

AN OVERVIEW OF FAMILIAL MEDITERRANEAN FEVER WITH EMPHASIS ON PYRIN AND COLCHICINE

<http://www.lebanesemedicaljournal.org/articles/56-1/indepth2.pdf>

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Dbouk HA, Uthman IW. An overview of familial Mediterranean fever with emphasis on pyrin and colchicine. *J Med Liban* 2008 ; 56 (1) : 35-41.

ABSTRACT : Familial Mediterranean fever (FMF) is the earliest known autoinflammatory disease, characterized by symptoms such as arthritis, peritonitis, pleuritis, erysipelas-like erythema, and most importantly amyloidosis. This disease is very common in populations of the Mediterranean area, and due to its high carrier frequency and occurrence rate in these populations, it has been the focus of much research work. Such research has allowed greater insights into the genetics of FMF, leading to the discovery of the responsible gene in 1997 and the determination of mutations and their effect on the phenotype of patients, as well as the interactions and roles of the pyrin protein, which seems to have various roles in regulation of innate immunity, inflammation, and apoptosis. Colchicine has been used as preventive treatment since 1972, and recent studies have allowed the determination of its mode of action.

INTRODUCTION

Familial Mediterranean Fever (FMF ; MIM 249100) is an autosomal recessive disease that is common in populations of the Mediterranean basin, mostly observed in Sephardic and Ashkenazi Jews, Arabs, Armenians, and Turks [1-2]. This disease is one of a subset of autoinflammatory disorders characterized by recurrent, leukocyte-mediated tissue infiltration, dysregulation of cytokine production, and systemic inflammation without evidence of high titer antibodies or apparent involvement of antigen-specific T-cells. This subset, referred to as periodic fever syndromes (PFSs), includes dominant diseases such as TNF receptor-associated periodic syndrome (TRAPS), familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and neonatal-onset multisystem

inflammatory disease (NOMID), and recessive diseases such as hyperimmunoglobulinemia D and PFS (HIDS) and FMF [3-6].

FMF is the longest recognized of the hereditary periodic fever syndromes, and was first described in 1945 by Siegal as benign paroxysmal peritonitis [7]. Studies of the origin of some of the more widespread FMF mutations (such as M694V and V726A) suggest that the original ancestors dated back to biblical times, and since these mutations are highly prevalent in modern-day descendants, it raises the possibility of selective advantage for carriers of these mutations over other populations historically, which may have appeared due to a greater, but controlled, inflammatory response against some pathogen endemic to the Mediterranean basin [2, 4, 8-9].

CLINICAL FEATURES

The disease is characterized by acute episodes of fever with elevated temperatures, usually preceded by chills, and lasting only 12 hours to 3 days. This fever is accompanied by abdominal (95% of patients), chest (25-80% of patients) or arthritic (35-75% of patients) – monoarticular in 70% of patients, oligoarticular in 26%, and polyarticular in 4% [10] – pain (caused by inflammation of the peritoneum, pleura, and joints respectively), myalgia (up to 20% of patients), along with erysipelas-like erythema lasting 2-3 days and appearing almost invariably on the extensor surfaces of the leg, over the ankle joint, or dorsum of the foot (3-46% of patients), as well as amyloidosis being observed in some patients (0.4-60% ; especially those having a more severe form of the disease) due to the accumulation of a cleavage product of one of the acute phase reactants, and this usually affects the kidneys [5, 8, 11-12] (Table I). Uncommon manifestations include headaches [13-14], meningeal irritation and increased concentrations of CSF proteins and cells [15-16], as well as transient microscopic hematuria.

An FMF episode is characterized by a massive influx of neutrophils into an anatomic compartment (up to 1,000,000 leukocytes/mm³ of synovial fluid) [17], and common laboratory findings include leukocytosis, an elevated erythrocyte sedimentation rate, increased acute-phase reactants, and Th1 polarization. In addition, interleukin levels in FMF patients are different from controls, both during attack phases and in asymptomatic periods [18-19]. The attacks usually end abruptly, with the clinical manifestations residing only for the duration of the attack, except for the arthritic symptoms which usually last longer and diminish gradually. However, the above

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TABLE I
DISTRIBUTION OF THE MAJOR CLINICAL SYMPTOMS AMONG FMF PATIENTS

SYMPTOM	PAIN			MYALGIA	ERYTHEMA	AMYLOIDOSIS
	ABDOMINAL	CHEST	ARTHRITIC			
Patient percentage	95%	25-80%	35-75%	Up to 20%	3-46%	0.4-60%

normal IL-12 levels, C-reactive protein (CRP), and Serum Amyloid A (SAA) in attack-free patients, as well as healthy heterozygous mutation carriers, show that there is a continuous state of subclinical inflammation even though disease symptoms are not manifested and the disease is considered inactive according to clinical criteria [18, 20-21].

FMF often manifests during infancy or adolescence, 80-90% of patients will have their first FMF exacerbation by age 20, while only 5% present after the age of 30 [1-2, 5, 22]. Acute episodes may be induced by environmental factors, stress (physical or emotional), and menstruation.

The Tel-Hashomer severity scale/index (Table II) is used to assess the severity of the disease based on certain criteria, including age at onset, frequency of attacks, presence of amyloidosis, as well as the different manifestations. This scale helps stratify patients according to severity of illness in order to determine the appropriate dose of colchicine [23].

GENETICS

In 1992, the gene responsible for FMF was mapped onto the short arm of chromosome 16 [24]. Five years later, the FMF gene was identified and cloned by two

TABLE II
CRITERIA USED IN TEL-HASHOMER SCALE

	POINTS/SCORING
Duration of attacks	
More than 72 hours	3 points
24-72 hours	2 points
Less than or equal to 24 hours	1 point
Frequency of attacks	
> 2 per month	3 points
1-2 per month	2 points
< 1 per month	1 point
Response to colchicine	
None	3 points
Partial	2 points
Complete	1 point
Mild disease	3-4 points
Moderate disease	5-6 points
Severe disease	7-9 points

independent consortia, one team at the National Institute of Health and the other consisting of a group of French laboratories [25-26], and this gene was termed *MEFV* (for MEditerranean FeVer).

The gene is located on the short arm (p) of chromosome 16, at locus 13.3. It consists of 10 exons, and a total of 3,505 nucleotides, of which 2,300 nucleotides encode a protein named pyrin or marenostrin (named as such by the International consortium and the French consortium respectively) [1, 25-26]. This gene is expressed differentially in different cell types, and is mostly observed in granulocytes or precursors cells that are differentiating towards becoming mature granulocytes, specifically neutrophils. The FMF phenotype is observed due to mutations in the *MEFV* sequence (Table III), and a wide range of disease-causing mutations are available and are present in all exons and even some intron regions (INFEVERS ; <http://fmf.igh.cnrs.fr/infervers>), and these contribute in different intensity to the severity of the disease phenotype [2, 27].

PYRIN PROTEIN

The product of the *MEFV* gene is a protein termed pyrin or marenostrin [25-26], and this protein is formed of 781 aminoacids, and weighs 86 kDa, with arginine and lysine constituting 13% of the amino-acid residues

TABLE III
DISTRIBUTION OF FMF MUTATIONS
IN THE LEBANESE POPULATION*

Mutation	STUDY	
	MANSOUR <i>et al.</i> [2001]	MEDLEJ-HASHIM <i>et al.</i> [2005]
M694V	27%	30.3%
M694I	9%	12.8%
V726A	20%	19.4%
E148Q	8%	8.3%
M680I	5%	7.4%
Other mutations	33%	41%

*The sum of percentages is not 100% because for the unidentified group, the percentages were considered among all studied patients, whereas for mutations, the percentages correspond to the number among patients with identified mutations.

[2]. This protein is expressed preferentially in granulocytes, specifically neutrophils, but also in activated monocytes, eosinophils, synovium, and skin, as well as differentiating granulocyte precursors (premyelocytic cells, at the point of secondary granule expression), which stresses the role of this protein as a key player during the inflammatory condition and the innate immune response [4, 6, 8].

The familial Mediterranean fever disease mutations do not lead to a truncated protein, since most of the mutations are missense mutations, that lead either to the change of a single amino-acid, or are in other cases do not lead to any change in the protein (Table IV). The native protein is nuclear in synovial fibroblasts and polymorphonuclear cells, while it is predominantly cytosolic in monocytes. Mutated pyrin isoforms showed variable localization, where all protein products containing exon 2 had a cytoplasmic localization, while those lacking exon 2 had cytoplasmic, nuclear, or mixed distribution in cells [17].

TABLE IV
LOCATION AND PERCENTAGE
OF FMF MUTATIONS DISTRIBUTION
IN *MEFV* GENE AND PYRIN PROTEIN

Location in Gene	Percentage	Protein Domain(s)
Exon 1	3.4%	PyrinD domain
Exon 2	28.4%	NLS bZIP basic domain
Exon 3	7.95%	B-box zinc-finger -helical domain
Intron 4	1.1%	–
Exon 5	11.3%	-helical domain
Intron 5	1.1%	–
Exon 7	1.1%	B30.2 domain
Intron 8	2.27%	–
Exon 9	2.27%	B30.2 domain
Exon 10	40.9%	B30.2 domain

The function of the *MEFV* gene product is still not adequately explored and completely known, however the protein structure and domains have been determined, and they give us a general insight into the structure, role, and function of this protein, through further studies and comparative analysis of these domains with other proteins containing the same domains.

The pyrin protein consists of six domains, and these are an N-terminal Pyrin-Death (PyD) domain (aminoacids 1-90), a bZIP basic domain (aminoacids 266-270), two nuclear localization signals (aminoacids 157-163 and 420-437), B-box zinc finger (aminoacids 375-407) and an -helical (coiled coil) domain (408-594), and the B30.2 domain (aminoacids 598-774) [4, 6, 8, 17]. These domains mediate the multiple interactions of the

pyrin protein and its functions in the regulation of inflammation, cytokine and chemokine production, and apoptosis [6].

The different domains show that the pyrin protein may have a multitude of actions. The bZIP domain, NLS, B-box zinc finger, and the coiled coil domains all suggest that pyrin may function as a nuclear effector molecule [8, 28]. In addition, these domains, as well as the remaining pyrin domains (PyD and B30.2) have essential roles through their interactions with other proteins, most importantly the ASC (apoptosis-associated speck-like protein with a caspase recruitment domain) and this catalyzes the activation of caspase-1 and hence the production of active IL-1 β which acts as a pyrogen and induces inflammatory gene expression, activates lymphocytes, and recruits leukocytes to the specific locations of its activity. A recent study has also shown that the B30.2 domain of pyrin interacts directly with caspase-1 to increase IL-1 β production [29]. In addition, pyrin was shown to associate with microtubules and actin filaments, thereby linking it to the regulation of inflammatory responses at the level of leukocyte cytoskeletal organization and directed migration [30-37].

Studies have shown that both truncated and FMF-associated pyrin lead to heightened inflammatory responses characterized by caspase-1 and IL-1 β activation, and IL-1-independent impairment of macrophage apoptosis through impaired caspase-8 activity [4, 31, 36, 38], or to decrease in IL-1 β production [4, 39]. These facts clearly point out that pyrin is an important modulator of innate immunity, and it has a critical role in inflammatory cytokine production, especially in response to endotoxins such as lipopolysaccharides (LPS), as well as possibly disrupting ASC-dependent apoptosis [4, 6, 17, 31-32, 36, 39-40]. In addition, various reports have shown that the ASC-pyrin interaction affects the NF κ B pathway, which is an important regulator of many intracellular signaling processes [6, 17, 36].

Overall, mutations in pyrin may lead to failure in regulation of caspase-1 activity or to varying NF κ B activity, resulting in dysregulated cytokine production, apoptosis, and inflammation [6].

GENOTYPE-PHENOTYPE ANALYSIS

The presence of a large number of mutations in the *MEFV* gene leading to different phenotypic forms of the FMF disease, has led to speculation about the role of specific mutations in the disease, as well as considerations of possible environmental factors, and background genes that affect the disease and lead to the wide variations between patients. A range of studies, in order to determine possible correlations between specific genotype mutations and the differential expression of specific phenotypic characteristics, have mainly focused on the five most common mutations, which are E148Q, M680I, M694V, M694I, and V726A [22, 41-42]. Table III shows the relative distribution (according to two differ-

ent studies) of these five mutations among Lebanese FMF patients.

Various studies have implicated the M694V mutation with the highest severity in both homozygotes for the mutation or heterozygotes with any other mutation [42, 43-44]. In addition, patients both homozygous and heterozygous for this mutation have been associated with earlier disease onset and more frequent arthritis, arthralgia, pleuritis, and erysipelas-like skin erythema [42-43, 45-48]. Studies have also shown that most patients displaying amyloidosis carry the M694V mutation, which has led to the association of this mutation with this phenotype [5, 12, 22, 44-45, 47, 49-50], and this has also been correlated to the / polymorphism in the SAA gene [22, 43, 49].

Other mutations have also been studied, and M694I homozygotes have also been associated with high severity score, as well as those patients with an M694V/M694I genotype [44, 51]. As for the E148Q mutation, most researchers consider it to be benign polymorphism [60, 52] and this belief was strengthened by a population-based approach [53] and pedigree analysis [54], which showed defective segregation in families. However, this claim was negated by a study showing that the E148Q mutation modified the clinical manifestations of rheumatoid arthritis and increased the disease severity [55]. The M680I mutation has been implicated in other forms of renal involvement, such as fibrillary glomerulonephritis [56].

These studies, although they might potentially shed light and allow for correlating genotype with possible disease outcome, cannot be considered as absolute conditions. Further studies have shown different phenotypes in patients with a common genotype (M694V/M694V) in two family members, and therefore different phenotypes cannot be explained solely by particular mutations [22, 41].

TREATMENT BY COLCHICINE AND ITS ANALOGUES

No abortive therapy has yet been described for FMF, however non-steroidal anti-inflammatory drugs (NSAIDs) are often used for symptom relief. Colchicine is currently the only administered treatment for patients with familial Mediterranean fever, and it has been widely used since its first application by Goldfinger in 1972 [57], although its mode of action and its exact functional pathway are still largely unidentified. Colchicine is given at a dose of 1 to 2 mg/day as a prophylactic treatment because it helps prevent amyloidosis and has been documented to decrease the frequency and the severity of attacks in patients. Colchicine, which is extracted from two plants of the lily family, is also given in a number of other diseases such as Behcet's disease, Sweet's syndrome, scleroderma, amyloidosis and liver cirrhosis, although the reasons for administering it differ in each syndrome [58].

Colchicine is an alkaloid formed of three hexameric

rings termed A, B and C. Its structure allows interaction with tubulin monomers and inhibits their assembly, and thus its function was believed to be mainly, and solely, the blocking of microtubule polymerization, thereby preventing neutrophil migration and acting as an overall anti-inflammatory agent. Colchicine also blocks cyclooxygenase-2 (COX-2) activity, as well as prostaglandin E2 and thromboxane A2 synthesis, leading to reduction of swelling and pain [59-60].

A recent study by Ben-Chetrit et al. has attempted to elucidate the pathway of colchicine in the cell and the range of its effects on FMF. It was shown that colchicine has two modes of action; early effects appear shortly after administration and require a low concentration of medication, and these include interaction with microtubules and inhibition of neutrophil chemotaxis mainly by altering the distribution of surface adhesion molecules (selectins) [22, 59-60]. Short-term effects also include modification of genes involved in the regulation of the cell cycle. The second group of effects, the long-term effects, requires about 12-24 hours to be observed and is usually the result of higher doses of colchicine, and these include changes in the expression of many genes that are essential for neutrophil migration, as well as suppression of caspase-1 and eNOS3 expression, thus exerting an anti-inflammatory response [59-60].

Colchicine is a very effective agent used as a prophylactic treatment against FMF disease episodes, as well as playing a preventive role against the development of amyloidosis. Colchicine cannot stop an acute FMF attack if administered during the attack, and its main roles are observed when it is given continuously, because the main anti-inflammatory effects can only be observed several hours after use. Furthermore, colchicine non-responsiveness may be attributed to differences in the level of transporters such as P-glycoprotein at the surface of granulocytes (which also explains the specific activity of colchicine on specific cell types) [59, 61-62]. In addition, drug discontinuation results in a relapse in most patients within a few days.

The main side effects of colchicine are diarrhea and malabsorption. Azoospermia and oligospermia may occur, but are relative. Colchicine does not seem to affect female fertility [60, 63]. Current studies are focusing on the design and production of new colchicine analogues and other agents that may play the same anti-inflammatory function with greater efficacy and a decreased range of side effects. Such colchicine analogues are already being tested *in vitro* on neutrophils from FMF patients [59]. In addition, several alternatives to colchicine have been recently suggested including interferon- [64], as well as allogenic bone marrow transplantation, which is still controversial [65-66].

CONCLUSION

Familial Mediterranean fever is an autoinflammatory disease involving mutations of the *MEFV* gene on chro-

mosome 16p13.3 that has a myriad of presentations that mimic other more common diseases often leading to misdiagnosis. The diagnosis of FMF is based on genetic analysis in addition to a thorough clinical assessment, and recent attempts to define specific genotype-phenotype correlations have been somewhat contradictory, providing opposing results at times concerning the same mutations. Several classification systems have been used to describe the severity of FMF, the most common the Tel-Hashomer classification [50, 67]. Colchicine is the only treatment modality that is of proven benefit, and novel research is elucidating the pathways of this drug [60], and thus helping to design others with fewer potential side effects.

The underlying mechanisms involved in the pathogenesis of FMF are still unclear, and further research is needed to gain more insight on the role of pyrin as well as other factors inducing the disease process. Studies using pyrin knockouts and model systems of FMF, as well as mapping the multitude of pyrin-associated proteins and their possible pathways and effectors, will lead to valuable knowledge for proper understanding and treatment of this disease.

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