

Fuchs Corneal Dystrophy: Aberrant Collagen Distribution in an L450W Mutant of the COL8A2 Gene

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PURPOSE. To characterize histologically Descemet's membrane in an early-onset Fuchs corneal dystrophy (FCD) COL8A2 mutant and compare these findings with corneas from late-onset FCD and normal corneas.

METHODS. A corneal explant from a patient with the L450W COL8A2 mutation and others with late-onset disease were studied with antibodies to collagens IV, VIIIA1, VIIIA2, fibronectin, and laminin. Transmission electron microscopy was performed on a portion of the explant. Control explants included eye bank corneas without known disease and surgical explants from unrelated conditions.

RESULTS. In normal corneas, a regular array of colocalized COL8A1 and COL8A2 was observed in the anterior half of Descemet's membrane. In the L450W mutant, Descemet's membrane was several times thicker than normal and traversed by refractile strands and blebs that stained intensely for COL8A2, a feature also observed in late-onset FCD. Both the $\alpha 1$ and $\alpha 2$ subtypes of collagen VIII were observed at high levels along the anterior edge of Descemet's, another abnormal feature also found in late-onset FCD. Ultrastructure of the L450W cornea revealed a well-formed anterior banded layer more than three times thicker than normal. An unusual, thin internal layer was rich in patches of wide-spaced collagen. The layer is a distinctive pathologic structure that is associated with FCD and is characterized by ~ 120 nm periodicity and the presence of collagen VIII. Depositions of collagen IV, fibronectin, and laminin were also greatly increased in the of posterior Descemet's membrane, yet another general feature shared between early- and late-onset disease.

CONCLUSIONS. Early-onset COL8A2 L450W disease involves massive accumulation and abnormal assembly of collagen VIII within Descemet's membrane, a process that is presumed to begin during fetal development. Both early- and late-onset subtypes of FCD appear to be the result of abnormal basement membrane assembly rather than a primary defect in endothelial metabolism. (*Invest Ophthalmol Vis Sci.* 2005;46:4504-4511) DOI:10.1167/iovs.05-0497

Fuchs corneal dystrophy (FCD) is a progressive degeneration affecting the endothelium that leads to a thickened Descemet's membrane with posterior excrescences known clinically

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cally as cornea guttata.¹⁻³ Descemet's membrane is formed by corneal endothelial cells that secrete an extracellular matrix that acquires a complex laminar structure as it thickens with age.⁴⁻⁶ The development of Descemet's membrane has been well characterized, with the secretion of lamellae noted at 4 months of gestation with detectable condensation into layered "banding" patterns by 8 months.^{5,6} In prenatal development, secretion of thin, short filaments is observed perpendicular to the plane of Descemet's membrane.^{4,6} In electron microscopic studies, these filaments have a striated appearance with banding patterns with 120-nm intervals.^{4,6-8} At birth, Descemet's membrane averages 3 μm in thickness and then continues to thicken with age, but no longer with cross-bridging, linking filaments. The deposition of this nonstriated, nonlamellar, amorphous material continues with age and is referred to as the postnatal⁶ or posterior nonbanded layer (PNBL).^{7,8} The PNBL grows in thickness from approximately 2 μm at 10 years of age to 10 μm at 80 years.

Descemet's membrane can be considerably altered in FCD. Goar⁹ noted from the histopathologic examination of a button with cornea guttata that Descemet's membrane was thickened two- to threefold. In most patients with FCD, the PNBL is thinner than normal, with an average of 1.8 μm , although in some patients it is absent.¹⁰ However, as noted in electron microscopic studies of FCD, Descemet's is greatly thickened by a posterior banded collagenous layer (PCL)⁷ or referred to as a posterior banded layer (PBL) that can range from 10 to 20 μm .¹⁰ In some corneas, a fibrillar layer is interspersed between the PBL and the endothelium.¹⁰⁻¹³ Wide-spaced collagen, which appeared in thin sections as patches with a periodicity of ~ 120 nm, was observed in the anterior and posterior banded layers. It was ultrastructurally identified by antibodies to type VIII collagen.¹¹ Key histopathologic features of Descemet's membrane in FCD are excrescences or warts that correspond to the guttata observed clinically. These excrescences can either project posteriorly or become incorporated in a multilaminar membrane (buried warts).^{7,8,14,15}

Magovern et al.¹⁶ presented a pedigree of FCD with autosomal dominant inheritance, with equivalent numbers of males and females affected. Many exhibited onset in childhood, progressing in their 20s and 30s to corneal clouding and edema.¹⁶ Histopathology demonstrated smaller warts that were mostly buried with both interspersed homogenous and collagenous material. A more homogenous layer was noted posterior to the buried warts, which bordered an attenuated endothelium. This family was re-evaluated for the genetic basis of the disease and a novel pathogenic L450W COL8A2 mutation was found.¹⁷ In the current study, the highly distinctive clinical pathology is confirmed as a corneal button from an affected family member of this pedigree was studied for ultrastructure and deposition of COL8.

MATERIALS AND METHODS

Patients

The study protocol was approved by the Joint Committee on Clinical Investigation at the Johns Hopkins University School of Medicine and

TABLE 1. The Antibodies

Antibodies	Origin	Source	Dilution
Type IV collagen	Mouse monoclonal	Developmental Studies Hybridoma Bank, Univ. of Iowa, Iowa City, IA	1:5
Type VIII collagen- α 1	Mouse monoclonal	Seikagaku America, East Falmouth, MA	1:50
Type VIII collagen- α 2	Rabbit polyclonal	P. F. Davis, New Zealand	1:3000
Fibronectin	Rabbit polyclonal	Dako, Carpinteria, CA	1:2000
Laminin	Mouse monoclonal	Dako	1:50

was in compliance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all study participants. Patients were recruited for study after initial evaluations of patients with FCD who presented to the Cornea Service at the Wilmer Ophthalmological Institute. Patient recruitment and participation followed institutional review board-approved procedures.

Specimen Preparation

For immunohistochemical study, the corneal buttons were bisected and immediately immersed in fresh 4% paraformaldehyde-PBS, fixed overnight at 4°C, transferred to 20% sucrose, embedded in optimal cutting temperature compound (Tissue-Tek; Sakura Finetek, Tokyo, Japan), and frozen with a mixture of methyl butane and dry ice. Blocks were cryosectioned at 9- μ m thickness. For ultrastructural studies, a portion of the corneal button was fixed in 1% glutaraldehyde and 4% formaldehyde, stained with tannic acid and lead citrate, embedded, and thin sectioned for transmission electron microscopy.

Immunohistochemistry

The antibodies and their dilutions are listed in Table I. After the sections were dried at room temperature for 10 minutes, they were successively incubated with blocking serum and mouse monoclonal or rabbit primary antibodies overnight at 4°C (Table 1) and anti-mouse or anti-rabbit secondary antibody conjugated with cy-3 (1:100; Jackson ImmunoResearch, West Grove, PA). For double labeling, the sections were incubated overnight with two primary antibodies from different species and then the secondary fluorescent antibodies labeled with either cy-2 or cy-3 (Jackson ImmunoResearch). Slides were stained with Hoechst (1:2000; Molecular Probes, Eugene, OR) for 60 seconds and mounted (ProLong; Molecular Probes). Specimens were viewed with a standard fluorescence or a laser confocal microscope (Ultra-view 2; Perkin Elmer, Boston, MA).

RESULTS

Refractile Structures within Descemet's Membrane

Observations of hematoxylin-eosin-stained sections under bright-field light microscopy revealed that Descemet's membrane of the *COL8A2* L450W mutant (Fig. 1B) was much thicker than that from a control cornea of a similar age (Fig. 1A) or one with late-onset Fuchs dystrophy (Fig. 1C). Descemet's membrane from the mutant also revealed a network of wrinkle-like structures that consisted of fibrous refractile bodies within the matrix. Because the posterior aspect of Descemet's in the mutant did not demonstrate posterior excrescences characteristic of FCD, it is possible that the guttata observed earlier by *in vivo* retroillumination of the cornea^{16,17} resulted from the distortion of light passing through Descemet's. These refractile structures stained very strongly with antibodies to the *COL8A2* protein, but correlated to a lesser extent with the *COL8A1* staining pattern. Similar refractile structures and their association with both α 1 and α 2 (Fig. 2) subtypes of collagen VIII were also observed in three different cases of late-onset FCD, which do not have mutations in *COL8A2*.

Differences in the Distribution of Collagen VIII α 1 and α 2 Subtypes

In normal cornea, Descemet's membrane showed fluorescent labeling in a periodic pattern with antibodies to *COL8A1* (Fig. 3A) and *COL8A2* (Fig. 3B). The periodic array of deposits was located in the anterior portion of Descemet's. In early-onset FCD, Descemet's was several times thicker and showed a very different pattern, with substantially stronger signals in specimens prepared and examined in parallel. An intense layer of fluorescent staining was seen in the anterior fetal layer of Descemet's membrane with antibodies to either *COL8A1* (Fig. 3C) or *COL8A2* (Fig. 3D). These images also revealed a wide, continuous band of staining with *COL8A1*, but the *COL8A2* protein appeared more closely associated with the irregular refractile fibrous structures (Figs. 3C, 3D). In late-onset FCD specimens, there were also differences in the distribution of the two collagen VIII subtypes. As with the early onset L450W mutant, both were found in the anterior fetal layer. *COL8A2* again showed a greater degree of association with the refractile

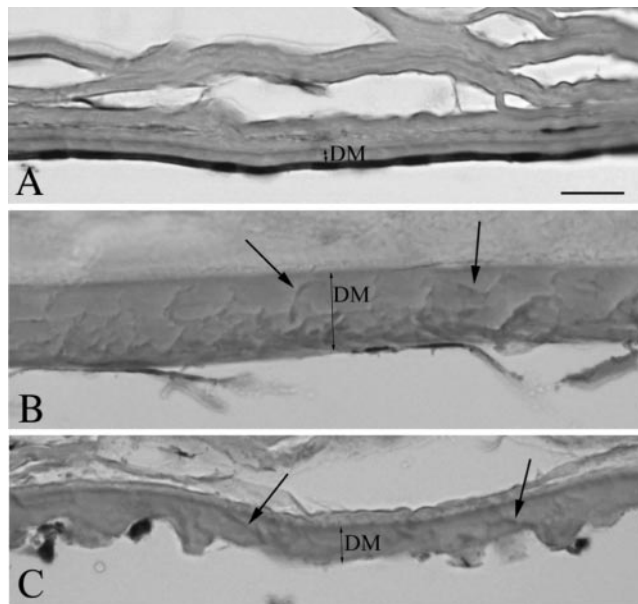


FIGURE 1. Morphologic changes in control and FCD corneas. Hematoxylin-eosin staining with bright-field microscopy. (A) Control cornea from a 30-year-old patient with keratoconus who had a healthy, normal Descemet's membrane (DM). Corneal endothelial cells were darkly stained and well aligned. (B) Early-onset FCD *COL8A2* L450W mutant, showing prominent network of wrinkle-like structures (arrows). Remaining endothelium on the posterior face (bottom) shows cytoplasmic degenerative changes. No posterior excrescences were visible in this or other sections. (C) Late-onset FCD cornea, showing refractile structures in the anterior and central portion of the DM (arrows). Prominent focal excrescences were present on the posterior surface of the DM. A few degenerated endothelial cells were present between the excrescences. Bar, 20 μ m.

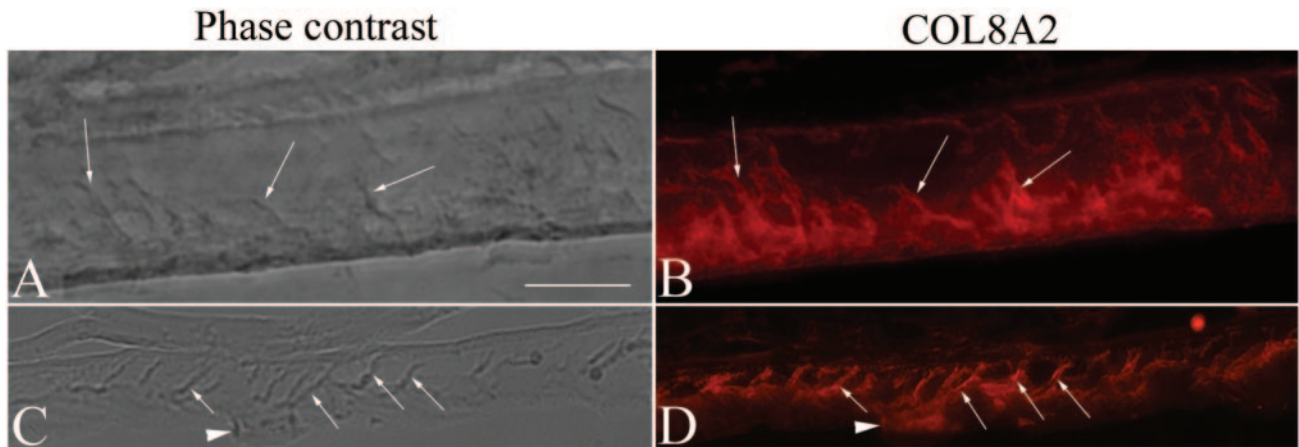


FIGURE 2. Phase contrast microscopy and immunocytochemistry for collagen VIII $\alpha 2$. Matched phase-contrast photomicrographs and immunofluorescent detection of collagen VIII $\alpha 2$ in (A, B) early-onset and (C, D) late-onset FCD corneas. *Arrows*: corresponding refractile and collagen VIII $\alpha 2$ -containing structures. *Arrowhead*: a focal excrescence in the late-onset FCD cornea. Bar, 20 μm .

fibrous structures (Fig. 3F, arrows), whereas COL8A1 showed a more uniform distribution (Fig. 3E). COL8A1 was associated with the most posterior layer of Descemet's, especially in the periphery and granular bodies within the posterior excrescences, whereas only low levels of diffuse COL8A2 signal were observed in the guttae or their posterior margin.

Differential Distribution of COL8A1 and COL8A2

In the normal cornea (Figs. 4A–C), double immunolabeling showed that the highly periodic structures in Descemet's membrane contained both COL8A1 (red) and COL8A2 (green; Figs. 4A, 4B). The merged image revealed a single shade of yellow for these structures, indicating that they were uniformly composed of a constant ratio of COL8A1 and COL8A2. These periodic structures may be composed of a defined heteromultimer of COL8A1 and COL8A2, and that the molecular structure of these arrays is highly ordered. Signal in the endothelial cells

appeared more granular, with some granules being red and others yellow, but with an overall stronger signal for COL8A1.

In contrast, Descemet's membrane of the L450W mutant loses this periodic structure, as well as the perfect register between red and green signals (Figs. 4D–F). Although the anterior fetal layer and the central layer (thick arrows) of collagen VIII stain with both subtypes, the signal appears instead as a granular mosaic, in which COL8A1 predominates in some bleb-like or fibrous structures, whereas COL8A2 predominates in other colabeled features (thin arrows). This suggests that the coassembly of COL8A1 and COL8A2 chains does not occur in a coordinate fashion, and that some areas receive more of one type than another. The differences in staining patterns between the two collagen VIII subtypes noted earlier indicate that these imbalances are not random.

In late-onset FCD, Descemet's membrane also shows dramatic disparities between the two subtypes (Figs. 4G–I). The

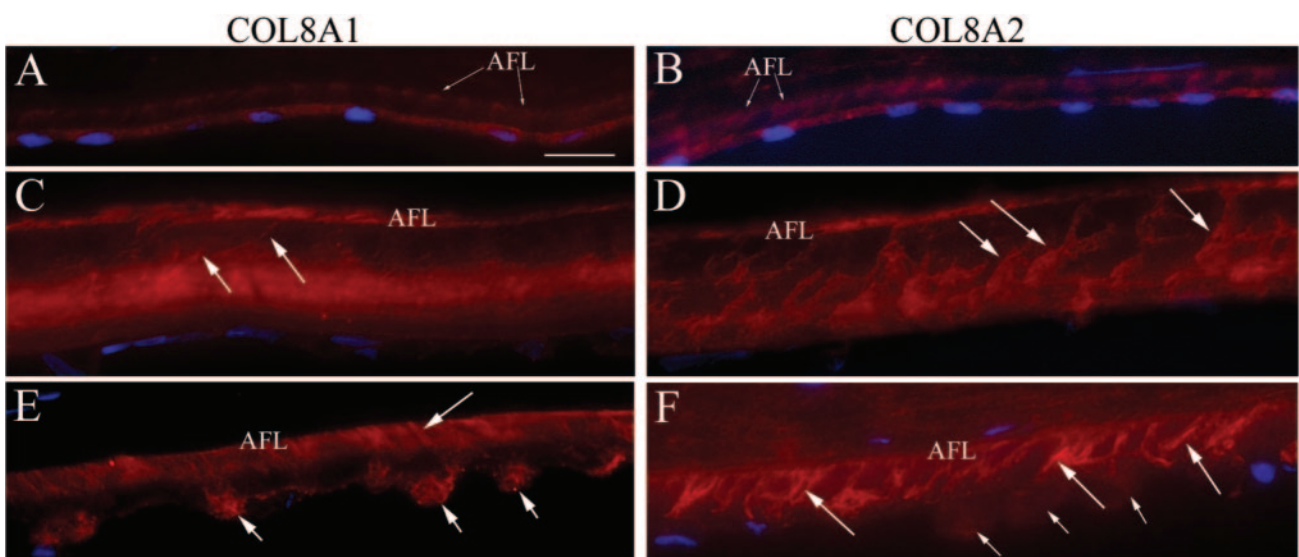


FIGURE 3. Collagen VIII $\alpha 1$ and $\alpha 2$ in normal and FCD corneas. (A, B) Normal cornea, showing corneal endothelial cells and DM stained with both antibodies. AFL: anterior fetal layer, marking the anterior edge of Descemet's, which is the first portion made during fetal development. (C, D) Early-onset FCD COL8A2 L450W mutant cornea. *Arrows*: $\alpha 1$ and $\alpha 2$ immunolabeling of thin and thick branchlike fibers in Descemet's membrane, respectively. (E, F) Late-onset FCD cornea. (E) *Short arrows*: posterior excrescences visible clinically as guttae. *Long arrows*: $\alpha 1$ -labeled brushlike fibrillar structures. (F) *Long arrows*: $\alpha 2$ -labeled brushlike thick fibers. *Short arrows*: locate excrescences negative for $\alpha 2$. Bar, 20 μm .

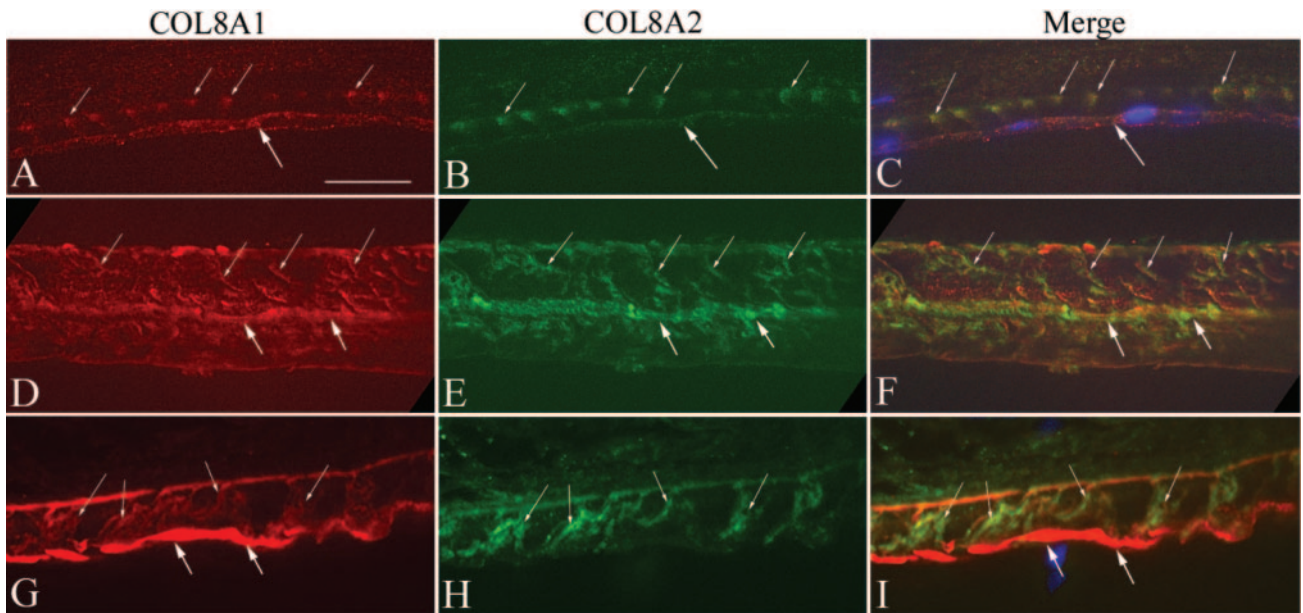


FIGURE 4. Confocal imaging of collagen VIII subtypes. Antibodies to collagen VIII $\alpha 1$ (red; COL8A1) and $\alpha 2$ (green; COL8A2) were used to double-label and produce merged images of normal, early-onset, and late-onset FCD corneas. Blue: Hoechst-stained nuclei. (A–C) Normal cornea. *Thin arrows*: colocalization of collagen VIII $\alpha 1$ and $\alpha 2$ in arrayed domains. Note the very uniform shade of yellow in the merged image. *Thick arrows*: endothelial cells that stain more strongly with the $\alpha 1$ antibody. (D–F) Early-onset FCD cornea. *Thin arrows*: signal for both collagen VIII subtypes in vertically arranged fibrillar structures. *Thick arrows*: central bandlike pattern observed in most sections. (G–I) Late-onset FCD. *Thick arrows*: posterior fibrillar layer of Descemet's, which shows a strong signal with $\alpha 1$, but little or no signal with $\alpha 2$. *Thin arrows*: fibrillar and globular structures which show mosaic staining for $\alpha 1$ and $\alpha 2$, with a relatively stronger signal for $\alpha 2$. Bar, 20 μm .

posterior layer shows an intense signal with COL8A1, but very little with COL8A2 (thick arrows). Although both label internal fibrous and bleblike structures, COL8A2 seems to predominate, but to varying degrees (thin arrows). Although the overall pattern is different from the early-onset disease, the presence of inhomogeneities is again strikingly different from normal corneas.

Segregated Distribution of $\alpha 1$ and $\alpha 2$ Collagen VIII Subtypes

Although the images shown in Figures 3 and 4 are typical of L450W disease, a few sections showed quite different patterns,

perhaps due to the orientation of the section or to local variations in the disease. Two examples in Figure 5 show either more localized distributions of collagen VIII (Figs. 5A–C) or a more dense and granular arrangement. A remarkable feature shared by these images is the fact that COL8A1 and COL8A2 are segregated from each other, with individual structures or subregions of a single structure giving different signals (arrows). Figure 5C shows the interior of Descemet's to be composed of a stratified grouping of blebs and strands composed almost entirely of COL8A1, whereas a more disordered, largely nonoverlapping set of features contains the COL8A2 subtypes.

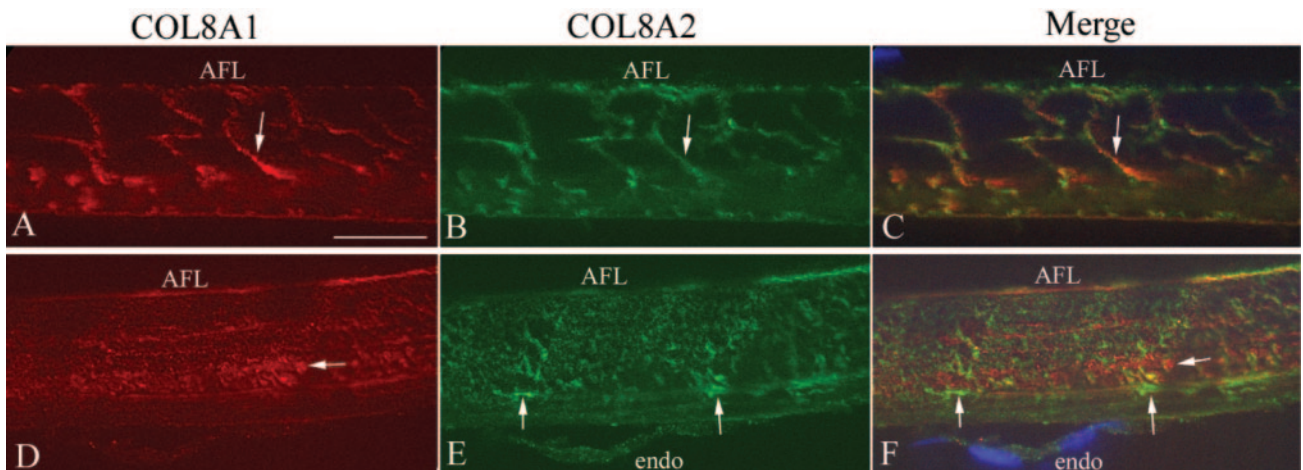


FIGURE 5. Distribution of collagen VIII subtypes. Confocal image of early-onset FCD corneas double-labeled with collagen VIII $\alpha 1$ and $\alpha 2$ antibodies. Red: COL8A1; green: COL8A2; blue: Hoechst-stained nuclei. (A–C) Image of a region with an atypical pattern. Both antibodies stained positively in the anterior fetal layer (AFL) and the vertically-arranged fibrillar structures (arrows) of Descemet's and were colocalized. Some globular structures stained preferentially with $\alpha 1$ antibody. (D–F) Region with a second atypical pattern. Most of the whorl-like or globular-like structures in Descemet's were not colocalized. (*horizontal arrows*: $\alpha 1$ labeling; *vertical arrows*: $\alpha 2$ labeling). Few remaining endothelial cells (endo) showed the positive staining with $\alpha 2$ antibody only. Bar, 20 μm .

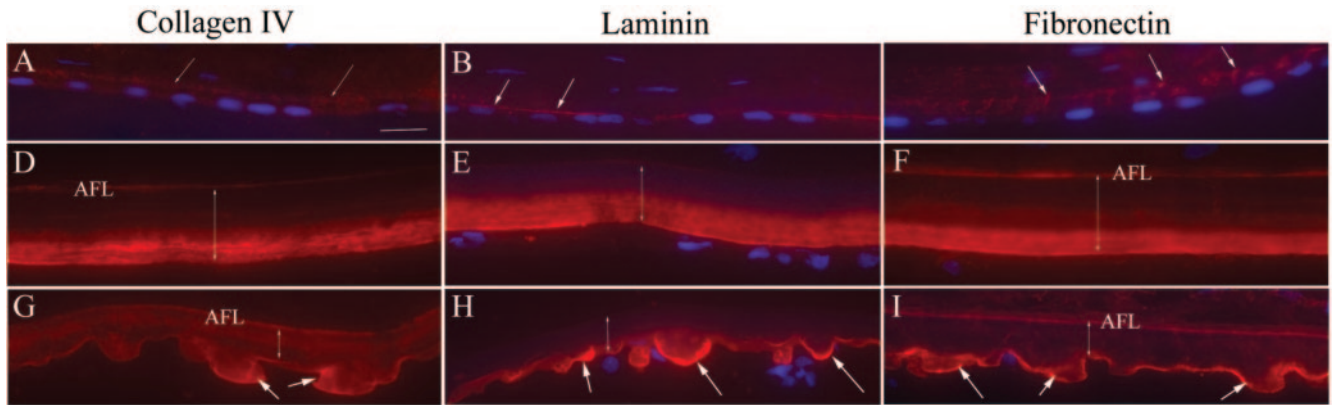


FIGURE 6. General basal lamina components: collagen IV, laminin, and fibronectin. In normal cornea (A–C), endothelial cells showed weak staining with all three antibodies. Collagen IV and fibronectin immunolabeling showed a weak linear pattern in the anterior Descemet's (*arrows*). In early-onset FCD (D–F), thick intensely labeled bands were observed in the posterior portion of Descemet's. Fibronectin labeling also revealed a prominent AFL (F). In the late-onset form (G–I), intense labeling was seen in the posterior layer of the DM as well as excrescences (*arrows*). An AFL was also noted with the fibronectin staining (D). Bar, 20 μm.

Collagen IV, Laminin, and Fibronectin

Antibodies to three additional proteins that are widely distributed in basal laminae revealed further differences between normal and pathologic specimens. In normal cornea (Figs. 6A–C), Descemet's membrane revealed a weak signal for collagen IV and fibronectin in a repeating structure that appears to coincide with that stained by collagen VIII. Laminin shows a more irregular and weaker pattern of staining. Endothelial cells show weak fluorescence with all three antibodies. In early-onset FCD cornea (Figs. 6D–F), intense signal similar to that observed for COL8A1 was seen as a continuous layer in the posterior half of Descemet's membrane. In the late-onset Fuchs cornea (Figs. 6G–I), intense labeling with all three antibodies was observed on and near the posterior surface of Descemet's membrane and within the excrescences. Collagen IV is also found widely distributed within Descemet's, whereas laminin

and fibronectin were found only in the posterior layer and to a lesser extent in the anterior fetal layer.

Ultrastructure of the COL8A2 L450W Mutant

Figure 8 presents sections of normal Descemet's membrane, late-onset FCD, and the early-onset L450W mutant. The normal cornea specimen (Fig. 8A) revealed the well-described anterior banded layer (ABL), which contained long-range arrays of vertical bands that corresponded to the large, regularly spaced domains of collagen VIII observed on immunohistochemistry of normal cornea (Fig. 5C). The PNBL (Fig. 8A) lacked this periodicity and occupied roughly the posterior half of Descemet's, which did not stain with antibodies to collagen VIII (Fig. 5C).

The late-onset FCD specimen (Fig. 8B) also had a layered appearance, labeled in the figure in accordance with the classic histologic assignments of ABL, posterior nonbanded layer, and

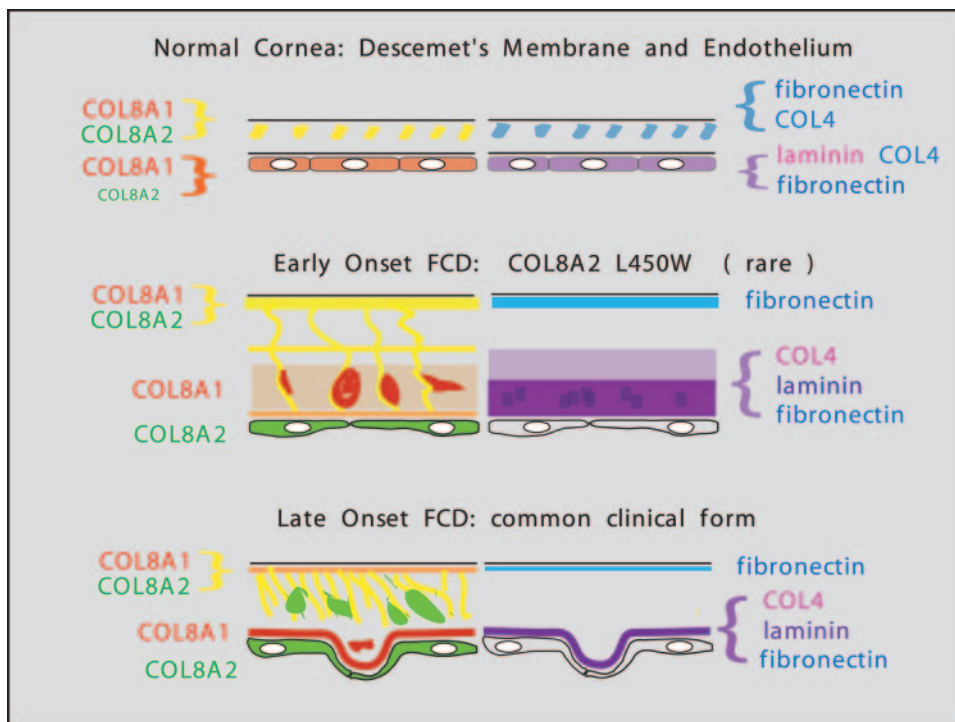


FIGURE 7. Summary of immunocytochemical results. Diagram of Descemet's membrane and underlying endothelial cells summarizes the observed distribution of collagen VIII subtypes (*right*) and more widely expressed extracellular components. *Top:* normal cornea. *Middle:* L450W mutant. *Bottom:* late-onset FCD. *Red:* collagen VIII α1; *green:* the α2 subtype, *yellow:* an equal mixture of the two, as in a merged micrograph. A similar scheme is used for collagen IV, laminin, and fibronectin.

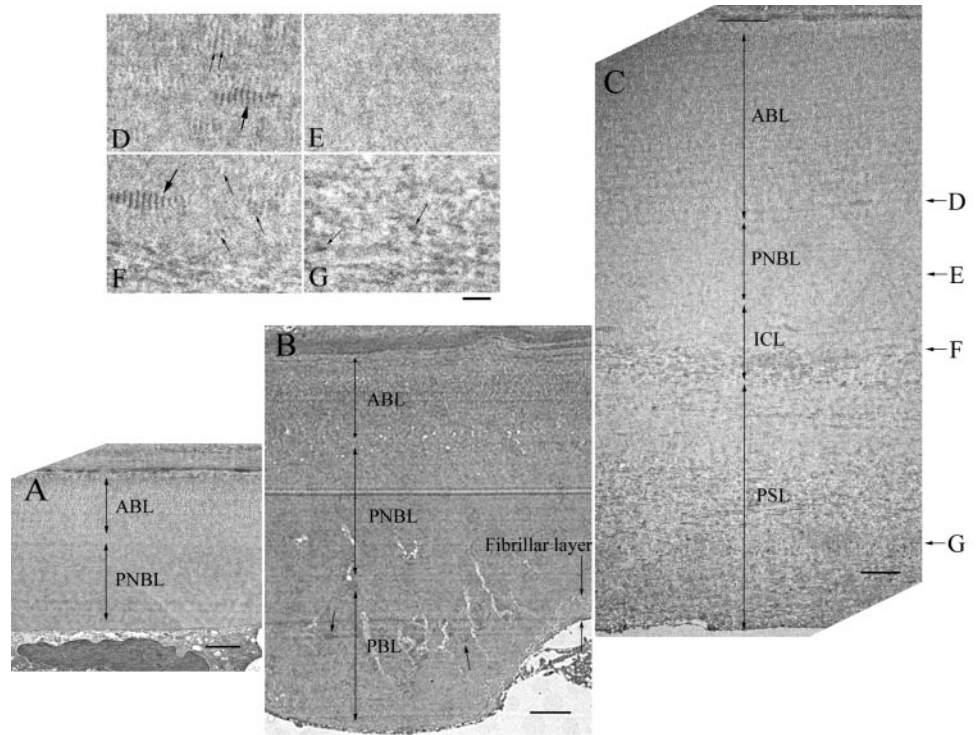


FIGURE 8. Ultrastructure of Descemet's membrane of normal, FCD, and L450W cornea. Transverse sections of Descemet's membrane from (A) normal control, (B) late-onset FCD, and (C) early-onset FCD *COL8A2* L450W mutant. Arrows and letters, to the right of (C) indicate layers of origin for the higher-magnification images (D-G). (D) ABL, at its bottom edge. Thick arrow: classic wide-banded ~ 120 nm periodicity collagen in an atypical location, to show alignment with repeat units of the ABL. (E) Detail of PNBL posterior nonbanded layer. (F) ICL showing disorganized wide-banded collagen (arrows), and adjacent electron dense fibrous material (bottom). (G) PSL. Arrow: electron-dense foci. Bar: (A-C) $2 \mu\text{m}$; (D-G) 500 nm .

posterior banded layer characteristic of FCD. There was an ABL, followed by a nonbanded layer that was less electron dense and had a fine-grained structure that lacked this regular periodicity. Posterior to this is another fine-grained layer containing short strips with transverse bands of ~ 120 nm periodicity. These are designated wide-spaced collagen and have been reported to contain collagen VIII.¹¹ They are a pathologic feature characteristic of FCD and not found in normal cornea. These strips presumably correspond to thin sections of irregular fibrous strands of structured collagen VIII. It is not clear how this ultrastructural morphology corresponds to the refractile collagen VIII fibrous strands and blebs, but it is possible that much of this excess collagen VIII in FCD is not structured, and appears amorphous in electron microscopy.

The section of Descemet's membrane from the L450W *COL8A2* mutant that is shown in Figure 8C was $35 \mu\text{m}$ thick. It began with a thin ($\sim 1\text{-}\mu\text{m}$) amorphous layer, followed by a well-ordered $10\text{-}\mu\text{m}$ ABL. This layer was much thicker than the 3 to $4 \mu\text{m}$ ABL observed in normal and late-onset FCD corneas. Posterior to this was a nearly uniform region very similar to the PNBL of normal and FCD corneas, except for the inclusion of rare strips of wide-spaced collagen. This was followed by a novel $\sim 2\text{-}\mu\text{m}$ internal collagenous layer (ICL) that was characterized by wide-spaced collagen strips with $\sim 120\text{-nm}$ spacing (Figs. 8C, 8F), as well as electron-dense fibrous material that corresponded to a narrow layer that stained strongly with antibodies to collagen VIII (Figs. 4B, 5F). After this was a novel posterior striated layer (Fig. 8C, PSL) $12 \mu\text{m}$ thick, that contained darker, horizontally striated material, and small, very electron-dense foci (Figs. 8C, 8G). Although most endothelial cells had been either lost at this late stage of the disease, or during surgery, degenerated endothelial cells were present in some sections (not shown) and had residual swollen mitochondria and cytoplasmic filaments. With regard to the ultrastructure of other sections, the image shown is representative, although there is some variation. Appearance and relative positions of boundaries between the ABL, PNBL, and PSL were quite constant. The thin ICL was a prominent feature in most sections, but varied in position within the PNBL.

DISCUSSION

Recently, we identified an L450W mutation in the *COL8A2* gene as the underlying genetic basis for a rare early-onset form of Fuchs' corneal dystrophy that can manifest in the first decade of life.¹⁷ The more common form of FCD is characterized by onset in the fourth decade or later. We have documented the distinctive ultrastructure and distribution of collagens, fibronectin, and laminin of Descemet's membrane in this early-onset FCD *COL8A2* mutant and contrasted it with late-onset FCD.

Our results with normal and L450W corneas are summarized in Figure 7. Normal Descemet's membrane appears to have regular periodic deposition of COL8A1 and COL8A2. Collagen VIII has been found in the anterior portion of Descemet's membrane in fetal and adult eyes.¹⁸ In the current study we found that COL8A1 and COL8A2 were precisely colocalized, and their relative amounts appeared constant, suggesting that they were coassembled in a structure with well-defined subtype composition. Whether such an orderly stoichiometry is established during the assembly of $\alpha 1$ and $\alpha 2$ chains into helical trimers or at a higher-order level of assembly is unknown. In both early-onset L450W disease and late-onset FCD, this regularly spaced distribution is disrupted. The L450W mutant no longer has a periodic structure, but instead an irregular mosaic, in which COL8A1 or COL8A2 predominates at different relative amounts in different areas of the membrane. Thus, the coassembly of COL8A1 and COL8A2 chains did not occur in a coordinated fashion, and some areas received more of one subtype than another. In late-onset FCD, Descemet's membrane also showed dramatic disparities between the two subtypes, especially in the posterior fibrous layer, which contained only COL8A1. Although the overall pattern of late-onset FCD is different from L450W, the presence of inhomogeneities is again a striking contrast with normal corneas.

Early-onset FCD shows greater thickening of Descemet's membrane and no histopathologic evidence of posterior excrescences. This absence of excrescences in tissue sections at

first seems at odds with earlier observations, when viewed with retroillumination and specular microscopy which characterized the guttata as smaller and more densely spaced.¹⁷ The clinical appearance of guttata in L450W disease appears to be an optical phenomenon due to light passing through a nonuniform distribution of refractile collagen bundles in the posterior layer. It was originally suggested by Magovern et al.¹⁶ that buried globular excrescences, known as warts, are responsible for the guttata that are noted with biomicroscopy.¹⁶

Ultrastructurally, Descemet's membrane in L450W disease reveals a surprisingly normal structure in its anterior ~30%. After an amorphous ~1- μm layer, there is a greatly thickened, 10- μm ABL, which is presumed to be assembled during fetal development and is thought to contain loosely stacked sheets of hexagonally arranged collagen VIII.⁵ The thickness of a normal ABL averages 3.1 μm .^{6,8} Antibody labeling does not show the pattern of regularly arrayed collagen VIII domains of normal Descemet's membrane, and it is possible that the weaker signal of this open matrix is obscured by the intense signal from the much more abundant pathologic collagen VIII deposits. Our L450W button was from a 31-year-old woman who first had FCD diagnosed as a 3-year-old and was originally reported by Magovern et al.¹⁶ The early onset of L450W disease may be a consequence of this unusually thick ABL, which is three times as thick as a normal ABL (3.1 μm).^{5,6} By contrast, in late-onset FCD,¹² the ABL is typically near normal thickness, and the ultrastructural pathology of Descemet's is first expressed in the postnatal layers, particularly in the posterior banded layer and the posterior excrescences.

In L450W disease, the ABL is followed by a nonbanded layer (PNBL) of fairly conventional appearance, although it contains widely spaced collagen. This is followed by the ICL, which contains an upper portion with a dense arrangement of wide-banded collagens of differing orientations (Figs. 8C, 8F). This upper portion is characterized by some classic, intensely stained wide-banded collagen strips, with most staining more weakly and embedded in an amorphous layer. This is always accompanied by an underlying layer of electron-dense fibrous material that contains collagen fibers with narrower ~20- μm spacing. The ICL corresponds to the intense midlevel band observed by immunocytochemistry, and its presence signals the abrupt onset of more severe disease in the composition and structure of Descemet's membrane.

After the ICL was the posterior striated layer (PSL) which spanned ~40% of Descemet's. It contained electron-dense striated and punctate features, as well as occasional classic banded collagen. There was no clear counterpart of this layer in normal FCD. In immunocytochemistry, this region was rich in collagen VIII. Because there were relatively few of the wide-banded collagen VIII strips, it seems likely that nearly all this excess collagen VIII was amorphous. This posterior region also abundantly stained for collagen IV, fibronectin, and laminin, which are key components of the basal lamina of many different tissues. All three components formed a similar stratified distribution and did not appear to be associated with the irregular collagen VIII deposits. In late-onset disease greatly increased amounts of these three extracellular matrix proteins and COL8A1 were associated with a thin posterior fibrillar layer (Fig. 8B), which formed the posterior aspect of the excrescences. Finally, we confirmed the findings of others that COL4,¹⁹⁻²³ fibronectin,^{21,24} and laminin^{19,20,22} are present in normal Descemet's membrane.

Taken together, these results suggest that both forms of FCD are probably involved in abnormal assembly and possibly the turnover of collagen within this specialized extracellular matrix. Although there were differences between early- and late-onset FCD, certain features, such as the accumulation of large amounts of collagen VIII in fibrous, refractile structures,

and in the anterior fetal layer, were found in both forms of the disease and may be its fundamental pathologic feature. The presence of large amounts of disorganized collagen VIII and the excessive growth of this anterior layer of Descemet's, which is presumed to be produced in early in fetal development, indicates that early-onset FCD first appears in utero and continues into juvenile development and adulthood.

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