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INVITED EDITORIAL
The Genetic Basis for Periodic Fever

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The periodic-fever syndromes are a genetically heterogeneous group of inflammatory disorders characterized by recurrent fever, abdominal pain, and polyserositis. The clinically distinct entities of familial Mediterranean fever (FMF; MIM 249100) and hyperimmunoglobulinemia D with periodic fever (MIM 260920) are autosomal recessive and genetically distinct. Two Turkish families with clinical features of FMF do not link to the FMF region on chromosome 16p13.3, suggesting genetic heterogeneity for FMF (Akarsu et al. 1997). Vertically transmitted periodic fevers with autosomal dominant inheritance are rarer than FMF; these include Hibernian fever (MIM 142680) and other families in which allelism to Hibernian fever remains to be tested. Vertical transmission in families of Mediterranean origin does not rule out autosomal recessive FMF exhibiting pseudodominance, given the relatively high carrier frequencies in certain ethnic groups and the consanguinity practiced in some of these. The molecular defects underlying FMF can now be detected after identification of the *MEFV* gene (French FMF Consortium 1997; International FMF Consortium 1997). Among the autosomal dominant periodic-fever families, the allelic relationships can now be inferred by linkage, given the recent localization of Hibernian fever to 12p13 (McDermott et al. 1998; Mulley et al. 1998). The era of laboratory-based diagnosis for the periodic fevers has begun.

Aksentijevich et al. (1999 [in this issue]) describe and expand the spectrum of mutations within *MEFV* from an ethnically diverse group. The initial reports based on non-Ashkenazi Jews, Armenians, Turks, and Arabs presented just four conservative missense mutations from *MEFV* exon 10 (M680I, M694V, M694I, and V726A), accounting for ~85% of FMF chromosomes from these populations (French FMF Consortium 1997; International FMF Consortium 1997). Bernot et al. (1998) described eight additional mutations in patients whose origin was expanded to Afghans, Druze, and French. Aksentijevich et al. reexamined earlier cases in which mutations were not detected and expanded their analysis to include Ashkenazi Jews and Italians. Five of the 16 mutations now known from all studies are found in exons other than exon 10. Eleven of the mutations found by Aksentijevich et al. accounted for 79% of the carrier chromosomes that they detected in 90 ethnically diverse (i.e., from the United States, Europe, and Israel), mutation-positive individuals. Carrier frequency is estimated to be as high as one in five in northern African non-Ashkenazi Jews and one in seven in Armenians from Los Angeles. The frequent occurrence of several *MEFV* alleles that are deleterious when homozygous (M694V, V726A, and E148Q), in different populations all of Mediterranean origin strongly suggests their maintenance in these populations by pressure from some form of balancing natural selection. This is most probably heterozygote advantage in response to an as yet undefined past or present environmental agent localized to that geographic region. None of the 16 mutations so far characterized will truncate the protein, so effects of truncation mutations can only be speculated.

An important new finding has been the *MEFV* mutation E148Q found in exon 2 (Bernot et al. 1998). It is embedded in multiple microsatellite marker haplotypes across ethnic groups, but these extragenic microsatellite haplotypes have now been shown by means of *MEFV* single-nucleotide polymorphisms (SNPs) to converge to a single ancestral haplotype (Aksentijevich et al. 1999). Similarly, multiple microsatellite haplotypes containing M694V or M680I mutations from different ethnic groups had been shown to converge to a single ancestral intragenic SNP haplotype between exons 3 and 10 (International FMF Consortium 1997). This convergence to ancestral haplotypes argues against recurrent mutation to the same FMF allele as the source of mutation present in the different ethnic groups, suggesting instead that each FMF allele originated from a single founder mutation. Gene migration represents an alternative to mutation as a source of genetic variation for a population, and genetic exchange has almost certainly

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accompanied Mediterranean commerce even prior to biblical times. Once introduced into adjacent populations living in similar environmental conditions, natural selection would promote further dispersal of the original founder mutation. Thus, the different ethnic groups eventually share that minute genome segment containing the *MEFV* mutation with its surrounding ancestral haplotype.

The power of intragenic SNPs for establishment of ancestral relationships of *MEFV* mutations has been clearly demonstrated. Their greater stability compared with microsatellite markers ensures their value for establishment of ancestral relationships between chromosomal segments. However, in the case of *MEFV,* their intragenic abundance compared with microsatellites is the crucial factor in their utility. Because the common *MEFV* mutations are themselves conservative missense substitutions, any exonic SNPs represent potential phenotypic modifiers for the 16 primary mutations so far identified.

Reduced penetrance of FMF in females has long been known; however, reduced penetrance of the E148Q, P369S, and K695R mutations in the Ashkenazim was a surprise finding. Therefore, the FMF carrier frequency within this group has been reassessed and adjusted upward to 21%, considerably higher than previously thought. E148Q was the most common mutation. The explanation for this newfound complexity will likely involve untangling of the interactions between elements of the genetic background (perhaps even intragenic SNPs) and/or environmental factors, and comparisons between affected and unaffected individuals homozygous for the same mutant alleles are likely to be instructive. Interactions could conceivably exist between pairs of mutant FMF alleles, between mutant FMF alleles and SNP variation at *MEFV,* or between mutant FMF alleles and SNP variation at other loci affecting inflammatory pathways. Asymptomatic homozygotes for the E148Q, P369S, and K695R mutations were observed within the Ashkenazim, prompting Aksentijevich et al. (1999) to recognize the difficulties in separating harmless exonic SNPs from pathological variants. Prognosis for these variants has now been thrown into confusion, except for the fact that the FMF phenotype within the Ashkenazi Jewish population is milder than that within the Sephardic Jewish population. There are precedents (e.g., from cystic fibrosis) suggesting that the phenotype associated with a primary mutation depends on variation found elsewhere in a gene (Kiesewetter et al. 1993).

Another unusual result presented by Aksentijevich et al. (1999) is detection of a complex allele with a double mutation in *cis* configuration in the Ashkenazim. Thus, some affected FMF family members unexpectedly carry not two, but three *MEFV* mutations. The complex allele contains E148Q and one of the next-most-frequent mutations in that ethnic group, either P369S or V726A. In the case of the complex allele E148Q-V726A, family study of ancestral SNP haplotypes demonstrated likely formation by a historic intragenic recombination event, rather than a double mutational hit. The same complex allele was also seen by Aksentijevich et al. (1999) in an Italian patient and by Bernot et al. (1998), who also observed the complex alleles E148Q-I692del and E167D-F479L. These observations suggest potential problems with the general utility of SNPs for detection of associations at the population level, for the purpose of mapping and identifying genes for polygenic disorders, even by means of dense SNP maps. The simpler, monogenic *MEFV* situation demonstrates different ancestral SNP haplotypes associated with different FMF mutations and intragenic scrambling of FMF alleles by recombination, problems that will be multiplied when extended to several loci affecting the same phenotype.

Finding double mutations is not new, so how frequent are these complex alleles? Complex alleles, the segregation of more than one mutation for the same gene within the same family for X-linked or autosomal dominant disorders, or segregation of mutations with similar phenotypic consequences at two loci within the one family (e.g., breast cancer genes *BRCA1* and *BRCA2*; Friedman et al. 1998) fortunately represent rarely encountered complications for diagnostic laboratories. Double mutations in *cis* configuration could arise simultaneously in the same individual, sequentially on the same chromosome in different generations, or by intragenic recombination, as now suggested for FMF. Mutation screens frequently terminate when "the" mutation (or two mutations for a patient with an autosomal recessive disorder) is detected. Perhaps the first example of such a complex allele was documented for X-linked Duchenne/Becker muscular dystrophy (Wilton et al. 1993), but examples are now also known for cystic fibrosis (Savov et al. 1995) and autosomal dominant angioedema (Verpy et al. 1996). Should the diagnostic laboratory be expected to detect novel double-mutant alleles or only those double-mutant alleles already characterized and known to be present in the ethnic group to which the patient belongs, as now described for *MEFV?*

Another feature of the work by Aksentijevich et al. (1999) is a neat demonstration of pseudodominance in an Israeli family of Ashkenazi ancestry in whom the mode of inheritance is not as it initially appeared. Pseudodominance is known for FMF, but in the case of the Ashkenazim it was considered unlikely because of low FMF gene frequency. The molecular tools now available led to detection of *MEFV* mutations in this family and to diagnosis of FMF. Vertical transmission was explained by carriers of *MEFV* mutations who married into the pedigree in successive generations. Reintroduction of the same mutant allele identical by descent could easily be

envisaged through an inbreeding loop; however, for FMF the same apparent vertical transmission is feasible by the introduction, into the pedigree, of any of the other common *MEFV* mutations known from that ethnic group. This was found to be the Ashkenazi family, which is far more plausible now that the FMF carrier frequency in the Ashkenazim has been revised upward to one in five.

Rapid screening methods are evolving for known *MEFV* mutations to facilitate transportability of testing from research laboratories to diagnostic laboratories (Bernot et al. 1998; Aksentijevich et al. 1999). The methodology involves digestion of PCR products in which mutations have created or destroyed restriction-endonuclease sites or amplification-refractory mutation screening (ARMS), which will significantly affect testing strategy when coupled with knowledge of the ethnic origin of each patient. The frequency of SNPs complicates single-strand conformation analysis to the extent that the *MEFV* mutations have been initially detected by direct sequencing. As other periodic fever genes are identified, mode of inheritance, as well as clinical evaluations, will further influence the mutation-testing strategy. The application of this technology (or perhaps DNA array technology in the future) will enable early confirmation or even presymptomatic diagnosis of periodic fevers, especially in populations in which the disorder is uncommon and in which it might otherwise remain undiagnosed and lead to unnecessary surgery or renal complications. Prognosis may be complicated by incomplete penetrance and variable expressivity of *MEFV* mutations; however, early treatment of FMF with colchicine is known to effectively reduce the chance of renal failure.

The task of identification of the *MEFV* gene was formidable, for what transpired to be a novel gene not represented in the expressed-sequence-tag databases. Aksentijevich et al. (1999) have shown that, far from representing the endpoint, gene identification was merely the milestone that signaled the beginning of an understanding of the molecular basis for periodic fevers. The current physical and genetic map of the human genes (Deloukas et al. 1998) contains half the estimated number of all human genes, which should facilitate the selection and testing of positional candidate genes for the other periodic fevers. The identity of these genes will lead to the characterization of molecular pathways for this group of disorders and to opportunities for study of how the inflammatory processes are regulated by each of these genes. They could be homologues of *MEFV;* they could be structurally unrelated to, but affect the same pathway as, *MEFV;* or their products may interact with pyrin (or marenostrin), the *MEFV* protein product. Alternatively, the different genes involved may affect different and independent pathways. The positional candidate approach to gene identification now has the potential to swiftly dissect the remaining genetic loci and to define the molecular basis for all the periodic fevers. Mutational and functional analyses of the genes involved may then lead to identification of the environmental triggers that interact with the different defective gene products and are responsible for the periodicity of the inflammatory process. Modification of environmental components that trigger the onset of periodic fever, together with the genetic identification of patients by laboratory-based diagnosis, may soon lead to better management for members of families with hereditary periodic fevers.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for FMF [MIM 249100], for hyperimmunoglobulinemia D with periodic fever [MIM 260920], and for Hibernian fever [MIM 142680])

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