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 nucleolus organizer (RNR1), 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. (Invest Ophthalmol Vis Sci. 2006;47:140–145) DOI:10.1167/iovs.05-0578

Fuchs corneal dystrophy (FCD) is a degenerative disorder specific to the corneal endothelium1–4 which affects roughly 4% of the population more than 40 years of age.5 It is distinguished from other corneal disorders by the progressive formation of guttae, which are microscopic refractile excrescences of Descemet’s membrane, a collagen-rich basal lamina secreted by the corneal endothelium.6–7 From onset, it often takes two decades for FCD to impair endothelial cell function seriously.5,8 leading to stromal edema and impaired vision.5,9,10 Currently, the only effective means of restoring vision in advanced cases is penetrating keratoplasty; FCD is one of the most common indications for this type of transplantation surgery.11

Although Fuchs first described this disease in the early 20th century, its pathogenesis remains poorly understood. With regard to its genetics, large families exhibiting autosomal dominant inheritance of FCD have long been reported in the literature.12–14 The first progress toward identifying loci and gene mutations associated with familial FCD has been provided by a rare subtype of FCD characterized by atypical histopathology and disease onset in early childhood.14 This rare condition is caused by point mutations in the COL8A2 gene.8,15 which encodes the α2 subtype of collagen VIII, a major component of Descemet’s membrane. Although COL8A2 disease has provided important evidence that implicates Descemet’s membrane as the site of primary defects that cause FCD, we still have no convincing evidence that COL8A2 mutations are involved in the more common late-onset form of this disease.8,16

For late-onset familial FCD, the existence of additional gender-related genetic or physiological factors are suggested by the consistent observation of a 2.5:1 ratio of affected females to males.5,8 This ratio is not explained by the greater frequency of women in older populations, or by simple autosomal dominant inheritance of the disease. Even though a combination of genetic, gender-related and environmental factors are thought to underlie most common diseases, large families with apparent dominant inheritance of FCD are surprisingly prevalent. On closer examination, as many as 50% of clinical patients with FCD have siblings, parents, or offspring who are also affected.5,8 In this study, we mapped the first genetic locus for late-onset FCD. In one large family it followed Mendelian inheritance as a single autosomal dominant trait.

METHODS

Patients

Family members for this study were recruited, examined and DNA samples obtained through procedures approved by the Institutional Review Board for Human Subjects Research at the Johns Hopkins University School of Medicine, according to the Declaration of Helsinki. Written consent was provided by each study participant, or by one of their parents.

Determination of Phenotype, Disease Severity, and Corneal Photography

Individuals were examined and photographed to document guttae by slit lamp biomicroscopy. A confocal specular microscope17 (ConfoScan3; Nidek Technologies, Vigonza, Italy) was used to view the guttae. Grading followed the scale proposed by Krachmer et al.,5 with Grade 0 indicating no disease, as defined by fewer than 11 central guttae. In this study, none of the unaffected cases had more than two central guttae. Grade 1, which represented definitive onset of the disease, was indicated by 12 or more central, nonconfluent guttae in at least one eye. Grade 2 patients exhibited a zone of confluent central guttae 1 to 2 mm in horizontal width,
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 599 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 Pedcheck18 identified Mendelian inconsistencies. Two-point linkage was performed using MLINK.19 SimWalk2 version 2.8020 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,21 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,22 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; FGF9 NM_002010; LOC387911 NM_001007537; MGC48915 NM_178540; MRP63 NM_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 guttata with a distinctive peaked appearance.8,17 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.5,8 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...
later supported by haplotype analysis of the genotype data.

Two affected women of generation III had husbands who were completely unrelated, a statement that was not known to be related, but it was possible the affected children were homozygous for the mutant allele because of distant consanguinity. Two copies of the dominant mutation might be expected to increase severity and to accelerate onset of the disease. We therefore modeled the pedigree as a recessive early-onset FCD trait, with an intermediate phenotype for the heterozygotes, which corresponded to late-onset disease. This was implemented in the linkage analysis program MLINK with the binary factors format. Using this model, we first obtained significant linkage to chromosome 13 with LOD scores of 3.08 at D13S175 and 4.44 at D13S1236.

Initial Genetic Linkage

To map the disease trait, DNA isolated from individuals marked by asterisks in Figure 1 was genotyped for 399 polymorphic markers on all autosomes. Because of the potential for incomplete penetrance due to a late age of onset, unaffected individuals younger than 50 were excluded from the analysis. In this first analysis, early onset FCD in the two children was assumed to be caused by homozygosity for the mutation. Their parents were not known to be related, but it was possible the affected children were homozygous for the mutant allele because of distant consanguinity. Two copies of the dominant mutation might be expected to increase severity and to accelerate onset of the disease. We therefore modeled the pedigree as a recessive early-onset FCD trait, with an intermediate phenotype for the heterozygotes, which corresponded to late-onset disease. This was implemented in the linkage analysis program MLINK with the binary factors format. Using this model, we first 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 descendants 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 haplotypes. 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...
the p-to-q order of the Marshfield linkage map. The horizontal bracket indicates all markers in the chromosome 13 linkage group.

Table 1. Genetic Linkage to 13pTel-13q12.13

<table>
<thead>
<tr>
<th>Marker</th>
<th>Location (Mb)</th>
<th>Location (cM)</th>
<th>Lod Score θ = 0</th>
<th>Lod Max θ Max</th>
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<tr>
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<tr>
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<td>32.15</td>
<td>25.08</td>
<td>( \sim )</td>
<td>0.97</td>
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</table>

Detailed results of the analysis in Figure 3 are provided for the first seven markers of the Marshfield chromosome 13 linkage map. Markers are ordered by physical map position in megabases according to the May 2004 genome assembly. Recombination distance in centimorgans is from the Marshfield linkage map.11 Lod scores are calculated with the assumption of no recombination (θ = 0), or the maximum lod score obtained when recombination distance (θ) is varied. As in Figure 3, linkage was computed using MLINK, with the assumption of fully penetrant dominant inheritance, and individuals III-10 and III-16 (see Fig. 1) were scored as formally unaffected. This last feature of the linkage model reflects the observation that III-10 and III-16 are unrelated to III-11 and III-15 and that their FCD has an independent genetic basis. The horizontal bracket indicates all markers in the chromosome 13 linkage group.
report in this study, the guttae were indistinguishable from
those described for conventional late-onset FCD.\textsuperscript{8,17} These
findings open the possibility that the incidence of FCD in
children is more frequent than previously thought. Another
alternative is that 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.

chromosome 13

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.
Acknowledgments

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References