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Linkage of Familial Hibernian Fever to Chromosome 12p13

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Summary

Autosomal dominant periodic fevers are characterized by intermittent febrile attacks of unknown etiology and by recurrent abdominal pains. The biochemical and molecular bases of all autosomal dominant periodic fevers are unknown, and only familial Hibernian fever (FHF) has been described as a distinct clinical entity. FHF has been reported in three families—the original Irish-Scottish family and two Irish families with similar clinical features. We have undertaken a genomewide search in these families and report significant multipoint LOD scores between the disease and markers on chromosome 12p13. Cumulative multipoint linkage analyses indicate that an FHF gene is likely to be located in an 8-cM interval between D12S77 and D12S356, with a maximum LOD score (Z_{max}) of 3.79. The two-point Z_{max} was 3.11, for D12S77. There was no evidence of genetic heterogeneity in these three families; it is proposed that these markers should be tested in other families, of different background, that have autosomal dominant periodic fever, as a prelude to identification of the FHF-susceptibility gene.

Introduction

Autosomal dominant periodic fever was first described by Bouroncle and Doan (1957), who reported a family in which abdominal pain and fever occurred in six sibships over five generations. Since then, dominant inheritance of periodic fever has been reported in several ethnic groups—for example, Austrians (Mache et al. 1996), Dutch (Zweers and Erkelens 1993) Finns (Karenko et al. 1992), Germans (Gertz et al. 1987; Hawle et al. 1989), Italians (Reich and Franklin 1970), Swiss (Federspiel and Tonz 1987), and Swedes (Bergman et al. 1968). However, only familial Hibernian fever (FHF) has been described as a separate clinical entity, originally in an Irish-Scottish family based in the United Kingdom (Williamson et al. 1982; McDermott et al. 1997) and subsequently in two Irish families with a similar clinical presentation (Quane et al. 1997). FHF is one of at least three hereditary-periodic-fever syndromes that are characterized by recurrent attacks of abdominal pain and fever and that may be associated with musculoskeletal manifestations (Kastner 1997); the other two genetic syndromes are familial Mediterranean fever (FMF) (Meyerhoff 1980) and hyperimmunoglobulinemia D (hyper-IgD syndrome) (Van der Meer et al. 1984). Although FHF clinically resembles FMF, the inheritance in FHF is dominant rather than recessive; the duration of attacks is generally longer, and there is a favorable response to high-dose steroids (McDermott et al. 1996). Amyloidosis, which is a major cause of morbidity in FMF without colchicine prophylaxis, appears to be relatively rare in FHF. The prevalence of FHF is unknown but is probably much lower than that of FMF; rates as high as 1/250 and 1/1,000 have been reported for FMF, in Iraqi and Sephardic Jews, respectively (Kastner 1997). A high index of suspicion is necessary to allow diagnosis of FHF; the age at onset and the clinical severity are variable, but disease usually occurs at <40 years of age.

The FMF gene, designated "*MEFV,*" has been mapped to the short arm of chromosome 16 (16p13.3) in Jewish and Armenian families (Pras et al. 1992; Shohat et al. 1992), as well as in Arabs, Druze Moslems, and Turks (Aksentijevich et al. 1993). The FMF-susceptibility gene (pyrin/marenostrin) recently has been identified as a member of the *RoRet* gene family (French FMF Consortium 1997; International FMF Consortium 1997), and missense mutations in exon 10 of this gene are thought to be responsible for the condition. To date, no susceptibility loci have been mapped for FHF, but X-linked inheritance is excluded, since there are at least

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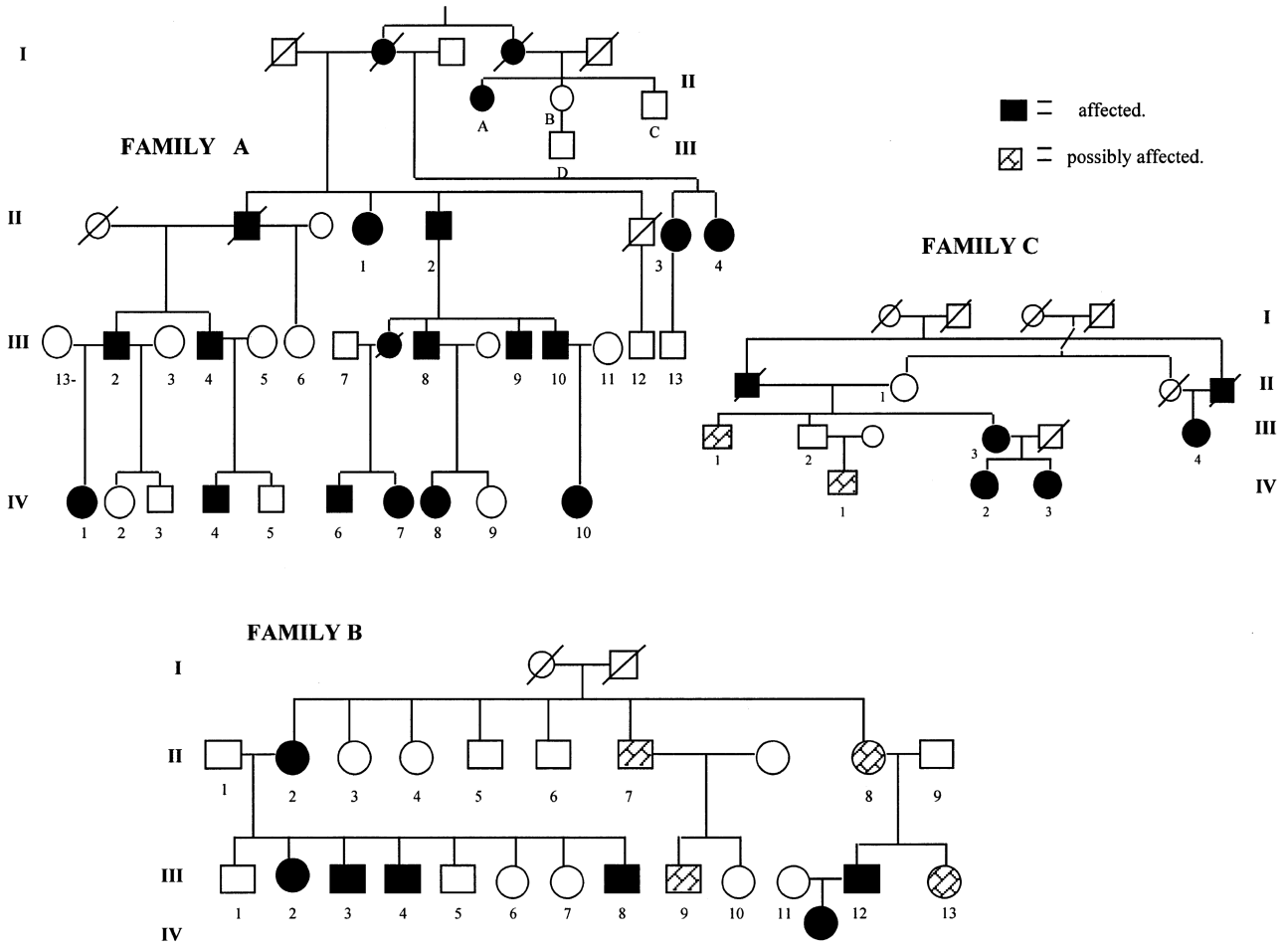

Figure 1 Pedigrees of the three families studied. To facilitate drawing of the pedigrees, not all family members have been included.

four cases of father-son transmission in the original FHF family. Linkage of FHF to *MEFV* has also been excluded (McDermott et al., in press). We have initiated a genome screen in the three families described above and report evidence for a susceptibility gene(s) localized to chromosome 12p13.

Subjects and Methods

Clinical Evaluation

The project was subject to hospital-ethical-committee approval in each participating center, and informed consent was obtained from all participating family members. Clinical history was ascertained for all available living family members, through interview, medical records, and examination. Affection status was determined by application of criteria described elsewhere (McDermott et al. 1997). Two cardinal signs and at least one minor criterion were required for a definite diagnosis of FHF. The major criteria were (i) recurrent febrile attacks with no other identifiable cause and (ii) either recurrent abdominal pains or localized myalgias associated with marked stiffness during febrile episodes. Minor criteria consisted of (i) discrete tender erysipelas-like skin lesions found anywhere during an attack, (ii) conjunctival injection, (iii) periorbital edema, (iv) positive family history (autosomal dominant), and (v) benefit from high-dose prednisolone in <72 h. Individuals fulfilling some criteria but fewer than two major and one minor were treated as unknowns for the purposes of linkage analysis.

Families.—The original extended family (family A) in which FHF was described and the two Irish families (families B and C) (fig. 1) with affected members fulfilling the criteria for FHF were studied. There were $\geqslant 16$ known affected individuals in five sibships over three generations in family A, with 4 instances of male-to-male transmission. In family B there were 8 definitely affected individuals and 3 possibly affected (of a total of 23), whereas there were 4 definitely affected members among the 8 individuals studied in family C. Samples

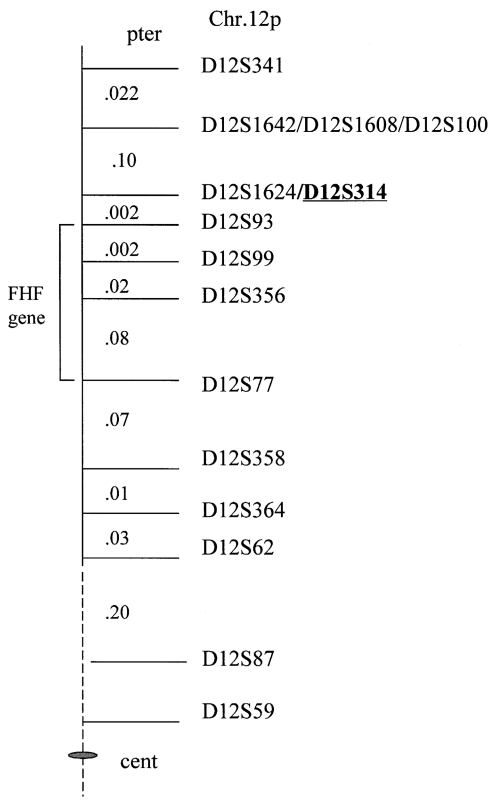

Figure 2 Schematic regional map of chromosome 12p. Markers used for linkage analysis in this study are indicated, and their respective distances (θ) are given (Kucherlapati et al. 1997). Marker D12S314 was used in the study by Mulley et al. (1997) but was not used in the present study.

were not taken from children <10 years of age. Clinical features, including pedigrees and laboratory data pertaining to these families, have been described elsewhere (Williamson et al. 1982; McDermott et al. 1997; Quane et al. 1997)

Search strategy and microsatellite-analysis genotyping.—Genomic DNA was extracted from peripheral blood by use of Puregene kits (Gentra Systems). For the purpose of genome screening, the Medical Research Council (MRC) mapping set of 200 fluorescently labeled markers defining a 10-cM resolution was used initially. Our strategy involved concentration on candidate-gene regions, and PCR primers were chosen from these regions, for simple-tandem-repeat–polymorphism amplification. PCR reactions were performed in a 10-μl volume containing 1 μl (~10 ng) DNA in 1 × *Taq* polymerase buffer (10 mM Tris-HCl pH 8.3, 2.0 mM $MgCl_2$, 200μM each dNTP, 50 mM KCl, and 0.1% Triton-X 100) containing 10 pmol of each primer and 0.25 units of *Taq* polymerase (Promega). A 96-well PTC100 MJR thermal cycler machine (M.J. Research) was used for PCR amplification by a touchdown protocol. The following is a brief resume of the protocol: denaturation at 94°C for 45 s, followed by touchdown annealing at various temperatures (descending progressively from 60°C to 46°C) for 45 s, with extension at 72°C for 45 s. The touchdown annealing temperatures were decreased by 3°C intervals, to 48°C, after every third cycle, and 14 cycles were then performed at 46°C (Don et al. 1991). Genotypings were performed by means of an Applied Biosystems (ABI) 373A automated DNA sequencer and 6.0% Sequagel 6 nondenaturing acrylamide (National Diagnostics). For electrophoretic multiplexing, an average of three to five were combined per lane, and allele sizes were calculated on the basis of an internal size standard in each lane (Genescan 350 or 500 Tamra; Perkin Elmer ABI). Amplified fragments were analyzed by Genescan and Genotyper software (Perkin Elmer ABI) on a Macintosh Quandra 650. It was usually necessary to dilute the final PCR product to one-fifth, after assessment on a 1% agarose gel, before the ABI loading for analysis. Additional pairs of fluorescently labeled primers were purchased commercially (PE-ABI) for chromosome 12p13–region markers, to obtain comprehensive coverage (Weissenbach et al. 1992; Gyapay et al. 1994). All genotypings were scored blinded to phenotype and, in the case of uncertainty about allele size, were performed at least twice. Allele classification and frequencies were taken from published sources (Généthon database).

Linkage Analysis

Two-point LOD scores between the disease locus and each individual marker were calculated by the MLINK program of the LINKAGE 5.2 package (Lathrop et al. 1985), by the U.K. Human Genome Mapping Project (HGMP) Resources Centre and FASTLINK, version 3.0 (Cottingham et al. 1993). A dominant-transmission model was specified, with age-specific penetrances of 10%, 70%, and 90% for individuals in age groups <15 years, 15–40 years, and > 40 years, respectively. The probability of a normal homozygote being diagnosed as affected was set at .001 for each age group (to allow for the possibility of phenocopies), and the frequency of the abnormal allele was set at .00001 (in view of the relative rarity of the disease). Recombination frequency (θ) was assumed to be equal for males and females. Homogeneity testing was performed by the program HOMOG (Terwilliger and Ott 1994). This analysis evaluates evidence for interfamilial genetic heterogeneity by comparing a likelihood including interfamilial heterogeneity versus a likelihood assuming homogeneity. The analysis was conducted by use of all two-point results from markers in the region. Sequential five-point multipoint analysis was performed by VITESSE (O'Connell and Weeks 1995), and results were plotted contiguous to each other (Terwilliger and Ott 1994, pp. 126–134). Marker order and intermarker distances were based on existing linkage

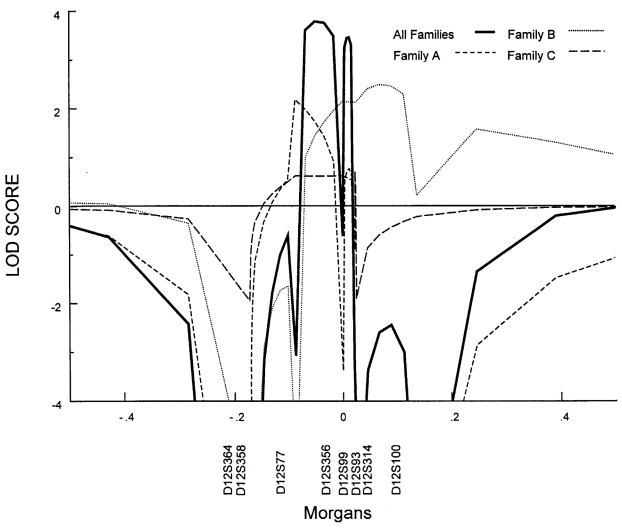

Figure 3 Multipoint linkage analysis between FHF and markers on chromosome 12p13 (D12S364, D12S358, D12S77, D12S356, D12S99, D12S93, D12S314, and D12S100), determined by the use of genotypes from all three families. Marker D12S356 was set at map position zero.

maps. The markers that were studied and the distances among them are given in figure 2 (Krauter et al. 1995; Kucherlapati et al. 1997). Haplotype analysis was also performed, to determine whether there was haplotype sharing between affected individuals within and between families.

Results

We had excluded approximately one-third of the genome (as containing the FHF gene), using 78 markers over nine specific chromosomes, when a pairwise positive LOD score was found for D12S358, on chromosome 12p13, in family A (maximum LOD score [Z_{max}] = 3.93 at maximum θ [θ_{max}] = .00). We have tested a total of 14 markers on the short arm of chromosome 12 (fig. 2). The FHF candidate region included six markers between and including D12S364 and D12S93 (i.e., markers D12S364, D12S358, D12S77, D12S356, D12S99, and D12S93), in all three families. Table 1 shows the cumulative two-point LOD scores for these six markers spanning the FHF candidate region. The two-point Z_{max} in the three families was at D12S77 (Z_{max} = 3.11 at θ = .12) (fig. 3). The two-point Z_{max} in individual families peak at D12S358, in family A (Z_{max} = 3.93 at θ_{max} = 0), at D12S356 in family B (Z_{max} = 1.90 at θ_{max} = 0), and at D12S77 and D12S356 in family C (Z_{max} = 0.56 at θ_{max}= 0). Results from homogeneity tests showed no significant evidence for heterogeneity, for any of the markers tested in this region. Additional alleles were seen for all categorized markers—D12S364 (16 alleles, instead of the reported 14), D12S358 (13 instead of 11), D12S77 (14 instead of 13), D12S356 (10 instead of 9), and D12S99 (12 instead of 10), and D12S93 (9). Additional alleles were assigned frequencies of .02, and known alleles were proportionately reduced in frequency, to enable total frequencies to equal unity.

For the multipoint analysis, the following map order was used: D12S364-(.05)-D12S358-(.07)-D12S77-(.08)-D12S356-(.02)-D12S99-(.02)-D12S93 (Krauter et al. 1995; Kucherlapati et al. 1997). Multipoint analysis with the complete set of markers spanning the disease interval was not possible, because of the high number of alleles per marker. Results from sequential five-point analysis are depicted in figure 2. Multipoint analyses revealed that the FHF gene was most likely to lie in the 8-cM interval between markers D12S77 and D12S356 (Z_{max} = 3.79). However, the 2-cM interval between D12S99 and D12S93 also provided strong support for linkage (Z_{max} = 3.46) and cannot be excluded as possibly containing the FHF gene. The 1-LOD support interval, therefore, includes ~10 cM. Intermarker distances were consistent with the published map. Different estimates of disease-allele frequencies did not significantly alter the LOD scores. Haplotype analysis for polymorphic markers in 12p13 did not reveal any association between the FHF locus and a particular haplotype (data not shown).

Discussion

We have used linkage analysis to map the locus/loci for autosomal dominant FHF. The data reported here

Table 1

Cumulative Two-Point LOD Scores for Chromosome 12p Markers Spanning the FHF Locus in the Three Families Studied

	LOD Score at Recombination Fraction of							
Marker	.00	.01	.05	.10	.15	.20	Z_{max}	θ_{max}
D12S364	−19.99	−8.83	−3.99	−2.04	−1.08	−.53		
D12S358	−.92	.61	2.22	2.72	2.78	2.63	2.79	.13
D12S77	−4.29	1.72	2.64	3.11	3.11	2.89	3.15	.13
D12S356	.44	.64	1.94	2.34	2.32	2.11	2.36	.11
D12S99	1.03	1.00	.92	1.13	1.32	1.33	1.38	.19
D12S93	−7.90	−3.35	−.77	.17	.55	.67	.68	.22

show tight linkage between FHF and polymorphic microsatellite markers from the centromeric region of chromosome 12p. During the course of this analysis, Mulley et al. (1997) described a multipoint LOD score of 6.14 at D12S356 in an unspecified number of "benign periodic fever" families (symbolized as "FPF," for "familial periodic fever"). They concluded that an FPF gene was located in the interval between D12S314 and D12S364, spanning an ~19-cM interval on the short arm of chromosome 12 (Mulley et al. 1997). Although no clinical details are available on the family material used, it appears that the conditions of FHF and FPF are genetically similar, since LOD scores are significantly positive for the same markers in both sets of families. Assuming that the same gene is present in the families that we have studied, then, we have further narrowed the critical interval, to ~8–12 cM. There remains the possibility of more than one susceptibility gene being involved, especially since two-point LOD scores maximize at markers 15 cM apart in the two larger families; alternatively, the result may be due a phenotype error in one of the families. Family C is too small to produce significant independent LOD scores in the absence of a larger number of markers, but preliminary data also support the possibility that this region is involved.

The current interval is very gene-rich site, and many candidate genes for FHF have already been mapped to the region, including genes with immune function, such as TNFRRP, CD4, and CD69 (Kucherlapati et al. 1997). Identification of the FMF gene has provided insight into the possible mechanism of unexplained fevers, and we therefore are also looking for *MEFV* homologues and genes with functional similarities in the linkage area. Despite the similarities in the symptoms and clinical features present in FHF and FMF, it remains to be proved that affected individuals are indeed suffering from similar categories of disease. All three families studied share Irish ancestry, but, as far as we are aware, they are unrelated, and, indeed, the grandmother, who appears to have been the carrier in family A, originally came from Scotland. We have not identified an ancestral chromosome with the markers used in this study, as has been the case for a subset of families suffering from FMF (Aksentijevich et al. 1993); this is compatible with the independent occurrence of mutations at the FHF locus in different populations and families. Our priority is to identify additional markers, from within the region, that may define an ancestral haplotype shared by two or more families and also to clarify the reason for the 15-cM distance between peak LOD scores in the families. Further narrowing of the linkage interval will be achieved by fine mapping, prior to our embarking on a "positional candidate cloning project" approach. The other immediate objective is to test these markers in European families with autosomal dominant periodic fever—but not specifically FHF—to detect whether genetic heterogeneity is a feature of this group of conditions.

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Electronic-Database Information

URLs for data in this article are as follows:

U.K. HGMP Resources Centre, http://www.hgmp.mrc.ac.uk

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