Enigmas in familial Mediterranean fever (FMF)

E. Ben-Chetrit
M. Levy

Department of Medicine Hadassah University Hospital, Jerusalem, Israel.

This study was supported by a grant for FMF research from The Hadassah Medical Relief Association, UK.

Please address correspondence to: Eldad Ben-Chetrit MD, Department of Medicine A, Hadassah University Hospital, POB. 12000, Jerusalem, Israel. E-mail: eldad@hadassah.org.il

Received on November 23, 2000; accepted on November 30, 2001.

Key words: FMF, colchicine, MEFV.

Introduction

Familial Mediterranean fever (FMF), is an autosomal recessive disease which primarily affects populations surrounding the Mediterranean basin (1). It is characterized by recurrent attacks of fever and peritonitis, pleuritis, arthritis or erysipelas-like skin lesion. The most notorious aspect of FMF is amyloidosis which deposits in the kidneys leading to proteinuria and end stage renal failure. Despite its striking symptoms pattern FMF was first recognized as a nosological entity only in 1947. It took another 25 years until colchicine was found to be the drug of choice for FMF (2). This alkaloid aborts FMF attacks and the development of amyloidosis is inhibited. Recently, the gene related to FMF (MEFV) was cloned and some of the mutations associated with the disease have been isolated (3, 4). This step provided molecular diagnosis of the disease. Nonetheless, a number of puzzling questions and observations, old and new concerning this fascinating disease remain to be explained. These include atypical clinical presentations, mode of inheritance, mode and rate of response to colchicine, and issues related to genotype-phenotype correlation. We will attempt to discuss these issues in the light of our current knowledge of the pathogenesis and genetics of FMF (Table I).

Autosomal dominant transmission

The mode of inheritance of FMF has been the subject to some controversies. The observation that in the vast majority of affected families, the disease occurs in members of one generation supports a recessive mode of inheritance. Furthermore, in families where the disease occurred in two or more successive generations, high prevalence of consanguinity could explain this observation. Genetic epidemiological studies have also supported the recessive mode of transmission of FMF (1). However, in 1954, Reimann published a pedigree tracing the occurrence of FMF in 5 successive generations of an Armenian family living in Lebanon (5). He suggested a dominant mode of transmission and did not consider the possibility of high consanguinity in this family which seems very likely. However, in a later study by Yuval *et al.* the issue of autosomal dominant inheritance of FMF was raised once more (6). In 2 out of 77 families which they studied, FMF occurred in 4 consecutive generations while no consanguinity was evident. Recently, Booth *et al*. described 5 families in whom a dominant inheritance of FMF was suggested (7). In this study, dominant FMF was associated with the pyrin variant E148Q/ M694I encoded on a single allele or with heterozygosity for the simple deletion mutation encoded pyrin 694. Sequencing the complete coding region failed to detect any abnormality in the second MEFV allele in any of the families. By haplotype analysis it was shown that consanguinity was not high in these families.

Whenever the possibility of dominant inheritance is raised we have to be concerned about the exact diagnosis of the patients. Some of them may have a dominant periodic disease such as Hibernian fever. In fact in two reports from Finland and from the USA the authors described patients with autosomal dominant FMF-like syndrome with amyloidosis which were found to be Hibernian fever (8, 9). However, the clinical picture in the patients of Booth *et al.* was indistinguishable from that of other FMF patients. Moreover, in addition to the mutations associated with FMF, they responded to colchicine treatment and some of them even had amyloidosis. At present we remain with the question whether FMF is an autosomal recessive or autosomal dominant hereditary disease. The enigma of two modes of transmission which are present in the same disease remained to be explained.

While it is inherently likely that different mutations will impair the function of a particular protein to differing extent it seems that the methionine

Table I. Enigmas in FMF.

Autosomal dominant transmission
Non-familial cases
Phenotype II FMF
Genotype - Phenotype in-correlation
Defective gene in neutrophils and polyserositis
None - response to colchicine

residue at position 694 may have an important role in the physiology of pyrin. Patients homozygous for the M694V mutation experience more severe disease and are more prone to develop amyloidosis (10,11). Homozygosity for deletion of M694 have not been identified, suggesting the possibility that such complex of mutations is not compatible with life. A "severe" mutation such as methionine deletion at position 694 may lead to 50% of the complement pyrin activity and this amount of pyrin may be not enough to prevent symptomatic disease. The presence of two mutations one of which is in 694 position - on the same allele may have a similar effect on pyrin activity as methionine deletion on a single allele. The variable penetrance of autosomal dominant FMF, indicates that susceptibility to FMF differs from patient to patient just as the case in "typical" dual allele MEFV mutations. Although, it remains likely that in areas where FMF is prevalent, in most families in which FMF affects successive generations, inheritance is pseudodominance, in regions where the disease is rare, the possibility of dominant inheritance should be considered.

Non-familial cases

In some families FMF appears sporadically without any family history for the disease. These cases are more prevalent among ethnic groups where the disease is uncommon. Since FMF is a prime example of hereditary disease one must explain the enigma of non-familial occurrence of this disease. Several explanations could be offered. First it is possible that other familial members bear mutations which do not cause clinical manifestations of FMF such as the cases with individuals homozygous for the E148Q sequence variant (12). Second, it may present a new appearance of mutations in sporadic FMF cases. In fact, many of the mutations found in populations where FMF is rare, are not detected in Turks, Armenians, Jews or Arabs where the disease is more prevalent (13). Third, one always should consider the possibility of misdiagnosis of FMF instead of another known or yet unknown periodic disease (14).

FMF-Phenotype II

Currently, in most FMF patients the development of amyloidosis is prevented by colchicine treatment. Amyloidosis still occurs in those who are untreated or noncompliant symptomatic FMF patients, usually in adolescence or early adulthood. Nevertheless, in some cases FMF first presentation (even in childhood) can be proteinuria or nephrotic syndrome due to renal amyloidosis. Namely, in these patients there is no history of typical recurrent attacks of fever, peritonitis, pleuritis or arthritis. It has been suggested to call this form of presentation phenotype II FMF (15). In a recent study Melikoglu *et al*. investigated the prevalence of "phenotype II" by looking for proteinuria among the asymptomatic relatives of patients with FMF complicated by amyloidosis (16). As a control they chose asymptomatic relatives of patients with juvenile chronic arthritis (JCA) who also had amyloidosis. They found only 2 asymptomatic individuals with significant proteinuria among the 461 screened FMF relatives and one out of 269 screened individuals from the JCA relatives group. Rectal biopsy was negative for amyloidosis in all instances of proteinuria. They concluded that "phenotype II" - if at all - is an infrequent occurrence among relatives of FMF patients with amyloidosis.

The type of amyloid fiber which deposits in FMF is AA - the typical substance of secondary amyloidosis. These fibers may deposit in the kidneys of patients having chronic inflammatory diseases such as osteomyelitis, endocarditis, rheumatoid arthritis, *etc.* The traditional pathogenic explanation is that in these diseases the process of chronic inflammation leads to chronic stimulation of many acute phase reactants including the serum amyloid A protein (SAA) the precursor of AA (17). Following many years of active disease the SAA fibers change their physical properties and adopt the pleated sheet configuration thus leading to amyloid deposition. According to this mechanism it was suggested that in FMF the recurrent attacks result in an elevation of SAA and amyloidosis (1). Indeed, in a recent study it was shown that the plasma concentration of SAA frequently exceeded 1000 mg/L (normal < 3) during FMF attacks (18). These extremely high levels of SAA may lead to AA amyloidosis. Now how can we explain phenotype II FMF amyloidosis which appears in early childhood and is not preceded with a history of FMF attacks? Here we face another enigma.

Lachmann *et al*. measured the SAA levels at two-weeks intervals on 10 occasions in 165 Turkish individuals of whom 42 had FMF (18). They found that in asymptomatic FMF patients the SAA levels were significantly higher than those found in normal healthy volunteers. Furthermore, they found higher SAA levels even in healthy individuals (in the study group), heterozygous for the E148Q mutations. In another study Akar *et al.* found in 8 asymptomatic individuals who were either homozygous or compound heterozygous for MEFV mutations, high SAA levels (19). It is therefore suggested that FMF has an active sub-clinical phase and the frequency and severity of this asymptomatic inflammatory process presumably related to the risk of AA amyloidosis. Occasional short clinical attacks may add to that risk.

Other hypotheses have been offered. First, that the phenotype II is the result of a particular genotype. Several studies (10,11,20), but not all (21), have claimed that there is a significant correlation between homozygosity for M694V and amyloidosis (the main manifestation of phenotype II). Second, that the early development of amyloidosis is a parallel genetic process which is not related to the inflammatory component of FMF. This idea of "two genes two diseases" had already been proposed by Eliakim many years ago (1).

Genotype-phenotype correlation

Familial Mediterranean fever can be expressed in variable degrees of severity. There are patients who have several attacks per month whereas others experience only few if any, attacks per year. Furthermore, there may be differences in clinical manifestations between FMF patients and even within the same

patient at various times.

Years ago it was observed that different ethnic groups vary in the extent of severity of the disease and the rates of amyloidosis (22). Jews originating in North-Africa have more severe disease compared with those originating from Iraq. The isolation of the MEFV gene and identification of the mutations causing disease opened the way for the investigation of genotype-phenotype correlation. Indeed, it was found that most of the FMF patients of North African origin bear the M694V mutation whereas the Iraqi Jews have more A726V and other mutations (3, 4). These observation led some investigators to relate the severity of the disease and the relatively high occurrence of amyloidosis to the presence of the M694V mutation. On the other hand, the A726V mutation was considered as a sequence alteration causing a milder disease with "protective effect" against amyloidosis (3). Recent studies confirmed the association between severe disease and homozygosity to M694V while the E148Q mutation was found to be associated with less severe disease (10,11,20,23,24). Other investigators however, claimed that there was no strict correlation between the mutations and the clinical manifestations of FMF (21).

The ability to make a molecular diagnosis of FMF based on PCR technique has brought further dilemmas. First, patients were recognized with typical FMF clinical manifestations and yet with no known MEFV mutations. On the other hand, there are individuals who have a molecular diagnosis of FMF (homozygotes or compound heterozygotes) and yet they are not symptomatic at all (12).

These observations reflect three further issues about the lack of genotype phenotype correlation which need to be addressed.

In cases where similar genotypes result in different clinical manifestations it has to be assumed that other genetic or environmental factors play an important role. Support for the role of environmental factor/s can be found in the observation that Armenian FMF patients living in the United States develop significantly less amyloidosis, compared with Armenian FMF patients living in Armenia (25). Support for a genetic modifier can be found in a recent study by Touitou *et al.* who reported that the presence of the Major Histocompatibility Complex class I chain-related gene A (MICA) A9 in FMF patients homozygous for M694V mutation was associated with more severe disease whereas the presence of MICA A4 lead to less frequent attacks (26). This is the first study identifying a new genetic modifier which may explain partially the variation in clinical manifestations of FMF in patients bearing similar mutations.

To explain cases of patients with typical clinical manifestations for FMF and yet lacking the known mutations, there are a number of answers. First, that the diagnosis is not accurate and these patients have another periodic disease such as those recently identified (27-29). Second, these patients may bear yet unidentified mutations. Although 28 different mutations have been reported, most probably more are to come. Third, it is possible that there are additional genes causing FMF other than MEFV. The possibility of gene heterogeneity has already been raised by Akarsu *et al.* (30). We have also suggested this explanation in our study of the "Chuetas" - a small community in Mallorca - in whom we could not find a known mutation in more than 60% of the FMF patients (31).

In cases where an asymptomatic individual has a genetic diagnosis of FMF made incidentally through genetic screening, one should assume low penetrance owing to yet unknown genetic modifiers. In patients homozygous for the E148Q sequence alteration yet asymptomatic, we hypothesized that it is a polymorphism rather than a disease causing mutation (12). This mutation can cause a full blown disease when other concomitant genetic or environmental factor/s exist.

Defective gene in neutrophils and polyserositis

A synonym for familial Mediterranean fever is "Recurrent Polyserositis" (1). This name was coined by Rachmilewitz *et al.* to denote that the main target of the disease are serosal tissues such as the peritoneum, pleura, pericard and synovium. It is therefore likely that the genetic defect should be present in enzymes or cytokines found exclusively in these tissues. Further support for this possibility can be found in the studies of Matzner *et al.* who showed that the amount of C5a inhibitor was lower in peritoneum and synovia from FMF patients compared with normal individuals (32). These results led Matzner to suggest that the cause for FMF attack is the genetic lack of C5a inhibitor which allows enhanced neutrophil chemotaxis by the C5a component of the complement at the inflammatory site.

The isolation of the MEFV gene revealed that this gene is expressed almost exclusively in mature neutrophils and not in the serous tissues (3, 4). This finding was unexpected and opened new questions as to the involvement of the serous tissues. Namely, what is the relationship between the defective gene in the neutrophils and the serous membranes inflammation ? Furthermore, what causes the characteristic periodicity of this disease?

Several hypotheses may be offered to explain at least some of these questions. One hypothesis which may explain the relationship between the gene defect in neutrophils and the serositis is as follows: In every individual the serosal tissues are repeatedly exposed to various non-significant physical stimuli. These initiate a cascade of cytokine excretion which reaches the neutrophils in order to recruit them for the potential inflammatory process, i.e. serositis. In healthy individuals the signals received by the neutrophils do not enhance the migration of these cells due to their (direct or indirect) depression by the marenostrin/pyrin, the product of the MEFV gene. In FMF patients, where this protein or its function is lacking the signals cause an effective migration of neutrophils to the stimulated serous tissues leading to a full blown attack**.** Why do the attacks last 24 to 96 hours and why are they periodic ? We suggest that the stimuli initiating the disease are delivered in "quanti-

ty units" which lead to the excretion of distinct amounts of cytokines able to act for a period of time. Furthermore, it can be assumed that there are counter balancing systems - as in other physiological processes in the body - which play a role in the rhythmicity of the disease.

Non-response to colchicine

Since 1972 colchicine is the drug of choice for FMF as it prevents the attacks of FMF and inhibits the development of amyloidosis in the majority of those treated properly. About 65% of FMF patients respond with complete remission and 20-30% experience significant improvement with a reduction in the number and severity of the attacks (15). A partial explanation for non-response can be found in the study by Peters who showed that in many cases of non-responders a thorough investigation reveals that the patients are actually non-compliant (33).

Colchicine exerts its effect in several ways. It inhibits the migration of neutrophils to the inflammatory area by disturbing microtubule motility (34). It has also been reported that colchicine can decrease the expression of endothelial adhesion molecules affecting the transfer of neutrophils through the endothelium (35). Another proposal for colchicine action is that it can up-regulate the MEFV gene in neutrophils (15, 36). The remaining question is why 5-10% of FMF patients who are true non-responders do not respond to colchicine treatment ?

Colchicine is absorbed in the small intestines (37). In a pharmacokinetic study where colchicine serum levels were measured following oral administration of the drug, two populations were evident: one where plasma concentrations peak appeared half an hour after ingestion, whereas in the second only two hours after the drug ingestion. This variation, in colchicine absorption may be due either to a primary change in intestinal absorption, or due to other factors such as food components or medications which may interfere with colchicine absorption (34). Enhanced metabolism may lower plasma levels despite taking colchicine regularly at recommended dose. The main metabolism of colchicine takes place in the liver by the isoform 3A4 of the cytochrome 450 system. It may be possible that the activity of this enzyme is inhibited or enhanced by other drugs or food components. In addition, variation in the delivery of colchicine to its effective targets may also exist. Whether these putative pharmacokinetic changes explain non-response of FMF patients to colchicine remain to be studied.

FMF continues to puzzle and fascinate investigators. Recent progress has given some answers as to its etiology. However, many questions regarding pathogenesis and the mechanism of action of colchicine remain unanswered. Their elucidation will provide added value to our understanding of inflammation.

References

1. ELIAKIM M,LEVY M,EHRENFELD M: *Recurrent polyserositis (Familial Mediterranean Fever)*. Elsevier/North Holland Biomedical Press, Amsterdam, 1981.
2. GOLDFINGER SE: Colchicine for familial Mediterranean fever. *N Eng J Med* 1972; 287: 1302.
3. THE INTERNATIONAL FMF CONSORTIUM: Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell* 1997; 90: 797-807.
4. FRENCH FMF CONSORTIUM: A candidate gene for familial Mediterranean fever. *Nat Genet* 1997; 17: 25-31.
5. REIMAN HA, MODAIE J, SEMERDJIAN S, SAHYOUN PF: Periodic peritonitis - heredity and pathology, report of seventy-two cases. *J Am Med Assoc* 1954; 154: 1254-9.
6. YUVAL Y, HEMO-ZISSER M, ZEMER D, SOHAR E, PRAS M: Dominant inheritance in two families with famlial Mediterranean fever (FMF). *Am J Med Genet* 1995; 57:455-7.
7. BOOTH DR, GILLMORE JD, LACHMAN HJ, *et al*.: The genetic basis of autosomal dominant familial Mediterranean fever. *Q J Med* 2000; 93: 217-23.
8. MACHE CJ, GORIUP U, FISCHEL-GHODSIAN N, CHEN X, SCHWINGSHANDL J: Autosomal dominant familial Mediterranean fever-like syndrome. *Eur J Pediatr* 1996; 155: 87-90.
9. GERTZ MA,PETITT RM,PERRAULT J, KYLE RA: Autosomal dominant familail Mediterranean fever-like syndrome with amyloidosis. *Mayo Clin Proc* 1987; 62: 1095-100.
10. SHOHAT M, MAGAL N, SHOHAT T, CHEN X, MIMOUNI A, LOTAN R, *et al*.: Phenotype-genotype correlation in familial Mediterranean fever: evidence for an association between Met694Val and amyloidosis. *Eur J Hum Genet* 1999; 7: 287-92.
11. BEN-CHETRIT E, BACKENROTH R: Amyloidosis induced end stage renal disease in familial Mediterranean fever is highly associated with point mutations in the MEFV gene. *Ann Rheum Dis* 2000; 60: 146-9.
12. BEN-CHETRIT E, LERER I, MALAMUD E, DOMINGO C, ABELIOVICH D: The E148Q mutation in the MEFV gene: Is it a disease-causing mutation or a sequence variant ? *Hum Mutat* 2000; 15: 385-6.
13. SAMUELS J, AKSENTIJEVICH I TOROSYANY, CENTOLA M, SOOD R,KASTNER D: Familial Mediterranean fever in the millenium. *Medicine* 1998; 77: 268-97.
14. GRATEAU G, DRENTH JP, DELPECH M: Hereditary fevers. *Curr Opin Rheumatol* 1999; 11: 75-8.
15. BEN-CHETRIT E, LEVY M: Familial Mediterranean fever. *Lancet* 1998; 351: 659-64.
16. MELIKOGLU M, OZDOGAN H, KORMAZ C, KASAPÇOPUR O, ARISOY N, AKKUS S, *et al*.: A survey of phenotype II in FMF. *Ann Rhem Dis* 2000; 59: 910-3.
17. UHLAR CM, WHITE HEAD AS: Serum amyloid A,the major vertebrate acute-phase reactant. *Eur J Biochem* 1999; 265: 501-23.
18. LACHMANN HJ, BOOTH DR, BOOTH SE, BYBEE A, GALLIMORE JR, SOYTURK M, *et al*.:Serial monitoring of acute phase response in healthy individuals with wild-type pyrin, heterozygous carriers of the FMF trait and patients with FMF. *Clin Exp Rheumatol* 2000; 18: 279 (abstract).
19. AKAR S, BOOTH DR, BOOTH SE, BYBEE A, SENGUL B, YAVUZSEN TU, *et al*.: The significance of paired MEFV mutations in individuals without symptoms of familial Mediterranean fever. *Clin Exp Rheumatol* 2000; 18: 283 (abstr).
20. DEWALLE M, DOMINGO C, ROSENBAUM M, BEN-CHETRIT E, CATTAN D, BERNOT A, *et al*.: Phenotype genotype correlation in Jewish patients suffering from familial Mediterranean fever. *Eur J Hum Genet* 1998; 6:95-7.
21. AKAR N, MISIROGLU M, YALCINKAYA F, AKAR E,CAKAR N, TUMER N, *et al*.: MEFV mutations in Turkish patients suffering from Familial mediterranean fever. *Hum Mutat* (online) 2000; 15: 118-9.
22. LEVY M,EHRENFELD M,LEVO Y, FISCHEL R, ZLOTNICK A, ELIAKIM M. Circulating immune complexes in recurrent polyserositis (familial Mediterranean fever, periodic fever). *J Rheumatol* 1980; 7: 886-90.
23. LIVNEH A,LANGEVITZ P, SHINAR Y, ZAKS N, KASTNER DL, PRAS M, *et al*.: MEFV mutation analysis in patients suffering from amyloidosis of familial Mediterranean fever. *Amyloid* 1999; 6: 1-6.
24. CAZENEUVE C, SARKISIAN T, PECHEUX C, DERVICHIAN M, NEDELEC B, REINERT P, *et al*.: MEFV-Gene analysis in Armenian patients with familial Mediterranean fever:diagnostic value and unfavorable renal prognosis of the M694V homozygous genotype-genetic and therapeutic implications. *Am J Hum Genet* 1999; 65: 88-97.
25. SCHWABE AD, PETERS RS: Familial Mediterranean fever in Armenian: analysis of 100 cases. *Medicine* 1974; 53: 453-62.
26. TOUITOU I, PICOT MC, DOMINGO C *et al*.: The MICA region determines the first modifier locus in familial Mediterranean fever.

Arthritis Rheum 2001; 44: 163-9.
27. DRENTH JPH, CUISSET L, GRATEAU G, VASSEUR C, VAN DE VELDE-VISSER SD, DE JONG JGN, *et al.*: Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndrome. *Nat Genet* 1999; 22; 178-81.
28. HOUTEN SM, KUIS W, DURA M, DE KONING TJ, VAN ROYEN-KERKHOF A, ROMEIJN JG, *et al.*: Mutations in MVK,encoding mevalonate kinase, cause hyperimmunoglobulinemia D and periodic syndrome. *Nat Genet* 1999; 22: 175-7.
29. MCDERMOTT MF, AKSENTIJEVICH I,GALON J *et al.*: Germline mutations in the extracellular domains of the 55kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. *Cell* 1999; 97: 133-44.
30. AKARSU AN, SAATCI U, OZEN S, BAKKALOGLU A,BESBAS N, SARFARAZI M: Genetic linkage study of familial Mediterranean fever (FMF) to 16p13.3 and evidence for genetic heterogeneity in the Turkish population. *J Med Genet* 1997; 34: 573-8.
31. DOMINGO C, TOUITOU I, BAYOU A, OZEN S, NOTARNICOLA C, DEWELLE M, *et al.*: Familial Mediterranean fever in the "Chuetas" of Mallorca: a question of Jewish origin or genetic heterogeneity. *Eur J Hum Genet* 2000; 8: 242-6.
32. MATZNER Y, AYESH S, HOCHNER-CELNIKER D *et al.*: Proposed mechanism of the inflammatory attacks in familial Mediterranean fever. *Arch Intern Med* 1990; 150: 1289-91.
33. PETERSRS: Non-response to daily colchicine attack suppression in familial Mediterranean fever (FMF). The 15th International Conference on FMF (Jerusalem, 1997): 8 (abstract).
34. BEN-CHETRIT E,LEVY M: Colchicine update 1998. *Semin Arthritis Rheum* 1998; 28: 48-59.
35. CRONSTEIN BN, MOLAD Y, REIBMAN J, BALAKANE E, LEVIN RJ, WEISSMANN G: Colchicine alters the quantitative and qualitative display of selectins on endothelial cells and neutrophils. *J Clin Invest* 1995; 96:994-1004.
36. CENTOLA M, WOOD G, FRUCHT DM *et al.*: The gene for familial Mediterranean fever, MEFV, is expressed in early leukocyte development and is regulated in response to inflammatory mediators. *Blood* 2000; 95: 3223-31.
37. WALLACE SL, ERTEL NH: Plasma levels of colchicine after oral administration of a single dose. *Metabolism* 1973; 22: 749-53.