The Genetics and Pathophysiology of IC3D Category 1 Corneal Dystrophies: A Review

ABSTRACT

Corneal dystrophies are a group of inherited disorders affecting the cornea, many of which lead to visual impairment. The International Committee for the Classification of Corneal Dystrophy (IC3D) has established criteria to clarify the status of the various corneal dystrophies, which includes the knowledge of the underlying genetics. In this review, we discuss the IC3D category 1 (2nd edition) corneal dystrophies, for which a clear genetic link has been established. We highlight the various mechanisms underlying corneal dystrophy pathology, including structural disorganisation, instability or maladhesion, aberrant protein stability and deposition, abnormal cellular proliferation or apoptosis, and dysfunction of normal enzymatic processes. Understanding these genetic mechanisms is essential for designing targets for therapeutic intervention, especially in the age of Gene Ther and gene editing.

INTRODUCTION

Corneal dystrophies are a diverse group of inherited disorders. Although some entities are relatively asymptomatic, dystrophies generally result in progressive visual impairment through corneal opacification caused by decompensation, deposition, or scarring.

A system for categorising the corneal dystrophies was developed by The International Committee for Classification of Corneal Dystrophies (IC3D), integrating current understanding of the pathology, clinical phenotype, and incorporating the underlying genetic cause. The most recent iteration of the IC3D guidelines (2nd edition) has identified 22 types of corneal dystrophy. The nature, phenotype, and causative genes for many of these dystrophies are well documented, and the natural progression in our knowledge is to clarify the pathophysiology and processes by which disease occurs. Understanding the genetic mechanisms underlying these disorders paves the way for therapeutic intervention. With advances in small
interfering RNA, Gene Ther, and gene editing technologies, there is the potential to treat these disabling conditions by targeting the problem at its genetic root.

This review concentrates on the IC3D category 1 dystrophies (2nd edition): “a well-defined corneal dystrophy in which the gene has been mapped and identified and the specific mutations are known”. Many review articles have previously summarised the corneal dystrophy phenotypes and histopathology. As such, this article takes a more mechanistic approach, discussing the commonalities and differences in the genetic pathophysiology, with an aim to provide a greater comprehension of the processes causing disease. Following identification of a dystrophy-associated gene, a mechanistic investigation and understanding is the integral next step in the pathway to effective therapeutic intervention. A brief summary of corneal anatomy is provided for reference purposes, and common themes of disease process are discussed.

**THE CORNEA**

The cornea is the transparent window at the anterior of the eye, and is responsible for the majority of its optical power. The cornea contains 5 major layers: epithelium, Bowman layer, stroma, Descemet membrane, and endothelium (Figure 1).

The corneal epithelium is comprised of 5-6 cell layers in 3 sections: superficial (squamous) cells, intermediate (wing/polyhedral) cells and basal (columnal) cells. These cells are connected to each other via desmosomes, while the basal cells are connected to the underlying basement membrane by hemidesmosomes. Corneal epithelial basal cells secrete components that make up the epithelial basement membrane, including type IV collagen, type VII collagen, and laminin 332, which are essential for cell adhesion. These fixtures are essential for maintaining the integrity of the epithelial layer. Underneath the basement membrane, Bowman layer is acellular and comprised of randomly distributed condensed collagen fibres (types I and V) and keratan sulfate proteoglycans.3,4

The stroma accounts for the majority of the corneal thickness and is comprised mainly of collagenous lamellae that act as an extracellular matrix (ECM). Each of the lamellae are stacked and offset at 90° in a
plywood-like pattern (Figure 1). This regular structure prevents light scattering and confers transparency to the cornea. Glycosaminoglycans and proteoglycans bridge the collagen fibres and help maintain the regular organisation required for corneal clarity. The keratocytes are located amongst the lamellae of the stroma, and are connected to each other via gap junctions. Descemet membrane is the acellular basement membrane of the corneal endothelium and contains glycoproteins, laminins 332, 411, and 511, and collagen types IV and VIII.

The corneal endothelium monolayer plays a primary role in the maintenance of corneal hydration, maintaining the water content in the stroma. It exchanges waste and nutrients with the aqueous humor and is often referred to as the ‘fluid pump’. Corneal endothelial cells do not readily regenerate, and instead neighbouring cells stretch to fill gaps.

MECHANISMS OF DISEASE IN THE IC3D1 CORNEAL DYSTROPHIES

The following section details, by known or presumed pathogenic process, the mechanisms thought to result in disease for the IC3D category 1 dystrophies (2nd edition). These pathophysiological mechanisms underlying corneal dystrophy include structural disorganisation, instability or maladhesion, aberrant protein stability and deposition, abnormal cellular proliferation or apoptosis, and dysfunction of normal enzymatic processes. This information is summarised for each disease gene in Table 1.

ABNORMAL CELL ADHESION

Epithelial Recurrent Erosion Dystrophies (EREDs)

**Collagen, type XVII, alpha 1 (COL17A1)** has recently been identified as a causative gene in a sub-group of autosomal dominant ERED. The **COL17A1** variant c.3156C>T has been independently identified by two groups as segregating with disease in 5 ERED families.5,6 The c.3156C>T variant causes a splice site alteration, leading to the truncation of exon 46 (p.(Gly1052_Thr1070delinsAla)).5 A second **COL17A1** variant, c.2816C>T, p.Thr939Ile, was shown to cause a similar ERED in a sixth family.5
COL17A1, a member of the collagen family, is an integral part of the hemidesmosome structure. The hemidesmosomes anchor epithelial cells to Bowman layer, and are comprised of transmembrane COL17A1 (which interacts with β4 integrin in the basal membrane), BP230, and plectin (which interacts with keratin intermediate filaments of the cytoskeleton). Keratins are proteins that make up intermediate filaments, which alongside microfilaments and microtubules, form the cytoskeleton of the epithelium. In the human cornea, COL17A1 is present within Bowman layer and around the epithelial cells.\textsuperscript{5-8} COL17A1 mutations cause skin pathologies resulting from poor epithelial attachment including the autoimmune disease, bullous pemphigoid,\textsuperscript{9} and the autosomal recessive disease, junctional epidermolysis bullosa.\textsuperscript{10} COL17A1 is important for corneal wound healing, as it is up-regulated during healing,\textsuperscript{11} and is important in regulating keratinocyte cell motility.\textsuperscript{12,13} The described functions of COL17A1 are entirely consistent with the phenotype described in these ERED families, which includes recurrent corneal erosion, opacities at Bowman layer, and flecks in the anterior stroma.

**CORNEAL OPACIFICATIONS**

Corneal opacifications are the result of aberrant protein deposits within the cornea. These can arise as a consequence of abnormal protein function, protein instability, or a disruption of the normal enzymatic processes. Protein deposits can be amyloidogenic (structured) or non-amyloidogenic (granular). Amyloids are self-assembled protein structures with a cross-beta sheet quaternary structure, rendering them extremely stable.\textsuperscript{14} Corneal opacifications can also result as a consequence of scarring.

**Structural Instability**

**Meesmann Corneal Dystrophy**

Meesmann Corneal Dystrophy (MECD, OMIM#122100, IC3D1), an autosomal dominant disease, is characterised by the presence of corneal opacities in the corneal epithelium, which result from small, transparent (in oblique light) intraepithelial cysts. Meesmann Corneal Dystrophy has been attributed to mutations in the *keratin 3 (KRT3, 3 reported mutations)* and *keratin 12 (KRT12, 22 reported mutations)*.
Mutations in *KRT12* are associated with the clinically similar, but more severe, Stocker-Holt variant. *KRT3* and *KRT12* are expressed by corneal epithelial cells. In the cornea, *KRT3* and *KRT12* form keratin heterodimers that provide structure and stability to the corneal epithelial cells. Heterodimerisation of *KRT3* and *KRT12* is facilitated by motifs at the N- and C-termini of the keratin proteins: the helix-initiation and helix-termination motifs. The mutations described in *KRT3* and *KRT12* are confined to these motifs. As a result of *KRT3*/KRT12 mutations, the intermediate filaments fail to assemble correctly, and the corneal epithelium lacks structural integrity. The fragile corneal epithelium is vulnerable to cyst formation, which are likely composed of aberrant KRT3/KRT12 proteins and other cellular debris (Figure 1).

The location of the mutations in these genes leads to variability in the disease phenotype. The recurrent European mutation in *KRT12* – p.Arg135Thr – causes a more mild phenotype than the p.Leu132Pro mutation, which is associated with the presence of microcysts and corneal scarring. Cells transfected with K12 bearing the p.Leu132Pro mutation produced less filamentous keratin than those with p.Arg135Thr K12, suggesting a greater disruption to normal keratin function. Interestingly, epidermolysis bullosa patients also present with mutations in helix-initiation and helix-termination motifs of keratin proteins. Current therapies under investigation are based around RNA-induced silencing of the aberrant *KRT12*, and the gene-editing tool CRISPR/Cas9 (clustered regularly-interspaced short palindromic repeats), where RNA is used to specifically target a locus for DNA cleavage.

**Gelatinous Drop-Like Corneal Dystrophy (GDLD)**

Gelatinous Drop-like Corneal Dystrophy (GDLD, OMIM# 204870) is an uncommon autosomal recessive disease. GDLD is characterised by subepithelial and stromal gelatinous amyloid deposits. These deposits disturb the smooth corneal surface and can lead to corneal erosions. GDLD has been attributed to mutations in *tumor-associated calcium signal transducer 2 (TACSTD2)*. *TACSTD2* is a cell surface receptor that transduces calcium signals, and contains a phosphatidylinositol 4,5-bisphosphate (PIP₅₂) domain which regulates binding to cytoplasmic molecules or the cell membrane. The role of calcium signalling in GDLD is not yet understood. Currently 28 *TACSTD2* mutations (including 11 indels) have been described in GDLD.
GDLD corneas and TACSTD2-depleted cell lines show decreased tight junctions, suggesting the problem lies with the weakening of the barrier system in the cornea. Important cell-junction proteins, including occludin, tight junction protein 1 and claudins (1,4 & 7), are absent or reduced in the cell junctions in GDLD corneas. The basement membrane integrins are also mislocalised. Gelatinous masses of amyloid deposits have been attributed to the increased barrier permeability of the corneal epithelium in the absence of TACSTD2, leading to the penetration and accumulation of the globular protein lactoferrin from ocular secretions (Figure 1). It is possible that lactoferrin has additional roles in GDLD, as it has also been detected in the nuclei of epithelial cells in GDLD patients.

GDLD is a rare disease that is most prevalent in the Japanese population, where a p.Gln118X founder mutation in TACSTD2 has been described. The p.Gln118X mutation in the thyroglobulin repeat domain leads to a truncated protein that lacks the transmembrane domain.

Protein Instability

TGFBI-Associated Corneal Dystrophies

Several corneal dystrophies can be attributed to the accumulation of transforming growth factor beta-induced (TGFBI) protein. In general, in this group of autosomal dominant corneal dystrophies, pathological deposits containing TGFBI may accumulate within or adjacent to either one or both layers of the anterior cornea: Bowman layer (Reis-Bücklers Corneal Dystrophy (RBCD), Thiel-Behnke corneal dystrophy (TBCD)) and the stroma (lattice corneal dystrophy type 1 (LCD1), granular corneal dystrophy type 1 (GCD1) and type 2 (GCD2)) (Figure 1). Within the literature, there are some varying descriptors regarding the exact nature of the deposits and associated scar tissue if present.

TGFBI is induced by TGFB and secreted to the extracellular matrix (ECM) to aid in cell adhesion and motility. TGFBI expression is highest in the corneal epithelial cells, but is also expressed by corneal keratocytes. At least 5 different autosomal dominant corneal dystrophies are associated with TGFBI, with 65 reported pathological mutations identified to date. It is worth noting that whilst TGFBI is expressed in multiple tissues, it is only in the cornea that pathological accumulation occurs. In these corneal dystrophies,
the aggregates of aberrant TGFBI protein result in poor cell adhesion, poor motility, and changes to the epithelial/Bowman layers of the cornea.

The secreted TGFBI protein contains 4 tandem fasciclin (FAS1) domains, which enable cell adhesion. These FAS1 domains are flanked by an upstream EMI (elastin microfibril interfacer family) domain (for protein-protein interaction) and a C-terminal Arg-Gly-Asp (RGD) domain facilitating binding to collagen types I, II and IV. The vast majority of the reported TGFBI mutations (49 identified to date) localise to the fourth FAS1 domain. TGFBI interacts with integrins, the transmembrane receptors important in cell signalling and cell-ECM interaction. It is through the integrins that a cell can respond to external stimuli by regulating the cell cycle, ECM shape, and cell motility. TGFBI also interacts with collagens and proteoglycans. In the cornea, wild-type TGFBI protein is monomeric and has no post-translational modifications. Corneal disease pathology is the result of the accumulation of mutant TGFBI protein as amyloid (structured) and/or non-amyloid (non-structured) aggregates.

Protein aggregates are thought to occur due to protein unfolding, often as a consequence of protein instability. As there are only two mutations in the FAS1-2 and FAS1-3 domains, studies have focused on the roles of amino acid substitutions within the FAS1-1 and FAS1-4 domains. Single amino acid substitutions in the FAS1-1 domain appear to have no impact on TGFBI protein stability. Mutations in the FAS1-4 domain have both decreased (p.Ala546Thr, amyloid-associated, LCD) and increased (p.Arg555Trp, non-amyloid associated, GCD) protein stability. Combined, these findings suggest that there are different mechanisms through which protein aggregation occurs.

Mutations in TGFBI highlight the importance in understanding the genotype in order to understand clinical presentation – although there are several examples of exceptions to this rule. The amino acid most commonly affected by mutations in TGFBI is p.Arg124, upstream of the FAS1 domain repeats. Intriguingly, different mutations affecting this site can lead to either amyloidogenic deposits causing LCD1 (p.Arg124Cys, 372 reported cases), or non-amyloidogenic deposits causing RBCD (p.Arg124Leu, 63 reported cases). Mutations involving p.Arg555 are also relatively common, typically causing either GCD1 (p.Arg555Trp, 375 reported cases) or TBCD (p.Arg555Gln, 66 reported cases). One example where the genotype-phenotype
correlation is not so clear is for the p.His626Pro mutation, which has been reported with an overlapping phenotype of RBCD and TBCD.³⁷,⁴⁰

*TGFBI* has also been implicated in a sixth corneal dystrophy - Epithelial Basement Membrane Dystrophy (EBMD, OMIM#121820) - with autosomal dominant mutations described in 2 families.⁴¹ EMBD presents as additional sheets of basement membrane extend abnormally into the corneal epithelium, intraepithelial pseudocysts comprised of migratory epithelial cells, and may result in corneal erosions. However, the majority of EMBD cases appear to arise as a degenerative disease or as a consequence of trauma.²

**Aberrant Enzyme Function**

**Macular Corneal Dystrophy (MCD)**

Macular Corneal Dystrophy (MCD, OMIM#217800) is an autosomal recessive disease. Patients present with central, superficial, whitish fleck-like opacities, extending out to the limbus, and involving all layers of the stroma down to Descemet membrane, progressively leading to a diffuse haze.² The cornea in these patients is thin, and in advanced disease, Descemet membrane greys and develops excrescences (guttate).² MCD is caused by mutations in the carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 6 (*CHST6*) gene.⁴² To date, 165 distinct mutations have been identified in *CHST6* and associated with MCD.¹⁵ *CHST6* encodes a Golgi transmembrane enzyme that catalyses the transfer of a sulfate group to N-Acetylg glucosamine residues on the glycosaminoglycan (GAG) keratan sulfate.⁴³,⁴⁴ Keratan sulfates are structural carbohydrates most prevalent in cornea. Keratan sulfate is predominantly found in the corneal stroma, whilst *CHST6* is mainly expressed by stromal keratocytes.⁴⁵ Keratan sulfates are adjoined to proteins (forming proteoglycans), and have roles in cell adhesion, ECM structure, and are also thought to be important in maintaining corneal hydration. The highest level of keratan sulfate in the chick cornea correlates with maximum corneal clarity during development, suggesting keratan sulfates are important in maintaining transparency of the cornea.⁴⁶ In MCD corneas, the keratan sulfate proteoglycan proteins are produced, but the GAG chains are not sulfated to become keratan sulfate.⁴⁷ The sulfate groups on proteoglycan GAG chains can bind water; the unsulfated keratan sulfate are predicted to be less water-soluble than sulfated keratan sulfate, which will affect secretion and deposition of the proteoglycans.⁴⁴
Furthermore, these unsulfated keratan sulfate proteoglycans may not be degraded by the cell at the same efficiency.44

Onset of MCD occurs in the first decade, and is characterised by punctate, grey, glycosaminoglycan deposits in the stroma and Descemet membrane (Figure 1), leading to Descemet thickening; corneal erosions rarely occur. These deposits lie underneath the epithelium, between the stromal lamellae, and within keratocytes and endothelial cells. MCD is classified into two subtypes: MCD type I, where antigenic keratan sulfate is absent in both patient serum and cornea, and MCD type II, where antigenic keratan sulfate in serum is normal, and the corneal accumulations react to antibodies for keratan sulfate.48 Mutations in CHST6 that cause MCD type II are outside of the coding region of the gene, in the regions that regulate its corneal-specific expression. These mutations appear to arise via homologous recombination with the highly homologous region upstream of the CHSTS gene.42 The presence of antigenic keratan sulfate in serum in MCD type II is attributed to non-corneal keratan sulfate degradation.44 As the main cellular component causing MCD are the stromal keratocytes,45 treatments must ensure the removal of all aberrant keratocytes (e.g. penetrating keratoplasty or deep anterior lamellar keratoplasty).

**Fleck Corneal Dystrophy (FCD)**

Fleck corneal dystrophy (FCD, OMIM#121850) is an early onset autosomal dominant disorder. Whilst patients are typically asymptomatic, they present with subtle, small, translucent disc-shaped opacities, or discrete, flat, grey-white, often ring-shaped opacities throughout the stroma (Figure 1).2 FCD is attributed to mutations in the phosphoinositide kinase, FYVE finger containing (PIKFYVE) gene. PIKFYVE is both a lipid and protein kinase, and is important in regulating signal transduction, cytoskeletal organisation, and membrane trafficking. 12 mutations in PIKFYVE have been described, and these mutations are restricted to 5 of the 41 coding exons.49

The opacities in fleck corneal dystrophy are the apparent result of keratocytes containing intracytoplasmic vesicles packed with lipids and glycosylaminoglycans.50 Murine embryonic stem cells deficient in PIKFYVE show enlarged endosomes.51 Mice deficient in PIKFYVE are embryonic lethal, attributed to incapability of the swollen vacuoles to update essential maternal nutrients in the developing
Whilst the exact mechanism of disease in FCD is not yet known, the pathology may be a consequence of failed breakdown of lipids and glycosaminoglycans, and accumulation of intracytoplasmic vesicles in the keratocytes.\textsuperscript{50,51}

**Pre-Descemet Corneal Dystrophy (PDCD) associated with X-linked ichthyosis**

Pre-Descemet Corneal Dystrophy (PDCD) encompasses a poorly defined range of corneal dystrophies for which there is no clear inheritance pattern. A sub-group of PDCD is associated with X-linked ichthyosis (XLI, OMIM\#300747) – a disorder leading to dry, scaling skin. Up to half of all XLI patients present with some type of ocular symptoms – PDCD is not present in all XLI individuals.\textsuperscript{52} The majority of patients with X-linked ichthyosis have a deletion of the \textit{steroid sulfatase (microsomal), isozyme S (STS)} gene. In one individual with XLI and PDCD, a microdeletion was identified which included \textit{STS}.\textsuperscript{53} Corneal opacities in XLI have been attributed aggregates of cholesterol sulfate, or alternatively to disruption of the stromal structure by elevated cholesterol sulfate levels.\textsuperscript{55}

**Schnyder Corneal Dystrophy (SCD)**

Schnyder corneal dystrophy (SCD, OMIM\#21800) is an autosomal dominant disease, with onset from childhood through to the second or third decade. Patients present with characteristic clinical features that change with age. Patients <23 years of age have a ring- or disc-like central corneal opacity and/or central comma-shaped subepithelial crystals. Patients aged 23-38 years also present with arcus lipoides, and those >38 years of age show a midperipheral panstromal haze. Approximately half of patients present with corneal crystals.\textsuperscript{2} In the SCD cornea, there are accumulations of intracellular and extracellular cholesterol and phospholipids, most notably unesterified cholesterol, causing opacity.\textsuperscript{56,57}

SCD has been attributed to mutations in \textit{UbiA prenyltransferase domain containing 1 (UBIAD1)}, with 25 reported mutations.\textsuperscript{15} The UbiA superfamily of prenyltransferases catalyse the transfer of isoprenyl groups to aromatic acceptors. UBIAD1 has a known role in converting vitamin K\textsubscript{1} to vitamin K\textsubscript{2},\textsuperscript{58} and more recently has been linked to the cholesterol biosynthetic enzyme HMG CoA (3-hydroxy-3-methylglutaryl coenzyme A)
HMG CoA reductase catalyses the reduction of HMG CoA to mevalonate, a rate-limiting step in the production of cholesterol and other sterols. UBIAD1 associates with the membrane domain of HMG CoA reductase in the presence of sterols, enabling the reaction to proceed towards cholesterol generation. In a negative feedback loop, sterol induced binding of UBIAD1 to HMG CoA reductase is inhibited by geranylgeraniol, which releases the UBIAD1 protein for translocation from the endoplasmic reticulum (ER) to the Golgi. The HMG CoA reductase is then targeted for ER-associated degradation and cholesterol synthesis is reduced. However, mutations identified within UBAID1 and associated with SCD resist the displacement of UBAID1 upon geranylgeraniol binding, preventing ER activated degradation of HMG CoA reductase. The constitutive activation of mutant UBAID1 is hypothesised to lead to cholesterol accumulation, and therefore the pathology leading to Schnyder corneal dystrophy (Figure 1).

**DISRUPTION OF STROMAL ORGANISATION**

**Posterior Amorphous Corneal Dystrophy (PACD)**

Posterior Amorphous Corneal Dystrophy (PACD, OMIM#612868) is an autosomal dominant disorder causing posterior stromal lamellar opacification, decreased central corneal thickness, and flattening of the cornea. Grey, sheet-like opacities are visible in the stroma, particularly in the posterior region, and these can cause indents to Descemet membrane and the endothelium. PACD is a rare disorder that has only been described in 13 families. Recently, copy number variant (CNV) analysis revealed large deletions in 3 unrelated PACD families; in affected family members from all 3 families, the genes *keratocan (KERA), lumican (LUM), decorin (DCN), epiphycan (EPYC)* and *coiled-coil glutamate rich protein 1 (CCER1)* are deleted on one chromosome copy, resulting in haploinsufficiency of these proteins. The genes encoding SLRPs are clustered on human chromosome 12. While the mechanism of PACD pathology has yet to be elucidated, several inferences can be made based on the functions of the affected genes. *CCER1*, a provisional protein-coding gene, and the...
SLRP, have effectively been eliminated as causative genes in PACD, as their expression is absent in the cornea. Lumican and keratocan are keratan sulfate proteoglycans in the cornea. Mice deficient in keratocan and lumican have aberrant collagen fibrils, though only lumican deficient mice have corneal opacities. In contrast, mice deficient in decorin, a dermatan sulfate proteoglycan, show no evidence of collagen fibril irregularities in the cornea. In PACD, LUM is hypothesized to be the causative gene, as loss of lumican in the cornea also reduces keratocan. LUM expression is restricted to the posterior stroma in the mature cornea. The loss of LUM (and potentially other SLRPs) is hypothesized to cause irregular stromal structure by disrupting collagen fibril spacing, leading to the pathology observed in PACD.

**Congenital Stromal Corneal Dystrophy (CSCD)**

Congenital stromal corneal dystrophy (CSCD, OMIM#610048) is a very rare autosomal dominant disorder, reported in 4 families. Patients present with flake-like whitish stromal opacities, accompanied by increased stromal thickness. CSCD has been attributed to mutations in the *decorin (DCN)* gene, a SLRP – this gene is included in the deletion region described for PACD. The reported mutations in *DCN* have all affected the C-terminus of the protein, including 3 deletions truncating the SLRP-specific “ear” repeat. Mice heterozygous for mutant decorin recapitulated human CSCD, with corneal opacities and abnormal fibril organisation, and disrupted normal expression of other SLRPs. In vitro studies have shown that the aberrant decorin protein is retained within the endoplasmic reticulum (ER), causing ER stress and consequently disrupting ECM component synthesis and assembly. It appears that in CSCD, the mutant decorin is acting in a dominant negative fashion, as mice deficient in decorin show compensation with another SLRP - biglycan (*BGN*).

**Cornea Plana (CNA2)**

While not classified in IC3D, Cornea Plana 2 (CNA2, OMIM#217300) is an autosomal recessive disorder attributed to homozygous or compound heterozygous mutations in another member of the SLRP family, keratocan (*KERA*). *KERA* is a keratan sulfate proteoglycan and and as previously mentioned is important in regulating the structure, and thus transparency of the cornea. 10 mutations in *KERA* have been reported, all of which affect the Leucine-rich repeat (LRR) domain of the protein — a structure predicted to be
important for protein function. The instability of KERA is likely to result in poor stromal structure and incorrect spacing of the collagen fibrils, leading to CNA2 with reduced corneal curvature, extreme hypermetropia, hazy corneal limbus and corneal clouding.

**Congenital Hereditary Endothelial Dystrophy (CHED)**

Congenital hereditary endothelial dystrophy (CHED, OMIM#217700) is an autosomal recessive disorder. Patients are often asymptomatic, but present with corneal clouding, thickening of the cornea (2-3 times the normal corneal thickness) and reduced endothelial cell count. CHED has been attributed to sequence variants in *solute carrier family 4, sodium borate transporter, member 11 (SLC4A11)*, although a quarter of families with CHED have no identifiable SLC4A11 mutation. Over 76 variants in SLC4A11 have been reported, and mutations are also associated with FECD, as well as corneal dystrophy and perceptive deafness (Harboyan syndrome, OMIM#217400).

SLC4A11 regulates the isoelectric point (pHi) of the corneal epithelium, by transporting the ions $\text{Na}^+$ coupled to $\text{OH}^-$. As expected for an ion transporter, SLC4A11 is located in the plasma membrane; however, several variant forms of SLC4A11 have been demonstrated to localise incorrectly, and instead are present in the cytoplasm, suggesting a loss of normal protein function. HEK293 cells expressing mutant SLC4A11 accumulate reactive oxygen species (ROS), and show reduced expression of the antioxidant protein nuclear factor, erythroid 2 like 2 (NFE2L2). Furthermore, these cells expressing mutant SLC4A11 indicate weak mitochondrial function and have increased apoptosis. One hypothesis is that the SLC4A11 mutations disrupt the function of this important transporter, leading to accumulated ROS and mitochondrial damage, followed by accelerated cell death in the endothelium. Clouding of the cornea has been attributed to fluid accumulation in the stroma, disrupting collagen fibril organisation. SLC4A11 is located basolaterally in the corneal endothelium and expression of SLC4A11 in HEK293 and *Xenopus laevis* oocytes causes osmotically induced cellular swelling. Based on these findings, a second hypothesis proposes that SLC4A11 forms the basolateral fluid pump, moving water from the stroma into the endothelium.
CELL PROLIFERATION

Posterior Polymorphous Corneal Dystrophy (PPCD)

There are three sub-types of posterior polymorphous corneal dystrophy (PPCD): PPCD1 (OMIM#122000), PPCD2 (OMIM#609140) and PPCD3 (OMIM#609141). These disorders are characterised by opacities at Descemet membrane and the corneal endothelium with distinct features including grey opacities, vesicular lesions (often encircled by circular grey opacities) and parallel grey-white endothelial bands with flaky white material, often extending across the entire cornea. PPCD3 is also associated with corneal curvature steepening. PPCD2 has been attributed to mutations in collagen, type VIII, alpha 2 (COL8A2), but the evidence is weak and has not been reproduced (summarised in [75]). The underlying genetics has only been clearly identified in PPCD3, an autosomal dominant disorder. PPCD3 has been attributed to mutations in zinc finger E-box binding homeobox 1 (ZEB1), with over 30 mutations identified to date. Recently, analysis of copy number variants (CNV) have also revealed heterozygous ZEB1 deletions in PPCD3 pedigrees. Interestingly, sequence variants in both ZEB1 and COL8A2 have been associated with Fuchs Endothelial Corneal Dystrophy (FECD).

As a zinc finger transcription factor, ZEB1 plays an important role controlling gene expression in normal development as well as contributing to disease when this regulation goes wrong. ZEB1 contributes to the epithelial-to-mesenchymal transition (EMT) process by repressing the expression of epithelial-specific genes. EMT is an important response during wound healing, as well as becoming an aberrant process that leads to cancer. The exact mechanism of ZEB1 regulation in the endothelial cells is not yet understood. In mice lacking ZEB1, increased expression of epithelial genes COL4A3 and E-cadherin accompanied abnormal corneal endothelial and keratocyte proliferation. In the human PPCD3 cornea, epithelial cell markers are increased and the epithelial-like endothelium is indicative of aberrant proliferation. Together, these results suggest that the haploinsufficiency of ZEB1 is causative of PPCD3.

CELL DEATH
Fuchs Endothelial Corneal Dystrophy (FECD)

Fuchs endothelial corneal dystrophy (FECD) is a group of related corneal dystrophies characterised by central cornea guttae (collagenous excrescences of Descemet membrane) spreading peripherally, and, in advanced stages, endothelial decompensation and stromal edema. Secondary swelling of the epithelium also occurs. Descemet membrane is thickened as ECM material accumulates and there is a gradual loss of endothelial cells. The genetics underlying FECD are complex, indicating a genetically heterogeneous disease. FECD is grouped broadly into early and late onset (classic) disease. Although it can present as an autosomal dominant trait, the majority of cases are sporadic. There is a significant skewing towards presentation in females at a ratio of 2.5:1.

Early onset FECD (FECD1, OMIM# 120252) has been attributed to mutations in collagen, type VIII, alpha 2 (COL8A2). COL8A2 is a component of collagen VIII, which is assembled into triple helices within the endoplasmic reticulum. Six COL8A2 mutations have been reported, all of which lie in the triple-helical domain, suggesting a possible interference with correct protein assembly. The unfolded protein response (UPR) is a cell’s defensive mechanism to prevent toxicity from incorrectly folded proteins. Two genetic knock-in mice have successfully recapitulated human FCED by introducing the previously identified Q455K and L450W mutations in COL8A2. In these mouse models, there is evidence to support a link between the COL8A2 mutations and UPR-mediated autophagy (cell death): enlarged rough endoplasmic reticulum were observed, alongside up-regulation of UPR genes and proteins, including damage-regulated autophagy modulator (DRAM1).

Several genes have been associated with late onset FECD. Mixed evidence suggests that heterozygous variants in solute carrier family 4, sodium borate transporter, member 11 (SLC4A11) may be associated with FECD4 (OMIM#613268). Inconclusive segregation analysis has been reported in these families (reviewed in [75]). As described elsewhere in this review, homozygous variants in SLC4A11 result in CHED, yet parents of CHED patients do not appear to present with late onset FECD. Whether unique variants segregate in FECD versus CHED, and whether these variants are sufficient to act in a dominant negative fashion leading to FECD remains to be examined.
Similarly conflicting evidence has been presented for sequence variants in **zinc finger E-box binding homeobox 1 (ZEB1)** (FECD6, OMIM# 613270),92-94 and it is not clear whether ZEB1 plays a role in late onset FECD pathology. Increased expression of ZEB1 has been identified in corneal endothelial cells derived from FECD corneas, and induces an EMT-like phenotype.95 ZEB1 regulates EMT by interacting with TGFB1 (transforming growth factor beta 1) and the miR200 microRNA family.96 In corneal endothelial cells TGFB1 induces the secretion of ECM proteins, including collagen and fibronectin which accumulate in FECD.97,98 It is proposed that the increased levels of ZEB1 in the FECD cornea facilitates an increased sensitivity to TGFB1, leading to increased ECM production, causing ER stress and leading to UPR-induced apoptosis.95

Recently, expansion of an intronic trinucleotide repeat (CTG18.1) within **transcription factor 4 (TCF4)** has been associated with FECD3 (OMIM#613267), with the expansion allele (>50 repeats) conferring a 30-fold risk of FECD.99,100 Transcription of the expanded TCF4 results in nuclear foci comprised of corresponding (CUG)n RNA and the splicing protein muscleblind-like 1 (MBNL1).101,102 RNA toxicity is proposed to lead to the endothelial cell death phenotype in FECD, either as MBNL1 is sequestered by these cells with consequences to additional transcript splicing or via the induction of apoptosis.102 As MBNL1 has previously been implicated in EMT,103 it is possible that this pathway may also be disrupted in these cells.

**CONCLUSIONS**

The number of genes clearly implicated in the causation of the corneal dystrophies now number 15 (Table 1), but there are a number of commonalities in the pathogenic processes underlying disease progression. This review has highlighted the pathways towards corneal malfunction and opacification, from a myriad of processes including structural disorganisation, instability or maladhesion, aberrant protein stability and deposition, abnormal cellular proliferation or apoptosis, and dysfunction of normal enzymatic processes. A thorough knowledge of the mechanisms and systems of disease genesis are a prerequisite for identifying therapeutic interventions and gene-related treatments for the corneal dystrophies.
REFERENCES


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