Familial Mediterranean fever (FMF) is an autoinflammatory disease mainly affecting populations surrounding the Mediterranean basin, with a genetic prevalence reaching 1–6% (1). Patients suffer from seemingly unprovoked bouts of fever, peritonitis, and arthritis, which resolve spontaneously. FMF has long been recognized as the sole "periodic fever". Diagnosis often took years to establish after the onset of symptoms, following costly and time-consuming elimination of other causes of inflammation. Biological tests were of little help, only demonstrating nonspecific increases in concentrations of acute-phase reactants. Identification of the responsible gene, MEFV, by positional cloning (2, 3) has revolutionized management of patients, who now benefit from specific diagnosis through mutation screening of MEFV. Systematic genetic characterization of patients with definite clinical criteria has revealed a limited number of recurring mutations, associated with the ancestral haplotypes (4) that were highlighted during the search for the gene. Five founding mutations, M694V, V726A, M680I, M694I (all four in exon 10), and E148Q (exon 2) in order of decreasing frequency, account for >70% of deleterious alleles. Several others are relatively prevalent, depending on the ethnic origin of the patient, e.g., A744S and I692del in Arabs, R761H in Armenians, and F479L in Greeks. Finally, approximately three-fourths of the remaining mutations were found only in single patients. Interestingly, no gross gene rearrangement has been found to date, strongly supporting the idea that such an alteration would lead to other phenotypes or would be lethal.

The number of laboratories providing genetic tests for FMF has flourished since the discovery of the gene in 1997. At least 15 of these laboratories are in Western Europe (5), although in this part of the world the disease is not endemic. This demonstrates that requests for testing have increased because of the increased awareness of doctors and patients. There is no consensual technical approach to date: all mutation detection methods have been used. Approximately one-half of the laboratories have adopted a two-step strategy starting with exon 10 screening (sequencing or denaturing gradient gel electrophoresis) and E148Q-specific determination (observation of restriction fragment length patterns). Rarer alleles are then nonsystematically searched for in non-exon 10 mutation-rich regions (exons 2, 3, and 5). Other laboratories perform targeted detection of either all five of the above-mentioned frequent mutations or of various combinations of one to four of these mutations. These strategies are tedious, not automated, and thus require several days to be completed.

FMF StripAssay, an new commercial assay for FMF genetic diagnosis, is described in two accompanying reports in this issue of Clinical Chemistry. Oberkanins et al. (6), from the Viennalab laboratory where the assay originates, present the development of this reverse hybridization assay, its specificity, and its potential use for routine purposes. Twelve of the most recurrent MEFV mutations are simultaneously investigated in an easy-to-use, half-day-long procedure. DNA from the patient and controls is multiplex-amplified using biotinylated primers; amplified products are hybridized to probed nitrocellulose stripped membranes and revealed by a streptavidin–alkaline phosphatase colorimetric assay. Mutations are readily identified by alignment of the generated purple bands on a matched-size decoder table provided by the manufacturer. A clear cutoff coloration between positive and negative bands is obtained.

The FMF StripAssay was also tested in an independent laboratory that routinely used classic methods but wished to implement a more efficient procedure. Tchernitchko et al. (7) evaluated the influence of several variables on the sensitivity and specificity of the reverse hybridization assay. Incubation temperature was found to be critical for specificity. Both the Austrian and French groups experimented with the new technique in a large sample of patients with FMF-like disease and obtained full concordance with reference methodologies. In our hands, the assay was also sensitive, but our results were compromised by small variations in the incubation times (I. Touitou and C. Lault, unpublished data).

The first FMF assay to be commercially available was Pronto™ FMF (Savyon Diagnostics). This test is based on a primer extension assay followed by ELISA, and it is designed for detection of four to six “oriental” MEFV mutations. The FMF StripAssay includes six additional rarer mutations but is slightly more expensive. Both assays appear to be reliable and convenient for large-scale and routine FMF testing. However, one must keep in mind the risk of erroneous interpretation of results inherent either to general pitfalls associated with mutation-specific-based protocols or to more specific ones related to FMF gene peculiarities. One pitfall is the existence of several complex MEFV alleles (mutations in trans on the same chromosome), e.g., V726A + E148Q in Druzes (8) and F479L + E167D in Greeks (9) and Armenians (10). FMF is a recessively inherited disease, and finding two mutations does not necessarily confirm the disease genetically. Phasing by analysis of the parents’ DNAs is mandatory, especially if one of the two mutations is E148Q, a low-penetrance mutation frequent (up to 4%) in the general population. Another pitfall is the existence of at least four “hot spots”, i.e., codons with more than one of the reported mutations (codons 694, 692, 680, and 148) (11). Detection of a positive signal with the M694V probe and no signal with both the M694I and the corresponding wild-type probes correctly pinpoints true M694V homozygotes in the vast majority of cases, but it does not preclude that the genotype of the tested patient is actually M694V/M694del or M694I. Yet another pitfall is the spectrum of FMF mutations. Whereas the two tests mentioned above cover patients with Arabic, Armenian, Jewish, or Turkish ancestry fairly well, it is becoming clear...
that FMF mutations may arise in patients with various origins. Rare mutations are preferentially found in ethnic groups not classically affected (1), and the sensitivities of the tests are expected to be very low in such populations. The final pitfall involves the accessibility of the tests, a pitfall specific to the disease and not to the test principle. The costs of the tests unfortunately make them unaffordable for countries where they would be the most useful!

What is the future of FMF assays? A few years of experience are needed to get a proper opinion on the sensitivity and specificity of these tests, provided that investigators abandon their in-house procedures. It may be reasonably anticipated that they will because the two protocols are already widely used for other diseases. Moreover, as commented above, there is no unified approach for FMF genetic testing throughout the world, and the accuracy of the diagnosis has not been assessed. The results of a quality-control trial set up 5 years ago for cystic fibrosis suggested that there are many laboratories (35%) that have a percentage of errors unacceptable in a routine testing setting (12). The development of a consensus testing strategy could facilitate and improve FMF genotyping, and such assays may turn out to accomplish harmonization among reported results. On the other hand, MEFV analysis remains noncontributive for a non-negligible set of patients. This observation is likely to be attributable to rare mutations and locus heterogeneity. Locus heterogeneity may be interpreted as non-MEFV genes responsible for periodic fevers other than FMF, e.g., tumor necrosis factor receptor-associated periodic syndrome (13), hyper-IgD syndrome (14,15), and familial cold autoinflammatory syndrome (16). Altogether, the viability of these assays lies in their capacity to evolve, become less expensive, and expand the number of examined mutations and inflammatory genes.

References


Isabelle Touitou

Laboratoire de Génétique Moléculaire et Chronosomique
Hôpital A de Villeneuve
34295 Montpellier cedex 5, France
Fax 33-4-6733-5862
E-mail isabelle.touitou@igh.cnrs.fr

DOI: 10.1373/clinchem.2003.025791