Clinical & Experimental Ophthalmology

WILEY

Clinical & Experimental Ophthalmology

REVIEW OPEN ACCESS

Genotype–Phenotype Correlations in Corneal Dystrophies: Advances in Molecular Genetics and Therapeutic Insights

Petra Liskova[1,2] | Pavlina Skalicka[1] | Lubica Dudakova[2] | Andrea L. Vincent[3,4]

[1]Department of Ophthalmology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic | [2]Department of Paediatrics and Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic | [3]Department of Ophthalmology, New Zealand National Eye Centre, Faculty of Medical and Health Science, The University of Auckland, Auckland, New Zealand | [4]Eye Department, Greenlane Clinical Centre, Te Toka Tumai Auckland, Te Whatu Ora, Health New Zealand, Auckland, New Zealand

Correspondence: Andrea L. Vincent (a.vincent@auckland.ac.nz)

Received: 18 September 2024 | **Revised:** 19 February 2025 | **Accepted:** 27 February 2025

Funding: This study was supported by UNIVERZITA KARLOVA, SVV 2600631, UNCE/24/MED/022 and Ministerstvo Zdravotnictví Ceské Republiky, MH CZDRO-VFN64165, NW24-06-00083, NW25-07-00303.

Keywords: corneal dystrophy | genetics | hereditary | molecular biology

ABSTRACT

Corneal dystrophies are a group of predominantly rare inherited disorders. They are by definition bilateral, relatively symmetrical, and without systemic involvement, affecting corneal transparency and/or refraction. Traditional classification of corneal dystrophies is based on slit-lamp appearance, affected corneal layer and histological features. Molecular genetics has provided ultimate proof for the existence of distinct corneal dystrophies and discarded duplicates in their terminology. Currently, there are at least 16 genes with identified pathogenic variants implicated in corneal dystrophies. Herein, we summarise contemporary knowledge on genotype–phenotype correlations of corneal dystrophies, including a critical review of some reported variants, along with the understanding of the underlying pathogenic dystrophic process; essential knowledge for the development of targeted therapies.

1 | Introduction

Corneal dystrophies (CDs) represent a heterogeneous group of hereditary disorders with variability in onset, progression, and severity. By definition, CDs are bilateral and relatively symmetrical, with no systemic involvement, affecting corneal transparency and refraction, frequently manifesting in the first or second decade of life. The identification of underlying genetic causes has widely impacted their classification and recognition in general. With the exception of Fuchs endothelial corneal dystrophy (FECD), CDs are rare disorders affecting fewer than five individuals per 10 000 [1].

Traditionally, CDs have been distinguished based on their clinical appearance and histological features, and many eponymous names were used, often for the same clinical entity. Molecular characterisation, however, has transformed our knowledge of many inherited diseases, with awareness that changes in one gene can cause different clinical phenotypes, but also that one apparent phenotype may be caused by changes in different genes [1].

The International Committee for Classification of Corneal Dystrophies (IC3D) is a specialised group focused on the classification and understanding of CDs [1]. The IC3D classification is organised by the layers of the cornea affected and incorporates genetic information where available. The IC3D regularly updates its classification system to reflect new discoveries in genetic research and clinical observations [1]. This review will therefore use the proposed IC3D nomenclature and any exceptions will be discussed.

Online Mendelian Inheritance in Man (OMIM, https://omim.org/) is a continuously updated catalogue of human genes and genetic disorders and traits, with a particular focus on the molecular relationship between genetic variation and phenotypic expression. In this review, we will refer to the OMIM number of each condition described. Standard nomenclature will be used; gene symbols are italicised (e.g., *SPARCL1*) and the protein is not italicised (e.g., SPARCL1).

The ACMG/AMP criteria refer to the guidelines established by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) for the interpretation of genetic variants. These criteria are used to classify genetic variants in a standardised manner: pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, benign, which is essential for understanding their potential role in disease and for informed clinical decisions. Various parameters are assessed for the variant classification, including population data, functional studies, computational predictions and segregation data, among others [2].

Although the genetic cause for many of the CDs has been characterised, continuous advancements in technology and in our knowledge of genomics have allowed identification of two further genes implicated in the pathogenesis of CDs in 2024 alone [3, 4]. In addition, innovative therapeutic approaches for CDs are under development, including cellular therapies, RNA interference, and antisense oligonucleotides (ASOs) [5], for which a prerequisite will be a genetic diagnosis at the DNA level.

This review summarises the latest understanding of CDs based on genotype–phenotype correlations, focusing on disorders that have robust genetic evidence supporting their delineation as separate clinical entities. This knowledge is crucial for patients and their family members: to determine the risk to subsequent generations, for prognosis regarding visual acuity, for planning any surgical intervention to optimise vision and minimise recurrence post-intervention, and for clearly understanding the aberrant pathway causing the corneal opacification – a necessity for targeted pharmacological success.

2 | Prevalence of CDs

A knowledge of the prevalence of disease within given populations and sex predominance may aid diagnosis, and this also helps with interpretation of genetic variants.

To decide on the rarity of each disease, we have retrieved publications from the PubMed database (https://pubmed.ncbi.nlm.nih.gov/ accessed 13 August 2024) by searching combinations of the following keywords: 'corneal dystrophy' 'mutation' and/or 'pathogenic variant' and abbreviation of the disease-associated genes, except for *TGFBI, CHST6*, and *UBIAD1*, which have been recently reviewed in other publications [6–8].

Estimates of the prevalence of CDs have substantial limitations as they are primarily based on information from two sources: corneal transplant registries and large case series from corneal surgeons, and are thus biased toward more severe cases. Hence, the number of individuals with CDs that do not require keratoplasty is difficult to assess. As our approach did not include exact numbers of affected individuals, only probands, nor deep assessment using ACMG/AMP criteria for the already reported variants, which is beyond the scope of this review, we show an approximate number of families (range), except for CDs observed in fewer than 10 families worldwide. These numbers may be underestimates as not all CD's or their genetic variants are published, nor reported in ClinVar [9] or gene-specific variant databases, and may be held in institutional databases.

As rare CDs are Mendelian diseases, they can occur in any population as a result of spontaneous mutational events, and except for X-linked endothelial corneal dystrophy, there seems to be no male-to-female ratio differences. The prevalence of recessive disorders such as Macular corneal dystrophy (MCD) and Congenital hereditary endothelial dystrophy (CHED) is known to be higher in communities where consanguinity is common [10, 11]. Some CDs have been observed in only a few families, and their incidence is influenced by local founder effects and geographically isolated frequencies of diseased alleles. For example, MCD is relatively common in the Icelandic population [12, 13], while PPCD1 and PPCD4 show higher prevalence in the Czech Republic compared to other countries, which is attributed to two different founder effects [14, 15].

Estimates on age-related FECD prevalence vary significantly, partly as there is no consensus on whether isolated guttae are part of the FECD spectrum or an independent, nonprogressive condition [16, 17]. A recent meta-analysis estimated the global prevalence of age-related FECD to be 7.33% [18]. Another study that used Medicare data of patients older than 65 years concluded that the prevalence of age-related FECD in the United States is 1.12% [19]. Disease occurrence is significantly higher in women and the white population compared with other ethnicities [18, 19]. Taken together, we conclude that age-related FECD is present in more than 2% of the population worldwide.

3 | Genetic Testing

Advances in genetic technologies have resulted in more comprehensive gene panels for a given disease, either isolated CDs or other ocular conditions. These next-generation sequencing panels are cost-effective, with a relatively quick turnaround. A decision to undertake genetic testing can only be made with appropriate and informed genetic counselling and consent of the patient(s) and their family, in either a dedicated ocular genetic service or through the general genetic service. Many variants may be identified using this technology, and it is crucial that correct interpretation, using ACMG/AMP criteria, is applied for a definitive genetic diagnosis, and segregation analysis performed in family members.

4 | Summary List of CDs

Well-defined CDs that will be further discussed in the current review are summarised in Table 1. For each condition, phenotype OMIM number (https://www.omim.org, accessed 2 August 2024), the associated gene, inheritance pattern and number of reported families as a proxy of the global prevalence are shown.

TABLE 1 | List of monogenic corneal dystrophies. The number of reported probands refers only to families linked to a genetic locus or families with an identified molecular genetic aetiology.

Epithelial and subepithelial	Abbreviation	OMIM no.	Gene	Inheritance	Reported probands
Meesmann corneal dystrophy, type 1	MECD1	#122100	*KRT12*	AD	20–30
Meesmann corneal dystrophy, type 2	MECD2	#618767	*KRT3*	AD	4
Epithelial recurrent erosion dystrophy	ERED	#122400	*COL17A1*	AD	10–20
Lisch epithelial corneal dystrophy	LECD	#620763	*MCOLN1*	AD	10–20
Gelatinous drop-like corneal dystrophy	GDLD	#204870	*TACSTD2*	AR	20–30
Epithelial-stromal					
Reis-Bücklers corneal dystrophy	RBCD	#608470	*TGFBI*	AD	> 100
Thiel-Behnke corneal dystrophy	TBCD	#602082	*TGFBI*	AD	> 100
Granular corneal dystrophy, type 1	GCD1	#121900	*TGFBI*	AD	> 100
Granular corneal dystrophy, type 2 (also known as Avellino)	GCD2	#607541	*TGFBI*	AD	> 100
Lattice corneal dystrophy, type 1 (classic) and variants	LCD	#122200 (includes #608471)	*TGFBI*	AD	> 100
Stromal					
Macular corneal dystrophy	MCD	#217800	*CHST6*	AR	> 100
Schnyder corneal dystrophy	SCD	#121800	*UBIAD1*	AD	80–100
Congenital stromal corneal dystrophy	CSCD	#610048	*DCN*	AD	9
Delayed onset stromal corneal dystrophy	DOSCD	Not yet given	*SPARCL1*	AD	1
Fleck corneal dystrophy	FCD	#121850	*PIKFYVE*	AD	10–20
Posterior amorphous corneal dystrophy	PACD	#612868	Deletion involving *CCER1, LINC00615, KERA, LUM, DCN, and EPYC*	AD	8
Endothelial					
Early-onset Fuchs endothelial corneal dystrophy, type 1	FECD1	#136800 (includes #609140)	*COL8A2*	AD	10–20
Fuchs endothelial corneal dystrophy, type 3	FECD3	#613267	*TCF4*	AD	> 1% of general population
Fuchs endothelial corneal dystrophy, type 4	FECD4	#613268	*SLC4A11*	AD	10–20

(Continues)

TABLE 1 | (Continued)

Epithelial and subepithelial	Abbreviation	OMIM no.	Gene	Inheritance	Reported probands
Posterior polymorphous corneal dystrophy, type 1	PPCD1	#122000	*OVOL2*	AD	20–30
Posterior polymorphous corneal dystrophy, type 3	PPCD3	#609141	*ZEB1*	AD	50–70
Posterior polymorphous corneal dystrophy, type 4	PPCD4	#618031	*GRHL2*	AD	6
Congenital hereditary corneal endothelial dystrophy/Harboryan syndrome with hearing loss	CHED	#217700/#217400	*SLC4A11*	AR	> 100
X-linked endothelial corneal dystrophy	XECD	%300779	Unknown	X-linked	1

Abbreviations: #, phenotype description, molecular basis known; %, phenotype description or locus, pathogenic variant unknown; AD, autosomal dominant; AR, autosomal recessive; OMIM, Online Mendelian Inheritance in Man.

All images of CDs in the following text were taken in patients with a diagnosis confirmed at the DNA level.

Table S1 provides further details about the known molecular genetic pathology, with genes causing CDs listed, and includes the name of the encoded protein, its known function, and presumed pathogenic mechanisms implicated in the associated CD. In some genes, especially those encoding transcription factors, the etiopathogenetic downstream mechanisms are likely to be cell-specific, involving a network of target genes that are not fully elucidated. Genes deleted in posterior amorphous corneal dystrophy (PACD) are not shown, as the primary driver of the pathology is not known.

The scope of this review covers those dystrophies in the last edition of IC3D [1] and therefore does not include anterior segment dysgenesis genes nor keratoconus, which is a complex disease. The genetic basis for keratoconus is not yet clearly identified, with multiple genetic associations identified in genome-wide association studies (GWAS) [20, 21]. Detailed surgical management is beyond the scope of this review, with many publications devoted to this topic.

5 | Epithelial and Subepithelial CDs

Meesmann Epithelial Corneal Dystrophy (MECD) presents with multiple, solitary transparent microcysts seen on retroillumination, which on direct illumination may appear as a diffuse grey, superficial opacity with a distinct border (Figure 1a,b). This is an example of a clinical entity that is in fact caused by pathogenic variants in 2 different genes.

MECD is due to dominant negative pathogenic variants in *KRT3* and *KRT12*, which encode corneal-specific cytoskeletal proteins, Keratin 3 and 12 [22]. The intermediate cytoskeletal filaments formed by heterodimers of K3-K12 stabilise the corneal epithelial cells with structural support. Protein misfolding results from the mainly missense pathogenic variants that are all located in helix initiation or helix termination motifs—functionally critical regions [23, 24]. A mouse model carrying the human *KRT12* p.Leu132Pro mutation demonstrated a cornea with a disorganised epithelium with fragile cells, delamination of the basal layer, and cytolysis generating cysts that occasionally rupture at the corneal surface, as well as apoptotic unfolded protein response (UPR) markers [25]. The UPR is a defensive mechanism of the cell to prevent toxicity from incorrectly folded proteins [26].

Epithelial recurrent erosion dystrophy (ERED) is characterised by an onset of recurrent painful epithelial erosions in the first decade of life that become less frequent with age, with resultant focal or diffuse subepithelial opacities (Figure 1c).

Gain-of-function mutations in *COL17A1* were described in 2015, with six unrelated families harbouring the c.3156C>T variant, which introduces a cryptic donor site resulting in aberrant pre-mRNA splicing [27–29]. In vivo confocal microscopy examination in two families showed brightly hyperreflective polymorphous intraepithelial opacities in the epithelium, although they were invisible on biomicroscopic examination.

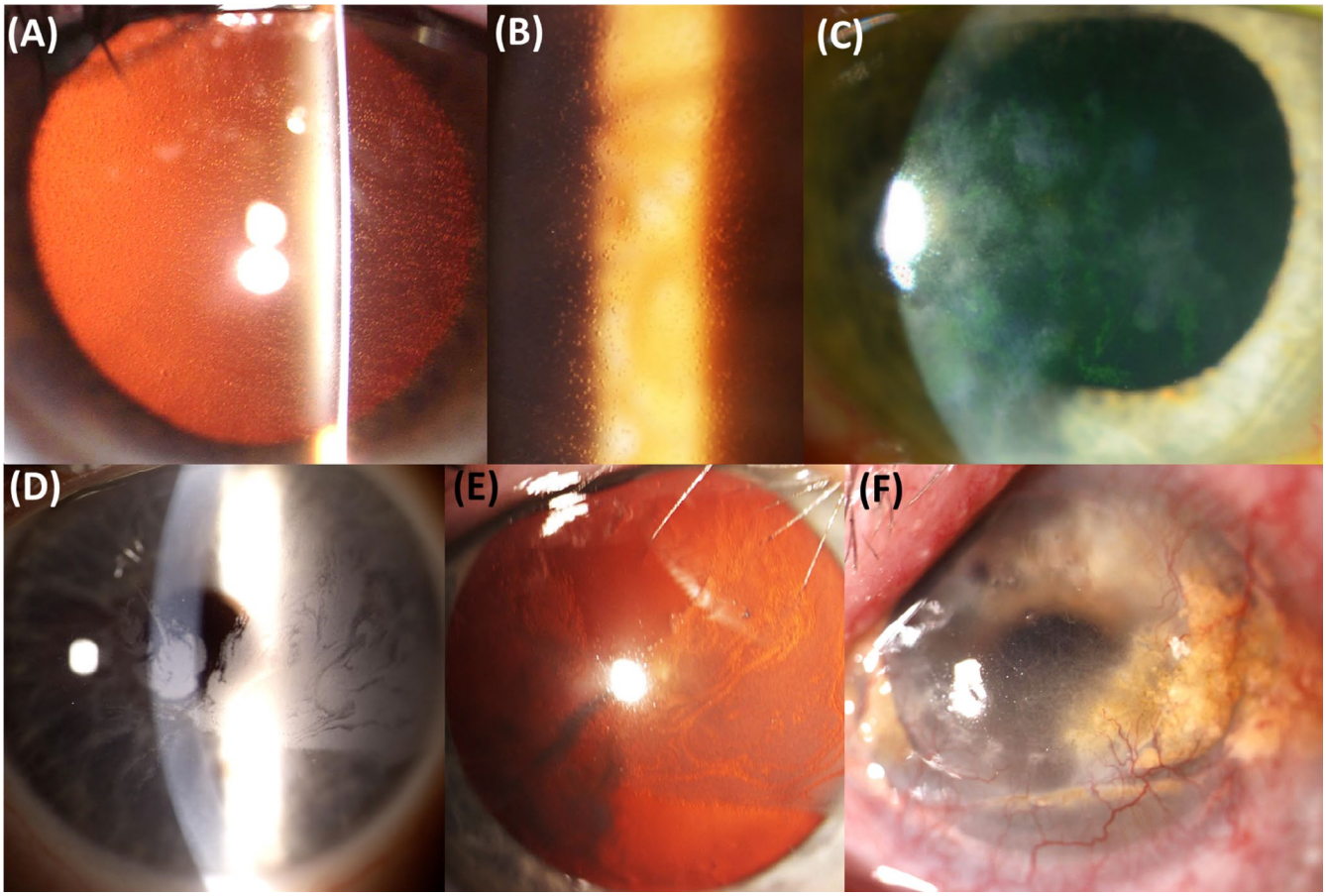

FIGURE 1 | Slit-lamp photographs of epithelial and subepithelial corneal dystrophies. Meesmann corneal dystrophy with tiny round opacities in the corneal epithelium (a, b); epithelial recurrent erosion dystrophy with subepithelial scarring and fibrosis (c); Lisch epithelial corneal dystrophy, note whorl-like, feathery grey opacities (d, e); gelatinous drop-like corneal dystrophy characterised by yellow-white deposits with superficial neovascularisation (f).

Areas with clinically visible disc-like opacities showed bowl-like epithelial thickening extending into the anterior stroma, with complete destruction of the Bowman layer and the subepithelial nerve plexus. The adjacent anterior stroma was remarkable for diffuse accumulation of anterior stromal extracellular matrix [28].

COL17A1, a member of the collagen family, is an integral part of the hemidesmosome structure and is expressed in the corneal epithelium [28, 30], suggesting a function biologically relevant to the ERED phenotype. Elucidating the genetic cause for ERED has allowed multiple eponymously named entities (Franceschetti corneal dystrophy, Dystrophia Smolandiensis, Dystrophia Helsinglandica) to be redefined under this umbrella term [31]. *COL17A1* is associated with other diseases characterised by compromised epithelial attachment. Autoimmunity against COL17A1 produces the skin-blistering disease bullous pemphigoid, whereas mutations in *COL17A1* cause the recessive, mechanically induced skin-blistering disease junctional epidermolysis bullosa [32], which manifests with corneal erosion in many patients [33]. COL17A1 is linked to keratinocyte mobility [34], and increased levels of COL17A1 have been observed during corneal wound healing [35], suggesting it plays a crucial role not only in maintaining epithelial attachment but also in recovering from injury [34].

Lisch Epithelial Corneal Dystrophy (LECD) is characterised by the presence of typically bilateral whorl-like, band-shaped, or feathery grey opacities in the corneal epithelium (Figure 1d,e). Patients with this condition remain asymptomatic as long as the visual axis is uninvolved. Some may experience mild visual disturbances, such as glare or blurred vision, depending on the extent and location of the opacities. The condition usually presents in childhood or early adulthood, but can be diagnosed later as well.

The affected corneal epithelium shows intracellular vacuoles, which are thought to be responsible for the opacities observed in LECD. The abnormal epithelial cells are postulated to originate from pathological stem cells [36].

LECD is an autosomal dominant disease with incomplete penetrance. Most recently it has been shown that heterozygous loss-of-function mutations in the *MCOLN1* gene are disease-causing [3]. This gene encodes Mucolipin Transient Receptor Protein Cation Channel 1, a transmembrane protein involved in the regulation of ion channels and lysosomal function within cells. This protein localises to the intracellular vesicular membrane, including lysosomes, and functions in the late endocytic pathway and in the regulation of lysosomal exocytosis. Biallelic pathogenic variants cause recessive mucolipidosis

type IV, a rare lysosomal storage disorder that includes visual impairment due to corneal clouding, retinal degeneration, and optic atrophy [37].

Gelatinous drop-like corneal dystrophy (GDLD) presents with central nodular, 'mulberry' or band-shaped grey deposits in the subepithelium, with subsequent vascularisation often occurring in the first two decades (Figure 1f).

GDLD is caused by biallelic variants in the single exon of the *TACSTD2* gene, encoding tumour-associated calcium signal transducer 2 protein, which is integral to the barrier function of the corneal epithelium by binding to the basal epithelial tight junctions, claudin 1 and 7 [38]. Corneal epithelial permeability is increased with the lack of TACSTD2, leading to progressive subepithelial deposition of amyloid, resulting in nodule development and subsequent destruction of the epithelial basement membrane and Bowman layer [39, 40].

6 | Epithelial-Stromal and Stromal CDs

6.1 | TGFBI-Associated Dystrophies

TGFBI is the primary gene responsible for 6 distinctly named epithelial-stromal dystrophies, with strong genotype–phenotype correlations, predominantly associated with two codons which are prone to mutation ('hot spots') in the gene. Less frequent variants can manifest with intermediate phenotypes with atypical features. Diseases associated with *TGFBI* pathogenic variants are the most common of the rare CDs, with over 4200 individuals reported in the literature [6]. The protein product, TGFBIp (transforming growth factor β-induced protein), is abundantly expressed in the epithelium, Bowman layer, stroma and endothelium of the normal cornea, as well as in other human tissues [41], but pathogenic variants in the gene are only reported as manifesting with corneal phenotypes. TGFBIp is a 683 amino acid-long secretory protein which has been demonstrated to interact with proteins in the extracellular matrix: collagen, proteoglycans, fibronectin and periostin, as well as integrins [42]. The protein has four homologous fasciclin 1 (FAS-1) domains, each of approximately 140 amino acids, an N-terminal cysteine-rich secretory EMI (Emilin-like domain) and an integrin-binding Arg-Gly-Asp (RGD domain) situated at the C-terminal [43]. ClinVar currently lists 25 pathogenic and 8 likely pathogenic variants, mostly missense single nucleotide substitutions, and only 1 insertion and 1 deletion associated with corneal disease (https://www.ncbi.nlm.nih.gov/clinvar, accessed 29 Aug 2024), although more are reported in a recent publication [6].

The location and type of amino acid substitution determine the clinical phenotype – age of onset, severity, the location in the cornea where the deposits occur, and the morphology of the deposits. Based on slit-lamp biomicroscopy and histological examinations, the protein deposits can be classified as amyloidogenic, where the protein deposits appear as thin and long amyloid fibres (lattice corneal dystrophy, LCD), nonamyloidogenic with a more spherical morphology of the protein aggregates (granular corneal dystrophy, GCD), or a mixed form, with both fibrils and discrete deposits present. For many of the phenotypes, homozygotes are also described, with more severe and earlier onset of disease.

In a healthy cornea, the full-length wildtype TGFBIp is processed by serine proteases into many shorter fragments [44]. Amino acid substitutions at the mutational hot spot in the first FAS1 domain (p.Arg124His, p.Arg124Leu, and p.Arg124Cys) do not alter the stability of the protein, but lead to a 1.5–3-fold increase in the level of full-length mutant protein in the cornea [44]. There is also abnormal accumulation of many proteolytic products in the dystrophic cornea with the p.Arg124Cys variant.

In contrast, substitutions within the fourth FAS1 domain (FAS1-4), where the majority of *TGFBI* mutations are located, including p.Arg546Thr, p.Arg555Glu and p.Arg555Trp, alter the overall stability of TGFBIp; p.Arg546Trp is the least stable mutant and forms amyloid fibrils, while the more stable variants generate non-amyloid amorphous deposits in vivo. Experimental data have demonstrated that both an increase and a decrease in the stability of FAS1-4 initiate the dystrophic process [44].

Reis-Bücklers corneal dystrophy (RBCD) presents in the first two decades with confluent irregular and coarse geographic-like opacities developing at the level of the Bowman layer and the superficial stroma, initially more centrally, and extending to the limbus and deeper into the stroma with time (Figure 2a). Recurrent corneal erosions may occur, with the opacities often developing later. The characteristic histology is that the Bowman layer is replaced with granular Masson trichrome-positive deposits in a sheet-like layer [45]. The genotype for RBCD is p.Arg124Lys, and recurrence post keratoplasty is frequent [46].

Thiel-Behnke corneal dystrophy (TBCD) also starts in childhood, with isolated flecks or irregularly shaped opacities at the level of the Bowman layer, and subsequently symmetrical, subepithelial honeycomb opacities, and the peripheral cornea is usually spared (Figure 2b). Recurrent corneal erosions are also frequent, but this phenotype is generally less severe than RBCD; however, over time, the opacities may progress to the periphery and to deeper stromal layers. The predominant mutation associated with TBCD is p.Arg555Gln, although other rarer alleles lead to atypical TBCD phenotypes. On electron microscopy, curly collagen fibres are observed with a diameter of 9–15 nm, which is unique to TBCD [1].

Granular corneal dystrophy, type 1 (GCD1) is the most common of the *TGFBI*-associated CDs, usually caused by the p.Arg555Trp variant. Initially manifesting in childhood with a vortex pattern of small granules superficial to the Bowman layer, the deposits become more discrete and well-defined, greyish white in appearance, with clear intervening stroma, although over time they may coalesce and extend into the deeper stroma, with frequent recurrence post keratoplasty (Figure 2c,d) [46].

Experimental evidence determined the p.Arg555Trp mutation results in increased resistance to proteolysis and reduced degradation of the mutant TGFBIp, which accumulates and aggregates as non-amyloid deposits via electrostatic interactions [47]. Other variants, such as p.Arg124Ser, may also cause GCD1, although phenotypically they are more severe with an earlier onset [1].

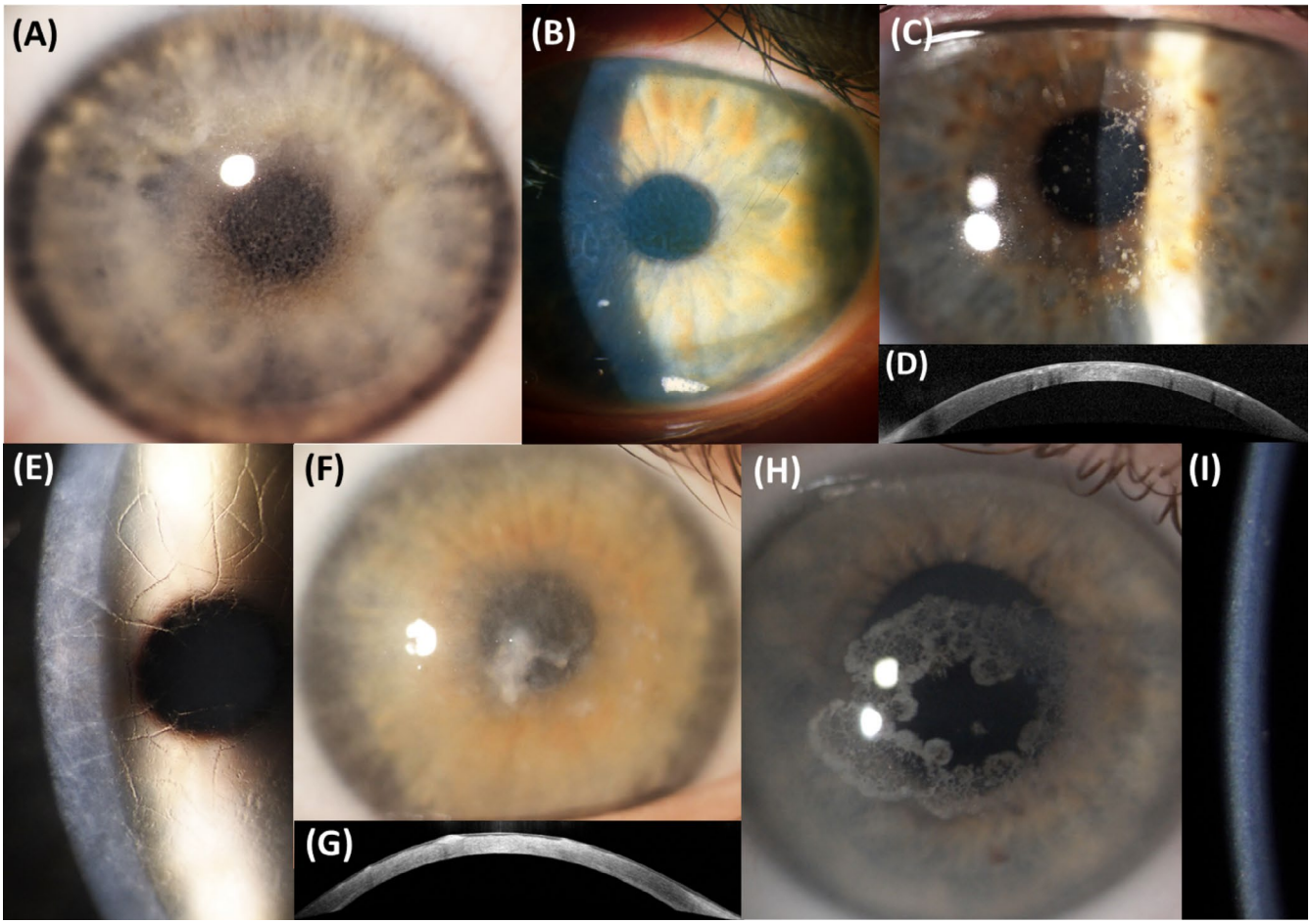

FIGURE 2 | Slit-lamp photographs and anterior segment optical coherence tomography (AS-OCT) imaging of stromal corneal dystrophies. Reis-Bücklers corneal dystrophy with reticular opacities in the superficial central cornea (a); Thiel-Behnke corneal dystrophy with subepithelial honeycomb-shaped corneal opacities in the superficial cornea (b); granular corneal dystrophy type 1 (c) and AS-OCT showing deposits resembling breadcrumbs in the same patient (d); fine translucent lattice lines and dots in a variant of lattice corneal dystrophy (e); central stromal opacities and diffuse corneal haze (f) and AS-OCT showing deposition of hyperreflective material in macular corneal dystrophy in the same patient (g); central ring opacity with superficial crystalline deposits in Schnyder corneal dystrophy (h); fine stromal opacities in fleck corneal dystrophy (i).

Granular corneal dystrophy, type 2 (GCD2) phenotypically has both granular and lattice-like deposits and is usually diagnosed in adolescence. As the deposits increase, their morphology ranges from crumb-like deposits, branching, ring-like, star shapes and spiny deposits beneath the Bowman layer. The most common variant causing GCD2 is p.Arg124His, with reduced proteolysis and resultant deposition of the mutant protein, both non-amyloid and amyloidogenic [46]. Refractive laser surgery may exacerbate GCD2, and although it is postulated that the cellular stress response is the driver, in a transgenic mouse model with the p.Arg144His mutation, external stressors, including epithelial debridement, did not result in increased deposition [48].

Lattice CDs (LCD) are subdivided into Classic LCD and LCD variants.

Classic LCD is associated with the p.Arg124Cys variant, whereas LCD variants are associated with *TGFBI* pathogenic changes, other than p.Arg124Cys, with many located in the 4th Fas-1 domain. A comprehensive list of these variants is provided in the most recent IC3D publication [1]. The appearance of LCD in the early phase of the disease is best examined with retroillumination at the slit lamp, which demonstrates subtle isolated lattice lines in the stroma, with thin branching refractile lines and/or white dots, starting centrally and spreading out, but sparing the periphery, and progressing deeper into the stroma (Figure 2e). The lattice lines may cross, and a diffuse ground glass haze develops in the subepithelium. Onset of classic LCD is usually in the first decade, with recurrent corneal erosions occurring and gradual vision reduction leading to surgery in the second or third decade [1, 46]. Scarring with more advanced disease may obliterate the appearance of the lattice lines. Mass spectrometry identified a short peptide fragment in the 4th FAS-1 domain that is enriched in the amyloid deposits in dystrophic patients [49]. Bioinformatic analysis also predicts that this region of TGFBIp has amyloidogenic potential. Most missense changes in this region are accompanied by an overall reduction in the net charge of the peptide, which is proposed to alter the secondary structure, with significant changes in amyloid formation kinetics and cytotoxicity of the fibrils [49].

MCD is an autosomal recessive disease which typically manifests in the first or second decade of life, although symptoms may sometimes present later in life. It is characterised by the

accumulation of abnormal deposits within the cornea, with progressive haze and clouding of the cornea, along with thinning (Figure 2f,g), often leading to significant visual impairment by the third or fourth decade of life. Photophobia can also occur, and occasionally recurrent corneal erosions [50, 51]. The carbohydrate sulfotransferase six gene, *CHST6*, encodes an enzyme designated corneal N-acetylglucosamine-6-sulphotransferase (CGlcNAc6ST), and different variants of MCD are described (MCD I, IA, and II) based on the immunoreactivity of specific sulphated epitopes of antigenic keratan sulphates (AgKS) in the cornea and the serum, although these are indistinguishable clinically [52].

More than 180 unique *CHST6* variants are reported; of these, around two-thirds are missense [8]. In MCD I, lack of *CHST6* expression in corneal cells, at least in the epithelium, causes the production of unsulphated keratan sulphate, as well as in the serum, leading to intra- and extracellular accumulation of abnormal materials, which results in the opacities in MCD [53]. Initially, all type II mutations were identified in the upstream region of *CHST6*, which may include a gene regulatory element that affects the transcription of *CHST6* and seems to promote the loss of cell-specific *CHST6* expression exclusively in corneal cells, but not in other keratan sulphate-rich tissues such as cartilage [54]. Subsequently, further variants were identified in the coding region of *CHST6* [8].

Schnyder corneal dystrophy (SCD) is an autosomal dominantly inherited dystrophy with corneal opacification due to abnormal deposition of phospholipids and cholesterol in the cornea, and crystals in the anterior and mid-stroma; however, crystals are only present in about 50% of affected individuals (Figure 2h) [1]. The *UBIAD1* (UbiA prenyltransferase domain containing 1) gene is demonstrated to be involved in vitamin K2 and CoQ10 biosynthesis. This sterol triggers the binding of UBIAD1 to 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) at endoplasmic reticulum (ER) membranes, which is regulated by an intracellular geranylgeranyl diphosphate molecule. Pathogenic variants in *UBAID1* means that HMGCRs are redundant and cannot be degraded through the usual mechanism of degradation via the ER, with resultant excess cholesterol accumulation in the cornea [7].

Congenital stromal corneal dystrophy (CSCD) is characterised by diffuse bilateral white flake-like stromal opacifications that typically present at birth or shortly after. Although nonprogressive, the resultant reduction in vision can be amblyogenic, and some patients have photophobia and strabismus [55]. Inherited in an autosomal dominant fashion, pathogenic variants in the *DCN* gene encoding Decorin, which belongs to a family of class I small leucine-rich proteoglycan proteins (SLRPs), are the main component of the extracellular matrix, together with collagen fibrils, and act as a bridging molecule between type I and type VI collagen. Decorin also interacts with and modulates multiple growth factors and signalling pathways, such as the TGF-B pathway [56]. While CSCD is typically associated with truncating mutations in *DCN* [55], a missense mutation p.Cys346Gly in *DCN* has been reported in a mother and son who presented with later-onset and milder corneal changes [57].

Most recently, an autosomal dominant corneal stromal dystrophy with delayed onset in a pedigree with eight affected individuals across three generations was described. Affected individuals had diffuse central stromal opacity, with reduced visual acuity in older family members. Linkage analysis in the family excluded all known loci causing corneal stromal dystrophies. Whole genome sequencing identified a novel heterozygous missense variant in exon 4 of *SPARCL1* (NM_004684) c.334G>A p.Glu112Lys, segregating with disease. The gene *SPARCL1* encodes SPARC-like protein 1 (SPARCL1), a matricellular protein expressed in the corneal stroma, which is involved in cell migration, cell adhesion, tissue repair and remodelling. Interestingly, SPARCL1 regulates *DCN* causing CSCD, suggesting a common pathogenic pathway [4].

Fleck corneal dystrophy (FCD) is a congenital or early-onset autosomal dominant dystrophy, with numerous tiny, dot-like white flecks scattered in all layers of the corneal stroma; the stroma located in between the flecks is clear, and the endothelium, the epithelium, Bowman layer and Descemet membrane are normal (Figure 2i) [58]. Patients with FCD are usually asymptomatic. The *PIKFYVE* (phosphoinositide kinase FYVE finger) gene encodes a protein that regulates vesicle size of early/late endosomes in Golgi-dependent transport [58]. Dysregulation of this protein results in dilated keratocytes with membrane-limited intracytoplasmic vesicles filled with complex lipids and glycosaminoglycans, corresponding to the punctate corneal stromal opacities observed. The majority of pathogenic variants described to date are frameshifts, observed in 5 exons of the 41-exon gene, in addition to a whole deletion of the gene and other splicing mutations. Therefore, the disease mechanism is loss of function, as these variants are predicted to affect the cytosolic chaperone CCTγ apical domain-like motif and spectrin repeats binding sites, important for multiprotein interactions and cytoskeleton structure [59].

PACD is a congenital, nonprogressive autosomal dominant disease. The typical PACD appearance is irregular sheet-like, with areas of opacification either at or just anterior to the Descemet membrane. Affected individuals also demonstrate diffuse corneal thinning and flattening; however, vision is usually not severely impacted [60]. PACD is associated with heterozygous deletions in the 12q21.33 locus. This region includes the SLRPs *KERA*, *LUM*, *EPYC* and *DCN*, of which all but *EPYC* are expressed in the corneal stroma and are known to have a role in corneal development, structure, function, and transparency [61]. The other two genes within the deleted region are an RNA coding gene, *LINC00615* and *CCER1*, a protein-coding gene not expressed in the cornea. As little is known about the function of these two genes, their significance in the pathogenesis of PACD is difficult to determine.

7 | Endothelial CDs

FECD is associated with excreted collagenous deposits, called guttae, that project posteriorly from the Descemet membrane. As FECD progresses, guttae grow in numbers and merge, leading to a thickening of the Descemet membrane. These changes put stress on corneal endothelial cells, which begin to undergo

cell death via apoptosis; all these changes result in corneal oedema and visual loss. FECD is the primary indication for all corneal transplantations performed worldwide [62] (Figure 3).

GWAS have identified 12 genomic loci associated with age-related FECD [63–65]. Of these, the most significant is common variation at 18q21.212, tagging the trinucleotide repeat expansion (termed CTG18.1) in an intron of the *TCF4* gene (transcription factor 4) [66]. Depending on ancestry, 17%–81% of individuals with age-related FECD have one or more expanded copies of CTG18.1, making it the most common trinucleotide repeat expansion disease in humans [66]. Currently, it is anticipated that at least two distinct pathogenic mechanisms, RNA toxicity and *TCF4* isoform-specific dysregulation, underpin the pathophysiology of FECD [67]. Apart from the *TCF4* locus, the causal variants driving GWAS signals remain elusive [63]. The heritability of the remaining 25% of cases of FECD may thus be complex and involve multiple genes. Traditional linkage and candidate gene screening approaches have pointed to other genes that may be implicated in the pathogenesis of late-onset FECD, including *ZEB1* [68], *SLC4A11* [69], *LOXHD1* [70] and *AGBL1* [71]. Some of these findings, however, require further validation; currently, convincing evidence at a functional level has been provided for mutations in *SLC4A11* [72].

Early-onset FECD is a distinct corneal dystrophy with an autosomal dominant mode of inheritance, usually symptomatic from the third to the fourth decade. Signs of the disease can be, however, detected much earlier; the youngest examined affected patient was 3 years old [73]. Slit-lamp examination initially shows small, shallow guttae positioned near the centre of the endothelial cells, compared to late-onset FECD in which the guttae generally appear first at cell–cell junctions [73].

Early-onset FECD is caused by heterozygous point mutations altering the collagen helix domain of the α2 chain of type VIII collagen (*COL8A2*), a major component of the Descemet membrane. To date, only three variants classified as pathogenic have been identified (NM_005202.4) c.1363_1364CA>GT p.Gln455Val, c.1349T>G p.Leu450Trp, and c.1363C>A p.Gln455Lys [73–75]. Studies of corneas obtained from heterozygous carriers of p.Leu450Trp show massive accumulation and abnormal assembly of collagen VIII within the Descemet membrane. The Col8a2^{Q455K} mouse model shows features strikingly similar to the human disease, including progressive alterations in endothelial cell morphology, cell loss and formation of guttae. The model supports the role of UPR and UPR-associated apoptosis in the pathogenesis of FECD caused by *COL8A2* mutations [76].

Posterior polymorphous corneal dystrophy (PPCD) is an autosomal dominant condition characterised by geographic opacities, vesicular lesions, Descemet membrane bands, best visualised by retroillumination and a reduced endothelial cell density (Figure 4a–g). Some patients also develop band keratopathy, peripheral iridocorneal adhesions, iris atrophy, and corectopia. In most patients, the condition is slowly progressive or static; about one-third develop corneal oedema necessitating keratoplasty and/or secondary glaucoma [14, 15, 77]. The condition can also manifest as congenital corneal oedema [78].

There are three subtypes: PPCD1 is caused by pathogenic gain-of-function regulatory variants in *OVOL2* (NM_004183.3) c.-61G>A, c.-274T>G, c.-307T>C, c.-339_361dup. A family carrying the c.339_361dup was previously described in the literature as autosomal dominant congenital hereditary endothelial dystrophy (CHED1) [15]. PPCD3 is caused by loss-of-function variants in *ZEB1*, summarised in [79], and PPCD4 is associated with gain-of-function regulatory *GRHL2* mutations (NM_024915.4) c.20+133del, c.20+257del, c.20+544G>T [14]. Each of these genes encodes a transcription factor that regulates cell state transition. Endothelial cells from corneas affected with PPCD have been demonstrated to exhibit epithelial-like characteristics as a result of alterations in the ZEB1-OVOL2-GRHL2 axis. Although it is not possible to reliably distinguish between the three PPCD subtypes by clinical examination, clinical markers such as corneal steepening and astigmatism are a feature of PPCD3. PPCD3 is also the most common form, with the highest rate of de novo origin observed in about one-third of probands; therefore, in sporadic cases with PPCD, the underlying mutation is most likely located in *ZEB1*. While PPCD1 and PPCD4 seem

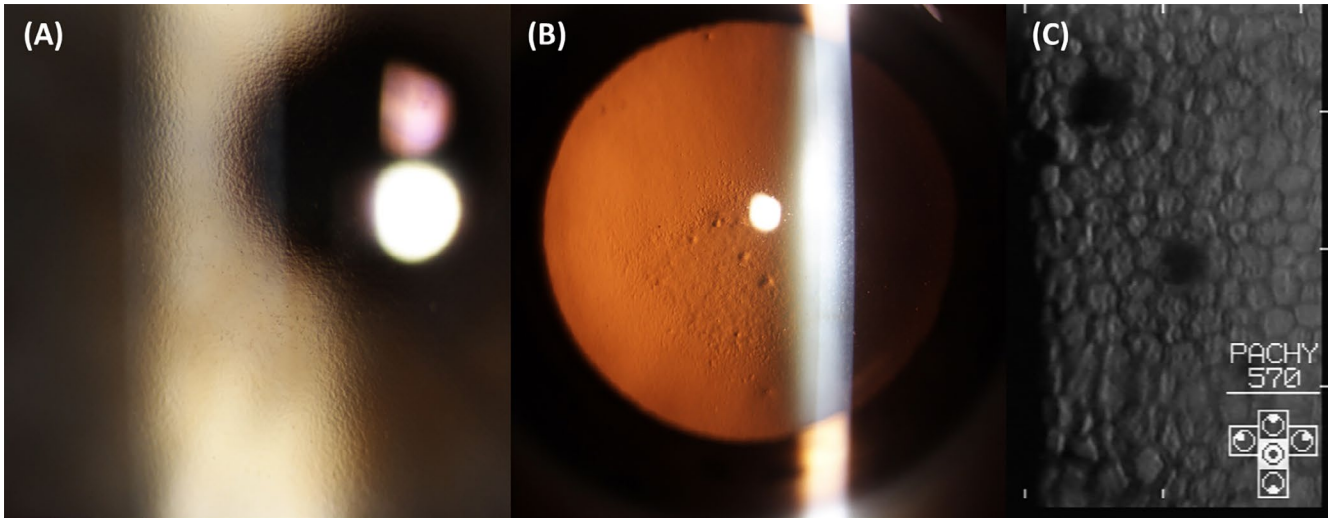

FIGURE 3 | Slit-lamp photographs and specular microscopy imaging in age-related Fuchs endothelial corneal dystrophy. Cornea guttata in slit-lamp examination using broad beam (a), after dilation of the pupil in retroillumination (b) and isolated guttae on specular microscopy (c).

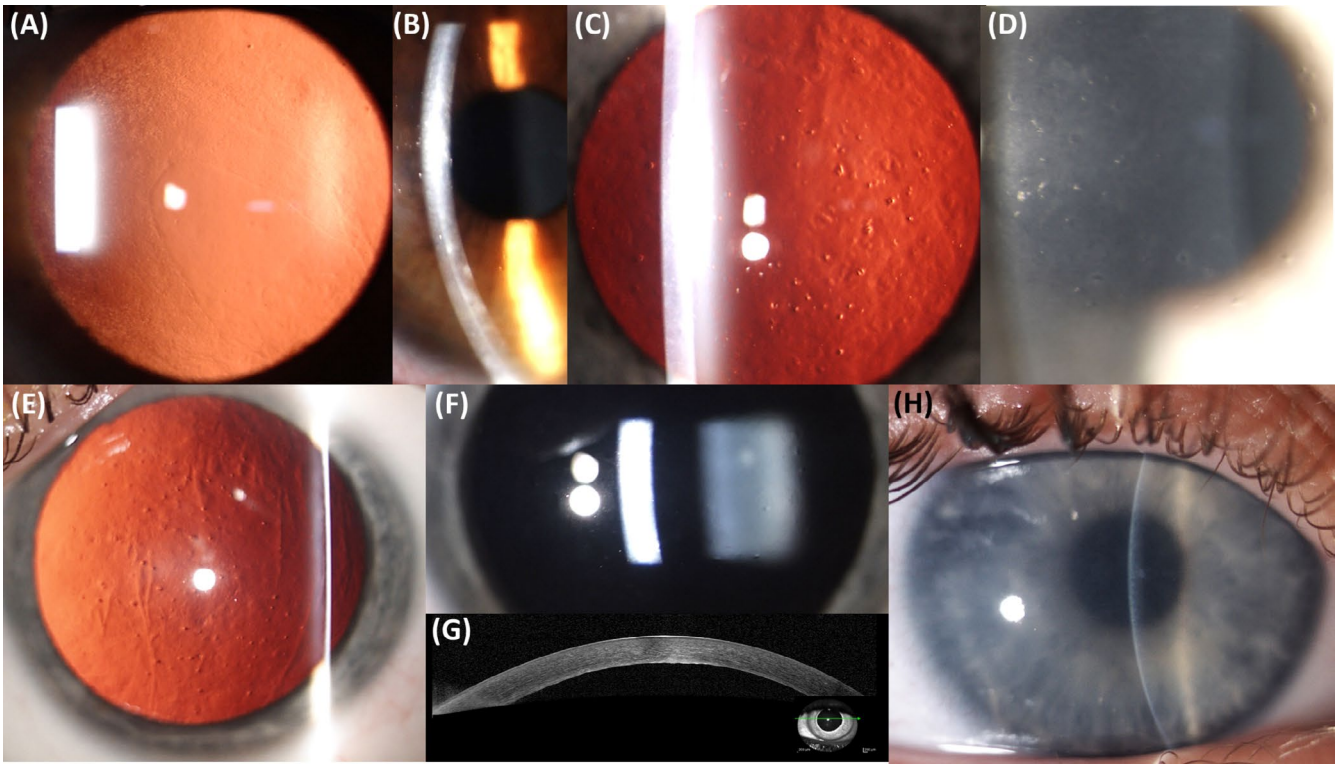

FIGURE 4 | Slit-lamp photographs and anterior segment optical coherence tomography (AS-OCT) of corneal endothelial dystrophies. Posterior polymorphous corneal dystrophy type 1: Irregular corneal surface with marked patchy opacification of the posterior corneal layers (a, b); type 3: Irregular posterior corneal surface with vesicle-like lesions (c, d); type 4 showing vesicle-like lesions and hyperreflective posterior corneal surface on AS-OCT (e–g) and congenital hereditary endothelial dystrophy characterised by diffuse ground glass appearance (h).

to be fully penetrant diseases, PPCD3 shows incomplete penetrance with around 5% of carriers with no evidence of disease [77–79].

Previously the *COL8A2* gene was also implied as causative for posterior polymorphous dystrophy type 2 (PPCD2). This was based on one child carrying p.Leu450Trp variant, associated with mulberry-like endothelial lesions, and guttae present in the surrounding endothelium [73], and two other individuals who had undergone penetrating keratoplasty, and their phenotype could not be reliably documented. As these two patients shared a common microsatellite haplotype as the originally mapped family with early-onset FECD, and both families originated in the same geographic region, the disease-causing variant was shown to be inherited from a common founder [75]. In summary, there is no robust evidence that PPCD2 can be differentiated as a separate clinical entity. Observed endothelial abnormalities in the three individuals described as having PPCD2, should be considered as phenotypic variability of *COL8A2*-associated corneal endothelial dystrophy.

CHED is an autosomal recessive disorder, typically presenting as corneal clouding at, or shortly after birth, causing both nystagmus and amblyopia. The cornea may gradually increase in thickness (double to triple that of normal values), accompanied by visual deterioration and photophobia, although discomfort is rare. The endothelial cell density is greatly reduced, but this is usually difficult to visualise clinically due to the corneal haze. There is no significant association with glaucoma, and the ocular examination is otherwise normal. Some patients with CHED eventually develop Harboyan syndrome characterised by progressive, postlingual sensorineural hearing impairment.

Biallelic pathogenic variants in *SLC4A11* cause both CHED and Harboyan syndrome. The ClinVar database [9], accessed 31 August 2024) lists 135 short pathogenic/likely (missense, splicing, nonsense and frameshifting) variants in *SLC4A11*. SLC4A11 encodes a membrane-bound multifunctional transporter at the basolateral cell membrane of endothelial cells, facilitating transendothelial fluid reabsorption in the aqueous humour with an impact on cell morphology and differentiation [80].

7.1 | X-Linked Endothelial Corneal Dystrophy

Although the gene for X-linked endothelial corneal dystrophy (XECD) has not been identified, available clinical and linkage data are convincing to separate this clinical entity from other hereditary CDs, although only one family has been identified to date. Genotyping was performed in 50 family members, of which 35 were affected or trait carriers, and the disease was mapped to the X chromosome, between markers DXS8057 and DXS1047. Consistent with X-linked inheritance, no male-to-male transmission was observed in the pedigree [81].

Most male patients exhibit only moon crater-like endothelial changes, decreased endothelial cell density, and band-shaped keratopathy; however, congenital corneal oedema may occur, leading to amblyopia and nystagmus. Corneal grafts are

necessary in 20% of males. In females, asymptomatic moon crater-like endothelial changes have been noted [81].

8 | Important Phenocopies

A phenocopy refers to a clinical presentation with a similar appearance, or features of a monogenic disorder, but is not caused by the same genetic factors. Important phenocopies of CDs are monoclonal gammopathy, which can lead to the deposition of paraproteins with diverse appearances, such as superficial reticular opacities and nummular lesions, diffuse posterior stromal opacity and superficial and stromal crystalline deposits. Stromal lattice lines misdiagnosed as LCD in a young patient have also been described [82]. An abnormal paraprotein band on plasma electrophoresis confirms the diagnosis. Amyloidosis, Finnish type (OMIM #105120) manifests with linear opacities in the corneal stroma, similar to LCD, and it is not confined to the cornea. This is a systemic amyloidosis caused by mutations in the gelsolin gene (*GSN*). Systemic features include peripheral neuropathies and amyloid infiltration of many organs [82, 83].

Punctate pre-Descemet opacities (previously termed pre-Descemet corneal dystrophy) are part of the clinical spectrum of X-linked ichthyosis (OMIM #308100), due to loss-of-function mutations in the steroid sulphatase (*STS*) gene [84]. Other metabolic keratopathies manifesting with bilateral corneal opacification include lecithin-cholesterol acyltransferase deficiency (OMIM #245900) and Fish eye disease (OMIM #136120), mucopolysaccharidoses (OMIM #607014, #607016, #607015, #309900, #252900, #252930, #252940, #253000, #253010, #253200, #253220) generalised gangliosidosis 1 (OMIM #230500), Fabry disease (OMIM #301500), mucolipidoses (OMIM #252605, #252650), galactosialidosis (OMIM #256540), and cystinosis (OMIM #219800) [85]. If suspected, referral to an appropriate medical team should be undertaken.

PPCD also needs to be distinguished from static posterior corneal vesicles, which are not clearly genetic in origin [86].

9 | Conclusion

In this review we have discussed our understanding of the pathophysiology in those CDs defined as separate clinical entities by molecular genetic approaches. Identification of the underlying molecular genetic cause confirms the clinical diagnosis and may have vast implications for patient management and potentially also for genetic preventive measures – such as preimplantation genetic diagnosis and family planning.

All patients with a suspected CD should undergo genetic testing, as a clinical descriptor alone is not always sufficient to confirm the diagnosis. As an example, in a male presenting with congenital corneal oedema and no family history, differential diagnosis includes autosomal recessive CHED, PPCD3 due to a de novo pathogenic variant [87], congenital glaucoma [88] and XECD [81].

Examination of first-degree relatives is important, as some CDs show variable expressivity with atypical or very mild phenotypes, or are clinically indistinguishable, for example, early signs of *TGBFI*-associated corneal dystrophy versus MCD. Thus, detection of advanced disease in relatives and/or refinement of the inheritance pattern can provide further clues to establishing a diagnosis.

Clinicians also need to be aware that CDs may have phenocopies. Observation of two rare CDs in one patient, or homozygosity for a pathogenic variant in dominant disorders or pseudodominance in recessive traits, may lead to confusion even for ocular genetic specialists, further highlighting the need for molecular genetic testing as an ultimate method to establish final diagnoses [89–91].

Children with unexplained bilateral corneal opacity should be referred for systemic assessment and monitored for visual deterioration. Children with CHED need to be under surveillance for hearing loss, and individuals with PPCD should be regularly reviewed for secondary glaucoma. Advanced CDs are mainly treated with allogenic corneal transplantation. The chance of recurrence needs to be considered individually for each condition; it is common, especially in *TGFBI*-associated CDs and GDCD.

In conclusion, CDs are likely very underdiagnosed. Although there is no reliable prevalence data, *TGFBI*-associated CDs, MCD and CHED are the most frequent of the rare corneal disorders encountered in clinical practice, while FECD is the most common but considered age-related in the majority of cases. A molecular genetic diagnosis correlated with the observed phenotype is a robust means of establishing a definitive diagnosis than clinical description alone, even when supported by histopathology data. Innovative therapeutic approaches for CDs are under development, and a prerequisite for intervention will be a conclusive genetic diagnosis.

Acknowledgements

Open access publishing facilitated by The University of Auckland, as part of the Wiley - The University of Auckland agreement via the Council of Australian University Librarians.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

References

1. J. S. Weiss, C. J. Rapuano, B. Seitz, et al., "IC3D Classification of Corneal Dystrophies-Edition 3," *Cornea* 43, no. 4 (2024): 466–527.

2. S. Richards, N. Aziz, S. Bale, et al., "Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology," *Genetics in Medicine* 17, no. 5 (2015): 405–424.

3. K. Patterson, J. X. Chong, D. D. Chung, et al., "Lisch Epithelial Corneal Dystrophy Is Caused by Heterozygous Loss-Of-Function Variants in MCOLN1," *American Journal of Ophthalmology* 258 (2024): 183–195.

4. F. L. Braddock, J. C. Gardner, N. Bhattacharyya, et al., "Autosomal Dominant Stromal Corneal Dystrophy Associated With a SPARCL1 Missense Variant," *European Journal of Human Genetics* 32, no. 12 (2024): 1583–1589, https://doi.org/10.1038/s41431-024-01687-8.

5. D. Roshandel, F. Semnani, A. Rayati Damavandi, et al., "Genetic Predisposition to Ocular Surface Disorders and Opportunities for Gene-Based Therapies," *Ocular Surface* 29 (2023): 150–165.

6. R. Jozaei, M. A. Javadi, I. Safari, et al., "Genetic Screening of TGFBI in Iranian Patients With TGFBI-Associated Corneal Dystrophies and a Meta-Analysis of Global Variation Frequencies," *Ophthalmic Genetics* 43, no. 4 (2022): 496–499.

7. J. Xie and L. Li, "Functional Study of SCCD Pathogenic Gene UBIAD1 (Review)," *Molecular Medicine Reports* 24, no. 4 (2021): 706, https://doi.org/10.3892/mmr.2021.12345.

8. J. Zhang, D. Wu, Y. Li, Y. Fan, Y. Dai, and J. Xu, "A Comprehensive Evaluation of 181 Reported CHST6 Variants in Patients With Macular Corneal Dystrophy," *Aging (Albany NY)* 11, no. 3 (2019): 1019–1029.

9. M. J. Landrum, J. M. Lee, M. Benson, et al., "ClinVar: Improving Access to Variant Interpretations and Supporting Evidence," *Nucleic Acids Research* 46, no. D1 (2018): D1062–D1067, https://doi.org/10.1093/nar/gkx1153.

10. B. Hemadevi, R. A. Veitia, M. Srinivasan, et al., "Identification of Mutations in the SLC4A11 Gene in Patients With Recessive Congenital Hereditary Endothelial Dystrophy," *Archives of Ophthalmology* 126, no. 5 (2008): 700–708.

11. M. A. Aldahmesh, A. O. Khan, B. F. Meyer, and F. S. Alkuraya, "Mutational Spectrum of SLC4A11 in Autosomal Recessive CHED in Saudi Arabia," *Investigative Ophthalmology & Visual Science* 50, no. 9 (2009): 4142–4145.

12. N. P. Liu, C. F. Smith, B. L. Bowling, F. Jonasson, and G. K. Klintworth, "Macular Corneal Dystrophy Types I and II Are Caused by Distinct Mutations in the CHST6 Gene in Iceland," *Molecular Vision* 12 (2006): 1148–1152.

13. N. P. Liu, S. Dew-Knight, M. Rayner, et al., "Mutations in Corneal Carbohydrate Sulfotransferase 6 Gene (CHST6) Cause Macular Corneal Dystrophy in Iceland," *Molecular Vision* 6 (2000): 261–264.

14. P. Liskova, L. Dudakova, C. J. Evans, et al., "Ectopic GRHL2 Expression due to Non-Coding Mutations Promotes Cell State Transition and Causes Posterior Polymorphous Corneal Dystrophy 4," *American Journal of Human Genetics* 102, no. 3 (2018): 447–459.

15. A. E. Davidson, P. Liskova, C. J. Evans, et al., "Autosomal-Dominant Corneal Endothelial Dystrophies CHED1 and PPCD1 Are Allelic Disorders Caused by Non-Coding Mutations in the Promoter of OVOL2," *American Journal of Human Genetics* 98, no. 1 (2016): 75–89.

16. J. S. Weiss and N. A. Afshari, "Corneal Guttae Alone Do Not Make a Diagnosis of Fuchs' Endothelial Corneal Dystrophy," *American Journal of Ophthalmology* 264 (2024): x–xii.

17. M. P. Fautsch, E. D. Wieben, K. H. Baratz, et al., "TCF4-Mediated Fuchs Endothelial Corneal Dystrophy: Insights Into a Common Trinucleotide Repeat-Associated Disease," *Progress in Retinal and Eye Research* 81 (2021): 100883.

18. F. Aiello, G. Gallo Afflitto, F. Ceccarelli, M. Cesareo, and C. Nucci, "Global Prevalence of Fuchs Endothelial Corneal Dystrophy (FECD) in Adult Population: A Systematic Review and Meta-Analysis," *Journal of Ophthalmology* 2022 (2022): 3091695.

19. R. B. Singh, U. P. S. Parmar, F. Kahale, B. H. Jeng, and V. Jhanji, "Prevalence and Economic Burden of Fuchs Endothelial Corneal Dystrophy in the Medicare Population in the United States," *Cornea* 43, no. 8 (2024): 1022–1027.

20. A. J. Hardcastle, P. Liskova, Y. Bykhovskaya, et al., "A Multi-Ethnic Genome-Wide Association Study Implicates Collagen Matrix Integrity and Cell Differentiation Pathways in Keratoconus," *Communications Biology* 4, no. 1 (2021): 266.

21. L. Y. Xu, X. D. Zheng, S. S. Yin, et al., "Association of Novel Loci With Keratoconus Susceptibility in a Chinese Genome-Wide Association Study," *Investigative Ophthalmology & Visual Science* 65, no. 5 (2024): 29.

22. A. D. Irvine, L. D. Corden, O. Swensson, et al., "Mutations in Cornea-Specific Keratin K3 or K12 Genes Cause Meesmann's Corneal Dystrophy," *Nature Genetics* 16, no. 2 (1997): 184–187.

23. W. H. McLean and C. B. Moore, "Keratin Disorders: From Gene to Therapy," *Human Molecular Genetics* 20, no. R2 (2011): R189–R197.

24. H. Hassan, C. Thaung, N. D. Ebenezer, G. Larkin, A. J. Hardcastle, and S. J. Tuft, "Severe Meesmann's Epithelial Corneal Dystrophy Phenotype due to a Missense Mutation in the Helix-Initiation Motif of Keratin 12," *Eye (London, England)* 27, no. 3 (2013): 367–373.

25. E. H. Allen, D. G. Courtney, S. D. Atkinson, et al., "Keratin 12 Missense Mutation Induces the Unfolded Protein Response and Apoptosis in Meesmann Epithelial Corneal Dystrophy," *Human Molecular Genetics* 25, no. 6 (2016): 1176–1191, https://doi.org/10.1093/hmg/ddw001.

26. J. D. Malhotra and R. J. Kaufman, "The Endoplasmic Reticulum and the Unfolded Protein Response," *Seminars in Cell & Developmental Biology* 18, no. 6 (2007): 716–731.

27. F. Jonsson, B. Bystrom, A. E. Davidson, et al., "Mutations in Collagen, Type XVII, Alpha 1 (COL17A1) Cause Epithelial Recurrent Erosion Dystrophy (ERED)," *Human Mutation* 36, no. 4 (2015): 463–473.

28. V. F. Oliver, K. A. van Bysterveldt, M. Cadzow, et al., "A COL17A1 Splice-Altering Mutation Is Prevalent in Inherited Recurrent Corneal Erosions," *Ophthalmology* 123, no. 4 (2016): 709–722.

29. L. S. Sullivan, X. Zhao, S. J. Bowne, et al., "Exclusion of the Human Collagen Type XVII (COL17A1) Gene as the Cause of Thiel-Behnke Corneal Dystrophy (CDB2) on Chromosome 10q23-q25," *Current Eye Research* 27, no. 4 (2003): 223–226.

30. J. A. McGrath, B. Gatalica, A. M. Christiano, et al., "Mutations in the 180-kD Bullous Pemphigoid Antigen (BPAG2), a Hemidesmosomal Transmembrane Collagen (COL17A1), in Generalized Atrophic Benign Epidermolysis Bullosa," *Nature Genetics* 11, no. 1 (1995): 83–86, https://doi.org/10.1038/ng0995-83.

31. J. A. Turunen, I. S. Tuisku, P. Repo, et al., "Epithelial Recurrent Erosion Dystrophy (ERED) From the Splice Site Altering COL17A1 Variant c.3156C>T in Families of Finnish-Swedish Ancestry," *Acta Ophthalmologica* 102, no. 3 (2024): 296–305.

32. L. Bruckner-Tuderman and C. Has, "Molecular Heterogeneity of Blistering Disorders: The Paradigm of Epidermolysis Bullosa," *Journal of Investigative Dermatology* 132, no. Suppl 3 (2012): E2–E5.

33. J. D. Fine, L. B. Johnson, M. Weiner, et al., "Eye Involvement in Inherited Epidermolysis Bullosa: Experience of the National Epidermolysis Bullosa Registry," *American Journal of Ophthalmology* 138, no. 2 (2004): 254–262.

34. S. Loffek, T. Hurskainen, J. Jackow, et al., "Transmembrane Collagen XVII Modulates Integrin Dependent Keratinocyte Migration via PI3K/Rac1 Signaling," *PLoS One* 9, no. 2 (2014): e87263.

35. I. K. Gipson, S. Spurr-Michaud, A. Tisdale, and M. Keough, "Reassembly of the Anchoring Structures of the Corneal Epithelium During Wound Repair in the Rabbit," *Investigative Ophthalmology & Visual Science* 30, no. 3 (1989): 425–434.

36. K. Kurbanyan, K. D. Sejpal, A. J. Aldave, and S. X. Deng, "In Vivo Confocal Microscopic Findings in Lisch Corneal Dystrophy," *Cornea* 31, no. 4 (2012): 437–441.

37. D. Gibson, V. Brar, R. Li, A. Kalra, A. Goodwin, and N. Couser, "The High Association of Ophthalmic Manifestations in Individuals With

Mucolipidosis Type IV," *Journal of Pediatric Ophthalmology and Strabismus* 59, no. 5 (2022): 332–337.

38. M. Nakatsukasa, S. Kawasaki, K. Yamasaki, et al., "Tumor-Associated Calcium Signal Transducer 2 Is Required for the Proper Subcellular Localization of Claudin 1 and 7: Implications in the Pathogenesis of Gelatinous Drop-Like Corneal Dystrophy," *American Journal of Pathology* 177, no. 3 (2010): 1344–1355.

39. S. Kinoshita, K. Nishida, A. Dota, et al., "Epithelial Barrier Function and Ultrastructure of Gelatinous Drop-Like Corneal Dystrophy," *Cornea* 19, no. 4 (2000): 551–555.

40. H. Kaza, M. R. Barik, M. M. Reddy, R. Mittal, and S. Das, "Gelatinous Drop-Like Corneal Dystrophy: A Review," *British Journal of Ophthalmology* 101, no. 1 (2017): 10–15.

41. J. Skonier, M. Neubauer, L. Madisen, K. Bennett, G. D. Plowman, and A. F. Purchio, "cDNA Cloning and Sequence Analysis of Beta Igh3, a Novel Gene Induced in a Human Adenocarcinoma Cell Line After Treatment With Transforming Growth Factor-Beta," *DNA and Cell Biology* 11, no. 7 (1992): 511–522.

42. J. S. Bae, S. H. Lee, J. E. Kim, et al., "Betaig-h3 Supports Keratinocyte Adhesion, Migration, and Proliferation Through alpha3beta1 Integrin," *Biochemical and Biophysical Research Communications* 294, no. 5 (2002): 940–948.

43. K. Runager, R. V. Basaiawmoit, T. Deva, et al., "Human Phenotypically Distinct TGFBI Corneal Dystrophies Are Linked to the Stability of the Fourth FAS1 Domain of TGFBIp," *Journal of Biological Chemistry* 286, no. 7 (2011): 4951–4958.

44. E. Korvatska, H. Henry, Y. Mashima, et al., "Amyloid and Non-Amyloid Forms of 5q31-Linked Corneal Dystrophy Resulting From Kerato-Epithelin Mutations at Arg-124 Are Associated With Abnormal Turnover of the Protein," *Journal of Biological Chemistry* 275, no. 15 (2000): 11465–11469.

45. T. Berger, J. S. Weiss, W. Lisch, and B. Seitz, "The Latest IC3D Classification of Corneal Dystrophies-Overview and Changes of the 3rd Edition," *Die Ophthalmologie* 121 (2024): 714–725.

46. K. E. Han, S. I. Choi, T. I. Kim, et al., "Pathogenesis and Treatments of TGFBI Corneal Dystrophies," *Progress in Retinal and Eye Research* 50 (2016): 67–88.

47. J. Underhaug, H. Koldso, K. Runager, et al., "Mutation in Transforming Growth Factor Beta Induced Protein Associated With Granular Corneal Dystrophy Type 1 Reduces the Proteolytic Susceptibility Through Local Structural Stabilization," *Biochimica et Biophysica Acta* 1834, no. 12 (2013): 2812–2822.

48. K. Yamazoe, S. Yoshida, M. Yasuda, et al., "Development of a Transgenic Mouse With R124H Human TGFBI Mutation Associated With Granular Corneal Dystrophy Type 2," *PLoS One* 10, no. 7 (2015): e0133397.

49. V. Anandalakshmi, E. Murugan, E. G. T. Leng, et al., "Effect of Position-Specific Single-Point Mutations and Biophysical Characterization of Amyloidogenic Peptide Fragments Identified From Lattice Corneal Dystrophy Patients," *Biochemical Journal* 474, no. 10 (2017): 1705–1725.

50. A. K. Nowinska, E. Wylegala, S. Teper, et al., "Phenotype and Genotype Analysis in Patients With Macular Corneal Dystrophy," *British Journal of Ophthalmology* 98, no. 11 (2014): 1514–1521.

51. S. Singh, S. Das, C. Kannabiran, S. Jakati, and S. Chaurasia, "Macular Corneal Dystrophy: An Updated Review," *Current Eye Research* 46, no. 6 (2021): 765–770.

52. S. Aggarwal, T. Peck, J. Golen, and Z. A. Karcioglu, "Macular Corneal Dystrophy: A Review," *Survey of Ophthalmology* 63, no. 5 (2018): 609–617.

53. R. D. Young, T. O. Akama, P. Liskova, et al., "Differential Immunogold Localisation of Sulphated and Unsulphated Keratan Sulphate Proteoglycans in Normal and Macular Dystrophy Cornea Using Sulphation Motif-Specific Antibodies," *Histochemistry and Cell Biology* 127, no. 1 (2007): 115–120, https://doi.org/10.1007/s00418-006-0228-8.

54. T. O. Akama, K. Nishida, J. Nakayama, et al., "Macular Corneal Dystrophy Type I and Type II Are Caused by Distinct Mutations in a New Sulphotransferase Gene," *Nature Genetics* 26, no. 2 (2000): 237–241, https://doi.org/10.1038/79987.

55. D. Williams, D. D. Chung, A. Hovakimyan, A. Davtyan, B. J. Glasgow, and A. J. Aldave, "Novel DCN Mutation in Armenian Family With Congenital Stromal Corneal Dystrophy," *Cornea* 42, no. 4 (2023): 464–469.

56. E. Kubo, S. Shibata, T. Shibata, H. Sasaki, and D. P. Singh, "Role of Decorin in the Lens and Ocular Diseases," *Cells* 12, no. 1 (2022): 74, https://doi.org/10.3390/cells12010074.

57. J. H. Lee, C. S. Ki, E. S. Chung, and T. Y. Chung, "A Novel Decorin Gene Mutation in Congenital Hereditary Stromal Dystrophy: A Korean Family," *Korean Journal of Ophthalmology* 26, no. 4 (2012): 301–305.

58. A. S. Nicot and J. Laporte, "Endosomal Phosphoinositides and Human Diseases," *Traffic* 9, no. 8 (2008): 1240–1249.

59. J. A. Gee, R. F. Frausto, D. W. Chung, et al., "Identification of Novel PIKFYVE Gene Mutations Associated With Fleck Corneal Dystrophy," *Molecular Vision* 21 (2015): 1093–1100.

60. A. J. Aldave, G. O. Rosenwasser, V. S. Yellore, et al., "Linkage of Posterior Amorphous Corneal Dystrophy to Chromosome 12q21.33 and Exclusion of Coding Region Mutations in KERA, LUM, DCN, and EPYC," *Investigative Ophthalmology & Visual Science* 51, no. 8 (2010): 4006–4012, https://doi.org/10.1167/iovs.09-4067.

61. F. J. Basbus, F. A. Cremona, F. Lucero Saa, P. A. Chiaradia, L. Francipane, and S. Menazzi, "Posterior Amorphous Corneal Dystrophy: New Chromosomal Breakpoints in the Small Leucine-Rich Proteoglycan-Coding Region," *Cornea* 41, no. 4 (2022): 491–495.

62. P. Gain, R. Jullienne, Z. He, et al., "Global Survey of Corneal Transplantation and Eye Banking," *JAMA Ophthalmology* 134, no. 2 (2016): 167–173.

63. N. A. Afshari, R. P. Igo, Jr., N. J. Morris, et al., "Genome-Wide Association Study Identifies Three Novel Loci in Fuchs Endothelial Corneal Dystrophy," *Nature Communications* 8 (2017): 14898.

64. B. R. Gorman, M. Francis, C. L. Nealon, et al., "A Multi-Ancestry GWAS of Fuchs Corneal Dystrophy Highlights the Contributions of Laminins, Collagen, and Endothelial Cell Regulation," *Communications Biology* 7, no. 1 (2024): 418, https://doi.org/10.1038/s42003-024-06046-3.

65. K. H. Baratz, N. Tosakulwong, E. Ryu, et al., "E2-2 Protein and Fuchs's Corneal Dystrophy," *New England Journal of Medicine* 363, no. 11 (2010): 1016–1024, https://doi.org/10.1056/NEJMoa1007064.

66. E. D. Wieben, R. A. Aleff, N. Tosakulwong, et al., "A Common Trinucleotide Repeat Expansion Within the Transcription Factor 4 (TCF4, E2-2) Gene Predicts Fuchs Corneal Dystrophy," *PLoS One* 7, no. 11 (2012): e49083.

67. N. Bhattacharyya, N. Chai, N. J. Hafford-Tear, et al., "Deciphering Novel TCF4-Driven Mechanisms Underlying a Common Triplet Repeat Expansion-Mediated Disease," *PLoS Genetics* 20, no. 5 (2024): e1011230.

68. S. A. Riazuddin, N. A. Zaghloul, A. Al-Saif, et al., "Missense Mutations in TCF8 Cause Late-Onset Fuchs Corneal Dystrophy and Interact With FCD4 on Chromosome 9p," *American Journal of Human Genetics* 86, no. 1 (2010): 45–53.

69. E. N. Vithana, P. E. Morgan, V. Ramprasad, et al., "SLC4A11 Mutations in Fuchs Endothelial Corneal Dystrophy," *Human Molecular Genetics* 17, no. 5 (2008): 656–666.

70. S. A. Riazuddin, D. S. Parker, E. J. McGlumphy, et al., "Mutations in LOXHD1, a Recessive-Deafness Locus, Cause Dominant Late-Onset Fuchs Corneal Dystrophy," *American Journal of Human Genetics* 90, no. 3 (2012): 533–539.

71. S. A. Riazuddin, S. Vasanth, N. Katsanis, and J. D. Gottsch, "Mutations in AGBL1 Cause Dominant Late-Onset Fuchs Corneal Dystrophy and Alter Protein-Protein Interaction With TCF4," *American Journal of Human Genetics* 93, no. 4 (2013): 758–764.

72. T. R. Tsedilina, E. Sharova, V. Iakovets, and L. O. Skorodumova, "Systematic Review of SLC4A11, ZEB1, LOXHD1, and AGBL1 Variants in the Development of Fuchs' Endothelial Corneal Dystrophy," *Frontiers in Medicine (Lausanne)* 10 (2023): 1153122.

73. J. D. Gottsch, O. H. Sundin, S. H. Liu, et al., "Inheritance of a Novel COL8A2 Mutation Defines a Distinct Early-Onset Subtype of Fuchs Corneal Dystrophy," *Investigative Ophthalmology & Visual Science* 46, no. 6 (2005): 1934–1939.

74. J. W. Mok, H. S. Kim, and C. K. Joo, "Q455V Mutation in COL8A2 Is Associated With Fuchs' Corneal Dystrophy in Korean Patients," *Eye (London, England)* 23, no. 4 (2009): 895–903.

75. S. Biswas, F. L. Munier, J. Yardley, et al., "Missense Mutations in COL8A2, the Gene Encoding the alpha2 Chain of Type VIII Collagen, Cause Two Forms of Corneal Endothelial Dystrophy," *Human Molecular Genetics* 10, no. 21 (2001): 2415–2423.

76. A. S. Jun, H. Meng, N. Ramanan, et al., "An Alpha 2 Collagen VIII Transgenic Knock-In Mouse Model of Fuchs Endothelial Corneal Dystrophy Shows Early Endothelial Cell Unfolded Protein Response and Apoptosis," *Human Molecular Genetics* 21, no. 2 (2012): 384–393.

77. P. Liskova, M. Palos, A. J. Hardcastle, and A. L. Vincent, "Further Genetic and Clinical Insights of Posterior Polymorphous Corneal Dystrophy 3," *JAMA Ophthalmology* 131, no. 10 (2013): 1296–1303.

78. C. J. Evans, P. Liskova, L. Dudakova, et al., "Identification of Six Novel Mutations in ZEB1 and Description of the Associated Phenotypes in Patients With Posterior Polymorphous Corneal Dystrophy 3," *Annals of Human Genetics* 79, no. 1 (2015): 1–9.

79. L. Dudakova, V. Stranecky, L. Piherova, et al., "Non-Penetrance for Ocular Phenotype in Two Individuals Carrying Heterozygous Loss-Of-Function ZEB1 Alleles," *Genes (Basel)* 12, no. 5 (2021): 677.

80. D. Malhotra, M. Jung, C. Fecher-Trost, et al., "Defective Cell Adhesion Function of Solute Transporter, SLC4A11, in Endothelial Corneal Dystrophies," *Human Molecular Genetics* 29, no. 1 (2020): 97–116.

81. E. Schmid, W. Lisch, W. Philipp, et al., "A New, X-Linked Endothelial Corneal Dystrophy," *American Journal of Ophthalmology* 141, no. 3 (2006): 478–487.

82. P. Skalicka, L. Dudakova, M. Palos, et al., "Paraproteinemic Keratopathy Associated With Monoclonal Gammopathy of Undetermined Significance (MGUS): Clinical Findings in Twelve Patients Including Recurrence After Keratoplasty," *Acta Ophthalmologica* 97, no. 7 (2019): e987–e992.

83. E. K. Schmidt, T. Mustonen, S. Kiuru-Enari, T. T. Kivela, and S. Atula, "Finnish Gelsolin Amyloidosis Causes Significant Disease Burden but Does Not Affect Survival: FIN-GAR Phase II Study," *Orphanet Journal of Rare Diseases* 15, no. 1 (2020): 19.

84. C. Hung, R. I. Ayabe, C. Wang, R. F. Frausto, and A. J. Aldave, "Pre-Descemet Corneal Dystrophy and X-Linked Ichthyosis Associated With Deletion of Xp22.31 Containing the STS Gene," *Cornea* 32, no. 9 (2013): 1283–1287.

85. G. C. M. Black, J. L. Ashworth, and P. I. Sergouniotis, *Clinical Ophthalmic Genetics and Genomics* (Elsevier Inc., 2022).

86. P. Liskova, N. J. Hafford-Tear, P. Skalicka, et al., "Posterior Corneal Vesicles Are Not Associated With the Genetic Variants That Cause Posterior Polymorphous Corneal Dystrophy," *Acta Ophthalmologica* 100, no. 7 (2022): e1426–e1430.

87. K. Cunnusamy, C. B. Bowman, W. Beebe, X. Gong, R. N. Hogan, and V. V. Mootha, "Congenital Corneal Endothelial Dystrophies Resulting From Novel De Novo Mutations," *Cornea* 35, no. 2 (2016): 281–285.

88. K. Yousaf, S. Naz, A. Mushtaq, et al., "Exome Sequencing Reveals SLC4A11 Variant Underlying Congenital Hereditary Endothelial Dystrophy (CHED2) Misdiagnosed as Congenital Glaucoma," *Genes (Basel)* 14, no. 2 (2023): 310.

89. L. Dudakova, P. Skalicka, A. E. Davidson, and P. Liskova, "Coincidental Occurrence of Schnyder Corneal Dystrophy and Posterior Polymorphous Corneal Dystrophy Type 3," *Cornea* 38, no. 6 (2019): 758–760.

90. Y. Bouyacoub, Y. Falfoul, M. Ouederni, et al., "Granular Type I Corneal Dystrophy in a Large Consanguineous Tunisian Family With Homozygous p.R124S Mutation in the TGFBI Gene," *Ophthalmic Genetics* 40, no. 4 (2019): 329–337.

91. N. Yamada, T. I. Chikama, N. Morishige, et al., "Homozygous Mutation (L527R) of TGFBI in an Individual With Lattice Corneal Dystrophy," *British Journal of Ophthalmology* 89, no. 6 (2005): 771–773.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.