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Molecular Genetics Analysis of *MEFV* Gene Mutations in Familial Mediterranean Fever (FMF) Among Palestinians in the West Bank

By

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ABSTRACT

Familial Mediterranean fever (FMF) is an autosomal recessive hereditary disease characterized by recurrent self-limited attacks of fever accompanied by peritonitis, pleurisy and arthritis with sterile infiltration of polymorphonuclear leukocytes into the serosal and synovial fluids. The most severe complication of FMF is nephrotic amyloidosis which leads to terminal renal failure at an early age. Life-long colchicine treatment is used for alleviation of acute attacks and for prevention of amyloidosis. The disease affects mostly ethnic groups living around the Mediterranean basin including non-Ashkenazi Jews, Armenians, Turks, and Arabs. Recently, the gene linked to FMF (*MEFV*, MEditerranean FeVer) has been mapped to the short arm of chromosomes 16. This gene encodes for the pyrin/marenostrin protein, which is thought to play a role in granulocyte-mediated inflammation.

In this study, *MEFV* gene mutations were investigated in sixty-seven Palestinian patients from different localities of the West Bank that were clinically diagnosed as having FMF. DNA was extracted from EDTA-blood samples and analysis of *MEFV* gene mutations was performed using Polymerase Chain Reaction (PCR), Amplification Refractory Mutation System (ARMS) and direct DNA sequencing.

Twelve missense mutations were identified in 71% of the 134 independent alleles, with both FMF alleles identified in 62.5% of the patients. Eight of these mutations are located in exon ten, two in exon two, and two in exon 3. Evidently, the M694V mutation appears to be the most common one accounting for 31% of all

detected mutations, followed by V726A, M694I, E148Q, and M680I mutation. The distribution profile of these mutations showed a degree of preference in the various localities. In comparison to other ethnic groups, our mutational profile showed a similarity to the Lebanese and Jordanian populations, but a difference from non-Ashkenazi Jews, Armenians, and Turks. A putative new mutation (R653H) which resulted in the substitution of histidine for arginine was identified in three patients. This mutation appeared in a heterozygous genotype in conjugation with the M694V, M694I or V726A mutations in the other allele. The R653H mutation was eventually identified in the parents and other siblings of the indicated patients.

The pyrin gene expression was investigated in colon cancer tumor cells using *in situ*-hybridization. This was based on previous studies that the gene might play a role in cancer biology. Positive identification was observed in two out of a total of thirty-three patient samples that were included in this section. These results suggest that either the gene expression is highly selective in colon cancer cells or that it is expressed in very low abundance in these cells.

This study is the first molecular genetics investigation of Familial Mediterranean Fever among Palestinians in the West Bank. It provides the mutations in the *MEFV* gene in Palestinian FMF patients. Moreover, our work helped in the establishment of a molecular diagnostic system for FMF mutations in suspected patients. This protocol will be valuable in prenatal testing for couples with family history. Clearly, the potential role of the *MEFV* gene in colon cancer requires more thorough investigation and testing.

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1. INTRODUCTION

1.1. Historical Background

In 1908, Janeway and Mosenthal described a 16 years old Jewish girl who had had recurrent attacks of fever and abdominal pain since infancy. The intervals between attacks were initially about one month and they varied in intensity. During strong attacks, the patient had intensive pain in the abdomen, shoulders, hips and feet, her temperature rose to 40℃, her pulse was rapid and her white blood cells count rose to 28,000 per mm^3. Appendectomy had no effect on her disease course. This detailed description seems to be the first documentation of Familial Mediterranean Fever (Janeway et al., 1908). In 1945 Siegal was the first to recognize this disorder as a distinct clinical entity at the Mount Sinai Hospital in New York and he called the condition "benign paroxysmal peritonitis" (Siegal, 1945). In 1948, Reimann reported the first three cases of peritonitis then he enlarged his series and was impressed by the periodicity of the symptoms and therefore introduced the term "periodic disease" (Reimann, 1948). In the early of 1950s, French investigators Mamou and Cattan described the disease in North Africa among Jews of Sephadric extraction. They were the first to mention the familial occurrence and the lethal nephropathy that may affect the patients (Cattan and Mamou, 1951). It also became obvious that the disease affected subjects of Mediterranean origin, due to this Heller and co-workers decided to call the disease "familial Mediterranean fever" (FMF) (Heller et al.,1958). In 1972, Goldfinger reported the suppressive effect of colchicine on the attacks in five patients (Goldfinger, 1972). FMF followed an autosomal recessive inheritance pattern, and since it has been described more than 50 years ago, the diagnosis of FMF has been based on clinical presentation. In 1997, a crucial advance in this

study was achieved with the cloning of the gene responsible for FMF (designated "*MEFV*"), which encodes a novel protein called "pyrin" or "marenostrin". Mutations in the *MEFV* gene correlated well with the clinical phenotypes observed in FMF patients (French FMF Consortium, 1997; International FMF Consortium, 1997).

1.2. Epidemiology

FMF occurs predominantly in populations originating from the Mediterranean basin. The vast majority of patients are Jews (Ehrenfeld et al., 1961, Armenians (Armenian and Khachadurian, 1973), Turks (Erek et al., 1978) and Arabs (Schwabe and Peters, 1974). However, the disease has also been described in subjects of other ethnic groups (Sohar et al.,1967). While some cases of FMF present during infancy, most patients experience their first symptoms during childhood or adolescence, with more than 80% of patients suffering an attack before the age of 20 years (Samuels et al.,1998). In a study of FMF among Arab children, the age at onset ranged between 4 months and 16 years with 24% of the patients started their illness below the age of two years and 88% were symptomatic before the age of 10 years (Rawashdeh & Majeed, 1996). FMF is more prevalent among males with male to female ratio was 1.76 to 1.00 (Eliakim et al.,1981).

1.3. Clinical Manifestations

Familial Mediterranean fever typically presents as acute episodes of fever accompanied by complains of abdominal pain, chest pain, or joint pain. In addition, symptoms were reported all over the body from the skin to the scrotum

as shown in figure 1 (Samuels et al., 1998). Attacks were frequently precipitated by activity (strenuous exercise), stress (anger, anxiety, fear), temperature (exposure to cold), and menses but were conspicuously absent during pregnancy (Schwabe and Peters, 1974). Some patients experience a prodromal period, during which chills or some other warning precedes an imminent attack. The actual attack usually lasts from 12 to 72 hours, with the arthralgia often lasting longer. The degree of temperature elevation as well as the anatomic distribution (abdominal, chest, or joint), may vary from one patient to another, even among the same family (Samuels et al.,1998).

1.3.1. Peritonitis

Abdominal pain is present in almost all patients; it represents the most constant feature and the central manifestation of the disease, with about 50% citing such pain as the first symptom (Sohar et al., 1967). Among the Arab patients about 93.7% present with peritonitis (Barakat et al., 1986). The clinical picture of acute peritonitis is manifested by fever, abdominal pain, exquisite tenderness often starting in one area and spreading over the whole abdomen. Moreover, involuntary rigidity, rebound tenderness of the abdominal wall and diminished peristalsis are common features of peritonitis, the patients frequently flex their thighs to relieve the pain and lie motionless to avoid pain. Crisis resolves spontaneously and is usually terminated within 24 to 42 hours. In the interval between attacks, most patients are entirely free of symptoms (Eliakim et al., 1981). FMF abdominal crisis may be misdiagnosed with acute appendicitis unless elective laparoscopic appendectomy is performed (Ressiman et al, 1994) and these crisis may also mimic gynecological disease (Eliakim et al., 1981).

Familial Mediterranean Fever
Signs & Symptoms

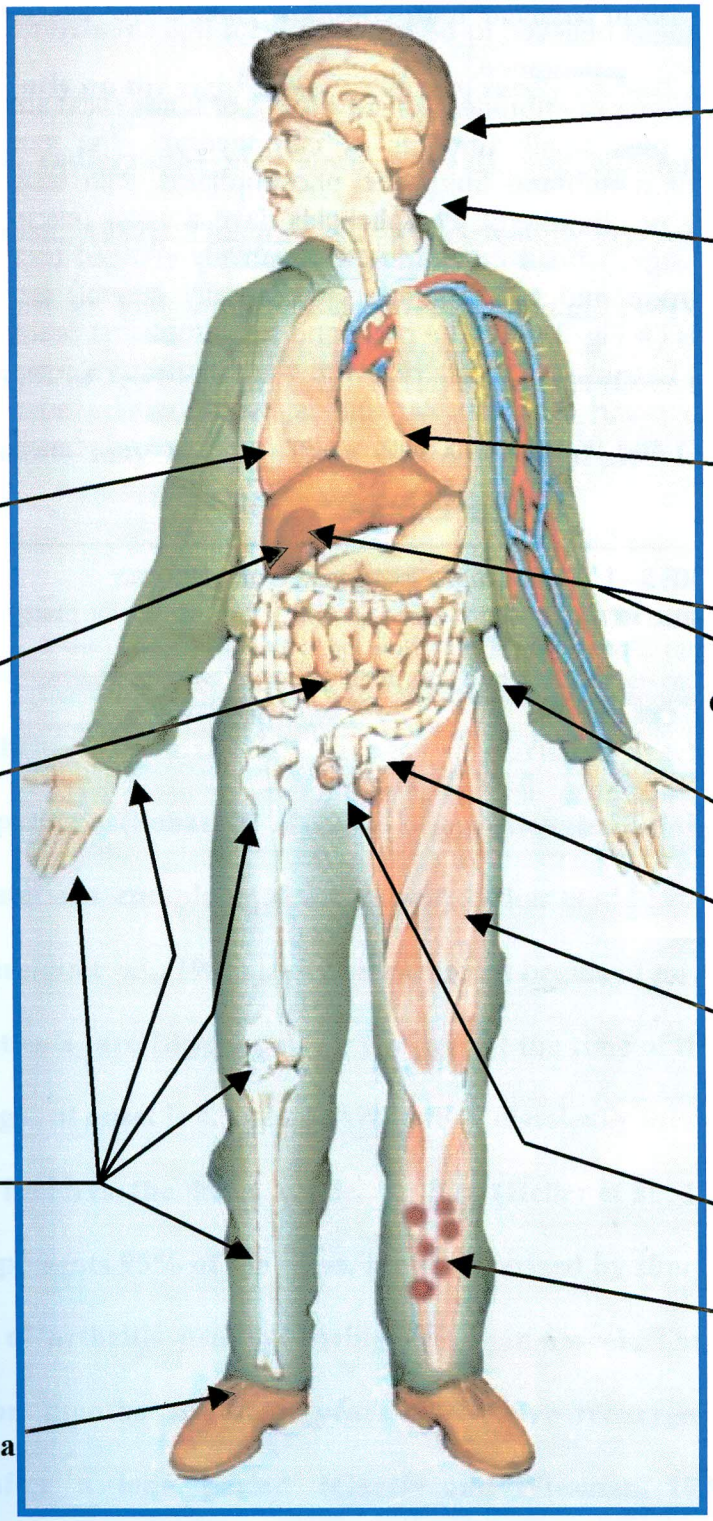

Figure 1

1.3.2. Pleurisy

Chest pain due to pleurisy is another frequent manifestation of FMF. The attack is characterized by a sharp stabbing pain localized in the lower side of the chest more frequently on the right than on the left, rarely on both sides (Eliakim et al., 1981). Chest radiographs may show a small lung effusions or atelectasis (Bruama and Giboa, 1987). The pleuritic attack usually disappears within 12 to 72h but it may last as long as seven days (Eliakim et al., 1981). Pleural attacks occur in up to 50% of Jewish, Arab, and Turkish patients (Ozer et al., 1971), and in Armenian patients may have an even higher rate of pleurisy (Schwabe and Peters, 1974).

1.3.3. Arthritis

While arthritis and arthralgia have been reported in nearly 75% of North African Jewish patients (Sohar et al., 1967), less than half of the patients from other FMF populations complain of joint pain (Sohar et al., 1967; Schwabe and Peters, 1974; Barakatet al., 1986). Joint involvement begins at an early age; more than 50% of patients are 10 years old or younger at the time of the initial attack, and the mean age at onset is 4.9 years. Arthritis is classically monoarticular, and most frequently involves the knee, ankle, or hip (Heller et al., 1966). The acute attack, which represents 95% of the cases, is characterized by short duration, self-limited episodes of arthritis usually lasting less than a week. The patient can be asymptomatic for months or even years and have recurrence of articular manifestations after a long period (Garcia and Wiesman, 1992). Migrating monopredominant arthritis was observed in some FMF children patients where

there was rapid cartilage and bone destruction in the involved joints (Miller and Emery, 1996). With arthritis, the affected joints often have sterile effusions with polymorphonuclear leukocyte-filled synovial fluid (Eliakim et al., 1981).

1.3.4. Cutaneous manifestations

Frysipelas-like plaques are a very characteristic feature and the most frequent skin lesion in FMF. They appear on the extensor surfaces of the legs below the knees, over the ankle joints or the dorsum of the foot. The skin becomes bright-red, hot, swollen and painful over an area of about 20-200 cm^2. The border may or may not be sharply defined. Rapidly the erythema fades away within 2-3 days without therapy (Azizi et al., 1976). It was reported that about 2.9% of Arab patients complain of Erysipelas-like erythema (Barakat et al.,1986).

1.3.5. Uncommon Manifestations

Myalgia or muscle pain is a classical manifestation of FMF and occurs in about 20% of the patients. Usually the pain is not severe and appears in the lower extremities after physical exertion, mostly in the evenings, lasts from a few hours to 2-3 days, and subsides with rest (Langevitz et al., 1994). A small percentage of FMF patients develop acute scrotal inflammation mainly among Jewish and Arab children patients. The attacks include gradual onset of pain over 12 hours, with scrotal swelling and edema (Majeed et al., 2000; Eshel et al., 1994). Pericarditis may be a feature of FMF. Pericarditis attacks lasts approximately for four days, accompanied by elevated temperature and other symptoms of FMF attacks at another site. The pericarditis resolved spontaneously and left no sequelae (Kees et al., 1997). Patients frequently complian of headaches with their attacks (Eliakim et

al., 1981) and recurrent meningitis may also occur, with a characteristic spinal fluid picture; pleocytosis of mixed cellular type including endothelial cells *(Mollaret cells)* during the attack, in the absence of any positive agent (Barakat et al., 1988). Splenomegaly, mild hepatomegaly and lymphadenopathy have also been observed in FMF patients (Eliakim et al., 1981). Fertility can be impaired in female FMF patients, possibly due to pelvic adhesion or the induction of early misscarriages by abdominal attacks (Dabestani et al., 1982; Kees et al., 1997). Ocular involvement has been reported in FMF patients (Akman et al., 2001). Certain forms of vasculitis also appear to occur more commonly in FMF patients than in the general population. Approximately 5% of FMF population have been reported to have Henoch-Schonlein purpura (HSP) and 1% have Polyarteritis nodosa (PAN) (Tekin et al., 2000).

1.3.6. Amyloidosis

The most severe complication of FMF is nephrotic amyloidosis which leads to terminal renal failure at an early age unless treated with colchicine (Pras et al., 1982; Zemer et al., 1986). The cleavage product of serum amyloid A (SAA), an acute-phase reactant produced by the liver, infiltrate the kidneys, adrenals, intestine, spleen, and liver (Sohar et al., 1967). FMF accounts for 3% of the diseases underlying secondary amyloidosis (Gertz et al., 1991). Amyloidosis had been divided into two types: type I amyloidosis; which was defined as amyloidosis developing subsequent to clinical features of FMF and Type II that was defined as amyloidosis developing as the initial manifestation (Saatci et al., 1997). While family history of amyloidosis was significantly more frequent in the amyloidosis group (Saatci et al., 1997), Type II amyloidosis was found to be uncommon among

relatives of patients with FMF and amyloidosis (Melikoglu et al., 2000). Male-to-female ratio was observed to be higher in the amyloidosis population than it was in FMF population without amyloidosis (Saatci et al., 1997) and it was also observed that pregnancy significantly suppress amyloidogenesis in mice (Shtrasburg et al., 2000).

Four stages of amyloidosis have been identified:

(1) Preclinical Stage: By definition, this stage can be detected only by biopsy methods in those FMF patients who show no signs of renal disease.

(2) Stage of Proteinuria: At this stage, no new symptoms are present. The patient continues to suffer his usual attacks and is not aware that his disease has undergone a change. The proteinuria, which may be intermittent at first but later constant and increasingly heavy, is an "incidental" finding.

(3) Stage of Nephrotic Syndrome: The first ominous sign that the patient is entering the nephritic stage is a change in blood proteins. Serum albumin decreases and the albumin-globulin ratio becomes inverted.

(4) Uremic Stage: Once the nephritic picture has developed, the rate of progress becomes accelerated, and uremia supervenes relatively quickly. The edema may persist into this phase of the disease or subside and disappear (Cattan, 1955).

The renal disease begins in the young and most patients die at an early age (Heller et al., 1961). Some correlation between amyloidosis and certain gene mutations has been observed below.

Ethnically related variation in the incidence of amyloidosis among FMF patients does exist. While early death due to systemic amyloidosis is indeed the

genetically-determined fate of patients of North African ancestry, such a fate is as rare in Jews from other ethnic communities (Pras et al., 1982; Ozdemir et al., 1969). Furthermore, Turkish FMF patients have a high incidence of amyloidosis ranging from 7 to 60% (Ozdimer et al., 1969; Saatci et al., 1993). Moreover, this incidence in Armenians varied between 0% in the USA and 24% in Russia (Yazici et al., 1997; Aviazian et al., 1977). Among Arab patients with FMF, the frequency of amyloidosis is also quite variable, ranging from 0 to 25% (Said et al., 1992).

1.4. Laboratory Diagnosis

During attacks, white blood cells count is increased and the Erythrocyte sedimentation rate is also invariably increased. In addition, acute phase reactants as C-reactive protein, serum amyloid A protein, haptoglobin, C3, C4, and fibrinogen are higher among FMF patients (Tunca et a., 1999; Eliakim et al., 1981). Moreover, impaired releases of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) (Ozyilkan et al., 1992), interleukin 1 (IL-1) (Rozenbaum et al.,1992), and IL-6 were observed during acute attacks (Gang et al., 1999). Mild and transient albuminuria and hematuria may be present during attacks, but persistent albuminuria is present only in patients suffering from renal amyloidosis (Eliakim et al., 1981).

1.5. FMF-Related Diseases

1.5.1. Hyperimmunoglobulinemia D Syndrome (HIDS)

The hyper-IgD syndrome is a autosomal recessive periodic disease was first described by van der Meer in 1984 (van der Meer et al., 1984). The disease is characterized by recurrent febrile attacks usually of very early onset (<1 year).

There is an even male-female distribution. Typical attacks occur every 4-8 weeks and last about 3-7 days with a large individual variation (van der Meer et al., 1984; Drenth et al., 1994). The attacks feature the acute onset of high spiking fever, sometimes preceded by chills. Abdominal pains, vomiting, and diarrhea are associated with the episodes. Headache and arthralgia occur frequently with attacks. On physical examination during the attacks, swollen, tender lymph nodes, most often in the cervical region, can be palpated. Especially in the young age-group, splenomegaly may be found. Non-destructive recurrent arthritis, mainly in the larger joints, can be demonstrated in many patients. During the attacks, most patients have erythematous macules and papules, with histologic signs of cutaneous vasculitis (Drenth et al., 1994).

Although FMF and HIDS share certain features, but lymphadenopathy, certain types of rash, and symmetric oligoarthritis are exclusively found in HIDS, while peritonitis, monoarthritis, and pleuritis are typical to FMF. Increased levels of IgG, IgM, IgA, and IgD have a much lower prevalence in FMF compared with prevalence reported in HIDS. Finally, molecular analysis, confirmed that the HIDS locus is not linked to the FMF susceptibility gene on chromosome 16p (Livneh et al., 1997).

1.5.2. Familial Hibernian Fever

Familial Hibernian Fever (FHF) is an autosomal dominant periodic disease was first described by Bouroncle and Doan in 1957. FHF is one of the hereditary-periodic-fever syndromes characterized by recurrent attacks of abdominal pain and fever and that may be associated with muscuskeletal manifestations. Although FHF clinically resembles FMF, the inheritance of FHF is dominant rather than

recessive; the duration of attacks is generally longer, and there is a favorable response to high-dose steroids. Other distinguishing features include episodic periorbital edema, and inguinal hernias in males (McDermott et al., 1997; Ostuni et al., 1996). Amyloidosis, which is a major cause of morbidity in FMF without colchicine prophylaxis, appears to be relatively rare in FHF (Williamson et al., 1982). In addition, the susceptibility locus of FHF has been mapped to chromosome 12p13 (McDermott et al., 1998).

1.6. Non-Molecular Diagnosis of FMF

FMF can be diagnosed according to clinical features following the criteria explained in table 1. The diagnostic criteria for FMF is based on the occurrence of recurrent, short-lived, febrile attacks accompanied by inflammation of one of the serous membranes, the development of AA amyloidosis, and the favorable response to colchicine treatment. These points do not hold for the other periodic fever syndromes. Minor criteria include recurrent febrile episodes, erysipelas-like erythema, and FMF in a close family relative. Either two of the major criteria, or one major and two minors, are sufficient to establish a definite diagnosis of FMF, while one major and one minor criterion constitute a probable diagnosis (Pras et al., 1998; Langevitz et al., 1997).

Another specific diagnostic test for FMF was performed by provoking the attacks using 10 mg of metaraminol, which is sympathomimetic agent that acts directly on adrenergic receptors and indirectly by releasing noradrenaline. The test was considered positive if one or more of the following symptoms developed within 48h; abdominal or pleuritic pain with or without fever, abdominal distension, or joint pain (Barakat et al.,1984).

Table 1. Criteria for the Diagnosis of FMF

Major criteria	Minor criteria
1.Recurrent fibrile episodes accompanied by peritonitis , synovitis or pleuritis	1. Recurrent fibrile episodes
2. Amyloidosis of A-A type without predisposing disease.	2. Erysiplelas-like erythema
3. Favorable response to continuous colchicine treatment	3. FMF in a first-degree relative

Definitive diagnosis : 2 major , or 1 major and 2 minor

Probable diagnosis : 1 major and 1 minor

(Langevitz et al., 1997)

1.7. Management

In 1972, Goldfinger was the first to describe the efficacy of prophylactic colchicine in treating FMF patients (Goldfinger, 1972). The daily recommended regimen of oral colchicine is 1-2mg/day (Dinarrello et al., 1974; Goldstein et al., 1974). Colchicine, an alkaloid extracted from the plant Colchicum autumnale, is widely used for a variety of diseases, including gouty arthritis (Harel et al., 1998). It inhibits mitosis of dividing cells and, in high concentration, is a general cellular poison (Guven et al., 1999).

Colchicine provides prophylaxis against amyloidosis in FMF patients whom amyloidosis has not developed but whose ethnicity places them at high risk. However, its effectiveness is limited in patients with clinical amyloidosis as colchicine does not alter the deleterious effects on cellular and function of amyloid fibrils that have been already deposited in the basement membrane (Ravid et al., 1974, Zemer et al., 1974).

The drug distributes rapidly from the plasma with a high concentration observed in granulocytes (Chappey et al., 1993) which might explain its efficiency in treating FMF patients. It has been reported that colchicine-inhibits polymorphonuclear (PMN) chemotaxis (Ehrenfled et al., 1980), modulates their cytokines production (Allen et al., 1991) and decreases the expression of adhesion molecules on their membranes (Molad et al., 1992). The accumulation of colchicine inside PMN cells can also modify other several functions of PMNs such as phagocytosis, lysosomal degranulation, adherence, and capping of membrane receptors (Dallaverde et al., 1982).

The low response to colchicine in some patients may be explained by the P-glycoprotein efflux pump (P-gly) which is an integral membrane protein that

serves as an energy-dependent transport peptide of diverse medications and substrates. Granulocyte population lack or at least have a lesser P-gly function compared with the lymphomonocyte population. Colchicine is one of the drugs reported to be affected by the P-gly efflux, the lack of this pump in granulocytes may cause colchicine retention in these cells (Ben-Chterit and Levy, 1998). In colchicine non-responders, it is possible that they may have an over-expression of the P-glycoprotein (Ben-Chterit and Levy, 1998).

While colchicine is effective in treating FMF, patients suffer from several side effects of the drug. The most common side effect is gastrointestinal symptoms, which are dose related. Patients receiving colchicine showed significantly higher percentage of lactose malabsorption compared with untreated patients (Fradkin et al., 1995). A number of uncommon side effects also have been attributed to colchicine therapy. The drug can induce a reversible myopathy with elevated creatine kinase levels, as well as neuropathy (Kuncle et al.,1987). In children treated with colchicine, neuromuscular phenomena of unknown etiology may develop (Harel et al,1998). While there are no large prospective inquiries into colchicine-induced male infertility, decrease in the sperm count are usually reversible with the cessation of the drug (Ehrenfeld et al., 1986). Chronic colchicine treatment for many years prior to pregnancy and continuous treatment during conception and pregnancy, under controlled conditions, do not harm mother or child (Rabinovich et al., 1992), but the abdominal attacks themselves might have an effect on female fertility (Ehrenfeld et al., 1987).

An alternative treatment for FMF is the alpha-Interferon (IFN-α) which is known to have pleiotropic effects including suppression symptoms in some autoinflammatory diseases. This agent may exert its therapeutic effect in FMF by

causing overexpression of a functionally deficient *MEFV*-gene product by stimulating proinflammatory pathways (Tunca et al.,1997). Low-fat diet is another option for FMF treatment as some patients were treated by a diet containing less than 20g fat per day (Eliakim et al., 1981).

1.8. Molecular Basis of Familial Mediterranean Fever

In the summer of 1997, the FMF gene was cloned and termed *MEFV* gene from the underlined bold letters of Familial MEditerranean FeVer. The first step of localizing the gene to a specific chromosome was accomplished by examining more than 100 polymorphic markers in a panel of non-Ashkenazi Jewish families. After this method placed *MEFV* on the short arm of chromosome 16 (16p13.3), linkage was confirmed in patients of other ethnic groups, including families of non-Ashkenazi Jewish (French FMF Consortium, 1997; Shohat et al., 1992), Armenians (Shohat et al., 1992), Arabs (Pras et al., 1994), and Turkish (Akarsu et al., 1997) heritage. A candidate intervals of approximately 200 kb between D16S3082 and D16S3373 was defined, then the interval was narrowed to 115 kb by the international consortium and to 60 kb by the French consortium (French FMF Consortium, 1997; International FMF Consortium, 1997). Within the minimal candidate interval, 10 exons, 3,505-nucleotide cDNA, encoding a 781 amino acids was identified as shown in figure 2 (International FMF Consortium, 1997). Both groups found the same 3 disease-associated conservative missense mutations in exon 10, while the French consortium identified a fourth, also in exon 10. These 4 mutations were not found in an aggregate total of 600 control chromosomes. The strict association of haplotype-bearing carrier chromosomes with specific mutations, and the absence of those mutations in a large panel of controls,

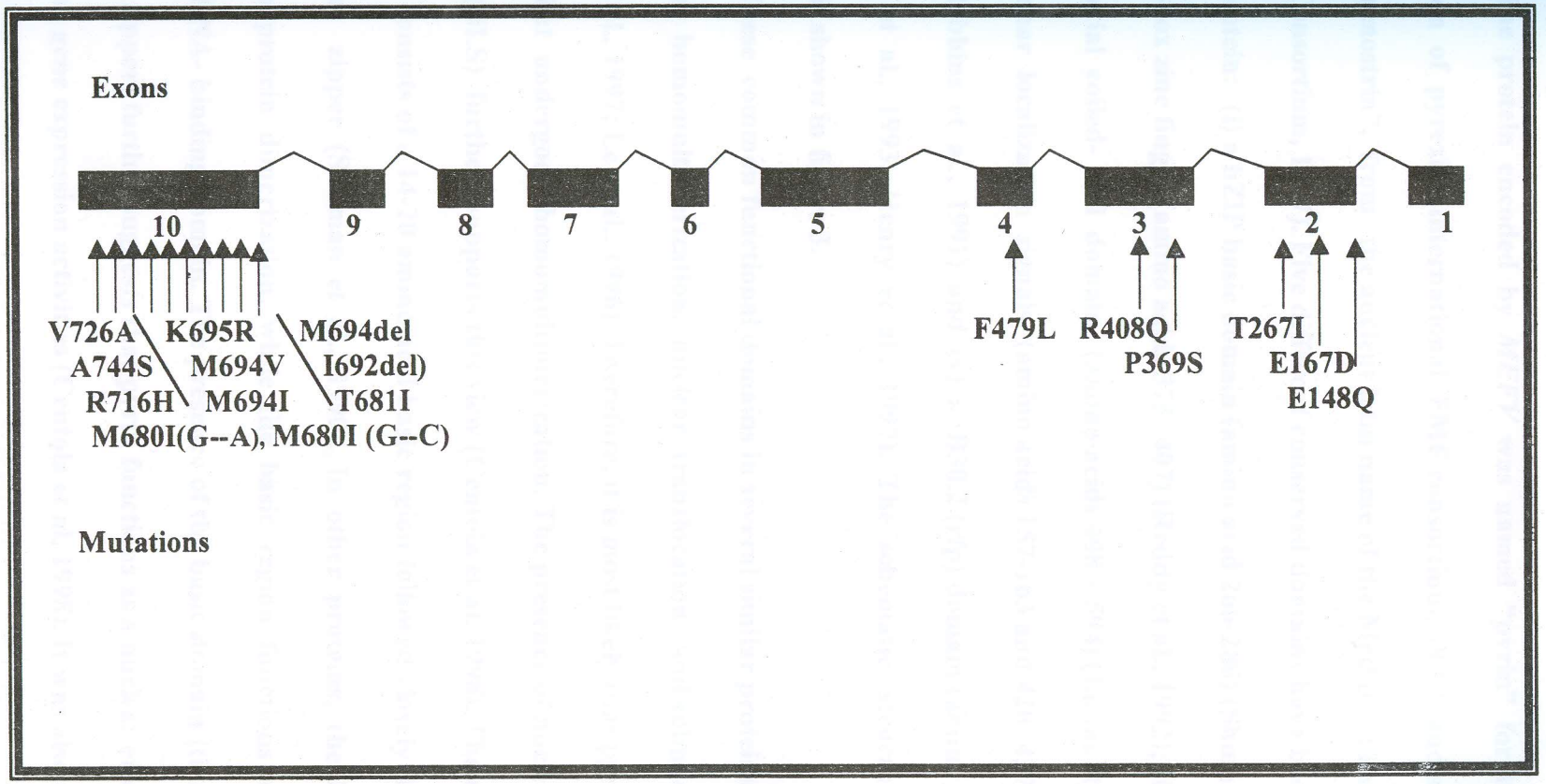

Fig. 2. Schematic Diagram of the *MEFV* Gene and Identified Mutations

is a strong evidence that this clone is in fact the FMF gene (French FMF Consortium, 1997; International FMF Consortium, 1997).

The protein encoded by *MEFV* was named "pyrin" for its role in the regulation of pyrexia (international FMF consortium, 1997), and also referred to as "marenostrin", from the ancient latin name of the Mediterranean Sea (French FMF Consortium, 1997). Five different conserved domains have been identified in pyrin protein: (i) a bZIP basic domain (amino acid 266-280) (Shuman et al., 1990) (ii) a B-box zinc finger (amino acids 375- 407) (Reddy et al., 1992); (iii) an ά-helical or potential coiled- coil domain (amino acids 408 – 594) (Lupas et al., 1997); (iv) two nuclear localization signals (amino acids 157-163 and 420- 437) (Nakai et al., 1992; Robbins et al., 1991) and (v) a B30.2 (rfp) domain (amino acids 598-774) (Vernet et al., 1993; Henry et al., 1997). The schematic structure of the pyrin protein is shown in figure 3.

These common functional domains in several similar proteins identify three activities; homomultimerization, nuclear translocation and subnuclear targeting (Cao et al., 1997; Le et al., 1996). Therefore, it is most likely that pyrin is a nuclear factor that undergoes homomultimerization. The presence of nuclear localization signals (NLS) further supports this view (Centola et al, 1998). The complete bZIP domain consists of a 14-20 amino acid basic region followed closely downstream by a leucine zipper (Shuman et al., 1990). In other proteins, the leucine zipper mediates protein dimerization, while the basic region functions as a sequence-specific DNA- binding domain. The presence of the basic domain (though not the leucine zipper) further suggests that pyrin functions as a nuclear effector molecule that alters gene expression activities (Centola et al, 1998). It was also suggested

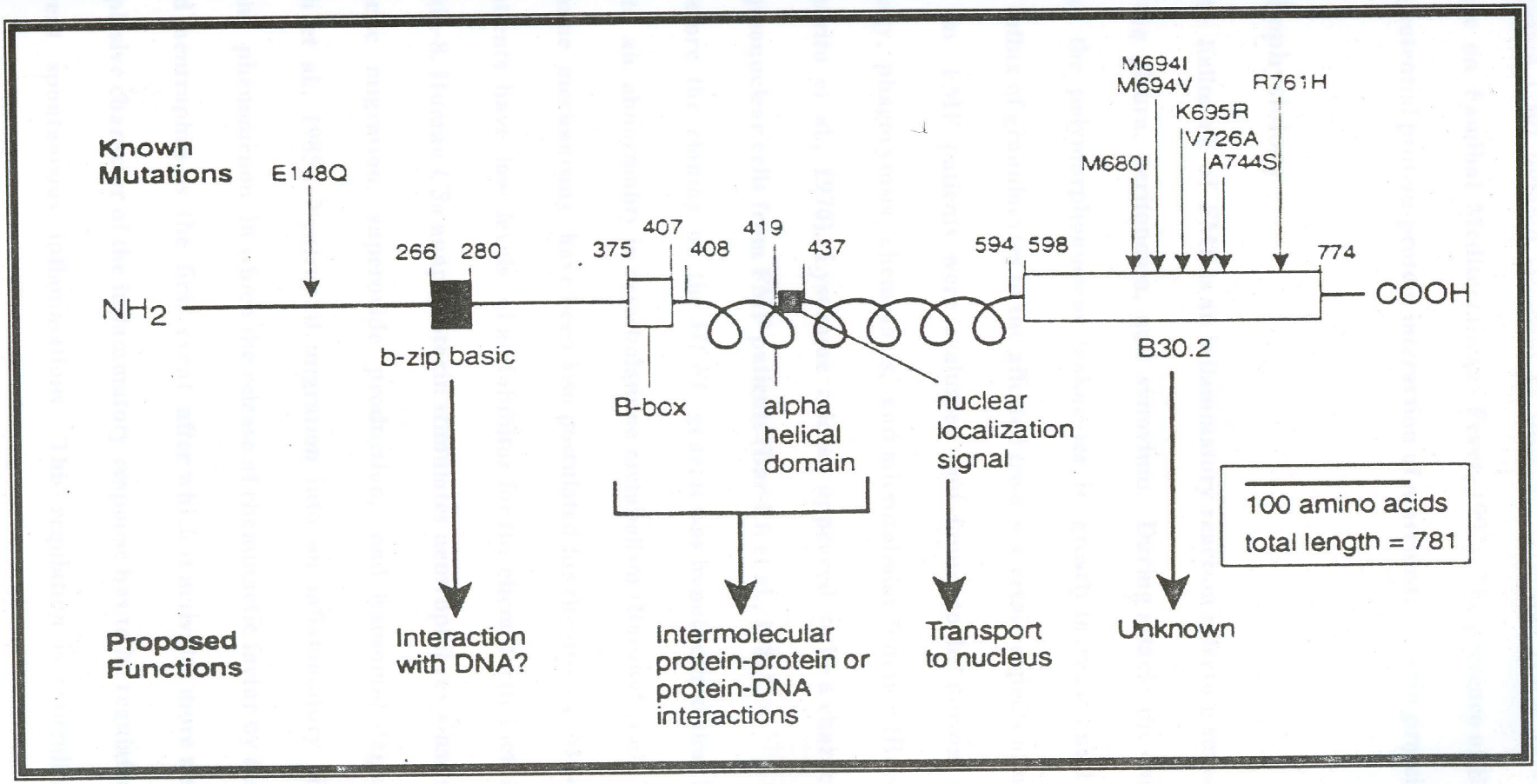

Fig. 3. Pyrin Protein Domains and Corresponding Functions

that pyrin may be a new member of the RoRet family of proteins, which have a ret finger protein (rfp) domain (B 30.2) at their C- terminus (First International Conference on Familial Mediterranean Fever, 1997). The presence of B30.2 may suggest a potential protein-protein interaction of marenostrin/pyrin protein.

1.9. Pathophysiology

The hallmark of FMF is an inflammatory reaction affecting serosal tissues such as the pleura, peritoneum, and synovium. During attacks the chemotactic activity of the polymorphonuclear leukocytes is greatly increased and there is a massive influx of granulocytes to the affected tissues. Certain aspects of neutrophil function in FMF patients were evaluated and found to be normal including morphology, phagocytosis, chemotaxis, and microtubular function (Bar-Eli et al., 1982; Territo et al., 1976). Lysozome release appeared to be a characteristic of polymorphonuclear cells from FMF patients (Bar-Eli et al., 1981).

Before the cloning of the *MEFV* gene, it was hypothesized that FMF may be due to an abnormality in catecholamine metabolism (Barakat et al., 1988). An autoimmune mechanisms have been also postulated for the disease. Most notably, FMF patients have low levels of an inhibitor for the chemotactic factor C5a and interleukin-8. Human C5a anaphylatoxin stimulates neutrophil response such as chemotactic migration, superoxide production, and lysosomal degranulation. (Johnson et al., 1985). Neutrophil migration into an inflammatory site is a self amplifying phenomenon in which the release of chemotactic factor by the arriving activated neutrophils is the first event after which it activates more neutrophils. This explosive character of the inflammatory response has to be regulated in order to prevent spontaneous inflammations. This regulation is controlled by an

inhibitor that is directed against the chemotactic factor C5a (Matzner et al., 1983, 1984a). The C5a-inhibitor also inhibits intrerleukin-8 (IL-8), a proinflammatory cytokine, that stimulates neutrophils and causes increased chemotaxis and neutrophil adhesion. IL-8 levels were shown to be increased in the sera and synovial fluid of various inflammatory conditions with neutrophil accumulation. Furthermore, the increased levels of IL-8 will induce expression of the cell adhesion molecule leukocyte function antigen-1 (LFA-1) on the neutrophils and its ligand Intercellular adhesion molecule-1 (ICAM-1) on the endothelial cells and enhances the adherence of neutrophils to endothelial cells and subendothelial matrix (Rot, 1992; Direskeneli et al.,1999).

The C5a-inhibitor was detected in the synovial and peritoneal fluids and in fibroblasts (Matzner et al., 1986). It has a protease activity that attacks C5a in a specific manner to produce a molecule that lacks the capacity for functional interaction with the appropriate neutrophil receptor (Ayesh et al., 1990) and consequently prevents the C5a from provoking a full scale inflammatory response (Ayesh et al., 1995). The C5a-inhibitor activity is deficient in FMF patients and was estimated in their fluids to be as less than 1% of that found in control peritoneal fluids (Matzner et al., 1984b,c). Moreover, gel filtration and ion exchange chromatography of peritoneal fluids from FMF patients failed to reveal a fraction with C5a-inhibitory activity (Matzner et al., 1990). It is postulated that FMF attacks due to the lack of the protection of the C5a-inhibitor, and therefore a bolus of C5a released accidentally into a serosal space will sometimes persist long enough to incite an inappropriate inflammatory response (Ayesh et al., 1995).

Marenostrin/pyrin protein is primarily expressed in granulocytes, the major cell population involved in acute inflammation, and it contains conserved

domains which are the characteristics of known transcription factors (The International FMF Consortium, 1997). Due to this, pyrin protein is probably involved in the down-regulation of mediators of inflammation (Ben-Chetrit & Levy, 1998) or in the up-regulation of the anti-inflammatory mediators, mainly the C5a inhibitor which may function as a tissue-specific inflammatory mediator that is dependent on pyrin/marenostrin for proper expression and secretion (Matzner et al., 2000). Consequently, it inhibits the development of an undesirable inflammatory response. On the other hand, dysfunction of the pyrin/ marenostrin protein due to mutations in the *MEFV* gene, would result in a decrease in C5a-inhibitor activity and in the uncontrolled inflammatory attacks characteristic of FMF (Babior and Matzner, 1997). The autosomal recessive inheritance pattern of FMF suggests that a missense mutation in both copies of the gene leads to a loss of the pyrin function (Samuels et al., 1998). *MEFV* mRNA expression was also detected in human tumor cell lines, mainly myeloid leukemic cell lines, in the colon cancer cell line SW 480, and also in three other colon cancer and three prostate cancer cell lines (Tidow et al., 2000). No clear explanation was provided to the significance of these findings.

1.10. Genetics

The cloning of *MEFV* gene lead to the identification of four missense mutations (M680I, M694V, M694I, V726A) in FMF patients, clustered within 46 amino acids of a predicted 781-amino acid protein. These four mutations accounted for about 85% of the carrier chromosomes originally studied by both the international and the French FMF consortium (French FMF consortium, 1997; International FMF Consortium, 1997). The haplotype analyses implied that all

chromosomes carrying the M694V mutation may have been derived from a single ancestral chromosome, which originated probably more than 2000yr ago (Aksentijevich et al.,1999). Other mutations were actually identified in the *MEFV* gene. The overall description of these mutations is outlined in table 2.

Most of the *MEFV* mutations are clustered within the highly conserved B30.2 (rfp) globular domain at the C-terminal end of the pyrin protein. All of the reported mutations, except for M694del (Booth et al., 1998), I692del (Bernot et al., 1998), and Y688X (new nonsense mutation) (Notarnicola et al., 2001), are missense mutations with no drastic effects such as frame-shift or splice mutations. The nature of the substitutions is also compelling. All mutations so far described in the tenth exon are conservative (M680I, M694V, M694I, K695R, V726A, A744S and R76I), and even the deletions (I692del and M694del) conserve the reading frame. Less conservative alterations (E148Q, E167D, T267I, P369S, R408Q, F479L) are located in the most N-terminal part of the protein (Bernot et al., 1998). The E148Q and E167D are not part of any recognizable motif or domain in the protein (International FMF Consortium, 1997), but the T267I falls within the bZIP basic domain. The P369S is located six amino acids N-terminal to the B-box zinc finger domain. The R408Q mutation lies in a region that comprises the C-terminal residues of the B-box zinc finger domain (Cazeneuve et al., 1999) and F479L resides within the α-helical (coiled-coil) domain (International FMF Consortium, 1997). It was also suggested that more drastic mutations particularly those affecting the C-terminal region could be lethal or would induce a very different phenotype in homozygous individuals (Bernot et al., 1998). This lead to the hypothesis that functional constraints imposed on the C-terminal region also restrict the nature of the mutations occurring in the rest of the protein. So far, only

Table 2. *MEFV* Gene Mutations

Mutation	Codon change	Amino acid change	Position	Exon	Reference
E148Q	GAG › CAG	Glu › Gln	148	2	Bernot et al. (1998)
E167D	GAG › GAC	Glu › Asp	167	2	Bernot et al. (1998)
T267I	ACA › ATA	Thr › Ile	267	2	Bernot et al. (1998)
P369S	CCC › TCC	Pro › Ser	369	3	Aksentijevich et al. (1999)
R408Q	CGG › CAG	Arg › Gln	408	3	Cazeneuve et al. (1999)
F479L	TTC › TTG	Phe › Leu	479	5	Bernot et al. (1998)
M680I-a	ATG › ATC	Met › Ile	680	10	International FMF Consortium (1997)
M680I-b	ATG › ATA	Met › Ile	680	10	Aksentijevich et al. (1999)
T681I	ACT › ATT	Thr › Ile	681	10	Booth et al. (1998)
Y688X	TAC› TAG	Tyr › non-Translated Amino acid	688	10	Notarnicola et al., 2001
I692del	AAT deletion (fromCodon ATA.ATG)	Ile del	692	10	Bernot et al. (1998)
M694del	TGA deletion (From Codon ATG.AAG)	Met › Lys	694	10	Booth et al. (1998)
M694V	ATG › GTG	Met › Val	694	10	International FMF Consortium (1997)
M694I	ATG › ATA	Met › Ile	694	10	French FMF Consortium (1997)
K695R	AAG › AGG	Lys › Arg	695	10	Bernot et al. (1998)
V726A	GTT › GCT	Val › Ala	726	10	International FMF Consortium(1997)
A744S	GCC › TCC	Ala › Ser	744	10	Bernot et al. (1998)
R761H	CGT › CAT	Arg › His	761	10	Bernot et al. (1998)

the M694 residue has been described as the target of multiple mutations (M694V, M6941, 694del) (Bernot et al., 1998; Aksentijevich et al., 1999).

A number of polymorphic variations have been identified in the *MEFV* gene as shown in table 3. Most of the polymorphisms are clustered in exons 2 and 5, one of which is a non-conservative amino acid polymorphism (R202Q: CCG/CAG) which changed an arginine codon into a glutamine codon (Bernot et al., 1998).

1.11. Population Genetics

FMF is almost completely restricted to non- Ashkenazi Jews, Armenians, Arabs, and Turks. Although patients with FMF have been reported from Germany, Poland, Australia, and Brazil, however, in most of these cases the exact ancestry was not disclosed or they could be cases of another form of periodic diseases (Ben-Chetrit &Levy, 1998). Carrier frequency was estimated among non-Ashkenazi Jews to be 1:5 to 1:7 (Daniels et al., 1995) with the M694V mutation predominates in North African Jews and V726A is common in other Jewish patients (Gershoni-Bruch et al., 1999). A high carrier frequency has also been estimated among Armenians to be 1/7 (Rogers et al., 1989) with the M680I represents the most common mutation in this group (Samuels et al., 1998). The exact frequency of FMF among Turks and Arabs is not available because formal epidemiological studies have not been performed. In a study of the mutations in families of Turkish origin, the M694V, M680I , and V726A mutations accounted for 85% of the disease alleles (Chen et al., 1998). In another study of Jordanian and Lebanese FMF patients, M694V and V726A reported to be the most common mutations in these populations (Medlej-Hashem et al., 2000; Mansour et al., 2001).

Table 3. *MEFV* Gene Polymorphic sites

Nuecleotide Variant	Codon	Location
306, T› C	102	Exon 2
414, A› G	138	Exon 2
495, C› A	165	Exon2
605, G› A	***202***	***Exon 2***
942, C› T	314	Exon 3
1356+44, A› G	...	Intron4
1422, G› A	474	Exon 5
1428, A› G	476	Exon 5
1503, C› T	501	Exon 5
1518, C› T	506	Exon 5
1530, T› C	510	Exon 5
1588-69, G› A	...	Intron 5
1759+8, C› T	...	Intron 8
1760-30, T› A	...	Intron 8
1764, G› A	588	Exon 9

(Cazeneuve et al., 1999)

A high FMF gene frequency was also reported among an Isreali-moslem Arab population having the V726A as the most common mutation, followed by M680I, M694V, and M694I (Shinawi et al., 2000). Definitely, more studies are needed to identify the full spectrum of mutations and their global distribution in the gene among the inflicted ethnic groups.

1.12 Genotype-Phenotype Relationship

The French FMF Consortium reported that four mutations (Met694Val, Met680Ile, Val726Ala and Met694Ile) accounted for 85% of the carrier chromosomes in their study group. The results of these initial studies lead to the hypothesis that phenotypic variations of the disease may be attributable to the existence of particular mutations. The Met694Val mutation is also known as the Mediterranean mutation since it was demonstrated in FMF patients from different ethnic backgrounds including non-Ashkenazi Jews, Turks, Armenians, and Arabs (French FMF Consortium, 1997).

Patients with M694V/M694V genotype express more severe disease, including earlier age of disease onset, higher frequency of attacks, higher prevalence of arthritis and erysipelas-like erythema, higher dose of colchicine necessary to control the disease (Shinar et al., 2000). The M694V/V726A genotype was found to be more severe than M694V/E148Q and V726A/V726A genotypes (Shinar et al., 2000). This classification is not absolute since some patients with the M694V/M694V genotype were reported to have mild disease onset, while some patients with the other genotypes had severe disease symptoms (Shinar et al., 2000).

Moreover, patients with the M694V homozygous genotype seems to have a higher prevalence of amyloidosis (Cazenevuve et al., 1999). Ethnic background is

one of the major contributors to the risk of this complication, but the possibility of an environmental influence cannot be ignored by the observation that amyloidosis was not found in Armenians living in the USA, but was obserevd in 25% of Armenian FMF patients living in Armenia (Yalcinkaya et al., 2000). In addition to the M694V homozygous genotype, amyloidosis has been found in association with various other genotypes of FMF patients with, including M694V/V726A, V726A/V726A-E148Q, M680I/V726A, M694V/T681I, M694I/unknown, and V726A/unknown (Shinar et al., 2000). It was reported also that amyloidosis is highly associated with 694 substitution (M694I or M694V) in the *MEFV* gene (Ben-Chetrit and Backnroth, 2001). An Arab kindred where all the affected individuals were homozygotes for M694I showed marked amyloidosis in addition to one patient with systemic amyloidosis who was homozygous for V726A (Mimoini et al., 2000). A possible explanation was provided based on a study for the genes encoding the major acute-phase serum amyloid Al (SAAl) and A2 (SAA2) proteins, which are precursors of the amyloid A (AA) proteins present in the amyloid fibrils found in AA amyloidosis (Liepnieks et al., 1995). The SAA1 *ala* genotype was found to be associated with sevenfold increased risk for renal amyloidosis compared with other SAA1 (Cazeneuve et al., 2000). Another explanation reffered amyloidosis to a polymorphic site in the *MEFV* gene, the Ala138Gly polymorphism significantly correlated with the incidence of amyloidosis (Akar et al., 2001).

The V726A mutation is known to be attributed by limited features of the classical disease, older age of onset, less protracted arthritis, and smaller dose of colchicine therapy (Touitou et al.,1998). This mutation was found to be frequent in Ashkenazi Jews, Druzes and Armenians, among whom amyloidosis occurs at lower frequencies (International FMF Consortium, 1997). Therefore, it was suggested

that the V726A mutation may be protective from amyloidosis (Pras et al., 1997). However, recent case reports have shown that homozygotes or compound heterozygotes with the V726A mutation may develop amyloidosis (Yalcinkaya et al., 1998).

Among the other mutations, the E148Q mutation deserves attention. It was reported to be frequent in patients from especially Ashkenazi Jewish ancestry. Recent reports have indicated that its frequency is lower in Turkish and Armenian FMF patients (Tekin et al., 2000). This mutation has been associated with mild disease (Bernot et al., 1998). Moreover, it was suggested that this mutation has a reduced penetrance (Mimoini et al., 2000), since most homozygotes for this mutation are asymptomatic and a high carrier rate for this mutation was found in populations known to have a low incidence of FMF (Shinar et al., 2000). Another molecular evidence for incomplete penetrance in of the disease phenotype was provided from three asymptomatic healthy relatives (one male and two females) of affected children whom were found to carry two mutated *MEFV* alleles of disease-causing mutations (i.e., V726A and M680I), which may point out to the existence of modifier factors (Yuval et al., 1995).

Interestingly, in a study of two unrelated British FMF patients associated with simple heterozygosity for M694V, were also consistent with autosomal dominant inheritance (Booth et al., 2000). The clinical features of dominantly inherited FMF were absolutely typical, including AA amyloidosis in a patient with pyrin M694V (Booth et al., 2000). This suggested that the methionine residue at position 694 makes a crucial contribution to pyrin's function, and that a 50% complement of normal pyrin activity did not prevent susceptibility to FMF (Booth et al., 2000).