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Characterization of Choriocapillaris and Choroidal Abnormalities in Alport Syndrome

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Purpose: To analyze the characteristics of the choriocapillaris and the choroid in patients with Alport syndrome (AS) and investigate their clinical and demographic associations.

Methods: Multicenter, cross-sectional study. Forty-two eyes with AS were consecutively enrolled. A cohort of 33 healthy eyes was included as controls. Demographics and medical history were collected for each participant. Each eye underwent 3 × 3 swept-source optical coherence tomography angiography (PLEX Elite 9000 2.0; Carl Zeiss Meditec, Dublin, CA, USA) and spectral-domain OCT (Spectralis HRA2; Heidelberg Engineering, Heidelberg, Germany). Choriocapillaris flow deficit (FD) number, mean FD size, total FD area, FD density, subfoveal choroidal thickness (CT), total CT, and choroidal vascularity index (CVI) were compared between AS and control eyes. Factors associated with the FD density and the CVI in AS were explored with multivariable linear mixed models.

Results: There was high intragroup variability in choriocapillaris and choroidal measurements in patients with AS. Choriocapillaris FD in patients with AS were more numerous compared to controls ($P = 0.02$). FD density in eyes with AS increased with older age (estimate = 0.31% for each year; 95% confidence interval [CI], 0.06−0.57; $P = 0.02$) and was higher in patients with a history of kidney transplant (estimate = 9.66% in case of positive history; 95% CI, 3.52−15.8; $P = 0.006$). The CVI was lower in eyes with dot maculopathy (estimate = −3.30% if present; 95% CI, −6.38 to −0.21; $P = 0.04$) and anterior lenticonus (estimate = −6.50% if present; 95% CI, −10.99 to −2.00; $P = 0.006$).

Conclusions: Patients with AS with kidney involvement requiring transplant may present with more severe choriocapillaris impairment. Lower choroidal vascularity was found in the presence of other ocular structural abnormalities.

Translational Relevance: An increased load of choriocapillaris flow deficits on optical coherence tomography angiography was found in patients with Alport syndrome who also had severe kidney disease requiring transplant.

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Introduction

Alport syndrome (AS) is a genetic disorder affecting 1 in 5000 to 10,000 people and accounts for 3% of pediatric chronic kidney disease in the United States. Progressive kidney failure, hearing loss, and ocular abnormalities are the hallmarks of the disease. AS is caused by mutations in the collagen IV gene; COL4A5, which encodes for the collagen IV α5-chain, is the most commonly mutated gene and is inherited in an X-linked fashion. Homozygous or compound heterozygous mutations in the COL4A3 and COL4A4 genes, which encode collagen IV α3- and α4-chains, respectively, have an autosomal recessive (AR) inheritance.[1] Female patients with heterozygous X-linked mutations (carriers) show various disease manifestations, with symptom onset delayed by about 30 years compared with X-linked affected males.[2]

The choroid is a densely vascularized structure providing oxygen and nutrients to the outer retina, as well as thermal regulation[3]; the choriocapillaris is the innermost choroidal meshwork of freely anastomosing capillaries and is essential for the vascular supply to the outer retina and the retinal pigment epithelium (RPE). Choroidal and choriocapillaris abnormalities play a key role in the pathophysiology of several chorioretinal disorders, including age-related macular degeneration, high myopia, and other degenerative conditions.[4,5] Previous works have described both choroidal thinning[6,7] and thickening,[8] along with choriocapillaris hypoperfusion,[9,10] in patients with AS; however, the clinical factors affecting the choroidal and the choriocapillaris changes in AS have never been explored.

The recent advances in noninvasive ocular imaging have renewed the interest in the quantitative analysis of the choroid. On the one hand, the choroidal vascularity index (CVI), calculated on structural transverse optical coherence tomography (OCT) scans, estimates the relative proportion of luminal vessels over the total choroidal surface. The CVI has shown better repeatability and intra- and interrater reliability than the choroidal thickness (CT).[11] On the other hand, the coupling of OCT angiography (OCTA) technology with longer-wavelength light sources has improved the lateral resolution and the signal-to-noise ratio of the commercial devices, resulting in a detailed depiction of the choriocapillaris network. Regions of undetectable choriocapillaris flow due to actual capillary depletion or blood circulation below the decorrelation threshold have been labeled and counted as flow deficits (FDs).[12] FD density is an indirect measure of capillary dropout and a prognostic biomarker in several macular diseases, such as age-related macular degeneration[13] and diabetic retinopathy.[14]

On the basis of clinical observations, we hypothesize that the degree of choroidal involvement in AS ranges from thick and enlarged choroidal vessels to thin choroids and from preserved choriocapillaris to marked choriocapillaris flow impairment. This article analyzes the choriocapillaris and choroidal metrics in patients with AS and determines whether there are any clinical and demographic associations. Our study may help understand the pathogenesis of the choroidal vascular depletion seen in a subset of patients with AS.

Methods

This is a cross-sectional study of consecutively enrolled patients with AS between June 2020 and November 2020 in the Department of Ophthalmology at Northwestern University (Chicago, Illinois) and the Department of Ophthalmology of the Medical University of Vienna (Vienna, Austria). The data were reported according to the Strengthening the Reporting of Observational Studies in Epidemiology recommendations (Supplementary Table S1).[15] The study was approved by the local institutional review boards (IRBs) (Northwestern University and the Medical University of Vienna, IRB STU00212848) and followed the tenets of the Declaration of Helsinki and the regulations of the Health Insurance Portability and Accountability Act. Written consent was obtained by each participant.

Patients were identified using data registries and personal communications between the Nephrology and the Ophthalmology Departments at the respective institutions. The diagnosis of AS was made in the presence of typical clinical features (kidney failure, hearing loss, lenticonus, retinopathy), histopathology compatible with AS on kidney biopsy, and/or genetic mutations of COL4A5 or COL4A3 or COL4A4 genes (available in 76% of the cohort). The family history helped in identifying the inheritance pattern when genetic testing was not available (24% of patients). Patients with a history of preterm birth (less than 37 gestational weeks); corneal, lens, or media opacity precluding imaging; any other retinal disease; and history of intraocular surgery were excluded from the study. Some characteristics of this cohort have been described in a sister publication.[16]

Demographics (age, gender) and medical history, history of kidney transplant, age at the time of kidney transplant, self-reported history of hearing impairment, pattern of inheritance, and type of mutation

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were collected for each participant. Each patient underwent a complete ophthalmic examination, including measurement of Snellen best-corrected visual acuity (BCVA), refractive error (as spherical equivalent), intraocular pressure, slit-lamp examination, and dilated ophthalmoscopy. Presence of anterior lenticonus and/or dot maculopathy (defined as scattered dots in the perifoveal areas, ranging from a few lesions in the temporal macula to perimacular annulus of densely packed dots) were reported.

A healthy control group matched in age, gender, and refractive error to the study population was enrolled following informed consent. All control participants had no systemic comorbidities and no evidence of ocular disease or media opacity in the recruited eye as evaluated by dilated fundus examination or multimodal imaging. Both eyes were imaged if eligible.

All the patients and controls underwent OCTA (PLEX Elite 9000 2.0; Carl Zeiss Meditec, Dublin, CA, USA). The FD number, the mean FD size, the total FD area, and the FD density were calculated; we did not correct the choriocapillaris OCTA slabs for uneven illumination, as no eye presented overlying retinal or RPE pathology potentially obscuring the choriocapillaris signal. All study participants underwent macular spectral domain OCT (SD-OCT; Spectralis HRA2; Heidelberg Engineering, Heidelberg, Germany). The central macular thickness, the CT, the CVI, and the

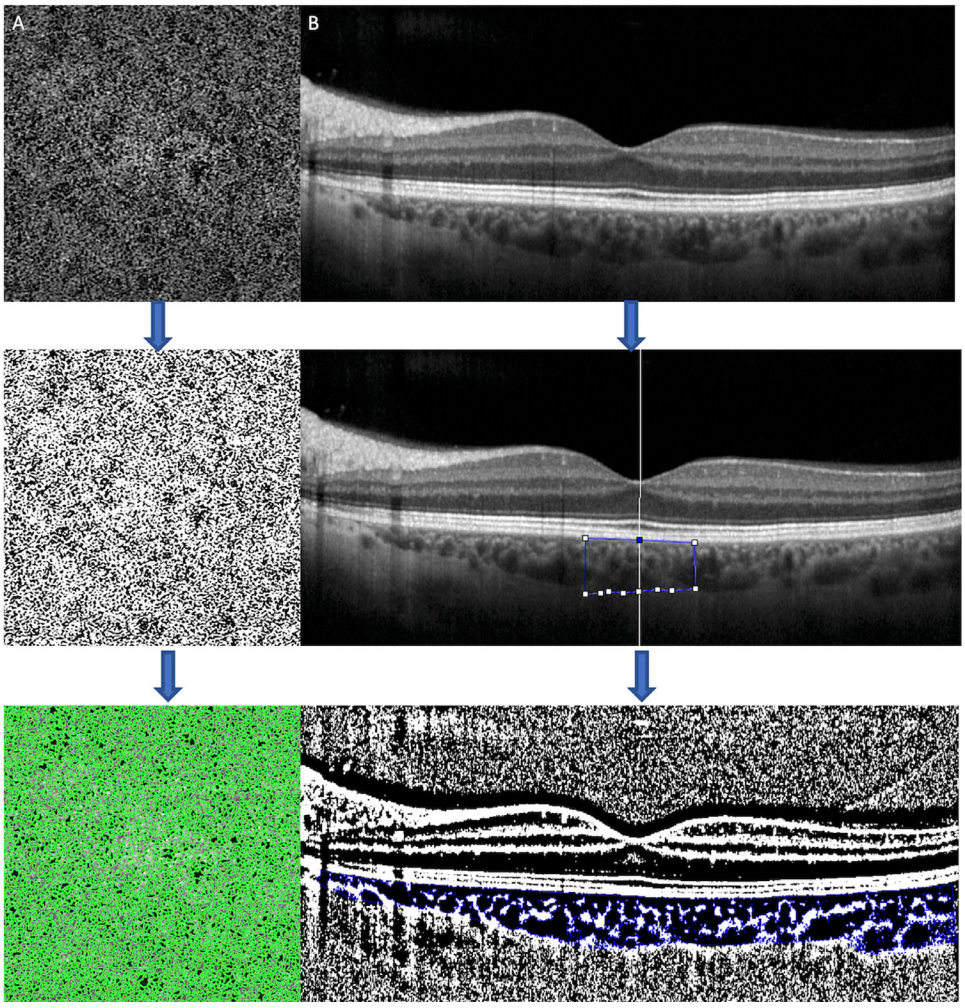

Figure 1. Image processing and analysis of choriocapillaris flow deficits (**A**) and choroidal vascularity index (**B**). (**A**) Choriocapillaris optical coherence tomography angiography slabs (*first row*) were binarized using the Phansalkar local threshold with a radius set at 4 pixels (*second row*). The flow deficits were identified with the "Analyze particles" tool and shadowed in *green* for graphical presentation (*third row*). (**B**) The choroidal vascularity index was calculated on enhanced-depth optical coherence tomography scan (*first row*). A 1500-µ-wide polygon centered on the foveal depression having as boundaries the retinal pigment epithelium (*upper boundary*), the sclerochoroidal junction (*lower boundary*), and two *vertical lines* perpendicular to the retinal pigment epithelium each at 750 µm from the foveal depression was used as region of interest (*second row*). Then, dark pixels in this area were highlighted (*blue* in the figure) and measured as choroidal luminal area (*third row*).

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total choroidal area (TCA), the luminal choroidal area (LCA), and the stromal choroidal area (SCA) were measured. Details on imaging processing and measurements are provided in the supplementary methods and Figure 1.

Statistical Analysis

Statistical calculations were conducted with the open-source programming language R. The cutoff point for statistical significance was set at $P < 0.05$. The BCVA was converted into logarithm of the minimum angle of resolution.

The primary outcome was to compare the choriocapillaris (FD number, mean FD size, total FD area, and FD density) and choroidal (subfoveal CT, total CT, CVI, LCA, SCA, and TCA) quantitative parameters in patients with AS with healthy controls with a focus on patients with AS with kidney failure requiring transplant.

The secondary outcome was to explore the demographic and clinical factors associated with the FD density and the CVI in patients with AS. For both descriptive and predictive statistics, only complete cases were used. Details on statistics are provided in the supplementary methods and Supplementary Figure S1.

Results

Forty-two eyes of 21 patients with AS and 33 eyes of 18 control participants were included; all patients could be categorized by inheritance pattern.

Nine male patients had X-linked disease (43%), 5 patients (24%, 3 males and 2 females) had AR disease, and 7 females (33%) were X-linked carriers. Genetic mutations were available for 16 participants (32 eyes, 76%); the detailed list of mutations in our cohort has been described in a sister publication by our group.[16] In summary, 9 participants had a missense mutation (18 eyes, 56%), 4 participants had a slice mutation (8 eyes, 25%), and 3 participants had a frameshift mutation (6 eyes, 19%).

Eleven patients (52%) had a history of kidney transplant at a mean age of 25 ± 11 years (range, 11–51); patients with a history of kidney transplant were slightly older with a marginally higher prevalence of males compared to the AS cohort who was never transplanted; these participants also had a higher prevalence of hearing loss, dot maculopathy, and anterior lenticonus. No patients had posterior lenticonus; all were phakic. A summary of our cohort's genetic, demographic, and clinical characteristics is presented in Table 1.

Choriocapillaris Analysis and Factors Associated with FD Density

Choriocapillaris FDs in patients with AS were more numerous compared to controls ($P = 0.02$) (Table 2, Fig. 2).

Patients with kidney transplant had a larger total FD area ($P = 0.01$), a larger FD mean size ($P = 0.02$), and a higher FD density ($P = 0.01$) compared to patients with AS who were never transplanted (Table 2, Fig. 3A, and Fig. 4). There was a significant inverse correlation between the FD number and the mean FD size ($r = -0.8$, $P < 0.001$); this negative relationship was also found when the log transformation of the FD number and mean FD size variables was used ($r = -0.9$, $P < 0.001$). The BCVA worsened as the FD density increased, but the association was not significant ($r = 0.1$, $P = 0.5$).

The FD density in eyes with AS increased with older age (estimate = 0.31% for each year, $P = 0.02$) and in patients with a history of kidney transplant (estimate = 9.66% in case of positive history, $P = 0.003$). Data suggested that patients carrying a splice mutation (estimate = 6.23% compared to those with missense mutation) and those carrying a frameshift mutation (estimate = 2.96% compared to those with missense mutation) had a higher load of FDs, but the associations were not significant ($P = 0.06$) (Table 3).

Choroidal Analysis and Factors Associated with Choroidal Vascularity

The qualitative inspection of the CT at different locations did not reveal regional disparities between patients with AS and controls (Fig. 3B); patients with AS with a history of kidney transplant had slightly thinner choroids compared to those who were never transplanted. Nevertheless, standard deviations were wide, and the difference was not statistically significant (Fig. 3C).

The subfoveal and total CT were similar to normal values ($P = 0.4$ and $P = 0.9$, respectively) and between AS patient groups (Table 2). The CVI, the LCA, the SCA, and the TCA also fell within normal limits, irrespective of history of kidney transplant (Table 2). The luminal area and the stromal area increased in parallel ($r = 0.88$, $P < 0.001$), and both correlated linearly with the total CT ($r = 0.9$, $P < 0.001$ for the LCA and $r = 0.88$, $P < 0.001$ for the SCA; Supplemen-

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Table 1. Demographic and Clinical Characteristics of Patients With AS, Divided by History of Kidney Transplant, and Healthy Controls

Characteristic	Controls (n =18)	Patients With AS (n = 21)	AS With History of Kidney Transplant (n = 11)	AS With No History of Kidney Transplant (n = 10)
Gender				
Male	9 (50)	12 (57)	7 (64)	5 (50)
Female	9 (50)	9 (43)	4 (36)	5 (50)
Age at inclusion, y				
Mean ± SD	40.3 ± 13.7	36.6 ± 12.9	38.8 ± 12.1	34.2 ± 13.5
Range	27–68	18–60	21–60	18–54
IQR	30–54	25–48	31–47.3	24–49
Pattern of inheritance				
X-linked	NA	9 (43)	5 (45)	4 (40)
X-linked carrier	NA	7 (33)	2 (19)	5 (50)
AR	NA	5 (24)	4 (36)	1 (10)
Type of mutation				
Missense	NA	9 (56)	5 (62)	4 (50)
Splice	NA	4 (25)	2 (25)	2 (25)
Frameshift	NA	3 (19)	1 (13)	2 (25)
Hearing impairment (% of patients)	NA	15 (71)	10 (91)	5 (50)
Ocular findings	n = 33 eyes	n = 42 eyes	n = 22 eyes	n = 20 eyes
Anterior lenticonus	NA	8 (19)	8 (100)	0 (0)
Dot maculopathy	NA	19 (45)	14 (64)	5 (25)
TTI				
Mean ± SD	5.73 ± 4.4	9.9 ± 5.3	10.4 ± 6.1	9.4 ± 4.1
Range	−7.2 to 11.2	−3.3 to 22.7	−3.3 to 22.7	5.3–19
IQR	−5.3 to 8.2	6.7–15	5.6–15	7–8.7
Refraction, diopters				
Mean ± SD	−2.4 ± 2.3	−1.5 ± 2.8	−1.9 ± 3.5	−0.9 ± 1.5
Range	−5.9 to 1.5	−9.1 to 3	−9.1 to 3	−3.6 to 1.8
IQR	−4 to −1.5	−2.4 to 0.4	−3.8 to 0.5	−1.83 to 0.3
BCVA, logMAR				
Mean ± SD	0.0 ± 0.01	0.13 ± 0.19	0.19 ± 022	0.07 ± 0.11
Range	0–0.10	0–0.70	0–0.70	0–0.30
IQR	0–0	0–0.22	0–0.38	0–0.13

Values are presented as number (%) unless otherwise indicated. Refraction was recorded as spherical equivalent. IQR, interquartile range; logMAR, logarithm of the minimum angle of resolution; NA, not assessed; SD, standard deviation; TTI, temporal thinning index.

tary Figs. S2A, S2B). Total CT negatively correlated with CVI values ($r = -0.39$, $P = 0.02$; Supplementary Fig. S2C). While the SCA was negatively related to CVI ($r = -0.6$, $P < 0.001$), the LCA did not ($r = -0.2$, $P = 0.3$; Figs. 3D, 3E).

The CVI was inversely correlated with the clinical presence of dot maculopathy (estimate = −3.30% if present, $P = 0.04$) and anterior lenticonus (estimate = −6.50% if present, $P = 0.006$; Table 3).

Discussion

This study investigated the choriocapillaris and the choroidal features of a large cohort of patients with AS. We found that patients with AS had more numerous choriocapillaris FDs compared to controls. Among eyes with AS, the degree of choriocapillaris involvement was heterogeneous, while the large choroidal

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Table 2. Choriocapillaris and Choroidal Parameters in Patients With AS and Healthy Controls

Characteristic	Controls ($n = 33$ Eyes)	Patients With AS ($n = 42$ Eyes)	AS With History of Kidney Transplant ($n = 22$ Eyes)	AS With No History of Kidney Transplant ($n = 20$ Eyes)	*P* Values of Statistical Comparisons[a]: Controls vs. Patients With AS	*P* Values of Statistical Comparisons[a]: AS With History of Kidney Transplant vs. AS With No History of Kidney Transplant
Choriocapillaris features						
FD number					**0.02**[b]	0.3
Mean ± SD	4165 ± 764	5161 ± 1759	4707 ± 2280	5642 ± 755		
Range	2584–5172	2086–10,032	2086–10,032	3732–6632		
IQR	3625–4897	3578–6257	3260–6079	5262–6228		
Total FD area, mm^2					0.3	***0.01***[b]
Mean ± SD	0.95 ± 0.19	0.88 ± 0.30	1.04 ± 0.32	0.71 ± 0.20		
Range	0.62–1.32	0.33–1.74	0.59–1.74	0.33–1.24		
IQR	0.82–1.09	0.63–1.11	0.79–1.19	0.61–1.78		
Mean FD size, μm^2					0.4	***0.02***[b]
Mean ± SD	245 ± 102	216 ± 170	296 ± 201	132 ± 62		
Range	126–512	57–743	73–743	57–333		
IQR	166–302	99–310	127–386	99–136		
FD density, %					0.3	***0.01***[b]
Mean ± SD	30.9 ± 6.2	28.5 ± 10.2	33.8 ± 10.3	23 ± 6.5		
Range	20.3–43.1	10.8–56.7	19.3–56.7	10.8–40.4		
IQR	26.8–36.6	20.6–36	25.6–38.6	19.9–25.5		
Choroidal thickness						
Subfoveal CT, µm					0.4	0.2
Mean ± SD	337.3 ± 96.3	350.8 ± 108.2	327.7 ± 100	378.9 ± 113.9		
Range	171–514	132–573	132–572	211–573		
IQR	265.8–417.5	287.8–406.8	287.2–369.5	302.8–461.2		
Total CT, µm					0.9	0.2
Mean ± SD	334.2 ± 89.1	343.3 ± 105.2	316.8 ± 93.9	373.9 ± 112.8		
Range	171–502.4	131.2–562.2	131.2–515	206.4–562.2		
IQR	280.9–389.7	276.2–399.1	260–374.6	301.6–454.3		
Choroidal components area						
LCA, mm^2					0.9	0.3
Mean ± SD	1.37 ± 0.40	1.38 ± 0.38	1.31 ± 0.41	1.47 ± 0.33		
Range	0.73–2.13	0.59–2.17	0.59–2.13	0.98–2.17		
IQR	1.14–1.49	1.16–1.54	1.16–1.45	1.29–1.61		
SCA, mm^2					0.7	0.8
Mean ± SD	0.74 ± 0.30	0.69 ± 0.26	0.68 ± 0.25	0.71 ± 0.27		
Range	0.27–1.45	0.32–1.35	0.32–1.35	0.34–1.34		
IQR	0.57–0.79	0.54–0.76	0.54–0.72	0.54–0.81		
TCA, mm^2					0.7	0.5
Mean ± SD	2.12 ± 0.69	2.08 ± 0.62	1.99 ± 0.63	2.18 ± 0.60		
Range	0.10–3.52	0.92–3.51	0.92–3.48	1.33–3.51		
IQR	1.71–2.20	1.67–2.31	1.66–2.29	1.90–2.41		
CVI					0.07	0.2
Mean ± SD	65.6 ± 4.1	67 ± 3.9	66 ± 3.9	68.3 ± 3.7		
Range	58.9–72.8	58.6–74.2	58.6–72.3	61.8–74.2		
IQR	62.6–69.6	63.6–70.6	63.1–68.8	65.5–71		

Patients with AS were divided according to their history of kidney transplant.
[a]All *P* values were adjusted for multiple comparisons.
[b]Statistically significant value ($P < 0.05$).

vessels appeared relatively preserved. We identified demographic and clinical factors, such as a history of kidney transplant and the presence of dot maculopathy and anterior lenticonus, as negatively associated with choriocapillaris and choroidal vascularity, respectively.

Type IV collagen is an essential component of basement membranes in various regions of the body.

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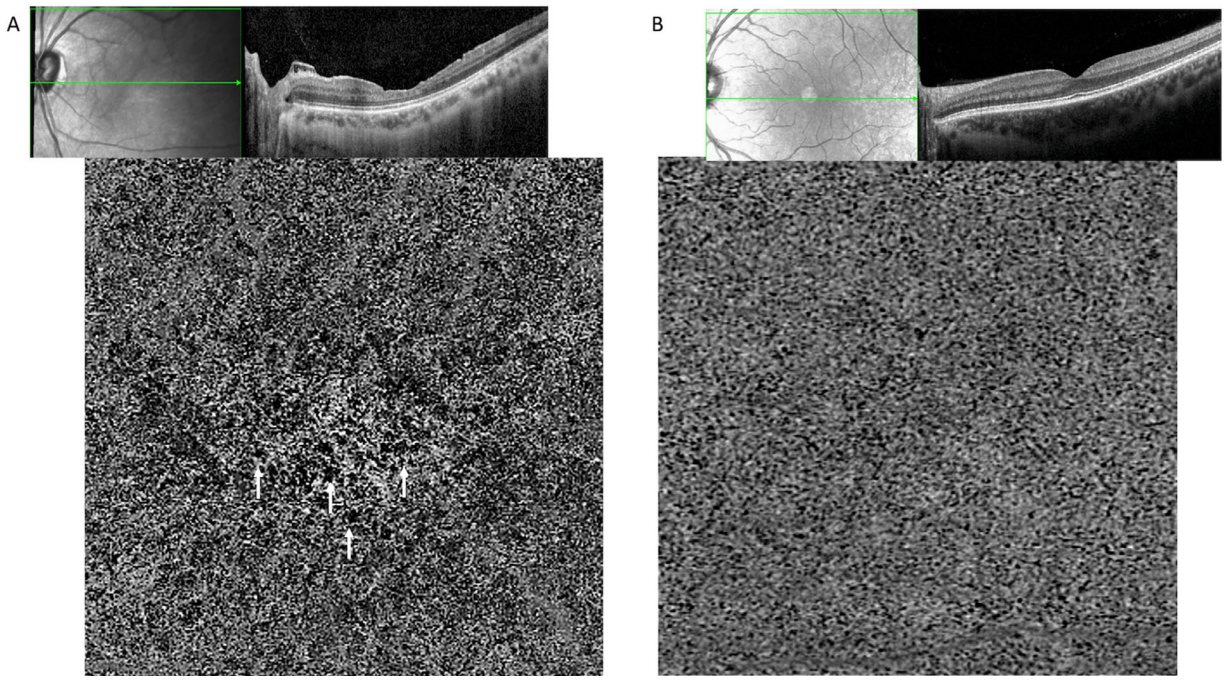

Figure 2. Choriocapillaris slab in a patient with AS and a healthy participant imaged with optical OCTA. (**A**) The patients with AS had a history of kidney transplant; the OCTA slab (3 × 3 mm) shows a high number of flow deficits (*black areas* as indicated by the *white arrows*). The structural OCT scan shows staircase maculopathy with temporal thinning. (**B**) The OCTA slab in the healthy control shows a considerably lower count of flow deficits, while the structural OCT reveals a normal foveal contour.

Mutations in the collagen IV gene severely affect their structure and stability. Ultrastructural analysis of the inner ear in patients with AS showed separation between the basilar membrane and the overlying basement membrane of the organ of Corti, causing progressive sensorineural hearing loss.[17] The persistence of immature isoforms of collagen IV in the glomerular basement membrane causes diffuse thickening and splitting of the lamina densa, leading to hematuria, proteinuria, and kidney failure early in life.[18] The absence of the collagen IV network in the crystalline lens leads to thinning of the capsule and dehiscence of the lens epithelium, clinically presenting as anterior or posterior lenticonus.[19] In the retina, the lack of the collagen IV may cause abnormal development of the macula and its vascularization; structural alterations of the foveal contour and changes in the shape and circularity of the foveal avascular zone have been recently described using OCTA.[16,20]

The α3(IV)–α4(IV)–α5(IV) collagen IV heterotrimer has been found within all five layers of Bruch's membrane (BM), namely, the RPE–basal lamina complex, the inner collagenous layer, the middle elastic layer, the outer collagenous layer, and the choriocapillaris basement membrane, with intense homogeneous immunofluorescence staining.[21,22] Collagen IV provides tensile strength and elasticity to the BM.[23] The BM has structural similarities to the glomerular basement membrane of the kidney, and an association between BM impairment and kidney disease has been described not only in AS[24] but also in other retinal affections, such as age-related macular degeneration with reticular macular disease.[25]

Functionally, the BM is involved in filtration and transport of fluid and macromolecules, ensuring photoreceptor and RPE survival. BM also provides anchorage to the choriocapillaris via the collagenous protrusions of the choriocapillaris basement membrane. A previous case report used noninvasive OCTA imaging to demonstrate disorganization of the choriocapillaris network in a patient with AS.[9] We compared the choriocapillaris perfusion metrics with healthy participants, and we found that patients with AS had significant alterations. We suspect choriocapillaris damage could result from primary BM degeneration and damage to the collagenous anchorage system in AS. Nevertheless, given the relatively low resolution of currently available imaging devices, our understanding of BM pathology in vivo is still limited; our hypothesis needs to be confirmed by ex vivo correlations.

Previous reports have found clinical associations between posterior segment findings, such as dot maculopathy or temporal retinal thinning, and early onset renal failure.[26,27] In our study, patients with AS requiring kidney transplant had increased area and density of choriocapillaris FDs compared to

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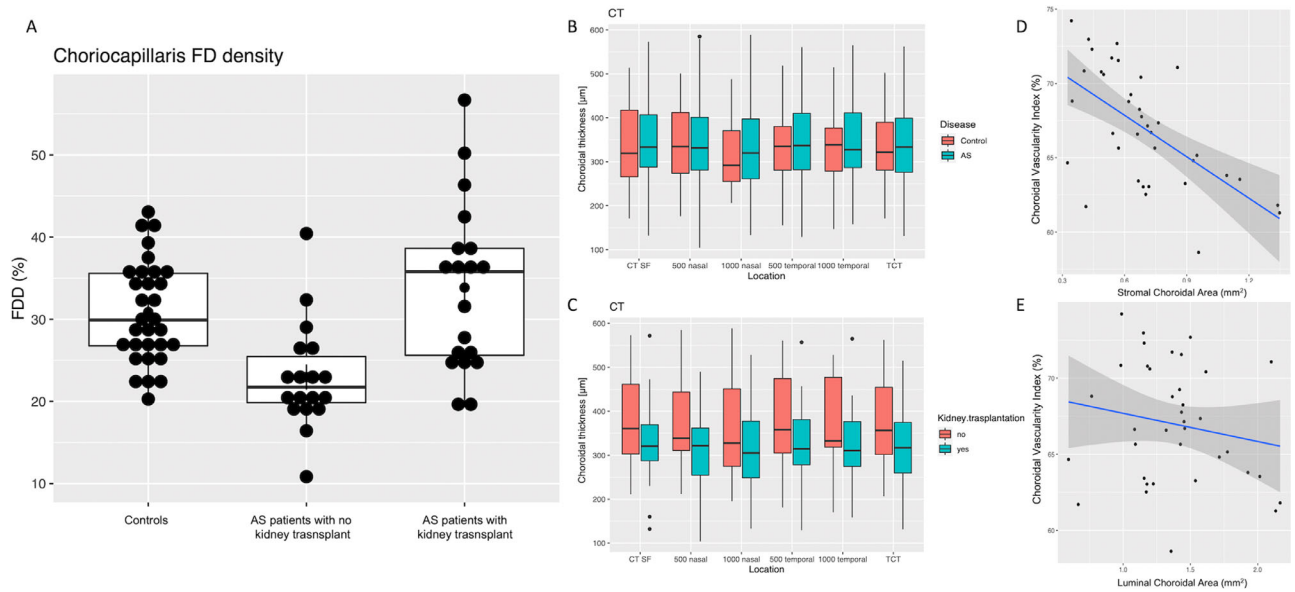

Figure 3. Choriocapillaris features, CT, and choroidal components in patients with AS, stratified by their history of kidney transplant (KT) versus healthy controls. (**A**) Boxplots comparing the choriocapillaris flow deficit density (FDD) between controls and patients with AS. (**B**) Boxplots comparing the choroidal thickness between controls and patients with AS. Locations refer to microns from the fovea. SF, subfoveal; TCT, total choroidal thickness. (**C**) Boxplots comparing the choroidal thickness between patients with AS with kidney transplant and patients with AS who had not had a kidney transplant. Locations refer to microns from the fovea. (**D**) Linear regression showing a high degree of correlation between the stromal choroidal area and the choroidal vascularity index. (**E**) Linear regression showing an absence of correlation between the luminal choroidal area and the choroidal vascularity index.

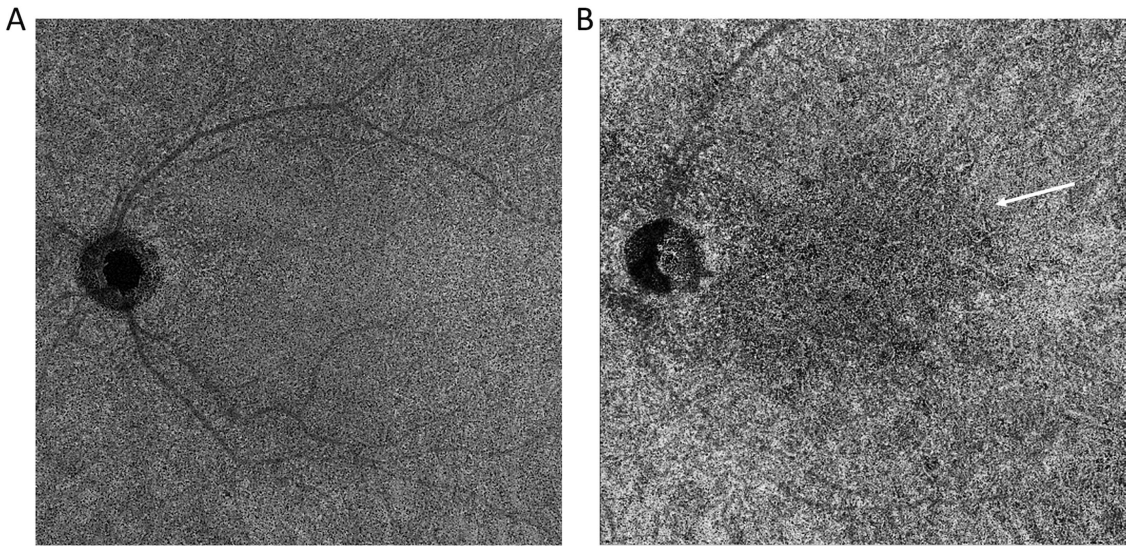

Figure 4. Widefield optical coherence tomography angiography of a healthy control and a patient with AS. (**A**) In the 12-mm × 12-mm slab, the choriocapillaris is seen as a homogeneous layer within the vascular arcades in a healthy participant. (**B**) The choriocapillaris shows a high density of flow deficits at the posterior pole in the patient with AS (*white arrow*).

patients who were never transplanted. The association between choriocapillaris impairment and kidney failure in patients with AS is intriguing and was significant even after correcting for age and other possible clinical confounders. It may imply a true association between glomerular basement membrane damage and choriocapillaris disruption. On the other hand, it may be spurious due to associated comorbidities in patients undergoing a kidney transplant, particularly hypertension and chronic use of medications (e.g., corticosteroids). A reduction of choriocapillaris perfusion has been indeed associated with

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Table 3. Association of Choriocapillaris FD Density and CVI With Clinical and Demographic Variables of Patients With AS

Variable	Estimate (SE)	95% CI	*P* Value
FD density			
Age (for each year)	0.31 (0.12)	0.06 to 0.57	***0.02***[a]
Female gender (reference: male)	3.35 (3.29)	−3.37 to 10.08	0.3
History of kidney transplant (reference: no)	9.66 (3.00)	3.52 to 15.80	***0.003***[a]
Splice mutation (reference: missense)	6.23 (3.25)	−0.45 to 12.90	0.06
Frameshift mutation (reference: missense)	2.96 (4.06)	−5.37 to 11.28	
Marginal $R^2 = 54\%$			
Conditional $R^2 = 91\%$			
CVI			
Age (for each year)	−0.05 (0.06)	−0.17 to 0.08	0.2
Splice mutation (reference: missense)	−0.75 (1.75)	−4.42 to 2.92	0.7
Frameshift mutation (reference: missense)	1.08 (2.24)	−3.70 to 5.85	0.6
Refraction (for each diopter)	−0.65 (0.34)	−1.34 to 0.04	0.07
Dot maculopathy (reference: no)	−3.30 (1.50)	−6.38 to −0.21	***0.04***[a]
Anterior lenticonus (reference: no)	−6.50 (2.19)	−10.99 to −2.00	***0.006***[a]
Marginal $R^2 = 43\%$			
Conditional $R^2 = 87\%$			

A separate multivariable model was run for each outcome; the variables included in the model were selected with a least absolute shrinkage and selection operator regression approach (see supplementary methods section). Estimates, standard error (SE), and 95% confidence intervals (CIs) are shown.

CMT, central macular thickness.

[a]Statistically significant value ($P < 0.05$).

systemic hypertension[28] and amyloidosis[29] complicated by kidney failure, and it has also been described in patients undergoing hemodialysis.[30] Given the cross-sectional nature of the study, we could not assess the temporal association between choriocapillaris depletion and kidney impairment to determine whether the choriocapillaris FDs precede or follow kidney failure. Similarly, we did not investigate other biomarkers of kidney function (such as glomerular filtration rate, serum creatinine, or creatinine clearance). Further studies are needed to clarify this point.

Consistent with the previous literature on healthy and diseased eyes, we found a close association between age and choriocapillaris FD density.[31] Areas devoid of capillary lumen within choriocapillaris lobules have been labeled "ghost vessels" in aging eyes. OCTA confirmed the age-dependent distribution of choriocapillaris FDs, more evident in the central macula.[32,33] Choriocapillaris FDs have been correlated with worse visual acuity[34] and impaired photoreceptor function.[35] Our data suggest that denser FDs were associated with worse BCVA, but our results were confounded by most eyes having high visual acuity. Indeed, only 10 eyes had a BCVA of 20/25 or worse, and statistical analysis with such a small sample was likely underpowered.

The CVI is a robust quantitative parameter of choroidal vascularity in posterior segment diseases. Normal CVI ranges between 50% and 70%,[11,36] and patients with AS fell within this interval. We found that both the luminal and the stromal components of the choroid correlated with the choroidal thickness. However, while the LCA was relatively constant in AS eyes, falling within a small range, the stromal component had higher variability, influencing the CVI values. This may be consistent with the preferential localization of the normal α1(IV) and α2(IV) subunits in the choroidal blood vessels[22] and the affected α3–α5(IV) subunits in the choroidal stroma.[21] In patients with AS, the CVI and the CT were negatively related, as previously found in eyes with reticular pseudodrusen[37] and polypoidal choroidal vasculopathy.[38] This association might seem counterintuitive. However, looking at the graphical distribution of the CVI, LCA, and SCA variables, we understand that the relative increase in the SCA with increasing choroidal thickness, rather than a genuine depletion of the luminal component, is most likely the reason for the progressive reduction of the CVI in eyes with thicker choroids. Lower CVI values were found in eyes with dot maculopathy and anterior lenticonus; these eyes had a marginally higher stromal

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component area (0.75 vs. 0.64 mm^2 for dot maculopathy and 0.77 vs. 0.67 mm^2 for anterior lenticonus) than eyes with preserved macular reflex and normal lens, respectively. It is possible that the relative ratio between the luminal and the stromal choroidal components is altered in patients featuring ocular abnormalities. Further studies are needed to understand whether an abnormal distribution of the choroidal components is a typical feature of AS eyes.

Our study has limitations. We were unable to evaluate the changes of the choriocapillaris and choroidal parameters with time. We did not stratify patients according to their systemic comorbidities, such as hypertension or renal function, or account for systemic treatments potentially affecting the choriocapillaris and the choroidal vascularity.[39] We found a negative association between the number of FDs and their mean size; this may suggest that FDs coalesce with increasing size.[31] Nevertheless, choriocapillaris measurements are still not easy to achieve, and a standardized, widely accepted approach has not been yet adopted. Our data showed a possible negative correlation between the CVI and the refractive error in eyes with AS. It should be noted the refractive error in patients with AS does not necessarily correspond to axial length, as other factors (such as the anterior and posterior corneal surface and crystalline lens) may significantly affect the final refractive error.[40] A more detailed analysis, including axial length measurements, is warranted to confirm our results. Finally, the variables presented in this study explained 54% and 43% of the FD density and the CVI variability in our cohort, respectively. We hypothesize other factors not investigated in this setting may have a role in determining the choriocapillaris and choroidal vascularity in patients with AS.

In conclusion, our study reports quantitative abnormalities of the choroid in a large cohort of patients with AS. We found that choriocapillaris damage in AS worsened with age and was associated with renal failure requiring transplant. Patients with dot maculopathy and anterior lenticonus had lower choroidal vascularity. Longitudinal investigations are necessary to evaluate the prognostic implication of these findings and the role of these noninvasive modalities in the assessment of progression in this rare disease.

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