No Pathogenic Mutations Identified in the COL8A1 and COL8A2 Genes in Familial Fuchs Corneal Dystrophy

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PURPOSE. To investigate the genetic basis of late-onset, familial Fuchs endothelial corneal dystrophy (FECD) through screening of the COL8A1 and COL8A2 genes, in which mutations have been associated with both early and late-onset, familial and sporadic FECD.

METHODS. DNA extraction, PCR amplification, and direct sequencing of the COL8A1 and COL8A2 genes was performed in affected and unaffected members of 15 unrelated families with two or more members with late-onset FECD.

RESULTS. Screening of the COL8A1 gene did not reveal sequence variants in any affected individuals from the 15 FECD families. In the COL8A2 gene, the previously identified mutations presumed to play a pathogenic role in cases of familial FECD (Arg155Gln, Leu450Trp, and Gln455Lys) were not discovered in any of the affected patients. A mutation previously considered causative of FECD (Arg434His) was shown not to segregate with the disease in the one family in which it was identified. Two previously identified single-nucleotide polymorphisms (SNPs), Pro575Leu and Pro586Pro, were identified in a single affected individual and three affected individuals (two families), respectively.

CONCLUSIONS. The Arg434His mutation in the COL8A2 gene, previously associated with FECD, has been shown not to segregate with the disease phenotype, and thus may not be considered a disease-causing mutation. The absence of pathogenic mutations identified in the COL8A1 or COL8A2 genes in affected members of 15 pedigrees with familial FECD indicates that other genetic factors are involved in the development of this autosomal dominant corneal dystrophy. (Invest Ophtalmol Vis Sci. 2006;47:3787–3790) DOI:10.1167/iovs.05-1635

Fuchs endothelial corneal dystrophy (FECD; MIM 136800; Mendelian Inheritance in Man; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) is a dominantly inherited disorder of the corneal endothelium and is one of the most common indications for corneal transplantation. In 2001, Biswas et al. published the results of linkage analysis in a family with an early-onset form of FECD, demonstrating linkage to a 6- to 7-cM region on the short arm of chromosome 1 (1p34.3-p32). The collagen, type VIII, alpha 2 (COL8A2) gene, which has been localized to this interval, was identified as both a positional and functional candidate gene, as it is highly expressed in Descemet’s membrane, the corneal endothelial cell basement membrane. A missense mutation (Gln455Lys) was identified in the COL8A2 gene that segregated with the disease phenotype in this family, as it was present in 12 affected members of the family and absent in 3 unaffected family members. A recently published report by Gottsch et al. also identifies a missense mutation in the COL8A2 gene in a five-generation pedigree with early-onset FECD. A genome-wide linkage analysis also demonstrated linkage to 1p34.3-p32, prompting the screening of the COL8A2 gene. All 17 affected individuals in whom COL8A2 gene screening was performed demonstrated a Leu450Trp mutation, which was not identified in five unaffected individuals or in >200 control chromosomes. Thus, convincing evidence exists to support the role of the COL8A2 gene in early-onset FECD.

Although Biswas et al. identified the Gln455Lys mutation in two additional families with an early-onset form of FECD, this mutation was not identified in 115 unrelated patients with the typical late-onset FECD. In addition, Gottsch et al. did not identify this mutation or the Leu450Trp mutation in the pedigrees of 62 pedigrees with late-onset FECD, leading to the conclusion that these two mutations are associated only with a rare early-onset subtype of FECD.

The COL8A1 gene, which encodes the alpha 1 chain of collagen type VIII, is also highly expressed in Descemet’s membrane and has been identified as a potential candidate gene for FECD (Hagstrom SA, et al. IOVS 2002;43:ARVO E-Abstract 1730; Fujimaki T, et al. IOVS 2003;44:ARVO E-Abstract 3868). A recently developed animal model has demonstrated that targeted inactivation of the COL8A1 and COL8A2 genes results in normal development of most organs, except for abnormalities of the anterior segment of the eye, including Descemet’s membrane and corneal endothelium. Thus, in this study, we sought to investigate further the role of the COL8A1 and COL8A2 genes in familial, late-onset FECD through screening of both genes in affected families.

MATERIALS AND METHODS

The researchers adhered to the tenets of the Declaration of Helsinki in the treatment of the subjects reported herein. Study approval was obtained from the institutional review board at The University of California, Los Angeles (UCLA MIRB 94-07-245-21).

Patient Identification and DNA Collection and Preparation

Patients who presented to one of the authors (AJA) with FECD and had at least one family member with a confirmed diagnosis of FECD were offered enrollment in the study. The diagnosis of FECD was based on
the presence of greater than 2 mm of confluent central corneal endothelial guttae in each eye (Krachmer grade 4 or higher), or histopathologically confirmed FECD after the performance of penetrating keratoplasty in one or both eyes. In addition, any patients who demonstrated endothelial changes associated with posterior polymorphous corneal dystrophy were excluded.

After an explanation of the nature and possible consequences of study enrollment were explain to each potential study subject, an informed consent to participate in the research was obtained from each affected proband. When available and interested in study participation, affected and unaffected relatives were enrolled after a slit lamp examination was performed to confirm their affected or unaffected status. Genomic DNA was obtained from peripheral blood samples and/or from buccal epithelial cells (CytoSoft brush, CP-5B; Medical Packaging Corp., Camarillo, CA). Genomic DNA was prepared from the buccal epithelial cells and peripheral blood leukocytes in a DNA spin protocol (QIAamp DNA Mini Kit; Qiagen, Valencia, CA).

**PCR Amplification and DNA Sequencing**

The coding regions of the **COL8A1** and **COL8A2** genes were amplified by polymerase chain reaction (PCR) with custom-designed primers (Primer 3; http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi; provided in the public domain by Massachusetts Institute of Technology, Cambridge, MA) shown in Table 1.

All primers were designed so that they would be positioned on intronic segments at least 80 nucleotides on either side of the intron-exon boundary, to ensure complete reading of the exons. PCR amplification and DNA sequencing reaction conditions used were as described previously. Nucleotide sequences, read manually as well as on computer (Mutation Surveyor ver. 2.2; Softgenetics, State College, PA), were compared with the published cDNA sequence for **COL8A1** (NM_001850) and **COL8A2** (NM_005202; http://www.ncbi.nlm.nih.gov/GenBank; provided in the public domain by NCBI, Bethesda, MD).

**RESULTS**

Fifteen affected probands with a family history of classic, late-onset FECD were enrolled in the study. The affected probands ranged in age from 37 to 81, with an average age of 61 years. Eight of the probands had only one affected family member, whereas seven had two or more affected family members. Nine probands reported having an affected parent, nine had an affected sibling, and two had an affected child.

Screening of the **COL8A1** gene in the affected individuals failed to reveal any coding region allelic variants. Screening of the **COL8A2** gene did not reveal the presence of any of the three mutations previously associated with either early-onset (Leu450Trp and Gln455Lys) or late-onset (Arg155Gln) familial FECD. As none of the other mutations in the **COL8A2** gene that has been associated with late-onset FECD (Arg454His) was identified in two affected siblings in one family (Fig. 1). However, this mutation was not identified in the siblings' affected mother (II-3), as expected; instead, it was identified in the siblings' father (II-2). Although this individual (II-2) demonstrated trace corneal endothelial guttae in one eye and 1+ guttae in the other, as the guttae were not confluent in either cornea, he did not meet the criteria established for classification of the affected phenotype and was thus classified as unaffected.

Two previously described polymorphisms were identified as well: c.1751C→T (Pro575Leu) was identified in the heterozygous state in a single affected proband and c.1765C→T (Pro586Pro) was present in two of three affected members of one family, and in one of two affected members of a second family (heterozygous in each).

**DISCUSSION**

Type VIII collagen is a short-chain collagen (as it possess a short triple-helical domain), composed of two α1 chains and one α2 chain, that is a major component of Descemet’s membrane. Although the function of type VIII collagen is not known, its close association with endothelial cells has led to its proposed role in determining cell phenotype. The selection of the **COL8A1** gene as a positional and functional candidate gene for an early-onset variant of FECD in two large families has led to the identification of two mutations (Leu450Trp and Gln455Lys) that satisfy many of the required criteria to be considered pathogenic. As none of the affected subjects in our study had a history of early-onset FECD, the fact that neither of these mutations was identified in this study is not surprising.

The association between mutations in the **COL8A1** gene and an early-onset variant of FECD led us to question whether mutations in the **COL8A1** gene might be associated with the more common late-onset variant of FECD. Whereas both the **COL8A1** and **COL8A2** genes have been selected for screening in conditions in which type VIII collagen is expressed in the diseased tissue of interest, we are unaware of any published reports of **COL8A1** screening in patients with a corneal endothelial dystrophy. The absence of identified coding region mutations in the **COL8A1** gene in this study, as well as in unpublished investigations (Hagstrom SA, et al. *IOVS* 2002;43: ARVO E-Abstract 3868), indicates that the **COL8A1** gene does not appear to play a role in the late-onset form of FECD.

Evidence to support the role of the **COL8A2** gene in the more common, late-onset form of the disease has been provided by the identification of three missense mutations in the **COL8A2** gene that has been associated with late-onset FECD (Arg454His) in two affected siblings in one family (Fig. 1). However, this mutation was not identified in the siblings’ affected mother (II-3), as expected; instead, it was identified in the siblings’ father (II-2). Although this individual (II-2) demonstrated trace corneal endothelial guttae in one eye and 1+ guttae in the other, as the guttae were not confluent in either cornea, he did not meet the criteria established for classification of the affected phenotype and was thus classified as unaffected.

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**Table 1. Primer Sequences Used for **COL8A1** and **COL8A2** Gene Amplification**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Forward Primer (5’–3’)</th>
<th>Reverse Primer (5’–3’)</th>
<th>Product Size (bp)</th>
<th>Exon Size (bp)</th>
</tr>
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<tbody>
<tr>
<td><strong>COL8A1</strong></td>
<td>4</td>
<td>GAAAGTGAGCATTGATTCTCTTATTCTGATT</td>
<td>TCTTACTCTGTCCTTTTAAGGGCTTG</td>
<td>667</td>
<td>328</td>
</tr>
<tr>
<td></td>
<td>5A</td>
<td>TGCCAGGCTGATTTTTTTTAAGGTC</td>
<td>aacgctgacctctgtgctggc</td>
<td>2311</td>
<td>1919</td>
</tr>
<tr>
<td></td>
<td>5B</td>
<td>tccttaagagcaacaggctctg</td>
<td>gtccaccccttttgccccacggcttc</td>
<td>2158</td>
<td>1907</td>
</tr>
<tr>
<td></td>
<td>5C</td>
<td>gaccccaagggaggctggattt</td>
<td>tttttggtgctctttttct</td>
<td>497</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>5D</td>
<td>ctgagggcttgaagagccagaags</td>
<td>tcgctgctggattgctaggcaggtg</td>
<td>2511</td>
<td>1919</td>
</tr>
<tr>
<td><strong>COL8A2</strong></td>
<td>1</td>
<td>TGGCAATTGCTGCTGAGCCTTT</td>
<td>AGGGTTGGTGGCTGTTTCTTCTTCT</td>
<td>2511</td>
<td>1919</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>GGCCATGCTGCAATCGGAGGATGAC</td>
<td>cccagactacccctgctgcttcg</td>
<td>2511</td>
<td>1919</td>
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<tr>
<td></td>
<td>2B</td>
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<td>2C</td>
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<td>cgcctggtcccttgtagagcagtc</td>
<td>2511</td>
<td>1919</td>
</tr>
</tbody>
</table>

* COL8A1 exon 5 and COL8A2 exon 2 were amplified as a large fragment by flanking primers (upper case) and sequenced with additional internal primers (lower case) due to their large size.
affected patients: Arg155Gln, Arg304Gln, and Arg434His2 (Table 2). The Arg155Gln mutation was identified in three affected individuals from one family, as well as in two sporadic cases of FECD, whereas the Arg304Gln and Arg434His mutations were identified only in sporadic cases. As none of the three mutations was identified in at least 150 control chromosomes, each was considered a presumed pathogenic variant.2 However, Kobayashi et al.10 identified the Arg155Gln mutation in 6.9% of unaffected Japanese control individuals, indicating that this mutation is not responsible for FECD, but is a polymorphism, the frequency of which may depend greatly on the population sampled.10 In addition, as the Arg304Gln mutation was identified in only 1 of 115 unrelated FECD patients reported by Biswas et al.,2 in none of the probands from 62 families with FECD reported by Gottsch et al.,4 in none of the 15 unrelated FECD patients reported by Kobayashi et al.,10 and in none of the patients from the 15 families that we report, the identification of this mutation in only 1 of more than 200 unrelated patients with FECD suggests that it most likely represents a rare polymorphism.

The Arg434His mutation also appears to represent a non-pathogenic polymorphism, further undermining previous conclusions that mutations in the COL8A2 gene are associated with the common form of FECD. In a study investigating the role of the COL8A2 gene in keratoconus and kerato-globus, we identified the Arg434His mutation in a 46-year-old African-American woman with keratoconus who had undergone corneal transplantation in her right cornea 17 years earlier. A slit lamp examination of her left cornea revealed features characteristic of keratoconus, but no evidence of FECD (depicted in Figure 2); demonstrated not to segregate with disease phenotype in a family with FECD (this report).

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933235/)

**Figure 1.** Pedigree of a family with late-onset Fuchs endothelial corneal dystrophy. **Filled symbols:** affected individuals; **unfilled symbols:** unaffected individuals; **arrowhead:** the proband. Asterisks: individuals in whom DNA collection and screening of the COL8A1 and COL8A2 genes were performed. Ages are shown below each symbol. R434H, mutant allele (Arg434His missense change in COL8A2); +, wild-type allele at codon 434 in COL8A2.

![Figure 2](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933235/)

**Figure 2.** Slit lamp photomicrograph of the left cornea of a 46-year-old woman with keratoconus in whom the Arg434His mutation was identified in the COL8A2 gene. A Fleischer ring is noted, but endothelial guttue are not present.

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**Table 2.** Reported Pathogenic COL8A2 Sequence Variants in FECD

<table>
<thead>
<tr>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Evidence for Pathogenicity</th>
<th>Evidence against Pathogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late-onset (classic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial FECD</td>
<td>c.471G→A</td>
<td>p.Arg155Gln</td>
<td>Present in three affected English individuals in one family; not present in 184 control chromosomes2</td>
</tr>
<tr>
<td>Sporadic FECD</td>
<td>c.471G→A</td>
<td>p.Arg155Gln</td>
<td>Present in 2 of 115 unrelated English FECD patients; not present in 184 control chromosomes2</td>
</tr>
<tr>
<td></td>
<td>c.918G→A</td>
<td>p.Arg304Gln</td>
<td>Present in 1 of 115 unrelated English FECD patients; not present in 150 control chromosomes2</td>
</tr>
<tr>
<td></td>
<td>c.1308G→A</td>
<td>p.Arg434His</td>
<td>Present in 1 of 115 unrelated English FECD patients; not present in 150 control chromosomes2</td>
</tr>
<tr>
<td>Early-onset (variant)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial FECD</td>
<td>c.1356T→G</td>
<td>p.Leu450Trp</td>
<td>Demonstrated to segregate with the disease phenotype in an American family4</td>
</tr>
<tr>
<td></td>
<td>c.1370C→A</td>
<td>p.Gln455Lys</td>
<td>Demonstrated to segregate with the disease phenotype in English and Australian families5</td>
</tr>
</tbody>
</table>
Two other previously described polymorphisms in the COL8A2 gene were identified in the patients we report with FECD. The Pro575Leu missense mutation identified in one affected proband has been identified previously in individuals with FECD, but is considered a nonpathogenic variant, as it did not segregate with FECD in affected families. In addition, as the Pro586Pro mutation does not produce a change in the encoded amino acid, has been identified previously in control individuals, and did not fully segregate with the disease phenotype in our families, it may also be considered a nonpathogenic variant.

Although the complete coding region for COL8A1 and COL8A2, including the intron–exon boundaries, were sequenced in this study, a promoter mutation, or a change in an intronic or untranslated noncoding region of COL8A1 or COL8A2 could play a role in the pathogenesis of FECD, although there is no evidence to support this possibility. Thus, although there is strong evidence to support a role for the COL8A2 gene in an unusual early-onset variant of FECD, no convincing evidence suggests that either the COL8A1 or COL8A2 gene plays any role in the development of the more common late-onset form of FECD.

References