Editorial

The impact of *MEFV* gene identification on FMF: an appraisal after15 years

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Familial Mediterranean fever (FMF) is a hereditary periodic fever disease characterised by recurrent attacks of serositis and fever (1). It was first described in 1945 by Siegal in New York (2). Reimann was the first to introduce the term "Periodic disease" in 1948 (3). Only 8 years later did Heller suggest the name Familial Mediterranean fever (FMF), although today we know that the disease may be sporadic and exists in countries far from the Mediterranean basin (4).

In 1997 we witnessed a major advance in the field of FMF investigation. The *MEFV* gene, which is associated with FMF, was identified and isolated by two independent consortia (5, 6). It was found that the gene encodes a protein named pyrin/marenostrin which plays an important role in the process of inflammation (7).

Fifteen years after this impressive discovery we ask: how has the identification of the *MEFV* gene affected our current knowledge and understanding of FMF as well as the management of FMF patients?

To evaluate the significance of the *MEFV* gene identification, we review its contribution to the following topics of FMF: Etiology and Pathogenesis, Genetics, Clinical features, Disease severity, Amyloidosis, Diagnosis, Treatment and Prognosis.

Etiology and pathogenesis

Over the years several theories have been proposed to explain the etiology of FMF. It was suggested that FMF is an autoimmune disease (vasculitis), and in a single study the authors even found autoantibodies – results which have not been confirmed in later studies (8, 9). An additional theory suggested that etiocholanolone (an endogenous steroid metabolite) is responsible for the fever characterising this disease (10). Studies by George *et al.* showed no role for this metabolite in the etiology of FMF (11). A group of investigators in Kuwait suggested that disturbed metabolism of cathecholamines may lead to FMF attacks. They even proposed the "metaraminol provocative test" for diagnosing FMF (12). Our findings did not confirm this suggestion (13). Deficiency in complement components, such as the C5a inhibitor, was also proposed as an etiology for FMF (14), but this theory, too, was not confirmed.

Following the August 1997 "revolution" with the isolation of the *MEFV* gene, we learned that pyrin/marenostrin is a key component of the innate immune system and its mutations lead to FMF due to modification in this protein function. This classifies FMF within the new family of autoinflammatory diseases which are characterised by apparently unprovoked inflammation without the involvement or production of autoantibodies or activated T-cells (15).

Studies following the identification of the *MEFV* gene indicated that pyrin modulates the production of the potent pro-inflammatory cytokine interleukin (IL)-1β probably through two major pathways: *i*. The N-terminal fragment of pyrin may regulate NF-κB activation through interactions with an adaptor protein named apoptosis-associated speck-like protein with a caspase-recruitment domain (ASC) and/or with p65 NFκB (16-17) *ii*. Pyrin regulates caspase-1/IL-1β activation through its interaction with ASC (18). Additionally, the C-terminal B30.2 domain of pyrin, also known as SPRY, directly interacts with caspase-1 to modulate IL-1β production. ASC works as an adaptor molecule that connects the stress-sensing component and pro-caspase-1 through its N-terminal PyD domain and

C-terminal CARD domain respectively (19). This brings two molecules of procaspase-1 into close proximity, leading to proteolytic activation and the subsequent release of the active catalytic domains, p20 and p10. Active caspase-1, in turn, cleaves the 31-kDa precursor form of IL-1β into its biologically active 17-kDa fragments.

The *MEFV* gene contains 10 exons. More than 200 sequence variants, almost all single nucleotide substitutions, have been recorded in Infevers, a database dedicated to auto-inflammatory mutations (http://fmf.igh.cnrs.fr/ISSAID/infevers/) (20). Only a small number of these variants are unambiguously pathogenic, such as M694V, a severe mutation with a founder effect, and no more than half of the reported *MEFV* variations are associated with the FMF phenotype. How mutations in the *MEFV* gene can result in so varied an inflammatory phenotype has been only partially elucidated. Most studies related to pyrin's function in the innate immune system have been focused on the regulation of the caspase-1 activation and subsequent IL-1β secretion. A growing body of evidence suggests, however, that FMF mutations may also lead to dysregulation in the *MEFV* expression affecting pyrin level.

Despite our current understanding of the pathogenesis of FMF, there is still no explanation for the periodicity of the attacks and their spontaneous recovery.

Genetics

Based on previous epidemiological studies by Sohar, Pras and Heller, an autosomal recessive transmission of FMF was proposed (21). Yuval *et al*. raised the possibility of an autosomal dominant transmission of FMF (22). However, it was thought that multiple intermarriage in the populations studied led to the incorrect conclusion of dominant inheritance which actually was pseudodominant. Following the isolation of the gene and its mutations, it was quite clear that FMF is an autosomal recessive disease. Still, Booth *et al*. described an FMF patient with the M694del mutation in whom the transmission of the disease was dominant (23). It was suggested that a single deletion – in contrast to missense mutation – is capable of causing FMF due to its serious effect on the peptide (pyrin) product. Recently, an autosomal dominant trait was also proposed by Booty *et al*. and Marek-Yagel *et al*. (24, 25). In these studies the authors thoroughly checked for the second mutation in groups of FMF patients who carried only a single mutation but without success. They concluded, therefore, that FMF transmission may be autosomal dominant, or digenic, or depend on modifier genes and environmental factors. It should be emphasised that most Mediterranean individuals with a single mutation are carriers by chance because of the high allele frequency in these populations. We still do not have a convincing explanation for the presence of FMF in patients who do not carry any *MEFV* mutation.

Clinical features

From the beginning, the clinical features of the disease were quite clear and included fever, serositis (peritonitis, pleuritis, pericarditis and synovitis) and erysipelas-like erythema. Following the *MEFV* gene isolation nothing has been added to this particular topic. Nevertheless, the isolation of the gene allowed us to find new clinical presentations – other than FMF – where the patients carry *MEFV* mutations and respond to colchicine treatment (26). Thus, the isolation of the *MEFV* gene widened the spectrum of clinical entities associated with it, in addition to FMF.

Disease severity

In 1982, an observational study by Pras *et al*. showed that Israeli Jews of North African origin experienced more severe disease than FMF patients of European or even Iraqi or Syrian extraction (27). The reason for these differences in severity among the various Jewish communities in Israel was not at all clear. Isolating the *MEFV* gene stimulated studies looking for a correlation between the genotype and phenotype of FMF. It was found that patients homozygous for the M694V mutation had more severe disease with earlier onset, more joint involvement, required a higher dose of colchicine for controlling the disease and were more prone to develop amyloidosis (28). Since it was shown that 70–80% of the Jews of North African origin carry the M694V mutation, it became clear why their disease was more severe. Jewish patients of other origins also bear the V726A or E148Q mutations which cause a milder form of the disease.

One may raise the question as to whether the *MEFV* gene is the entire story in FMF. The answer of course is "not really" since we encounter FMF patients who carry identical mutations and yet have different disease severity. On the other hand, siblings with FMF may display different genotypes. Therefore, it is suggested that additional environmental and other genetic modifiers play a role in the final clinical presentation of the disease. For example, FMF patients in Armenia have more severe disease and amyloidosis compared with their relatives who live in the USA (29). Similarly, Turkish FMF patients have more severe disease when living in Turkey compared with those living in Germany (30).

Amyloidoisis

In 1955 Mamou and Cattan were the first to recognise the kidney involvement by amyloidosis in FMF (31). The presentation of FMF as nephrotic syndrome due to amyloidosis without previous typical FMF attacks (phenotype II) was described in 1962 by Blum *et al*. (32). Following the identification of the *MEFV* gene, several points became clearer. First, as already mentioned, a correlation was found between homozygousity for several mutations such as M694V and amyloidois. Second, it was found that the gender of the patient and serum amyloid A (SAA) gene polymorphisms also play an important role in the development of amyloidosis. Last but not least, it was found that the country where the FMF patient lives plays an important role in the development of amyloidosis (33). This was explained by the conditions of the health system and additional environmental factors which differ among these countries.

Diagnosis

The diagnosis of FMF is based on clinical grounds. If the patient's origin fits and he has a family history of the dis-

ease, the diagnosis is further supported. In atypical cases in which the clinical presentation or the origin of the patient is not typical, the colchicine therapeutic test is used. In this trial we give the patient colchicine and compare the frequency of the attacks before and after treatment. If the treatment significantly reduced the rate of attacks and following cessation of colchicine the patient develops an acute attack, the diagnosis of FMF is quite definite. Genetic confirmation of the diagnosis is the main improvement gained from the identification of the FMF gene. Indeed, mutations screening of the *MEFV* gene is a valuable and specific way to make the diagnosis or to confirm it in such atypical cases. Nevertheless, genetic testing is helpful only when the results show 2 or more mutations, In cases where a single mutation (heterozygote) or no mutation is found, the genetic test does not contribute to the process of FMF diagnosis. In these cases one should perform the colchicine therapeutic test. In countries where FMF is rare, genetic testing is much more helpful for diagnosis, especially in atypical presentations.

Treatment

Colchicine has been the drug of choice since 1972 (34). Following the isolation of the *MEFV* gene, the basic treatment with colchicine has remained the same. Nevertheless, the understanding of the pathogenesis of FMF and the essential role of IL-1 in the cascade of inflammation led to the use of anti IL-1 agents in the 5% of FMF cases resistant to colchicine. On the other hand, the *MEFV* identification raised some dilemmas. Should we screen the siblings in families with an FMF patient? If we detect asymptomatic individuals with 2 clear FMF mutations, should we treat them?

Prognosis

FMF prognosis depends on the development of amyloidosis. If the diagnosis is made early and treatment with colchicine is started, the chances of developing this complication are virtually nil.
The identification of the gene has the potential to improve the prognosis, since in atypical cases it can accelerate the diagnosis and treatment of FMF patients.

Conclusion

In reviewing the above aspects of FMF, it seems that the isolation of the *MEFV* gene has had an enormous impact on almost all fields of this disease. Fifteen years after the identification of the gene, we better understand the etiology and pathogenesis of the disease and have improved the care and health of our patients (earlier treatment, as well as less pain, absenteeism and complications). Nevertheless, there are still unanswered questions and dilemmas to be solved over the next 15 years

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