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Familial Mediterranean Fever at the Millennium: Clinical Spectrum, Ancient Mutations, and a Survey of 100 American Referrals to the National Institutes of Health

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Abbreviations used in this article: ELE, erysipelas-like erythema; FHF, familial Hibernian fever; FMF, familial Mediterranean fever; FPF, familial periodic fever; HIDS, hyperimmunoglobulinemia D and periodic fever syndrome; NIH, National Institutes of Health; NSAID, nonsteroidal anti-inflammatory drug; RT PCR, reverse transcriptase polymerase chain reaction; SNP, single nucleotide polymorphism.

Introduction

At the age of 46 years, El now leads a healthy, active life. But almost every week throughout his childhood and many of his adult years, this Armenian-American man found himself curled up in a fetal position, incapacitated by high fevers and "piercing, violent" abdominal pains-often for 2 days at a time. While some of his teachers doubted him, surgeons believed him all too well and removed his appendix at age 3 years, only to witness a recurrence of his fever, chills, and abdominal pain the following week. Knowing that El's father suffered from recurrent episodes of fever and severe chest pain, a family physician diagnosed the father and son with familial Mediterranean fever (FMF; OMIM 249100[1]). "But he said there was nothing we could do." Nearly 30 years later, a rheumatologist prescribed daily doses of colchicine for the 2 men; their attacks have disappeared, with symptoms only returning when "I forget to refill the prescription on time." [We have encoded the initials of all patients described in this article to keep their identities confidential.]

([1]) The 6-digit OMIM number refers to Mendelian Inheritance in Man, a continually updated catalog of genetic disorders available in print [168] or CD-ROM version and electronically (Online Mendelian Inheritance in Man, OMIM), through the World Wide Web (http://proxy.library.upenn.edu:2082/omim/).

Such a clinical history illustrates many of the classic signs and symptoms seen in FMF, the most common and best understood of the hereditary periodic fever syndromes. The textbook FMF case, with each episode typically lasting for up to 3 days, manifests with fever and some combination of severe abdominal pain, pleurisy, arthritis, and a characteristic ankle rash-all from the accumulation of neutrophils at the symptomatic sites. Between these attacks, most patients are completely asymptomatic. If untreated with prophylactic colchicine, some patients later develop widespread amyloidosis that often leads to kidney failure and other organ damage. FMF follows an autosomal recessive pattern of inheritance, and is most prevalent within Jewish, Armenian, Turkish, and Arab populations. Although many patients have a positive family history for their symptoms, a significant proportion of them have no known affected relatives.

Since the disease was first described more than 50 years ago, the diagnosis of FMF has been based on clinical presentation. But a crucial advance in the study of FMF-the 1997 cloning of the responsible gene [50,66]-has permitted the diagnosis to become more precise and clinicians to identify which specific mutations (8 have already been identified) are present in a symptomatic patient.

In light of this discovery, we review the current knowledge of FMF and offer a comprehensive evaluation of this disease as it presents in the United States. After describing the historical and clinical pictures, including some recently identified manifestations, we provide a brief synopsis of the positional cloning effort that led to the identification of the gene, MEFV (short for MEditerranean FeVer). A description of what is known about MEFV and its protein product, pyrin/marenostrin, is followed by our present concept of the pathophysiology of FMF. We then include a mutational analysis of a series of 100 American patients referred to us for the evaluation of periodic fevers and other FMF-like symptoms; we use this genetic information to explore the sensitivity and specificity of a widely used set of clinical criteria for identifying FMF patients. In attempting to address a broad range of clinical presentations of FMF in the United States, we provide genotype-phenotype correlations and analyses of trends among different ethnic populations of FMF patients. We also discuss other periodic fever syndromes and speculate on their possible pathogenetic relationships to FMF.

We conclude with a discussion of future research, dealing with the function of the wild-type pyrin protein, the pathophysiology of FMF attacks, and the possibility of adjunctive therapies. We postulate that such findings might shed more light on neutrophil biology and on acute inflammation of all causes.

History, Genetics, and Epidemiology

Case reports of patients with symptoms of FMF first appeared in the literature in

the beginning of the 20th century. In 1908, Janeway and Mosenthal [69] described a 16-year-old Jewish girl who suffered from recurrent episodes of fever, abdominal pain, and leukocytosis. Not until 1945, however, did anyone document the constellation of symptoms and laboratory findings as a clinical entity, when Siegal [136] compiled 10 cases of "benign paroxysmal peritonitis." In the years since, the literature has referred to the disease as "recurrent polyserositis" [39], "periodic peritonitis" [132], "recurrent hereditary polyserositis" [16], "periodic disease" (the heading used in MEDLINE, National Library of Medicine, Bethesda, MD), and "familial Mediterranean fever" [63,139].

The words "familial mediterranean fever" refer to 3 of the classic aspects of the disease: an autosomal recessive inheritance pattern, a Mediterranean ancestry, and a history of recurrent fever. Clinicians, however, must be careful not to exclude the disease from a differential diagnosis, even if a patient fails to exhibit all 3 of these characteristics. Since heterozygote FMF carriers demonstrate no evidence of disease, it should be no surprise that only half of the patients report a family history of similar symptoms [63,131]. Although many of the patients, including Sephardi Jews, Armenians, Turks, and Arabs, have ancestors who emigrated from the "Mediterranean" region, the disease is uncommon in most other Mediterranean populations; in addition, the clinical symptoms are now well documented in groups of people who lack any Mediterranean background [79,144]. Finally, some patients do not realize that they are febrile during their attacks (but when such patients are studied closely, fever subsequently is observed).

Reports of patients with both clinical and genetic diagnoses of FMF now include a greater variety of ethnic groups (see later description of Italians), but the majority of cases continue to occur in 1 of 4 groups: Jews, Armenians, Turks, and Arabs. The genetic advances in recent years support the notion that the disease has existed at least since biblical times, and suggest that the diversity of patients who currently suffer from FMF might share common ancestors; yet the prevalence varies significantly among these populations, and even among subpopulations. For instance, the Sephardi Jews, who are descended from Jews who fled Spain after the Inquisition in 1492, have a prevalence of FMF ranging from 1:250 to 1:1,000 and an estimated carrier frequency of 1:8 to 1:16 [163]. A different study estimated the carrier frequency in this population to be between 1:5 and 1:7 [33]. The former, from a population-based study, might underestimate the frequency because of underreporting or mild cases that do not receive medical attention; the latter, a family-based study, may overestimate the frequencies if unrecognized inbreeding had occurred. Thus, the true Sephardi frequency probably lies between the 2 approximations. The prevalence among Ashkenazi Jews, traced back to Eastern Europe, has been reported to be as low as 1:73,000, with a carrier frequency of 1:135 [163]. The Armenian-American population is also believed to have a high carrier frequency of 1:7 [121].

Family and population-based studies established an autosomal recessive pattern of inheritance for FMF years ago. In recent years, this pattern has been confirmed with each genetic advance, from chromosomal linkage to haplotype analysis, and, finally, to

specific genetic mutations [50,66]. A 1992 twin study [135] provided another argument supporting recessive inheritance: only 3 of 11 dizygotic twin pairs with at least 1 affected twin demonstrated concordance (close to 25%), while all 10 monozygotic pairs showed full concordance. A number of cases have appeared in the literature over the years raising the possibility of dominant inheritance for FMF, but these families either represented examples of pseudodominance attributable to high gene frequencies within some communities [117], or instances of FMF-like diseases [94] whose suspected mutation does not lie in the MEFV gene. In 1 large Ashkenazi Jewish family presumed to exhibit dominant inheritance [163], in which neither of these explanations seemed likely, we recently found evidence for recessively inherited mutations in MEFV (Aksentijevich et al, manuscript in preparation).

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 [131,139]. Although there have been case reports of first attacks presenting in the elderly, physicians should hesitate to make the diagnosis of FMF if the onset of symptoms occurs after age 40 years [124]. The male:female ratio of cases has consistently been reported to be about 1.5-2.0:1.0, raising the possibility that the mutation has reduced penetrance in females. Many women report that attacks occur more commonly with their menses, suggesting that female sex hormones might influence the patients' disease [132]. In addition, many women find that their pattern of attacks disappears during pregnancy, only to return after delivery [132,139].

Clinical Picture

The common presentation

Familial Mediterranean fever typically presents as acute episodes of fever accompanied by complaints of abdominal pain, chest pain, or joint pain. While the acute inflammation typically involves the peritoneum, the pleura, and the joints, patients have reported symptoms at almost every location in the body from the skin to the scrotum (Figure 1). The frequencies of the common manifestations of FMF are shown in Figure 1, as found in our cohort of 47 American patients with at least 1 identifiable MEFV mutation. Although studies have yet to suggest any specific molecular triggers of these episodes, and many patients report attacks that occur without any identifiable provocation, the flares are often precipitated by menses, emotional stress, or strenuous physical activity.

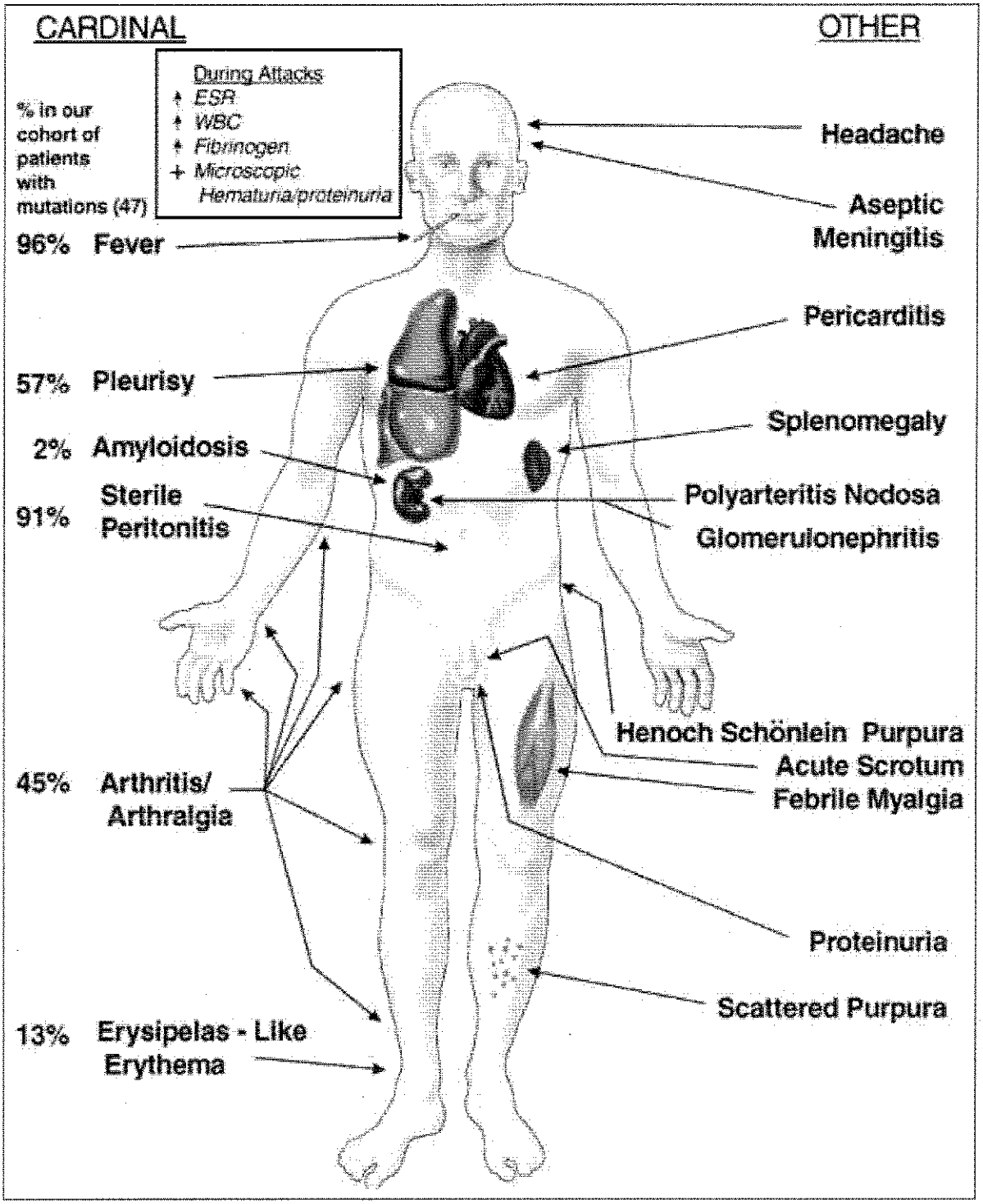


Figure 1. The spectrum of clinical manifestations seen in familial Mediterranean fever. Illustration of the male anatomy is not intended to imply any sex-related manifestations or risks with the exception of acute scrotum. (Adapted from [72]. Artwork by Jeffrey Aarons.)

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 or arthritis often lasting longer. The degree of temperature elevation, as well as the anatomic distribution of inflammation (abdominal, chest, or joint), may vary from 1 attack to the next; manifestations vary from 1 patient to another, even among individuals within the same family.

Abdominal pain: Nearly all FMF patients experience abdominal pain during at least some of their attacks, with about 50% citing such pain as the first symptom [139]. The pain may be diffuse or localized to a quadrant. The intensity of abdominal pain ranges from mild bloating to severe peritonitis with boardlike rigidity, direct and rebound tenderness, and air-fluid levels on upright X-ray films. The peritoneal inflammation slows peristalsis and thus patients present with constipation far more frequently than with diarrhea. A patient's past history often includes an appendectomy, exploratory laparotomies, or laparoscopies-usually with negative findings other than a sterile, inflamed peritoneal lining. One study [118] has even recommended elective laparoscopic appendectomies for FMF patients to prevent subsequent unnecessary emergency surgeries.

Although the differential diagnosis for patients with abdominal pain is extensive, the physician's first thought in these patients usually falls upon acute appendicitis-especially because most FMF patients first present before the age of 20 years. The spontaneous resolution of fever and pain, or the recurrence after an appendectomy, usually alerts the physician to search for another cause. In women, gynecologic evaluation is often required to rule out endometriosis, pelvic inflammatory disease, and ovarian cysts. Even if many attacks have been documented previously, the diagnosis is still difficult because some diseases, such as porphyria, can present with episodic abdominal pain and fever. Porphyria, however, is dominantly inherited, and causes hypertension and elevated porphyrins in the urine during attacks [34]. Hereditary angioedema might be suspected if a patient complains of abdominal pain without noticeable fever; but this disease is also inherited in an autosomal dominant pattern, and diagnostically low C1 esterase inhibitor levels can be measured [56,157]. Pancreatitis secondary to familial hypertriglyceridemia may also result in fluctuating abdominal pain similar to that seen in FMF, but these patients usually have pathognomonic triglyceride levels greater than 1,000 mg/dL [70].

Chest pain: Pleural attacks occur in up to 50% of Jewish, Arab, and Turkish patients [16,101,139], and Armenian patients may have an even higher rate of pleurisy [131]. The pleurisy is usually unilateral, and examination often reveals diminished breath sounds and a friction rub; chest radiographs may show a small effusion or atelectasis [25].

Joint pain: While arthralgia and arthritis have been reported in nearly 75% of North African Jewish patients with familial Mediterranean fever [139], less than half of

patients from the other FMF populations complain of joint pain [16,101,131]. Arthritis is classically monarticular, and most frequently involves the knee, ankle, or hip [51,61]. Some patients, including many in our cohort described later, report diffuse arthralgia, often involving the feet and upper extremities. Sacroiliitis [27] can occur either with or without spinal involvement, but is usually HLA-B27 negative [77,80]. With arthritis, the affected joints often have sterile effusions with polymorphonuclear leukocyte-filled synovial fluid, but the discomfort may be unaccompanied by any swelling or warmth. During the acute attack, X-ray films do not provide any evidence of bony changes. The acute arthritic attack typically resolves in only a few days, but can last for up to 1 month-long after other symptoms of the attack have disappeared [51].

A small percentage of acute arthritic episodes, however, develop into a protracted arthritis [61,128,138,161] with effusions that persist for months, although this is rare with the use of colchicine. Such patients often develop synovitis in a second joint and marked muscle atrophy, as well as juxta-articular osteoporosis, lytic erosions, or osteonecrosis. While most patients with arthritis recover completely without functional impairment, patients with protracted hip arthritis are frequently left with functional disability. Repeated joint aspiration of the hip may help to prevent osteonecrosis and the need for surgery [138]. Patients with chronic knee effusions may require chemical or arthroscopic synovectomy [128,161]. Spondyloarthropathy is refractory to treatment with colchicine, but responds to nonsteroidal anti-inflammatory drugs (NSAIDs) and second-line antirheumatic agents [77]. The most common spinal involvement is fusion of the lumbar vertebrae; however, there have been case reports of neck pain from joint fusion in the cervical spine [77,142].

The long list of possible causes of intermittent joint pain includes some with monarticular episodes similar to that seen in FMF. Such localized arthritides warrant joint aspiration and cultures to rule out crystal deposition and infection. With more diffuse arthralgia, however, the differential includes a broader spectrum of rheumatologic diseases. The prevalence of joint pain is higher in children, and thus one must consider the diagnosis of systemic-onset juvenile rheumatoid arthritis. This disease, however, presents with a characteristic evanescent rash, lymphadenopathy, and a quotidian fever pattern instead of the 1- to 3-day FMF episodes. Moreover, longstanding juvenile rheumatoid arthritis usually leads to chronic arthritis and radiographic changes, while these outcomes are much less common in FMF.

Erysipelas-like erythema: Many experts consider erysipelas-like erythema (ELE) to be the most characteristic cutaneous lesion in FMF [8], although the published frequency in FMF patients ranges from 3% to 46% [16,139]. The tender lesions appear as erythematous, warm, swollen areas 10-15 cm in diameter, and usually occur below the knee on the anterior leg or the dorsum of the foot (unilaterally or symmetrically).

Laboratory findings: Common laboratory findings during flares include leukocytosis

with a left shift, an elevated erythrocyte sedimentation rate, and increased acute-phase reactants (C-reactive protein, serum amyloid A, fibrinogen, haptoglobin, C3, and C4). Patients may also have transient, albuminuria and microscopic hematuria during episodes [44].

Uncommon manifestations

A small percentage of FMF patients develop acute scrotal inflammation, usually unilaterally, and this may be the first presentation in prepubertal boys [46,84,96]. In these cases, attacks include the gradual onset of pain over 12 hours, with scrotal swelling and edema, and swelling and tenderness of the involved groin; torsion can occur, although most cases only involve inflammation of the tunica vaginalis. If testicular radionuclide scintigraphy does not demonstrate decreased perfusion, conservative management is adequate.

Up to 20% of FMF patients complain of myalgia, which may take 2 forms [78]. In the milder type, the muscle pain usually lasts less than 2 days and occurs mostly in the evenings; affected patients complain only of transient lower extremity pain with physical exertion that resolves with rest and NSAIDs. On rare occasions patients develop protracted febrile myalgia, with more than a month of fever, excruciating muscle pain, abdominal pain, arthritis, diarrhea, and purpura. Corticosteroid therapy results in a dramatic improvement in these patients, but should only be used after myopathy and neuropathy from colchicine toxicity has been ruled out by a high erythrocyte sedimentation rate and normal creatine kinase levels.

Patients frequently complain of headaches with their attacks [44]. There are sporadic reports of meningeal irritation with increased cerebral spinal fluid protein and variable numbers of leukocytes in the fluid [18,53,109,130]. There are also reports of febrile convulsions and electroencephalogram abnormalities [53]. Fertility can be impaired in female FMF patients, possibly due to pelvic adhesions or the induction of early miscarriages by abdominal attacks [40,113]. Nonuremic pericarditis has been reported [31,74], and infrequently is complicated by cardiac tamponade [167].

Certain forms of vasculitis also appear to occur more commonly in FMF patients than in the general population. Henoch-Schonlein purpura (HSP) has been reported in 5%-7% of children with FMF [52,100,125,139]; polyarteritis nodosa (PAN), which by itself can present with fever and abdominal pain, occurs in less than 1% of FMF patients but is more frequent than in the general population [57,100,125,147]. Moreover, episodic scattered purpuric lesions appear on the face, trunk, or extremities, and much more frequently in children [88]. Although various types of glomerulonephritis have been reported in FMF patients [45,126], such as postinfectious, diffuse mesangial proliferative (with IgA or IgM deposits), and type II (immune complex) rapidly progressive glomerulonephritis, there is not enough evidence to be certain that

glomerulonephritis is more prevalent in FMF patients than in the general population.

Periods between attacks

Except for those with protracted arthritis, most patients remain free from fever and inflammation between their episodes; rarely patients report a low level of baseline discomfort or fever. Physical examination often reveals splenomegaly, even without amyloidosis [131]. Laboratory values during these quiescent periods may include a mild anemia, elevated fibrinogen levels, and elevated serum immunoglobulins [44].

Some patients develop adhesions and intestinal obstructions from repeated attacks or prior exploratory surgeries and laparoscopies. One recent study reported that these obstructions occur in 3% of FMF cases and often require surgery [29,139]. Thus, abdominal "attacks" without other typical symptoms for a particular patient should alert the physician to consider an obstruction.

Amyloidosis

The most dangerous manifestation of familial Mediterranean fever results from the deposition of amyloid A protein. This protein is presumed to be a cleavage product of serum amyloid A (SAA), an acute-phase reactant produced by the liver. Amyloidosis in FMF has been reported to infiltrate the kidneys, adrenals, intestine, spleen, and liver; less commonly it has affected the lung, thyroid, heart, stomach, and testes [62,73,139]. The most common clinical manifestation of FMF-related amyloid is the development of the nephrotic syndrome-and eventually uremia. Intestinal malabsorption and adrenal insufficiency are uncommon. Furthermore, FMF amyloidosis does not result in neuropathy or arthropathy as is seen in other forms of amyloidosis. Most patients who develop amyloidosis do so by the age of 40 years.

Due in large part to the widespread use of colchicine, only a minority of FMF patients now present with amyloidosis. The prevalence of amyloidosis among the FMF population has been thought to be independent of the frequency, duration, and intensity of flares [62]. This view is largely based on the (uncommon) observation of Phenotype II patients, in whom amyloid nephropathy presents before the first attack of fever and inflammation. However, recent data indicate that there probably is a general correlation between disease severity and amyloidosis (E. Pras, personal communication).

Even before the use of colchicine, however, amyloidosis was far from a universal long-term consequence of the disease; studies have implicated both heredity and the environment as factors affecting the risk of developing amyloidosis. The genetic

predisposition has been well documented, with 1 recent study of more than 600 FMF patients estimating a 6-fold increased risk of amyloidosis in patients with a family history of amyloidosis and consanguinity [125]. Ethnicity appears to contribute to the risk of developing amyloidosis [94,107]. The frequency has been reported to be higher in Jews of North African ancestry and in Turks [99,101]. A recent study, however, documented amyloidosis in only 7% of Turks-and attributed the higher frequencies from earlier reports to selection bias from nephrology centers [162]. The disposition to amyloidosis is less common in Iraqi and Ashkenazi Jews and Arabs [6,16,126,154]. The possibility of an environmental influence on amyloid pathophysiology is supported by the observation that Armenians living in Armenia have a much higher reported incidence of amyloidosis than Armenian-Americans [1,131], even before the introduction of colchicine prophylaxis.

Routine urinalyses are imperative for patients with FMF, as albuminuria appears early in the course of renal amyloidosis. Confirmed proteinuria necessitates a renal or rectal biopsy to confirm the diagnosis; a positive biopsy will have the characteristic apple-green appearance when stained with Congo red and viewed under polarized light. One study [24] reported that the sensitivity of such tests for FMF amyloidosis was 88% by renal biopsy and 75% by rectal biopsy, but only 19% with a gingival specimen. Bone marrow biopsies [143] and abdominal fat tissue aspirates [148] have also been considered as diagnostic approaches to look for amyloidosis. The former procedure has a sensitivity comparable to rectal biopsy [143], while the latter is much less sensitive [148]. Hemodialysis and renal transplantation have prolonged the lives of patients with amyloidosis, as long as colchicine is used to prevent amyloid from also depositing in the grafts [86].

Therapy

In a 1972 letter to the editor of The New England Journal of Medicine, Goldfinger [58] was the first to note the efficacy of prophylactic colchicine in FMF. After hearing that an FMF patient had experienced an end of his abdominal attacks with the initiation of colchicine for gout, Goldfinger tested the drug on a young woman whose FMF attacks had driven her to attempt suicide. Based on the woman's favorable response, he used the drug in 4 other patients, all of whom saw a virtual end to their devastating episodes.

A daily regimen of oral colchicine (1-2 mg/day) has remained the mainstay of treatment for FMF since 1974, when independent randomized, placebo-controlled trials established its efficacy [37,59,166]. Most patients experience significant improvement, while many patients report an end to their attacks. This regimen has been shown to have essentially the same efficacy in children younger than 16 years old, without significant side effects or eventual impact upon their normal growth, development, or fertility [164]. Some patients find that a minimal dose (0.6 mg/day) helps with the acute flares, but most only find maximum relief at 1.2-1.8 mg/day; these higher doses

are also recommended for the prevention of amyloidosis.

Although intermittent dosing may abort acute attacks in some cases [159], continuous daily treatment is urged for the prevention of amyloidosis [125,165]. A prospective study of 960 Israeli amyloid-free patients reported a 2% rate of amyloidosis in the 906 patients who complied with their daily regimen for 11 years, as compared to 49% of the 54 patients who admitted noncompliance over 9 years [165]. FMF patients already exhibiting proteinuria should still be placed on colchicine to stabilize or decrease the amount of protein loss [85]. As mentioned previously, renal transplant patients should also follow a daily colchicine regimen to prevent graft amyloidosis [86].

While colchicine is efficacious in treating FMF, it is not without its side effects. Many patients taking colchicine complain of diarrhea and general gastrointestinal upset, some to the point that the drug is not a therapeutic option. Patients are usually able to tolerate colchicine, however, if it is introduced with gradually increasing doses. In addition, colchicine has been shown to induce lactose intolerance in FMF patients, with 1 study demonstrating a 3-fold increase in FMF patients who use colchicine [48]. A lactose-free diet in the colchicine-treated patients induced an improvement in symptoms. Antiflatulents such as simethicone also help some patients. Colchicine has relatively minor long-term effects on the gastrointestinal tract, as evidenced by jejunal biopsies of FMF patients [60].

A number of uncommon side affects also have been attributed to colchicine therapy. The drug can induce a reversible myopathy with elevated creatine kinase levels, as well as a neuropathy, most frequently in older patients with impaired renal function [75]. The effects on male and female fertility are not yet conclusive. While there are no large prospective inquiries into colchicine-induced male infertility, decreases in the sperm count are usually reversible with the cessation of the drug [41]. Abdominal attacks themselves might have an effect on female fertility, but colchicine is not thought to affect women's ability to conceive [40,113]. While there is concern over the potential risk of colchicine causing birth defects if used during pregnancy because the drug is known to arrest mitotic and meiotic chromosomal segregation in vitro, none of the studies to date (consisting of a total of 231 pregnancies) has conclusively demonstrated that colchicine is responsible for cases of trisomy 21 or other birth defects [108,113]. Amniocentesis, however, is still suggested to screen for chromosomal defects if either parent is taking the drug [108,113]. The concentrations of colchicine excreted in breast milk are low enough that lactating women can continue the medication safely while nursing their babies [22]. It is advisable to check a patient's complete blood count periodically to guard against the rare possibility of a cytopenia.

While oral colchicine therapy carries few risks, the toxicities are much more frequent when patients receive intravenous therapy while concurrently taking the drug orally-and have led to multiple organ failure and death [111,137,155]. Thus, intravenous

colchicine should not be given as supplementary therapy during a breakthrough attack; the only advisable use is during the perioperative period when oral dosing is not possible.

One study from Turkey [151] concluded that interferon-alpha warrants consideration as a potential adjuvant therapy for acute flares in patients who still experience frequent or occasional FMF attacks. The drug, used by 7 colchicine-resistant patients in a total of 21 consecutive attacks, halted the symptoms in 18 of the 21 events; on average, the patients experienced only about 3 hours of symptoms per episode. Although each patient experienced some fever and flu-like symptoms with the interferon, all of them asked for a supply of the drug at the end of the trial. Still, interferon has yet to undergo a placebo-controlled double-blind study.

Positional Cloning of MEFV

In the summer of 1997 the FMF gene was independently cloned by 2 international consortia, 1 led by our group at the National Institutes of Health (NIH) [66], and the second led by several French laboratories [50]. Although the gene had long been suspected to play a role in controlling inflammation because of the periodic nature of the attacks, none of the biochemical or clinical changes seen in patients permitted identification of the FMF gene. Instead, the 2 successful consortia applied the techniques of positional cloning, which do not depend on any specific pathogenic hypothesis; this approach entails identifying the specific chromosome on which the gene resides, and then narrowing the candidate interval to smaller and smaller regions on that chromosome.

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, centromeric to the gene for hemoglobin alpha [103], linkage was confirmed in patients of other ethnic groups, including families of non-Ashkenazi Jewish [133,50], Armenian [133], Arab [104], and Turkish [2] heritage. Our group then progressively refined the candidate interval to a region spanning 1 million base pairs, flanked on the telomeric end by the genes responsible for autosomal dominant polycystic kidney disease and tuberous sclerosis, and on the centromeric end by the gene that is mutated in Rubinstein-Taybi syndrome [4,47,81,140].

In order to identify additional markers that would narrow the interval even more, our group developed a highly redundant map of overlapping clones (cosmids, bacterial artificial chromosomes [BACs], P1-derived artificial chromosomes [PACs], and small-insert yeast artificial chromosomes [YACs]) completely spanning the million base pair region [141]. Using new microsatellite markers isolated from these clones, we found intrafamilial recombinants that narrowed the candidate interval to the 285 kb between

D16S468/D16S3070 and D16S3376 (Figure 2) [11]. The French consortium utilized newly identified microsatellites from Genethon to define intrafamilial recombinants flanking a somewhat larger but consistent interval [49].

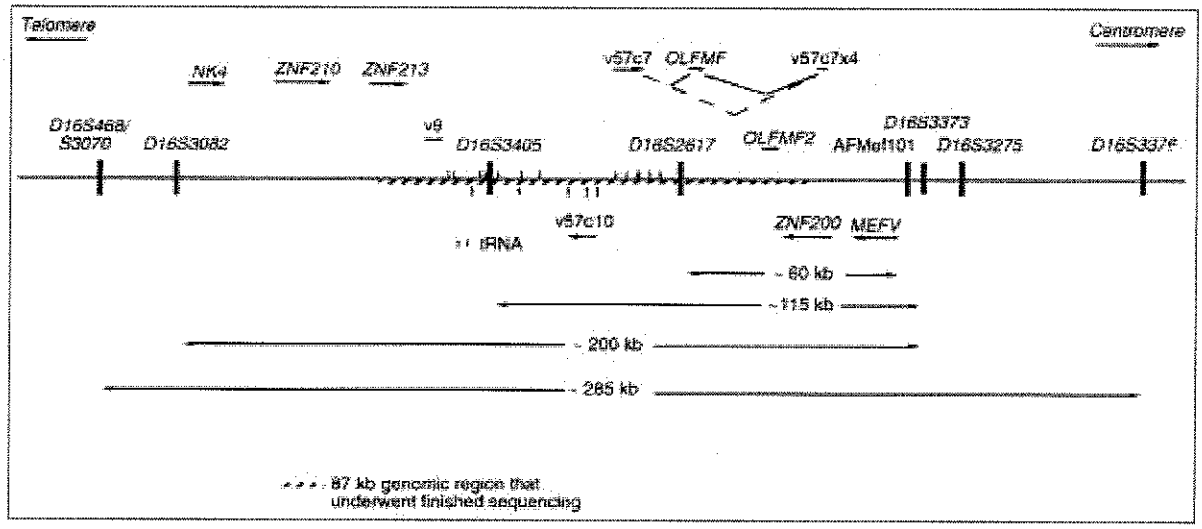


Figure 2. Integrated genetic/physical/transcript map of the MEFV region of chromosome 16. Physical distances on the map are drawn to scale. Transcripts were identified by exon-trapping, direct cDNA selection, and genomic sequencing. RNA polymerase II-directed genes are depicted as horizontal arrows pointing towards the 3[prime] end of the gene. Genes transcribed 5[prime]-3[prime] from telomere to centromere are shown above the line, genes transcribed 5[prime]-3[prime] from centromere to telomere are shown below the line. tRNA genes are depicted as vertical arrows placed above or below the line, conforming to the aforementioned convention regarding transcriptional orientation. ZNF200, ZNF210, and ZNF213 are all zinc finger genes. OLFMF is an olfactory receptor gene found to splice with v57c7 exons by reverse transcriptase polymerase chain reaction in fetal brain cDNA; OLFMF2 is an olfactory receptor pseudogene. NK4 is expressed in activated natural killer cells [32], v9 is a caspasc pseudogene, and MEFV is the gene causing familial Mediterranean fever (FMF). The 2 v57 genes are transcripts of unknown function. The [similar]285 kb interval between D16S468/D16S3070 and D16S3376 is the minimal candidate interval defined by the International FMF Consortium based on intrafamilial recombinants [11]. The [similar]200 kb interval between D16S3082 and D16S3373 was the candidate interval defined by historical recombinants for which our transcript map was assembled. The [similar]115 kb and [similar]60 kb intervals represent the final minimal candidate intervals, defined by historical recombinants, for the International [66] and French [50] FMF Consortia. The shaded 87 kb region was subjected to finished sequencing by the International FMF Consortium; an [similar]240 kb interval from D16S468/D16S3070 to D16S3275 was completely sequenced by the French FMF Consortium [50].

Additional narrowing of the candidate interval was made possible by the observation that carrier chromosomes from many of the non-Ashkenazi Jewish patients, as well as some of the Arabs, Armenians, and Turks, shared common microsatellite markers and defined a specific haplotype [4,11,49,81]. In this microsatellite haplotype analysis, the carrier chromosomes that contained only some of the same microsatellites

comprised "historical recombinant" haplotypes that further narrowed the candidate interval. As opposed to studying recombinations only within a family panel, this approach takes into account all of the meioses occurring over the many generations between the founder chromosome and our current patient pool.

This type of analysis defined candidate intervals of approximately 200 kb both for the French consortium [49] and our group [11]. During the final year of the search, the 2 groups utilized exon amplification, genomic sequencing, and direct cDNA selection to identify all of the genes encoded in this part of chromosome 16. Our transcript map (Centola et al, submitted manuscript) for the 200 kb between D16S3082 and D16S3373 is shown in Figure 2. Through this intensive study, additional markers were found that narrowed the interval to 115 kb for our group [66] and 60 kb for the French consortium [50].

Within this minimal candidate interval, we identified a 10-exon, 3,505-nucleotide cDNA, encoding a predicted protein of 781 amino acids [66], while the French consortium reported a partial clone encoding 477 amino acids identical to the C-terminal sequence we predicted. 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.

Each of the mutations was associated with distinct microsatellite haplotypes. In our studies, all FMF carrier chromosomes bearing the "A" haplotype (designated the "MED" haplotype by the French consortium) encoded a substitution of valine for methionine at position 694 (M694V). This haplotype was seen in a large percentage of carrier chromosomes of North African Jews, as well as some from Iraqi Jews, Armenians, Arabs, and Turks. Carrier chromosomes bearing the haplotype we designated as "C" (and the French designated as "ARM3/DRUZE") encoded a substitution of alanine for valine at position 726 (V726A), and were found in Armenians, Druze, and Iraqi and Ashkenazi Jews. The J and K haplotypes (equivalent to the French "ARM2") had an isoleucine for methionine exchange at position 680 (M680I), which only appeared in some Armenian chromosomes. The fourth mutation identified by the French, which resulted in a substitution of isoleucine for methionine at position 694 (M694I), appeared on a distinct Arab haplotype named "ARA2."

The strict association of haplotype-bearing carrier chromosomes with specific mutations, and the absence of those mutations in a large panel of controls, is strong evidence that this clone is in fact the FMF gene. Moreover, both our group and the French consortium found a small number of chromosomes bearing disease-associated haplotypes (presumably descended from the ancient chromosomes on which the mutations arose), which were noncarrier chromosomes by pedigree analysis. If the nucleotide substitutions we identified in FMF patients are truly mutations, rather than

polymorphisms on the disease-associated haplotype, then these haplotype-positive noncarrier chromosomes should not harbor the putative mutations; they did not [50,66]. Other supportive evidence includes the clustering of missense mutations in 1 region of exon 10, and the inability to find disease-associated mutations in other genes in the region (shown in Figure 2) despite intensive analysis. The identification of MEFV stands as 1 of the more difficult positional cloning projects, given that 1) all of the originally identified mutations are conservative missense substitutions, 2) the gene was not present in any of the existing public expressed sequence tag (EST) databases, 3) there were no chromosomal deletions or rearrangements to guide the search, and 4) the tissue distribution of expression of the gene is relatively restricted (see below).

The analysis that led to the identification of MEFV also gives rise to interesting historical speculation. Because some FMF patients from different ethnic groups share microsatellite haplotypes and mutations, it is likely that they are descended from common ancestors. Moreover, we found that certain M694V-bearing haplotypes that appeared distinct based on their microsatellite typings were identical at a series of single nucleotide polymorphisms (SNPs) within MEFV (Figure 3A). Haplotypes F and G were seen in a small percentage of North African Jewish carrier chromosomes. Although clearly distinct from the A haplotype by microsatellite typings, the F and G haplotypes were the same as the A haplotype for 11 intragenic SNPs, as well as for the M694V mutation. The B haplotype was observed in the Iraqi Jewish population. It, too, was easily distinguishable from the A haplotype at the level of microsatellites, yet was identical to the A haplotype for the 7 SNPs 3[prime] to exon 2, and at the M694V mutation. Similarly, the Armenian haplotypes J and K, which differed at microsatellites centromeric to MEFV, converged within exon 2 before coding for the M680I mutation (Figure 3B).

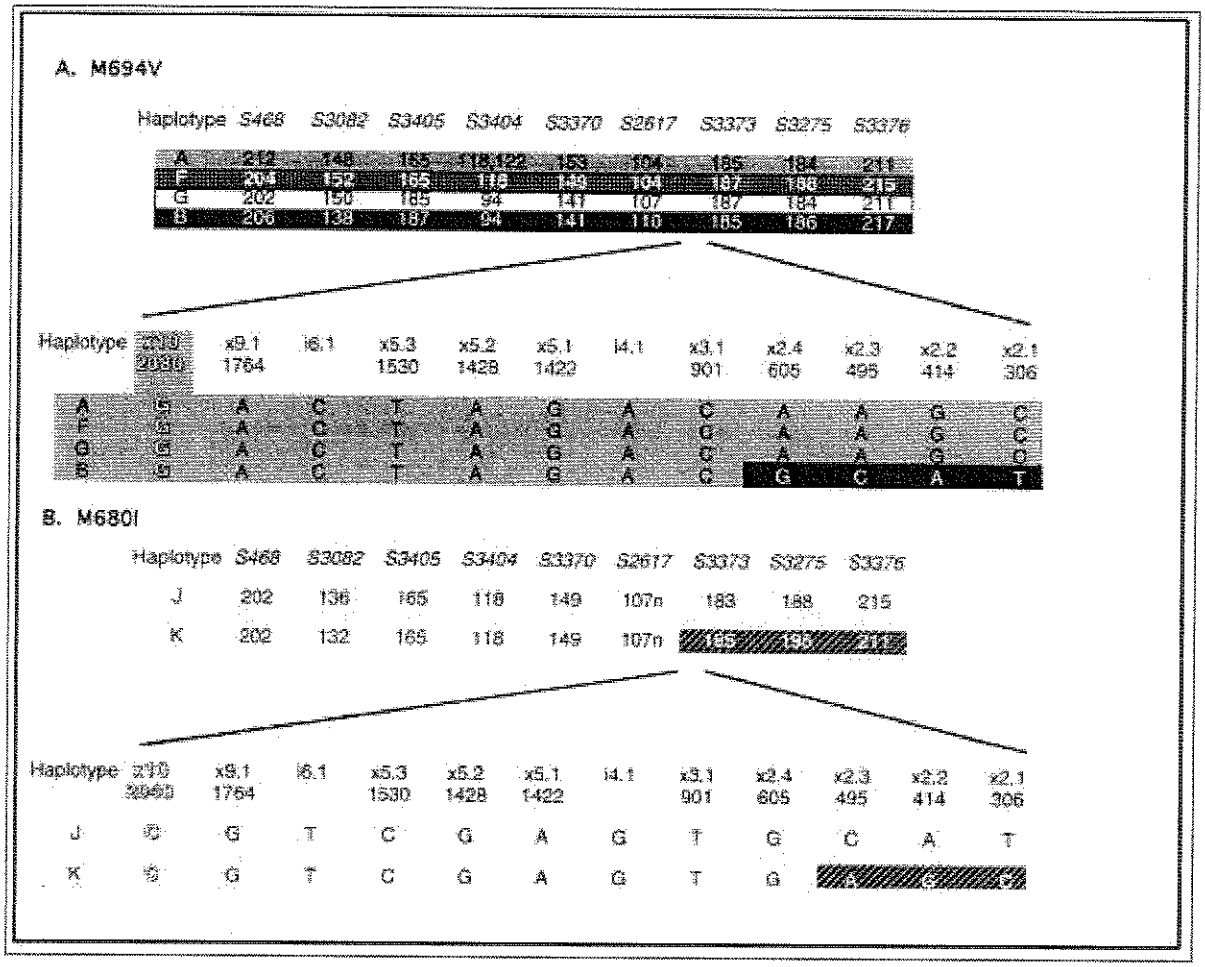


Figure 3. Haplotype convergence at MEFV. A) M694V, upper panel: The typings at 9 microsatellite loci are shown for 4 apparently independent FMF carrier haplotypes. The 4 haplotypes, A, F, G, and B, are each shaded differently. The A haplotype is the major founder haplotype in North African Jews, and is also observed in Iraqi Jewish, Armenian, Arab, and Turkish FMF patients. The B haplotype is seen in the Iraqi Jewish population. The F and G haplotypes are uncommon carrier haplotypes in the North African Jewish population. Lower panel: Typings for the same 4 haplotypes for single nucleotide polymorphisms (SNPs) identified within specific exons (denoted by "x") or introns (denoted by "i"). The first polymorphism in exon 2 is denoted "x2.1," etc. For SNPs within exons, the nucleotide position in the cDNA (relative to the translational start) is also given. Haplotypes A, F, and G are the same at all of the SNPs, and haplotype B converges beginning at the SNP in exon 3. At nucleotide 2080 in exon 10, all 4 haplotypes have a G, whereas 600 normal chromosomes all had A at this position. This results in a substitution of valine in haplotypes A, B, F, and G for methionine in normals.; B) M680I, upper panel: Microsatellite typings in the J and K Armenian carrier haplotypes, which differ at D16S3373, D16S3275, and D16S3376. Lower panel: MEFV intragenic SNP typings for the 2 haplotypes. The 2 haplotypes converge in exon 2. At nucleotide 2040 of exon 10, both haplotypes have a C, whereas 600 normal chromosomes all had G at this position. This results in the substitution of isoleucine in haplotypes J and K for methionine in normals.

If, in fact, most M694V-bearing chromosomes are descended from a common ancestor, the association of this mutation with the Iraqi Jewish B haplotype suggests that this ancestor may have lived at least 2,500 years ago, before the relative isolation of the Iraqi Jewish population that began with the Babylonian captivity. In addition to its radiations into Iraq, Turkey, and Armenia, this mutation spread from the eastern Mediterranean to Spain, and then to North Africa with the expulsion of the Sephardim in 1492. Furthermore, the observation of the V726A mutation and the C (ARM3/DRUZE) haplotype in Armenian, Ashkenazi, Iraqi Jewish, and Druze chromosomes suggests a second ancient founder chromosome. The presence of multiple founder mutations with high prevalence in modern-day Mediterranean populations raises the possibility of a selective advantage for carriers of these mutations, much like the heterozygote advantage of sickle cell trait against malaria.

The Protein: Pyrin/marenostrin

The protein encoded by MEFV, which our group named "pyrin" for its role in the regulation of pyrexia, is also referred to as "marenostrin" by the French consortium, in allusion to Mare Nostrum, the ancient Latin name for the Mediterranean Sea. Its 781 amino acids predict a positively charged protein weighing 86 kDa, with arginine and lysine constituting 13% of the residues [66]. Messenger RNA studies, described in more detail below in the Pathophysiology section, indicate that pyrin should be present almost exclusively in neutrophils and their precursors; the wildtype function of the protein is believed to be that of a direct or indirect downregulator of inflammation, specifically in neutrophils.

The exact mechanism of pyrin's action, and its inability to perform properly when missense mutations are present, remains a mystery at this time. Computer-assisted searches for homologies between the amino acid sequence of pyrin and of other proteins of known function have shed some light on this question. Pyrin's sequence includes a B30.2 domain from residues 598 to 774 (Figure 4), which appears in several proteins that act inside the nucleus [64,153]. Among them are Stat-50, an interferon-inducible transcription regulator [149]; PwA33, a protein that binds lampbrush chromosomes in the newt embryo [19]; and xnf7, a nuclear phosphoprotein in the Xenopus embryo [115]. Another member of the B30.2 family is Ro52/SSA, a ribonucleoprotein that is autoantigenic in systemic lupus erythematosus and Sjogren syndrome [67]. However, other proteins with B30.2 domains do not act in the nucleus, such as butyrophilin, a secreted molecule involved in the transport of milk proteins out of mammary epithelial cells [68], and the major stonefish poison (stonustoxin) [55]. In addition to the B30.2 domain, pyrin's sequence includes 2 overlapping potential nuclear localization signals, which further suggests that the protein works inside the nucleus. Two other sites on the protein, an alpha-helical region and a B-box zincfinger [114], might allow it to interact with other proteins. Genes encoding proteins with a tripartite structure comprised of a cysteine-rich region containing a RING finger and/or B-box, an alpha-helical domain, and a B30.2 domain, belong to a multigenic family we have termed the RoRet family [66]. The proteins encoded by these genes include pyrin, the

above-mentioned Ro52/SSA. xnf7, and PWA33, the ret finger protein (rfp) [145], and the newly cloned MID1 protein, mutations of which cause Opitz G/BBB syndrome [112]. Based on the foregoing analysis of functional motifs and structural homologies, pyrin may function as a transcription factor.

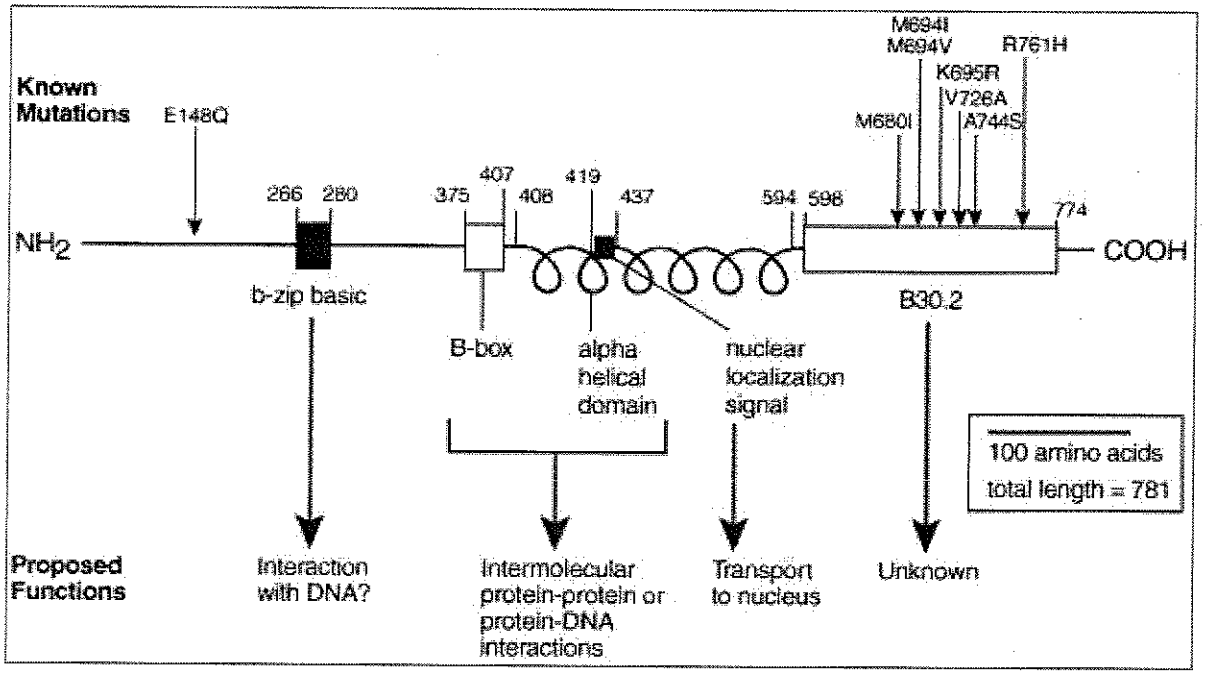


Figure 4. Pyrin's identified domains and corresponding functions. The amino and carboxy termini are indicated by NH_2 and COOH, respectively. The numbers above the protein segments refer to their amino acid positions on the primary protein structure. Known mutations are indicated by the 1-letter amino acid code.

At the very least, the homology search has demonstrated that MEFV does not encode the C5a inhibitor reportedly deficient in FMF serosal fluids [7,9,90,91]. Purified inhibitor protein has been partially sequenced and does not match pyrin; in addition, pyrin does not include the serine protease domain necessary for the inhibitor to function [43]. Although the expression studies described below may be of some help, pyrin's function may only become apparent after intracellular localization studies, the identification of its macromolecular interactions through immunoprecipitation or yeast 2-hybrid studies, and/or the development of mouse knock-out or knock-in models.

Pathophysiology

It has long been known that neutrophils accumulate in areas of inflammation during FMF attacks, but the molecular mechanism by which this happens has remained obscure. With the recent discovery of the gene and its protein product, the story is gradually becoming less of a mystery.

Few functional differences have been found between the neutrophils from FMF patients and those from normal subjects. In vitro studies have shown that FMF neutrophils demonstrate normal phagocytosis, chemotaxis, microtubular function, and morphology by light and electron microscopy [13,36,146]. Neutrophils from asymptomatic FMF patients do release more lysozyme in response to heat and hypotonic conditions, and they have been shown to have increased chemiluminescence [5,12]; such findings, however, have not explained the pathophysiology of FMF.

Some laboratories have studied the immunologic abnormalities seen in FMF patients, and have postulated potential autoimmune mechanisms for the disease. But those changes are now thought to be secondary to-and not the primary defect in-the pathophysiology of FMF [42]. Patients can have a polyclonal increase in serum immunoglobulins [44]; in addition, in vitro studies have shown that the induction of interleukin-1 and tumor necrosis factor-alpha is reduced in patients during attacks [122,129]. Most notably, patients reportedly have low levels of the inhibitor for interleukin-8 and the chemotactic factor C5a [7,90,91]; this inhibitor had long been postulated to be the product of the FMF gene. With the positional cloning of MEFV, advocates of the C5a inhibitor hypothesis now suggest that pyrin might be a transcriptional activator of the inhibitor. The integrated hypothesis [9] suggests that FMF mutations might lead to a deficiency of the chemotactic inhibitor, and subclinical serosal injury could activate the chemotactic C5a and attract granulocytes to perpetuate a spiral of C5a production-and thus unopposed inflammation. This hypothesis, however, has yet to be investigated.

Another pathogenetic hypothesis derives from the observation that some patients associate FMF attacks with stress. Reasoning that FMF might be due to a defect in catecholamine metabolism [17], Barakat and colleagues [14] infused the sympathomimetic agent metaraminol into patients to stimulate endogenous catecholamine release and simulate FMF-like symptoms. Although metaraminol-induced symptoms resembled most patients' attacks, and could be prevented with colchicine, these observations did not lead to additional biochemical or genetic insights. Thus, 1 study reported that FMF patients have increased levels of dopamine beta-hydroxylase [15], but other reports did not confirm this finding [20,30]. Moreover, the metaraminol test has never come into general use, because of concerns about its safety [28].

Since the discovery of the FMF gene, studies of the pathophysiology of the disease have shifted toward understanding the molecular and cellular role of MEFV and its protein product. Where is the wild-type protein pyrin expressed, what does the protein do, and how does its altered state (or its absence) lead to the FMF phenotype?

Based on the clinical manifestations of FMF attacks, it was reasonable to assume that MEFV mRNA and pyrin might be preferentially expressed in neutrophils and/or serosal cells lining the peritoneum, the pleura, and the synovia. Yet studies on a wide

range of normal tissues have shown that the gene is expressed almost exclusively on peripheral blood leukocytes [66], and is absent in lymph nodes, spleen, thymus, or any normal nonhematologic cell type. Using more sensitive assays, we have subsequently found clear evidence for expression in bone marrow (Centola et al, manuscript in preparation). However, by reverse transcriptase polymerase chain reaction (RT PCR), we could not detect MEFV message in several synovial samples. The French consortium did detect message by RT PCR in a synovial sample taken from a patient with rheumatoid arthritis, but it is unknown whether this represents expression in synoviocytes or infiltrating leukocytes. MEFV expression is not detectable in a peritoneal fibroblast cell line [9].

Further experiments indicate that, among white cells, there is expression only in granulocytes, and not in lymphocytes or monocytes [66], which is consistent with the key role that the granulocytes play in episodes of FMF. It is interesting to note that, among a panel of malignant cell lines, MEFV expression was detected in the SW480 adenocarcinoma, which produces the granulocyte growth factor GM-CSF [76]. Our current hypothesis is that in normal neutrophils, the product of the FMF gene acts as a downregulator of neutrophil-mediated inflammation. The autosomal recessive inheritance pattern of FMF suggests that a missense mutation in both copies of the gene leads to a loss of this function, specifically in the granulocytes in which the gene is expressed.

The nature of mutations demonstrated in certain other periodic diseases suggests a model for understanding how MEFV mutations cause episodic illness. In sickle cell anemia a specific missense mutation in beta-globin encodes a protein that functions adequately under baseline conditions, but, with hypoxia or other metabolic stresses, undergoes conformational changes leading to abnormal red cell morphology, microvascular occlusion, and sickle cell crises [156]. Similarly, in hyperkalemic periodic paralysis, missense mutations in the muscle sodium channel gene SCN4A produce a transporter that malfunctions with a dietary potassium load [110]. While the paradigm is clear, the nature of the physiologic factors that trigger or uncover pyrin dysfunction in FMF neutrophils remains unknown.

Obligate heterozygotes might have had a selective advantage by allowing a greater, but controlled, inflammatory response against some pathogen endemic to the Mediterranean basin. Of course, genetic drift likely accounts at least in part for the high frequency of the M694V mutation in North African Jews, but the high frequency of several MEFV mutations in multiple eastern Mediterranean populations strongly favors a selection hypothesis. To date, the only hint of a specific advantage is the report of a decreased prevalence of asthma in obligate heterozygotes [26], but this did not achieve statistical significance. In the era of antibiotics and improved sanitation, it may be difficult to document a heterozygote advantage against an infectious agent. Moreover, even a subtle advantage compounded over many generations can produce a high gene frequency. In addition, more severe nonsense mutations of the gene might lead to a complete loss of function, and perhaps a phenotype much different from FMF, such as

generalized, uncontrolled inflammation; this is the case for beta-globin, where nonsense mutations cause thalassemia instead of sickle cell disease. We are currently investigating the phenotypes associated with more severe loss of function mutations by creating pyrin knock-out mice. Knock-in mice, whose wild-type murine MEFV-homolog has been replaced with mutant human MEFV, may permit the experimental identification of the elusive selective factor, if such a factor truly exists.

As noted in the previous section, pyrin has sequence motifs that suggest it could be a transcription factor. If so, it could act either as a repressor of a proinflammatory molecule or a transcriptional upregulator of an anti-inflammatory molecule (Figure 5). However, as yet there are no experimental data directly addressing the possible role of pyrin as a transcriptional regulator, and it is possible that it exerts a more downstream effect.

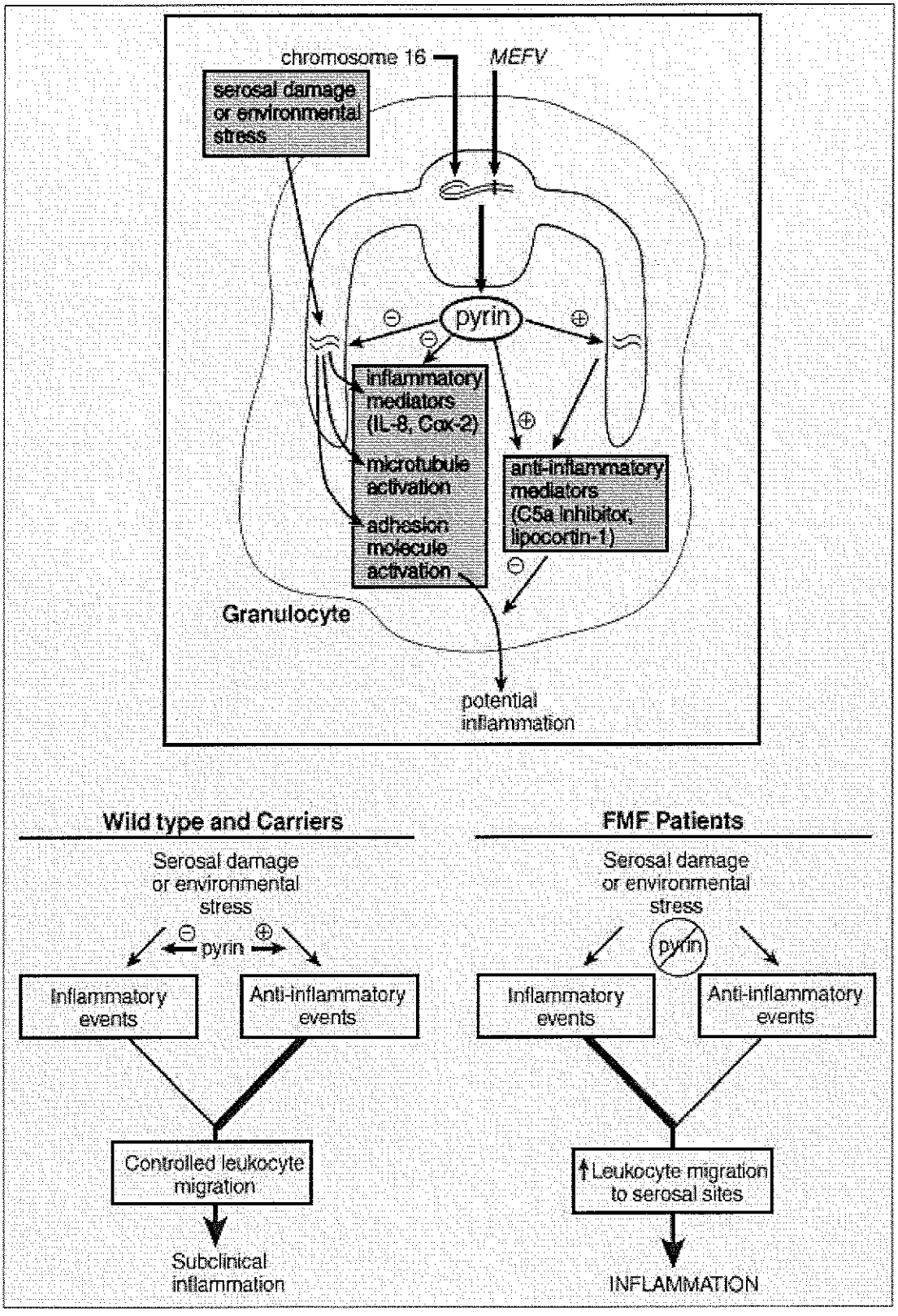


Figure 5. Pyrin and the proposed pathophysiology of inflammation in FMF. This hypothesis is based on the assumption that the normal function of pyrin is to

downregulate granulocyte-mediated inflammation, either by downregulating inflammatory mediators, microtubules, or adhesion molecules, or by upregulating anti-inflammatory mediators, such as the C5a inhibitor [7,9,90,91] or lipocortin-1 [134]. The mediators depicted in the Figure areintended only as examples of possible targets for pyrin's action.

Furthermore, how a mutation in a granulocyte-specific gene usually causes serosal inflammation remains an enigma. It is possible that an exogenous triggering agent with tropism to certain serosal/synovial membranes may be responsible; alternatively, pyrin may alter the expression of adhesion molecules on the neutrophils to target them to certain tissues.

Genotype-Phenotype Analysis

Because the aforementioned 4 mutations accounted for about 85% of carrier chromosomes originally studied by each consortium, we hoped that testing for these known mutations might provide a highly sensitive screen for FMF. Other authors have expressed similar optimism [9,21]. On the other hand, it should be noted that the panels of families selected for linkage analysis were skewed both with regard to ethnic background and severity of disease, and thus it would not have been surprising if these 4 mutations accounted for a smaller percentage of a broader sample. Thus, we set out to search for the mutations present in a pool of 100 unrelated American patients referred to the NIH Clinical Center with possible FMF. Our aims were to 1) estimate the relative frequencies of the known mutations in the United States, 2) investigate the ethnic distributions of various mutations, and 3) identify genotype-phenotype correlations, if present.

We asked each patient (48 patients in person, 52 by phone) a series of standard questions that began with date of birth, ethnicity, and age of onset of symptoms. We inquired whether there is a family history of the symptoms, and whether the specific attacks include fever, abdominal pain, chest pain, joint pain, ELE, pericarditis, vasculitis, or any atypical manifestations. Finally, we asked for each patient's duration and frequency of attacks, both before and after the initiation of colchicine therapy, as well as the actual colchicine dose. We also reviewed the patients' medical records; for those who visited the Clinical Center, we obtained a complete blood count, serum electrolytes, liver enzymes, acute phase reactants, complement levels, and a routine urinalysis. To evaluate a patient's severity of disease, we considered their duration of attacks, frequency of attacks, and general response to colchicine (Table 1). We did not calculate a severity score based on the recently published Tel Hashomer Key [106] because of its emphasis on amyloidosis and ELE, which are relatively uncommon in the United States, and because of the difficulties in evaluating the colchicine dose, given the wide disparities in prescribing habits among referring physicians.

Duration of attacks	
> 72 hours:	3 points
24–72 hours:	2 points
≤ 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	

Table 1. Disease severity scoring system

Finally, we obtained a blood sample from which we extracted genomic DNA to search for MEFV mutations. Several of the patients with textbook FMF stories lacked any of the 4 known mutations on either chromosome, and many of them only tested positive for a mutation on 1 chromosome. In total, 86 of the 100 patients met a recently published set of clinical criteria for FMF, derived from the experiences of the Sheba Medical Center in Israel (Table 2) [83]. The 97% specificity calculated for the Israeli population may overestimate the specificity in American patients (because the pretest probability of FMF is much lower in the United States), and hence some of our clinically positive, mutation-negative patients might have a different disease. Nevertheless, the finding of patients who met clinical criteria but had only 1 demonstrable MEFV mutation suggested that we had not yet identified all the mutations.

Major Criteria

Typical attacks (≥3 of the same type, rectal temp. ≥38 °C, at tacks lasting 12 hr to 3 d):

1. Peritonitis
2. Pleuritis (unilateral) or pericarditis
3. Monarthritis (hip, knee, ankle)
4. Fever alone

Minor Criteria

1–3. Incomplete attacks (typical attacks with 1 or 2 of the following exceptions: 1) temperature <38 °C, 2) attacks lasting 6–12 hours or 3–7 days, 3) no signs of peritonitis during abdominal attacks, 4) localized abdominal pain, 5) arthritis in joints other than hip, knee, or ankle) involving 1 or more of the following sites:
   1. Abdomen
   2. Chest
   3. Joint
4. Exertional leg pain
5. Favorable response to colchicine

Supportive criteria

1. Family history of FMF
2. Appropriate ethnic origin
3. Age <20 yr at disease onset

4–7. Features of attacks
   4. Severe, requiring bed rest
   5. Spontaneous remission
   6. Symptom-free interval
   7. Transient inflammatory response, with 1 or more abnormal test result(s) for white blood cell count, erythrocyte sedimentation rate, serum amyloid A, and/or fibrinogen

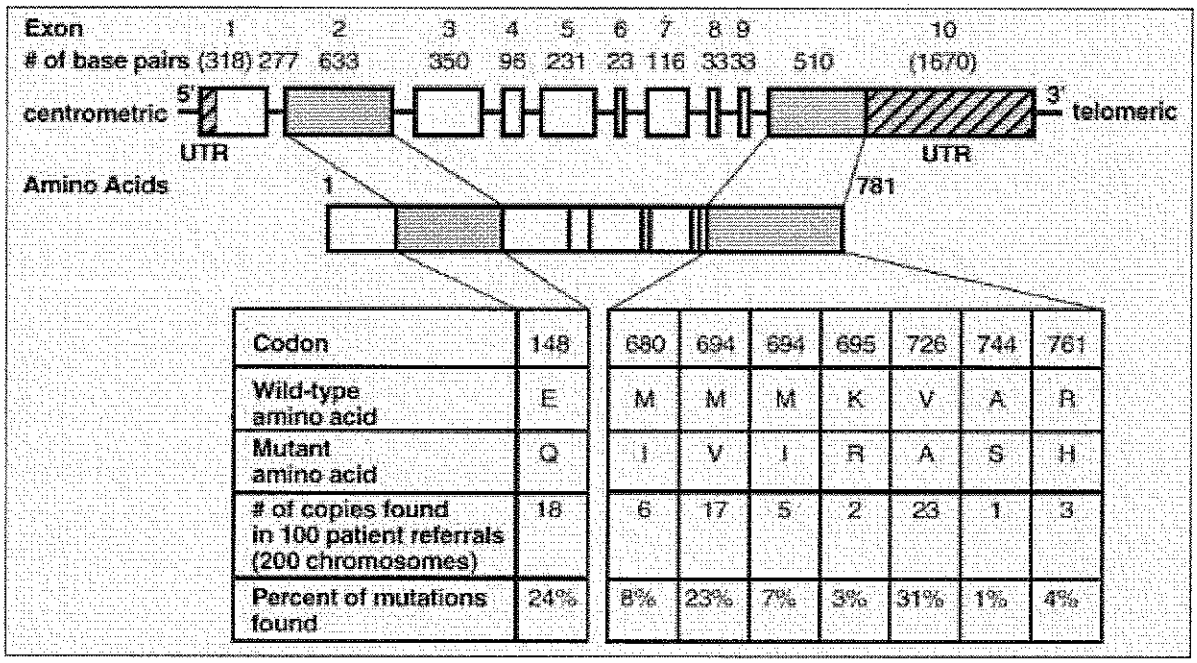
8. Episodic proteinuria/hematuria
9. Unproductive laparotomy or removal of white appendix
10. Consanguinity of parents

*An FMF diagnosis requires ≥1 major criteria, or ≥2 minor criteria, or 1 minor criterion plus ≥5 supportive criteria, or 1 minor criterion plus ≥4 of the first 5 supportive criteria.

Table 2. Criteria for diagnosing familial Mediterranean fever*

By genomic sequencing, we identified 4 new mutations and developed restriction enzyme or PCR assays for each (Aksentijevich et al, manuscript in preparation). Three of these are located in exon 10 with the 4 original mutations: they result in amino acid substitutions of arginine for lysine at position 695 (K695R), serine for alanine at position 744 (A744S), and histidine for arginine at position 761 (R761H). Perhaps more noteworthy is the identification of a novel mutation in exon 2 that substitutes glutamine for glutamic acid at position 148 (E148Q).

Armed with reliable screening tests for all 8 mutations, we assayed each mutation on all 100 patient referrals. In our cohort, we found that the V726A and M694V defects, and the new E148Q mutation in exon 2, were the most common mutations. In addition, we found 4 FMF patients with a complex allele (an individual chromosome with 2 mutations), consisting of the E148Q and V726A mutations on the same chromosome (Aksentijevich et al, manuscript in preparation). The relative locations and frequencies of the 8 different mutations in our pool of affected chromosomes appear in Figure 6.



Codon	148	680	694	694	695	726	744	761
Wild-type amino acid	E	M	M	M	K	V	A	R
Mutant amino acid	Q	I	V	I	R	A	S	H
# of copies found in 100 patient referrals (200 chromosomes)	18	6	17	5	2	23	1	3
Percent of mutations found	24%	8%	23%	7%	3%	31%	1%	4%

Figure 6. Locations and frequencies of FMF mutations in our cohort. The genomic DNA sequence has 10 exons, with untranslated regions (UTR) on exons 1 and 10. The total nucleotide lengths of exons 1 and 10 are given in parentheses, with the coding length not in parentheses. Intron lengths are not drawn to scale. We found 4 chromosomes with mutations at both the 148 and 726 positions, and counted these chromosomes in both columns.

General findings

Genotype analysis using the 8 mutations revealed missense errors on both chromosomes from 24 of the 86 patients meeting the clinical criteria, and we found only 1 mutation for 23 others. Thus, a total of 47 patients had at least 1 of the currently identified mutations. The remaining 39 cases of clinically suspected FMF, as well as the 14 patients who did not meet clinical criteria, did not have any demonstrable mutations.

We used these figures to calculate the sensitivity of the current genetic diagnosis in the United States population. The 24 patients with 2 known mutations provide 48 mutation-positive chromosomes, and there are 23 positive chromosomes from those with 1 identified mutation (24 + 24 + 23 = 71). If all of the latter group do indeed have FMF, there should be 2 mutated genes per patient, or 23 more than have been identified. Even if we exclude the patients who have clinical FMF but have no identifiable mutations, only 76% of the carrier chromosomes (71/94) have been identified with our 8 mutations. If we include the 39 clinically positive patients without any known mutations, adding 78 mutation-negative chromosomes to the denominator, then the 8 known mutations account for only 41% of the predicted total (71/172). If FMF includes only those individuals with mutations in MEFV (known or unknown), then some of the 39 patients meeting clinical criteria but without demonstrable mutations probably do not have true FMF. Hence, the proportion of chromosomes known to be mutated is between 41% and 76%-much lower than the 85% reported in the original sample of mostly North African Jews. At the very least, we have yet to find mutations in a substantial number (at least 24%) of carrier chromosomes after screening each sample for all 8 mutations (Table 3).

Patients Positive by Clinical Criteria (n = 86)	Chromosomes with Mutations Found	Chromosomes with No Mutations Found
Mutation + / + (n = 24)	24 + 24	0
Mutation +/ ? (n = 23)	23	23
Mutation ? / ? (n = 39)	0	39 + 39
Total chromosomes	71	101

*Percentage of carrier chromosomes identified to date: 41% < X < 76%.

Table 3. Categories of clinically positive patients*

We analyzed the 2 "positive" groups (the 47 with a mutation and the 86 meeting clinical criteria) in relation to each other and to their negative counterparts (53 without a known mutation and the 14 low-suspicion referrals) in a number of categories (Figure 7A). The percentages of our 47 patients with at least 1 identified mutation reporting

fever (96%), abdominal pain (91%), chest pain (57%), joint pain (45%), and erysiplelas-like erythema (13%) are consistent with the range of frequencies cited earlier in this paper. In addition, we found that about half of the mutation-positive patients recall a family history of similar symptoms, which is typical for patients with this recessively inherited disease. These values do not differ significantly with the broader cross-section of clinically positive patients, or with the genetically or clinically negative patients (except for those with ELE), suggesting that the presence or absence of these symptoms alone are not enough to rule in or rule out an FMF diagnosis.

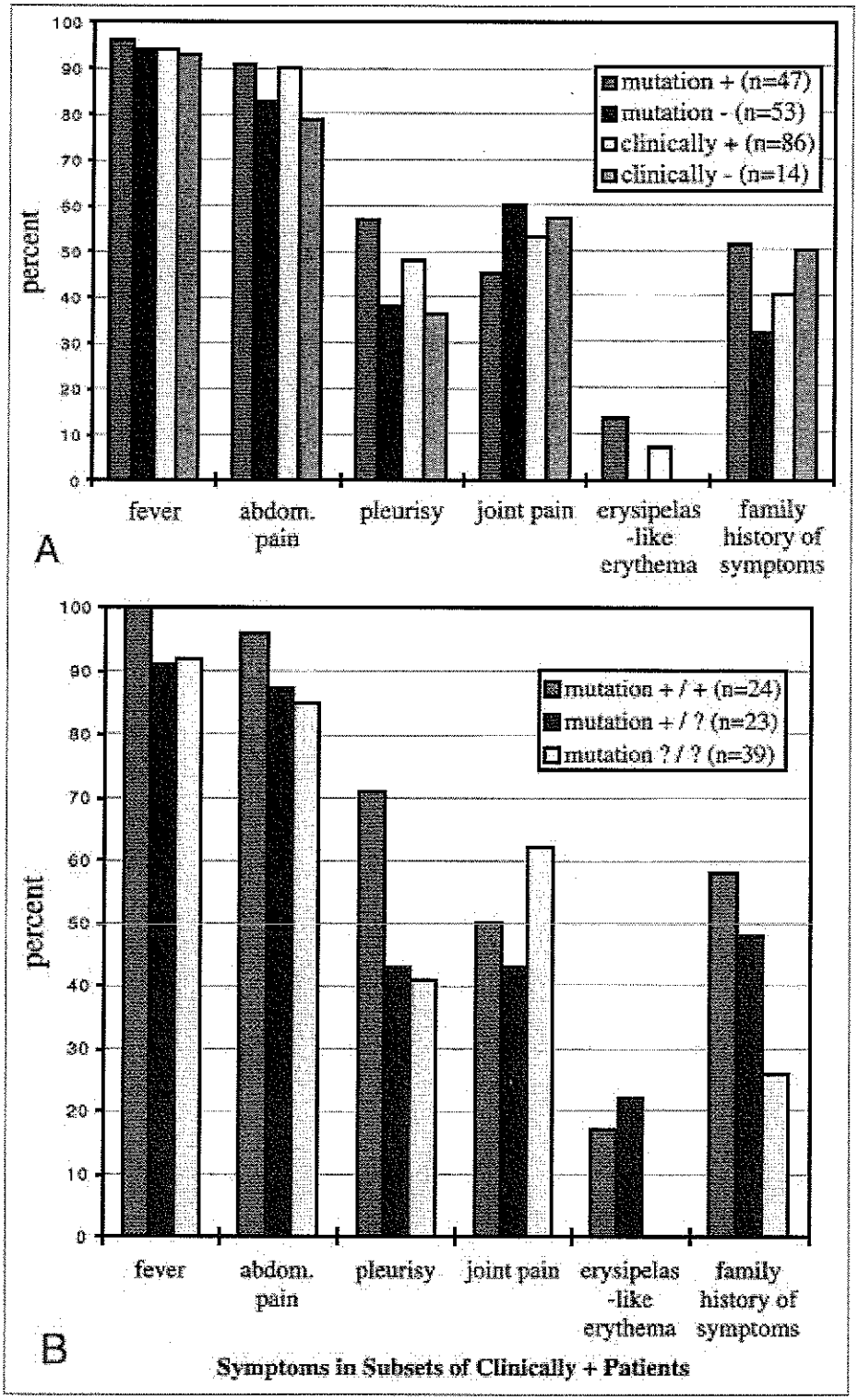


Figure 7. Frequency of cardinal FMF features in our cohort of 100 referrals. A) The 4 categories represent our patients in whom we found at least 1 mutation [47], those without any known mutations [53], those who met the clinical criteria [86], and those who did not meet the clinical criteria [14]. B) The 3 subsets in this graph comprise the clinically positive patients: those with both mutations found [24], those with only 1 identified mutation [23], and those without any identified mutations [39].

We also stratified the clinically positive patients into 3 groups to see if the single-mutation or mutation-negative patients have a different clinical picture from those with 2 known mutations (Figure 7B). There is a slightly higher frequency of pleurisy in the patients with 2 known mutations; we also found a higher frequency of joint pain, fewer positive family histories, and no ELE in the clinically positive patients without any known mutations. However, even with these exceptions it appears that all 3 subgroups of clinically positive patients have similar sets of symptoms.

The intervals between the acute flares vary widely from patient to patient, from 1 per week to 1 every 5-6 months; the mean and median values from the scattered values do not separate the groups. Gender also does not help us to discern 1 group from another, as about 40% of both our clinically positive and genetically positive patients are male; the small size of our patient pool is not enough to challenge the well-documented male:female ratio of 1.5-2.0:1.0. In addition, our severity scoring system, described in Table 1, does not single out any of the permuted populations; all 7 subgroups described in Figure 7 and Figure 8 have average severity scores between 6.0 and 6.6 (on a scale of 3 to 9), representing moderate to severe disease in this scoring system.

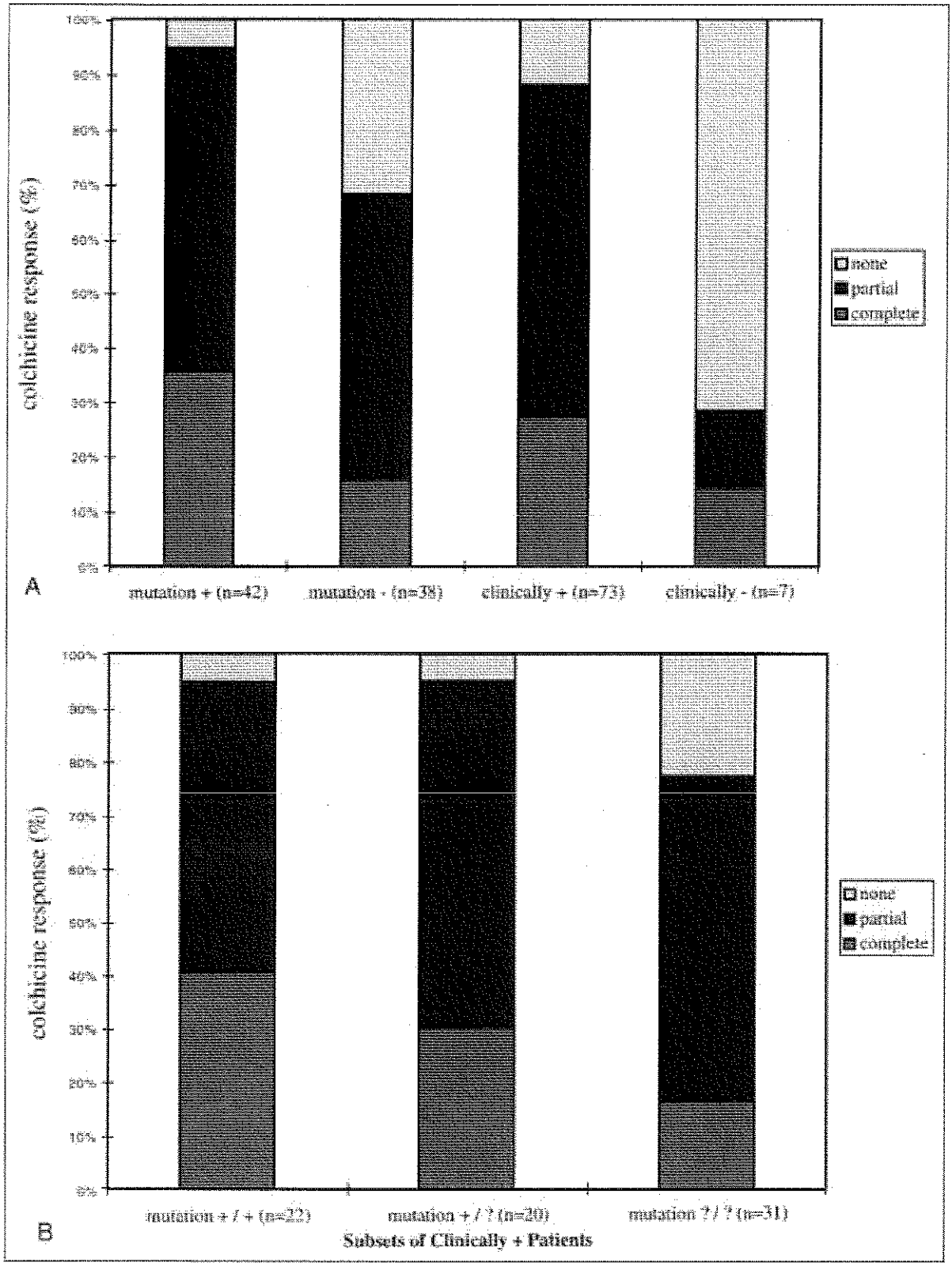


Figure 8. Response to colchicine therapy in our cohort of 100 referrals. The numbers of patients depicted in this graph are fewer than in Figure 7 because we excluded the patients not taking colchicine. A) The 4 categories represent our patients in whom we

found at least 1 mutation [42], those without any mutations [38], those who met the clinical criteria [73], and those who did not meet the clinical criteria [7]. B) The 3 subsets in this graph comprise the clinically positive patients: those with both mutations found [22], those with only 1 identified mutation [20], and those without any identified mutations [31].

Other aspects of the patients' histories, however, do provide information that help distinguish the different subgroups of patients from each other. The duration of attacks in the patients, not depicted in the graphs, do differ substantially between the mutation-positive and -negative groups (and similarly between the clinically positive and negative groups). Both the clinically positive and mutation-positive groups have mean and median durations of about 3 days, while the negative groups have mean and median values of 5-6 days per attack; both the positive and negative groups have a small number of patients whose attacks last more than 30 days, and they were excluded from mean calculations.

The response to colchicine in our cohort appears to be among the more reliable diagnostic factors in our study. More than 30% of the mutation-negative patients-and more than 70% of the clinically negative patients-who had attempted colchicine therapy are completely refractory; only 5% and 12% of the genetically and clinically positive patients, respectively, do not get any relief from symptoms when using colchicine (Figure 8A). Furthermore, when we divide the clinically positive patients into the 3 groups of 2, 1, or 0 identified mutations (Figure 8B), all but 2 of the colchicine-resistant patients do not have any identified mutations-suggesting they may not in fact have FMF. One of the colchicine-resistant patients who does have an MEFV mutation is BQ, a 37-year-old man of Armenian descent who has a 17-year history of nausea, vomiting, gas, abdominal pain, and occasional mild fevers lasting 3-5 days, as infrequently as 3 times per year. Although he has 1 copy of the V726A mutation, his history is equivocal; he may in fact be an FMF-carrier who suffers from an irritable bowel. The other exception is NI, a 40-year-old man of Arab descent homozygous for the M694I mutation, who reports classic attacks that have decreased in severity since his childhood; he has been noncompliant with colchicine in the past.

It is important to comment on the large percentage of patients in all of the subgroups who report only partial improvement from colchicine therapy. Although prophylactic colchicine is well established in preventing febrile attacks and amyloidosis in FMF, some patients do not take-or cannot tolerate-doses high enough to prevent their attacks completely. These patients cover the range of known MEFV mutations, and we are planning to offer them a pilot study of adjuvant therapy to be used simultaneously with colchicine.

Although some of the patients without demonstrable mutations probably do not have FMF, we are confident that we will ultimately identify MEFV mutations in several. The cases summarized below are prime examples of why currently unknown defects in MEFV may still be responsible for symptoms in the "mutation?/?" group included in Figure

7B and Figure 8B:

"KK, a 22-year-old non-Ashkenazi Jewish man without any known family history of FMF, was 13 years old when he began having monthly attacks of severe abdominal pain (without fever) lasting 12 hours at a time; colchicine has reduced the severity and duration of his attacks."

"ML is a 43-year-old Ashkenazi woman without a family history of FMF, who at the age of 11 years started experiencing 2-day episodes every 2-4 weeks of fever; a painful, rigid, and distended abdomen; and diffuse arthralgia; colchicine has decreased the severity, duration, and frequency of her attacks."

"BC, a 5-year-old Sephardi girl whose father and many cousins have clinically diagnosed FMF, suffers from 2-day episodes every few weeks of fever and abdominal pain. She has persistently had 3+ proteinuria-raising the possibility of amyloidosis. She has been unable to tolerate more than 0.5 mg of colchicine per day because of gastrointestinal side effects."

"OB, a 36-year-old man of Arab descent with a history of episodic fever, abdominal pain, and chest pain, has deteriorating renal function and positive amyloid biopsies as well as a family history of renal and liver failure. He often stops taking colchicine, especially during periods without acute flares lasting up to 2 years."

It is important to note that DNA from these patients has not been sequenced for all of MEFV, including the promoter region. Ultimately, we expect to find new mutations in many of these individuals. By screening other patients for such newly found mutations, we hope to improve upon the percentage of identified mutations.

By mutation

The availability of a mutationally characterized cohort of American FMF patients also afforded us the opportunity to examine genotype-phenotype relationships. Initial mutational studies have emphasized the high frequency of the M694V mutation in the North African Jewish population [50,66], a group that tends to have more severe disease and a higher risk of amyloidosis [107]. Moreover, the Iraqi Jewish and Arab Druze populations, which tend to have less severe FMF [106,123,150], have a higher frequency of the V726A mutation [66,105]. These observations have led to the hypothesis that M694V may cause a more severe form of FMF [35,66,105], with V726A being a milder mutation or possibly even protective [66]. On the other hand, the observation that monozygotic twins are sometimes discordant for specific symptoms and for severity of attacks [133] emphasizes that there are also nongenetic determinants of the overall

phenotype. Moreover, we and others have observed patients from the same family with different symptom clusters.

In general, our genotype-phenotype analysis does not demonstrate significant mutation-specific differences in the following categories: age of onset, frequency or duration of attacks, the relative prevalence of certain symptoms, or the response to colchicine. In regard to the characteristic ELE, our cohort includes only 6 patients with this symptom, but 4 of the 8 known mutations are represented. We do have isolated cases of some of the rarer manifestations of FMF, including aseptic meningitis and scrotal pain, but such findings are not common enough in our cohort to associate them with specific mutations.

Our 2 patients with the K695R mutation both have relatively mild disease. ON, a 66-year-old Ashkenazi man, experienced typical attacks of fever and abdominal and chest pain from his late 20s to early 30s before going into spontaneous remission; his symptoms returned 30 years later, after coronary artery bypass surgery. KL, an 11 year-old Caucasian girl, has had attacks from infancy that last only a few hours at a time. Perhaps this mutation results in a more stable pyrin product that responds relatively well to moderate stresses or environmental triggers.

All 4 of our patients with a copy of the V726A/E148Q complex allele have a similar constellation of symptoms, which includes fever, abdominal pain, and chest pain, and none of them has any joint pain or amyloidosis. However, the courses of their disease do not appear to be more or less severe than those of persons with single-mutation chromosomes in terms of the frequency, duration, or severity of attacks.

Among our mutation-positive patients, we have 15 who are M694V heterozygotes, but only 1 who is homozygous for this mutation. We did not find that patients with 1 copy of this mutation have more frequent or severe attacks. Our sole patient who is homozygous for M694V, DI, does suffer from a severe, destructive arthritis [95] with mild fevers and occasional ELE; but her symptoms do not include high fevers, abdominal pain, or chest pain. Recent data from groups in Israel and France indicate that M694V homozygotes generally do have more severe disease and a higher likelihood of developing amyloidosis ([35,105]; E. Pras, personal communication).

Nevertheless, patients with other genotypes may also develop amyloidosis. Only 2 of our patients have proven amyloidosis, neither of whom is homozygous for M694V: 1 is a M694V/E148Q heterozygote, and we have not identified either of the mutations in the other. The fact that our pool includes only 2 cases of amyloidosis credits the widespread prophylactic use of colchicine, but clouds any conclusions we might have made from mutation-associated amyloidosis risk. A 1998 letter in The New England Journal of Medicine provides additional evidence that patients with mutations other than M694V

are at risk for amyloidosis; the authors report 4 Turkish children with amyloidosis who are heterozygous for V726A [160]. The available data indicate that the risk of amyloidosis is not confined to FMF patients with the M694V mutation, nor prevented by the V726A mutation, but much larger samples, possibly stratified ethnically or geographically, will be required to quantitate this risk for specific MEFV genotypes.

By ethnicity

Our cohort includes a diverse spectrum of ethnic backgrounds (Figure 9). Not surprisingly, 26% of mutation-positive patients are of Armenian ancestry, and 17% are Arab. However, it is noteworthy that 19% of our mutation-positive patients are of Italian ancestry, and 21% are Ashkenazi Jewish-while only 8% are non-Ashkenazi Jewish, and 6% are of Turkish descent. We also have found MEFV mutations in patients of non-Jewish Assyrian, Cuban, and northern European heritage.

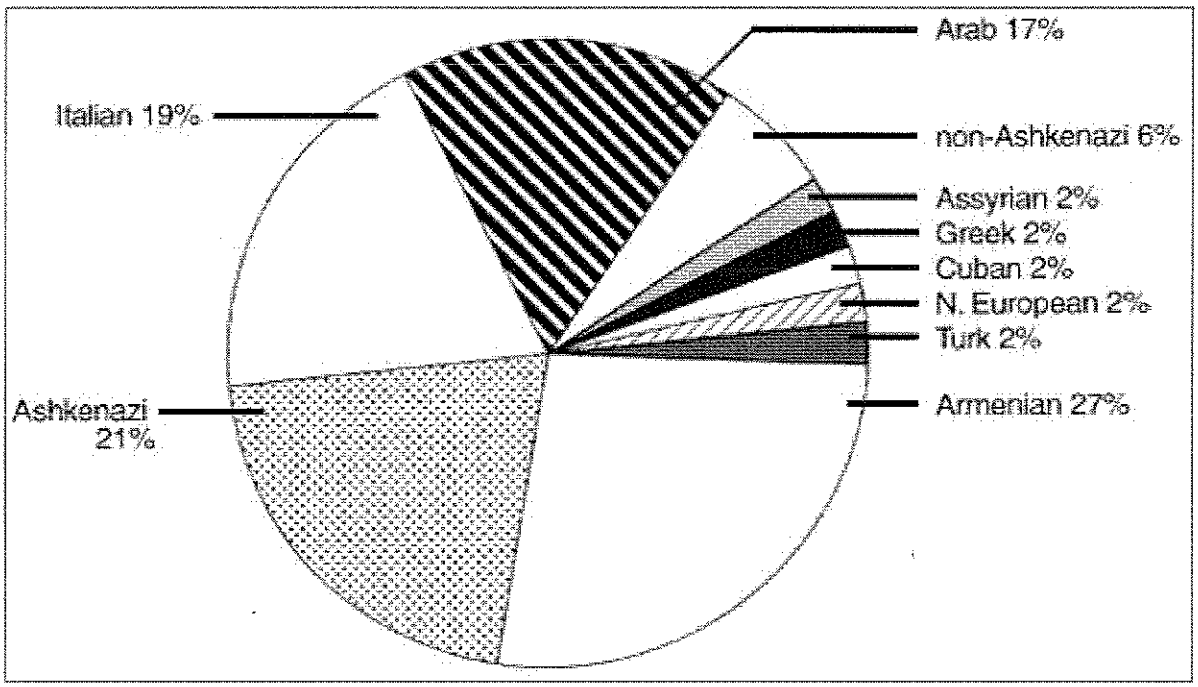


Figure 9. Ethnic breakdown of our referrals with at least 1 identified mutation. "Non-Ashkenazi" denotes non-Ashkenazi Jewish.

None of our ethnic subpopulations has a range of symptoms that clearly distinguishes 1 from another. All 4 Turkish patients (3 with mutations, 1 with a high clinical suspicion) report significant joint involvement in their disease; however, we have too few patients in this subpopulation to report a trend. Furthermore, we do not find that the different ethnic groups are unique in terms of their mutational repertoire, or that the mutations themselves are associated with certain ethnic groups. As shown in Figure 10, each of the more frequent ethnic groups in our cohort has a wide distribution of mutations. Three of the less common mutations in our analysis appear in only 1 ethnic group (M694I in 5 Arabs, A744S in 1 Arab, and the V726A/E148Q complex allele in

4 Ashkenazi Jews), but our sample size is too small to conclude that such mutations are specific to these ethnic groups.

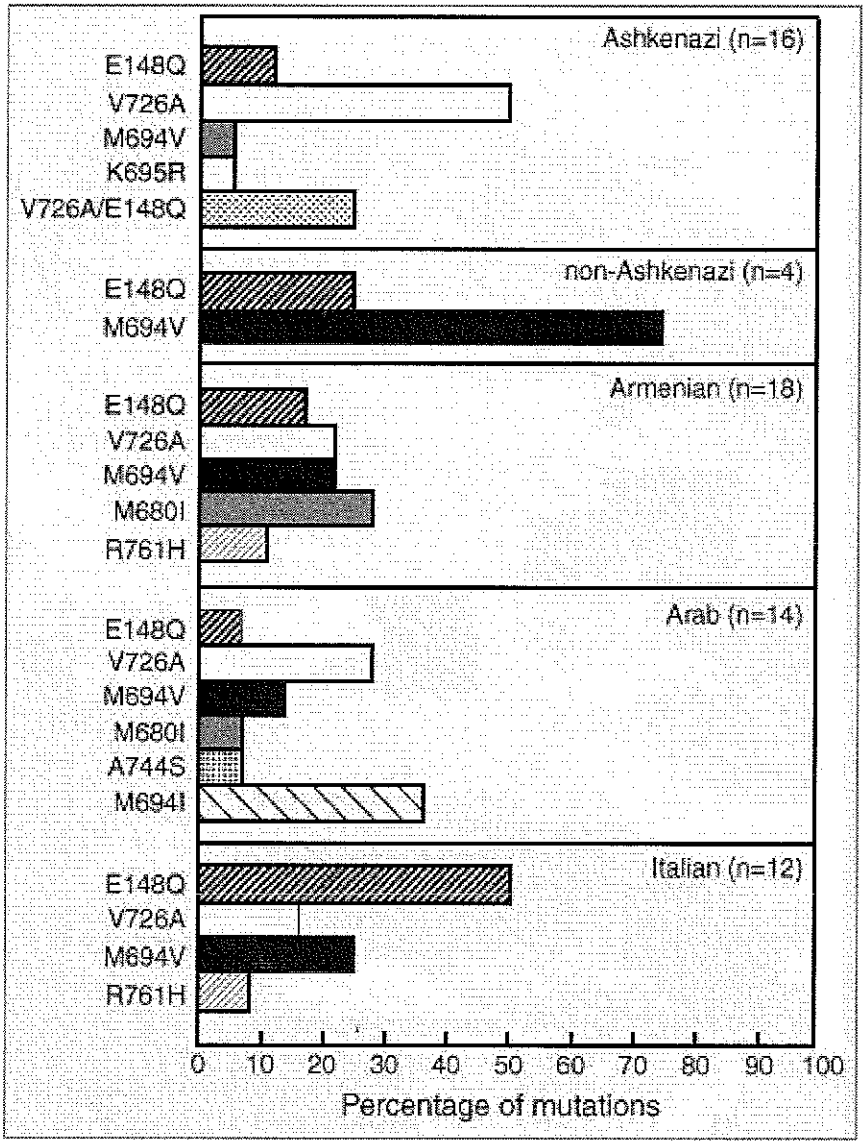


Figure 10. Frequencies of specific mutations within ethnic subgroups. We included the ethnic groups with the highest number of total mutation-positive chromosomes; the numbers of chromosomes with identifiable mutations are provided next to the names of each ethnicity.

While the literature only includes sporadic reports of suspected FMF in Italians [102,116], we have identified a relatively high prevalence of Italian patients with FMF mutations. Most of our patients presented with attacks typical of the ones seen in the previously described FMF-prone ethnic groups. In addition, we have found 4 of our 8 known mutations in this subset; no ethnicity tests positive for more than 6.

The fact that our cohort includes significantly more Ashkenazi than non-Ashkenazi

Jewish patients should not be surprising, considering the demographics in this country. While Israeli studies indicate that the FMF carrier frequency is higher in non-Ashkenazi than in Ashkenazi Jews, the latter population is far more numerous in the United States, and here we only include our American referrals.

(Figure 11) illustrates that a high percentage of our Jewish, Armenian, Arab, and Italian referrals meet clinical criteria for FMF and have at least 1 detectable mutation in MEFV. The 25 patients of northern European or Irish ancestry contrast sharply, with discernibly lower percentages meeting clinical criteria, and only 1 mutation-positive patient between the 2 groups. Two-thirds of the Irish patients do fulfill clinical criteria for FMF: DC is a 23-year-old woman who has had 3- to 4-day episodes of high fever and chills, diffuse abdominal pain, pleurisy, and ankle pain since she was 2 years old. These episodes occurred every 2-4 weeks until she began colchicine therapy, which decreased the occurrence to every 3 months; her father and brother also have similar symptoms. The paucity of mutations in 2 non-Mediterranean populations could simply indicate that FMF-like symptoms in these ethnic groups are caused by MEFV mutations not yet identified. However, in light of the fact that a clinically distinct periodic fever syndrome, familial Hibernian fever (FHF; OMIM 142680), has been identified in the Irish population, and that the causative gene appears to map to a different chromosome (see below), it is tempting to speculate that at least some of the Irish cases (and possibly the other northern European ones as well) are attributable to mutations in another gene.

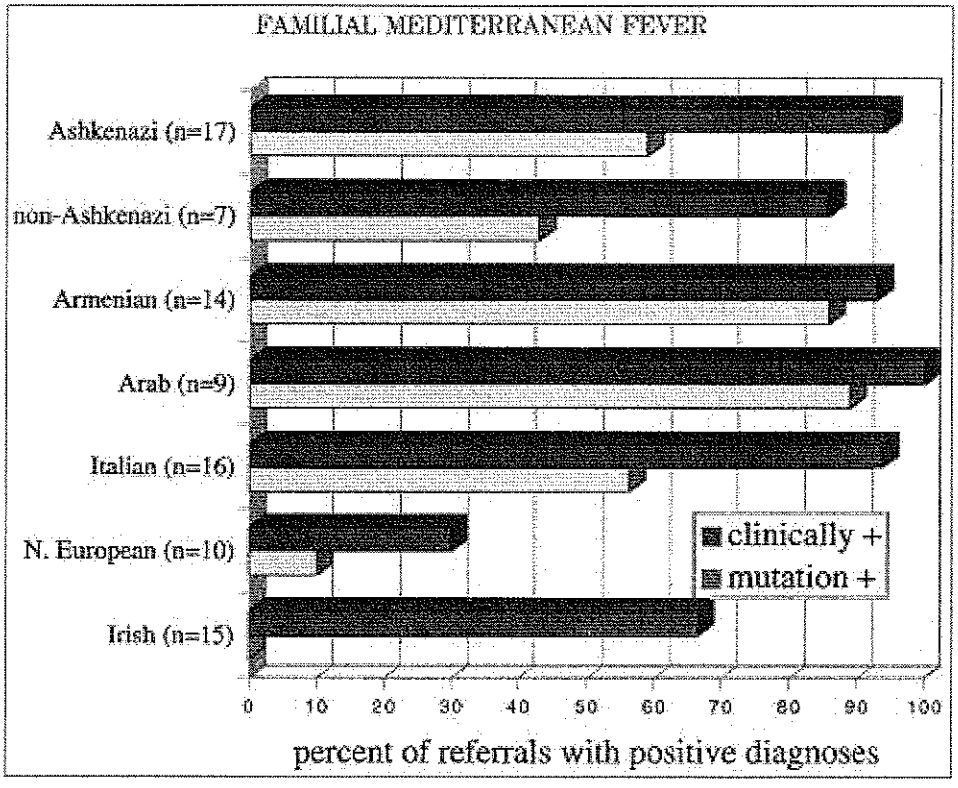


Figure 11. Percentage of clinically positive and mutation-positive referrals within ethnic subgroups. We included the ethnic groups with the highest number of referrals in our

cohort; the number of referrals is provided next to the name of each ethnicity.

Atypical presentations that widen the scope of disease

We also report atypical presentations in some of the patients with mutations that now broaden the scope of what should be suspected as possible FMF. First, our data indicate that, unlike the Israeli experience, joint involvement in American FMF patients is just as likely to present as diffuse arthralgia as it is monarticular arthritis. About half of the mutation-positive patients with joint involvement report diffuse arthralgia, including pain in the smaller joints in the hands, but no erythema or swelling. At the other end of the spectrum, but distinctly unusual, is the aforementioned DI, a 16-year-old girl of Assyrian and Armenian descent homozygous for M694V, who has a migrating acute monarthritis that has led to widespread joint destruction. Her episodes also include mild fevers, and the arthritis with massive swelling persists each time until high doses of NSAIDs or methotrexate control the disease; in the last 11 years, she has developed severe erosion of her hip joints and significant radiographic changes in her knees, ankles, wrists, and interphalangeal joints [95].

Although most patients are virtually asymptomatic between attacks, we have identified some patients who reported a baseline fever and abdominal discomfort (in addition to acute inflammatory flares lasting a few days) until they started taking colchicine. MA, a 19-year-old Italian-American female, had been a healthy, athletic teenager until she began having a daily fever of up to 101 [degree sign]F and abdominal discomfort for more than 2 years, punctuated by 3-day episodes of higher fever and piercing right lower quadrant pain. Only when these acute and chronic symptoms persisted after an appendectomy and an ovarian cystectomy did physicians entertain an FMF diagnosis; her symptoms have diminished with prophylactic colchicine. We have also received referrals for persistent fever and serositis lasting for months at a time in patients with ethnic backgrounds classic for FMF who do not test positive for any of the known missense mutations; such patients may have more severe loss of function mutations.

While patients typically present in childhood, and most have had their first attack by the age of 20 years, a handful of our patients experienced their initial episode well into their adult life. DN, a 63-year-old Ashkenazi woman who harbors an E148Q mutation, was 50 years old when she first experienced the 1- to 2-day episodes of high fever and arthralgia of the hand, ankle, and spine; she was asymptomatic between the episodes. Over the last 13 years, these episodes have progressed from once every 3 months to once per week; we have only recently identified her single known mutation, and will follow her response to colchicine. ME is another Ashkenazi woman with an E148Q mutation who had her first attack at age 50 years; her 2- to 3-day episodes of fever and abdominal pain occurred every 3-6 months until colchicine gave her complete relief. In addition, 1 patient with classic symptoms and a V726A mutation had her first attack at 38 years of age, and a V726A/M694V heterozygote woman did not present until 32 years of age. Perhaps these cases of late onset represent a "non-penetrant" female segment of the FMF population, in which the symptoms only manifest with the

withdrawal of the ameliorating effect of estrogens. This hypothesis might predict an equalization of the gender ratio for FMF with age.

FMF-like Diseases

In our present cohort of 100 referrals with suspected FMF, we have a total of 39 individuals who meet the clinical criteria for FMF, but in whom we have not found MEFV mutations. While we are confident that some of these individuals will ultimately be found to have mutations, at this point it is reasonable to consider the possibility that some will not. What other conditions might sometimes meet FMF criteria? We have already suggested that some of our Irish patients might have mutations in the Hibernian fever gene, and there are other hereditary periodic fever syndromes in the differential diagnosis; 1 study even reported genetic heterogeneity within the Turkish population, identifying 2 families that were unlinked to the region on chromosome 16 that contains MEFV [2].

FMF shares many clinical features with the recessively inherited hyperimmunoglobulinemia D and periodic fever syndrome (HIDS; OMIM 260920) [38,152]. These patients present with week-long fevers, accompanied by abdominal pain, lymphadenopathy, skin eruptions, and/or a symmetrical oligoarticular arthritis. White counts and acute phase reactants are elevated during attacks. An HIDS patient presenting primarily with abdominal or arthritic attacks could satisfy the clinical criteria for FMF. Patients with HIDS by definition have elevations in the serum IgD level (>100 IU/mL, or >14 mg/dL), but 13% of FMF patients also have modest increases in IgD levels, and thus the serologic test is not always helpful [82]. There are clinical differences between FMF and HIDS, such as the lymphadenopathy, skin eruption, and symmetry of arthritis in HIDS, and the occurrence of monarthritis, peritonitis, and pleurisy in FMF. Moreover, HIDS patients do not respond to colchicine. Families with HIDS do not show linkage to chromosome 16p [82], providing genetic evidence that the 2 disorders are distinct. Although we have obtained IgD levels on many of our patients without demonstrable MEFV mutations, we have not yet diagnosed anyone at the NIH with HIDS. Aside from our present series, we have encountered 1 patient with suspected HIDS; but her IgD levels were only 2- to 3-fold the upper limit of normal, and she is a cigarette smoker, a factor known to cause elevations in IgD [10].

One patient from our cohort and 2 of his siblings appear to manifest a recessively inherited periodic fever syndrome apparently distinct from both FMF and HIDS. TI is a 13-year-old Mennonite boy who has experienced monthly, 5- to 7-day episodes of fevers greater than 105 [degree sign]F, severe abdominal and chest pain, and swollen elbows with effusions. His leukocyte count and erythrocyte sedimentation rate are elevated during attacks, and there is a definite autosomal recessive pattern of inheritance in the family. Features of his illness that are atypical for FMF include the duration of his attacks, his persistent cervical lymphadenopathy, his lack of response to colchicine, and his ethnic background. To date we have found no MEFV mutations, and his IgD levels are

normal.

Among the dominantly inherited periodic fever syndromes that might be mistaken for FMF, FHF is the best characterized. To entertain this explanation in cases without a family history, one would need to invoke the possibilities of reduced penetrance or de novo mutation. Perhaps in part because "Hibernian" means "Irish," this disorder has not been reported in any other ethnic groups. The true incidence of FHF may also have been underestimated because of the absence of amyloidosis in the original case report [158], although amyloidosis has subsequently been noted in 1 of the members of this family [92]. Episodic fever, abdominal pain, and localized myalgia are common, with attacks often lasting longer than in FMF. Other distinguishing features include episodic erythematous patches, conjunctivitis, unilateral periorbital edema, and inguinal hernias in males [72,92,98,158]. The susceptibility locus has now been mapped to chromosome 12p13 [93].

Recently, a locus causing a dominantly inherited periodic fever syndrome in a large Australian family of Scottish ancestry was also mapped to chromosome 12p13, and named "Familial Periodic Fever" (FPF) [97]. There are also isolated reports in the literature of dominantly inherited FMF-like syndromes, for which a susceptibility locus has not been mapped. These include a 2-generation Finnish family without amyloidosis [71], a 2-generation Austrian family without amyloidosis [87], a 3-generation American family of non-Jewish German ancestry with amyloidosis [54], and a 2-generation non-Jewish Swedish family with widespread amyloidosis in 1 of the 4 affected members [23].

Our group has seen only 1 patient with known FHF, and this occurred before MEFV was cloned and we embarked on our series of 100 patients. DD is a 27-year-old Irish woman with a 14-year history of 3- to 5-week episodes of fevers with fatigue; blurred vision and eye pain; oral ulcers; an erythematous, tender rash; lymphadenopathy; arthralgia; myalgia; and occasional abdominal pain. She reports symptom-free intervals of 1-2 months. Although her episodes do not respond to colchicine, she has found that steroids ameliorate her attacks. When DD first presented to the NIH Clinical Center, she was 2 weeks postpartum, and was in the midst of an attack after a prolonged remission during her pregnancy. On physical exam, she had bilateral conjunctival injection and tender shoulders; her leukocyte count was 29,000/mm^3 with 94% neutrophils, and her acute phase reactants were elevated.

As previously noted, the disease in some of our Irish and other northern European referrals may be attributable to FHF or FPF. In the case of SU, a 6-year-old patient of northern European descent who did not meet the clinical criteria for FMF, family linkage studies are consistent with a dominant susceptibility locus in the FHF/FPF region of chromosome 12p, but not the FMF region of chromosome 16. SU's FMF-like symptoms include fevers up to 105 [degree sign]F, sharp abdominal pains, and diffuse arthralgia; she has shown some improvement with colchicine. Her father recently died of

complications of amyloidosis, and her aunt has the nephrotic syndrome with amyloid on renal biopsy. KD is a 40-year-old Puerto Rican man who meets clinical criteria for FMF, but for whom we do not have linkage data; he has suffered each month since birth from week-long attacks of 106 [degree sign]F fevers, abdominal pain, and diffuse arthralgia. He recently underwent a liver transplant for hepatic amyloidosis (documented to be AA amyloid), but does not have proteinuria. Neither he nor his affected daughter have benefited from colchicine, but steroids have helped to some extent.

At present it is not clear what percentage of our 39 clinically positive, mutation-negative patients will ultimately be found to have MEFV mutations, or other hereditary periodic fever syndromes. When the genes causing HIDS, FHF, and some of the other periodic fever syndromes are eventually cloned, efforts may focus on determining if these diseases are caused by mutations in proteins that have homology to pyrin or act in the same biochemical pathway. Such findings might reveal novel information about the general processes involved in both inherited and acquired forms of acute inflammation.

The Current State of FMF Diagnosis

The present study demonstrates an evolving but incomplete role for genetic testing in the diagnosis of FMF. Inherent in this discussion is the question of whether, when we speak of FMF, we are referring only to those episodic inflammatory conditions caused by mutations in MEFV, or whether we are referring to any recessively inherited disorder characterized by episodic fever and serosal inflammation. In the former case, once the MEFV mutational catalog is more complete, genetic criteria would ultimately define FMF, and clinical criteria might be applied to identify related conditions. In the latter case, again with the proviso of a thorough knowledge of the mutational repertoire, clinical criteria would define FMF, and genetic analysis would identify the subtype, much as in the hyperlipidemias (perhaps disease attributable to MEFV mutations would be called subtype I). The choice is somewhat arbitrary, and will ultimately be a matter of convention among physicians treating FMF. Approaching the issue from a geneticist's view, and bearing in mind the geographic clustering of both what has traditionally been called FMF and the MEFV mutations we are now finding, we would favor restricting the diagnosis of FMF to those patients who ultimately will be proven to have mutations in MEFV.

Taking our narrower definition, the present series indicates that screening for the 8 currently known mutations is not sufficiently sensitive to identify many cases of FMF, at least when applied to the American referral population. At best, our sensitivity is 76% for detecting chromosomes bearing mutations (based on the assumption that the 47 individuals with demonstrable mutations are the only ones with FMF). Assuming that this optimistic Figure istrue, the probability that a person with 2 MEFV mutations is negative for all 8 mutations on both chromosomes would be (1-0.76)(1-0.76) = (0.24)(0.24) = 0.06; the probability that we would find both mutations would be (0.76)(0.76) = 0.58. In

the worst-case scenario that our sensitivity to detect MEFV mutations on a given chromosome is 41%, substituting 0.41 for 0.76 in the above calculations, roughly one-third (0.35) of FMF patients would test completely negative for all 8 mutations, and we would find both mutations in less than one-fifth (0.17). If we require mutations on both chromosomes to make the diagnosis, neither set of assumptions results in a satisfactory sensitivity. Under optimistic assumptions, and requiring only mutation, the sensitivity would be satisfactory, but, particularly in populations with high carrier frequencies, specificity would likely become an issue [65].

In light of these data, we favor a diagnostic algorithm that makes use of both clinical data and MEFV mutational analysis. In our series, all of the patients who are mutation-positive meet clinical criteria for FMF, thus underscoring the importance of clinical judgment in making the diagnosis. However, particularly among individuals of atypical ethnic backgrounds, the clinical criteria are likely to identify some individuals who have hereditary periodic fever syndromes that will ultimately be genetically distinguishable from FMF.

At present, whether or not molecular diagnostics are readily available, a therapeutic trial of colchicine would be advisable for all patients meeting clinical criteria. Our data indicate a much higher response rate in mutation-positive individuals, and a positive response may be of tremendous benefit to the patient, regardless of the specific genetic diagnosis.

Even if molecular diagnostics are available, however, we do not advocate their use in all patients clinically suspected of having FMF. We have no data that would cast doubt on the diagnosis in a patient of typical ethnic background and classic attacks, who is seen by a physician experienced with FMF. However, particularly in Western countries, where FMF is relatively uncommon, it is reasonable to expect that mutational analysis will become the norm. By definition, we would consider anyone with 2 copies of MEFV mutations to have FMF (bearing in mind the need to demonstrate that E148Q and V726A are on opposite chromosomes by family studies, when these mutations are found in the same individual). To date we have not encountered asymptomatic individuals with 2 mutations, though such cases must exist, at least in the presymptomatic state. There is currently no consensus regarding colchicine treatment in such individuals, and such decisions are likely to be strongly influenced by regional data on amyloidosis risk.

The situation is more complex for individuals with 1 MEFV mutation. If the molecular diagnostic test is performed on the basis of a strong clinical history, then finding even 1 mutation increases the confidence in the diagnosis, and raises the expectation that eventually the second mutation will be found. If the test is performed because the patient has an ethnic background with a high carrier frequency, yet the clinical history is only questionable for FMF, 1 identified mutation is unlikely to resolve the diagnosis. Although it has been suggested that 1 copy of certain mutations might be

sufficient to confer disease [65], there are currently no data to support this view. As molecular testing is more broadly applied in populations with a high FMF carrier frequency, but among asymptomatic individuals, the value of finding a single mutation will be substantially diminished. For example, if the carrier frequency in the Armenian community is taken as 14% (1:7), then the disease prevalence is roughly 30-fold lower (0.5%), and the number of patients with false-positive tests (that is, persons with 1 mutation who are carriers) will far outnumber the true-positive patients in such high-risk populations. Among individuals who test negative for all 8 mutations (we estimate that this amounts to 6%-35% of FMF patients in the United States), clinical judgment remains the only guide.

Future Research

The most pressing issues in the study of FMF revolve around the question of how pyrin controls inflammation on the molecular level, and how the mutations permit episodes of the fever syndrome. Development of antibodies to pyrin will soon facilitate the study of its subcellular localization and tissue expression; immunoprecipitation with these antibodies, and the use of the yeast 2-hybrid system, should shed light on its interaction with other molecules and help to explain the pathophysiology. In addition, we hope to solve the crystal structure of pyrin in order to visualize the effects of specific mutations on protein conformation; we are currently producing large quantities of pyrin in a baculovirus system. We are also investigating the kinetics of MEFV expression during neutrophil differentiation: by using hematopoietic stem cells from bone marrow to determine the stage at which MEFV is turned on, we may be able to begin to understand its role in granulocytes.

Mouse knock-out models may shed light on what a severe loss-of-function mutation would do. We also expect knock-in mice to help us study what triggers attacks and leads to amyloidosis, and possibly to help explain the heterozygote advantage of MEFV mutations. Other clues may arise from the study of the Chinese Shar-Pei dog, as many of these canines have a presentation similar to FMF patients, including amyloidosis [119,120]. The canine MEFV homolog is a logical candidate gene for Shar-Pei fever.

Another major avenue of ongoing research is the search for the remaining mutations on the FMF gene. Only when a more complete repertoire of the mutations has been identified, or technologic methods make it easier and faster to sequence the entire gene in any suspected patient, will the sensitivity of genetic testing be sufficient for general use. Furthermore, the continual identification of new mutations might better define the range of phenotypes found in FMF patients who carry specific mutations. Are there more chromosomes with more than 1 mutation in the FMF gene, in addition to the V726A/E148Q allele, and do they lead to different phenotypes? Are there carrier chromosomes encoding mutations more dramatic than the conservative missense mutations that have been identified thus far?

We also hope to glean information from our ongoing studies of gene expression in FMF patients with the different mutations. We are collecting RNA from these patients to look for both qualitative and quantitative differences in the expression of the FMF gene during their different clinical states (quiescent or during an active attack, on or off colchicine). In addition, we hope to determine if and how the different mutations affect the production of pyrin.

Finally, with some patients gaining either partial or no relief from colchicine, adjuvant or alternative therapies need to be developed to add to the sole therapy of more than 25 years. With the potential for gene therapy several years away, the only reported attempt at an alternative is the limited, controlled trial with interferon-alpha [151]; this drug, however, carries a number of side effects, and we are exploring pilot studies with less toxic drugs.

Summary

Regarded as the most common and best understood of the hereditary periodic fever syndromes, familial Mediterranean fever (FMF) is a recessively inherited disease of episodic fever with some combination of severe abdominal pain, pleurisy, arthritis, and a characteristic ankle rash. The flares typically last for up to 3 days at a time, and most patients are completely asymptomatic between attacks; if untreated with prophylactic colchicine, some patients later develop amyloidosis and renal failure. The recent cloning of the FMF gene on the short arm of chromosome 16p, and the subsequent finding that its tissue expression is limited to granulocytes, has helped to explain the dramatic accumulation of neutrophils at the symptomatic serosal sites; the wildtype gene likely acts as an upregulator of an anti-inflammatory molecule or as a downregulator of a pro-inflammatory molecule.

For nearly half a century, FMF was thought to cluster primarily in non-Ashkenazi Jews, Arabs, Armenians, and Turks, although the screening of the 8 known mutations in an American cohort has identified substantial numbers of people from the Ashkenazi Jewish and Italian populations in the United States who also have this disease. Nevertheless, the symptoms often go unrecognized and patients remain undiagnosed for years, not receiving the highly efficacious colchicine therapy; their histories often include multiple laparotomies, laparoscopies, and psychiatric evaluations.

The combinations of clinical manifestations among FMF patients are quite heterogeneous, but our American cohort did not establish any connections between individual mutations and specific clinical pictures-as is seen in other diseases like cystic fibrosis, in which distinct genotypes target certain organ systems. Specifically, the data from our American series are insufficient to evaluate the hypothesis that the

M694V/M694V genotype confers a more severe phenotype, or increases the risk of amyloidosis; but both our data and the recent literature [160] indicate that amyloidosis can occur in FMF patients with only 1 copy, or no copies, of the M694V mutation. It appears that specific MEFV mutations are probably not the sole determinants of phenotype, and that unknown environmental factors or modifying genes act as accomplices in this disease.

Although we hope the discovery of the FMF gene will allow the diagnosis of FMF to become genetically accurate, the reality is that both clinical and genetic tools must still be used together unless mutations are identified on both of a patient's chromosomes. Physicians should be careful not to rule out the diagnosis in patients of high-risk ethnic backgrounds just because of atypical clinical features, as our data indicate that MEFV mutations are sometimes demonstrable in such patients. At the same time, physicians cannot yet rely solely on a genetic diagnosis because we have not yet identified a sufficient spectrum of mutations, and it is not currently feasible to examine every patient's full DNA sequence for the entire gene; screening an ethnically consistent and clinically positive patient for the 8 known mutations frequently identifies a mutation on only 1 chromosome, and genetic analysis of other classic cases will often reveal none of the 8 mutations. Still, our data suggest that ethnic background is an important predictor of finding 1 of the presently known mutations, and the knowledge of ancestries atypical for FMF can suggest the diagnosis of other hereditary periodic fever syndromes.

As the list of FMF-associated MEFV mutations is expanded, and/or new sequencing technologies permit more rapid screening, the value and interpretation of genetic testing for FMF will become more straightforward. Moreover, as the pathophysiology of this disorder becomes less of a hypothesis and more of an understood entity, it is likely that treatment options will broaden beyond the use of daily prophylactic colchicine. The cloning of MEFV has opened the door to a new pathway regulating inflammation; this advance has already dramatically affected the diagnostic approach to FMF, and we hope that basic research on the pyrin pathway will shed light on inflammation in many other clinical settings.

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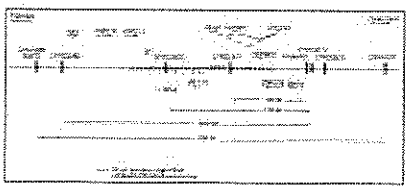

☐ Figure 2