EXTENDED REPORT

A decision tree for genetic diagnosis of hereditary periodic fever in unselected patients

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Familial Mediterranean fever (FMF) is an autoinflammatory disease that shows autosomal recessive transmission. FMF is the most frequent of all hereditary periodic fever syndromes, mainly affecting populations from the Mediterranean basin, such as Sephardic Jews, Armenians, Turks and Arabs, with a prevalence ranging from 1 in 200 to 1 in 1000.^{1,2} Its major clinical features are recurrent episodes of fever, accompanied by abdominal pains reflecting acute peritoneal inflammation. The main complication of FMF is renal amyloid A amyloidosis. As this severe complication can be effectively prevented by treatment with colchicine, it is critical that diagnosis of FMF is established early.³ Owing to the absence of specific biological tests, diagnosis of FMF has long been exclusively based on phenotypic grounds, and a set of currently widely used clinical criteria was proposed in Israel in 1997.⁴ The identification in the same year of MEFV, the gene responsible for FMF, allowed the development of a new diagnostic tool.^{5,6} MEFV is located on the short arm of chromosome 16 and encodes pyrin marenostin, a protein associated with innate immunity.⁷ To date, >70 MEFV mutations have been associated with symptoms of FMF, although only five of them (M694V, V726A, M694I, M680I and E148Q) account for 70–80% of the cases in Mediterranean countries.^{8,9} The value of molecular diagnosis of FMF has been well established in patients classically affected by this disease or fulfilling a particular clinical criteria—that is, in selected populations.^{10–14} In unselected cases, the diagnosis of other hereditary periodic syndromes is only rarely considered, and except in expert centres requests for genetic diagnosis almost exclusively focus on the FMF

Background: The diagnostic value of molecular analysis of the familial Mediterranean fever (FMF) gene (Mediterranean fever (MEFV)) has been well established only in patients selected on the basis of ethnic background or clinical criteria. Genetic diagnosis for other hereditary periodic fever syndromes has been poorly evaluated.

Objective: To determine the diagnostic contribution of genetic tests for hereditary periodic syndromes in a large, unselected series of patients.

Methods: A retrospective study was conducted on 1941 patients referred to us for FMF genetic tests between 1997 and 2005. MEFV genotypes were compared with clinical data to appraise criteria for FMF diagnosis. Genetic tests for tumour necrosis factor receptor-associated periodic syndrome (TRAPS), hyperimmunoglobulinaemia D syndrome (HIDS) and cryopyrin-associated periodic syndromes (CAPS) were also reviewed.

Results: 71% of the 1574 patients with enough data had a clinical diagnosis of FMF according to the widely used Israeli criteria. Two MEFV mutations were found in only 409 patients of this subgroup (sensitivity=37%) and in 15 (3.3%) of the patients with an improbable clinical diagnosis of FMF (specificity=97%). Molecular diagnosis for alternate hereditary periodic syndromes was carried out in 456 of the patients having a non-conclusive FMF genetic test. A positive diagnosis was obtained in 31 of these patients (TRAPS (n=19), HIDS (n=4) and CAPS (n=8)).

Conclusions: First-line MEFV mutation screening in patients with clinically typical FMF may be appropriate only in particular areas. To optimise genetic diagnosis, we propose a decision tree, which, with the advice of an expert practitioner, could help redirect test indications towards non-FMF hereditary periodic syndromes.

gene. To date, only two studies have been carried out to evaluate the diagnostic value of molecular tests for FMF in unselected populations, even though in clinical practice such an evaluation could be of great interest in atypical cases.^{2,15} We thus retrospectively reviewed data, from a large series of 1941 unselected patients, that had been referred to our laboratories for genetic diagnosis of FMF between 1997 and 2005. MEFV genotypes were correlated with the presence or absence of symptoms fulfilling recognised clinical criteria.⁴ When only one or no MEFV mutation was detected, and if the clinical presentation of the patient was suggestive, we carried out molecular diagnosis for one or more of the three other hereditary periodic fevers: tumour necrosis factor receptor-associated syndrome (TRAPS),¹⁶ hyperimmunoglobulinaemia D syndrome (HIDS),^{17,18} or cryopyrin-associated syndromes (CAPS), which comprise familial cold urticaria (FCU), Muckle-Wells syndrome and chronic infantile neurological cutaneous and articular syndrome.^{19,20}

METHODS

Samples

We included in this study all patients referred to our laboratories for genetic diagnosis of FMF up to December 2005. We did not require that patients fulfil published clinical

Abbreviations: CAPS, cryopyrin-associated periodic syndrome; FCU, familial cold urticaria; FMF, familial Mediterranean fever; HIDS, hyperimmunoglobulinaemia D syndrome; MEFV, Mediterranean fever; PCR, polymerase chain reaction; TRAPS, tumour necrosis factor receptor-associated periodic syndrome

criteria as a condition for molecular testing. For each patient, epidemiological data (including sex, ethnic background, familial history and age of onset of inflammation signs) and main clinical data (including fever, abdominal, articular, thoracic, cutaneous and neurosensorial signs, duration and frequency of episodes, presence of amyloidosis, and response to colchicine) were recorded by the prescribing doctor using a standard form.

We retrospectively assess the simplified clinical criteria set for the diagnosis of FMF for each patient.⁴ We chose to use the simplified criteria as they were easily retrieved from our standard medical chart, show similar diagnostic value to the extensive ones,⁴ and are more specific than the Tel Hashomer criteria.²¹ These criteria are divided into five main types (1–4, typical episodes of peritonitis, pleuritis or pericarditis, monoarthritis, fever alone; 5, incomplete abdominal episodes) and 4 minor criteria (1–2, incomplete episodes involving chest or joints; 3, exertional leg pain; 4, favourable response to colchicine). The requirements for diagnosis of FMF are ≥ 1 major criteria, or ≥ 2 minor criteria. A comprehensive description of these criteria is provided in the supplementary material (available at <http://www.annrheumdis.com/supplemental>). All patients gave written informed consent before blood sampling, the research protocol was approved by the ethics committee of the CHU of Montpellier, France.

Detection of mutation

Genetic diagnosis of FMF has been available in our laboratories since the cloning of the *MEFV* gene. A specific examination of E148Q (in exon 2) and a generic search for mutations in exon 10 were systematically conducted. When one mutation was identified in this screening step, rare mutations in exons 3, 5 and sometimes 2 were subsequently searched for. We used various combinations of restriction enzyme digestions, an amplification-refractory mutation system and denaturing gradient gel electrophoresis of polymerase chain reaction (PCR) fragments, as previously described.²² Direct sequencing of both strands was carried out where necessary. A test was considered positive when at least two mutations were found in the *MEFV* gene. It was considered non-conclusive in all other cases.

Genetic diagnosis of TRAPS and HIDS has been available in our laboratories since 2003, and that of CAPS since 2004. DNA samples, dating back to 1997 from patients with an inconclusive *MEFV* test, were reanalysed for non-FMF periodic fever genes as we estimated, on the basis of the clinical charts, that complementary tests could be relevant. Tumour necrosis factor receptor superfamily type 1A (TNFRSF1A) exons 2, 3 and 4, mevalonate kinase exons 2 and 9, cold-induced autoinflammatory syndrome 1 exon 3, and the corresponding intronic boundaries of these three genes, were amplified using the M13-tagged primers (supplementary material; available at <http://www.annrheumdis.com/supplemental>). In all, 50 ng genomic DNA, 2.5 mM magnesium chloride, 0.25 μ M each of forward and reverse primers, 0.025 U of Taq Gold polymerase and 0.2 mM dNTP were used for amplification reactions as follows: an initial denaturation at 95°C for 9 min, followed by 30 cycles of denaturing at 94°C for 30 s, annealing at 58°C and extension at 72°C for 1 min, with a final 9-min extension at 72°C. PCR efficiencies were verified on a 5% acrylamide gel, and 1–4 μ l of amplified product was directly submitted to sequencing in both directions with M13 universal primers using the Big Dye Terminator v3.1 kit (Applied Biosystems, Applied Biosystems, France), following the manufacturer's instructions. Sequencing products were run on an ABI Prism Genetic Analyzer 3100 (Applied Biosystems). Mevalonate kinase exon 11 was amplified using 1176 and 1381 primers,²³ and the PCR

fragments were digested with BsmA1 to examine V377I, the most frequent HIDS mutation.

Statistics

Statistical comparisons were carried out using the χ^2 method or Fisher's exact test. A p value >0.05 was considered significant.

RESULTS

Samples from 1941 patients were sent to our laboratories for genetic diagnosis of FMF between 1997 and 2005. FMF was genetically confirmed in 27% patients. The 1941 patients came from 1512 unrelated families, which were used to calculate the genotype distribution. In patients genetically confirmed to have FMF, M694V/M694V was the most frequent genotype (39.2%), followed by M694V/V726A (9.0%), E148Q/M694V (7.6%) and M680I/M694V (5.9%). No other genotype frequency exceeded 5%. In 16.5% of the patients, only one mutation was identified, which in 43% of such patients was M694V. No mutation was found in 56.5% of the patients.

The referring doctors were from diverse specialties (internal medicine 33%, paediatrics 33%, genetics 8%, gastroenterology 7%, infectiology 5%, each of the others $<5\%$). Patients came from various ethnic groups: non-Ashkenazi Jews 20.1%, Arabs 13.4%, Turks 8.7%, Armenians 5.5%, French not from these four groups 14.9%, other 29.8% and not determined 7.6%, with a 0.93 male:female ratio. Patients primarily had recurrent episodes of fever (82.6%) and abdominal pain (77.5%). The other signs of inflammation reported were synovitis (59.1%), pleuritis (31.5%) and erysipelas-like erythema (19.7%). Age of disease onset was <20 years in 66.7% of the patients. Amyloid A amyloidosis was documented in 1.1% patients. Treatment with colchicine had been tested in only 41.5% patients, with a positive response seen in 70.8% and no or little effect observed in the others. We had sufficient phenotypic data to retrospectively determine clinical criteria in 1574 of 1941 patients.⁴ FMF was clinically defined in 1118 of 1574 (71%) patients.

These clinical results were compared with the genetic diagnostic data (table 1). Among the expected results obtained was the finding that the genetic diagnosis was positive in 409 of 1118 (overall sensitivity = 37%) patients with a clinical diagnosis of FMF, and that no mutation was found in 441 of 456 (overall specificity = 97%) patients with an improbable clinical diagnosis of FMF. On the other hand, genetic testing failed to confirm diagnosis in 709 of 1118 (63%) patients with a clinical diagnosis of FMF, although 15 of 456 (3.3%) genetically confirmed patients were not detected when using Livneh's criteria *MEFV*. Table 2 shows the characteristics of the 15 patients with a genetic diagnosis of FMF, but with an improbable clinical diagnosis.

The diagnosis value of *MEFV* testing according to ethnicity was also dealt with (table 1). The sensitivity of the molecular analysis of the *MEFV* gene, and to a lesser extent, the specificity, dramatically varied according to the origin of the patient ($p<10^{-4}$). As expected, the highest rates of positive genetic diagnosis were obtained in patients from the four mainly affected populations (48.5%), but molecular diagnosis also pinpointed 16.2% patients from other Mediterranean countries (Italy, Greece and Spain, but not Portugal). A small number of French patients positive for *MEFV*, with no known Mediterranean ancestry, were also identified (5/234, 2.1%).

The possibility that an alternative hereditary periodic fever syndrome was present was investigated for a subset of the patients with a non-conclusive genetic diagnosis of FMF ($n = 456$). One or more additional genes was analysed after the request of the referring practitioner or on an internal

Table 1 Comparison of clinical and genetic criteria of familial Mediterranean fever

Reference	Study n*	Clinical criteria†	Genetic criterion‡		Sensitivity (%)§	Specificity (%)¶
			Yes	No		
Samuels <i>et al</i> ²	100 (52; 48)	Yes	24	62	28	100
		No	0	14		
Tchernitchko <i>et al</i> ²⁴	208 (0; 208)	Yes	0	208	0	ND
		No	ND	ND		
This study	1574 (752; 822)	Yes	409 (351; 58)	709 (235; 474)	37 (60; 11)	97 (91.6; 99.7)
		No	15 (14; 1)	441 (152; 289)		

FMF, familial Mediterranean fever; MEFV, Mediterranean fever; ND, not done.

*n, number in the total population, followed in brackets by the number of patients in the subgroups from (the four mainly affected ethnic groups; patients from other origin).

†Clinical criteria as defined by Livneh.

‡Genetic criterion as defined by the identification of at least two MEFV mutations.

§Percentage of MEFV-positive patients among those with a clinical FMF.

¶Percentage of MEFV-negative patients among those negative for the clinical criteria.

decision for 225 and 231 patients, respectively. In 231 patients, we did not use available clinical diagnostic criteria (HIDS²⁵ and FCU²⁶) as a basis for selecting patients. Instead, a complementary procedure was carried out when at least one typical sign was recorded—that is, periorbital oedema, myalgia, late onset, long episodes or effectiveness of corticoids for TRAPS; aphtosis, post-vaccinal reactivity or cervical adenopathy for HIDS; neurosensorial symptoms or urticaria for CAPS. A diagnosis was genetically confirmed in 19 of 289 (6.6%) patients tested for TRAPS, 4 of 202 (2%) patients tested for HIDS and 8 of 96 (8.3%) patients tested for CAPS. A mutation in both TNFRSF1A and cold-induced autoinflammatory syndrome 1 was identified in a female patient and her mother (this family is described in a companion paper,²⁷ see page 1530). Note that 17 of these 31 patients initially referred for FMF genetic diagnosis were eventually identified in our laboratories as having an alternate recurrent fever. Tables 3–5 show the epidemiological, clinical and genetic characteristics of these 31 patients.

Although no clear phenotype–genotype correlation can be drawn from this relatively small set of patients, several interesting observations can be made. Firstly, none of the patients with non-FMF periodic fever had renal amyloidosis. Secondly, none of the 19 patients with TRAPS showed pathognomonic periorbital oedema. Thirdly, 10 of these 19 patients have an episode duration ≤ 5 days, considerably shorter than usual report durations. Finally, none of the

patients with HIDS mutations had cervical adenopathies or post-vaccinal hyper-reactivity, two symptoms frequently associated with this syndrome.

DISCUSSION

Until the identification of the *MEFV* gene, FMF was a diagnosis of exclusion that was made based on clinical grounds. The diagnostic value of molecular analysis of *MEFV* has been mainly investigated in selected populations that fulfil clinical criteria or come from classically affected ethnic groups, with genetic confirmation rates ranging from 55% to 89%.^{10–14} Molecular tests allowing the routine diagnosis of FMF have now been developed in many laboratories in non-classically affected countries, such as France. We determined the diagnostic contribution of genetic tests for hereditary periodic syndromes in patients referred to our laboratories up to December 2005. We are aware that such a laboratory-based retrospective study has limitations, especially because we used a clinical form filled out most of the times by non-expert practitioners. However, the aim of the study was to assess unselected patients, and this form was comprehensive and standardised. The epidemiological and clinical characteristics of our 1941 patients show the heterogeneity of cases referred by doctors from various specialities. Their requests for genetic diagnosis of FMF seem to have been justified in more than two thirds of the cases, as 71% of the informative patients had clinical FMF according to published criteria.⁴

Table 2 Characteristics of the 15 patients with a positive genetic diagnosis of familial Mediterranean fever and an improbable clinical diagnosis according to Livneh's criteria

Ethnic background	Sex	Age at inclusion in the study	Major criteria	Minor criteria	Other signs	Genotype
Arab	M	14	No	Incomplete joint episodes	Familial FMF, atypical abdominal signs	M694I/M694I
Arab	M	11	No	No	Splenomegaly, myalgia, atypical articular signs	M694I/M694I
Arab	M	20	No	Incomplete chest episodes	No	M694I/M694I
Arab	F	38	No	Incomplete chest episodes	Atypical abdominal signs	M694I/V726A
Armenian	F	36	No	No	Familial FMF, atypical articular signs	R761H/E148Q
Jewish	M	21	No	No	Familial FMF, Crohn's disease	M694V/M694V
Jewish	F	34	No	Incomplete joint episodes	No	M694V/R761H
Jewish	M	13	No	Incomplete chest episodes	No	M694V/M694V
Jewish	F	23	No	Favourable response to colchicine	Atypical abdominal and articular signs	E148Q/M694V
Jewish	M	4	No	Incomplete joint episodes	Familial FMF	M694V/M694V
Turkish	M	55	No	Favourable response to colchicine	Splenomegaly, atypical abdominal and articular signs	E148Q/M694V
Turkish	M	9	No	No	Familial FMF, myalgia, atypical abdominal and articular signs	M680I/M694V
Turkish	M	6	No	Incomplete chest episodes	Familial FMF	M694V/V726A
Turkish	M	8	No	Incomplete joint episodes	No	M694V/M694V
French	F	52	No	Incomplete joint episodes	No	E148Q/E148Q

F, female; FMF, familial Mediterranean fever; M, male.

Table 3 Characteristics of the 19 patients with a genetic diagnosis of TRAPS

Patients*	Ethnic background	Sex	Age at onset (years)	Episode duration (days)	Episode frequency (per month)	Fever	Abd	Art	Tho	Cut	Myalgia	Peri-orbital oedema	Other signs	Cortico-steroids efficacy	TNFRSF1A genotype
1a	French	F	<1	1–2	<1	+	+	+	+	–	–	–	–	+	C30R
1b	French	M	<1	1–2	<1	+	+	+	–	–	–	–	–	+	C30R
2a	Greek	F	4	14	1	+	+	+	–	–	–	–	–	NT	C73W
2b	Greek	F	2	2	2	+	+	+	–	–	+	–	Pharyngitis	NT	C73W
2c	Greek	F	2	10	1–2	+	+	+	–	–	+	–	–	NT	C73W
3	French	F	1	15	1–2	+	+	+	–	+	+	–	Conjunctivitis	–	H69fs
4	Spaniard	F	6	ND	>2	+	+	+	+	–	+	–	–	–	L39F
5	Arab	M	2	3–4	1	+	+	+	–	+	–	–	Splenomegaly	NT	P46L
6	Turkish	M	10	ND	>2	–	+	+	–	–	+	–	–	+	P46L
7	French	M	43	5	2	+	–	+	–	–	–	–	–	NT	P46L
8a	Sefardi-Jews	F	ND	Chronic	Chronic	–	–	+	–	–	–	–	Aphthosis	NT	R92Q
8b	Sefardi-Jews	M	10	1	<1	–	+	–	–	–	–	–	Conjunctivitis	NT	R92Q
9	Italian	M	26	10	<1	+	–	+	–	–	+	–	Aphthosis	+	R92Q
10	French	M	19	3	<1	+	+	+	–	–	+	–	–	–	R92Q
11	French	M	32	Chronic	Chronic	+	–	+	+	+	–	–	–	NT	R92Q
12	Jewish	F	12	2	<1	+	+	+	+	–	–	–	–	ND	R92Q
13	French	M	ND	3–5	<1	+	+	+	–	–	+	–	–	NT	R92Q
14	French	M	10	10	<1	+	–	+	+	–	–	–	–	NT	R92Q
15	French	M	24	4	1–2	ND	–	–	+	–	+	–	Aphthosis	NT	R92Q

Abd, abdominal signs; Art, articular signs; Cut, cutaneous signs; F, female; M, male; ND, not determined; NT, not treated; Tho, thoracic signs; +, presence of symptoms; –, absence of symptoms.

*Patients' relationships: 1a and 1b are siblings; 2a is the mother of 2b and 2c; 8a and 8b are siblings.

We genetically confirmed the diagnosis of FMF in 27% of the patients. Probably the routine strategy that we and others use—that is, a partial examination of the MEFV gene focusing on mutational hot spots—cannot account for the inconclusive FMF test results obtained in many patients. Indeed, this low value is consistent with two previous studies, conducted in unselected populations, in which the authors yielded a positive diagnostic value in 24 of 100 (24%) and 133 of 303 (44%) patients, respectively.^{2–15} As expected, both we and Grateau *et al*¹⁵ observed higher genetic confirmation rates in patients with a definite diagnosis of FMF. Grateau *et al*'s study used the Tel Hashomer criteria, which are more sensitive but less specific. The genetic approach for diagnosis of FMF was also more effective in patients belonging to the 4 (48.5%) classic ancestries, and most of all in clinically and ethnically typical patients (60%). These values of clinically and ethnically typical patients fall within ranges previously reported for selected populations.^{10–14} Genetic diagnosis was also helpful in 3.3% cases that did not fulfil clinical criteria, and in 7.2% of our patients from other ancestries. Tchernitcko *et al*²⁴ reported a series of 208 western European Caucasian patients meeting Livneh's criteria, including 142 French. None of them had two demonstrable MEFV mutations. We found 5 of 234 positive western European Caucasian (French) patients, but the difference between the two studies was not significant. Restricting genetic diagnosis to those cases that strictly fulfil the clinical criteria or that are from classically affected ethnic backgrounds may not be rational, as a diagnosis of FMF would have been missed in 15 and 58 of our patients, respectively. We concede, however, that genetic confirmation of FMF was not made in 441 of 456 (97%) patients without a clinical diagnosis of FMF.

In patients with a non-conclusive genetic diagnosis of FMF, other hereditary periodic fevers should be considered as a preferred strategy, especially in patients with non-Mediterranean ancestry. We carried out complementary molecular tests in 456 patients with phenotypes compatible with TRAPS (n = 289), HIDS (n = 202) or CAPS (n = 96), and found TRAPS, HIDS and CAPS mutations in 6.6%, 2% and 8.3% of the tested patients, respectively. Altogether, we found that 31 of 456 (6.8%) of the patients referred to us for genetic diagnosis of FMF actually had a different hereditary periodic syndrome. The significance of some sequence variants such as E148Q in MEFV, R92Q and P46L in TNFRSF1A receptor,²⁸ or V198M in CAPS is, however, currently debated because they are frequent in the general population. They may correspond to functional polymorphisms, and are recognised at least as low-penetrance mutations. In our family with both TRAPS and CAPS mutations, the two patients with combined mutations presented with quite atypical, overlapping and severe symptoms, whereas the three patients with only one variant were either totally asymptomatic or had a much milder disease.²⁷ This suggests that the phenotype of the patient may depend on his or her genetic background or result from digenic inheritance, which is consistent with the transmission pattern observed in our family. Accumulation of clinical data related to these variants will help elucidate their true significance. An accurate diagnosis is critical for targeting novel and effective therapeutic agents to these conditions, such as anti-interleukin 1 in CAPS, anti-tumour necrosis factor in TRAPS, and possibly simvastatin and anti-interleukin 1 in HIDS.^{29–32} The diagnosis of patients in whom we could identify no mutation remains unclear. They may have been carrying a rare mutation that escaped our

Table 4 Characteristics of the four patients with a genetic diagnosis of hyperimmunoglobulinaemia D syndrome

Patients	Ethnic background	Sex	Age at onset (years)	Episode duration (days)	Episode frequency (per month)	Fever	Abd	Art	Tho	Cut	ADP	Aphthosis	Post-vaccinal reactivity	Other signs	IgD (UI/L)	MVK genotypes
1	French	M	5	3	<1	+	+	+	+	–	–	–	–	–	>400	I268T/V377I
2	French	M	3	3–5	<1	+	+	–	–	–	–	–	–	Pharyngitis	ND	I268T/V377I
3	French	F	<1	2	1–2	+	–	+	–	–	–	ND	–	–	>800	V377I/S329N
4	French	F	<1	3–4	1–2	+	+	+	–	+	–	–	–	Conjunctivitis	normal	V377I/V377I

Abd, abdominal signs; ADP, cervical adenopathies; Art, articular signs; Cut, cutaneous signs; F, female; M, male; MVK, mevalonate kinase; ND, not determined; Tho, thoracic signs; +, presence of symptoms; –, absence of symptoms.

Table 5 Characteristics of the eight patients with a genetic diagnosis of cryopyrin-associated periodic syndrome

Patient*	Ethnic background	Sex	Age at onset (years)	Episode frequency (per month)	Episode duration (days)	Fever	Abd	Art	Tho	Urticaria	Deafness	Meningitis	Other signs	CIAS1 mutation
1a	French	M	3	<1	>7	+	+	+	-	+	-	-	Oedema	V198M
1b	French	F	5	<1	>7	-	-	+	-	+	-	-	Oedema	V198M
3a	Italian	F	15	2	1-2	+	+	+	+	+	-	-	Myalgia, Uveitis	R260W
3b	Italian	M	7	1-2	1-2	+	-	+	-	+	+	-	Myalgia, Uveitis	R260W
4	Italian	F	8	1-2	1	+	-	+	-	+	-	-	Bi-polar aphthosis	R260W
5	French	F	0-1	1	14	+	-	+	-	+	+	-	Conjunctivitis	T348M
6	French	F	<1	>2	1	+	-	+	-	+	-	-	-	D303N
7	French	F	<1	1-2	1-2	+	-	+	-	+	-	-	Conjunctivitis	D303N

Abd, abdominal signs; Art, articular signs; F, female; M, male; Tho, thoracic signs; +, presence of symptoms; -, absence of symptoms.

*Patients' relationships: 1b is the mother of 1a; Sibships: 3a and 3b.

screening strategy, a mutation in a yet unknown gene, or may have had another inflammatory condition. The low number of genetically confirmed diagnosis of periodic diseases was probably caused by the fact that samples were freely sent to our laboratories. As observed by Simon *et al*,²⁴ a low yield is reached when tests are carried out on the basis of a minimal clinical suspicion.

In conclusion, molecular screening of MEFV mutations proved to be of positive diagnostic value in 27% of 1941 unselected patients having an inflammatory disorder of unknown origin who were referred for routine genetic diagnosis of FMF. Even if this value had been increased to 60% by testing only the most typical patients, 73 of our clinically (n = 15) or ethnically (n = 58) atypical patients

would have been missed had they not been genotyped. At the same time, consistent with previous studies conducted in Western countries,^{2, 15, 24} the diagnosis of FMF was not genetically confirmed in most of our patients, strongly suggesting that most of them had another disease that sometimes meets the clinical diagnostic criteria of FMF. It is important for clinicians to realise that there is a considerable number of patients with definite autoinflammatory syndromes without genetic diagnosis. This suggests that the widely used Israeli clinical criteria may only be appropriate in particular areas. We thus propose that in such cases, molecular tests should be carried out only after an expert's advice. This expert consultation could point to the use, when appropriate, of molecular tests for non-FMF conditions

Figure 1 Decision tree for genetic testing in patients with unexplained recurrent fever.

including TRAPS, HIDS and CAPS, potentially allowing a correct diagnosis to be obtained in as many as 6.8% more patients. In light of these results, we propose a decision tree for determining the indications of molecular diagnosis in patients with periodic fever in uncommonly affected areas (fig 1).

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