The genetic basis of autosomal dominant familial Mediterranean fever


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Summary

Familial Mediterranean fever (FMF) is classically an autosomal recessive periodic inflammatory disease occurring in Mediterranean and Middle Eastern populations. It is caused by mutations affecting both alleles of MEFV, a gene that encodes pyrin (marenostrin), an uncharacterized neutrophil protein. Occasional reports of autosomal dominant FMF have often been discounted, on the basis that asymptomatic FMF carriers are common in certain populations, and give rise to pseudo-dominant inheritance. We performed comprehensive MEFV genotyping in five families in whom FMF appeared to be inherited dominantly. Transmission proved to be pseudo-dominant in two cases, but true dominant inheritance of FMF with variable penetrance was supported by the genotyping results in the other three families. The disease in these cases was associated with heterozygosity for either pyrin ΔM694 alone or the compound pyrin variant E148Q/M694I, the latter occurring in two unrelated families. Complete MEFV sequencing failed to identify any coding region abnormality in the other allele in any of these cases, and, in the largest kindred, single-allele disease transmission was further supported by analysis of silent single nucleotide polymorphisms, which proved that affected individuals had at least three different complementary alleles. Studies of two further unrelated British patients with FMF associated with simple heterozygosity for pyrin ΔM694 were also consistent with autosomal dominant inheritance. The clinical features of dominantly inherited FMF were absolutely typical, including AA amyloidosis in a patient with pyrin ΔM694. These findings extend the spectrum of FMF, and suggest that the methionine residue at position 694 makes a crucial contribution to pyrin’s function, and that a 50% complement of normal pyrin activity does not prevent susceptibility to FMF.

Introduction

Familial Mediterranean fever (FMF) is an inherited inflammatory disease that is principally recognized in Jewish, Armenian, Turkish and Arab populations. The characteristic intermittent clinical episodes of fever, peritonitis, pleurisy, rashes and arthritis are variable in their pattern, frequency, intensity and age of onset, as is the proportion of FMF patients in different ethnic groups who develop AA amyloidosis. The recent identification of the FMF gene, MEFV, which is apparently expressed only in neutrophils and encodes a protein called pyrin or marenostrin, should enable the precise molecular
basis of the disease to be unravelled, and is already used as a diagnostic aid. About 20 mutations in MEFV have now been associated with FMF, and paired MEFV mutations, presumed to involve both alleles, have been reported in the vast majority of patients with FMF. These findings accord with the autosomal recessive mode of inheritance that is usually evident clinically, and which has been defined in large population studies. Although there have been occasional reports suggesting that FMF can also be inherited dominantly, these have tended to be discounted, on the basis that asymptomatic FMF carriers are very common in certain populations, and therefore give rise to pseudo-dominant inheritance. However, few family studies incorporating MEFV genotyping have been performed.

We report here comprehensive analysis of MEFV in five families from different ethnic backgrounds. The findings are compatible with the existence of dominantly-inherited FMF, and demonstrate that this syndrome has a heterogeneous genetic basis. The mutations associated with dominant FMF shed light on aspects of disease susceptibility and the structure-function relationships of pyrin.

Methods

Patients

We studied five families who had been previously evaluated in our clinics. All symptomatic family members met the Tel Hashomer criteria for clinical diagnosis of FMF. Eight were tested for amyloidosis using radiolabelled serum amyloid P component scintigraphy. Two families were Turkish; the other three were British, Jordanian Arabic and Punjabi Indian, respectively (Figures 1–3). In addition, we reviewed the clinical features and MEFV sequence of two further British patients with classical FMF associated with simple heterozygosity for pyrin M694, and performed a limited family study in one of these cases.

Genotyping

MEFV exons and flanking intronic sequences were amplified from genomic DNA as previously described. PCR products were sequenced using big dye terminator sequencing chemistry and an ABI 310 sequencing machine. Exon 10 was sequenced in all cases, and additional regions of MEFV were characterized as necessary to elucidate the basis of FMF in individual cases. The complete coding sequence for pyrin was analysed in representative patients in each of the five families, and all identified mutations were sought in each available family member, whether FMF symptoms were present or not. The mutation encoding pyrin E148Q was routinely sought in every individual by gel electrophoresis of the 5’ exon 2 amplicon after MvaI digestion.

Results

MEFV mutations were identified in each of the five families: as pairs in four cases and as a solitary finding in one. Inheritance proved to be pseudo-dominant in two of these families (Figure 1) due to patients with FMF having married carriers. Pyrin E148Q and M694I were encoded on opposite MEFV alleles in a Jordanian family, as were M694V and M680I in a Turkish family. One individual in the latter kindred had this well-recognized FMF-associated genotype without ever having experienced symptoms, indicating incomplete penetrance.

In the remaining three families, the observed autosomal dominant pattern of inheritance of FMF was authenticated by finding, in a single allele, either a novel compound MEFV mutation (Figure 2), or simple deletion of M694 (Figure 3). The other allele had the wild-type coding sequence in all cases. The compound pyrin variant E148Q/M694I was present in both a Turkish and an Indian family. All of the individuals with FMF in each of these three families carried the respective mutations, and no other mutations were identified despite obtaining the complete coding sequence of MEFV in each proband. Asymptomatic individuals were identified with each

Figure 1. Pseudo-dominantly inherited FMF in two families. a Pyrin variants E148Q and M694I encoded by mutations in opposing MEFV alleles in a Jordanian family. b Pyrin variants M694V and M680I encoded by mutations in opposing MEFV alleles in a Turkish family. Open boxes are asymptomatic FMF gene carriers. Black boxes are FMF patients with compound dual allele MEFV mutations. Shaded boxes are asymptomatic carriers of compound dual allele MEFV mutations.
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Figure 2. Autosomal dominant FMF with variable penetrance in three families due to complex mutations in a single MEFV allele. a) Pyrin E148Q/M694I encoded by two mutations in a single allele in an Indian family. b) Pyrin E148Q/M694I encoded by two mutations in a single allele in a Turkish family. Open boxes are healthy individuals with wild-type MEFV. Black boxes are FMF patients with compound single-allele MEFV mutations. Shaded boxes are asymptomatic carriers of compound single allele MEFV mutations. The four individuals marked * additionally had one MEFV allele encoding pyrin E148Q alone, which had no bearing on disease transmission.

Autosomal dominant inheritance and incomplete penetrance.

The fortuitous presence of several previously described wild-type single nucleotide polymorphisms in exons 2 (codon 202 G or A) and 5 (codons 474 G or A, 476 G or A and 510 C or T) of MEFV encoded pyrin of the apparently normal MEFV allele. Three different haplotypes were identified in individuals with FMF, having been brought into the family through three different marriage unions, providing strong additional evidence for dominant, single-allele disease transmission.

Two patients among these five kindreds had AA amyloidosis, the distribution and extent of which was confirmed by SAP scintigraphy. One was the proband of the Jordanian family who had pyrin E148Q and M694I encoded on separate MEFV alleles, and the other was a British patient with pyrin ΔM694 alone. Identifiable visceral deposits were limited to the spleen and kidneys in both cases, and both had proteinuria as the sole clinical manifestation of their amyloid disease. Sub-clinical amyloid deposits were excluded in six other cases. All of the patients among the five families, plus the two addi-
tional patients with pyrin ΔM694, responded very well to regular prophylactic therapy with colchicine.

**Discussion**

The results of MEFV analysis in this study provide compelling evidence for autosomal dominant inheritance of FMF. Dominant FMF was associated with the previously undescribed compound pyrin variant E148Q/M694I encoded on a single allele, or heterozygosity for the simple deletion mutation encoding pyrin ΔM694. All affected individuals had one or other of these pyrin variants, one of which, E148Q/M694I, occurred in independent Indian and Turkish kindreds, and the other, ΔM694, in three unrelated British families. Sequencing of the complete coding region failed to detect any abnormality in the second MEFV allele in any of the three families or the two additional patients with ΔM694. The possibility that inheritance could have been pseudo-dominant due to a covert mutation in the second MEFV allele, for example in its control region, or in another gene, is remote. Not only are no such mutations known, but coding region mutations on both MEFV alleles are found in the vast majority of FMF patients, indicating that FMF-causing mutations outside the MEFV coding region cannot be sufficiently prevalent to have caused pseudo-dominant inheritance in the numerous generations of our study population. Furthermore, MEFV haplotyping in the large Turkish family with E148Q/M694I showed that affected individuals had at least three different complementary alleles as a result of various marriages. All of the individual point mutations in MEFV associated with dominant FMF have previously been recognized as solitary pathogenic mutations.

The possibility that these patients had some other dominantly-inherited periodic fever syndrome, which by chance segregated with MEFV mutations, is also exceedingly remote. The clinical features of FMF in these families were classical in every respect, including the response to colchicine. Other autosomal dominant periodic fever syndromes, such as the Muckle-Wells syndrome, familial cold urticaria and familial Hibernian fever do share some component features with FMF, but their overall clinical pattern is distinct.12 Among these disorders, familial Hibernian fever might resemble FMF closely enough to cause diagnostic confusion, but it has only been reported in about a dozen families, and none of Mediterranean or Indian origin. Familial Hibernian fever is due to mutations in the gene for tumour necrosis factor receptor 1,13 and these were excluded in each of the three families by testing by Dr M.F. McDermott (St Bartholomew’s and the Royal London Hospital School of Medicine and Dentistry).

It remains likely, at least in regions where FMF is prevalent, that in most families in which FMF affects successive generations, inheritance is pseudo-dominant due to the marriage of an FMF patient to a carrier. This was the situation in two families reported here. Interestingly, the onset of clinical symptoms was unusually late in the Jordanian family with pyrin M694I and E148Q encoded on different alleles. The proband only began to have attacks of FMF aged 38 years and was found to have amyloidosis within one year; neither of his two children with the same genotype developed symptoms of FMF until they were nearly 30 years of age.

While it is inherently likely that different mutations will impair the function of a particular protein to differing extents, several findings indicate the particular importance of the methionine residue at position 694 in the physiological role of pyrin. Three different pathogenic mutations involving M694 have been identified, and heterozygous deletion of this residue alone may cause FMF. Homozygotes for ΔM694 have not been identified, but patients who are homozygous for M694V are reported to have particularly severe and early-onset FMF disease, and possibly a greater propensity to develop AA amyloidosis. Recognition that MEFV mutations affecting only a single allele can give rise to FMF suggests that a 50% complement of normal pyrin activity is not sufficient to prevent disease susceptibility. Pyrin appears to be expressed only in neutrophils and their precursor cells, and it likely to be involved in down-regulating or inhibiting their pro-inflammatory activity. Although pyrin has not yet been characterized, the MEFV mutations which cause FMF presumably disrupt its structure sufficiently to reduce its function and lead to neutrophil activation and migration in situations that would not normally produce these effects. It is possible that all clinical attacks of FMF are ultimately triggered by unknown exogenous factors, but the variable penetrance of autosomal dominant FMF indicates that susceptibility to FMF differs from patient to patient. Even individuals with ‘typical’ dual allele MEFV mutations often remain healthy for prolonged periods between clinical attacks, or may never experience symptoms. Physical and emotional stress, menstruation and diet have been reported to increase susceptibility to FMF attacks, but the mechanisms by which these and other constitutional factors modulate clinical expression of the disease are not known.

The paucity of studies in which MEFV has been completely sequenced means that it is difficult to quantify the potential of simple single allele mutations, other than that encoding pyrin ΔM694, to cause FMF. Whilst it is clear from the high prevalence of the carrier state in some populations, such as Armenians and North African Jews, in which up to
1:7 individuals have the trait, that most heterozygotes are outwardly healthy, the possibility cannot yet be excluded that some individuals are unusually susceptible to the effects of other common simple single-allele mutations. It may be significant that in our own experience of genotyping more than 100 individual FMF patients, most of the few cases in whom we could only identify a single MEFV mutation had pyrin M694V, supporting the hypothesis that disruption of this particular residue impairs the normal function of pyrin to an especially severe degree.

Although MEFV analysis clearly represents a major breakthrough in the diagnosis of FMF, some caution is required in its interpretation: patients with typical pathogenic dual-allele mutations do not necessarily develop FMF, and can acquire other inflammatory diseases, and FMF can be caused by rare MEFV mutations in exons that are at present not examined routinely. In particular, selective screening for only three or four mutations that are prevalent in the eastern Mediterranean basin, for example by RFLP analysis, may be wholly inadequate for evaluating individuals from other regions. Furthermore, DNA sequencing cannot distinguish whether multiple mutations are on the same or opposing alleles, and this and the associated clinical significance can only be defined in appropriate family studies.

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