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The genetics of Fuchs' corneal dystrophy

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Abstract

Fuchs' corneal dystrophy (FCD) is a common late-onset genetic disorder of the corneal endothelium. It causes loss of endothelial cell density and excrescences in the Descemet membrane, eventually progressing to corneal edema, necessitating corneal transplantation. The genetic basis of FCD is complex and heterogeneous, demonstrating variable expressivity and incomplete penetrance. To date, three causal genes, *ZEB1*, *SLC4A11* and *LOXHD1*, have been identified, representing a small proportion of the total genetic load of FCD. An additional four loci have been localized, including a region on chromosome 18 that is potentially responsible for a large proportion of all FCD cases. The elucidation of the causal genes underlying these loci will begin to clarify the pathogenesis of FCD and pave the way for the emergence of nonsurgical treatments.

Keywords

cornea guttata; corneal endothelium; Descemet membrane; epithelial–mesenchymal transition; FCD; Fuchs' corneal dystrophy; guttae; oxidative stress; unfolded protein

Fuchs' corneal dystrophy (FCD; MIM 136800), first described by the Austrian ophthalmologist Ernst Fuchs in 1910, is a common hereditary disease of the corneal endothelium [1–4,101]. The first clinical signs appear in approximately the fourth decade of life with the formation of excrescences in the central Descemet membrane termed *guttae* (Latin: drops), eventually progressing in some cases to corneal edema [5–7]. Clinically, the progression is typically graded on a 0–5 scale first used by Krachmer *et al.*, with grade 0 indicating an absence of guttae, grade 1 representing 12 or more central guttae, increasing up to grade 5, denoting confluent central guttae with corneal edema [8]. The prevalence of FCD is generally considered to be approximately 4% in individuals above 40 years of age, although higher estimates have been reported in some populations; prevalences of 9, 6.5 and 21.6% were reported in Icelandic, Singaporean and inbred American populations, respectively [9–12]. The disease is typically inherited in an autosomal dominant fashion, although females are more typically affected than males by a two-to-one ratio, precluding strictly Mendelian population-wide autosomal dominant inheritance with high penetrance [1,4,8,13–15].

Two major genetic techniques have been used to investigate the genetic basis for FCD. Linkage analysis, which uses a panel of single nucleotide polymorphism (SNP) or short tandem repeat (STR) markers to identify a causal haplotype in familial cases, possesses the

ability to identify regions harboring rare mutations, provided those mutations have a large effect (i.e., high penetrance). Linkage analysis is often followed by whole-exome genetic sequencing, which investigates all regions of the human genome that encode expressed proteins and can therefore find the causal mutation within a linkage interval, provided the mutation resides in a coding region. The second technique is the genome-wide association study (GWAS), which uses a SNP panel, often larger than those used in linkage studies, to genotype a cohort of unrelated individuals at hundreds of thousands or millions of SNPs. Because of the increased resolution provided by a larger panel, along with a sample size that is not limited by the constraints of a familial study, GWAS can detect regions of interest with greater resolution than linkage analysis (several kilobases for GWAS vs several megabases for linkage). GWAS can also identify smaller effects, identifying loci that are associated with predisposition to a disease, but which are not necessarily sufficient for pathogenesis. A major drawback of GWAS is its inability, due to the use of a panel of common SNPs, to identify an association between rare alleles and disease. To date, traditional techniques of linkage analysis, genetic sequencing and GWAS have succeeded in identifying three FCD genes and four causal FCD loci, along with numerous linkage peaks and susceptibility loci. However, the complex and heterogeneous genetic basis for FCD has thus far prevented the elucidation of the majority of the genetic load of FCD.

Pathology

FCD results primarily from a decrease in function of the corneal endothelium. A decrease in endothelial cell density, including discontinuity of the endothelial cell layer in some cases, has been observed using electron microscopy [16]. Function of the remaining endothelial cells may also be impaired, although data are mixed whether barrier function, pumping function, or both are impaired. In a study of FCD patients without epithelial edema, Burns *et al.* found an increase in endothelial permeability, but no difference in pump rate [17]. By contrast, Wilson *et al.* found no difference in permeability, but decreased pump rate in FCD endothelium [18].

Studies on isolated FCD corneas suggest that pump site density could vary based on disease stage. Geroski *et al.* found that in corneas with moderate guttae (i.e., relatively early-stage disease), Na⁺/K⁺ ATPase pump density was significantly increased [19]. By contrast, FCD corneas with a high density of guttae but without edema have a moderately reduced density of Na⁺/K⁺ ATPase, and edematous FCD corneas have a severely reduced density [20,21]. Recently, Kopplin *et al.* found that central corneal thickness increases with increasing severity of FCD, supporting a loss of pump and/or barrier function in FCD endothelium, even before the development of corneal edema [22]. Central corneal thickness was increased even in early-stage (Krachmer grades 1–3) FCD, suggesting that increased pump density may not completely compensate for decreased barrier function in early FCD.

Endothelial cell morphology is also altered, with the appearance of intracellular vacuoles, expression of epithelial characteristics such as desmosomes, along with cytoplasmic processes and collagen fibril production characteristic of fibroblasts [16]. The Descemet membrane is markedly thickened, and excrescences extend posteriorly, displacing the cytoplasm of overlying endothelial cells [16,23].

FCD loci identified by genetic linkage analysis

FCD1

The first locus linked to FCD, *FCD1*, was localized to a 26.4-Mb interval between 13pTel and 13q12.13 (Tables 1 & 2) [24]. Sundin *et al.* described a four-generation pedigree of a Caucasian family, recruited after examination of an affected proband. Consistent with the

previously reported 2:1 sex ratio in individuals affected by FCD, females represented the majority of affected individuals in the family, with 21 females and only 11 males, although the overall number of females in the pedigree ($n = 41$) was similarly greater than the number of males ($n = 24$). A total of 13 affected and three unaffected individuals across three generations were recruited for genotyping, and linkage was determined using 399 STR markers from the MD10 genotyping panel, obtaining significant two-point logarithm of odds (LOD) scores of 3.91 at D13S1236 and 3.80 at D13S1304.

Slit lamp biomicroscopy and specular microscopy revealed phenotypically typical late-onset FCD. Notably, two children in the fourth generation of the pedigree were diagnosed with clinically significant disease: one 13-year-old girl with a Krachmer grade of 1, and one 10-year-old boy, with a Krachmer grade of 2. In each of these cases, both parents were affected, although genotyping data did not support the possibility that either pair of parents was consanguineous. The phenotype of the children, like the phenotype of other affected family members, was typical of late-onset FCD and unusual only in its age of onset.

Sequential retroillumination photographs taken over a 34-month timespan demonstrated a significant progression of *FCD1*-linked disease. The number of guttae in each cornea was observed to increase an average of 29% over the course of 30 months, exhibiting an exponential increase over time. A significantly increased density of guttae was also noted in the inferotemporal quadrant of the cornea relative to the other three quadrants.

FCD2

Sundin *et al.* localized *FCD2*, the second locus for FCD, to 18q21.2–18q21.32 in three large families [25]. *FCD2* was the first locus identified in more than one familial case of FCD, suggesting the possible existence of a common locus for FCD on chromosome 18. Linkage analysis was performed on 43 affected and 33 unaffected individuals using 1107 STR markers, and maximum two-point LOD scores of 3.41, 2.89 and 2.45 were obtained for the three families. While the disease in all three families linked to the same region on chromosome 18, the disease-linked haplotype was different in each family, leaving open the possibility of heterogeneity of the locus.

Consistent with previously reported cases of FCD, the families linked to chromosome 18 exhibited autosomal dominant inheritance and an affection rate that was biased toward women, with an observed total of 27 affected women and 19 affected men in three families. Unlike *FCD1* and similar to more typical cases of late-onset FCD, none of the affected individuals had two affected parents and the youngest affected individual was 32 years old. Interestingly, the phenocopy rate in the three *FCD2*-linked families was 22%, significantly higher than the general population affection rate of FCD, suggesting the possibility of a multigenic cause in these families. In addition, 10% of individuals with the disease haplotype were unaffected, further highlighting the complexity of inheritance associated with this locus.

When two families whose disease links to the *FCD2* locus were examined using retroillumination photography, the distribution of guttae was found to be similar to *FCD1*, although the course of the disease appears to be different [26]. Like individuals linked to *FCD1*, those affected by FCD linked to chromosome 18 had increased density of guttae in the inferotemporal region, suggesting that this may be a common characteristic of FCD. In contrast to *FCD1*, disease caused by *FCD2* appears to progress more slowly, with a regression model suggesting that individuals with disease linked to *FCD2* experience a 5% annual increase in number of guttae versus a 24% annual increase for *FCD1*.

FCD3

FCD3, the third locus for FCD, was localized to 5q33.1–5q35.2, with a maximum two-point LOD score of 3.39 [27]. A three-generation family comprising 26 individuals was collected, with 17 of those individuals examined by slit lamp biomicroscopy. Ten individuals were diagnosed with FCD, of whom eight were female and two were male. A SNP array was used to localize the causal locus to chromosome 5, and a STR panel refined the region to a 27-Mb interval. Analysis of Krachmer scores and ages of affected individuals revealed that *FCD3* causes disease that is mild and progresses more slowly than *FCD1*- or *FCD2*-linked disease.

FCD4

A fourth FCD locus, *FCD4*, was recently identified in the region 9p22.1–9p24.1 [28]. Seventeen individuals from a four-generation pedigree were recruited, and an initial attempt to localize their disease under the assumption of Mendelian inheritance did not identify any regions of significant linkage. In some affected individuals, causal mutations were identified in *ZEB1*, mutations in which have previously been linked to posterior polymorphous corneal dystrophy (PPCD) [29]. Reanalysis of the linkage data conditioned to the presence of the *ZEB1* mutation identified the *FCD4* locus on chromosome 9, with a maximum two-point LOD score of 2.43. The linkage region was refined using STR markers, resulting in maximum LOD scores of 3.09 at D9S168 and 3.20 at D9S256, and a linkage interval spanning 14.3 Mb between recombinations at D91681 and D9S1684. Notably, not all affected individuals in the family possessed the *FCD4* haplotype. Either the *FCD4* haplotype or the *ZEB1* mutation was sufficient to cause a disease phenotype, and individuals who possessed both the causal *FCD4* haplotype and the causal *ZEB1* allele exhibited more severe disease than individuals with only one causal factor, demonstrating an interaction between the two pathogenic alleles [28].

Other linkage peaks

Using SNP linkage panels on a large cohort of FCD-affected individuals and their family members, Afshari *et al.* identified four new putative dominant FCD loci [30]. Linkage was performed using 92 individuals from 22 families, consisting of mostly small pedigrees but also including one large five-generation pedigree of 63 individuals, of whom only 22 were used for the study. The four loci identified were on chromosome 1, with a maximum two-point heterogeneity LOD (HLOD) score of 2.02 at rs760594, chromosome 7 (HLOD 1.92, rs257376), chromosome 15 (HLOD 2.48, rs352476) and chromosome 17 (HLOD 2.10, rs938350). Although this study did not identify dominant linkage peaks above the typical LOD threshold of 3.0, the authors speculate that testing a greater number of families may have given a significant result.

In a follow-up study, Li *et al.* identified additional linkage peaks using 215 individuals from 64 families [31]. Six linkage peaks were identified with maximum dominant HLOD scores greater than 2: chromosome 5 (HLOD 2.22, rs1301475), chromosome 8 (HLOD 2.65, rs2466216), chromosome 10 (HLOD 3.37, rs1889974), chromosome 15 (HLOD 3.53, rs235512), chromosome 20 (HLOD 2.17, rs674630) and chromosome X (HLOD 2.08, rs1990383). While only two of the peaks identified by Li *et al.* reach an HLOD threshold of 3.0, these linkage peaks and the peaks identified by Afshari *et al.* nevertheless may represent promising targets in understanding the causality of FCD.

Causal genes

ZEB1

ZEB1, encoding the Zinc finger E-box-binding homeobox 1 transcription factor also known as TCF8, was initially identified as the causal gene in a family affected by PPCD (MIM

122000) [29]. Subsequently, Mehta *et al.* screened 74 Chinese individuals with FCD for *ZEB1* mutations, identifying a novel variant, p.N696S, which was present in one sporadic case and absent in 93 control individuals [32]. While this represented the first mutation in *ZEB1* suggested to possibly cause FCD, segregation data were unavailable, and functional analyses were not performed to confirm the connection.

Riazuddin *et al.* then identified a causal mutation in *ZEB1* in a large multigenerational family whose disease also linked to the *FCD4* locus on chromosome 9 [28]. The initial mutation identified was the missense mutation p.Q840P, and four additional pathogenic mutations (p.N78T, p.P649A, p.Q810P and p.A905T) were discovered when the exonic region of *ZEB1* was sequenced in 384 unrelated FCD-affected individuals. Three of these mutations (p.Q810P, p.Q840P and p.A905T) occurred at sites that are highly evolutionarily conserved in vertebrates, while the remaining two occur at moderately conserved sites. An *in vivo* assessment of the functionality of the *ZEB1* variants using zebrafish embryos revealed that, unlike wild-type *ZEB1*, injection of human mRNA containing each of the FCD-linked mutations was unable to fully rescue developmental abnormalities caused by morpholino oligonucleotide knockdown of endogenous *ZEB1*. Two variants, p.N78T and p.Q810P, partially rescued the phenotype, while the other three variants demonstrated phenotypes identical to morpholino injection alone. Importantly, while PPCD and FCD are both caused by mutations to *ZEB1*, all PPCD-causing alleles identified thus far have contained frameshift, nonsense, or lost-start-codon mutations, while FCD-causing alleles contain missense mutations [28,29,32,33]. Studies of PPCD corneas from individuals harboring *ZEB1* mutations have noted overexpression of COL4A3, mutations in which cause cor-neal dystrophy associated with Alport syndrome (MIM 120070), suggesting a potential role for COL4A3 dysregulation in the pathogenesis of FCD [29,34].

SLC4A11

A second FCD gene, *SLC4A11*, encoding a sodium-borate cotransporter, was initially identified as underexpressed in FCD corneal endothelium compared with control endothelium in a study using serial analysis of gene expression (SAGE) [35]. Later, Vithana *et al.* implicated recessive *SLC4A11* alleles in congenital hereditary endothelial dystrophy (CHED), while Desir *et al.* identified other recessive alleles in Harboyan syndrome, which is characterized by corneal dystrophy and perceptive deafness [36,37].

Vithana *et al.* sequenced the *SLC4A11* gene in 89 FCD-affected individuals, of whom 64 were of Chinese ethnicity and 25 were of Indian ethnicity [38]. Four previously unreported mutations were identified, which were absent in 354 ethnically matched controls: p.S33SfsX18 in a Chinese sporadic case, p.E399K in an Indian sporadic case, p.G709E in a Chinese familial case, and p.T754M in a Chinese sporadic case. Whereas the mutations in CHED were inherited in an autosomal recessive fashion [36], the alleles that caused FCD acted in an autosomal dominant pattern [38].

Functional analysis of the three missense mutations in *SLC4A11* revealed significantly decreased total SLC4A11 protein in p.E399K and p.G709E transfected HEK293 cells, and decreased mature glycosylated SLC4A11 expression in p.E399K, p.G709E and p.T754M mutants when compared with wild-type-transfected controls. Confirming the previous SAGE results by Gottsch *et al.*, Vithana *et al.* detected SLC4A11 expression in the corneal endothelium using reverse-transcription PCR [35,36].

Additional FCD mutations were identified in an American cohort by Riazuddin *et al.* after sequencing all coding regions of *SLC4A11* in 192 FCD-affected and 192 unaffected control individuals [39]. The missense mutations p.E167D, p.R282P, p.Y526C, p.V575M, p.G583D, p.G742R and p.G834S were identified in FCD-affected individuals and were

absent from all 192 sequenced control individuals, representing a statistically significant enrichment of these alleles in FCD cases. Four of the mutation positions, p.E167, p.R282, p.G583 and p.G742, were conserved across all species with an *SLC4A11* ortholog, while two, p.V575 and p.G834, were conserved only in mammals; p.Y526 was poorly conserved. Sorting Intolerant From Tolerant (SIFT) and PolyPhen predicted that five of the mutations are pathogenic; p.E176D and p.Y526C were predicted to be benign. Similar to the results of Vithana *et al.* [38], HEK293T cells transfected with two of the variants (p.R282P and p.G583D) expressed decreased mature SLC4A11 protein relative to controls, while two others expressed increased immature protein (p.Y526C and p.V575M). Subsequent research by Liu *et al.* demonstrated that short hairpin RNA silencing of *SLC4A11* in cultured human corneal endothelial cells decreased cell proliferation and viability and increased apoptosis, suggesting a possible causal mechanism for CHED and FCD due to *SLC4A11* mutations [40].

LOXHD1

A third casual gene for FCD was recently identified on chromosome 18 by Riazuddin *et al.* [41]. The first causal mutation was identified in a family whose disease was previously linked to the *FCD2* locus, although *LOXHD1* does not reside in the *FCD2* critical interval [25]. Due to the high phenocopy and impenetrance rate in individuals with *FCD2*-linked disease, Riazuddin *et al.* expanded the conservative linkage interval, defined by two recombination events at each boundary, by 5 Mb on each side, and designed a custom exon-capture array to sequence a total of 36 Mb on chromosome 18 [41]. One affected and one unaffected individual were sequenced in three families whose FCD linked to the *FCD2* locus. In two of three *FCD2*-linked families, no coding causal mutation was identified, but sequencing did identify a p.R547C change in *LOXHD1*, a variant that was not previously identified by the 1000 genomes project or the 1500 exomes from the University of Seattle Human Exome Variant database. The p.R547C variant was also not found in 384 unaffected control individuals and was predicted to be pathogenic by PolyPhen 2.

LOXHD1 transcript was identified by reverse-transcription PCR in human cultured corneal endothelial cells at a level fivefold less than β -actin or GAPDH. It was also observed in the epithelium and endothelium of harvested mouse corneas by immunofluorescence. Immunostaining of the affected proband with the p.R547C mutation demonstrated increased *LOXHD1* staining in the Descemet membrane and corneal endothelium compared with non-*LOXHD1* Fuchs cornea and a keratoconus-affected control, along with apparent aggregation of *LOXHD1* protein.

Riazuddin *et al.* subsequently sequenced the coding region of *LOXHD1* in 207 unrelated individuals with sporadic FCD, identifying 13 additional mutations that were absent in 192 control individuals and predicted to be damaging by the PolyPhen 2 algorithm [41]. To evaluate the functional significance of these additional mutations, two randomly selected variants, p.R157C and p.R751W, along with the original p.R547C variant, were transfected into cultured retinal pigment epithelium cells. All three mutants demonstrated punctate immunostaining for *LOXHD1*, reminiscent of the FCD phenotype and similar to the aggregates observed in the cornea of the affected proband.

Genetic associations

TCF4

In addition to the loci and genes causally linked to FCD, Baratz *et al.* identified an association between FCD and alleles of *TCF4*, a transcription factor located on chromosome 18 that resides within the *FCD2* locus (Table 3) [25,42]. Baratz *et al.* performed a GWAS, correlating common SNP alleles with FCD status. One hundred and thirty cases were

genotyped, along with 260 controls that were matched by age and sex with the cases. A second cohort, composed of 150 cases and 150 controls, was used to replicate findings.

Of 338,727 SNPs, 11 demonstrated an association with FCD status, but only one reached the threshold for genome-wide significance: rs613872, located in the third intron of *TCF4*. The minor (G) allele at this locus was significantly enriched in FCD cases (T = 0.63, G = 0.37) versus controls (T = 0.86, G = 0.14), a finding that was even more pronounced in the replication group (cases: T = 0.57, G = 0.43; controls: T = 0.85, G = 0.15). Notably, the odds ratio associated with the minor allele was 5.5 for heterozygotes and 30 for homozygotes. A risk model, calculated using multiple logistic regression of four FCD-associated SNPs at the *TCF4* locus, predicted FCD status with 76 and 78% accuracy in the discovery group and the replication group, respectively.

Since the publication of the initial GWAS results by Baratz *et al.*, several studies have replicated the association between rs613872 and FCD in different populations. The largest replication study was performed by Li *et al.*, with 450 FCD cases and 360 unaffected controls [31]. Confirming the results of Baratz *et al.*, the minor allele of rs613872 was significantly enriched in FCD cases (cases: T = 0.53, G = 0.47; controls: T = 0.81, G = 0.19). The odds ratio associated with the minor allele was 8.0 under a dominant model.

Riazuddin *et al.* genotyped nine SNPs in 170 affected individuals and 180 controls, along with three large families previously linked to *FCD2* on chromosome 18 [43]. In this cohort, a similar enrichment of the G allele of rs613872 was identified (cases: T = 0.612, G = 0.388; controls: T = 0.870, G = 0.130; odds ratio [OR]: = 4.2). The *TCF4* SNP rs613872 did not fully segregate in any of the three *FCD2* families, suggesting independence between the *FCD2* locus and *TCF4*. However, rs613872 did partially segregate in one *FCD2*-linked family, preventing a strong conclusion from being drawn. Eight out of ten affected individuals were heterozygous for rs613872, while all four unaffected individuals were homozygous for the major allele. Of the two phenocopies, one was the daughter of two affected individuals, one of whom was not genotyped for rs613872.

Likewise, the association between rs613872 and FCD was replicated by Kuot *et al.* in an Australian cohort of 105 FCD cases and 275 unaffected controls [44]. The allelic distribution was nearly identical in this population to those studied by Baratz *et al.* and Riazuddin *et al.* (cases: T = 0.58, G = 0.42; controls: T = 0.85, G = 0.15; OR = 4.05) [41,42]. Three other *TCF4* SNPs were also significantly associated with FCD: rs9954153, rs2286812 and rs17595731. In a group of individuals drawn from the inbred population of Tangier Island (VA, USA), Eghrari *et al.* also described similar findings [11]. The severity of disease was significantly higher in individuals heterozygous or homozygous for the minor allele of rs613872, and the minor allele of rs613872 corresponded with a lower prevalence of subclinical disease and a greater frequency of clinical disease.

Another study was conducted in a Chinese population by Thalamuthu *et al.* [45]. Fifty seven individuals affected by FCD, along with 121 unaffected controls, were genotyped for 18 SNPs within *TCF4*. While the minor allele of rs613872 was not present in the genotyped cohort, two other SNPs in *TCF4* were significantly associated with FCD affection with an odds ratio of >2. Upstream, the frequency of the T allele of rs17089925 was enriched (0.6228 for cases; 0.4174 for controls; OR: 2.381), as was the T allele of rs17089887, located in intron 3 (0.6491 for cases; 0.4504 for controls; OR: 2.567).

Across several different cohorts from diverse populations, polymorphisms near *TCF4* have been consistently linked with an increased prevalence of FCD. However, a causal mutation in *TCF4* has not been identified, and it remains possible that the association represents linkage between the *TCF4* markers and a more distant causal gene.

CLU & TGFBI

CLU, encoding the molecular chaperone clusterin, plays a role in extracellular matrix interaction [46] and is overexpressed in FCD cornea relative to pseudophakic bullous keratopathy cornea and normal controls [47]. Similarly, *TGFBI* encodes TGF- β -induced protein, an extracellular matrix protein mediating cell adhesion, mutations in which are known to cause a variety of corneal dystrophies [48,49]. In their study of 105 FCD cases and 275 controls in an Australian cohort, Kuot *et al.* identified a significant association between FCD affection and the minor allele of rs17466684 near the 5'-UTR of *CLU* (cases: G = 0.78, A = 0.22; controls G = 0.87, A = 0.13; OR: 1.85) [44]. An association was also identified between FCD and one *TGFBI* haplotype, spanning SNPs from rs756463 to rs10043360 (cases: TAAAT = 0.11; controls: TAAAT = 0.05; OR: 2.29). Notably, both *CLU* and *TGFBI* demonstrate increased expression in guttae of FCD cornea [50]. Although the associations observed by Kuot *et al.* have not yet been replicated in any other cohort, the localization of *CLU* and *TGFBI* to guttae suggests that these proteins may play a role in FCD, supporting the conclusion that variations in either gene may lead to pathogenesis.

Diseases with similarity to the FCD phenotype

COL8A2 endothelial dystrophy

An early-onset corneal dystrophy caused by mutations to *COL8A2* has been referred to as 'early-onset FCD', nomenclature that is not supported by clinical and histopathological evidence (Table 4). Of particular note is the fact that children of two FCD-affected parents exhibit an early-onset variant of FCD, suggesting the existence of a true early-onset variant caused by the same alleles as late-onset FCD, through homozygosity, compound heterozygosity at a single FCD locus, or inheritance of FCD alleles at two independent loci [24,51]. Conflating early-onset dystrophy caused by *COL8A2* mutations with these instances of early-onset FCD muddles the understanding of both diseases. Analysis of the similarities and differences between *COL8A2* dystrophy and FCD can clarify which aspects of corneal physiology are commonly disrupted in hereditary endothelial dystrophy, while also elucidating pathways uniquely affected by each disease.

Biswas *et al.* linked an early-onset endothelial dystrophy to the 7-cM interval 1p32.3–1p34.3 and used a positional candidate approach to identify coding mutations in the *COL8A2* gene, encoding the $\alpha 2$ subunit of collagen VIII, a major component of the Descemet membrane [52]. Four potential pathogenic mutations were identified: R155Q, present in one familial case and two sporadic cases and absent in 92 control individuals, R304Q and R434H, each present in one sporadic case and absent in 75 controls, and Q455K, present in one familial case of PPCD and two familial cases of a novel early-onset endothelial dystrophy with similarities to FCD. However, a later study by Kobayashi *et al.* detected the R155Q variant in unaffected Japanese individuals (five out of 72 tested chromosomes), suggesting that it is a non-pathogenic allele [53]. Furthermore, the R304Q and R434H mutations have not been replicated in any other population, although a Q455V mutation has been associated with a similar early-onset dystrophy in a Korean population, supporting the pathogenicity of the Q455K allele observed by Biswas *et al.* [54].

An additional mutation was identified by Gottsch *et al.* in a family originally described by Magovern *et al.* [55,56]. Corneal disease in a large six-generation family was initially linked to 1p32–1p34.3, and the mutation L450W was identified in all 21 members with early-onset corneal dystrophy, along with one family member diagnosed with PPCD. The same mutation was subsequently linked to a phenotypically similar dystrophy in a British family by Liskova *et al.* [57]. Gottsch *et al.* examined the progression and structural features of the early-onset disease, comparing the family with the L450W mutation with 62 families affected by late-onset FCD [55]. Members of the early-onset family reached a severe

Krachmer grade of 3 or higher approximately 40 years younger than individuals with late-onset FCD, a significant difference. Specular microscopy of *COL8A2* dystrophy and late-onset FCD patients revealed striking differences between the two phenotypes. Whereas guttae in early-onset *COL8A2* dystrophy appeared to be relatively broad with low elevation, guttae in FCD were very sharply raised with a greater elevation. In one individual with early-onset disease, large 'mulberry-like' excrescences, characteristic of PPCD, were present with no apparent guttae.

In a subsequent study, Gottsch *et al.* compared the histological features of early-onset *COL8A2* dystrophy caused by the L450W mutation, FCD of unknown cause, and normal control corneas [58]. The Descemet membrane was thickened in both *COL8A2* dystrophy and FCD, although the degree of thickening was greater in the *COL8A2* mutant. *COL8A2* immunostaining of the L450W variant cornea revealed abnormal collagen structures, which colocalized to refractile structures in the Descemet membrane. Strikingly, the cornea affected by *COL8A2* dystrophy lacked excrescences of the Descemet membrane, a pathognomonic feature of FCD. A second histological study, investigating two additional L450W corneas from the same family, found shallow atypical guttae in one individual, but did not observe excrescences in another more severely affected individual [59].

COL8A2 dystrophy has been modeled in murine transgenic systems, first through *Col8a2* knockout and *Col8a1/Col8a2* double knockout mice and later using the *Col8a2*^{G257D} mutant mouse and a homozygous *Col8a2*^{Q455K/Q455K} knock-in mouse. Hopfer *et al.* noted several anterior segment abnormalities in collagen VIII knock-out mice. Stromal thinning was noted in *Col8a1*^{-/-} and *Col8a1*^{-/-}/*Col8a2*^{-/-} mice, but not in the *Col8a2*^{-/-} single knockout. In contrast to human *COL8A2* dystrophy, the Descemet membrane exhibited thinning in the *Col8a1*^{-/-}/*Col8a2*^{-/-} double knock-outs and to a lesser degree in the *Col8a1*^{-/-} and *Col8a2*^{-/-} single knockouts. A keratoglobus-like deepening of the anterior chamber was observed in *Col8a1*^{-/-} and *Col8a1*^{-/-}/*Col8a2*^{-/-} mice, but not in the *Col8a2*^{-/-} single knockout. Notably, no guttae were observed in any of the collagen VIII knockout strains.

Investigating the phenotype of the *Col8a2*^{G257D} mouse, Puk *et al.* replicated many of the findings from collagen VIII knockouts. Both heterozygous and homozygous mutants exhibited reduced corneal thickness, with thinning of the epithelium, stroma and endothelium. Like collagen VIII knockouts, both *Col8a2*^{G257D/+} and *Col8a2*^{G257D/G257D} mice had keratoglobus-like elongation of the anterior chamber. Both the corneal thinning and anterior segment enlargement were more severe in the homozygote, suggesting a semidominant mode of inheritance. No guttae were observed in either heterozygous or homozygous *Col8a2*^{G257D} mice.

A later study by Jun *et al.* described a *Col8a2*^{Q455K/Q455K} knock-in mouse expressing a mutation identified in human *COL8A2* dystrophy patients. In contrast to *Col8a2* knockout mice, no corneal thinning or anterior segment enlargement was observed in the *Col8a2*^{Q455K/Q455K} knock-in mouse, and guttae were observed in the homozygous knock-in. However, the phenotype of the heterozygous knock-in mouse, analogous to human cases of *COL8A2* dystrophy, was not documented.

The endothelial dystrophy linked to mutations in *COL8A2*, which was originally described as an early-onset variant of FCD, represents a phenotypically distinct disease. When compared with classic late-onset FCD, the early age of onset and atypical-pathological features of the *COL8A2* disease indicate that it is not representative of FCD, but instead is its own distinct endothelial dystrophy. While *COL8A2* endothelial dystrophy, like FCD, causes endothelial degeneration that eventually leads to stromal and epithelial edema, individuals affected by *COL8A2* dystrophy typically lack the characteristic guttae of FCD

[55,59]. Guttatae have also not been observed in *Col8a2* knockout mice and *Col8a2*^{G257D} mutant mice, and have been described only in the homozygous *Col8a2*^{Q455K/Q455K} knock-in. Furthermore, individuals with *COL8A2* mutations experience an earlier onset of disease with very high penetrance and an equal sex ratio, in contrast to the late-onset, incomplete penetrance, and bias toward females seen in FCD. Although *COL8A2* is not a classic late-onset FCD gene, it may nonetheless offer some insight into the pathogenesis of FCD.

EDICT syndrome

Another autosomal dominant disease with some phenotypic similarity to FCD is EDICT syndrome (MIM 614303), first described by Akpek *et al.* and later linked to 15q22.1–15q25.3 by Jun *et al.* [60,61]. Individuals affected by EDICT syndrome demonstrate a severe, early-onset syndrome of the anterior segment, characterized by endothelial dystrophy, iris hypoplasia, early-onset cataract, and stromal thinning. Similar to FCD patients, individuals with EDICT syndrome develop a thickened Descemet membrane with posterior excrescences. Other corneal abnormalities are also present in the form of overlapping endothelial cells, along with endothelial aggregates that stain positive for cytokeratin, also seen in FCD and PPCD [61]. Recently, a candidate gene approach identified a +57C>T variant in *MIR184* as the causal mutation for EDICT syndrome, a result later confirmed by whole-exome sequencing [62]. *MIR184* encodes the microRNA miR-184, which, like all microRNAs, regulates gene expression through mRNA degradation and translational inhibition. While the mutation in miR-184 is likely to affect the expression of a variety of targets, leading to the complex and severe phenotype of EDICT syndrome, analysis of its effects could shed light on the causation of the Fuchs-like endothelial phenotype.

Snowflake vitreoretinal degeneration

Similar to EDICT, snowflake vitreoretinal degeneration (SVD; MIM 193230) is an autosomal dominant syndrome with some similarities to FCD. SVD causes early-onset cataract, liquefaction of the vitreous humor, retinal detachment, and endothelial dystrophy [63]. In a family with SVD, cornea guttata segregated with the disease phenotype. Guttatae were observed in four out of five prospectively examined affected individuals, and guttatae were not observed in any individuals without SVD. The causal mutation for SVD has been identified as R162W in *KCNJ13*, encoding the inwardly rectifying potassium channel Kir7.1 [64]. While it remains possible that a FCD-causing mutation on chromosome 2 cosegregates with the causal mutation for SVD, Kir7.1 may interact with members of same pathogenic pathways that cause the endothelial dystrophy in FCD.

Extraocular phenotypic characteristics

Hearing loss

Recently discovered FCD genes, along with a self-reported hearing study, have raised the possibility that hearing loss is a component of the FCD phenotype. Recessive *SLC4A11* alleles have been associated with Harboyan syndrome (MIM 217400), characterized by congenital corneal dystrophy along with perceptive deafness, developing in the first to third decade of life [37,65–68]. *SLC4A11* has been described as a sodium-borate cotransporter, although in cells grown in borate-free medium, it is permeable to sodium and hydroxide ions [69]. *SLC4A11* protein is expressed both in the corneal endothelium and in the lateral wall of the cochlea, which contains the stria vascularis, an epithelial tissue containing cells which, like the corneal endothelium, are derived from the neural crest [36,70–72]. Similar to the corneal endothelium, the stria vascularis is involved in maintaining fluid homeostasis in the cochlea, suggesting that defective ion transport by *SLC4A11* in analogous tissues may be the cause of both ocular and auditory pathology in Harboyan syndrome [73]. *SLC4A11*

has also been localized to fibrocytes in the spiral ligament, which have been implicated in a similar role in maintaining fluid homeostasis [74,75]. The phenotype of *Slc4a11*-knockout mice demonstrates increased thickness of the basal corneal epithelium, along with collapse of the vestibular membranous labyrinth and hearing loss [75]. Taken together, these findings raise the question of whether the dominant mutations in *SLC4A11* that cause FCD also result in an auditory phenotype.

A second hearing-related FCD gene was identified in *LOXHD1*. The function of *LOXHD1* is unknown, although it is predicted to contain 15 polycystine-1, lipoxigenase, α -toxin (PLAT) domains. Mutations coding premature terminations in *LOXHD1* have been identified in autosomal recessive hearing loss, and *LOXHD1* protein localizes to cochlear hair cells in the ear [76,77]. Although substantially less is known about *LOXHD1* than about *SLC4A11*, the involvement of *LOXHD1* with both FCD and hearing loss likewise supports a possible hearing phenotype in FCD.

FCD and sensorineural hearing loss have also been associated in one case linked to a mutation in mitochondrial DNA, with a phenotype of early-onset corneal dystrophy and bilateral hearing loss, followed by tremor and deterioration of motor control [78]. While the majority of familial FCD cases demonstrate autosomal rather than mitochondrial inheritance, the concurrence of FCD and sensorineural hearing loss in this case provides further evidence of a link between endothelial dystrophy and hearing loss.

In an observational study, Stehouwer *et al.* examined the prevalence of hearing disability in severely affected FCD patients, compared with controls who had undergone cataract extraction [79]. Individuals with FCD who had undergone or were planning keratoplasty were recruited, and each was matched with two cataract surgery patients matched for age and sex. Individuals were provided with a questionnaire that included the Hearing Handicap Inventory for the Elderly Screening test, which determines self-reported hearing disability and records factors that could affect hearing, such as a history of noise exposure. A total of 72 FCD patients and 144 controls were collected, of whom 50% were male and 50% were female. Stehouwer *et al.* found a significantly increased risk of hearing disability in severe patients, with an unadjusted OR of 1.59 (95% CI: 0.89–2.83) and an OR after adjustment for age, noise exposure and diabetes mellitus of 1.97 (95% CI: 1.04–3.75). This study provides further evidence for hearing loss as a copathology in FCD, although an empirical study using objective measurement of hearing acuity is necessary to draw a strong conclusion. While current evidence falls short of a proof of association between FCD and hearing loss, further investigation is warranted.

Pathways

Epithelial–mesenchymal transition

Epithelial–mesenchymal transition (EMT) may represent a mechanism for renewal and repair of the endothelium. Stem cell markers have been detected in cells of the peripheral endothelium, transition zone and trabecular meshwork, while additional markers of proliferation and maturation are present after endothelial wounding, suggesting that these cells may be activated and mobilized to fill gaps in the endothelium [80]. Defects in EMT could impair this mobilization and cause a defect in endothelial replacement, potentially leading to FCD. Consistent with this view, FCD genes discovered thus far have implicated the EMT pathway as a potential causal pathway in FCD. *ZEB1*, mutations in which cause FCD and PPCD, acts as a transcriptional repressor, downregulating E-cadherin and inducing a transition to a mobile, fibroblastoid phenotype [81]. Likewise, the E2-2 protein encoded by *TCF4* induces EMT through indirect mechanisms, including upregulation of *ZEB1* expression [82]. TGFBI likewise induces EMT [83], as does silencing of *CLU*, suggesting

that CLU suppresses EMT [84]. Furthermore, silencing of the FCD gene *SLC4A11* decreases proliferation, which is inversely correlated with motility in human corneal endothelial cells, suggesting a possible impact of *SLC4A11* mutations on EMT [40,85].

EMT may also link the endothelial dystrophy of EDICT syndrome to FCD, as miR-184 regulates several members of the EMT pathway. miR-184 competes with miR-205, disinhibiting SHIP2, a regulator of the EMT-promoting Akt pathway [86–88]. miR-184 may also induce EMT by impairing miR-205 inhibition of *ZEB1* expression [89,90]. Although functional studies are necessary to elucidate the role of EMT impairment on endothelial dystrophy, the implication of mutations in *ZEB1* and *MIR184* in FCD, PPCD and, EDICT syndrome, along with associations between FCD and variants of *TCF4*, *TGFBI* and *CLU*, suggests that EMT may represent a common pathway for the pathogenesis of endothelial disease. Notably, impairment in EMT leading to defective migration of replacement endothelial cells from the periphery could explain the fact that guttae first appear in the central cornea.

Oxidative stress & apoptosis

FCD endothelium exhibits increased markers of oxidative stress and apoptosis relative to control endothelium, leading to the suggestion that apoptosis as a result of oxidant–antioxidant imbalance is a causal mechanism for FCD [91–95]. Due to the production of oxygen radicals in mitochondria as a result of electron transport, mitochondria are particularly sensitive to a deficient antioxidant response, suggesting mitochondrial oxidative stress as one possible mechanism for the pathogenesis of FCD [96]. Indeed, in a SAGE study comparing FCD corneal endothelium with control endothelium, Gottsch *et al.* identified significant underexpression of several mitochondrial genes involved in electron transport and oxidative phosphorylation, including NADH dehydrogenase subunits 1, 2 and 4, cytochrome b, cytochrome c oxidase subunit III, and ATP synthase F0 subunit 6 [35]. Oxidative damage to mitochondria, resulting in decreased expression of mitochondrial genes, has the potential to impair survival of FCD endothelial cells and decrease the ability of remaining cells to maintain corneal deturgescence and clarity [97].

Supporting this hypothesis, FCD corneal endothelium exhibits reduced expression of antioxidant peroxiredoxins, and FCD endothelium and Descemet membrane have increased levels of advanced glycation end products associated with oxidative stress [93,98]. In a study by Jurkunas *et al.*, additional members of antioxidant pathways were underexpressed in FCD endothelium relative to controls: thioredoxin reductase 1 (necessary to replenish peroxiredoxin), metallothionein 3 (a reactive oxidant species scavenger), and superoxide dismutase 2 [95]. In addition, decreased expression was observed in genes associated with oxidative stress response: dual-specificity phosphatase 1, oxidative stress responsive 1, and angiopoietin-like 7. Neutrophil cytosolic factor 2, involved in reactive oxidant species metabolism, was significantly increased, while the expression of transcription factor Nrf2, which coordinates increased expression of antioxidant genes, was significantly decreased. Jurkunas *et al.* also found an increase in oxidative DNA damage in FCD corneal endothelium, with a marker for oxidative lesions localizing to endothelial mitochondria. Furthermore, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), a marker of apoptosis, colocalized to cells with DNA damage in FCD endothelium, while both TUNEL and oxidative DNA damage were absent in control corneas. Importantly, markers for oxidative damage and apoptosis were also absent in pseudophakic bullous keratopathy cornea, suggesting a deficit in antioxidant activity that is specific to FCD. Treatment of cultured human corneal endothelial cells with hydrogen peroxide caused a decrease in Nrf2 and a similar pattern of oxidative DNA damage, mimicking the changes seen in FCD cornea. Likewise, *ex vivo* treatment of mouse corneal endothelium with hydrogen peroxide caused polymegathism and pleomorphism, similar to FCD changes in cell morphology.

A follow-up study by Azizi *et al.* compared the response of cultured FCD and control human corneal endothelial cells to oxidative stress [99]. Supporting the earlier study by Jurkunas *et al.*, FCD cells had a significantly higher level of oxidative DNA damage than control cells. Furthermore, FCD endothelial cells exhibited a significantly increased level of p53 expression and significantly increased apoptosis after treatment with the oxidant *tert*-butyl hydroperoxide, suggesting that p53-mediated apoptosis after oxidative stress may represent a major contributor to cell death in FCD corneal endothelium. The *in vitro* and *ex vivo* studies on oxidative stress, combined with the association of FCD with alleles of *CLU*, a chaperone involved in oxidative stress response, suggest that oxidative stress response genes may play a causal role in the development of FCD pathology.

Protein folding

Unfolded protein response has also been implicated as a possible pathogenic mechanism in FCD. Engler *et al.* examined unfolded protein response in FCD corneas, corneas with non-Fuchs corneal dystrophies, keratoconus corneas, and normal controls, testing the hypothesis that the production of misfolded protein is increased in FCD [100]. In a masked examination of ten corneas with FCD and nine corneas with non-Fuchs' corneal dystrophy using transmission electron microscopy, all FCD corneas exhibited a prominent rough endoplasmic reticulum versus only a third of corneas affected by other dystrophies. FCD corneas also exhibited increased immunofluorescent staining for GRP78, phospho-eIF2 α and CHOP, markers for unfolded protein response, while keratoconus corneas exhibited decreased staining relative to normal controls. Similarly, the apoptosis markers caspase 3 and caspase 9 were both significantly increased in FCD cornea, suggesting that increased misfolded protein in FCD may lead to an increased unfolded protein response and caspase-dependent apoptosis. Notably, *CLU* is involved in protein folding, while pathogenic alleles of *LOXHD1* form misfolded aggregates in the corneal endothelium, supporting a possible role for improper protein folding in FCD. Unfolded protein response represents a possible explanation for the difficulty in identifying a common pathway for FCD: it is possible that pathogenic mutations could cause misfolding and/or protein aggregation in a wide variety of unrelated targets.

Expert commentary

To date, four FCD loci including regions on chromosomes 5, 9, 13 and 18 have been localized through linkage analysis of large families. Linkage analysis of a cohort including one large family and numerous small families has found another nine linkage peaks, on chromosomes 1, 5, 7, 8, 10, 15, 17, 20 and X. In addition, three causal FCD genes have been identified: *SLC4A11*, *ZEB1* and *LOXHD1*. Mutations in other genes, causing distinct endothelial dystrophies with some similarity to FCD, have also been discovered. One gene, *COL8A2*, has been implicated in the causation of a similar corneal dystrophy with earlier onset but a unique histopathological phenotype, while mutations in *MIR184* and *KCNJ13* have been shown to cause ocular syndromes with FCD-like endothelial dystrophy. Furthermore, a GWAS has identified an association between the minor allele of the intronic *TCF4* SNP rs613872 and FCD, while another study has found associations with rs17466684 near *CLU*, as well as with one haplotype of *TGFBI*. However, no causal relationship has been identified underlying these three genes.

Little functional data exist to explain how the heterogeneous causal mutations that have been identified, along with the presumed causal mutations underlying FCD-associated markers, contribute to the single disease of FCD. *ZEB1*, *TCF4*, *CLU* and *TGFBI* are involved in the regulation of EMT, but *LOXHD1* and *SLC4A11* are not known to have any effect on that process. *CLU* is also involved in antioxidant response, and *ex vivo* and *in vitro* studies of FCD corneal endothelium have suggested that deficits in antioxidant pathways may

contribute to the FCD phenotype. Finally, CLU is involved in normal protein folding, and pathogenic variants of LOXHD1 show misfolding and aggregation of LOXHD1 protein, while increased unfolded protein response has been observed in FCD corneas *ex vivo*. Identification of the causal genes located in FCD loci will clarify the mechanisms involved in the pathogenesis of FCD, while continued functional analysis will suggest new candidate genes by elucidating the abnormal cell biology in FCD.

Five-year view

In the next 5 years, we will likely see a significant clarification of the genetic basis for FCD. Until now, the expense of genetic sequencing has greatly limited progress by making it cost prohibitive to sequence the exome or genome of every collected FCD-affected individual. Expense has also necessitated whole-exome instead of whole-genome sequencing, thus causing possible noncoding causal mutations to be ignored. Notably, the SNP rs613872, which is strongly associated with FCD, is located in an intronic region of *TCF4*, and therefore could not have been identified by whole-exome approaches. The rapidly decreasing cost of next-generation DNA sequencing, a trend that is likely to continue with the introduction of third-generation sequencing technologies, will result in a greater availability of whole-exome and whole-genome sequence data. Several research groups have collected DNA samples from large cohorts of FCD-affected individuals and unaffected controls, and inexpensive whole-genome sequencing will allow the identification of both common and rare disease-causing alleles, including noncoding variants, in these populations. Perhaps most notably, these advances may allow the causal alleles on chromosome 18 to be identified. Although FCD-causing mutations in *LOXHD1* have been found, the causal mutations associated with the *TCF4* SNP rs613872, as well as the majority of familial disease linked to the *FCD2* locus, have not yet been identified. Chromosome 18-linked disease may represent a substantial proportion of the genetic load of FCD, so the identification of one or more causal mutations would represent a significant advance in our understanding of FCD genetics [25,42].

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Key issues

- Fuchs' corneal dystrophy (FCD) is a genetic disorder of the corneal endothelium, which represents a major cause of corneal transplantation in the developed world.
- The genetic basis of FCD is complex and heterogeneous, with variable expressivity and incomplete penetrance.
- Only three FCD genes (*ZEB1*, *SLC4A11* and *LOXHD1*) have been identified, representing a small proportion of the total genetic load.
- A common causal mechanism uniting the three causal genes has not been identified, although genetic and molecular biology approaches have implicated epithelial-to-mesenchymal transition, oxidative stress response and protein folding as three potential pathways involved in FCD pathogenesis.
- Four causal FCD loci and nine additional linkage peaks for FCD have been isolated, along with three genes associated with FCD. However, the causal relationships that underlie these associations have not yet been identified.
- Future research will identify the causal genes located in FCD loci in order to determine causal mechanisms and devise nonsurgical treatments.

Table 1

Fuchs' corneal dystrophy linkage peaks determined through genetic linkage analysis.

Chromosome	Linkage peak	Maximum dominant HLOD	Ref.
1	rs760594	2.02	[30]
5	rs1301475	2.22	[31]
5 (<i>FCD3</i>)	D5S209–D5S425	3.39 [†]	[27]
7	rs257376	1.92	[30]
8	rs1380229	2.26	[31]
9 (<i>FCD4</i>)	D9S168–D9S1869	3.48 [†]	[28]
10	rs1889974	3.37	[31]
13 (<i>FCD1</i>)	D13S1236–D13S1304	3.91 [†]	[27]
15	rs352476, rs235512	2.48, 3.53	[30,31]
17	rs938350	2.10	[30]
18 (<i>FCD2</i>)	D18S487–D18S1134	3.41 [†] , 2.89 [†] , 2.45 [†]	[25]
20	rs674630	2.17	[31]
X	rs1990383	2.08	[31]

HLOD: Heterogeneity logarithm of odds; LOD: Logarithm of odds.

[†]LOD score.

Table 2

Causal loci for Fuchs' corneal dystrophy.

Chromosome	Identified genes	Other diseases caused by mutations in the same gene	Ref.
5q33.1–5q35.2	None		[27]
9p22.1–9p24.1	None		[28]
10	<i>ZEB1</i>	Posterior polymorphous corneal dystrophy	[28,32]
13pTel–13q12.13	None		[24]
18q21.2–18q21.32	<i>LOXHD1</i> [†]	Autosomal recessive sensorineural hearing loss	[25,41–43]
20	<i>SLC4A11</i>	Congenital hereditary endothelial dystrophy and corneal dystrophy with perceptive deafness (Harboyan syndrome)	[37–39]

[†] Although mutations in *LOXHD1* cause a portion of FCD2-linked disease, additional FCD2-linked familial cases exist which do not have *LOXHD1* mutations, suggesting locus heterogeneity.

Table 3

Genes associated with Fuchs' corneal dystrophy, for which a causal relationship has not been established.

Gene	Association peak	Replicated?	Ref.
<i>TCF4</i>	rs613872 (within <i>FCD2</i>)	Yes	[31,42,43,45]
<i>CLU</i>	rs17466684	No	[44]
<i>TGFBI</i>	rs756463–rs10043360	No	[44]

Table 4

Genes that harbor mutations causing endothelial dystrophies similar to Fuchs' corneal dystrophy.

Gene	Gene name	Function	Disease	Ref.
<i>COL8A2</i>	Collagen VIII $\alpha 2$ subunit	Structural/connective pression	Early-onset <i>COL8A2</i> endothelial dystrophy	[52,53,55]
<i>MIR184</i>	MicroRNA184	Regulation of gene expression	EDICT syndrome	[60–62]
<i>KCNJ13</i>	Potassium inwardly rectifying channel J13	Ion channel	Snowflake vitreo-retinal degeneration	[63,64]