samuels et al., 1998).

The analysis of the typical patients revealed an autosomal recessive model of inheritance pattern (Onen, 2006).

The predisposing gene is MEditerranean FeVer (MEFV) gene, localised on chromosome 16p13.3 (Cantarini et al., 2012; Chae et al., 2009), and encoding a 781-amino acid protein pyrin/marenostrim. Met694Val, Val726Ala, Met680Ile, Met694Ile (conservative mutations clustered in exon 10) and Glu148Gln (clustered in exon 2) are considered as the most common mutations related to FMF (Cantarini et al., 2012; Chae et al., 2009).

FMF is recognised by three phenotypically independent manifestations: type 1, commonly associated with recurrent short episodes of inflammation and serositis; type 2, in which amyloidosis represents the first clinical manifestation of the disease in an otherwise asymptomatic individual; type 3, also noted as “silent homozygous or compound heterozygote state”, characterized by two MEFV mutations detection in asymptomatic patients (Camus et al., 2012). Moreover, Met694Val homozygosis is generally associated with a severe form of the disease, while mutations Glu148Gln and Val726Ala have been correlated with reduced penetrance and milder form of the disease. Furthermore, it has been observed that also compound homozygotes for Val726Ala/

Abbreviations: AP, abdominal pain; E, erysipelas-like erythema; FMF, Familial Mediterranean Fever; F, fever; FUO, fever of unknown origin; JP, joint pain; MEFV, MEditerranean FeVer; N, number; PI, pathogenicity index; PCR, polymerase chain reaction; TP, thoracic pain; SD, standard deviation

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Glu148Gln are as severely affected as Met694Val homozygotes, providing evidence to the wide allelic variability in the disease expression (Gershoni-Baruch et al., 2002). Additionally, although the number of mutations (either as heterozygote, compound heterozygote, homozygous) recognised as related to FMF is increased, in parallel, it is well clear that diagnostic interpretation can be very complex as some FMF patients may show none or only one of the known MEFV mutations (Federici et al., 2012), and, conversely, the carriage of MEFV variants is not always accompanied by clinical symptoms. Moreover, patients carrying MEFV mutations can also display aspecific clinical manifestations as well as report significant changes in disease behavior over time. Furthermore, the penetrance of disease-causing mutations varies by MEFV mutations, contributing negatively to diagnostic dilemma, especially in populations where the disease is rare (Gershoni-Baruch et al., 2002). Finally, although MEFV genotyping confirms the diagnosis of FMF, due to the lack of a clear and univocal genotype-phenotype correlation, FMF diagnosis remains often difficult to establish (Gershoni-Baruch et al., 2002).

Thus, in order to provide a new possible tools for FMF diagnosis, we aimed to collect and analyze demographic data regarding patients affected by FMF, investigating also the impact of genetic factors on the disease phenotype as well as the disease inheritance model. Finally, we speculated on the real disease genetic testing usefulness.

2. Materials and methods

2.1. Subjects and experimental design

A total of 44 patients and 63 first-degree patients' relatives, who had been referred to the Genetics and Pediatric Immunology Unit, Department of Pediatrics, University of Messina, between September 2010 and December 2015, were enrolled in the study. The patients have been chosen among people referred for fever of unknown origin (FUO). The diagnosis of FUO has been made according to Petersdorf and Beeson (Petersdorf and Beeson, 1961) criteria modified by Durack and Street (Durack and Street, 1991).

Adult patients were diagnosed clinically according to Tel-Hashomer diagnostic criteria (Tunca, 1998).

Pediatric patients were considered positive if satisfying two or more of the five following proposed criteria: fever episodes, abdominal pain, chest pain and oligoarthritis (of 6–72 h duration) and a positive family history for FMF (Yalcinkaya et al., 2000).

At the same time, epidemiologic (*e.g.*, gender, consanguinity of parents, familial history, and age of onset of disease) and main clinical data (*e.g.*, fever, abdominal, joint and thoracic pain and, erysipelas-like erythema) were also recorded.

Demographic data and other clinical characteristics of the study subjects were also collected using telephone interviewes.

All patients underwent to genetic analysis of the FMF locus on 16p13.3.

The genotyping has been extended also to 63 first-degree patients'relatives (Fig. 1).

Institutional Review Board approved the study and informed consent was obtained from the patients and parents and informed assent from the children and adolescents.

2.2. Mutational analysis

Blood samples (2–5 mL) were collected from all subjects. Genomic DNA extraction, Polymerase chain reaction (PCR), and reverse dot blot hybridization were performed using FMF Strip Assay kit (Viennalab) according to the official protocol. The common MEFV gene mutations located in exon 2: p.Glu148Gln (c.442G > C); exon 3: p.P369Ser (c.1105C > T); exon 5: p.PheF479Leu (c.1437C > G); and exon 10: p.Met680Ile (c.2040G > C), p.Met680Ile (c.2040G > A), p.Met694Val (c.2080A > G), p.Met694Ile (c.2082G > A), p.Ile692del (c.2076_2078del), p.Lys695Arg (c.2084A > G), p.Val726Ala (c.2177T > C), p.Ala744Ser (c.2230G > T), and p.Arg761His (c.2282G > A) were detected.

We classified patients with mutation in the MEFV gene into 3 groups according to heterozygote, homozygous, and compound heterozygote genotypes status.

Additionally, patients were classified according to the allele status for genotype–phenotype correlations study.

2.3. Data analysis

Analyses were performed using the software Statistica (StatSoft®, version 10). Shapiro–Wilk normality test was used to assess data distribution patterns. Continuous variables were expressed either as mean ± standard deviation (SD) or as percentage. Group comparisons were carried out using a non-parametric Mann–Whitney *U* test for continuous variables and a Fisher's exact test for dichotomous variables. The prevalence of symptoms was compared between negative, positive for one and two mutations using a χ^2 test for trend. A p value < 0.05 were considered statistically significant.

A pathogenicity index (PI) was formulated and applied in order to carry out the pathogenicity of investigated genetic variants. Herein, we define PI as the ratio of the percentage of patients carrying mutations and showing clinical symptoms to the total percentage of patients. Thus, the following formula was adopted: $PI = \nu \times 100/\rho$ (ν: percentage of patients carrying the allelic arrangement investigated and showing mutation-related clinical symptoms; ρ: percentage of all patients carrying the same allelic arrangement).

3. Results

We enrolled 107 subjects, all of Italian origin, came from southern Italy (Sicily and Calabria). Particularly, the study group consisted of 58 males (54.20%) and 49 females (45.8%) with FMF; male/female ratio of 1.18. The mean age at disease onset was 27.4 ± 18.83 years old (range 8.57–46.23 years). The age at symptoms onset was between 0 and 10 years old in 20 patients (18.69%), followed by 10–19 years old in 17 patients (15.88%), 20–30 years old in 17 (15.88%), and major than 30 years old in 53 patients (49.53%).

Demograghic and clinical data are summarized in Table 1.

3.1. MEFV mutations in FMF patients

Out of 107 patients, 9 distinct mutations were detected. Particularly, 92 (85.98%) were heterozygous, 8 (7.47%) were homozygous genotypes, and 7 (6.54%) were compound heterozygous.

The most common genotypes were p.Met680Ile/wt with a frequency of 32.7% and p.Met694Val/wt with a frequency of 20.6%, followed by p.Glu148Gln/wt (15%), p.Met680Ile/Met680Ile (5.6%), p.Val726Ala/wt (5.6%), p.Arg761His/wt (3.7%), p.Ala744Ser/wt (3.7%), p.Glu148Gln/Met680Ile (2.8%); p.Met694Ile/wt, p.Pro369Ser/wt, p.Met680Ile/Met694Val (1.9%). Additionally, p.Met694Ile/Arg761His, p.Arg761His/Arg761His, p.Glu148Gln/Pro369Ser, p.Lys695Arg/wt, and p.Glu148Gln/p.Glu148Gln were observed as rare mutations of the MEFV gene in our cohort, at 0.9% frequency, respectively.

In regard to allelic frequency, we detected the following frequency distribution: wt with a frequency of 42.99%, followed by Met680Ile (24.30%), Met694Ile (11.21%), Glu148Gln (10.28%), Arg761His (3.27%), Val726Ala (2.80%), Ala744Ser (1.87%), Pro369Ser (1.40%), Met694Ile (1.40%), and Lys695Arg (0.47%).