Familial Mediterranean Fever: Genetic Update

Alessandra Soriano MD[1] and Elon Pras MD[2]

[1]Periodic Fevers Research Centre, Department of Internal Medicine, Catholic University of Sacred Heart, Rome, Italy
[2]Gertner Institute of Human Genetics, Sheba Medical Center, Tel Hashomer, affiliated with Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

KEY WORDS: familial Mediterranean fever (FMF), pyrin, *MEFV* gene, genotype, phenotype

IMAJ 2014; 16: 274–276

The gene causing familial Mediterranean fever was cloned in 1997 simultaneously by two groups [1,2]. Called *MEFV,* this relatively small gene is composed of 10 exons and encodes a 781 amino acid protein named pyrin. MEFV is expressed in polymorphonuclear cells and macrophages and FMF is thus a disease of the white blood cells. Supporting the hematopoietic origin of FMF was a report from 2002 [3] describing an allogenic bone marrow transplantation in a child who suffered from FMF and congenital dyserythropoietic anemia. Although the procedure was performed for congenital anemia, the child was cured of both diseases.

Most of the mutations that cause classical disease are found in exon 10 in the region of the B30.2 domain. To date, more than 150 MEFV mutations have been described, most of which are missense mutations. Very few deletions or insertions have been described. The most common alterations in this gene are M694V, V726A and E148Q; the first two are undoubtedly mutations; however, some concern has been raised with regard to the third. M694V is a severe mutation, which in the homozygous state is associated with severe disease manifested by early age of onset, frequent attacks, increased frequency of arthritis, erysipelas-like erythema and amyloidosis, as well as a relatively high dose of colchicine needed to stabilize the disease. Interestingly, in 2010 a Turkish study found that these patients do not respond well to colchicine [4]. Only 36% of the patients homozygous for M694V showed a complete response to treatment, in comparison to much higher response rates in patients with other mutations; 18% of the M694V homozygous patients were defined as non-responders compared to only 3% of the M694V/V726A patients. A study from the Sheba Medical Center showed that only 25% of the M694V homozygous patients (10/40 patients) did not report any attack in the year prior to the initiation of this study, while 30% of them reported more than one attack every 2 months. This was on maximal tolerable colchicine therapy [5].

Familial Mediterranean fever (FMF) is a periodic autoinflammatory disorder caused by mutations in the *MEFV* gene

FMF = familial Mediterranean fever

Shortly after the discovery of *MEFV,* it became clear that the carrier rate for the disease is very high in the Mediterranean region; a study by Shohat et al. [6] found a carrier frequency of 8% and 5% for M694V among North African Jews and Iraqi Jews, respectively, and a carrier rate of 5% for the V726A mutation in Iraqi Jews, implying an overall carrier rate of 8%–10% in these two ethnic groups. Similar results were obtained in other studies; for example, a study from Turkey found a combined carrier rate of 10% for the three mutations M694V, M680I and V726A [7]. The finding of high carrier rates with more than one mutation in a limited geographic area raises the question of a selective advantage for the heterozygotes. The hypothesis of an advantage over an infectious agent prevalent in the Middle East is attractive but has yet to be proven.

Since the discovery of the gene, considerable progress has been made in our understanding of the cellular pathways through which pyrin acts: in short, pyrin binds to two other proteins – PSTPIP1 and ASC – and converts caspase-1 into its active form. Caspase-1 converts pro IL-1β into IL-1β. The latter is a pro-inflammatory molecule responsible for producing many of the disease symptoms and signs. Interestingly, in some of the genes involved in this pathway, mutations were found in other autoinflammatory diseases, such as pyogenic arthritis, pyoderma gangrenosum and acne – PAPA syndrome (*PSTPIP1*), cryopyrin-associated period syndromes – CAPS (*NLRP3*) and deficiency of IL-1 receptor antagonist – DIRA (*IL1RN*). The identification of these pathways provided the theoretical support for treating FMF patients with IL-1 antagonists, including anakinra, canakinumab and rilonacept, which are used mainly in colchicine-resistant patients [8-10].

Over the years, there have been a few attempts to produce transgenic mice models for FMF. Most of these have failed, mainly because the mice equivalent of the *MEFV* gene does not contain the B30.2 domain. Chae et al. [11] created a transgenic mouse containing a human-mice hybrid of the *MEFV* which included the B30.2 domain with the V726A mutation. Mice homozygous for this hybrid gene were smaller compared to heterozygous and wild-type mice, and showed marked evidence of inflammation, including arthritis, enlarged lymph nodes and spleen, all heavily infiltrated with polymorphonuclear cells. In contrast to the disease in FMF patients, in these mice the disease was not periodic, but continuous.

The finding that patients with the same genotype suffer from different symptoms and disease severity suggested that the final phenotype is not determined by *MEFV* alone, but by a combination with other modifier genes and environmental factors. Factors like menstrual period in women, increased physical activity and psychological stress can trigger FMF attacks and are considered environmental factors. In a study published in 1974, Schwabe and Peters [12] showed a very low frequency of amyloidosis among Armenians living in Los Angeles compared to Armenians who lived in Armenia. The authors related their findings to environmental factors [12]. One of the first genes shown to affect the disease course was the *SAA* gene. Cazeneuve and co-authors [13] showed that homozygosity for the alpha allele at the SAA1 locus predisposes for the development of amyloidosis. These results were later confirmed in other studies [14].

In 20–25% of patients only one mutation can be identified in *MEFV*

Another gene affecting the phenotype is the *MICA* gene, a gene involved in different inflammatory diseases. Touitou et al. [15] showed that a certain variant in this gene, A9, predisposes to a more severe disease. Recently, Berkun and colleagues [16] showed that FMF patients carrying mutations in *NOD2/CARD15* gene (previously associated with Crohn's disease) are prone to a more severe disease course and high rates of colchicine resistance. These modifier genes were found using the "candidate gene" approach. A more systematic approach is to perform genome-wide association studies in search for genes affecting the phenotype. Such GWAS have helped to find modifier genes in diseases such as cystic fibrosis, BRCA1 and BRCA2-related breast cancer, thalassemia and sickle cell anemia. It is most probable that in the near future we would see such studies in FMF.

FMF was considered as a prototype of recessive diseases, and indeed in 60–70% of the patients two mutations are found. However, in 20–25% of patients only one *MEFV* gene mutation can be detected even when complete sequencing of the gene is performed [17-19]. These patients suffer from classical disease, although much milder than in patients with two mutations. A number of rigorous studies, which included complete sequencing of the gene and extensive areas of the promoter and multiple ligation-dependent probe amplification in search for deletions and duplications, failed to identify a second mutation [20-21]. Thus, it became clear that FMF in these patients is caused by a single *MEFV* mutation. Candidate genes involved in other autoinflammatory disorders and closely related to FMF, such as *PSTPIP1*, *ASC* and *NLRP3,* were screened in these patients for mutations but none were found. According to our calculations approximately 1 of 50 carriers of the *MEFV* M694V mutation will present with clinical symptoms of FMF.

Modified genes and environmental factors have an important role in determining the final phenotype of the patients

GWA = genome-wide association studies

E148Q, an evolutionary conserved amino acid change, was initially described as a mild low penetrance mutation. However, this variant is found in about 3–25% of control samples. Its prevalence in the Israeli population is about 5%, but in the Far East it is very common and is found in 25% of normal individuals. In a population where FMF is extremely rare, such a high carrier rate raises doubts whether it is a real mutation. One of the most common ways to differentiate between a mutation and a polymorphism is to assess its frequency in patients versus controls. This is especially important in cases where no functional assay is available. A number of studies performed on large cohorts of FMF patients failed to show such a difference for E148Q [22,23]. We performed the largest study of its kind, with 412 FMF patients and 1400 controls, but did not detect a significant difference between the groups. E148Q appeared in 7% of patients compared to 5.81% of controls ($P = 0.23$) [24]. This issue is still controversial and some investigators claim that E148Q is indeed a pathological mutation [25]. In addition to E148Q, there are other variants of unknown significance including P369S and R408Q.

Recently, a group from the Netherlands described three families with patients who suffered from periodic fever, arthritis, pleuritis and an urticarial rash, and who had an autosomal dominant mode of inheritance. The investigators presumed that they were dealing with a new autoinflammatory disease. They performed exome sequencing in one of the patients and surprisingly found a heterozygous missense mutation in position 577 in *MEFV*. Screening of the other patients revealed another missense mutation exactly in the same spot. A few of these patients responded to colchicine, others to IL-1 blockers, while some did not respond to either of these medications [26]. Thus, the spectrum of the diseases associated with *MEFV* gene is expanding and perhaps we should start using terminology such as *MEFV*-associated diseases, analogous to tumor necrosis factor receptor-associated periodic syndrome (TRAPS) and cryopyrin-associated periodic syndromes (CAPS).

Over the years, an increased rate of *MEFV* carriers has been found in complex multifactorial diseases such as Behçet disease [27,28], Henoch-Schönlein purpura [29,30], polyarteritis nodosa [31], PFAPA syndrome (periodic fever, aphtous stomatitis, pharyngitis, cervical adenitis) [32], ankylosing spondylitis [33], and multiple sclerosis [34], thus implicating this gene and its pathways in the development of these disorders. Another interesting association was recently described between fibromyalgia and the *MEFV* R202Q variant [35]. This last finding awaits further confirmation.

FMF is mainly a clinical diagnosis and although the identification of *MEFV* has greatly advanced our understanding about the pathophysiology of the disease, it did not resolve all the diagnostic problems [36,37]. In fact, some new questions

have emerged; for example, how do we define patients with two mutations but no symptoms, or patients with typical disease but no *MEFV* mutations? Indeed, none of the diagnostic criteria proposed over the years have incorporated the mutation as a criterion [38-40]. What we are still lacking is a gold standard test, something similar to the sweat test in cystic fibrosis, or angiography in pulmonary embolism.

Despite the great steps in our understanding of this disease, we still have a number of questions for the future: what is the role of additional genes in the development of the final phenotype in FMF? What is the pathophysiology of the disease in patients with one *MEFV* mutation? What is the molecular basis of the disease in patients without any *MEFV* mutation?

We need additional data about the inflammatory pathways involved in the disease, the chain of events leading to the formation of amyloidosis, and the mechanism of action of colchicine.

Corresponding author:
Dr. E. Pras
Gertner Institute of Human Genetics, Sheba Medical Center, Tel Hashomer 52621 Israel
Phone: (972-3) 530-2998
Fax: (972-3) 530-2914
email: epras@post.tau.ac.il

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