Linkage of a Mild Late-Onset Phenotype of Fuchs Corneal Dystrophy to a Novel Locus at 5q33.1-q35.2

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PURPOSE. To identify the disease locus associated with autosomal dominant Fuchs corneal dystrophy (FCD) in a large family and to compare the progression of severity in families mapped to the FCD1 and FCD2 loci.

METHODS. Seventeen individuals in a large family were examined by slit lamp biomicroscopy. Blood samples were collected, DNA was extracted, and a genome-wide scan was performed with a microarray SNP chip. After initial generation of a genome-wide, two-point parametric LOD score, linkage was confirmed and the critical interval was established by genotyping of short tandem repeat (STR) microsatellite markers.

RESULTS. A genome-wide linkage scan localized the disease interval to the long arm of chromosome 5, with a maximum two-point parametric LOD score of 3.41. Haplotype analyses refined the critical interval to 5q33.1-q35.2, spanning a 27-Mb (29-cM) region. Clinical examination of affected individuals in this family revealed an early onset of FCD at approximately age 40, after which progression of the disease was significantly attenuated compared to the FCD1- and FCD2-linked families.

CONCLUSIONS. Late-onset FCD is linked to a novel locus on 5q33.1-q35.2 and is associated with a milder severity in age at onset and rate of progression than the FCD1 and FCD2 loci. Correlation of individual genotypes with unique rates of disease progression will provide important tools for disease management, as well as for identifying the underlying genetic lesion, offer insight into the pathomechanism of FCD. (Invest Ophthalmol Vis Sci. 2009;50:5667–5671) DOI:10.1167/iovs.09-3764

Fuchs corneal dystrophy (FCD) is a hereditary, progressive disorder of the posterior cornea that affects up to 4% of individuals older than 40 years and represents the highest indication for corneal transplantation in the United States.1,2 Clinically, FCD is marked by the development of guttae, excrescences of Descemet’s membrane that generally appear in the fourth or fifth decades of life and increase in number over time.3,4 As the disease progresses, patients present with decreased visual acuity secondary to corneal edema and endothelial cell loss, with end-stage disease evidenced by the formation of painful epithelial bullae.5 This course of disease is aggravated by intraocular surgery or trauma and anterior segment inflammation, contributing to corneal decompensation and the need for corneal transplantation.5

Nearly a century after Fuchs’ initial description of this phenotype,6 its etiology is still not well understood. Although an early-onset FCD has been causally associated with mutations in COL4A2,7 the molecular mechanisms underlying the more common late-onset form are yet to be elucidated. Previously, we reported two loci associated with late-onset FCD in large families: an FCD1 locus on chromosome 15 and a FCD2 locus on chromosome 18.8,9 More recently, mutations in SLC4A11, a gene associated with congenital hereditary endothelial dystrophy,10 have been identified in a few sporadic cases of late-onset FCD.11

Here, we report a large family with late-onset FCD. A genome-wide scan localized the disease to the long arm of chromosome 5 with suggestive LOD scores, a finding substantiated with short tandem repeat (STR) microsatellite markers. Haplotype analyses refined the critical interval to a 27 Mb (29 cM) region. Clinical examinations concluded that the severity of the disease was relatively milder, and normal progression of the disease was slower compared with the affected individuals in families linked to the FCD1 and FCD2 loci. The identification of a third FCD locus in the human genome provides another entry point for determining the genetic basis of the disorder while, for the first time, offering potential differential clinical phenotyping data that may be of value in patient management.

MATERIALS AND METHODS

Recruitment

Family members were recruited through one proband presenting to our clinic with FCD. An extended pedigree was subsequently developed through interviews, and the patients were examined in locations proximal to their area of residence. Recruitment, examination, and procedures for DNA sample collection were 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, in the case of minors, by one of their parents.

Determination of Phenotype and Disease Severity

Individuals underwent detailed ophthalmic examination that included slit lamp biomicroscopy. Severity was graded on a scale developed by Krachmer et al.,3 with >12 central guttae in one eye indicative of disease (grade 1), or in both eyes of individuals older than 60 years. Progression of confluency was defined by area and divided into three categories: horizontal width <2 mm (grade 2), from 2 to 5 mm (grade 3), and >5 mm (grade 4). The development of stromal and/or epithelial edema elevated this score to grade 5. Documentation included retroillumination photography performed with a digital camera (D2xs; Nikon, Tokyo, Japan) and photo slit lamp (Carl Zeiss Meditec, Inc., Dublin, CA).

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Data Analysis

All clinical severity data were entered into a spreadsheet program for statistical analyses (Excel; Microsoft, Redmond, WA). Exclusion criteria included a history of ocular inflammation, surgery, or trauma, factors that could serve as strong confounding variables. Differences in progression over time were examined by comparing clinical data with those from one family associated with the FCD1 locus and three families with the FCD2 locus. Severity was recorded according to the Krachmer scale, allowing calculation of the mean age at each level of FCD severity. We used the t-test to assess differences in severity across genotypes.

Genotyping

A SMP microarray (ver. 5.0; Affymetrix, Santa Clara, CA) was used for genome-wide linkage analyses. Genomic DNA (500 ng) was used in a multiplexed SNP genotyping assay. Briefly, genomic DNA was digested with StyI and NspI, followed by ligation with adaptor sequences. These sequences acted as primer binding sites in PCR amplification. Amplified products were pooled and purified with magnetic beads, washed with ethanol and eluted in Tris (pH 8.0), digested into 50- to 100-bp fragments, and end-labeled by terminal deoxynucleotidyl transferase (TdT). The unincorporated labeling reagent was washed before the labeled products were loaded onto the arrays and were hybridized in an oven rotating at 60 rpm, 50°C for 20 hours. The arrays were then scanned (GeneChip Scanner 3000 7G; Affymetrix). The output signal files were checked for quality control (QC; BRLMM analysis tool 2.0 [BAT 2.0]; Affymetrix). The default threshold score was set at 0.05 and was used as a cutoff for SNPs to be excluded. Cell files were processed further to generate genotypes using a genotyping console (BAT 2.0; Affymetrix) and locally written Perl scripts. SNP genotypes were incorporated into the family pedigree files to generate PED files using Plink.12 Once incorporated, the same software was used to identify parentage inconsistencies. Pedigrees were analyzed assuming an autosomal dominant mode of inheritance, 0.02 disease allele frequency, and 90% penetrance by MERLIN.13 SNP allele frequencies from an ethnically matched control population were obtained from HapMap (hapmap.org).

The critical interval was delineated by genotyping STR markers. Multiplex PCR was carried out in a 25-μL mixture containing 40 ng genomic DNA, 10 μM dye-labeled primers pairs, 0.5 μL 10× PCR buffer (GeneAmp Buffer II; Affymetrix), 0.5 μL 10 mM dNTP mix, 2.5 mM MgCl₂, and 0.2 U Taq DNA polymerase (AmpliTaq Gold; Applied Biosystems, Inc. [ABI], Foster City, CA). Amplification was performed in a PCR System (GeneAmp PCR System 9700; ABI); primers and PCR conditions are available on request. PCR products were pooled and mixed with a loading cocktail containing size standards (HD-500; ABI), separated in a genetic analyzer (model 3100; ABI), and analyzed (GeneMapper software: ABI).

Linkage Analysis

Two-point linkage analyses were performed with the FASTLINK version of MLINK from the LINKAGE Program Package (http://www.hgmp.mrc.ac.uk/ provided in the public domain by Human Genome Mapping Project Resources Centre, Cambridge, UK). Maximum LOD scores were calculated using ILINK under an autosomal dominant model with a 0.02 disease allele frequency and 90% penetrance. The marker order and distances between the markers were obtained from the Genéthon database (http://www.genethon.fr/ provided in the public domain by the French Association against Myopathies, Evry, France) and the National Center for Biotechnology Information chromosome 5 sequence maps (http://www.ncbi.nlm.nih.gov/mapview/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD). For the initial genome scan, equal allele frequencies were assumed, whereas for fine mapping, allele frequencies were estimated from 96 unrelated and unaffected individuals.

RESULTS

As part of our ongoing effort to map and clone new loci for adult onset FCD, we identified a large, three-generation family, MO, through a proband examined at The Wilmer Eye Institute (Fig. 1: individual 21). On the conclusion of interviews and review of medical records, we were able to recruit and examine through slit lamp biomicroscopy a total of 17 individuals. Of these, we found 10 (eight female, two male) to fulfill the phenotypic criteria for an FCD diagnosis (12 or more central guttae in one or in both eyes of individuals above 60 years of age). The initial slit lamp examination of individual 22 detected central corneal guttae, although their number did not meet the minimum criteria for Krachmer grade 1. Hence, for the purpose of initial linkage analyses, this person was designated as unaffected. In addition, given that the father of individual 10...
was found positive for FCD, we cannot rule out the possibility of her inheriting the pathogenic mutation paternally; hence, alleles of individuals 10 and 11 were not used in linkage and haplotype analyses. Last, even though the older members of family MO were certain that deceased individuals 1, 3, and 8 were positive for FCD, there were no medical records available to confirm the disease; hence, we designated the status of these individuals as unknown.

We first asked whether this family may be linked to any of the known FCD loci. Therefore, we genotyped all available individuals with STR microsatellite markers that span the critical intervals of early- and late-onset FCD loci. Both haplotype analyses and two-point LOD scores excluded family MO from FCD1, FCD2, and the COL8A2 locus (data not shown). We therefore initiated a genome-wide scan by genotyping all appropriate family members by using an SNP microarray (5.0 platform; Affymetrix) that contains 500K SNPs spaced across the whole genome. Of the SNPs, 96% passed the initial QC and were investigated for parentage inconsistencies. A further 4560 SNPs were excluded due to non-Mendelian transmission likely attributed to bad genotyping calls, leaving us with ~475 K SNPs. As linkage analysis (two-point or multipoint) of the entire SNP set requires exceptional computational power, we decided to simplify our dataset while maintaining uniform coverage across the genome. Hence, we selected every fifth consecutive SNP, and performed two-point linkage on ~95 K SNPs.

As SNPs are bi-allelic, a family consisting of 17 members would not have enough power to attain significant linkage (LOD > 3); hence, we investigated further all regions with suggestive linkage (LOD > 2). From the genome-wide scan, we observed suggestive linkage only with SNPs located on the long arm of chromosome 5. The highest LOD scores were obtained with rs17451810, rs4958561, and rs778816, yielding scores of 2.71, 2.68, 2.66, 2.70, 2.64, and 2.68, respectively. Reconstruction of a candidate disease haplotype localized the putative critical interval to 27 Mb on 5q.

To determine whether this signal was truly indicative of a candidate disease haplotype, we performed two-point linkage on consecutive SNP, and performed two-point linkage on 5q.

### Table 1. Two-Point LOD Scores for a Series of Consecutive 5q STR Markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>cM</th>
<th>Mb</th>
<th>0.00</th>
<th>0.01</th>
<th>0.05</th>
<th>0.10</th>
<th>0.30</th>
<th>Z&lt;sub&gt;max&lt;/sub&gt;</th>
<th>θ&lt;sub&gt;max&lt;/sub&gt;</th>
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<tbody>
<tr>
<td>D5S436</td>
<td>147.5</td>
<td>145.1</td>
<td>-0.53</td>
<td>0.01</td>
<td>0.66</td>
<td>0.93</td>
<td>0.59</td>
<td>0.95</td>
<td>0.93</td>
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<tr>
<td>D5S434</td>
<td>150.3</td>
<td>147.2</td>
<td>-0.67</td>
<td>-0.19</td>
<td>0.51</td>
<td>0.77</td>
<td>0.45</td>
<td>0.77</td>
<td>0.77</td>
</tr>
<tr>
<td>D5S470</td>
<td>153.2</td>
<td>150.2</td>
<td>0.81</td>
<td>0.95</td>
<td>1.12</td>
<td>1.10</td>
<td>0.50</td>
<td>1.15</td>
<td>0.07</td>
</tr>
<tr>
<td>D5S209</td>
<td>156.5</td>
<td>152.5</td>
<td>0.86</td>
<td>0.99</td>
<td>1.18</td>
<td>1.18</td>
<td>0.58</td>
<td>1.20</td>
<td>0.07</td>
</tr>
<tr>
<td>D5S2093</td>
<td>164.2</td>
<td>162.7</td>
<td>2.29</td>
<td>2.21</td>
<td>2.14</td>
<td>2.02</td>
<td>0.91</td>
<td>2.25</td>
<td>0.00</td>
</tr>
<tr>
<td>D5S671</td>
<td>172.7</td>
<td>167.6</td>
<td>2.88</td>
<td>2.82</td>
<td>2.60</td>
<td>2.32</td>
<td>1.05</td>
<td>2.88</td>
<td>0.00</td>
</tr>
<tr>
<td>D5S425</td>
<td>179.1</td>
<td>171.4</td>
<td>3.41</td>
<td>3.35</td>
<td>3.11</td>
<td>2.80</td>
<td>1.33</td>
<td>3.41</td>
<td>0.00</td>
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<tr>
<td>D5S2108</td>
<td>182.4</td>
<td>173.5</td>
<td>0.55</td>
<td>0.79</td>
<td>1.10</td>
<td>1.15</td>
<td>0.56</td>
<td>1.14</td>
<td>0.10</td>
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<tr>
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<td>187.8</td>
<td>174.9</td>
<td>1.30</td>
<td>1.32</td>
<td>1.35</td>
<td>1.28</td>
<td>0.52</td>
<td>1.35</td>
<td>0.05</td>
</tr>
<tr>
<td>D5S2030</td>
<td>190.9</td>
<td>177.7</td>
<td>-0.02</td>
<td>-0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

LOD scores were calculated at different θ values for each marker with individual 22 designated as unaffected (top row) and then as affected (bottom row).
two have a history of inflammatory ocular disease, in addition to FCD. Individual 21 had recurrent bilateral birdshot choroidopathy with a chronic anterior uveitis; 24 had a history of recurrent nodular scleritis and underwent argon laser trabeculoplasty for elevated intraocular pressures. As chronic anterior segment inflammation may distort the FCD phenotype, individuals 21 and 24 were not included in the severity analysis.

DISCUSSION

Here, we report a new locus for late-onset FCD in a large family, with the critical interval mapped to 5q33.1–35.2. Haplotype analyses refined the interval to a 27 Mb (29 cM) region with a maximum two-point parametric LOD score of 3.41. Although the maximum LOD score of 3.41 is only slightly higher than the traditional limiting value of 3.0, it represents the maximum theoretical obtainable value for this family. Identification of the third FCD locus strongly suggests heterogeneity for late-onset FCD, contrary to early-onset that is casually associated with \( \text{COL8A2} \). The milder phenotype noted in the present family may be a consequence of environmental and/or other genetic factors. Sequential retroillumination photography of additional families linked to \( \text{FCD3} \) are needed to quantitatively determine the rate of progression. At the same time, it is important to identify additional \( \text{FCD3} \)-linked families to determine whether the slower progression of the phenotype is a characteristic of the locus or of the particular mutation carried by family MO.

\( \text{FCD3} \) maps to a gene-rich region that harbors 97 annotated genes, suggesting that traditional positional cloning approaches will be challenging. Nonetheless, the eventual identification of the genetic lesion in \( \text{FCD3} \) will illuminate the etiopathology of FCD and potentially inform the variable expressivity and clinical progression that typifies this disorder.

Acknowledgments

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FIGURE 2. Photographic documentation of Fuchs corneal dystrophy associated with the \( \text{FCD3} \) locus. Individual nonconfluent guttae are visible on slit beam and specular reflection of corneal endothelium of individuals 22 (A) and 25 (B). Of note, individual 25, a 47-year-old woman, is the daughter of individual 22, a man with the same distribution of guttae at age 73.

FIGURE 3. Development of disease severity over time in relation to \( \text{FCD1} \) and \( \text{FCD2} \). Data points represent average age per level of severity within each locus. Individuals with the disease haplotype in each family began to demonstrate early, subclinical signs of disease in the fourth decade of life, with slower progression over time evidenced in the family linked to 5q. Individuals older than 60 years in the \( \text{FCD3} \)-linked family experienced significantly reduced severity relative to those associated with the \( \text{FCD1} \) (\( P < 0.0001 \)) and \( \text{FCD2} \) (\( P < 0.001 \)) loci.
References