Fuchs corneal dystrophy (FCD) is a degenerative disorder specific to the corneal endothelium which affects roughly 4% of the population more than 40 years of age. 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. From onset, it often takes two decades for FCD to impair endothelial cell function seriously, leading to stromal edema and impaired vision. 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.

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. 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. This rare condition is caused by point mutations in the COL8A2 gene, 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.

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. 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. 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 guttatae by slit lamp biomicroscopy. A confocal specular microscope (ConfoScan3; Nidek Technologies, Vigonza, Italy) was used to view the guttatae. Grading followed the scale proposed by Krachmer et al., with Grade 0 indicating no disease, as defined by fewer than 11 central guttatae. In this study, none of the unaffected cases had more than two central guttatae. Grade 1, which represented definitive onset of the disease, was indicated by 12 or more central, nonconfluent guttatae in at least one eye. Grade 2 patients exhibited a zone of confluent central guttatae 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 2.8920 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 guttae 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
did not have the unusual, fine-grained appearance that we and others have characterized in patients with FCD with COL9A2 mutations.\textsuperscript{8,15}

**Initial Genetic Linkage**

To map the disease trait, DNA isolated from individuals marked by asterisks in Figure 1 was genotyped for 599 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.\textsuperscript{19} 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\textsuperscript{18,20} 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 599 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...
Figure 3. Genome-wide genetic linkage of FCD. Maximum LOD scores were obtained for 399 polymorphic STR markers. Calculations used the same methods and fully penetrant dominant trait model described in Table 1. Scores are plotted as individual vertical bars, with 3.0 being the minimum necessary to establish linkage. Markers are ordered by chromosome, beginning with chromosome 1. Each chromosomal set follows 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>
<td>D13S1316</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.21 Lod scores are calculated with the assumption of no recombination (θ = 0), or the maximum lod score obtainable 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.

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 of 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 COL8A2.7,8,15 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.25

Possible difficulties in identifying the FCD1 mutation are its dominant nature and its mild, late-onset phenotype.22 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.22,26 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.8,14,15 In the two children we
report 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. Sectioned 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