Linkage of Late-Onset Fuchs Corneal Dystrophy to a Novel Locus at 13pTel-13q12.13

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PURPOSE. To identify the gene locus underlying the inheritance of late-onset Fuchs corneal dystrophy (FCD) in a large white kindred.

METHODS. Genotypes of small tandem repeat polymorphisms were obtained from 17 affected and 3 unaffected family members, followed by genetic linkage analysis.

RESULTS. In this family, classic late-onset FCD appeared to be inherited as a single, dominant Mendelian trait. In two exceptional sibships, however, children aged 10 and 13 years had FCD. In each sibship, both parents were found to be affected, opening the possibility that this unusually early age of onset was the result of homozygosity for an FCD mutation. Genotype results, however, were not consistent with consanguinity of the parents, who appear to have independent cases of FCD. A whole-genome linkage scan mapped FCD to a single locus at 13pTel-13q12.13, with significant two-point LOD scores of 3.91 at D13S1236 and 3.80 at D13S1304. The 26.4-Mb disease interval contains the chromosome 13 nucleolar organizer (RNRI), the centromere, and 44 annotated protein-encoding genes. So far, exons of 10 of these genes have been screened, but no mutations have been found.

CONCLUSIONS. FCD1 is the first genetic locus to be identified for late-onset FCD, a common disease of the aging cornea. The exceptional early onset of the disease observed in two children is unusual and might be the result of digenic interaction between FCD1 and an independent late-onset FCD mutation.

whereas in grade 3, this area of confluence had expanded to 2 to 5 mm, and in grade 4 its width was greater than 5 mm. Grade 5 was the same as grade 4, but also exhibited edema of the corneal stroma and/or epithelium.

**Genotyping and Linkage Analysis**

Blood (−10 mL) was collected and frozen at −20°C. DNA was extracted (Qiagen, Santa Clara, CA), and genotyped for 399 polymorphic linkage markers, using the MD10 set of fluorescent primers (Applied Biosystems, Inc. [ABI], Foster City, CA) using an automated DNA sequencer (Prism 377, ABI). Data processing and genotype assignment were performed on computer (GeneScan 3.1 and Genotyper 2.1, respectively; ABI). RelCheck confirmed pedigree relationships, and Pedcheck identified Mendelian inconsistencies. Two-point linkage was performed using MLINK. SimWalk2 version 2.80 was used for multipoint linkage and haplotype analysis. Unaffected individuals under 50 years of age were excluded from the analysis because of the late-onset nature of the disease. Marker allele frequencies were estimated by counting alleles of all genotyped members of the pedigree. Genetic markers and linkage distances were from the Marshfield maps, and map positions were from the physical genome map.

**Screening Gene Exons for Mutations**

Coding exons and their flanking splice-sites, the most common sites for disease-causing mutations, were PCR amplified from genomic DNA with custom primer pairs, purified and then sequenced with an automated sequencer (model 3730; ABI). Trace chromatogram data were viewed on computer (Sequencher 3.0 software) and manually screened for heterozygous sequence variants. Experimentally determined nucleotide sequences were compared with published sequences by alignment and coassembly, using the software. Genes already screened and their cDNA accession numbers are CRYL1 NM_015974; GJA3 NM_021954; GJB2 NM_004004; GJB6 NM_006783; FG9 NM_002010; LOC387911 NM_001007537; MGC48915 OM_178540; MRP63 OM_024026; SGCG NM_000231; and TUBA2 NM_006001.

**RESULTS**

**The Family and Its Clinical Phenotypes**

A four-generation white pedigree with 20 affected individuals was assembled after discussions with an initial proband (Fig. 1, arrow). FCD was diagnosed using slit lamp biomicroscopy and assigned to severity grades (see Methods and Fig. 1). The distribution of individuals confirmed or presumed affected was generally consistent with Mendelian inheritance of a single dominant locus. In addition to apparent transmission from parent to child, the ratio of affected to unaffected individuals approximated a 1:1 ratio in the full pedigree. The excess of patients with FCD in the linkage pedigree (see Fig. 4) is an artifact of preferentially selecting those affected, who tend to be more informative because of the late onset of the disease.

The presence of late-onset disease in this family was established by two criteria. First, in all cases that were studied in detail and assigned a grade of severity, the clinical appearance of the posterior cornea by slit lamp and specular microscopy revealed lesions indistinguishable from those of common late-onset FCD. At early to intermediate stages, this was characterized by large, individual guttate with a distinctive peaked appearance. Second, the age-severity profile of this family was found to be generally consistent with that of late-onset FCD, which typically takes about two decades to progress from onset to end-stage disease. Those affected in generation II, who ranged from 74 to 84 years, all showed advanced FCD at grades 3 or 4, whereas in generation III, affected siblings of
Two affected women of generation III had husbands who were completely unrelated, a statement that was
had grade 1 FCD. Although at first we suspected distant con-
sequence, whereas in the other family, a 13-year-old girl (IV-5)
obtained significant linkage to chromosome 13 with LOD
scores of 3.08 at D13S175 and 4.44 at D13S1236.

Revised Genetic Linkage Model
Analysis of the genotype data for familial relationships and the
construction of haplotypes for chromosome 13 confirmed that the fathers of each sibship (III-10 and III-16) were not consanguineous with their wives. This favored the alternative explanation that III-10 and III-16 had independently developed FCD, perhaps because of an unlinked mutation. In this second model, the affected children were assumed to have had early-onset FCD because they had inherited one dominant FCD1 mutant allele from their mothers and a second, unmapped FCD gene from their fathers.

For purposes of linkage calculation, the two fathers (Fig. 1, sectored symbols) were classified as unaffected because they did not carry the same FCD1 mutation inherited by descend-
ants of the founders, I-1 and I-2. Maximum LOD scores for each of the 399 markers (Fig. 3) revealed only one region with scores higher than 3.0. Scores for individual markers were calculated with MLINK using the same parameters described in Table 1, where linkage data is presented in more detail. As shown in Table 1, the first five markers of the chromosome 13 linkage group all gave maximum LOD scores at \( \theta = 0 \), indicating that crossovers between these markers and the disease locus were not detected. The \( -\infty \) score at D13S1304 indicated obligatory crossovers between D13S1243 and D13S1304.

Finally, linkage analysis was also performed by treating the five-marker conserved haplotype as a single allele. Examination of the pedigree haplotypes (Fig. 4) yielded 13 different haplo-
types. MLINK analysis with 13 alleles of equal frequency and complete penetrance gave a maximum LOD score of 4.05 at \( \theta = 0 \).

Genes in the Disease Interval
The linkage data (Table 1) and haplotype map (Fig. 4) formally place the disease interval between the P telomere and D13S1304. Although they constitute most of the interval, there is no assembled DNA sequence available for the centromere and the entire short (p) arm of chromosome 13. As one proceeds down the q arm of 13, the first genome assembly DNA sequence begins at 17.9 Mb from the p telomere, and the first known gene, TUBA2, is located at 18.65 Mb. Further down, there are a total of 44 known or predicted genes within the disease interval (Fig. 5). We have sequenced the exons of 10 of
these (Fig. 5, stars; see also Methods), but have not yet found FCD-associated mutations.

**DISCUSSION**

**FCD1**, the first locus for late-onset FCD, is defined as a 26.4-Mb interval extending from the 13p telomere to 13q12.13 (Fig. 5). One complication associated with this large interval is that the entire p arm of this acrocentric chromosome and the centromeric region have no sequence or clone contigs in the May 2004 assembly of the human genome sequence. Although assembled sequence is not available, 13p12 is known as the site of the *RNR1* nucleosilus organizer (Online Mendelian Inheritance in Man [OMIM] 180450; http://www.ncbi.nlm.nih.gov/Omim/provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD), which is composed of hundreds of tandem repeats of the 5s, 18s, and 28s ribosomal RNA genes. Otherwise, there are no known genes mapping to this interval, which contains large blocks of highly repetitive DNA. Another technical difficulty we have encountered is that no polymorphic genetic markers are available for this large, unknown region of chromosome 13. Therefore, although the family may exhibit crossover events that define an upper boundary for FCD1 within 13p or 13q, we cannot detect them. Although it seems unlikely that this interval contains candidate genes, we still cannot formally rule out that 13p contains functionally significant loci. There are a few genome database and literature references to protein-coding genes in 13p, and we have examined them in detail. However, all appear to be incorrect, and either are the result or typographical errors, or are in old publications reporting gene locations that have since been remapped elsewhere.

The portion of the FCD1 interval that is represented in the sequenced genome assembly spans 13q12.11-q12.13 and covers 7.6 Mb. It includes 44 known and predicted protein-encoding genes (Fig. 5). We have screened the exons of 10 of these (see Methods for details), but so far have found no variants beyond common polymorphisms. Of early interest were two closely related genes, LOC387911 and MGC48915. Each of these has internal GXX collagen helix repeats and a C-terminal C1Q domain, features shared by other basal lamina collagens including *COL8A2*. Unfortunately, they contain no coding region mutations associated with FCD. These two candidate genes also do not appear to be major components of the cornea or Descemet’s membrane. They are not represented in expressed sequence tag (EST) clones from cornea, or in SAGE libraries from the corneal endothelium.

Possible difficulties in identifying the FCD1 mutation are its dominant nature and its mild, late-onset phenotype. The defect could be a noncoding region promoter mutation that causes a modest change in mRNA levels. Another possibility is that the FCD1 mutation is a gene deletion that causes a phenotype through haploinsufficiency, which in turn causes a dominant mode of inheritance, with a potentially lethal homozygous phenotype. Unfortunately, conventional exon screening usually does not detect the deletion of a whole exon or gene. Testing this possibility will require an examination of the relative kinetics of PCR for each gene amplimer using quantitative PCR, or the observation of apparent non-Mendelian inheritance of polymorphisms within the gene. It may also be necessary to examine the disease chromosome for other large-scale rearrangements, such as inversions. Such chromosome breakage and nonhomologous reunion are known to cause dominant gain-of-function alleles by fusing coding sequences of a gene with novel promoter elements. An intriguing feature of this particular pedigree is the finding that two parents with late-onset FCD can have a child with the early stages of classic FCD. This has yet to be reported elsewhere and was noted only during the course of a detailed examination of this particular family. Families with mutations in *COL8A2* show consistent early-onset of FCD, but it is a form of the disease with a very distinct clinical appearance and histopathologic abnormalities. In the two children we...
In this study, the guttae were indistinguishable from those described for conventional late-onset FCD. These findings open the possibility that the incidence of FCD in children is more frequent than previously thought. Another alternative is that FCD1 has a tendency toward earlier onset and that this tendency is sensitive to unlinked genetic modifiers or environmental factors. In two instances, it is possible that digenic interaction with dominant FCD-causing mutations in other genes has accelerated onset of the disease by two to three decades.

Figure 4. Haplotype analysis of the FCD1 disease locus. Predicted haplotypes generated by Simwalk are displayed underneath individuals who were genotyped. The only exceptions are II-5 and II-6 (italics), for whom haplotypes were deduced from genotypes of their children. Boxed numbers: allele haplotypes found on disease-associated chromosomes. The smaller box, with markers D13S1316 through D13S1243, contains a core haplotype common to all affected individuals descended from I-1 and I-2. Sectored symbols: FCD-affected individuals who are not descended from I-1 and I-2. Intervals where crossovers have occurred are indicated by an x.

Figure 5. Genes in the FCD1 disease interval. Ideogram of human chromosome 13, with FCD1 interval indicated by vertical bracket. 13pTel, 13qTel indicate p and q telomeres, with nucleotide positions of 0 and ~114 million base pairs, respectively. Right: the 7.6-Mb FCD cytological interval, with genes mapped by nucleotide position (May 2004 human genome assembly). Shaded area: region within the disease locus, whereas 13pTel and D13S1304 mark the outer boundaries of the interval. No protein coding genes are known to exist in the interval between TUBA2 and 13pTel.
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References