Retinal Basement Membrane Abnormalities and the Retinopathy of Alport Syndrome

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PURPOSE. To determine the effects of X-linked and autosomal recessive Alport syndrome on retinal basement membranes and how these result in the characteristic perimacular dot-and-fleck retinopathy, lozenge, and macular hole.

METHODS. The type IV collagen chains present in the normal retina were determined immunohistochemically. Ten patients with Alport syndrome underwent retinal photography and optical coherence tomography to determine the thickness of the internal limiting membrane (ILM) by segmentation analysis, the layers affected by the retinopathy, and any correlates of the lozenge and macular hole. Bruch's membrane was examined directly by electron microscopy in a donated Alport eye.

RESULTS. The $\alpha3\alpha4\alpha5$ type IV collagen network was present in the normal ILM and in the retinal pigment epithelium basement membrane of Bruch's membrane. In Alport syndrome, the ILM/nerve fiber layer and Bruch's membrane were both thinned. The dot-and-fleck retinopathy corresponded to hyper-reflectivity of the ILM/nerve fiber layer in the distribution of the nerve fiber layer. The lozenge and macular hole corresponded to temporal macular thinning. The thinning across the whole retina was principally due to thinning of the ILM/nerve fiber layer and inner nuclear layer.

CONCLUSIONS. The Alport dot-and-fleck retinopathy results primarily from abnormalities in the ILM/nerve fiber layer rather than in Bruch's membrane. Thinning of the ILM/nerve fiber layer contributes to the retinopathy, lozenge, and macular hole, possibly through interfering with nutrition of the overlying retina or clearance of metabolic by-products. (*Invest Ophthalmol Vis Sci.* 2010;51:1621–1627) DOI:10.1167/iovs.08-3323

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Alport syndrome is an inherited disease that occurs in 1 of 5,000 to 50,000 live births¹ and is characterized by renal failure, hearing loss, lenticonus, and retinopathy.² Eighty-five percent of families have X-linked inheritance³ with mutations in the *COL4A5* gene,⁴ and most of the others have autosomal recessive disease with mutations in the *COL4A3* or *-4* genes.⁵ All mutations result in the loss of the $\alpha3(IV)$ - $\alpha5(IV)$ collagen network from affected basement membranes⁶ and the subsequent development of thinning or lamellation.^{7–9}

The main ocular abnormalities in Alport syndrome are anterior lenticonus, and the central and peripheral retinopathies that occur in at least 50% males and 20% females with X-linked inheritance and also commonly in autosomal recessive disease (Figs. 1a–d; Refs. 2, 9–19). Anterior lenticonus results from bulging of the lens through a thinned capsule. It eventually affects vision, but is corrected with lens replacement. The central retinopathy comprises whitish yellow perimacular dots and flecks that are present from early adolescence and are more common when renal failure, hearing loss, and lenticonus are also present.²⁰ The central and peripheral retinopathies do not affect vision or require treatment and may occur independently.^{12,18}

The central retinopathy ranges from a few scattered dots and flecks in the temporal macula to, in the most severe cases, a perimacular annulus of densely packed dots between the foveal perimeter and the outermost vascular arcades. These form curvilinear streaks that appear to reflect the arrangement of the bundles in the nerve fiber layer.¹⁴ Sometimes the dots and flecks produce an abnormal tapetal-like reflex,¹⁹ and their demarcation from the perifovea results in a dull macular reflex or “lozenge.”¹² The lozenge is associated with severe Alport syndrome with early-onset renal failure. Retinopathy involving the fovea is uncommon. Macular holes associated with Alport syndrome are rare, but they are typically larger than those found in other conditions^{21,22} and respond poorly to attempts at surgical closure.

Few studies have addressed the pathogenesis of the perimacular dot-and-fleck retinopathy and other retinal abnormalities in Alport syndrome. Ophthalmoscopy and fluorescein angiography suggest that the superficial retina is affected^{11,14} but the $\alpha3(IV)$ - $\alpha5(IV)$ collagen chains have been demonstrated only deep in the retinal pigment epithelium (RPE) basement membrane of Bruch's membrane.²³ A further report has described unexplained thinning of the temporal inner macula in one individual.²⁴ In the present study, we examined the pathologic events underlying the retinal features of Alport syndrome.

METHODS

Patients and Clinical Features

Ten patients from two centers with renal biopsy-proven Alport syndrome were studied. All had been examined previously, and their

FIGURE 1. Alport retinopathy. (a) Typical perimacular dots and flecks, (b) peripheral retinopathy, (c) dull macular reflex (lozenge), and (d) red-free photograph demonstrating the dots and flecks in (c) more obviously.

retinal features were known. The mode of inheritance in each individual was determined from linkage to the *COL4A5* (X-linked) or *COL4A3/COL4A4* (autosomal recessive) loci in family studies.¹⁷

Four males and three females were from four families with X-linked Alport syndrome, and three males were from three families with autosomal recessive syndrome (Table 1). The median age of the participants was 35 years (range, 14–74).

Clinical features, in particular, an early age at onset of renal failure (≤ 30 years) and the presence of a clinically detectable hearing loss were noted. All participants were examined for the oil-droplet sign of anterior lenticonus by an ophthalmologist with a handheld retinoscope and, for the central and peripheral retinopathies, by direct ophthalmoscopy, slit lamp biomicroscopy with a 78-D lens, and indirect ophthalmoscopy with a 20-D lens. Optic fundi were photographed with a nonmydriatic camera (Carl Zeiss Meditec, Oberkochen, Germany).

Localization of Dots and Flecks to Retinal Layers

Two of the males with X-linked Alport syndrome, one with central retinopathy (patient 1) and one without (patient 4), underwent OCT with the Topcon machine (OCT-1000, software, ver. 2.11; Topcon, Tokyo, Japan). In addition, two of the males and one of the females with X-linked Alport syndrome, all with central retinopathy, were examined by HD-OCT (Cirrus; Carl Zeiss Meditec). Individual retinal dots were localized after examination of the surfaces of the internal limiting membrane (ILM) and the RPE basement membrane and on cross sections.

Total Retinal Thickness

All 10 patients with Alport disease were studied to determine the total retinal thickness, with multiple 5-mm vertical and horizontal OCT scans (Stratus OCT, software version 4.0; Carl Zeiss Meditec). The retinal thickness was measured in the right eye at nine locations where the most central location, R1, corresponded to the foveola; R2 to R5 to the inner macula; and R6 to R9 to the outer macula. Thinning was defined as less than the average thickness for the region -2 SD derived from 30 normal eyes, and thickening, greater than the mean $+2$ SD. Differences between patients and normal subjects were analyzed by using Student's unpaired *t*-test (two-tailed).

Thickness of Individual Retinal Layers Determined by Segmentation Analysis of OCT Images

The OCT images obtained with the Stratus OCT unit (Carl Zeiss Meditec) were analyzed further. The images from eight eyes with central retinopathy (three from the males and three from the females with X-linked and two from the females with recessive disease) were overlaid with retinal boundaries by a single operator, as described previously.²⁵ Thicknesses of the following retinal layers were determined: retinal nerve fiber layer (RNFL); ganglion cell layer (GCL),

together with the inner plexiform layer (IPL) because the outer boundary of the GCL was difficult to visualize; inner nuclear layer (INL); outer plexiform layer (OPL); outer nuclear layer (ONL); photoreceptor inner/outer segment junction (IS/OS); RPE; and Bruch's membrane-choroid complex (BMCC).

Ultrastructural Examination of Affected Retina

Another eye, from a 36-year-old male donor with X-linked Alport syndrome, who had early-onset renal failure and retinopathy, was examined postmortem by electron microscopy, as described previously.²⁶ The mean Bruch's membrane thickness was estimated from 50 random measurements and compared with the mean thickness in a normal retina. Bruch's membrane included the RPE membrane, inner collagenous zone, elastic layer, outer collagenous zone, and basement membrane of the choroidal capillaries.

Type IV Collagen Chain Composition of Basement Membranes in Normal Human Retina

A normal human retina was examined for the $\alpha 1(\text{IV})$ - $\alpha 6(\text{IV})$ collagen chains, as described previously.²³ Briefly, serial cryosections oriented parallel to the optic nerve-foveal plane were fixed, blocked, and incubated with rat monoclonal antibodies against type IV collagen isoform-specific peptides ($\alpha 1$, H11; $\alpha 2$, H22; $\alpha 3$, H31; $\alpha 4$, H43; $\alpha 5$, H53; and $\alpha 6$, H63, kindly provided by Yoshifumi Ninomiya, Okayama University Medical School, Japan) at 1:75 dilution for 1 hour followed by goat anti-rat biotinylated secondary antibody (1:500; ICN Pharmaceuticals, Aurora, OH) for 30 minutes. The reaction was amplified with an ABC kit for 30 minutes (Vector Laboratories, Burlingame, CA) and counterstained with methyl green (Vector Laboratories). Purified rat IgG (ICN Pharmaceuticals) was used as the negative control. The specimens were examined and photographed (BH-2 microscope; Olympus Optical, Tokyo, Japan).

This study had the approval of respective institutional research committees (Austin Health, Northern Health, and the University of Iowa) and participants provided signed, informed consent, in accordance with the Declaration of Helsinki.

RESULTS

Type IV Collagen Chain Composition of Normal Human Retinal Basement Membranes

The $\alpha 3(\text{IV})$, $\alpha 4(\text{IV})$, and $\alpha 5(\text{IV})$ collagen chains were all present in both the normal human ILM and the RPE basement membrane of Bruch's membrane (Figs. 2A–C). In contrast, the $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$ chains predominated in the retinal and choroidal vessel walls.

Clinical Features

Two of the males with X-linked disease had early-onset renal failure, three were deaf, two had lenticonus, three had central retinopathy (Fig. 1a), and two (of the three examined) had peripheral retinopathy (Fig. 1b). Patients 1 and 2 had a retinal lozenge that was more obvious in red-free images (Figs. 1c, 1d). None of the three females with X-linked disease had renal failure, hearing loss, or lenticonus, one (of the three examined) had central retinopathy and two (of two) had peripheral retinopathy. All three patients with autosomal recessive Alport syndrome had early-onset renal failure, hearing loss, lenticonus, and both the central and peripheral retinopathies.

Localization of Dots and Flecks to Retinal Layers

In the patient with the central retinopathy, the ILM/NFL was hyperreflective in an annulus around the fovea that corresponded to the location of the dots and flecks in Topcon OCT (Fig. 3a). The RPE-BMCC was much less hyperreflective than the ILM (Fig. 3b). On lateral view, the ILM surface was irregu-

TABLE 1. Average Thickness of Retinal Regions (R1–R9) from Right Eye in 10 Individuals with Alport Syndrome

Patient	Clinical Features	R1 (Fovea)	R2 (SIM)	R3 (TIM)	R4 (IIM)	R5 (NIM)	R6 (SOM)	R7 (TOM)	R8 (IOM)	R9 (NOM)
X-linked Alport syndrome										
1.	M, 38 y, RF < 30, HL, LC, CR, PR	200*	263†	217†	255†	281	238*	215†	244	279
2.	M, 27 y, RF < 30, HL, LC, CR, PR	276‡	312	267*	317	323	284	247	270	291‡
3.	M, 14 y, H, P, HL, CR, not tested for PR	289‡	321	283*	309	‡356	272	231†	242	305‡
4.	M, 31 y, H, P, no HL, no LC, no CR, no PR	182†	292	273*	293	289	248*	233†	243	269
X-linked Alport syndrome carriers										
5.	F, 74 y, H, P, no HL, no LC, but has CR and PR	223	282*	269*	274*	272*	240*	218†	248	262
6.	F, 56 y, H, P, no HL, no LC, no CR but has PR	178†	264†	250†	270*	270*	258	237†	247	273
7.	F, 55 y, H, no HL, no LC, no CR, not tested for PR	239	333	318	333	342‡	290	279	283	309‡
Autosomal recessive Alport syndrome										
8.	M, 47 y, RF < 30, HR, LC, CR, PR	137†	250†	216†	230†	225†	253	237†	259	263
9.	M, 32 y, RF < 30, HR, LC, CR, PR	196*	277*	252†	278	279	254	231†	246	274
10.	M, 31 y, RF < 30, HL, LC, CR, PR	217	275*	253†	273	298	252	226†	243	276
Number of Alport eyes with thinning < 2 SD (%)		3 (30)	3 (30)	5 (50)	2 (20)	1 (10)	0 (0)	8 (80)	0 (0)	0 (0)
Mean thickness ± SD in 10 Alport eyes		214 ± 46 (NS)	286 ± 27 (P = 0.0089)	259 ± 30 (P < 0.0001)	283 ± 31 (P = 0.0341)	293 ± 38 (NS)	258 ± 18 (NS)	235 ± 18 (P < 0.0001)	252 ± 14 (NS)	280 ± 16 (P < 0.0001)
Normal ± SD in 30 non-Alport eyes		228 ± 20	308 ± 20	311 ± 22	301 ± 19	296 ± 19	268 ± 16	279 ± 19	248 ± 15	247 ± 17

Patients 1 and 2 had retinal lozenge. Thicknesses are expressed in micrometers. H, hematuria; P, proteinuria; RF, renal failure; HL, hearing loss; LC, lenticonus; CR, central retinopathy; PR, peripheral retinopathy; TIM, temporal inner macula; SIM, superior inner macula; NIM, nasal inner macula; IIM, inferior inner macula; TOM, temporal outer macula; SOM, superior outer macula; NOM, nasal outer macula; IOM, inferior outer macula.

* Thickness < mean of 30 normal eyes – 1 SD.

† Thickness < mean of 30 normal eyes – 2 SD.

‡ Thickness > mean + 2 SD.

FIGURE 2. Immunohistochemistry of normal human retina demonstrating the $\alpha 3(IV)$ collagen chain in both the ILM and the RPE basement membrane of Bruch's membrane. (A) Purple-blue immunostaining of $\alpha 1(IV)$ chain shows strong labeling of retinal and choroidal vasculature. (B) Immunostaining of $\alpha 3(IV)$ demonstrates a different pattern with strong labeling of the ILM and Bruch's membrane. (C) Strong immunostaining of $\alpha 3(IV)$ collagen of the innermost aspect of Bruch's membrane which corresponds to the RPE basement membrane. Bar: (A, B) 60 μm ; (C) 30 μm . PR, photoreceptors.

lar, and sparse macular drusen were also evident between the RPE basement membrane and the outer collagenous zone of Bruch's membrane (Figs. 3c, 3d). The patient without central retinopathy had no increased reflectivity of the ILM or the RPE, compared with normal persons. These changes were also evident with the Cirrus OCT.

Total Retinal Thickness

The mean thickness of the Alport retinas was reduced in four of the nine macular regions (superior, temporal, and inferior inner macula, and temporal outer macula) in the 10 patients who were examined (Table 1). The temporal outer and inner macula was thinned ($<\text{mean} - 2 \text{ SD}$) in 8 (8/10, 80%) and 5 (5/10, 50%) eyes, respectively, in the males and females with X-linked and autosomal recessive Alport syndrome (Table 1; Fig. 4a). Less marked thinning ($<\text{mean} - 1 \text{ SD}$) was present in the temporal inner and outer macula in 9 (9/10, 90%) and 8 (8/10, 80%) eyes, respectively. The location of this thinning corresponded to the Alport-associated lozenge and occurred even when no lozenge was obvious. The youngest age at which thinning was evident was in a 14-year-old boy with X-linked disease and central retinopathy but without a lozenge (patient 3). However, thinning also occurred with mild disease

FIGURE 3. Retinal OCT demonstrating (a) increased reflectivity of the ILM/NFL corresponding to dots and flecks in a parafoveal annulus (Topcon OCT); (b) lack of reflectivity of RPE (Topcon OCT).

FIGURE 4. OCT of retina of a male with X-linked Alport syndrome (Figs. 1c, 1d) demonstrating (a) retinal thickness in regions R1 and R9 and total retinal thinning of the temporal inner and outer macula (223 μm versus normal of 311 μm ; and 229 μm versus normal of 279 μm , respectively; Topcon OCT); (b) a lateral view through the macula with cavitation of the central macula extending into the temporal inner macula (right; Topcon OCT); (c) surface view showing cavitation extending temporally.

and without a clinically obvious retinopathy (patient 4). Two of the three females with X-linked disease who had the retinopathy also had extensive thinning. The female without retinopathy had none (patient 7).

All three individuals with autosomal recessive Alport syndrome and both the central and peripheral retinopathies had a thinned temporal inner and outer macula. One of these patients had the most widespread thinning observed in this series.

Some individuals had thinning and thickening in different macular regions. The nasal outer macular was thickened in three patients and the average thickness overall in this region was increased.

Lateral View of the Retina through the Macula

In patient 1, who had X-linked Alport syndrome and central retinopathy, OCT demonstrated an irregular surface and large lateral defect that corresponded to the thinned temporal inner and outer macula and the clinically obvious lozenge (Figs. 4b, 4c; Cirrus OCT). In patient 4, with X-linked Alport syndrome but without the central retinopathy or lozenge, OCT demonstrated thinning of the temporal outer macula but no defect.

Thickness of Individual Retinal Layers Determined from Segmentation Analysis of OCT Images

Overall, retinal thinning appeared to be due to thinning of the ILM/NFL and INL (Table 2). The average thickness of these layers was less than the mean -2 SD in five and seven regions, respectively, in the eight eyes of eight patients with X-linked or autosomal recessive disease. However retinal thinning was most pronounced in the temporal outer macula, and only the average ILM/NFL was significantly thinned in this region. The average BMCC width was thinned in only one region, the inferior inner macula.

Ultrastructural Examination of Affected Retina

Overall, Bruch's membrane was thinner in the Alport retina (1.6 μm) than in the normal one (2.0 μm , $P < 0.05$). However the RPE and the choriocapillaris basement membranes were not obviously thinner, lamellated, or irregular (Fig. 5).

Occasional drusen were observed between the RPE basement membrane and the outer collagenous zone. These were approximately 8 μm high and 50 μm wide and granular in

TABLE 2. Average Thickness of Retinal Layers (ILM–RPE) in Regions R1–R9 in Eight Individuals with Alport Syndrome

	R1 (Foveola)	R2 (SIM)	R3 (TIM)	R4 (IIM)	R5 (NIM)	R6 (SOM)	R7 (TOM)	R8 (IOM)	R9 (NOM)
ILM/NFL									
Alport syndrome	No layer	34 ± 8.6 (NS)	27.2 ± 15.7* (P = 0.0347)	24.2 ± 16.2* (P = 0.0044)	24.2 ± 16.2 (NS)	47.2 ± 12.3 (NS)	47.9 ± 9.3* (P = 0.0034)	33.8 ± 18.7 (NS)	12.6 ± 14.8* (P = 0.0003)
Normal, SD	No layer	35.8 ± 4.2	34.2 ± 4.5	33.2 ± 2.3	22.9 ± 2.7	51.3 ± 3.4	56.7 ± 6.4	39.4 ± 1.6	39.4 ± 4.7
GCL+IPL									
Alport syndrome	68.6 ± 11.0* (P < 0.0001)	82.5 ± 18.1 (NS)	87.9 ± 34.0 (NS)	75.1 ± 18.2 (NS)	74.8 ± 18.9* (P = 0.0248)	61.7 ± 13.7† (P = 0.0102)	75.1 ± 28.6† (P = 0.0042)	55.2 ± 15.2† (P = 0.0015)	59.4 ± 21.2† (P = 0.0889)
Normal, SD	51.7 ± 7.3	83.4 ± 9.0	85.1 ± 10.7	81.1 ± 8.9	85.0 ± 7.9	51.8 ± 7.7	57.0 ± 8.8	42.9 ± 6.7	51.7 ± 6.6
INL									
Alport syndrome	16.9 ± 12.8* (P = 0.0003)	27.9 ± 13.7* (P = 0.0014)	32.7 ± 12.8* (P = 0.0870)	30.8 ± 6.6* (P < 0.0001)	23.3 ± 10.4* (P < 0.0001)	24.9 ± 10.0* (P = 0.0283)	30.3 ± 15.5 (NS)	26.7 ± 7.7* (P = 0.0465)	25.5 ± 10.3* (P = 0.0413)
Normal, SD	7.1 ± 2.5	36.8 ± 2.6	37.0 ± 2.7	36.8 ± 2.0	34.4 ± 2.5	29.2 ± 1.9	30.2 ± 4.9	30.7 ± 3.9	29.8 ± 2.6
OPL									
Alport syndrome	35.4 ± 9.4† (P < 0.0001)	39.0 ± 5.3† (P = 0.0007)	35.3 ± 8.5 (NS)	41.4 ± 6.8† (P < 0.0001)	37.3 ± 4.9† (P = 0.0053)	36.1 ± 7.1† (P < 0.0001)	32.1 ± 9.1 (NS)	36.0 ± 6.4† (P = 0.0080)	33.3 ± 4.8† (P = 0.0273)
Normal, SD	16. ± 4.6	34.9 ± 1.7	35.6 ± 2.3	34.9 ± 2.2	33.7 ± 2.4	27.9 ± 1.6	30.0 ± 2.1	30.9 ± 4.0	29.9 ± 3.4
ONL									
Alport syndrome	94.3 ± 14.8* (P = 0.0003)	85.5 ± 9.0 (NS)	79.4 ± 9.3 (NS)	80.0 ± 9.4 (NS)	81.6 ± 12.3 (NS)	80.9 ± 11.8† (P = 0.0368)	79.3 ± 12.3† (P = 0.0300)	71.9 ± 7.2† (P = 0.0469)	72.3 ± 9.6 (NS)
Normal, SD	112.5 ± 10.4	84.7 ± 10.5	85.0 ± 11.1	81.2 ± 9.7	86.8 ± 8.3	73.8 ± 7.1	71.9 ± 6.9	69.3 ± .0	73.6 ± 7.8
RPE									
Alport syndrome	15.8 ± 2.2 (NS)	12.2 ± 1.8 (NS)	15.3 ± 6.2† (P = 0.0158)	13.2 ± 2.2 (NS)	14.6 ± 3.2 (NS)	11.9 ± 2.8 (NS)	12.0 ± 3.0 (NS)	11.7 ± 1.8 (NS)	13.0 ± 3.6 (NS)
Normal, SD	15.2 ± 1.6	12.7 ± 3.5	11.9 ± 2.2	12.7 ± 3.0	12.9 ± 3.0	13.9 ± 3.1	12.8 ± 2.1	13.5 ± 2.9	13.7 ± 3.3
BMCC									
Alport syndrome	63.2 ± 3.2 (NS)	66.5 ± 3.5 (NS)	65.5 ± 3.9 (NS)	66.6 ± 2.2 (NS)	65.3 ± 3.0 (NS)	67.7 ± 2.5 (NS)	67.9 ± 3.0 (NS)	68.5 ± 1.8† (P = 0.0878)	67.0 ± 3.6 (NS)
Normal, SD	63.5 ± 2.0	67.5 ± 3.4	68.1 ± 2.3	67.2 ± 3.0	66.9 ± 2.8	66.3 ± 3.2	67.6 ± 1.6	66.6 ± 2.9	66.4 ± 3.2

Thicknesses are expressed in mean micrometers ± SD. There is no ILM/NFL at R1. TIM, temporal inner macula; SIM, superior inner macula; NIM, nasal inner macula; IOM, inferior inner macula; TOM, temporal outer macula; SOM, superior outer macula; NOM, nasal outer macula; IOM, inferior outer macula.

* Thickness < mean of 30 normal eyes – 2 SD.

† Thickness > mean + 2 SD.

FIGURE 5. Drusen between the RPE basement membrane and the outer collagenous zone of Bruch's membrane demonstrated in an electron micrograph from a male with X-linked Alport syndrome. Layers of Bruch's membrane are indicated. Original magnification, $\times 2500$.

appearance. The overlying RPE cells were attenuated and contained many lipofuscin granules, and the inner collagenous zone was filled with debris. The elastic lamina was within normal limits.

DISCUSSION

This study demonstrated the presence of the $\alpha 3(\text{IV})$ – $\alpha 5(\text{IV})$ collagen chains in the normal ILM as well as the RPE basement membrane of Bruch's membrane. In addition, it identified three major abnormalities that help explain the retinal characteristics of Alport syndrome. First, the ILM/NFL was hyperreflective in a pattern that corresponded to the distribution of the dot-and-fleck retinopathy. The perifovea was spared this effect, which occurred only where the NFL was present. Although foveal drusen were noted beneath the RPE basement membrane by electron microscopy and OCT, their locations differed from the clinically observed dots and flecks. Second, the temporal macula was thinned corresponding to the location of the dull macular reflex (lozenge) and the macular hole. Third, the ILM and Bruch's membrane were themselves thinned, suggesting that these are the primary defects that result in the retinopathy, the thinned temporal macula and lozenge, and, potentially, the macular hole.

Mutations in the *COL4A3*, *-4*, and *-5* genes in Alport syndrome commonly result in the loss of the $\alpha 3\alpha 4\alpha 5(\text{IV})$ collagen heterotrimer from affected basement membranes that subsequently become thinned or lamellated. This study demonstrated that the $\alpha 3(\text{IV})$ – $\alpha 5(\text{IV})$ collagen chains were present in both the ILM/NFL and the RPE basement membrane, whereas they have only been described previously in Bruch's membrane. The ILM is not a true membrane but results from fusion of the foot processes of the glialike Müller cells. It forms a physical barrier that protects the retina from toxins and from traction from the vitreous as the eye moves. Bruch's membrane primarily regulates the passage of nutrients and metabolites between the RPE and underlying choriocapillaris.

Reports have suggested that the Alport dot-and-fleck retinopathy is located either in the superficial retina or simultaneously in the superficial and deep layers.^{11,14} In the present study, the retinopathy principally affected the ILM/NFL. The hyperreflectivity evident on OCT of the ILM/NFL corresponded precisely with the location of the retinopathy. It was absent from the perifovea and occurred in an annulus with an inner margin that also represented the inner limit of the NFL. The retinopathy may thus originate in the Müller cells (the retinal glial cell equivalents) and could even be due to prominent Müller cell foot plates or Gunn's dots,²⁷ but its precise nature awaits histologic determination. The thinned ILM/NFL is likely to be important in the pathogenesis of the retinopathy. A

thinned ILM may be more susceptible to tractional forces from the vitreous, interfere with the transport of nutrients, or impair the clearance of waste products.

Fewer and less prominent drusen were noted between the RPE and Bruch's membrane at the foveola. These were barely visible on retinal photography, and their distribution did not correspond with the dot-and-fleck retinopathy. They were more obvious on retinal cross section with OCT and on ultrastructural examination of the Alport retina. Drusen usually result from an inability to clear metabolic by-products and degraded material and occur normally after middle age at the foveola, the most metabolically active part of the retina. They may result in overlying photoreceptor degeneration and Müller glial cell activation.²⁸ The RPE-Bruch's membrane choriocapillaris complex resembles the glomerular filtration barrier, and drusen occur in many forms of glomerular disease including mesangiocapillary, poststreptococcal, and membranous glomerulonephritis.^{29–31}

The Alport retina was consistently thinned at the temporal inner and outer macula, resulting in an irregularly hollowed surface obvious on OCT cross section. This effect was partly explained by thinning of the ILM/NFL and the INL demonstrated on segmentation analysis. The demarcation between the perimacular retinopathy and the thinned macula resulted in the dull macular reflex or lozenge and corresponded with the location of a possible macular hole. The thinning was likely to represent the consequences of both the defective ILM/NFL and the effects of mechanical traction. The macular hole in Alport syndrome affects the full thickness of the retina, and neighboring tissue is also abnormal, making it less amenable to surgery.

ILM/NFL thinning in the Alport retina was suggested by segmentation analysis of the retinal layers imaged by OCT, and Bruch's membrane was observed to be thinned in electron micrographs of an Alport eye. The RPE membrane also appears thinned in a bull terrier model of autosomal dominant Alport disease,³² and the lens capsule is thinned in human disease. In contrast, the glomerular and cochlear membranes are lamellated because substitution of the $\alpha 3(\text{IV})$ – $\alpha 5(\text{IV})$ chains with the $\alpha 1(\text{IV})$ – $\alpha 2(\text{IV})$ isoforms predisposes to increased proteolysis and mechanical remodeling.^{7–9,33} The type IV collagen chain composition of the Alport retina is not known.

The retinal abnormalities in Alport syndrome result from thinning of the ILM/NFL and the RPE basement membrane of Bruch's membrane. The dot-and-fleck retinopathy is due to an abnormal ILM/NFL, and its distribution is identical with that of the NFL itself. The thinning of these membranes may interfere with the nutrition and integrity of the overlying retinal layers and contribute not only to the development of the dot-and-fleck retinopathy but also to overall retinal thinning and the appearance of the lozenge and development of a macular hole.

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