RHEUMATOLOGY

Rheumatology 2017;56:209–213
doi:10.1093/rheumatology/kew058
Advance Access publication 5 May 2016

Concise report

Autosomal dominant familial Mediterranean fever in Northern European Caucasians associated with deletion of p.M694 residue—a case series and genetic exploration

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Abstract

Objective. This study was undertaken to characterize the phenotype and response to treatment in patients with autosomal dominant FMF caused by *MEFV* p.M694del mutation and to use haplotype reconstruction to investigate the possibility of common ancestry.

Methods. *MEFV* gene was analysed in 3500 subjects with suspected FMF referred to a single UK centre between 2002 and 2014. Patients with p.M694del underwent additional screening of the *SAA1* gene as well as haplotype reconstruction of the *MEFV* locus.

Results. The p.M694del variant was identified in 21 patients, sharing an identical disease haplotype that appears to have arisen about 550 years ago. The *SAA1.1* allele was found in four patients, including two with AA amyloidosis. The clinical features comprised typical FMF symptoms with median age at onset of 18 years; three patients presented with AA amyloidosis, of whom two had had symptoms of FMF in retrospect. Fifteen patients had received colchicine treatment, all with excellent responses.

Conclusion. The p.M694del variant is associated with autosomal dominantly inherited FMF in Northern European Caucasians. Symptoms may develop later in life than in classical recessive FMF but are otherwise similar, as is the response to colchicine treatment. The 14% incidence of AA amyloidosis may reflect delay in diagnosis associated with extreme rarity of FMF in this population. The common haplotype suggests a single founder living in about 1460.

Key words: FMF, MEFV gene, colchicine, haplotype, AA amyloidosis, dominant inheritance

Rheumatology key messages

- FMF is the commonest recessively inherited autoinflammatory disease of the Eastern Mediterranean region.
- In a Northern European Caucasian population FMF was associated with dominant inheritance caused by p.M694del.
- The FMF associated p.M694del mutation was introduced into the British population in the mid-15th century.

Introduction

FMF is the commonest inherited autoinflammatory disease (AID) affecting mainly Eastern Mediterranean populations and characterized by illness lasting up to 3 days of fever, serositis and arthritis. The FMF gene *MEFV* [1] encodes a 781-amino-acid protein known as pyrin or marenostrin, expressed predominantly in myeloid cells. Over

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Submitted 29 May 2015; revised version accepted 23 February 2016

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300 variants have been reported to date, and of these 56% are known to be pathogenic and associated with the FMF phenotype [2]; the most common are p.M694V, p.M680I and p.V726A. The p.M694V variant has been associated with particularly severe disease, early onset and a higher risk of AA amyloidosis [3–7], the most serious complication of FMF [6, 8–10]. Regular prophylactic colchicine has proven very effective in suppressing FMF-related inflammation, thereby both preventing and treating AA amyloidosis.

FMF is typically inherited in an autosomal recessive fashion, although there have been rare reports of dominant inheritance with specific heterozygous mutations [11, 12] and complex alleles. These include deletion of the methionine residue at position 694 described previously in three British Caucasian families, but the associated phenotype has not been reported in detail [11].

Our objective was to describe the clinical features and response to treatment in 21 patients, who were identified in a single specialist centre to be heterozygous for the p.M694del variant. All patients were of Northern European heritage, prompting us to performed haplotype reconstruction to investigate the possibility of common ancestry.

Materials and patients

Between 2002 and 2014, 3500 subjects with a suspected diagnosis or with a family history of FMF were referred to the National Amyloidosis Centre and underwent sequencing of the *MEFV* gene. Serial measurements of the acute phase reactants serum amyloid A (SAA) and CRP were performed monthly in all individuals.

Informed consent was provided by all subjects. Ethical approval was obtained from Royal Free Hospital Research Ethics Committee (Reference number 06/Q0501/42) in accordance with the Declaration of Helsinki.

Analysis of *MEFV* and *SAA1* genes

Exons 1, 2, 3, 5, 8, 9 and 10 of the *MEFV* gene (NCBI Reference Sequence: NG_007871) were amplified from whole-blood genomic DNA by PCR and sequenced in both forward and reverse directions using the ABI 3130*xl* Genetic Analyzer (Fisher Scientific UK Ltd, Loughborough, UK). Patients who were heterozygous for MEFV p.M694del underwent sequencing of exon 3 of the *SAA1* gene to assess the prevalence of *SAA1* alleles (NCBI Reference Sequence: NC_000011.9) [13].

Haplotype reconstruction of *MEFV* locus

The single nucleotide polymorphism genotyping and haplotype reconstruction were performed concurrently on the 23 DNA samples available from all the 21 subjects with M694del and two healthy relatives of patient 21. Genotyping was carried out using the HumanCytoSNP-12 BeadChip (Illumina, San Diego, CA, USA), according to the manufacturer's instructions. Whole genome haplotypes were reconstructed with 41 043 informative markers utilizing Allegro as described [14]. A common disease haplotype was established by inspection of all haplotypes reconstructed around the disease locus on chromosome 16p13.3. The age of the mutation was calculated with DMLE+ v2.3 software [15]. The disease allele frequency was estimated being 1/100 000; based on this value, the proportion of population sampled was 0.35. The population growth rate per generation derived from historical data for the last 100 years was estimated as 0.084. As most patients had South-West British ancestry the Welsh population has been taken as reference for the calculations.

Histology and immunohistochemistry

Sections of thickness 6 μm of kidney biopsies from three unrelated patients (subjects 9, 18 and 20) who presented with nephrotic syndrome were stained for amyloid with Congo red and viewed under crossed polarized light [16]. Confirmation of AA-type amyloid was sought immunohistochemically using monoclonal antibodies specific to SAA (Euro-Diagnostica, Malmö, Sweden).

Radiolabelled serum amyloid P component scintigraphy

The three patients with AA amyloidosis underwent whole-body anterior and posterior scintigraphic imaging 24 h after administration of ^{123}I-labelled serum amyloid P (SAP) using a GE Infinia Hawkeye gamma camera (GE Healthcare, Little Chalfont, UK), as previously described [17]. The SAP results were interpreted by a panel of physicians with experience of over 29 000 SAP scans (supplementary Fig. S1, available at *Rheumatology* Online).

Results

Analysis of *MEFV* and *SAA1* genes

The *MEFV* p.M694del mutation was found in 21 patients (12 M, 9 F). No other variants were detected in 20 subjects, while one patient carried the common exon 2 polymorphism E148Q. *SAA1* genotyping results are included in Table 1; four patients were found homozygous for the *SAA1.1* (SAA1-α) allele [18], including two who had AA amyloidosis (subjects 18 and 20). All patients were of North European descent.

Histology

Extensive amyloid deposits were identified on the renal biopsy obtained from the three subjects stained with monoclonal anti-SAA antibodies (supplementary Fig. S1, available at *Rheumatology* Online). There was no staining with antibodies against other amyloid fibril proteins known to be associated with renal amyloidosis.

Clinical characteristics in subjects with p.M694del

Clinical details of the cohort are included in Table 1. Fourteen patients (subjects 1–13 and 15) had a history of similar symptoms in at least one relative (supplementary Fig. S2, available at *Rheumatology* Online); two children aged 11 and 17 (subjects 5 and 6), who were identified to have the variant as part of family screening,

Table 1 Clinical characteristics in patients with p.M694del

Kindred/subject	Family history (1 = yes 0 = no)	Age at onset/ diagnosis, years	Attacks, days/ frequency per month	FMF symptoms/ clinical interventions	Triggers	*SAA1* genotype	Treatment	Median SAA/CRP pre-treatment, mg/l[a]	Median SAA/CRP Post-treatment, mg/l
Family 1/subject 1	1	17/62	3/1	Fever, abdominal pain/ cholecystectomy, hysterectomy	Menstrual attacks, stress, fatty food	1.1/1.5	Initially OCP, recently colchicine 1 mg	20/26	6/10
Family 1/subject 2	1	14/26	3/1	Fever, abdominal pain	Menstrual attacks	1.1/1.5	Initially OCP, recently colchicine 1 mg	Not done	3/4
Family 1/subject 3	1	18/38	2.5/1	Fever, abdominal pain	Menstrual attacks	1.1/1.5	OCP	71/49	5/1
Family 2/subject 4	1	18/38	6/0.3	Fever, abdominal pain/ appendectomy aged 12 years	None	1.1/1.5	Colchicine 2 mg	440/105	2/1
Family 2/subject 5	1	None/11	None	None	None	1.1/1.5	None	1	Not done
Family 2/subject 6	1	None/17	None	None	None	1.1/1.5	None	181/38	Not done
Family 3/subject 7	1	Unknown	Unknown	Never seen	Unknown	1.5/1.5	Unknown	Unknown	Unknown
Family 3/subject 8	1	7/32	2.5/1	Fever, abdominal pain, arthralgia	None	1.1/1.5	Colchicine 2 mg	278/62	14/4
Family 4/subject 9	1	6/46	3/0.5	Fever, abdominal pain, chest pain, AA amyloidosis age 47, CKD stage 4	None	1.1/1.5	Colchicine 1.75 mg	1167/302	41/33
Family 4/subject 10	1	10/58	2.5/1	Fever, abdominal pain, chest pain, erysipeloid erythema	Menstrual attacks, fatty food	1.1/1.3	Colchicine 1 mg	107/52	3/2
Family 5/subject 11	1	Unknown	Unknown	Never seen	Unknown	1.1/1.1	Unknown	Unknown	Unknown
Family 5/subject 12	1	35/64	2.5/0.3	Fever, abdominal pain, erysipeloid erythema	None	1.1/1.1	Colchicine 1.25 mg	Not done	3/3
Family 5/subject 13	1	31/34	2.5/1	Fever, abdominal pain, occasional chest pain	Menstrual attacks	1.1/1.5	Colchicine 1 mg	Not done	3/1
None/subject 14	1	13/61	3.5/0.5	Fever, abdominal pain, chest pain/cholecystectomy	None	1.1/1.5	Colchicine 1mg	923/113	9/2
None/subject 15	1	25/43	2/1	Fever, abdominal pain, chest pain	None	1.1/1.5	Colchicine 1 mg	Not done	14/6
None/subject 16	Unknown	Unknown	Unknown	Never seen	Unknown	1.5/1.5	Unknown	Unknown	Unknown
None/subject 17	0	42/50	1.5	Fever, abdominal pain/ appendectomy age 49	None	1.5/1.5	Colchicine 500 μg	673/140	5/1
None/subject 18	Unknown	8/59	3/1	Fever, abdominal pain, chest pain, AA amyloidosis age 60, CKD stage 1/appendectomy age 25, cholecystectomy age 55,	Stress	1.1/1.1	Colchicine 1.5 mg	346/68	9/4
None/subject 19	1	47/56	2.5/2	Fever, abdominal pain, arthralgia	None	1.1/1.5	Colchicine 1 mg	782/131	3/1
None/subject 20	1	None/79	None	None, AA amyloidosis age 79, CKD stage 4	None	1.1/1.1	Colchicine 1 mg	42/17	10/5
None/subject 21	0[b]	20/44	2.5/0.5	Fever, abdominal pain, occasional chest pain	None	1.1/1.5	Colchicine 1 mg	140/49	4/1

[a]The pre-treatment acute phase proteins were measured during febrile episodes. [b]DNA available on two mutation negative relatives; for pedigrees see supplementary Fig. S2, available at *Rheumatology* online. CKD: chronic kidney disease; OCP: oral contraceptive pill.

denied any symptoms at the time of their diagnosis and remain asymptomatic 8 years later, although elevated acute phase proteins in subject 6 may suggest subclinical inflammation. The mother of subject 20 had died of renal failure of unknown cause raising the possibility of AA amyloidosis although she was not known to have had symptoms suggestive of FMF. Four patients reported no family history (subjects 14, 17, 19 and 21) and one was adopted and unaware of any family details (subject 18).

Clinical assessments were made on the 18 patients who had attended our centre: median age at diagnosis with FMF was 45 years (range 11–64) with a median delay of 20 years since the onset of symptoms (range 3–51). Peritonitic abdominal pain and associated fever were the commonest symptoms occurring in 16 patients (89%); seven (39%) had had pleuritic symptoms; two had experienced arthritis typical for FMF; and recurrent erysipelas like erythema occurred in two others. The median age of onset of symptoms was 18 years (range 6–48); median attack duration was 2.5 days (range 1–6), with a median of one attack per month (range 0.3–2). Six patients (33%) had undergone emergency surgery for abdominal crises prior to diagnosis of FMF; three had an appendectomy (subjects 4, 17 and 18) and three had cholecystectomy (subjects 1, 14 and 18). Five of the eight symptomatic women reported attacks associated with menstruation. Three patients (14%) presented with nephrotic syndrome due to AA amyloidosis, in whom the diagnosis of FMF was made subsequently; patient 18 had stage 1 chronic kidney disease and patients 9 and 20 had stage 4 chronic kidney disease. At initial assessment their serum albumin was 36, 37 and 33 g/l, respectively, and 24 h urinary protein loss was 4.7, 2.8 and 7.3 g, respectively. SAP scintigraphy revealed small to moderate total body amyloid load in their spleen and kidney. They had no family history of kidney disease.

Laboratory and clinical response to therapy

Colchicine treatment was administered in 15 patients. Their response and pre/post-treatment levels of SAA and CRP are given in Table 1. The acute phase proteins were measured during febrile attacks on four to eight occasions to serve as a pre-treatment baseline period before colchicine was administered. Median SAA was 278 mg/l (range 42–1167) and CRP 62 mg/l (range 26–302). Median age at starting colchicine therapy (range 0.5–2.5 mg daily) was 50 years (range 27–79). All 15 patients reported remission of symptoms and normalization of SAA concentration to healthy values of <4 mg/l. The three subjects with AA amyloidosis have commenced colchicine since the diagnosis of FMF and have maintained normal SAA values since. The follow-up SAP scans revealed gradual regression of amyloid associated with improvement in renal function in subject 18 while renal function remains stable but poor in patients 9 and 20.

One patient reported that the oral contraceptive pill provided adequate relief of FMF symptoms (subject 3) and two others, subsequently receiving colchicine, reported that the oral contraceptive pill had been very effective in preventing FMF attacks when they were younger.

Haplotype reconstruction

All subjects with p.M694del showed an identical disease haplotype surrounding the deletion (supplementary Fig. S3, available at *Rheumatology* Online), which differed from the haplotypes of the two healthy relatives of patient 21 (data not shown). The disease haplotype stretched from rs734138 to rs7202780, covering a region of about 0.5 million bases (supplementary Table S1, available at *Rheumatology* Online). Based on this data we estimated that the mutation has been introduced into the population with the highest probability 22–23 generations ago (95% CI: 9–58 generations) (supplementary Fig. S4, available at *Rheumatology* Online). Thus, based on an estimate of 25 years per generation, this particular founder mutation appeared within the Northern European population about 550 years ago.

Discussion

FMF typically presents with recurrent episodes of serositis associated with fever lasting for >3 days. It is the commonest genetic disease of the Eastern Mediterranean region, in particular affecting Armenians, Turks, North African and Ashkenazi Jews, and Arabs. However, the severity and frequency of FMF symptoms vary enormously from patient to patient, and in some cases at different stages during life. The relationship between severity of symptoms and risk of AA amyloidosis also varies, and while it does occur rarely in cases with apparently subclinical disease, patients with more severe symptoms, notably in association with the p.M694V variant, appear to be most at risk [3, 5]. There is good evidence that position M694 of the pyrin molecule is critically important for its normal function. The p.M694 is located in the putative binding site of caspase-1 and the substitution or deletion of this residue may interfere with the inhibitory interaction between pyrin and caspase-1, and thus promote IL-1β generation [19]. There are multiple pathogenic mutations at this position: M694V, M694I, M694K, M694L and M694del (fmf.igh.cnrs.fr/ISSAID/infevers/) and there is a consensus that homozygosity for p.M694V is associated with particularly severe disease, earlier onset, colchicine resistance and a higher rate of complications including AA amyloidosis [4–7].

In the Northern European Caucasian population, FMF is an extremely infrequent and rarely considered diagnosis. We report that M694del is the commonest mutation found in symptomatic British patients and results in autosomal dominant FMF. The clustering of 12 patients from the South West of England suggested they might be related. Haplotype comparison in all 21 cases implies that the mutation originated from a single ancestor in the mid-15th century.

AA amyloidosis, the most feared complication of FMF, had developed in three of our patients. Previous studies have suggested that homozygosity for the *SAA1.1* polymorphism in Caucasians carries a 7-fold increased risk for development of AA amyloidosis, compared with other *SAA1* genotypes [13]. Analysis of the *SAA1* gene among

our cohort revealed that four patients were homozygous for the *SAA1.1* allele, including two with AA amyloidosis.

The clinical features of FMF associated with the p.M694del variant in this Northern European Caucasian population were typical of FMF, with the exception of autosomal dominant inheritance. Dominant FMF is unusual, but well recognized and interestingly may be commoner in atypical populations. Three different mutations, p.T577N, p.T577S and p.T577A, have also been reported to cause dominant FMF in British-Chinese, Turkish and British patients, respectively [12]. Unlike p.M694del the phenotype associated with these variants was very broad ranging from classical FMF to symptoms overlapping with other AID including urticarial rash, arthritis, hepatosplenomegaly, conjunctivitis, severe anaemia with growth retardation and delayed psychomotor development.

Conclusion

In summary p.M694del is a cause of dominant FMF in the British population associated with variable penetrance and tendency for symptoms to begin later in life than the classical disease, but nonetheless with considerable morbidity, and three patients (14%) have developed AA amyloidosis. This may well reflect persistent inflammation, early onset of symptoms and delayed diagnosis due to a lack of awareness of FMF in this population, and emphasizes the importance of considering FMF in all atypical populations particularly as all of the patients responded extremely well to daily colchicine, which effectively protects against AA amyloidosis.

Funding: No specific funding was received from any funding bodies in the public, commercial or not-for-profit sectors to carry out the work described in this manuscript.

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

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