Published in final edited form as:
Exp Eye Res. 2012 February ; 95(1): 24–34. doi:10.1016/j.exer.2011.08.002.

Molecular Bases of Corneal Endothelial Dystrophies

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Abstract

The phrase “corneal endothelial dystrophies” embraces a group of bilateral corneal conditions that are characterized by a non-inflammatory and progressive degradation of corneal endothelium. Corneal endothelial cells exhibit a high pump site density and, along with barrier function, are responsible for maintaining the cornea in its natural state of relative dehydration. Gradual loss of endothelial cells leads to an insufficient water outflow, resulting in corneal edema and loss of vision. Since the pathologic mechanisms remain largely unknown, the only current treatment option is surgical transplantation when vision is severely impaired. In the past decade, important steps have been taken to understand how endothelial degeneration progresses on the molecular level. Studies of affected multigenerational families and sporadic cases identified genes and chromosomal loci, and revealed either Mendelian or complex disorder inheritance patterns. Mutations have been detected in genes that carry important structural, metabolic, cytoprotective, and regulatory functions in corneal endothelium. In addition to genetic predisposition, environmental factors like oxidative stress were found to be involved in the pathogenesis of endotheliopathies. This review summarizes and crosslinks the recent progress on deciphering the molecular bases of corneal endothelial dystrophies.

Keywords

Apoptosis; Congenital Hereditary Endothelial Dystrophy (CHED); Fuchs Endothelial Corneal Dystrophy (FECD); genetics; guttae; oxidative stress; Posterior Polymorphous Corneal Dystrophy (PPCP); Sodium Biocarbonate Transporter-like Protein 11 (SLC4A11)

1. Introduction

The word “dystrophy” was adopted from the Greek (*dys* = wrong, difficult; *trophe* = nourishment) and entered the medical discourse in 1884 through Wilhelm Erb (Erb, W., 1884). The term “corneal endothelial dystrophy” refers to a group of diseases that are characterized by a slowly progressive endogenous degeneration of corneal endothelium and are at least in part due to genetic predisposition. The corneal endothelial dystrophies are

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congenital hereditary endothelial dystrophy 1 (CHED1), congenital hereditary endothelial dystrophy 2 (CHED2), posterior polymorphous corneal dystrophy (PPCD) and Fuchs endothelial corneal dystrophy (FECD) (Weiss, J.S. et al., 2008). In all cases, degeneration of corneal endothelium ultimately leads to severely impaired vision or blindness, but the molecular pathologies remain largely unknown. Recent efforts have shed more light on the molecular mechanisms of these diseases, and the latest genetic findings enforce the idea of a gradual continuum between endothelial dystrophies.

2. Fuchs Corneal Endothelial Dystrophy

2.1 Overview

Fuchs corneal endothelial dystrophy (FECD, **MIM# 136800**) is a corneal condition that primarily results from degeneration of corneal endothelium. It is characterized by progressive corneal endothelial cell loss, morphological changes in the hexagonal endothelial mosaic, and a concomitant formation of extracellular matrix deposits called guttae. As corneal endothelial cell numbers become critically low, the cornea becomes edematous, leading to loss of vision (Wilson, S.E. and Bourne, W.M., 1988). In the majority of cases, FECD is a slowly progressive disorder of aging that affects approximately 4% of the population over the age of 40 in the United States (Krachmer, J.H. et al., 1978). Currently, the only treatment modality to restore lost vision is corneal transplantation in the form of penetrating keratoplasty or Descement's stripping endothelial keratoplasty. Hence, FECD is the second most common indication for corneal transplants performed in the United States for patients over the age of 60 (Darlington, J.K. et al., 2006).

2.2 History and hallmarks

FECD was first described in 1910 by Ernst Fuchs as a "dystrophia epithelialis corneae" (Fuchs, E., 1910). Although in an advanced stage the pathological changes of FECD manifest in all corneal layers, it was not until the 1920s that the root cause of the disease was determined to be in the corneal endothelium (Friedenwald, H. and Friedenwald, J.S., 1925; Gifford, S., 1926; Kraupa, E., 1920). Microscopic investigations revealed the histological hallmark of FECD to be diffuse thickening of Descemet membrane (DM) and an increasing accumulation of extracellular excrescences, so-called guttae, on DM (Hogan, M.J. et al., 1974; Vogt, A., 1921). Graves was the first to describe the formation of guttae, originating in the central cornea and spreading towards the periphery (Graves, B., 1924). The accumulation of guttae is accompanied by a distinctive endothelial cell loss, and the number of cells is inversely proportional to the number of guttae (Waring, G.O., 3rd et al., 1982). When the number of remaining endothelial cells becomes critically low, the overall endothelial pump capacity becomes insufficient to keep the cornea in its natural state of deturgescence (McCartney, M.D. et al., 1989; Wilson, S.E. et al., 1988). The consequence is stromal and epithelial hydration, and corneal edema, leading to corneal opacity and, in turn, loss of visual acuity (Adamis, A.P. et al., 1993; Waring, G.O., 3rd et al., 1978). In addition, this pathologic progression involves very characteristic changes in endothelial cell morphology, clinically known as polymegethism (variation in cell size) and pleomorphism (variation in cell shape) (Polak, 1974; Waring, G.O., 3rd et al., 1978).

2.3 Associated cellular phenotypes

Histologic and ultrastructural studies of FECD specimens have revealed endothelial cell abnormalities such as large intracellular vacuoles often filled with melanin-type deposits (Hidayat, A.A. and Cockerham, G.C., 2006; Waring, G. et al., 1974). Moreover, extracellular pigment dots have been found around guttae. In many cases, endothelial cells develop a dilated, rough endoplasmic reticulum, as well as swollen mitochondria (Kayes, J. and Holmberg, A., 1964; Zhang, C. et al., 2006). Interestingly, FECD endothelial cells tend

to lose their phenotypic boundaries and undergo metaplasia, exhibiting both fibroblastic and epithelial morphology (Iwamoto, T. and DeVoe, A.G., 1971; Offret, G. et al., 1977), as well as the presence of epithelial cell markers (Hidayat, A.A. and Cockerham, G.C., 2006).

2.4 Clinical presentation

Clinically, two forms of FECD have to be differentiated. A rare early-onset form of FECD starts in the first decade of life and progresses through the second and third decades (Magovern, M. et al., 1979). It is characterized by a massively thickened DM at birth, causing corneal decompensation at a very early age (Gottsch, J.D. et al., 2005; Zhang, C. et al., 2006). The more typical late-onset form of FECD progresses through four clinically defined stages that span a course of two-to-three decades (Eghrari, A.O. and Gottsch, J.D., 2010; Elhalis, H. et al., 2010; Klintworth, G.K., 2009). Late-onset FECD has female predominance at a ratio of 2.5-3:1 (Cross, H.E. et al., 1971; Krachmer, J.H. et al., 1978; Waring, G.O., 3rd et al., 1978; Wilson, S.E. and Bourne, W.M., 1988).

2.4.1 Stage 1—In this stage, corneal biomicroscopy reveals isolated corneal guttae that are nonconfluent, and the patients are usually asymptomatic. Specular or confocal microscopy serves as a useful tool to detect isolated guttae (Fig. 1) and to perform morphometric analysis, which aids in diagnosis and staging of FECD.

2.4.2 Stage 2—In this stage, corneal guttae begin to coalesce. The progression of guttae deposition is accompanied by endothelial cell loss and residual cell thinning, enlargement and loss of hexagonal shape (Arffa, R., 1991; Miller, C. and Krachmer, J., 1988). Resulting corneal stromal edema causes painless decrease in vision.

2.4.3 Stage 3—Continued loss of endothelial cells leads to a compromise in barrier and pump function, and full corneal edema, including the epithelial cell layer, ensues. Patients experience a significant compromise in vision that leads to corneal transplantation.

2.4.4 Stage 4—Due to chronic edema, the cornea becomes densely opaque, vascularized, and scarred.

2.5 Genetics

The International Committee for Classification of Corneal Dystrophies (IC3D) classification system sorts FECD as category 1, 2 or 3, depending on the degree of genetic information available (Weiss, J.S. et al., 2008).

2.5.1 Early-onset FECD (Category 1)—Collagen type VIII is secreted by corneal endothelium as a major constituent of the posterior layer of DM. It exists in two isoforms, α1 and α2, which interact with each other to establish the highly ordered, three-dimensional collagen lattice (Shuttleworth, C.A., 1997). Within the past decade, two mutations in the *Col8A2* gene encoding the α2 polypeptide of collagen type VIII on chromosome 1p34.3-p32 were identified (Biswas, S. et al., 2001; Gottsch, J.D. et al., 2005). When studying early-onset FECD in several multigenerational families, with the youngest individual being diagnosed at the age of three, the mutations were found to be inherited in an autosomal dominant trait, as was observed in earlier studies of early-onset FECD (Magovern, M. et al., 1979; Rosenblum, P. et al., 1980). Notably, both mutations have also been found in posterior polymorphous dystrophy (PPCD) patients (Biswas, S. et al., 2001; Gottsch, J.D. et al., 2005). However, the PPCD phenotype is uniquely different from early-onset FECD (see Section 4). In early-onset.FECD, both mutations give rise to a distinct phenotype characterized by small, round guttae associated with cell centers instead of sharply peaked guttae at the cell edges, seen in late-onset FECD. Study patients commonly developed

severe symptoms, although the course of the disease spanned 25 years—comparable to late-onset FECD (Gottsch, J.D. et al., 2005). The Gln455Lys (Biswas, S. et al., 2001) as well as the Leu450Trp (Gottsch, J.D. et al., 2005) mutations affect the triple helical domain of α2 collagen VIII. This might have a substantial influence on the tertiary structure and, hence, the collagen lattice in DM (Levy, S.G. et al., 1996). As collagen type VIII has been found to be involved in terminal differentiation of vascular endothelium (Sage, H. and Iruela-Arispe, M.L., 1990), an aberrant basement membrane might impair corneal endothelial terminal differentiation.

Hopfer and co-workers generated a type VIII collagen knockout mouse to gain insight into the collagen's function in corneal dystrophies (Hopfer, U. et al., 2005). As expected, corneal stroma and DM were significantly thinned; however, no guttae formation or corneal opacification was detected. Notably, endothelial cells were reduced in number and showed polymegethistic and pleomorphistic changes. *In vitro* corneal endothelial cells had a lower capability to proliferate than did those in wild type mice. The authors suggested that type VIII collagen is important for cell proliferation and migration during eye development. However, although there are some phenotypic similarities, collagen type VIII knockout mouse pathology probably does not reflect that of FECD (Hopfer, U. et al., 2005).

2.5.2 Late-onset FECD (Categories 2 and 3)

2.5.2.1 SLC4A11: *SLC4A11* encodes the NaBC1 protein, a member of the so-called solute carrier family 4, which functions as a voltage-gated, sodium borate cotransporter (Park, M. et al., 2004). In recent years, different mutations of the *SLC4A11* gene on chromosome 20p12 have been identified (Fig. 2) (Riazuddin, S.A. et al., 2010a; Vithana, E.N. et al., 2008). Interestingly, *SLC4A11* mutations have also been found in recessive congenital hereditary dystrophy type 2 (CHED2) (Jiao, X. et al., 2007; Kumar, A. et al., 2007; Paliwal, P. et al., 2010; Ramprasad, V.L. et al., 2007; Vithana, E.N. et al., 2006). The heterozygous mutations included missense mutations as well as one deletion. Mutated proteins showed defects in posttranslational modification, failed to localize to the cell surface and accumulated in the endoplasmic reticulum instead (Riazuddin, S.A. et al., 2010a; Vithana, E.N. et al., 2008).

2.5.2.2 TCF4 and TCF8: The *TCF4* gene encodes the E2-2 protein, which belongs to the family of class I basic helix-loop-helix (bHLH) transcription factors, and is able to either repress or activate gene expression by binding to E-boxes in target promoters (Cisse, B. et al., 2008; Flora, A. et al., 2007; Murre, C. et al., 1994). A variety of single nucleotide polymorphisms in the *TCF4* locus have been found to be associated with sporadic late-onset FECD (Baratz, K.H. et al., 2010). However, no mutation within the coding region or exon-intron boundaries has been identified to date. Although the *TCF4* gene is located on chromosome 18q21, it seems to be independent of the *FCD2* chromosomal locus (see below) (Riazuddin, S.A. et al., 2011).

E2-2 is known to upregulate *TCF8* (Sobrado, V.R. et al., 2009). Interestingly, a recent study identified five loss-of-function mutations in the *TCF8* gene on chromosome 9 that are linked to late-onset FECD. One of these mutations was inherited in a large family and represents the first mutation to be associated with familial late-onset FECD (Riazuddin, S.A. et al., 2010b). Notably,*TCF8* mutations have also been demonstrated in PPCD patients (Aldave, A.J. et al., 2007b; Krafchak, C.M. et al., 2005). The *TCF8* gene expresses the ZEB1 protein, which belongs to the zinc finger transcription factor family and has both gene repressive and enhancing activities (Vandewalle, C. et al., 2009). *TCF8* expression has been shown in corneal endothelium, and ZEB1 binding sites are found in the promoter regions of various collagen genes (Krafchak, C.M. et al., 2005).

Both genes, *TCF4* and *TCF8*, play important roles in epithelial-mesenchymal transition, by repressing E-cadherin expression (Eger, A. et al., 2005; Sobrado, V.R. et al., 2009), as well as in other vital developmental processes (Higashi, Y. et al., 1997; Jan, Y.N. and Jan, L.Y., 1993; Tanaka, A. et al., 2010; Zhuang, Y. et al., 1996). Given that TCF4 is involved in TCF8 regulation and that the two genes' biological functions are similar, it has been suggested that their mutations act within the same pathway, leading to a high risk of FECD development (Baratz, K.H. et al., 2010; Li, Y.J. et al., 2011; Riazuddin, S.A. et al., 2011; Thalamuthu, A. et al., 2011). However, the exact mechanism of pathogenesis remains speculative.

2.5.2.3 Chromosomal loci: A rising number of chromosomal loci (*FCD1*, *2*, *3* and *4*) have been associated with late-onset FECD through investigation of multigenerational pedigrees. *FCD1* is located on chromosome 13 at 13pTel-13q12.13 and contains 44 protein-encoding genes (Sundin, O.H. et al., 2006b). *FCD2* is confined to chromosome 18 at 18q21.2-q21.3, spanning at least 28 genes. It displays the most common locus found to date, although there might be independent mutations involved. There were no phenotypic differences reported when *FCD2* patients are compared to common cases (Sundin, O.H. et al., 2006a). The *FCD3* locus is centered on chromosome 5q33.1-q35.2 and contains 97 annotated genes. Compared to *FCD1* and *FCD2*, it presents clinically as a milder phenotype (Riazuddin, S.A. et al., 2009). Recently *FCD4* was detected on chromosome 9 at 9p24.1-22.1 and shown to interact genetically with the *TCF8* mutation, leading to a more severe form of FECD (Riazuddin, S.A. et al., 2010b). This diversity suggests strong heterogeneity for late-onset FECD, although—in contrast to *FCD2—FCD1*, *3* and *4* seem to be familial instead of common mutations.

A linkage study including many small FECD families revealed chromosomes 1, 7, 15, 17 and X as potentially being involved in FECD. The authors concluded that FECD can be inherited in both an autosomal dominant and complex fashion (Afshari, N.A. et al., 2009).

2.6 Molecular pathology

2.6.1 Extracellular matrix proteins and guttae formation—Recent studies comparing the proteome of normal human corneal endothelial cell-DM complexes to FECD identified marked overexpression of clusterin (CLU) and transforming growth factor ß-induced protein (TGFBIp) (Elhalis, H. et al., 2010; Jurkunas, U.V. et al., 2009; Jurkunas, U.V. et al., 2008a).

CLU is a pro-aggregative, chaperone-like glycoprotein (Silkensen, J.R. et al., 1994) that is generally overexpressed in many tissues undergoing stress, mainly oxidative stress (Viard, I. et al., 1999). The secretory form of CLU is known to promote cell survival, while the nuclear form is known to target cells for apoptosis (Criswell, T. et al., 2005; Yang, C.R. et al., 2000). Both forms of CLU were found to be upregulated in FECD endothelium; immunohistochemistry revealed the most intense clusterin staining intracellularly where the secretory form of CLU is synthesized. CLU was also detected in the centers of the guttae, where old cell debris is located (Jurkunas, U.V. et al., 2008a). Endothelial cells staining most strongly for CLU were clustered around guttae, possibly because of the pro-aggregative properties of secretory CLU in protecting the cells against degenerative processes. The exact role of CLU in the pathogenesis of FECD is still under investigation. TGFBIp is an extracellular matrix adhesion molecule that interacts with collagens, integrins and fibronectins (Runager, K. et al., 2008). Immunohistochemistry has demonstrated a colocalization of TGFBIp and clusterin in the centers of guttae (Jurkunas, U.V. et al., 2009). Interestingly, TGFBIp tends to be localized more towards DM and is partly responsible for its gradual thickening. TGFBIp is thought to interact with CLU during guttae formation

(Jurkunas, U.V. et al., 2009) and might represent a defence mechanism against pro-apoptotic stimuli.

2.6.2 Apoptosis, oxidative damage, and oxidant-antioxidant imbalance—

Apoptosis is a genetically programmed cellular mechanism leading to cell death. It is seen during normal development and wound healing but is also known to be involved in the pathogenesis of many diseases (Nickells, R.W. and Zack, D.J., 1996; Yan, Q. et al., 2006). Apoptotic cell death has been demonstrated in FECD endothelium, as seen by terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) and DNA fragmentation assays (Borderie, V.M. et al., 2000; Li, Q.J. et al., 2001).

One of the major inducers of apoptosis is macromolecular (such as DNA) damage due to oxidative stress. Corneal endothelium is arrested in a post-mitotic state and does not divide, thus its genome is especially susceptible to reactive oxygen species-induced apoptosis. Jurkunas and co-workers detected significantly greater levels of oxidative DNA damage in FECD cells than in normal age-matched controls by measuring levels of oxidized guanosine base, 8-hydroxyguanine (8-OHdG) in corneal endothelium (Jurkunas, U.V. et al., 2010). Oxidative DNA damage colocalized with TUNEL labelling in endothelial cells located next to corneal guttae (Fig. 1B), indicating that macromolecular damage due to oxidative stress is involved in apoptosis in FECD. Studies investigating the uptake of a vital mitochondrial dye and staining with anti-8-OHdG antibody detected the majority of staining in the mitochondrial DNA (mtDNA), suggesting that mtDNA damage may be a key component of alterations seen in FECD (Jurkunas, U.V. et al., 2010). Similarly, earlier studies showed decreased numbers of mitochondria in FECD endothelium and a decrease in cytochrome oxidase, the major respiratory chain enzyme, in the central areas of FECD corneal buttons (Johns, D.R., 1995; Tuberville, A.W. et al., 1986). Gene array analyses that revealed underexpression of mitochondrial genes, such as SOD2, Prx3, and others, have emphasized further the deficiencies in normal mitochondrial functioning in FECD (Gottsch, J.D. et al., 2003; Jurkunas, U.V. et al., 2010).

Additional studies have corroborated the involvement of oxidative stress in the pathogenesis of FECD. Lipid peroxidation, advanced glycation end-products and their receptors, as well as other reactive oxygen species' (ROS) by-products, have been detected in FECD specimens (Buddi, R. et al., 2002; Wang, Z. et al., 2007). Engler and co-workers showed an upregulation of the unfolded protein response (UPR) in FECD endothelium. Rough endoplasmic reticulum was enlarged, and the levels of three UPR marker proteins (GRP78, phospho-eIF2α, and CHOP) were found to be elevated (Engler, C. et al., 2010). It has been shown that oxidative stress can induce UPR, which in turn triggers apoptosis, and it has been speculated that protein misfolding and accumulation is due to oxidation (Holtz, W.A. et al., 2006; Szegezdi, E. et al., 2006).

The evidence that FECD endothelium is susceptible to cellular damage due to chronic oxidative stress was further supported by detection of oxidant-antioxidant imbalance in FECD endothelium. Proteomic and PCR array analyses detected generalized downregulation of antioxidants and oxidative-stress related genes (Jurkunas, U.V. et al., 2010; Jurkunas, U.V. et al., 2008b). One of the classes of antioxidants that was diminished in FECD was thioredoxin-dependent antioxidants, peroxiredoxins, which scavenge intracellular ROS and function in the cytosol, mitochondria, and peroxisomes. Additionally, the levels of thioredoxin reductase, metallothionein 3 and superoxide dismutase 2, as well as nuclear ferritin, glutathione *S*-transferase π and heat shock 70, were significantly decreased in FECD samples (Gottsch, J.D. et al., 2003; Jurkunas, U.V. et al., 2010). Such generalized downregulation of antioxidants points to the diminished transcriptional activation of the antioxidant response element (ARE) in the promoter regions of oxidative stress defence

genes (Ishii, T. et al., 2000). Most antioxidants are upregulated by NF-E2-related factor 2 (Nrf2) during oxidative stress, by a complex interaction of Nrf2 with its binding partners (Ishii, T. et al., 2000). However, western blot analysis has demonstrated diminished production of Nrf2 protein in FECD endothelium, pointing toward a general dysfunction of the Nrf2 pathway that in turn manifests in the oxidant-antioxidant imbalance seen in FECD (Jurkunas, U.V. et al., 2010).

There is increasing evidence supporting the notion that, in addition to genetic factors, chronic oxidative stress contributes to the cellular and molecular damage in susceptible human corneal endothelium, which in turn leads to the pathologic findings of FECD (Fig. 3). Oxidative stress combined with genetic factors and post-mitotic arrest of corneal endothelium leads to a combination of oxidative mitochondrial damage, oxidant-antioxidant imbalance, endothelial morphological changes and apoptosis as seen in FECD (Fig. 3). It is likely that early and late-onset FECD are distinct corneal conditions with the similar clinical and phenotypic characteristics but different genetic risk factors. The role of oxidative stress in endothelial cell loss in the early-onset FECD needs further investigation.

3. Congenital hereditary endothelial dystrophy

Two types of congenital hereditary endothelial dystrophy (CHED) have been described: CHED1, which is autosomal dominant, and CHED2, which is autosomal recessive. Clinically, both disorders present with diffuse bilateral ground-glass appearance and markedly thickened corneas. Slit-lamp examination reveals corneal thickness up to three times greater than normal. The swollen corneas are due to scant or completely degenerated corneal endothelial cells. The major differences between CHED1 and CHED2 are onset of presentation, mode of inheritance, genetic mutations, and associated conditions. Overall, vision tends to be better in CHED1 patients than in those with CHED2.

3.1 CHED1 (MIM#121700)

CHED1 is a category 2 dystrophy by IC3D classification. It is autosomal dominant, and the genetic locus has been identified on chromosome 20p11.2-q11.2 pericentromeric region. Clinically, infants with CHED1 have clear corneas at birth and develop corneal clouding during the first or second year of life. The presentation is often asymmetric (Weiss, J.S. et al., 2008) and clouding ranges from haze to milky-white in appearance, with focal white spots. The endothelial appearance in asymptomatic patients can have moon crater-like changes and peau d'orange-like endothelial alterations. Light microscopy shows deposition of disorganized collagen fibrils in the posterior collagenous layer, which is thought to be secreted by metaplastic endothelial cells. As a result, there is diffuse thickening of DM without presence of guttae, as is seen in other corneal dystrophies. Corneal endothelium, for the most part, is atrophic with vacuolization, multilayering, and melanin deposition (Rodrigues, M.M. et al., 1975; Waring, G.O., 3rd et al., 1978).

3.1.1 Genetics of CHED1—Genetic study of a large British family with autosomal dominant and fully penetrant inheritance of CHED1 served as the basis for identifying the chromosomal locus (Toma, N.M. et al., 1995). Two-point linkage analysis of this seven-generation family revealed significant linkage to chromosome 20. The identified locus was within the 30 cM region of the same chromosome linked to posterior polymorphous corneal dystrophy (PPCD) (Heon, E. et al., 1995; Toma, N.M. et al., 1995). The linkage of both disorders to overlapping regions in chromosome 20 has sparked a debate—that the two disorders are allelic variants.

3.2 CHED2 (MIM#217700)

CHED2 is a category 1 dystrophy, by IC3D classification, since the gene causing it has been identified. It is autosomal recessive, often asymmetric, and more common and severe than CHED1. In CHED2, corneas are edematous and have a diffuse ground glass appearance that is evident at birth or in the neonatal period. Nystagmus is often present due to early and severe loss of vision. When associated with deafness, CHED2 is part of Harboyan syndrome (Desir, J. et al., 2007). Even though CHED1 and CHED2 are similar histopathologically, subtle differences exist in DM composition. The DM in CHED2 consistently exhibits increase in total thickness due to widening of the non-banded zone (five-to-eight times thicker than normal), while the fetal anterior-banded zone is of normal thickness and morphology. The endothelium is usually attenuated and absent (Cockerham, G.C. et al., 2002; Klintworth, G.K., 2009; McCartney, A.C. and Kirkness, C.M., 1988; Paliwal, P. et al., 2010).

3.2.1 Genetics of CHED2—Earlier studies have shown that CHED1 and CHED2 are genetically distinct conditions (Callaghan, M. et al., 1999); even though CHED2 has been mapped to the same chromosome as CHED1, it is a clearly distinct region (Hand, C.K. et al., 1999). Kanis et al. studied a consanguineous pedigree in a multigenerational Saudi Arabian family and reported that autosomal recessive CHED is not an allelic variant of CHED1 or PPCD (Kanis, A.B. et al., 1999). It was not until 2006 that a mutation in the *sodium bicarbonate transporter-like protein 11 (SLC4A11)* gene on chromosome 20p12 was detected (Vithana, E.N. et al., 2006).

Screening of affected CHED2 families from Myanmar, Pakistan, and India revealed seven different mutations in the *SLC4A11* gene, which encodes a bicarbonate transporter-related protein-1 (BTR1) or NaBC1 (as discussed in Section 2.5.2.1). BTR1 belongs to the family of bicarbonate transporters that is membrane bound and functions as a sodium-coupled borate cotransporter by transporting Na and OH in the absence of borate (Vilas, G.L. et al., 2011) (Fig. 2). The detected mutations were postulated to lead to loss of function of the protein. Transiently transfected HEK273 cells with the mutant BTR1 showed defective processing of the mutated protein through the endoplasmic reticulum, failure to reach mature size, and deficiency in cell surface processing and localization (Vithana, E.N. et al., 2006).

Since the first report of this gene defect, multiple studies have described several new mutations in the *SLC4A11* gene in different cohorts of CHED2-affected families (Fig. 2). Approximately 25 different mutations in different locations of the *SLC4A11* gene have been detected; the majority of these are homozygous—pointing to the presence of a common ancestor from which the mutations have originated (Aldave, A.J. et al., 2007a; Jiao, X. et al., 2007; Ramprasad, V.L. et al., 2007; Sultana, A. et al., 2007).

3.2.2 Molecular pathology of CHED2—To correlate the underlying genotype to the resultant phenotype, animal models of the *SLC4A11* mutations and BTR1 biochemical analyses have been studied. In a study by Lopez et al., generation of *SLC4A11*-deficient mice led to significant abnormalities in the audio-vestibular system, which is consistent with findings seen in Harboyan syndrome, where CHED is accompanied by hearing loss (Lopez, I.A. et al., 2009). However, the knockout mice did not exhibit significant differences in DM composition and endothelial cell size, shape, or density at 3, 5, and 10 months of age.

Subsequently, Groger and colleagues (Groger, N. et al., 2010) generated mice containing a mutation that led to a truncation and cytoplasmic localization of the BTR1 protein, which is consistent with previous studies showing that accumulation of BTR1 in the intracellular compartments leads to a loss-of-function effect (Hemadevi, B. et al., 2008; Vithana, E.N. et al., 2006). The mutant mice exhibited enlarged and abnormal endothelial cells with

intracellular vacuolization thought to indicate a disturbance of intracellular osmotic balance. The mutant mice also exhibited formation of salt crystals in corneal endothelial cells and increased sodium chloride concentrations in corneal stroma as compared to wild type. The authors concluded that genetic defects in *SLC4A11* disrupt the fluid flux across corneal endothelium necessary for maintenance of healthy corneal hydration. While this study provides novel insights into the role of BTR1 in corneal water balance, the mechanism by which the mutation leads specifically to attrition and atrophy of corneal endothelium needs further investigation.

To understand the structural basis of *SLC4A11* mutations that give rise to both CHED and FECD, biochemical characterization and development of the folding model of BTR1 protein have been performed (Vilas, G.L. et al., 2011). The protein was found to have a cytosolic N-terminal domain, a membrane domain organized as 14 transmembrane segments, and a short cytosolic C-terminal domain (Fig. 2). The mutations found in the transmembrane region are likely to be involved in protein misfolding and defects in ion permeation. The mutations that mapped to the extramembranous loops were shown to affect conduction of ions to the translocation pore, a major transporter function. The cytoplasmic domain mutations were found to be less important in the folding and, thus to the functioning of BTR1. Moreover, the study showed that diseased alleles affected the degree of BTR1 protein folding and its ability to localize to the plasma membrane, where its major function as cotransporter is performed. Interestingly, lowering the temperatures of the cell culture was able to rescue point mutation-induced BTR1 retention in the endoplasmic reticulum and induce protein maturation, especially when the mutation was caused by A269V. The authors concluded that there might be some hope for thermal rescue of misfolded BTR1 protein by applying cooling packs to patients during sleep, thus preventing the onset of corneal disease.

4. Posterior polymorphous corneal dystrophy

Posterior polymorphous corneal dystrophy (PPCD) is an uncommon, nonprogressive disorder that affects corneal endothelium and DM (Henriquez, A.S. et al., 1984). It was first described in 1916 by L. Koeppe (Koeppe, L., 1916), and is typically a bilateral autosomal dominant dystrophy. Isolated unilateral cases have been described with similar phenotypes but unclear hereditary patterns (Weiss, J.S. et al., 2008). The prevalence of this rare disorder in the general population is unknown. Clinically, PPCD is characterized by the presence of deep, corneal, vesicular, band-shaped, and placoid or diffuse lesions, usually asymmetric. Patients are often asymptomatic until middle age, and visual impairment only occurs in a small percentage of patients due to corneal edema. Associated features are peripheral iridocorneal adhesions, glaucoma, and a tendency to recur in a graft following perforating keratoplasty (Klintworth, G.K., 2009; Patel, D.V. et al., 2005; Weiss, J.S. et al., 2008). According to IC3D criteria, PPCD is classified as either category 1 or 2. (Weiss, J.S. et al., 2008). Three types of PPCD with different genetic loci have been recognized: PPCD1 (20p11.2-q11.2), PPCD2 (1p34.3-p32.3) and PPCD3 (10p11.2) (Weiss, J.S. et al., 2008).

Corneal endothelium of PPCD often contains lesions with a vesicular shape that have doughnut-like appearance in specular microscopy—circular dark rings with lighter centers in which abnormal endothelium resides (Fig. 4) (Laganowski, H.C. et al., 1991; Weiss, J.S. et al., 2008). Nests of abnormal cells often cluster next to normal endothelium. Band-shaped or “railroad track” areas are seen as chains of overlapping vesicles, creating a shallow trench with irregular edges. Pits, excrescences, troughs, and ridges were present in DM. Endothelial polymegethism and pleomorphism were also noted in PPCD patients (Fig. 4) (Cheng, L.L. et al., 2005; Patel, D.V. et al., 2005).

4.1 Cell biology of PPCD

Histologic and electron microscopy studies have demonstrated that the major morphologic feature of PPCD is the replacement of endothelial cells by squamous epithelium that is commonly stratified and has signs of aberrant keratinization (Fig. 4) (Krachmer, J.H., 1985). These epithelial-like cells form one-to-five layers and are joined by abundant desmosomes; moreover, the cells characteristically have surface microvilli and intracytoplasmic filaments (Henriquez, A.S. et al., 1984; Krachmer, J.H., 1985). DM appears with multiple layers of collagen that show irregular thickening and manifestations of focal fusiform or nodular excrescences. In PPCD, the posterior non-banded portion of DM is extremely thin and sometimes absent (Klintworth, G.K., 2009; Weiss, J.S. et al., 2008). The abnormal endothelial cells may extend onto the trabecular meshwork, leading to secondary glaucoma in severe cases (Krachmer, J.H., 1985).

To characterize corneal endothelium affected by PPCD, immunohistochemical analyses of post-keratoplasty corneal buttons of PPCD patients were performed. The abnormal endothelium of PPCD corneas were positive for a wide spectrum of cytokeratins, with cytokeratin 7 and cytokeratin 19 predominating (Jirsova, K. et al., 2007). The researchers concluded that the pattern of cytokeratin expression found in the abnormal cells is most likely related to a metaplastic process during which endothelial cells are transformed into epithelial-like cells, but the exact mechanisms of which have not been determined.

Merjava and colleagues (Merjava, S. et al., 2009) demonstrated changes in collagen IV and VIII chains in PPCD corneas, which may contribute to the morphological differences and increased proliferation of abnormal endothelium. Increased levels of α1 and α2 chains of collagen IV were found in DM of PPCD patients, with a stronger signal on the endothelial side of DM. In addition, the α1 chain of collagen VIII was found on the stromal side as well as on the endothelial side in PPCD, while it was detected predominantly on the stromal side in control tissues.

4.2 Genetics of PPCD

PPCD is an autosomal dominant condition with variable penetrance. The first genetic locus of PPCD (**PPCD1, MIM#122000**) was mapped to the long arm of chromosome 20p11, rendering PPCD1 a category 2 dystrophy (Heon, E. et al., 1995), since the exact mutation in this region has not been identified. Although mutations in the *visual system homeobox 1* gene (*VSX1*, located on chromosome 20) was thought to be associated with PPCD1 (Heon, E. et al., 2002), Aldave and colleagues (Aldave, A.J. et al., 2005) reported that the two identified sequence variants, Gly160Asp and Asp144Glu missense mutations, do not appear to be associated with PPCD1. In an analysis of two large families in the Czech Republic, Gwilliam and colleagues (Gwilliam, R. et al., 2005) excluded *VSX1* as the disease-causing gene, leading to general uncertainty regarding the role of the *VSX1* gene in the pathogenesis of PPCD1. According to genetic analysis of four families with PPCD, from the Czech Republic and the U.S., the common support interval for PPCD1 was a 2.4 cM region between markers D20S182 and D20S139, which included 26 mapped genes (Gwilliam, R. et al., 2005; Heon, E. et al., 1995; Yellore, V.S. et al., 2007). However, a recent study has reported the absence of a presumed pathogenic coding-region mutation in the common PPCD1 interval after the screening of 26 positional candidate genes between these markers in one of the families studied (Aldave, A.J. et al., 2009).

A missense mutation in *COL8A2*, a gene that encodes the α2 chain of type VIII collagen, located on the short arm of chromosome 1, has been termed **PPCD2** (**MIM#609140**) (Biswas, S. et al., 2001). Mutations in the *COL8A2* gene were identified in families with FECD and in 2 of 15 patients with PPCD2 (Biswas, S. et al., 2001). In contrast to this

finding, other studies have not identified any presumed pathogenic mutations in the *COL8A2* gene in a large number of individuals affected with PPCD (Kobayashi, A. et al., 2004; Yellore, V.S. et al., 2005), leading to general doubts about the effect of this gene in the pathogenesis of PPCD2.

After exclusion of chromosome 20 and chromosome 1 loci in a large affected family, Shimizu and colleagues (Shimizu, S. et al., 2004) mapped a PPCD locus in the 8.55 cM region on chromosome 10 (**PPCD3, MIM#609141**), and demonstrated that this dystrophy is genetically heterogeneous. Frameshift and nonsense mutations in the human zinc finger transcription factor 8 gene (*TCF8*, also known as *ZEB1*) were described by Krafchak and colleagues (Krafchak, C.M. et al., 2005) in approximately 50% of families with PPCD, as well as in 25% of the affected probands screened by Aldave et al. (Aldave, A.J. and Sonmez, B., 2007). An analysis of ocular features of six patients with PPCD caused by mutations in *TCF8* demonstrated a variable spectrum of phenotype and incomplete penetrance (Liskova, P. et al., 2010).

One of the largest published series to date, by Aldave and colleagues (Aldave, A.J. et al., 2007b), identified 8 different *TCF8* mutations in 8 of 32 PPCD-screened family members. The authors also evaluated the prevalence of inguinal, umbilical or abdominal hernias in affected individuals, both with and without *TCF8* mutations, as well as in their unaffected relatives. All PPCD-affected men (100%) with *TCF8* mutations had a history of hernia, while only 20% of PPCD-affected men without *TCF8* mutations, and none of the unaffected men, had the disorder. An increased prevalence of inguinal hernias and hydroceles in affected men with *TCF8* mutations was also found by Krafchak and colleagues (Krafchak, C.M. et al., 2005).

Liskova and colleagues (Liskova, P. et al., 2007) screened the coding regions of three genes implicated in PPCD (*VSX1*, *COL8A2* and *TCF8*) in six Czech and four British families. Four novel pathogenic mutations within the *TCF8* gene were detected in four of the 10 families, emphasizing the role of the *TCF8* gene in the pathogenesis of PPCD. No disease-causing mutations were identified in the other six families, indicating that the disorder is probably caused by an as yet unidentified gene(s) (Liskova, P. et al., 2007). In addition, clinical and molecular analyses of 11 probands from New Zealand identified a novel mutation in the *TCF8* gene in only 1 affected individual, confirming genetic heterogeneity of the dystrophy (Vincent, A.L. et al., 2009).

5. Summary

Histologic similarities have been detected between various corneal endothelial dystrophies, especially PPCD and CHED, by noting fibroblast-like cells, degenerated endothelial cells, and melanocyte-like cells in the posterior portion of the cornea and DM (Chan, C. et al., 1982). Likewise, the similarities between FECD, PPCD and age-related changes are immense, as guttae-type excrescences and pleomorphism, as well as polymegethism, are present in several conditions.

The reason for such similarities most likely lies in the fact that corneal endothelium as a cell type has a similar ability to respond to intrinsic (genetic defect-induced) and extrinsic (exposure to UV light and aging) stressors, regardless of the etiology. Therefore, clinical signs and/or morphological characteristics might not be sufficient to differentiate between various forms of dystrophies and corneal endothelial conditions; and the true future of corneal dystrophy classification lies in the genetic analysis. The IC3D classification is a breakthrough first attempt to compartmentalize the multitude of dystrophies based on genetic and inheritance patterns. One should be cautious though, not to underestimate the

impact of the interplay between environmental and genetic factors that most likely account for the multitude of phenotypic variations and redundancies in corneal endotheliopathies.

Acknowledgments

Supported by Grants: NIH/NEI R01-EY020581 and Research to Prevent Blindness (UVJ)

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Abbreviations

8-OHdG	8-Hydroxyguanine
ARE	Antioxidant Response Elements
bHLH	Basic helix-loop-helix
BTR1	Bicarbonate Transporter-related Protein-1
CHED	Congenital Hereditary Endothelial Dystrophy
CLU	Clusterin
DM	Descemet Membrane
FECD	Fuchs Endothelial Corneal Dystrophy
IC3D	International Committee for Classification of Corneal Dystrophies

MIM	Mendelian Inheritance in Man
Nrf2	NF-E2-related Factor 2
PPCD	Posterior Polymorphous Corneal Dystrophy
PRX	Peroxiredoxin
ROS	Reactive Oxygen Species
SLC4A11	Sodium Bicarbonate Transporter-like Protein 11
SOD	Superoxide dismutase
TGFBIp	Transforming Growth Factor β-induced Protein
UPR	Unfolded Protein Response

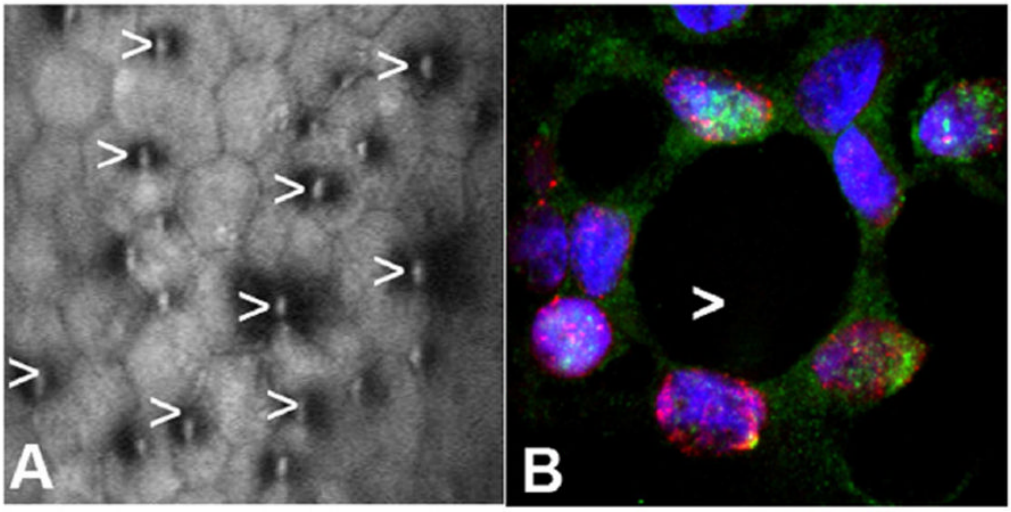

Fig. 1.

A. Confocal microscopy of a patient with FECD. Black areas (arrowheads) represent corneal guttae that are scattered in between corneal endothelial cells, disrupting a normally continuous layer of hexagonally shaped cells. **B**. Magnified view of one corneal gutta (arrowhead) with a center devoid of corneal endothelial cells. The remaining endothelial cells cluster around the gutta, staining positive for TUNEL (red), a marker of apoptosis, and positive for 8-OHdG (green), a marker of oxidative DNA damage.

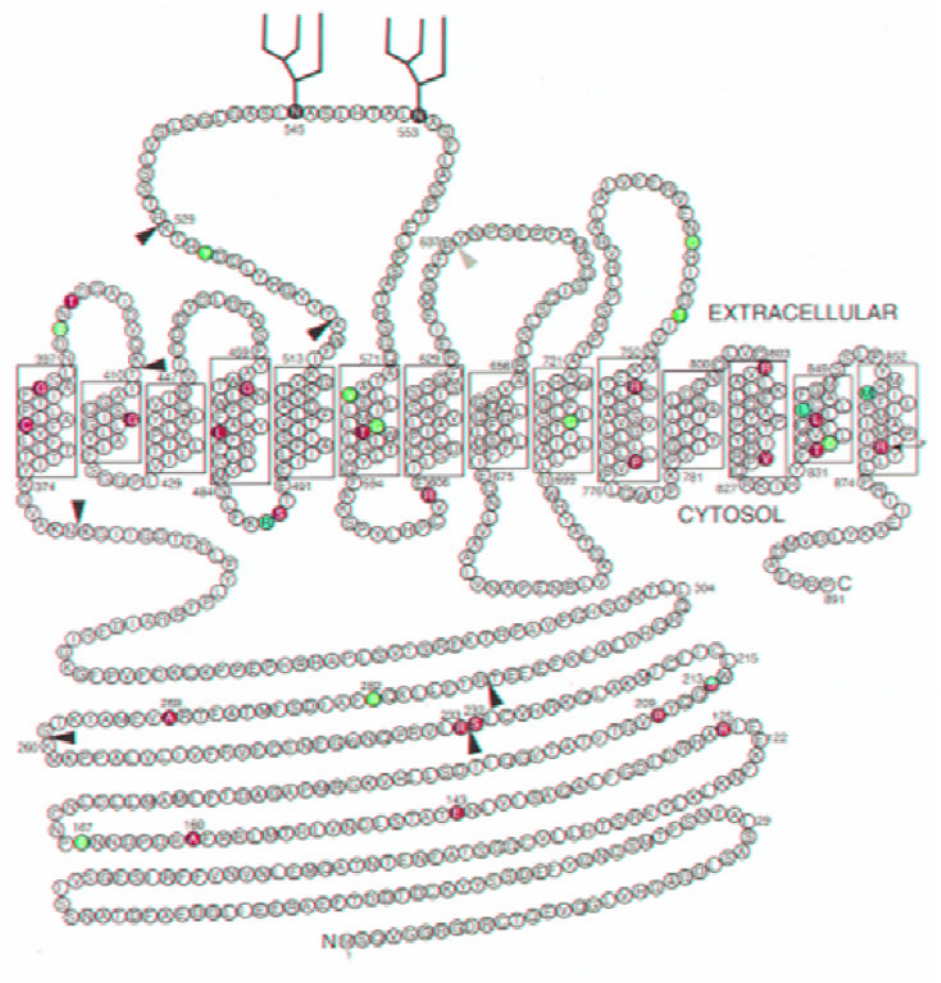

Fig. 2.

[1]Topology model for human SLC4All. Numbers indicate amino acid position. Predicted N-glycosylation sites are in black, and the branched structures represent oligosaccharide moieties. Black and gray arrowheads indicate trypsin cleavage sites identified through partial digestion of Myc-SLC4A11 and SLC4A11-Myc, respectively, as described in Vilas et al., 2011 (Vilas, G.L. et al., 2011). Identified point mutations causing CHED2 (blue filled), FECD (red filled), and Harboyan syndrome (orange filled) are indicated (see also Vilas et al. 2011, Refs. 4-6, 11,12,34,37,39,41,42). S213 was identified as mutated in both Harboyan syndrome and CHED2 and is shown in filled blue and orange, accordingly. Asterisks indicate residues where two different point mutations have been found to cause disease.

[1]Reproduced by permission from Vilas, GL, Morgan, PE, Loganathan, SK, Quon, A, and Casey, JR. Biochemistry. 2011;50:p. 2160.

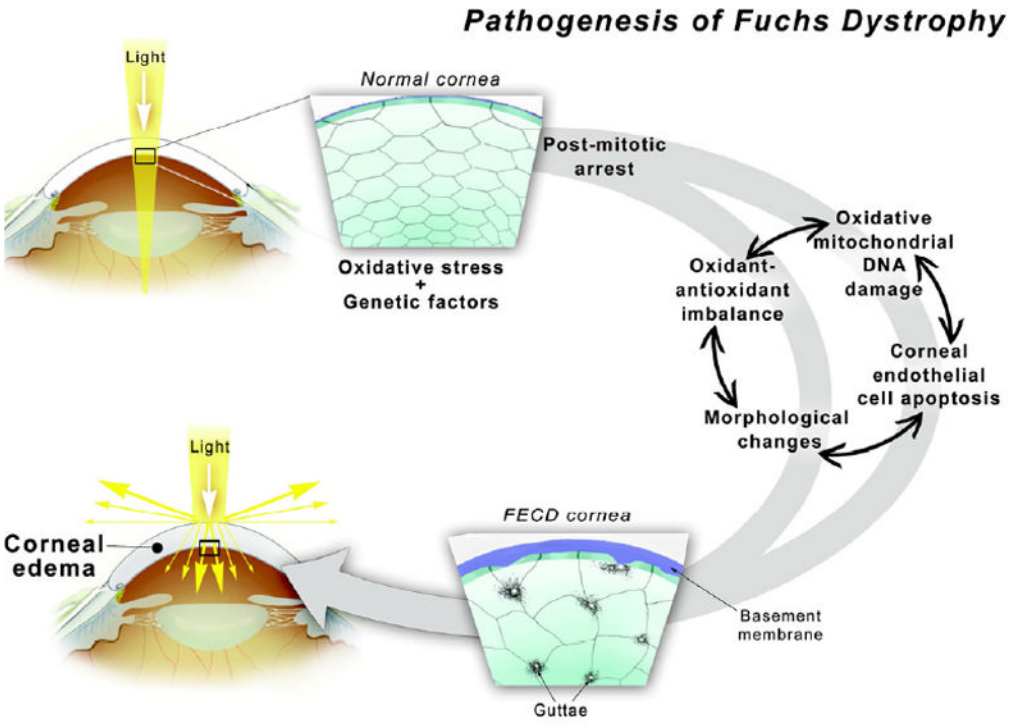

Fig. 3.

Diagram of the pathogenesis of FECD. Oxidative stress and genetic factors combined with endothelial cell post-mitotic arrest may lead to oxidant-antioxidant imbalance, oxidative mitochondrial DNA damage, endothelial morphological changes and apoptosis, and cause the corneal edema seen in FECD.

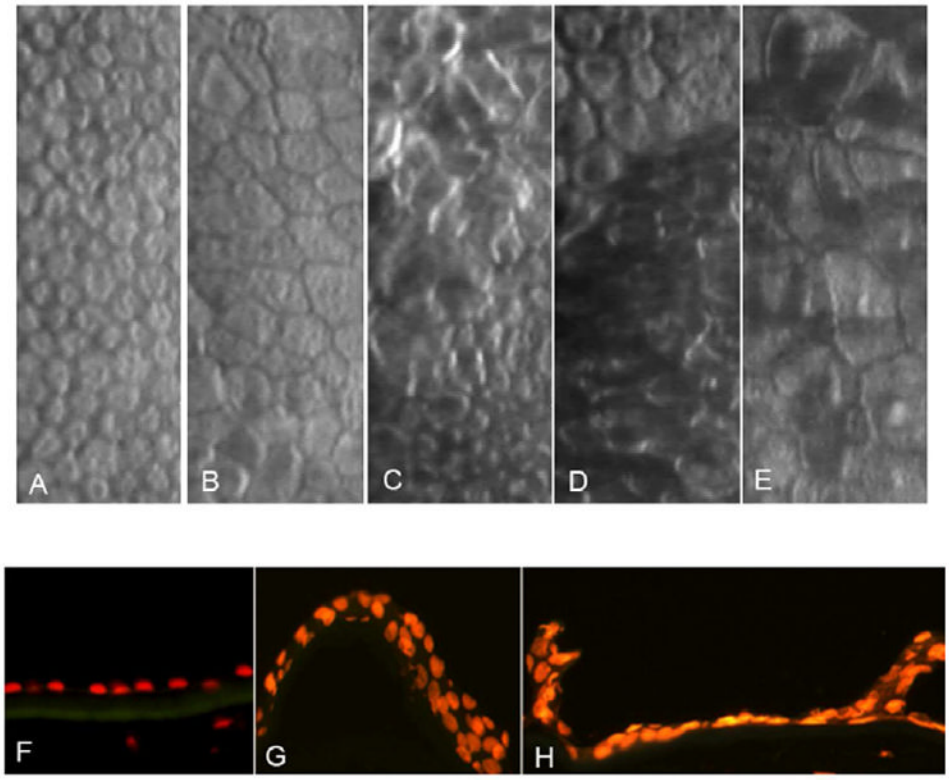

Fig. 4.

Specular photomicrographs of normal (A) and PPCD endothelium (B-E). PPCD endothelium displays pleomorphism, polymegethism, and vesicular lesions. Light microscopy detects oen layer of regular flat cells in normal endothelium (F). In PPCD, endothelium is composed of multilayered cells with prominent round nuclei and numerous projections (G,H). Red-propidium iodide. Courtesy of P. Liskova, M.D., Laboratory of the Biology and Pathology of the Eye, Charles University, Prague).