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**Letter to the Editor – Clinical Case Note**

# Screening of the *COL8A2* gene in an Australian family with early-onset Fuchs' endothelial corneal dystrophy

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## Conflict of Interest Statement

None of the authors has any conflict of interest related to this work.

Early-onset Fuchs' endothelial corneal dystrophy (FECD) is a rare, and almost always familial disease with autosomal dominant inheritance (1). To date, mutations in only the *COL8A2* (*alpha 2 type VIII collagen*) gene (MIM 120252; 1p34.2) have been reported to cause this disease (1, 2). So far, only one Australian family has been reported to develop the disease due to the p.Q455K mutation in *COL8A2* (1). To determine whether mutations in this gene cause the disease in other similar cases in Australia, we screened the entire coding region of the *COL8A2* gene for causative mutations in an affected Caucasian family. The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Southern Adelaide Clinical Human Research Ethics Committee (Adelaide).

Multiple members of the three-generation family (Fig. 1) were independently examined by six ophthalmologists at different times and institutions. Available clinical information is given in Table 1. Two (II.2 and III.2) of the family members were clinically diagnosed with advanced stage disease following their referral to ophthalmologists. Patients II.2 and III.2 underwent unilateral corneal grafting at the ages of 49 and 48, respectively, to prevent vision loss. The histopathological findings confirmed a diagnosis of early-onset FECD (Table 1) (2). Patient II.2 also had keratoconus (since 15 years of age), blue dot congenital cataract and Map dot fingerprint corneal degeneration. Patient IV.2, the youngest member of the family, was clinically diagnosed with early-onset FECD at 21 years of age, but has not undergone any corneal transplantation as yet. She had visual acuities of 6/9 (right eye) and 6/12 (left eye). Clinical details were not available for individual III.4 but other family members reported that he was diagnosed with endothelial corneal dystrophy, keratoconus and Herpes Simplex Virus (HSV) keratitis. His son (IV.3) is also reported to have endothelial corneal dystrophy but has not been examined. It was also reported that patient I.1 had bilateral corneal transplants at the age of 62; therefore, he was inferred to have FECD. Neither individual I.1 nor IV.3 was available for this study. Overall, the family displayed an autosomal dominant mode of inheritance of the disease (Fig. 1).

Four affected family members were recruited (through the Eye Clinic, Flinders Medical Centre, Adelaide) to participate in this study following written informed consent. Blood samples were collected and genomic DNA was extracted as previously described (3).

The two coding exons and their flanking sequence incorporating the 5'UTR (untranslated region), splice sites and stop codon of the *COL8A2* gene were amplified by polymerase chain

reaction (PCR) using gene-specific primers (Supplementary Table1) in each DNA sample. The PCR products were sequenced as previously described (3).

We identified only one intronic SNP (rs274754; G/A) in individuals II.2 and IV.2, as reported in the dbSNP 135 database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp). The minor allele frequency of the G allele of this SNP in the 1000Genomes Project (www.1000genomes.org) is 0.491. This SNP is highly unlikely to be causative as it is a common polymorphism and is present in individuals without early-onset FECD.

Neither of the previously reported disease-causing mutations in *COL8A2* (p.L450W and p.Q455K) (1, 2), were observed in this family and no novel mutations were identified. This is only the second study to investigate this gene in Australian cases with early-onset FECD. Our finding suggests that mutation in this gene does not contribute to the disease and as yet undetermined gene/s are likely to account for the disease in this Caucasian Australian family.

Our results are supported by other studies that also reported absence of mutation in the *COL8A2* gene in familial or sporadic early-onset FECD in French and Indian cases (4, 5). Given the heterogeneity of FECD, it is likely that mutations in genes other than *COL8A2* lead to the early-onset disease (2). Two members (II.2 and III.4) of the studied family also have other corneal conditions (Table 1), suggesting that there may be genetic overlap between FECD and other diseases including keratoconus. Keratoconus has never been reportedly associated with familial or sporadic early-onset FECD in patients with *COL8A2* mutations, which indicates a further distinction between the present family and those reported in previous studies.

In summary, mutations in the *COL8A2* gene do not contribute to all cases of early-onset FECD. We hypothesize that mutations in as yet undetermined gene/s are likely to account for the disease in some cases with early-onset disease.

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## Figure legends

**Figure 1.** The pedigree of the Caucasian Australian family with familial early-onset FECD. Squares = males; circles = females; white shapes = unaffected; black shapes = clinically confirmed as affected by FECD; * = DNA available for genetic screening of *COL8A*2; Grey shape = Affected by Keratoconus only.

**Table 1:** Clinical and histopathological findings in individuals from the Caucasian Australian family with early-onset Fuchs' endothelial corneal dystrophy (FECD). KC = Keratoconus; HSV = Herpes Simplex Virus; LE = left eye; RE = right eye; DM = Descemet's membrane; EM = Electron microscopy.

| **Individual** | **Diagnosis** | **History & Clinical Findings** | **Corneal Histopathology** |
|---|---|---|---|
| I.1 | FECD | Penetrating keratoplasty in both eyes, presumed for FECD | Not available |
| II.2 | KC<br>FECD | Both eyes:<br>Central corneal thinning<br>Fleischer rings<br>Abnormal endothelium<br>Corneal oedema<br>Blue-dot congenital cataracts<br>Map-dot fingerprint corneal degeneration<br>Penetrating keratoplasty LE 1991, RE 2001 | LE: Attenuated endothelium, no posterior nodularity of DM, thickened 3 layered DM and stromal deposits of unknown significance on EM.<br>RE: Reduced endothelial density, stromal oedema, subepithelial bullae. |
| III.2 | FECD | Corneal oedema RE<br>Abnormal endothelium both eyes<br>Penetrating keratoplasty RE 2002 | RE: Attenuated endothelium, thickened 4 layered DM on EM |
| III.4 | FECD<br>KC<br>HSV keratitis | Not examined | Not available |
| IV.2 | FECD | Both eyes:<br>Corneal guttata<br>Corneal oedema<br>Reduced endothelial density | Not available |
| IV.3 | FECD | Not examined | Not available |

**Supplementary Table 1.** Primers and PCR conditions used for mutation screening of the *COL8A2* gene. Primer sequences, primer annealing temperature in degrees centigrade, and the use of Q solution are indicated. Primers were designed using the Primer3 software (http://frodo.wi.mit.edu/primer3/).

| **Amplimers** | **Forward Primer 5'>3'** | **Reverse Primer 5'>3'** | **Annealing Temp (°C)** | **Q Solution Added?** |
|---|---|---|---|---|
| 1 | ATTCGGAATTGCTTCTCAGC | GCCCTCTGGGGTATTAGGAA | 57 | No |
| 2.1 | TCCTCTCCCGTGTACCTCAT | CCTGGTTTTCCAGGGATAGTA | 57 | No |
| 2.2 | CAGGAATACGAGGGGACCA | AGTCCTGGCATCCCATAGC | 56 | Yes |
| 2.3 | GGAGACAAGGGTGAGTCTGG | AGGAAGTCCCCTCTCACCTG | 57 | Yes |
| 2.4 | TGACCAGGGGCCTAGTGG | CGATGCCAGTCTCATCGAA | 57 | Yes |
| 2.5 | CTGGCTCCCCTGGAATCA | CCGGCACGTTGTTCTTGTA | 57 | Yes |
| 2.6 | CGTGAAATTTGACCGGACTC | CATGCAGGGAGAAAGCAAGT | 57 | No |