Characterization of microvascular abnormalities using OCT angiography in patients with biallelic variants in *USH2A* and *MYO7A*

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SYNOPSIS/PRECIS

OCT angiography detected decreased capillary density in retinal plexuses of *USH2A* and *MYO7A* patients. Choriocapillaris defects were detected in *MYO7A* but not *USH2A*. Decreased vessel density was associated with abnormal macular sensitivity and ellipsoid zone loss.

ABSTRACT

Aims: Using optical coherence tomography angiography (OCTA) to characterize microvascular changes in the retinal plexuses and choriocapillaris (CC) of *MYO7A* and *USH2A* patients, and correlate with genotype, retinal structure and function.

Methods: Twenty-seven molecularly-confirmed *USH2A* (n=21) and *MYO7A* (n=6) patients underwent macular 6x6mm OCTA using the AngioVue. Heidelberg spectral-domain OCT scans and MAIA microperimetry were also performed, the preserved ellipsoid zone (EZ) band width and mean macular sensitivity were recorded. OCTA of the inner retina, superficial capillary plexus (SCP), deep capillary plexus (DCP), and CC were analysed. Vessel density (VD) was calculated from the *en face* OCT angiograms of retinal circulation.

Results: Forty eight eyes with either *USH2A* (n=37, mean age: 34.4±12.2 years) or *MYO7A* (n=11, mean age: 37.1±12.4 years), and 35 eyes from 18 Age-matched healthy participants were included. VD was significantly decreased in the retinal circulation of *USH2A* and *MYO7A* patients compared to controls (p<0.001). Changes were observed in both the SCP and DCP, but no differences in retinal perfusion were detected between *USH2A* and *MYO7A* groups. No vascular defects were detected in CC of the *USH2A* group, but peripheral defects were detected in older *MYO7A* patients from the fourth decade of life. Vessel density in the DCP showed strong association with macular sensitivity and EZ width (Spearman's *rho*=0.64 and 0.59, respectively, p<0.001).

Conclusion: OCTA was able to detect similar retinal microvascular changes in *USH2A* and *MYO7A* patients. The CC was generally affected in *MYO7A* patients. OCT angiography may further enhance our understanding of inherited eye diseases, and their phenotype-genotype associations.

INTRODUCTION

Usher syndrome (USH) is an autosomal recessive disorder characterized by congenital sensorineural hearing loss and retinitis pigmentosa, with or without vestibular dysfunction.[1] USH has an estimated prevalence of ~1:12,000-28,000, and is thought to be responsible for more than 50% of patients with combined deaf-blindness.[2-4] Retinitis pigmentosa (RP) is a progressive pigmentary degeneration of rod and cone photoreceptors leading to sight loss.[5] Patients commonly present with nyctalopia, then constriction of peripheral vision, and eventually loss of central vision in the late stages of the disease. Fundus examination classically reveals the triad of pigmentary changes (bone spicules) at the midperiphery, waxy pale optic disc, and generalized arteriolar attenuation.

Usher syndrome has been classically divided into three clinical subtypes, based on age of onset, severity, and vestibular features.[6 7] Usher type 1 (USH1) is the most severe form of the disease with profound hearing and vestibular function loss, with RP presenting during childhood. Usher type 2 (USH2) is the most common type, which is characterized by partial hearing loss and presentation of RP in adolescence/early adulthood, with intact vestibular function. Hearing loss tends to be non-progressive in patients with USH1 and USH2.[6] In contrast, type 3 Usher syndrome (USH3) exhibits slowly progressive hearing loss and variable degree of vestibular dysfunction with RP.[7]

To date, USH has been associated with at least 19 loci, with 16 causative genes identified.[8] Mutations in *MYO7A* and *USH2A* genes are the most common causes of USH, responsible for more than 50% of USH1 and 70% of USH2, respectively.[9-13] *MYO7A* and *USH2A* genes encode the proteins myosin VIIa and usherin, respectively. Both proteins are essential for the structural and functional integrity of the cochlear hair cells of the inner ear and photoreceptors in the retina.[14 15] Myosin VIIa is further expressed in the retinal pigment epithelium (RPE),[16] as well as in the vestibular utricule and macula.[17] Mutations in *USH2A* have also been associated with non-syndromic autosomal recessive RP,[18] whereas *MYO7A* have been associated with non-syndromic hearing loss.[19]

Several therapeutic approaches have been introduced for the treatment of USH,[20] including gene augmentation therapy,[21] nonsense suppression therapy,[22] as well as ciliary neurotrophic factor (CNTF)-releasing cell therapy.[23] However, reliable and objective measurements are needed to assess disease progression and establish outcomes parameters of future clinical interventions. Optical coherence tomography angiography (OCTA)[24] has recently been introduced as a non-invasive functional extension of OCT technology that has the ability to provide depth-resolved visualization and quantification of retinal and choroidal microvasculature.[25 26] OCTA was used to investigate individual retinal plexuses, as well as choriocapillaris (CC) in patients with RP.[27-30] A decreased vascular density was generally observed in the superficial capillary plexus (SCP), deep capillary plexus (DCP), and CC of RP patients. Retinal perfusion has been reported to be correlated with visual function.[31 32] However, these

studies included either a molecularly unconfirmed or genotypically- heterogeneous cohort of patients. Given the known variation between degeneration rate and genotype, this heterogeneity inhibits the evaluation of vascular change as a metric of disease.

In this study, we aim to characterize vascular changes in the superficial and deep retinal capillary plexuses, as well as choroidal circulation in a cohort of patients with mutations in *MYO7A* and *USH2A* genes. We will also investigate the relationship between vascular, structural, and functional changes in these patients.

MATERIALS AND METHODS

Study Population

Twenty-seven patients with confirmed biallelic variants in *USH2A* (n=21) and *MYO7A* (n=6) were recruited from Moorfields Eye Hospital between May 2017 and December 2018. Eighteen age-matched healthy participants were also included in this prospective observational study. The study was approved by the national research ethics committee and all participants gave informed consent to participate. All study procedures adhered to the tenets of the Declaration of Helsinki. Exclusion criteria for patients included the presence of any retinal disease other than RP, or the inability to obtain reliable OCTA scans. Healthy control participants were selected based on (i) lack of any anatomically-modifying retinal disease such as glaucoma, diabetic retinopathy, and/or retinal detachment, (ii) ability to obtain OCTA scans, (iii) LogMAR best corrected visual acuity (BCVA) ≤0.3, and (iv) no history of major eye trauma or surgery. Detailed medical history was taken from patients, and comprehensive ophthalmological examination was performed including LogMAR BCVA and slit lamp examination. Both eyes from each participant were included.

OCT and OCTA Acquisition

High resolution OCT line scans were acquired from patients using Spectralis HRA+ OCT machine (Heidelberg Engineering, Inc., Heidelberg, Germany). The scanning protocol acquired 20°x20° (~6.3 mm) volumetric scans (193 B-scans, each consisting of 1024 A-scans). Image volumes were inspected to ensure the horizontal foveal scan was acquired.

All participants underwent OCTA scanning using a commercially available spectral-domain OCT system (Avanti RTVue XR machine, OptoVue, Inc., Fremont, CA, USA). This system has an acquisition speed of 70k A-scans/second, macular 6x6 mm scans were acquired. Each data set was created by orthogonal registration and merging of 1 x-fast and 1 y-fast scans. Blood flow was detected using a commercial version of split-spectrum amplitude-decorrelation angiography (SSADA) algorithm.[24] Scans collected were either the traditional 304x304 scans or the high density (HD) 400x400 scans.

Microperimetry

Patients were tested for macular sensitivity using Macular Integrity Assessment (MAIA, CenterVue SpA, Padova, Italy). The default (Expert Exam) mesopic protocol was used. The stimulus was Goldmann size III, with presentation time of 200 ms. Sensitivity measures were obtained by 4-2 strategy at 37 points distributed in 3 concentric circles covering an area of 10 degrees in diameter (Figure 1). Mean macular sensitivity (MS) was calculated from the measured sensitivities at the 37 points using the on instrument software.

All microperimetry results underwent a fixation-based quality-control check to ensure reliability. Examinations with high fixation loss rate (>30%) were excluded. Macular Integrity Index is a numerical value indicating the likelihood of a statistically significant alteration in macular sensitivity when compared with age-matched normal values. Mean sensitivity and macular sensitivity values and maps were collected and qualitatively assessed for co-localization with OCT and OCTA values.

Data Processing

Spectralis structural OCT images were viewed and analysed using the Heidelberg Eye Explorer (HEYEX) software. The B-scan passing through the center of the fovea was selected, and an expert grader manually identified the extension of ellipsoid zone (EZ) band from the cross-sectional OCT image, and the width of the preserved EZ was measured.[33]

OCT angiography scans were checked for image quality and segmentation using the ReVue software (OptoVue, Inc., Fremont, CA, USA). Low quality scans (Q less than 5/10, significant motion artifacts, defocused, or non-centered) were excluded from analysis. Due to the significant pathological changes in USH retinas, automated segmentation failed frequently and manual correction was needed. The retina was segmented at the inner limiting membrane (ILM), inner plexiform layer (IPL), and Bruch's membrane (BM). As the segmentation of the outer boundary of outer plexiform layer (OPL) was technically difficult due to the extensive retinal degeneration and poor visualization, a line 25 µm above the segmented BM (BM-25 µm) was used as an outer boundary of the inner retinal slab. *En face* OCT angiograms were generated by maximum projection of flow signal at specific slabs (Figure 1). The inner retinal slab was defined between ILM and 25 µm above BM. The retinal circulation was then subdivided into superficial and deep capillary plexuses. The SCP lies between the ILM and 9 µm above the IPL (IPL-9 µm). The DCP was detected between IPL-9 um and BM-25 µm. Choriocapillaris (CC) slab was defined between BM-9 µm and BM+31 µm.

En face angiograms were then exported using the "Export Angio" tool in ReVue software into PNG files for analysis using custom Matlab and ImageJ routines. Due to retinal degeneration, larger vessels from the superficial layers were partially displaced and appeared in the DCP slab, potentially interfering with accurate quantification of flow signal. Thus, areas occupied with large vessels were identified and excluded from the

analysis of DCP angiograms. Vessel density (VD) was measured from threshold *en face* angiograms within a 6-mm diameter annular region outside the manually-centered 0.6 mm-diameter foveal avascular zone (FAZ) (Figure 1). VD was defined as the percentage of the area occupied by pixels containing flow signal. FAZ was excluded from analysis to avoid the normal variability of FAZ area between subjects.

Statistical Analysis

All statistical analyses were performed on SPSS v. 25.0 (IBM Corporation, Armonk, NY, USA) and Microsoft Excel 2017 (Microsoft Office, Microsoft Corporation, Redmond, WA, USA). Vessel density, width of the preserved EZ, and mean macular sensitivity were presented as mean and standard deviation (SD) of the included participants in each group. Age-matching between groups was assessed using analysis of variance (ANOVA) test. Generalized estimating equations (GEE) was used to investigate the differences between groups. GEE tests account for the within-subject correlation between both eyes. *P* values were adjusted for multiple comparisons using Holm-Bonferroni correction. Spearman's rank correlation coefficient (*rho*) was used to assess the correlation between VD, EZ, and MS.

RESULTS

Participant Characteristics, Clinical Data, and Genetic Findings

Forty eight eyes from 27 patients (mean age ± SD, 35.0 ± 12.1 years) with bialleleic variants in either *MYO7A* or *USH2A* genes were included (see supplemental Table 1 for details of mutations). Thirty five eyes from 18 healthy participants (mean age ± SD, 34.0 ± 10.4 years) were included. The majority of patients had clinical manifestations of USH1 or USH2, with the exception of 3 *USH2A* patients who had non-syndromic autosomal recessive RP (ARRP) with no associated hearing loss (see Table 1). Patients and control groups were age-matched (p = 0.25, ANOVA). No statistically significant difference in central vision was found between USH2A and *MYO7A* patients (mean LogMAR ± SD, 0.31 ± 0.18 and 0.19 ± 0.23, respectively, p = 0.26).

TABLE 1. Characteristics of enrolled participants

	Control	*USH2A*	*MYO7A*
No. of participants	18	21	6
Gender			
Male	11	11	2
Female	7	10	4
Phenotype	N/A		

USH1	N/A	N/A	6
USH2	N/A	18	N/A
ARRP	N/A	3	N/A
No. of eyes	35	37	11
Age	34.0 ± 10.4	34.4 ± 12.2	37.1 ± 12.4
Range	23-61 years	19-56 years	18-52 years
LogMAR	N/A	0.19 ± 0.23	0.31 ± 0.18
Axial Length (mm)	N/A	23.69 ± 1.40	23.53 ± 0.91

Age, LogMAR and axial length are given as mean ± standard deviation. USH1 and USH2: Usher syndrome type 1 and 2, respectively, ARRP: autosomal recessive retinitis pigmentosa, LogMAR: logarithm of the minimum angle of resolution, N/A: not applicable

OCT Angiography, Structural OCT, and Microperimetry

Qualitative evaluation of OCT angiograms of retinal circulation revealed decreased capillary density in the inner retina, SCP, and DCP of USH patients, compared to controls (Figure 1). The decreased perfusion was more pronounced at the peripheral regions of the angiograms, with relatively preserved vascularity at central regions. Quantitatively, statistically significant reductions in vessel density were observed in patients compared to controls in all retinal plexuses ($p < 0.001$, GEE) (Table 2 and Figure 2). However, no significant differences were observed in retinal angiograms between *MYO7A* and *USH2A* groups (Table 2). In contrast, CC seemed to behave differently in *USH2A* and *MYO7A* patients. Patients with *USH2A* mutations had relatively intact CC layer throughout different age groups (19-55 years) (Figure 3, B1-3), except one USH2 patient (56 years old) who had marked retinal degeneration and RPE loss which associated with localized loss of CC. On the other hand, CC angiograms of *MYO7A* patients showed microvascular changes starting from third or fourth decade of life (Figure 3). Areas of lost CC was observed at the periphery of the 6 mm angiogram, exposing the deeper and less dense large choroidal vessel layers (Figure 3, white arrows in C2 and C3). A ring of increased flow signal was detected around the intact CC island, at the transitional area between preserved and lost CC (Figure 3, yellow arrow heads in C2 and C3).

Cross-sectional structural OCT images of USH patients revealed photoreceptor loss, evident by the extensive degeneration of EZ and outer nuclear layer (ONL) at the periphery of the scanned retina (Figure 1). USH patients showed an average of 1442 ± 1163 um of preserved EZ band at the horizontal B-scan at the center of the fovea. No significant difference was observed in the mean EZ between *MYO7A* and *USH2A* patients.

The microperimetry detected decreased mesopic macular sensitivity (mean ± SD, 15.6 ± 8.2 dB) in all patients, especially at the peripheral areas (Figure 1), with all patients identified as having abnormal Macular Integrity index, except for one patient who had "suspect" integrity. *MYO7A* patients had a slightly lower mean macular sensitivity than *USH2A* patients; however, the differences were found not to be statistically significant ($p = 0.66$, GEE).

Correlation between Vascular, Structural, and Functional Parameters

Areas of degenerated retina on structural OCT spatially co-localized with areas of hypoperfusion on OCTA and decreased sensitivity on the retinal sensitivity map (Figure 1). Vessel density of retinal plexuses significantly correlated with mean sensitivity and the width of the preserved EZ (Figure 2). The deep plexus showed slightly stronger associations with OCT (Spearman's *rho* = 0.59, $p < 0.001$) and microperimetry (*rho* = 0.64, $p < 0.001$) than the superficial plexus.

TABLE 2. Vessel density, EZ width and retinal sensitivity measurements

		Control	*USH2A*	*MYO7A*	Control vs. *USH2A*		Control vs. *MYO7A*		*USH2A* vs. *MYO7A*	
					% change	*P* value	% change	*P* value	% change	*P* value
OCT Angiography	**Inner (%)**	75.51 ± 3.09	51.51 ± 7.77	47.91 ± 5.99	-31.78%	**< 0.001**	-36.54%	**< 0.001**	-6.98%	0.58
	SCP (%)	61.10 ± 4.74	48.37 ± 7.26	46.30 ± 4.51	-20.84%	**< 0.001**	-24.22%	**< 0.001**	-4.27%	0.43
	DVC (%)	27.67 ± 4.06	13.79 ± 4.97	12.11 ± 4.88	-50.16%	**< 0.001**	-56.21%	**< 0.001**	-12.15%	0.55
Structural OCT	**EZ Width (µm)**	NA	2169 ± 1454	2341 ± 1755					7.94%	0.70
MAIA microperimetry	**Mean Sensitivity (dB)**	NA	16.1 ± 8.2	12.1 ± 4.9					-13.57%	0.31

P values were based on Generalized Estimating Equation (GEE, accounting for the inter-ocular subject correlation), and adjusted for multiple comparisons using Holm-Bonferroni method. SCP: superficial capillary plexus, DCP: deep capillary plexus, EZ: ellipsoid zone, MAIA: Macular Integrity Assessment microperimetry, NA: not applicable

DISCUSSION

Arteriolar attenuation is a classic sign in Usher-related retinitis pigmentosa. In our cohort of *MYO7A* and *USH2A* patients, OCTA was able to detect decreased perfusion in retinal plexuses, especially in the more peripheral areas, with a relatively preserved central macula. The deep plexus was found to be more affected than the superficial layer. Although the choriocapillaris in *USH2A* patients appeared to be relatively preserved until late stages of the disease, in *MYO7A* there was marked early loss of this layer compared

to healthy controls. Outer retinal degeneration and decreased macular sensitivity were also detected, which significantly correlated with the measured vessel density in the retinal OCT angiograms. Although, *MYO7A* patients had significantly worse VA than *USH2A* patients, no significant differences were observed in OCT, OCTA, or microperimetry parameters.

RP is characterized by progressive degeneration of rod photoreceptors, which typically begins at the mid-periphery, sparing the cones at the centre of the fovea until late stages of the disease. This explains the peripheral loss of photoreceptor bands (ONL, external limiting membrane, and EZ) on cross-sectional OCT, with a relatively preserved island of retinal tissue at the center. The death of photoreceptors can also explain the decreased macular sensitivity measured by mircoperimetry. Vascular changes in retinal circulation would then be expected secondary to the decreased metabolic needs in the degenerated regions. Decreased perfusion was observed in the OCTA of the unsegmented retinal slab, as well as the superficial and deep capillary plexuses.

Based on published experiments on oxygen metabolism in the retina, the deep and to lesser extent, intermediate retinal capillary plexuses were suggested to be partially responsible for supplying photoreceptors with oxygen.[34] Previous work has not suggested a linkage between the superficial plexus and photoreceptor metabolism.[35] Thus, we would expect the deep and intermediate capillary plexuses to be affected in USH, but not the superficial plexus.[28] However, the commercial OCTA machines follow the 2-layer segmentation scheme, which divides the retinal circulation into two plexuses; superficial and deep, incorporating most of the intermediate capillary plexus with the superficial slab. Therefore, changes were detected in both the superficial and deep plexuses. Additionally, the extensive thinning of the outer retina would allow higher oxygen diffusion to retinal layers, resulting in reflex autoregulatory constriction of retinal vessels and reduction in the measured vessel density.

Quantification of CC by OCTA is inherently technically-challenging due the effect of shadowing, projection, and motion artifacts.[36] Therefore, CC slabs were assessed qualitatively for the purpose of this study. Unlike the retinal circulation, the choriocapillaris was different in the two USH groups with the CC in *MYO7A* patients affected earlier and more severely than the *USH2A* group. Choroidal blood flow has been long considered to be only minimally regulated by oxygen levels.[37] Thus, changes in metabolic demand secondary to neural retinal degeneration would not be expected to affect choroidal blood flow. The retinal pigment epithelium (RPE) is essential for the development and maintenance of the CC integrity via RPE-specific factors such as vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF).[38 39] The usherin protein, encoded by the *USH2A gene*, has been found to be expressed specifically in the inner segments of the photoreceptors but not the RPE.[15] Thus, in *USH2A* patients, prior to photoreceptor degeneration, the structure of the RPE is initially intact, maintaining the viability of CC layer. In contrast, the myosin VIIa protein is mainly expressed in the RPE, therefore, in *MYO7A* disease the RPE will be directly affected with subsequent earlier

defects seen in the choriocapillaris.[16 40] The increased flow signal at the edges of lost CC needs to be interpreted cautiously. These regions might be corresponding to areas where RPE was disrupted prior to the loss of CC, leading to increased OCT signal reaching the CC, and consequently increased signal on the OCT angiograms, without actual increase in CC blood flow.

There are contrasting reports in the literature either suggesting significant hypoperfusion of the CC in RP,[30 31 41 42] whilst others have found no difference between disease and healthy groups.[43-45] This disparity may be due to the genetically- and phenotypically heterogeneous patients, representing a wide-spectrum of pathophysiological mechanisms. Different modalities, including fluorescein angiography (FA),[46] color Doppler ultrasonography,[47] functional MRI,[48 49] bidirectional laser Doppler velocimetry,[50] laser speckle flowgraphy,[32] and confocal laser Doppler flowmetry have shown altered ocular blood flow in RP.[51]

In the retinal circulation, various OCTA studies reported decreased flow signal in the superficial and deep plexuses,[31 42-45 52 53] with the DCP being reported to be more severely affected in several studies.[42 43 53] Moreover, Toto[31] and Koyanagi[52] and colleagues observed significant changes in the more peripheral parafoveal regions, but not the central foveal region, which are consistent with our findings. However, one study by Rezaei *et al.* [30] qualitatively assessed superficial and deep retinal layers and observed vascular defects only in the periphery of the deep plexus, but not the superficial layer in the severe group of RP patients. Additionally, they reported no vascular changes in the mildly affected subjects of their RP cohort.[30] The discrepancy in the findings can be attributed to the variations in the OCT machine, image processing methods, OCTA slab definition, as well as the differences in RP patients' characteristics.

This study provides valuable information on the correlation of vascular perfusion defects with structural and functional abnormalities, providing an insight into the residual vascular function within the degenerating retina. This is particularly important for understanding and planning therapeutic interventions for Usher syndrome. There were several limitations including the cross-sectional design and modest number of participants, especially in the MYO7A cohort, but these are rare disease cohorts. Larger studies with longitudinal data might be needed to confirm these results and better predict disease pathophysiology and progression. In addition, using OCTA is inherently associated with disadvantages as image quality is highly dependent on patient's condition and cooperation.[54] Thus, understanding of OCTA image artifacts is essential for appropriate interpretation and processing of the angiograms, as well as developing advanced image analysis tools.[55 56] Algorithms to remove projection and motion artifacts, as well as machine learning and averaging techniques have shown promising results for reliable segmentation, visualization, and quantification of OCT angiograms.[57-59]

In conclusion, OCTA was able to characterize microvascular changes in retinal and choroidal circulations in the macula of USH patients. Decreased vessel density was detected in the SCP and DCP of *MYO7A* and *USH2A* patients. Choriocapillaris defects

were detected earlier and more commonly in *MYO7A* than *USH2A* patients. OCTA vascular alterations in the retina correlated strongly with OCT photoreceptor degeneration and retinal sensitivity. Our findings suggest that OCTA might provide better understanding of the pathophysiology of retinitis pigmentosa, as well as the associations between vascular abnormalities and specific gene mutations.

Contributorship

Study conception and design: AMH, ARW, AMD, MM. Collection of data: AMH, AM, JSG, JMN, VT, SH. Statistical analysis: AMH. Interpretation of data: AMH, AMD, MM. Writing the article: AMH. Critical revision of the article: AMH, AM, JSG, JMN, VT, SH, ARW, AMD, MM. Final approval of the article: AMH, AM, JSG, JMN, VT, SH, ARW, AMD, MM.

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Competing Interests

There are no competing interests for any author.

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FIGURE LEGENDS

FIGURE 1. Optical coherence tomography (OCT), OCT angiography (OCTA), and microperimetry sensitivity maps of a healthy control, *USH2A* and *MYO7A* patients. (A1-C1): *En face* OCT angiograms of the inner retina. Retinal circulation was further divided into superficial capillary plexus (SCP, A2-C2) and deep capillary plexus (DCP, A3-C3). Boundaries of the segmented slabs correspond to the green lines in the cross-sectional OCT image in A4. Angiograms show capillary loss in all retinal slabs in *USH2A* (B1-B3) and *MYO7A* (C1-C3) patients compared to the healthy participant (A1-A3). Microvascular changes were more pronounced in the DCP, and in the peripheral parts of the angiograms, corresponding to areas of outer retinal degeneration on OCT (B4,C4), and areas of decreased macular sensitivity on microperimetry sensitivity maps (B5,C5). Clinical data including age, gender, visual acuity (VA) and/or MAIA microperimetry mean sensitivity are provided on A4-C4 panels.

FIGURE 2. Vessel density measurements and their relationship with macular structure and function. (A): box and whisker plots demonstrating the differences in OCT angiography vessel density of the inner retinal slab, as well as the superficial capillary plexus (SCP) and deep capillary plexus (DCP) between healthy participants, *USH2A* and *MYO7A* patients. Significant reductions in vessel density was found in all plexuses between patients and healthy participants. However, no significant differences were observed between *USH2A* and *MYO7A* groups. (B and C): scatter plots illustrating the positive correlation between macular perfusion at the level of the DCP, and macular sensitivity and the width of ellipsoid zone (EZ), respectively.

FIGURE 3. Optical coherence tomography angiography (OCTA) of the choriocapillaris (CC) in a healthy control, as well as *USH2A* and *MYO7A* patients at different age groups. OCT angiograms of *USH2A* patients (B1-B3) were comparable to control angiograms (A1-A3). Similarly, no significant changes can be detected in CC of young *MYO7A* (C1). However, peripheral CC loss was observed in the angiograms of *MYO7A* patients at the fourth decade of life or later (white arrows, C2 and C3). Areas of higher flow signal were also detected at the edges of the relatively preserved central CC island (yellow arrow heads, C2 and C3). Visual acuity (VA) and MAIA microperimetry mean sensitivity of the patients are added at the right bottom corners of the angiograms (B1-B3 and C1-C3).