REVIEW ARTICLE

Genetics of corneal endothelial dystrophies

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Abstract

The corneal endothelium maintains the level of hydration in the cornea. Dysfunction of the endothelium results in excess accumulation of water in the corneal stroma, leading to swelling of the stroma and loss of transparency. There are four different corneal endothelial dystrophies that are hereditary, progressive, non-inflammatory disorders involving dysfunction of the corneal endothelium. Each of the endothelial dystrophies is genetically heterogeneous with different modes of transmission and/or different genes involved in each subtype. Genes responsible for disease have been identified for only a subset of corneal endothelial dystrophies. Knowledge of genes involved and their function in the corneal endothelium can aid understanding the pathogenesis of the disorder as well as reveal pathways that are important for normal functioning of the endothelium.

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Introduction

The cornea is a convex, transparent structure on the anterior of the eye, forming part of the ocular surface, and constituting the major refractive device for light rays entering the eye. The adult human cornea has a thickness of about 500 μm, and is made up of five layers. Anterior to posterior, they are: (i) the epithelium 50–60 μm thick, consisting of five–six cell layers of squamous, nonkeratinized cells; (ii) the Bowman's layer; an acellular structure of 8–12 μm thickness consisting of randomly arranged collagen fibrils; (iii) the stroma, which makes up the bulk of the cornea and is essentially a collagenous matrix, made up of collagen lamellae with interspersed keratocytes; (iv) the Descemet membrane (DM) representing the basement membrane of the corneal endothelial cells; and (v) the endothelium, a single layer of polygonal cells made up of simple squamous epithelium. The DM is about 8–12 μm thick, and has an anterior banded zone formed *in utero*, about 3 μm thick and a homogeneous posterior nonbanded zone formed after birth, that increases in thickness with age, reaching up to 10 μm in thickness at age 80 years (Waring *et al*. 1982). The endothelium produces additional extracellular matrix known as the posterior collagenous layer, posterior to the normal DM, as a response to different types of diseases including developmental disorders, trauma and inflammation. The endothelial cells are 5–6 μm in height and 18–20 μm in diameter (Forrester *et al*. 2002). The endothelium forms a barrier between the corneal stroma and the aqueous humor and functions as a pump to remove excess water from the stroma. The net effect is to maintain stromal hydration at about 78%. Dysfunction of the endothelium causes excess water to enter the stroma, with resultant disruption of collagen fibrils and opacification of the cornea. The human endothelium has little regenerative capacity and cells are continually lost with increasing age. The endothelial cell density is about 3000–4000 cells/mm^2 at birth and can reduce to 1000 cells/mm^2 after age 50 (Waring *et al*. 1982). Reduction in the number of cells is accompanied by enlargement of the remaining endothelial cells to fill up the gaps and maintains a continuous monolayer.

Corneal dystrophies are a heterogeneous group of hereditary corneal disorders that are generally bilateral, noninflammatory conditions resulting in the formation of corneal opacities. Traditionally, corneal dystrophies have been categorized on the basis of the corneal layers that are primarily affected. This forms the basis for a revised classification system that incorporates genetic aspects of the disorder as well

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(Weiss *et al*. 2008). The major types of corneal dystrophy are: (i) epithelial and sub-epithelial dystrophies, (ii) Bowman layer dystrophies; (iii) stromal dystrophies; and (iv) Descemet membrane and endothelial dystrophies. Each group of corneal dystrophies is genetically and clinically heterogeneous (reviewed by Klintworth 2009). The knowledge of the genetics of dystrophies generated over the last few decades has opened up a new dimension in the understanding of their pathogenesis and called for revised approaches to their classification. Identification of the underlying gene mutation serves as a diagnostic support in cases of unusual or ambiguous clinical phenotypes and provides a method for unifying all variant phenotypes with a common underlying genetic basis instead of being considered as separate entities. The purpose of this article is to provide an overview of the current knowledge of the genetics of dystrophies of the corneal endothelium. There are four different corneal endothelial dystrophies described till date (listed in table 1) that are genetically heterogeneous both between and within themselves.

Posterior polymorphous corneal dystrophy (PPCD)

PPCD is a rare autosomal dominant disorder characterized by metaplastic corneal endothelial cells that have morphologic features of epithelium. The endothelium, which is normally a single layer of cells that do not divide further, develops into a multilayered stratified squamous epithelium that has abnormal proliferation, sometimes spreading to adjoining tissues such as the iris. Epithelial-like features of these cells include the presence of tonofilaments, desmosomes and microvilli (Waring *et al*. 1982; Klintworth 2009). The clinical features of this disease are highly variable and affected members of the same family can display extremes of severity of disease. Most individuals affected by PPCD, however, do not have any symptoms and retain normal vision. The age at onset of symptoms is variable and may be in early childhood in severe cases or in adulthood. Clinically evident lesions in the posterior cornea at the level of the DM include clusters of vesicles or blister-like lesions, band-shaped irregularities, or more extensive irregularity involving the entire DM and the variable presence of stromal edema. The DM is defective especially in the posterior nonbanded zone, which is very thin and in the presence of a posterior collagenous layer of variable morphology (Waring *et al*. 1982). The anterior banded zone of the DM, however, appears normal suggesting that the pathology occurred in the postnatal period. The gene expression pattern of the endothelium of PPCD corneas is also altered, and they express cytokeratins

Table 1. Genetic aspects of corneal endothelial dystrophies.

Phenotype	Inheritance	Locus	Gene	References
Posterior polymorphous corneal dystrophy (PPCD)	AD	Chromosome 20p11.2 (*PPCD1*)	Not known. Disputed role for *VSX1*	Heon *et al*. 1995, 2002; Gwilliam *et al*. 2005
	AD	Chromosome 1p34-32 (*PPCD2*)	*COL8A2*	Biswas *et al*. 2001
	AD	Chromosome 10p13 (*PPCD3*)	*TCF8/ZEB1*	Shimizu *et al*. 2004; Krafchak *et al*. 2005
Fuchs endothelial corneal dystrophy (FECD) early-onset	AD	Chromosome 1p34-32	*COL8A2*	Biswas *et al*. 2001
FECD late-onset	AD	Chromosome 13pTel-q12 (*FCD1*)	Not known	Sundin *et al*. 2006a
	AD	Chromosome 18q21.2-21.3 (*FCD2*)	Not known	Sundin *et al*. 2006b
	AD	Chromosome 5q33-35 (*FCD3*)	Not known	Riazuddin *et al*. 2009
FECD late-onset	Complex	Chromosomes 1, 7, 15, 17 and X	Not known	Afshari *et al*. 2009
	Complex	Chromosome 20p13	*SLC4A11*	Vithana *et al*. 2008
Congenital hereditary endothelial dystrophy (CHED)	AD	Chromosome 20p11-q11 (*CHED1*)	Not known	Toma *et al*. 1995
	AR	Chromosome 20p13-p12	*SLC4A11*	Hand *et al*. 1999; Vithana *et al*. 2006
X-linked endothelial dystrophy (XECD)	X-linked	Xq25	Not known	Schmid *et al*. 2006

characteristic of epithelium: CK7, CK8, CK18, CK17, CK19, CK4, CK13, CK6 and CK16, expressed in proliferating cells (Jirsova *et al*. 2007). Overgrowth of endothelial cells into the iris and trabecular meshwork results in glaucoma, found in a subset of patients with PPCD (Cibis *et al*. 1977).

Genetics of PPCD

Three genes have been identified as causing *PPCD* till date. The first locus, PPCD1, was mapped to a 30 cM interval on chromosome 20q in a large family (Heon *et al*. 1995), overlapping with the locus for the autosomal dominant form of congenital hereditary endothelial dystrophy (CHED) which was mapped to a 2.7 cM region within the *PPCD1* locus, raising the idea that the two disorders might be allelic (Toma *et al*. 1995). The *VSX1* gene located in the *PPCD1* interval was selected as a candidate on the basis of its expression in ocular tissues, with high levels of expression in the retina including the inner nuclear layer and the retinal ganglion cells (Heon *et al*. 2002). VSX1 is a member of a group of paired-like homeodomain transcription factors. Mutations were detected in the *VSX1* gene in two probands, each with PPCD and autosomal dominant keratoconus (including keratoconus in association with PPCD in one case) (Heon *et al*. 2002). Apart from the corneal phenotype, heterozygous mutation carriers showed reduction in electroretinographic (ERG) responses, although retinas were clinically normal, suggesting a subclinical defect in retinal function. Two other cases with mutation of the *VSX1* gene have been reported in two studies, in which the phenotypes of the affected individuals were PPCD in combination with other defects involving the retinal, auditory and craniofacial tissues (Mintz-Hittner *et al*. 2004; Valleix *et al*. 2006). The status of these *VSX1* sequence alterations, however, is questionable. One of the *VSX1* sequence changes described as pathogenic (Heon *et al*. 2002; Valleix *et al*. 2006) has been found at a frequency of 0.3% in normal control populations (Heon *et al*. 2002; Valleix *et al*. 2006), making it uncertain as to whether it is in fact a rare variant unrelated to disease. In the study by Mintz-Hittner *et al*. (2004) the presumed pathogenic variant identified was found to be absent in over 300 normal controls of which only 12 were of the same ethnic origin as the affected family. Thus, its frequency in an ethnically matched population is not reliably known; this leaves open the possibility that the sequence change in question is a population-specific variant that is nonpathogenic. Further, there is other evidence that goes against *VSX1* as the *PPCD1* gene. Families that were mapped to the *PPCD1* locus did not have *VSX1* mutations (Heon *et al*. 1995; Gwilliam *et al*. 2005; Hosseini *et al*. 2008). Mapping of PPCD in Czech families refined the critical interval to a 2.7 cM region on chromosome 20 overlapping with the dominant CHED (*CHED1*) locus, but excluding the *VSX1* gene (Gwilliam *et al*. 2005). Attempts to identify the gene by screening of coding sequences of all positional candidate genes located in the refined *PPCD1* critical interval in a *PPCD1* family failed to identify the PPCD gene at this locus (Aldave *et al*. 2005; Yellore *et al*. 2005; Aldave *et al*. 2009).

The second PPCD locus reported is the collagen VIII alpha-2 (*COL8A2*) gene on chromosome 1p34-32, coding for the alpha-2 chain of collagen VIII. Collagen VIII is a major component of the DM, and is present in the abnormal posterior collagenous layer of the DM in endothelial disorders including Fuchs dystrophy (Levy *et al*. 1996). *COL8A2* was identified as the gene for early-onset Fuchs endothelial corneal distrophy (FECD) and screening of this gene in families with PPCD identified mutations in two affected members of a single family (Biswas *et al*. 2001). There is another report on PPCD associated with a *COL8A2* mutation. Evaluation of a family with early-onset FECD revealed a disease-causing *COL8A2* mutation, *L450W*; one individual in the family who was a carrier of the same mutation was reported to have a phenotype of PPCD (Gottsch *et al*. 2005). The involvement of *COL8A2* in PPCD has not been substantiated further since no pathogenic mutations were found in additional families screened for mutations (Biswas *et al*. 2001; Yellore *et al*. 2005). The role of *COL8A2* in PPCD is not clear from available data which suggest that it could be, if at all, a rare cause of the disease.

A third *PPCD* locus, PPCD3 was mapped on chromosome 10p to an 8.2 cM interval containing the homeodomain transcription factor-8 gene (*TCF8*; also known as Zinc finger E-box binding homeobox 1; *ZEB1*) (Shimizu *et al*. 2004). Mutations in *TCF8* were found in the original family mapping to the *PPCD3* locus (Krafchak *et al*. 2005) as well as in about one-third to one-half of probands with PPCD (Krafchak *et al*. 2005; Aldave *et al*. 2007b; Liskova *et al*. 2007b), suggesting that *TCF8* mutations are a more frequent cause of PPCD than the other genes known till date. ZEB1 is an E-box binding transcription activator or repressor involved in differentiation and development of various tissues. Apart from the corneal manifestations, Krafchak *et al*. (2005) noted a high proportion of patients with *TCF8* mutations having additional abnormalities such as hernias and hydroceles, and skeletal deformities. Similar observations were made in more recent studies, in which a significantly higher frequency of abdominal and inguinal hernias was found in mutation-bearing individuals with PPCD as compared with control individuals having no *TCF8* mutations (Aldave *et al*. 2007b; Nguyen *et al*. 2009). *Tcf8/Zeb1*$^{-/-}$ mice showed craniofacial and skeletal defects reinforcing the notion that this transcription factor has a developmental role in the formation of bone and connective tissue (Takagi *et al*. 1998). One possible corneal target for *TCF8/ZEB1* action is the *COL4A3* (collagen IV alpha-3) gene that has a TCF8-binding site in the promoter. *COL4A3* is not normally expressed in the corneal endothelium but ectopic expression of COL4A3 was demonstrated in the corneal endothelium of individuals with *TCF8* mutations (Krafchak *et al*. 2005). Alterations in gene

expression patterns were also seen in a *Zeb1*-mutant mouse model in which expression of *COL4A3*, cytokeratins and E-cadherin showed a shift from epithelial expression in wild-type mice to corneal endothelial and stromal expression in mutant mice. Moreover, the altered pattern of gene expression in corneal keratocytes and endothelium of *Zeb1* mutant mice was accompanied by an abnormal proliferation of the endothelial cells (Liu *et al*. 2008), indicating that the *Zeb1* gene may serve to repress the epithelial phenotype.

Fuchs endothelial corneal dystrophy (FECD)

Fuchs endothelial corneal dystrophy is typically a late-onset disorder appearing in the 5–6th decades of life or later, with females being affected more frequently than males. It is the most common corneal dystrophy in western countries, accounting for about 10% of all corneal transplants in North America (Godeiro *et al*. 2007; Ghosheh *et al*. 2008), but appears to have a much lower prevalence in the Middle East (al Faran and Tabbara 1991) and Asia (Chen *et al*. 2001; Pandrowala *et al*. 2004). It is characterized by the presence of guttae, which are excrescences in the DM described as a 'focal, refractile accumulation of collagen posterior to the Descemet membrane' (Waring *et al*. 1982). In later stages of the disease, bilateral corneal edema develops due to degeneration of the corneal endothelium, with consequent loss of vision. FECD is progressive and the extent of corneal edema increases to involve the entire stroma and the epithelium. In advanced stages of the disease, sub-epithelial and stromal scarring occurs. Ultrastructural changes in the endothelium and DM have been described and involve progressive development of guttate changes in the DM, and thickening of the posterior collagenous layer (Waring *et al*. 1982; Klintworth 2009).

Genetics of FECD

FECD is complex in etiology, and genetic as well as environmental factors are likely to play a role in its causation. Familial clustering of the disease is often found with complex inheritance being more common. Mendelian forms of FECD with autosomal dominant inheritance also occur, though less commonly, both in case of late-onset as well as early-onset disease.

Mapping and linkage studies have been used to identify the disease locus in families with early-onset autosomal dominant FECD (AD-FECD). The first locus mapped for AD-FECD is on chromosome 1p34-32 and the *COL8A2* gene within this region was found to have mutations in these families (Biswas *et al*. 2001). Other studies confirmed the role of *COL8A2* in early-onset AD-FECD. Linkage mapping and mutational analysis of a large family identified the L450W mutation in *COL8A2* to be the cause of early-onset FECD described by Magovern and coworkers (Magovern *et al*. 1979; Gottsch *et al*. 2005). The same mutation was identified in another British family with early-onset FECD (Liskova *et al*. 2007a). The corneal phenotype of this family shared features with that of the family mapped by Gottsch *et al*. (2005) and was distinct from that of late-onset FECD (Zhang *et al*. 2006; Liskova *et al*. 2007a). A different mutation in *COL8A2* (Q455V) was identified in Korean families with early-onset FECD (Mok *et al*. 2009).

Late-onset AD-FECD in large multiplex families has been mapped to three separate loci at chromosomes 13pTel-q12 (*FCD1*), 18q21.2-21.3 (*FCD2*), and 5q33-35 (*FCD3*) (Sundin *et al*. 2006a,b; Riazuddin *et al*. 2009). The features of disease mapped to the *FCD3* locus were found to be milder with slower progression as compared with *FCD1* and *FCD2* phenotypes (Riazuddin *et al*. 2009). Different approaches have been used to identify the underlying genes in cases of the more common, complex forms of late-onset FECD. Mapping of the disease locus for late-onset FECD in multiple families showed modest linkage to loci on chromosomes 1, 7, 15, 17 and X (Afshari *et al*. 2009). Notably, none of these loci coincide with those identified in late-onset AD-FECD. The approach of candidate gene screening for FECD has been informative only in a subset of patients. Candidate genes selected for screening have included genes involved in other forms of endothelial dystrophy. Although the *COL8A2* gene was implicated in a fraction of patients with late-onset disease (Biswas *et al*. 2001), this has been contradicted by subsequent studies in which the sequence changes reported to be pathogenic for late-onset FECD were found in the normal population; in addition, there was no evidence for association of *COL8A2* mutations with late-onset FECD in a series of families screened in two different studies (Kobayashi *et al*. 2004; Aldave *et al*. 2006). A small fraction of patients (4/89) with late-onset FECD were found to have heterozygous mutations in the *SLC4A11* gene, which is responsible for CHED2 (AR-CHED; see following section); none of these mutations occurred in the control population, and corresponding mutant proteins were shown to be defective in localization and turnover in relation to the wild type (Vithana *et al*. 2006). These findings need to be extended to other patient populations to assess the contribution of *SLC4A11* mutations to FECD. Other candidates tested include the *TCF8/ZEB1* (PPCD3) gene and the *COL8A1* (collagen VIII, alpha-1 chain) gene. No significant role was found for either of these genes in FECD (Urquhart *et al*. 2006; Mehta *et al*. 2008; Vithana *et al*. 2008).

Congenital hereditary endothelial dystrophy (CHED)

CHED is a bilateral disorder involving degeneration of the corneal endothelium. It occurs in two forms, autosomal dominant (AD-CHED; CHED1) and autosomal recessive (AR-CHED; CHED2). It manifests at birth or in infancy as diffuse 'ground glass opacification of the cornea, markedly thickened cornea due to edema, and a thickened DM. There are no clear-cut differences between clinical features of the dom-

inant and recessive forms of CHED except that the recessive form may manifest earlier and is associated with nystagmus (Kirkness *et al*. 1987). Histologically, the endothelium is atrophic and may show greatly reduced cell count, altered morphology, and thickening of the DM due to abnormal secretion by the endothelial cells.

Genetics of CHED

The locus for AD-CHED was mapped to the pericentromeric region of chromosome 20 (Toma *et al*. 1995) although the gene has not been identified till date. The *CHED1* locus is contained within the larger interval for *PPCD1*. This locus including *PPCD1* as well as *CHED1* was excluded as the locus for CHED2 by two separate studies carried out on families of Irish and Saudi Arabian origins (Callaghan *et al*. 1999; Kanis *et al*. 1999). The *CHED2* locus was mapped to an interval of 8 cM on chromosome 20p13 and shown to be about 25 cM away from the *CHED1* locus on chromosome 20 (Hand *et al*. 1999). Subsequently, the *CHED2* gene was identified as the bicarbonate-transporter related *SLC4A11* (solute carrier family 4, member 11; also known as BTR1- bicarbonate transporter related protein 1, or NaBC1-sodium-borate cotransporter 1) by mapping and positional candidate approaches in families from Myanmar (Vithana *et al*. 2006) and confirmed in families of Indian origin (Jiao *et al*. 2007).

The *SLC4A11* gene is a distant member of the bicarbonate transporter family expressed in several different tissues (Parker *et al*. 2001). In the cornea, it is expressed in the endothelium and epithelium, as well as vascular endothelia and epithelial cells of kidney, pancreas and brain (Damkier *et al*. 2007). It was identified as the homologue of the borate transporter *BOR1* in *Arabidopsis* and shown to be capable of borate transport *in vitro*, functioning as an electrogenic sodium-borate cotransporter (Park *et al*. 2004). Borate is an essential micronutrient for plant and animal cells and is required for normal growth and development of various organisms such as *Xenopus laevis* (Fort *et al*. 2002) and in metabolic processes in chick and rat models (Hunt 1994). It has also been shown to have mitogenic effects on mammalian cell lines (Park *et al*. 2004). The precise role of borate or of *SLC4A11* in the corneal endothelium is not clear at present.

Mutations of *SLC4A11* also cause Harboyan syndrome (corneal dystrophy with perceptive deafness; CDPD) in which CHED is accompanied by sensorineural hearing loss appearing in about the second decade of life (Desir *et al*. 2007). AR-CHED appears to be genetically homogeneous since the same locus accounts for the disorder in families from different populations with about 67 different mutations identified in the *SLC4A11* gene so far indicating the high degree of allelic heterogeneity in CHED2 (Vithana *et al*. 2006; Aldave *et al*. 2007a; Desir *et al*. 2007; Jiao *et al*. 2007; Kumar *et al*. 2007; Ramprasad *et al*. 2007; Sultana *et al*. 2007; Hemadevi *et al*. 2008; Shah *et al*. 2008; Aldahmesh *et al*. 2009). In contrast to the AR-CHED phenotype arising from *SLC4A11* mutations in humans, a mouse knockout for *slc4a11* did not show any detectable abnormality in the corneal endothelium of *slc4a11*$^{-/-}$ mice; the endothelial cell size, number and morphology were comparable with wild-type mice, as was corneal clarity and thickness (Lopez *et al*. 2009). The mouse knockout, however, showed defects in auditory responses (Lopez *et al*. 2009), mirroring the auditory phenotype of Harboyan syndrome. The lack of a corneal endothelial disorder in the knockout mice suggests that *SLC4A11* function is either qualitatively or quantitatively different between mice and humans.

X-linked corneal endothelial dystrophy (XECD)

Schmid *et al*. (2006) described a new form of corneal endothelial dystrophy in a large family with males more severely affected, and an absence of male–male transmission. Clinical manifestations included 'moon-crater like' changes in the endothelium in all affected, variable presence of visual loss ranging from no change in visual acuity to moderate or severe loss of vision, and epithelial band keratopathy. Congenital clouding of the cornea was an occasional feature. Microscopic changes in the cornea included changes in the epithelium and Bowman layer, an irregularly thickened DM and areas of abnormal endothelial cells forming multilayers with endothelial cell loss in other areas (Schmid *et al*. 2006). Linkage and haplotype analysis under an X-linked dominant model mapped the disease locus to a 4.7 cM critical region on Xq25 (Schmid *et al*. 2006).

Summary

The genetic basis for many of the corneal endothelial dystrophies is not known as yet. Filling the lacunae would require more gene mapping efforts followed by identification of the disease gene on multiplex families both for Mendelian disorders such as CHED1 and PPCD as well as complex forms of late-onset Fuchs endothelial dystrophy. Methods of identifying the disease gene may need to incorporate extended screening so as to cover the entire genomic sequence within the interval in cases where no pathogenic changes are identifiable upon screening of only coding regions of known genes (such as in PPCD1). This is feasible by second generation sequencing technologies. It is possible that the disease is caused by uncharacterized genes or parts thereof that could be responsible, or an unusual mechanism involving functional or regulatory elements within the introns, or by copy-number changes. Gene expression profiling of diseased tissue, while having the potential to provide valuable information on primary as well as secondary genes involved in the pathology, is severely limited in these disorders due to decline in numbers of endothelial cells as the diseases progress. Defining and restricting phenotype groups for analysis of complex forms of FECD may enhance the detection of linkage in families. From the studies on AD-FECD as discussed above, specific loci appear to be associated with

phenotypically distinct forms of disease. Such heterogeneity may complicate the analysis of complex FECD. Understanding of the genetics of corneal endothelial disorders is likely to provide valuable insights into the molecular pathways that govern the normal development and functioning of the endothelium. This may in turn contribute towards unravelling the causes of endothelial dysfunction in more common conditions.

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