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ORIGINAL ARTICLE

A novel cluster of patients with Familial Mediterranean Fever (FMF) in southern Italy

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ABSTRACT

Background Familial Mediterranean Fever (FMF) is an autosomal recessive autoinflammatory disorder characterised by recurrent attacks of fever and serositis (peritonitis, pleuritic or synovitis) affecting mainly populations of Mediterranean origin.

Aim To describe a relatively new cluster of FMF subjects from Apulia and Basilicata regions (southern Italy).

Patients and methods Subjects were screened for FMF using the Tel-Hashomer criteria and genetic analysis. Demographic data were taken from patients' files and direct interviews. Patients were investigated about attack duration, intensity and site, body temperature, skin manifestations and overall quality of life before and after treatment with colchicine. Inflammatory parameters were also measured between these periods.

Results Forty-nine subjects had FMF (M : F = 26 : 23, age 38 years ± 2 SE) and followed-up up to 8 years. The age at disease onset was 22·1 years ± 1·2SE and the diagnostic delay was 15·5 years ± 1·9SE. The majority of patients (82%) suffered from abdominal pain, and 35% had undergone prior abdominal surgery or laparotomy. Severity score (ISSF) was mild in 43% of patients and intermediate in 57% of patients. Serum amyloid A (SAA) was increased in 20% of patients (16·9 ± 3·7, normal range < 6·4 mg/dL). In over 95% of patients, inflammation markers, duration and intensity of febrile painful attacks, quality of life and ISSF score improved dramatically following colchicine treatment.

Conclusion The Apulia region represents a new endemic area for FMF. Clinical presentation of FMF can be misleading and requires a complete and early workup to recognise the disease and avoid unjustified surgery. Colchicine remains the gold standard therapy to prevent FMF attacks and fatal long-term complications.

Keywords Colchicine, FMF, periodic fevers, serum amyloid A.

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Introduction

The Case #1

On February 2008, a 36-year-old tradesman from Altamura (a town 45 Km South-West of Bari, with a population of about 70 288) was referred to the Department of Internal Medicine due to recurrent attacks of fever of unknown origin, (about 14 h long, up to 38 °C), abdominal pain and malaise, since adolescence. The attacks frequency was about once a month. On physical examination, the patient had tender abdomen with guarding and rebound, and achy joints. Abdominal ultrasonography disclosed splenomegaly of 134 mm, and abdominal plan film showed signs of partial intestinal obstruction. Typical acute phase reactants [i.e. white-cell count, C-reactive protein (CRP), serum amyloid A (SAA) and the erythrocyte sedimentation rate (ESR)] were increased. The family history was apparently mute.

The association of periodic short-term febrile attacks with abdominal pain with symptom-free intercritical periods suggested the diagnosis of Familial Mediterranean Fever (FMF). Genetic analysis confirmed the *MEFV* gene mutations E148Q/R761H as compound heterozygosity. A trial with colchicine 1 mg/day resulted in complete response and to date the patient is totally asymptomatic.

Familial Mediterranean Fever (FMF) is a hereditary autosomal recessive autoinflammatory disorder. The gene associated with the disease (*MEFV* gene Chr 16p13·3) was firstly identified in 1997 by two groups [1,2]. The gene encodes a protein called pyrin (marenostrin), consisting of 781 amino acids and is

expressed predominantly in the cytoplasm in cells of myeloid lineage [3]. The MEFV gene is located on the short arm of chromosome 16p13·3 and consists of ten exons [4]. Most of the variants are located on exons 2 and 10. The current number of sequence variants for MEFV gene is > 300 [5].

FMF affects mainly people of the Mediterranean basin, including Turks, Armenians, Arabs and non-Ashkenazi Jews. Symptoms of FMF comprise recurrent bouts of fever with serosal inflammation. Colchicine is the drug of choice for FMF, firstly suggested for this indication in 1972 [6]. It controls the painful attacks of FMF and prevents the development of secondary amyloidosis [7]. The diagnosis of FMF is generally based upon clinical criteria according to Tel-Hashomer criteria [8]. Genetic testing is used to confirm the diagnosis in atypical cases.

In this study, the genotypic and phenotypic features of a novel cluster of patients with FMF living in southern Italy are described and related to the therapeutic outcome of the patients on follow-up of up to 8 years.

Subjects and methods

Subjects

Starting from the case #1, an initial number of 84 patients from Apulia and Basilicata regions were screened for FMF according to clinical features and the Tel-Hashomer criteria [8] previously discussed [4]. The requirements for the diagnosis of FMF are recurrent attacks of at least one major criteria (i.e. generalised peritonitis, or unilateral pleuritis or pericarditis, or monoarthritis of the hip, knee, ankle, or fever alone, or favourable response to colchicine), or at least two minor criteria (i.e. incomplete attacks involving one or more of the following sites: abdomen, chest, joint, exertional leg pain), or ≥ 1 minor criterion plus ≥ 4 supportive criteria (family history of FMF, appropriate ethnic origin, age of less than 20 years at disease onset, features of attacks, episodic proteinuria/haematuria, unproductive laparotomy or appendectomy, consanguinity of parents).

All patients gave written consent after being fully informed about the features of the disease and the need of medication and follow-up.

All the patients underwent a complete clinical evaluation and laboratory workup to exclude infections, malignancy, connective tissue diseases, immunodeficiency syndromes, other autoinflammatory and autoimmune diseases.

Clinical features

Patients were investigated about their attack duration (hours/days) and intensity (measured as Visual Analogue Scale, VAS in mm ranging from 0 to 100), rate of body temperature, site of attacks (i.e. abdominal pain, chest pain), skin manifestations, quality of life (QoL). The severity of disease was evaluated following the instructions of the FMF Arthritis Vasculitis and Orphan disease Research in paediatric rheumatology (FAVOR) by a recently validated questionnaire: international severity scoring system for FMF (ISSF) [9]. Symptomatic patients were subsequently treated with colchicine (*Colchicina Lirca*®, Acarpia Lda, Portugal) to prevent the attacks and complications of FMF. A clinical, laboratory follow-up was started thereafter, consisting of monthly telephone interviews and a 6-month outpatient visits. The data reported here are based on clinical follow-up of at least 1 year. During follow-up, features of febrile attacks, painful manifestations, ISSF and QoL were recorded by specific questionnaires and clinical notes.

Laboratory evaluations

The following parameters were investigated during the acute febrile attacks before and after treatment with colchicine: white blood cell number, CRP, ESR and SSA levels. These parameters were also measured during the attack-free period to evaluate subclinical inflammation.

Genetic analyses

Genetic analyses were part of the general clinical workup of patients with periodic febrile attacks observed at the Division of Medicine Clinica Medica 'A. Murri' at the Hospital Policlinico in Bari, Italy. Genotype profile was technically performed at the Section of Human Genetics, University of Bari Medical School or at the Division of Human Genetics, Hospital of Matera, Italy. After full information of the aim of the study, patients signed the specific informed consent for genetic testing. A venous blood sample was collected in EDTA-K3. Molecular analysis of the MEFV gene was performed following these steps:

- DNA isolation, starting from 25 μL of blood, using the Promega extraction kit (DNA IQ™ System, cod.C6701; Promega Italy S.r.l., Milan, Italy).
- Polymerase chain reaction (PCR) and reverse hybridization.

Genetic tests aimed at detecting 12 mutations in the *MEFV* gene. The mutations analysed were as follows: E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H. The patients who tested negative or carried a single mutation detected by reverse dot blot and had a clinical suspicion of Familial Mediterranean Fever – were analysed with a scanning approach designed to study all the MEFV coding exons. For these patients, fluorescent sequencing was performed on the coding regions and splice junctions of MEFV with BigDye Terminator version 3.1 chemistry on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequencing data were analysed with Sequencher 4·6 (Gene Codes, Ann Arbor, MI, USA).

Statistical analysis

Results are given as means ± SEM. Contingency tables were evaluated by chi-squared or Fisher's exact tests. Comparison of continuous variables was performed using the paired or unpaired *t*-test as appropriate. Statistical analyses were carried out using the NCSS software package [10]. A probability (*P*) value of <0·05 was considered statistically significant.

Results

Patients

Among the 84 patients who presented with recurrent fever, painful serositis and skin eruptions, 49 subjects were diagnosed with FMF. The remaining 35 patients had infections (n = 18), malignancies (n = 12) and connective tissue diseases (n = 5) (Fig. 1). Among the 49 FMF patients, 26 were females (age 41·0 ± 3·0 years, a body mass index [BMI] of 24·8 ± 1·1 Kg/m^2) and 23 males (age 30·0 ± 3·8 years, BMI 22·8 ± 1·1 Kg/m^2). Age range was 5–69 years, and males were significantly younger than females (P = 0·03). BMI was comparable between males and females.

The age distribution of patients with FMF according to gender is depicted in Fig. 2. Age range was 5–69 years in males and 16–69 years in females. The percentage of males was significantly higher than that of females in the youngest age range (0–20 years) (80% vs. 20%, P < 0·01) as the four youngest patients were males, with an age of 5 (N = 2), 9 (N = 1) and 14 years (N = 1). By contrast in the adult range (21–69 years), the percentage of females was significantly higher than that of males (61% vs. 39%, P = 0·04).

The genetic and clinical analysis of FMF was extended to 24 relatives of patients with FMF and detected 2 asymptomatic carriers with two mutations each, nine asymptomatic heterozygotes and 13 noncarrier healthy subjects.

MEFV gene mutations

MEFV gene mutations in the patients with FMF are listed in Table 1. The most frequent combination of mutations was E148Q/R761H (compound heterozygosity) (43%) followed by K695R (heterozygotes) (10%) and E148Q (8%) heterozygotes. These three mutations accounted for 61% of overall mutations. The M694V mutation was found in only 6/49 patients (12%) as compound heterozygosity. *MEFV* gene mutations in the asymptomatic carriers are listed in Table 2. The most represented mutation was P369S (36%), followed by K695R, V726A and V726A/P369S.

Inflammatory markers and serum amyloid A (SAA)

Typical inflammatory markers in the whole group of patients with FMF were 1·5 ± 0·4 fold increased during the acute attacks. SAA levels were elevated (11·6 ± 3·7, cut-off < 6·4 mg/dL) in 20% (10/49) of patients with FMF during the attack-free

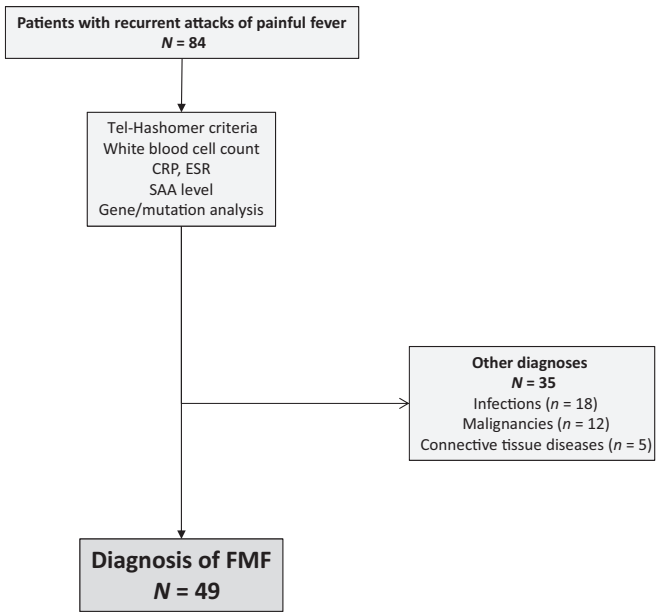

Figure 1 Flow chart illustrating the workup of patients suspected of Familial Mediterranean Fever (FMF). Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; SAA, serum amyloid A.

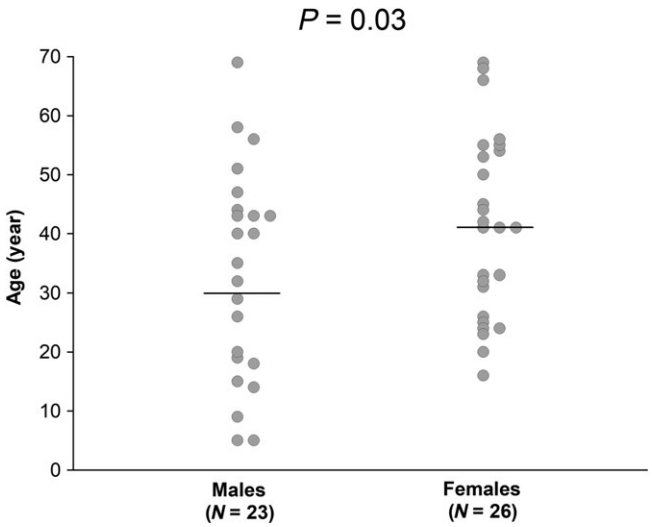

Figure 2 Age range distribution of FMF in both sexes. Both symptomatic and asymptomatic patients are included (N = 53). Note the prevalence of younger male patients because of four subjects aged 5 (N = 2), 9 and 14 years.

Table 1 PCR and sequencing analysis of entire coding region of the *MEFV* gene in FMF patients

MEFV mutations	Mutation type	N.	Per cent
E148Q/R761H	Compound H.	21	42·9
K695R	Heterozygosity	5	10·2
E148Q	Heterozygosity	4	8·2
E148Q/R761H/A744S	Compound H.	3	6·1
P369S	Heterozygosity	3	6·1
M694V/V726A/N270D	Compound H.	2	4·1
R202Q	Heterozygosity	2	4·1
R202Q/M694V	Compound H.	2	4·1
E148Q/A744S	Compound H.	1	2·0
E148Q/M694V	Compound H.	1	2·0
R202Q/I591T	Compound H.	1	2·0
V726A/R761H	Compound H.	1	2·0
P369S/R202Q/R408Q	Compound H.	1	2·0
M680IGA	Heterozygosity	1	2·0
M680IGC/M694V	Compound H.	1	2·0
Total		49	100

Table 2 PCR and sequencing analysis of entire coding region of the *MEFV* gene in asymptomatic carriers of MEFV mutation

MEFV mutations	Mutation type	N.	Per cent
E148Q/R761H	Compound H.	1	9
V726A/P369S	Compound H.	1	9
P369S	Heterozygosity	4	36
V726A	Heterozygosity	2	19
K695R	Heterozygosity	3	27
Total		11	100

period. Eighty per cent of patients with elevated SAA levels were compound heterozygotes for E148Q/R761H mutations.

Prior abdominal surgery

A history of prior abdominal surgery was recorded in 17/49 (35%) patients. The most frequent surgical procedures were appendectomy (65%), laparotomy (24%) and cholecystectomy (13%) (Fig. 3). As expected, none of the surgical interventions was associated with symptom improvement of FMF. The group undergone surgery was significantly older (43·3 years ± 4·4 years vs. 28·6 ± 4·6 years, $P = 0·03$), had a greater ISSF score (3·5 ± 0·4 vs. 2·5 ± 0·2, $P = 0·02$) and greater delay in diagnosis (24·4 ± 5·2 vs. 12·2 ± 1·7 years, $P < 0.01$) than the surgery-free group. M694V mutation was present in heterozygosity in 37% of surgical group vs. 0% among the surgery-free group.

Clinical manifestations

The clustering of most frequent FMF symptoms is depicted in Fig. 4. The most frequent clinical association was fever and abdominal pain (16/49, 33%). The least frequent symptoms were the full associations of abdominal pain, plus fever, arthralgia and erysipelas-like erythema, eventually associated with fatigue.

International severity scoring system for FMF (ISSF)

According to the novel severity scoring system ISSF, 43% (21/49) of patients were scored as 1–2 (mild disease), and 57% (28/49) were scored as 3–4–5 (intermediate disease). No patient was scored as severe disease (≥ 6).

When patients were stratified according to the most representative MEFV mutations, E148Q/R761H was present in 52% (11/21) of patients with mild disease and in 36% (10/28) of patients with intermediate disease. The distribution of MEFV mutations according to ISSF score is depicted in Table 3.

The group with mild disease showed a shorter delay in FMF diagnosis than the group with intermediate disease, but this difference was not significant (11·8 years ± 2·1 vs. 17·6 ± 2·8, NS).

Therapeutic outcome

Before starting therapy, the correlation between QoL and ISSF score showed a highly significant inverse relationship, implying a worse quality of life with increasing symptom severity ($r = -0·81$, $P < 0·001$).

In Fig. 5, the therapeutic outcome is depicted. In particular, 48 patients were put on a daily dose of colchicine ranging from 0·5 mg/day (in two children) to 1 mg/day (44 adults), 1·2 mg/day and 1·5 mg/day in another two adults. One girl with *MEFV* gene compound heterozygosity mutation R202Q/M694V did not respond to colchicine. She had undergone three explorative laparotomies; at another national FMF center she started biological drugs, that is a short course with anakinra (1 vial/week s.c.) and subsequently canakinumab (1 vial/month s.c.), with impressive improvement of symptoms.

The therapy with colchicine produced a dramatic improvement of major clinical outcomes. Body temperature as well as severity of attacks were significantly ameliorated after therapy. Changes of individual values of body temperature and pain before and after colchicine are depicted in Fig. 6(a–b).

Such improvement also influenced QoL, which shifted from very poor to optimal. At follow-up, the clinical improvement was observed within 3 months, as also confirmed by a telephone interview and outpatients visits.

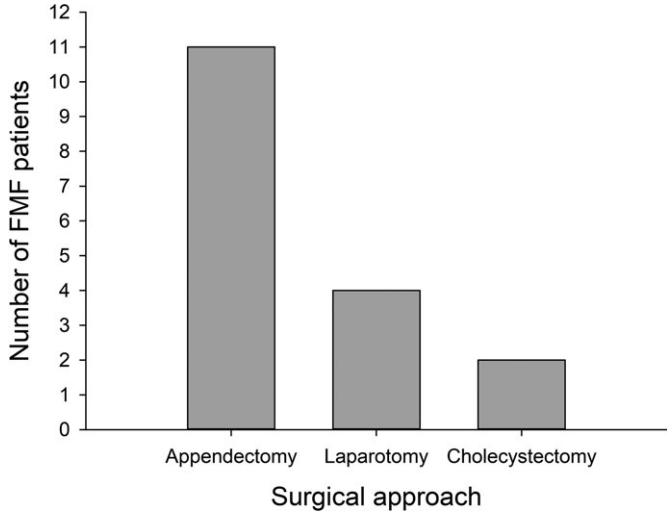

Figure 3 Distribution of surgical intervention in the 16 FMF symptomatic patients.

Changes of individual values of QoL before and after colchicine are depicted in Fig. 6(c).

We did not notice any side effects upon long-term treatment with colchicine (longest follow-up of 96 months).

Discussion

Starting from the case #1 of FMF in our geographical area [11], we have focused on various aspects of FMF [3,4,7,12]. At a later stage, we were informed that very few patients (about 4–5, totally unaware of the wide FMF occurrence in Altamura) were on follow-up at two other national italian centres for FMF and amyloidosis.

The novel cluster of patients with FMF in southern Italy, in Apulia (Altamura) and Basilicata (Matera) and especially its unique genetic components raise a question regarding their ethnic origin. In a study by La Regina *et al*., where 71 FMF subjects were collected from all over Italy, M694V was the most frequent mutation (16%), as in all Mediterranean populations, followed by E148Q (14%) and the common M680I (G > C) mutation (14%). The prevalence of M694I and of the rare form of M680I (M680I G > A) were also relatively high in Italians (10% and 8% respectively). Other rare exon 10 mutations were also found: A744S, R761H [13]. When we looked for possible sources for the R761H and the K695R mutations (found among our patients), we found that among Turkish Aegean patients with FMF allelic frequencies for the most common mutations were 47·60% (M694V), 16·75% (E148Q), 12·95% (V726A) and 11·94% (M680I G/C). The remaining alleles (10·76%) showed rare mutations which were R761H, P369S, A744S, K695R, F479L, M694I [14]. In another study from Cyprus, of the 268 identified alleles, V726A (27·61%) was the most frequent followed by M694V (19·40%). The missense mutations R761H (3·73%) and A744S (2·24%) were identified as the rarest [15]. These studies and others [16,17] show that the most prevalent mutations (R761H and K695R) in our study (in Apulia and Basilicata) are actually very rare in the neighbouring countries and islands as well as in relatively far states such as Iran. This observation suggests that the origin of the FMF population in Altamura and Matera is probably from small closed families who carried these rare mutations and emigrated together from neighbouring countries or islands (Turkey, Cyprus etc) and

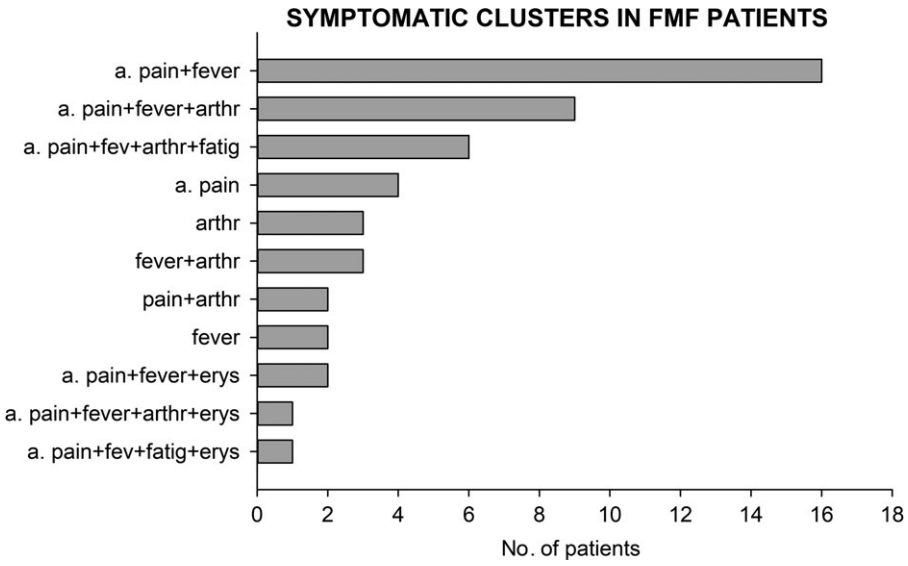

Figure 4 Distribution of solitary and clusters of symptoms in FMF patients. Legend: a. pain, abdominal pain; arthr, arthralgias; erys, erysipela-like dermatitis; fatig, fatigue.

Table 3 International severity scoring system for FMF (ISSF) according to *MEFV* mutations

MEFV mutation	Score 1–2 (mild)	Score 3–4–5 (intermediate)
E148Q/R761H	11	10
K695R	3	2
E148Q	1	3
E148Q/R761H/A744S	–	3
P369S	1	2
M694V/V726A/N270D	–	2
R202Q	2	–
R202Q/M694V	–	2
E148Q/A744S	–	1
E148Q/M694V	1	–
R202Q/I591T	1	–
V726A/R761H	–	1
P369S/R202Q/R408Q	1	–
M680IGA	–	1
M680IGC/M694V	–	1
Total	21	28

settled in these two towns. The history of Altamura offers several fascinating anthropological, geographical, religious and cultural insights and is closely connected with that of the Emperor Frederick II of Swabia. Around 1230, Frederick II issued an edict in which he declared the city of Altamura a free zone, thus encouraging the immigration of populations from neighbouring districts including Greeks, Moors and Jews. ('Founder Effect'). The relative paucity of M696V carriers further supports this notion.

In this study we found a high prevalence of E148Q (exon 2) and R761H (exon 10) mutations. E148Q is part of the five most common mutations found among Middle Eastern FMF patients, which also include M694V and I, M680I and V726A. The compound heterozygotes: E148Q and R761H mutations appeared in the vast majority of the patients with FMF (43%) in Altamura and Matera. Their occurrence was associated with mild to intermediate phenotype as defined by the ISSF score. This finding is in accord with the available literature [18,19] which showed that the carriage of the E148Q mutation attenuates the severity of the disease. The presence of four more cases of asymptomatic FMF patients, two of whom displayed the E148Q/R761H mutations further supports this observation. A long-term follow-up appears to be the most reasonable approach in these asymptomatic subjects, as unknown factors could act as phenotypic triggers.

By contrast, the M694V mutation has been associated with more severe phenotype [20]. In this study, M694V appeared as compound heterozygosity in 12% of the patients who had intermediate ISSF score. A practical issue emerging from this initial survey is that FMF symptomatic patients have an increased risk of abdominal surgery. Of note, all patients carrying the M694V mutation had undergone prior surgery before the ultimate diagnosis of FMF, and this group of patients represented 37% of the surgical group. This finding is also in accord with previous publications about recurrent intestinal obstruction and abdominal surgeries in patients with FMF carrying the M694V mutation [21].

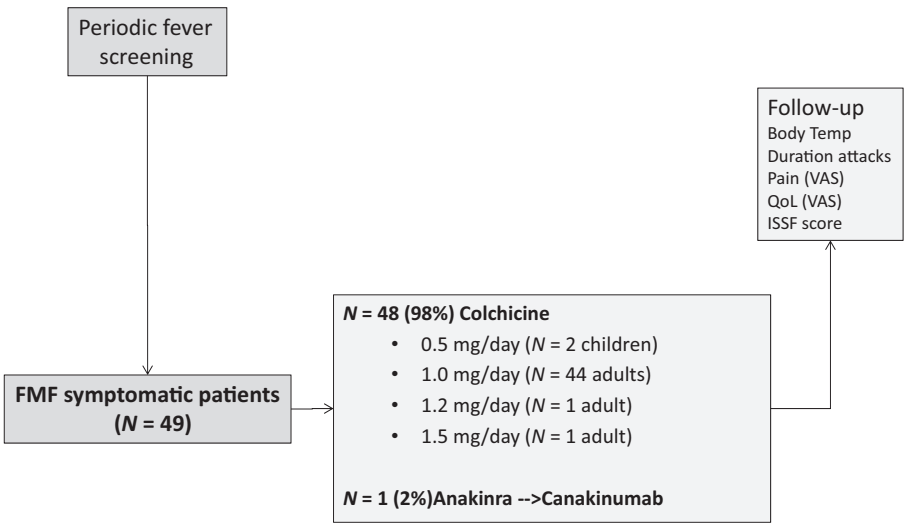

Figure 5 Therapeutic approach in the group of symptomatic FMF patients.

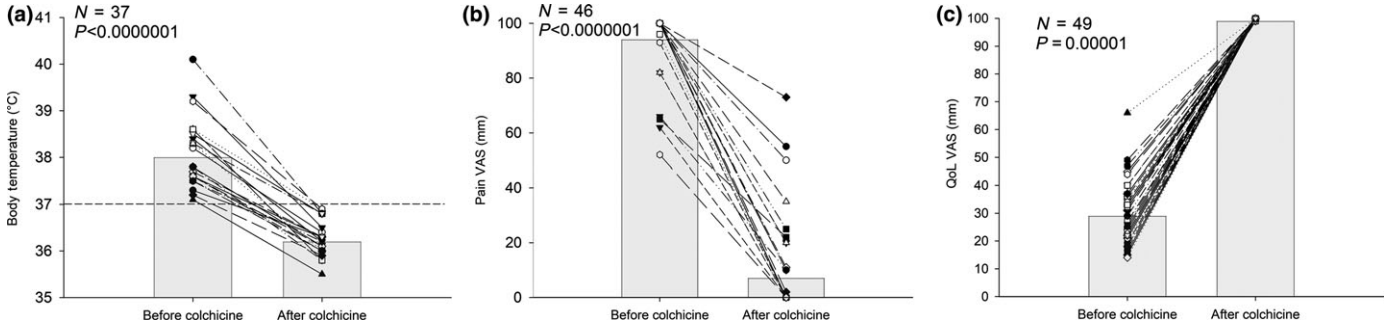

Figure 6 Therapeutic outcome with colchicine in the group of symptomatic FMF patients: peak body temperature (a) and overall pain intensity (b) decreased while QoL (c) increased significantly.

We assume that such unnecessary surgical approach in patients with FMF is caused by the lack of prior knowledge and awareness to this disease. These invasive procedures could expose the patients to the risk of postsurgical morbidity and complication without any impact on their symptoms. Interventional informative programmes are in progress in our area involving emergency departments, surgical divisions, general practitioners and patients' associations to avoid these unnecessary surgeries.

According to clinical manifestations, our patients suffered especially from abdominal pain and arthralgia with a low severity index. In line with these findings, the results of biochemical tests which revealed only a slight increase in inflammatory markers and an increase of SAA only in one-third of patients. Moreover, the late age of disease onset also reflects low penetrance and mild disease severity. In addition, none of those who carried this MEFV mutation developed renal amyloidosis – in our cohort. Different clinical presentations could be the consequence of different individual genetic backgrounds as well as additional environmental factors, two aspects which can modulate the penetrance of the mutation itself. Beside this aspect, one should consider the diagnostic delay which was particularly long in this study (15 years.). A possible explanation could be the 'missing knowledge' (lack of awareness) about FMF in a naïve area.

We confirm that also in our setting, colchicine is the mainstay of treatment for symptomatic FMF patients. In this adult cluster, colchicine reduced symptoms almost totally, and only one young female (R202Q/M694V mutation) was colchicine resistant (crFMF) requiring a biological approach. We expect more cases to be identified in this particular geographical area and merging with additional regional and southern Italian cases.

In summary, our study suggests that the Apulia and Basilicata regions represent an endemic area for FMF. Clinical presentation of FMF can be misleading and requires a complete and early workup to recognise the disease and avoid unjustified surgery. Our cohort displayed mild-intermediate score for severity of FMF. Colchicine remains the gold standard therapy to prevent FMF attacks and fatal long-term complications.

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