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

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ABSTRACT

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

Methods Twenty-seven patients with molecularly confirmed USH2A (n=21) and MYO7A (n=6) mutations underwent macular 6×6 mm 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 (MS) 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 patients with USH2A and MYO7A mutations compared with 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. VD in the DCP showed strong association with MS 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 patients with USH2A and MYO7A mutations. The CC was generally affected in MYO7A mutations. 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 characterised by congenital sensorineural hearing loss and retinitis pigmentosa (RP), 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 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 generalised arteriolar attenuation.

USH has been classically divided into three clinical subtypes, based on age of onset, severity and vestibular features.5–7 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 characterised 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 USH2.8 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 utricle 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 therapy22 as well as ciliary neurotrophic factor-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 visualisation 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. A decreased vascular density was generally observed in the superficial capillary plexus (SCP), deep capillary plexus (DCP) and CC of patients with RP. Retinal perfusion has been reported to be correlated with visual function. 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 characterise 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, Heidelberg, Germany). The scanning protocol acquired 20°×20° (~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, Fremont, California, USA). This system has an acquisition speed of 70 k A-scans/s, and macular 6×6 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. Scans collected were either the traditional 304×304 scans or the high density 400×400 scans.

Microperimetry

Patients were tested for macular sensitivity (MS) 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° in diameter (figure 1). Mean 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 MS when compared with age-matched normal values. Mean sensitivity and MS values and maps were collected and qualitatively assessed for colocalisation 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 centre 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.

OCT angiography scans were checked for image quality and segmentation using the ReVue software (OptoVue, Fremont, California, USA). Low quality scans (Q less than 5/10, significant motion artefacts, defocused or non-centred) 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 was technically difficult due to the extensive retinal degeneration and poor visualisation, 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 SCP and DCP. 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. 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 thresholded en face angiograms within a 6 mm diameter annular region outside the manually centred 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, Armonk, New York, USA) and Microsoft Excel 2017 (Microsoft Office, Microsoft, Redmond, Washington, USA). VD, width of the preserved EZ and mean MS were presented as mean and SD of the included participants in each group. Age-matching between groups was assessed using analysis of variance (ANOVA) test. Generalised estimating equations (GEE) were 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 biallelic variants in either MYO7A or USH2A genes were included (see online supplementary 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 three patients with USH2A mutations who had non-syndromic autosomal-recessive RP 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 groups (mean LogMAR ±SD, 0.31±0.18 and 0.19±0.23, respectively, p=0.26).

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 patients with USH, compared with 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 VD were observed in patients compared with 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 groups. Patients with USH2A mutations had relatively intact CC layer throughout different age groups (19–55 years) (figure 3, B1–B3), except one patient with USH2 (56 years old) who had marked retinal degeneration and RPE loss which associated with localised loss of CC. On the other hand, CC angiograms of patients with MYO7A mutations showed microvascular changes starting from third or fourth decade of life (figure 3). Areas of lost CC were 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
Correlation between vascular, structural and functional parameters

Areas of degenerated retina on structural OCT spatially colocalised with areas of hyperperfusion on OCTA and decreased sensitivity on the retinal sensitivity map (figure 1). VD 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.

DISCUSSION

Arteriolar attenuation is a classic sign in Usher-related RP. In our cohort of patients with MYO7A and USH2A mutations, 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 CC in patients with USH2A mutations appeared to be relatively preserved until late stages of the disease, in MYO7A, there was marked early loss of this layer compared with healthy controls. Outer retinal degeneration and decreased MS were also detected, which significantly correlated with the measured VD in the retinal OCT angiograms. No statistically significant differences were observed between USH2A and MYO7A groups in terms of VA, OCT, OCTA or microperimetry parameters.

RP is characterised 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 centre. The death of photoreceptors can also explain the decreased MS measured by microperimetry. 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 SCP and DCP.

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. Previous work has not suggested a linkage between the superficial plexus and photoreceptor metabolism. Thus, we would expect the deep and intermediate capillary plexuses to be affected in USH, but not the superficial plexus. 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 VD.

Quantification of CC by OCTA is inherently technically challenging due the effect of shadowing, projection and motion artefacts. Therefore, CC slabs were assessed qualitatively for the purpose of this study. Unlike the retinal circulation, the CC was different in the two USH groups with the CC in patients with MYO7A mutations affected earlier and more severely than the USH2A group. Choroidal blood flow has been long considered to be only minimally regulated by oxygen levels. Thus, changes in metabolic demand secondary to neural retinal degeneration would not be expected to affect choroidal blood flow. The RPE is essential for the development and maintenance of the CC integrity via RPE-specific factors such as vascular endothelial growth factor and pigment epithelium-derived factor. 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. Thus, in USH2A mutations, prior to photoreceptor degeneration, the structure of the RPE is initially intact, maintaining the viability of CC layer. In contrast,

Table 2  Vessel density, EZ width and retinal sensitivity measurements

|                          | Control | USH2A | MYO7A | Control vs USH2A | % change | P value | Control vs MYO7A | % change | P value | USH2A vs MYO7A | % 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 |
| DCP (%)                  | 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 GEE (accounting for the interocular subject correlation) and adjusted for multiple comparisons using Holm-Bonferroni method.

DCP, deep capillary plexus; EZ, ellipsoid zone; GEE, generalised estimating equation; MAIA, Macular Integrity Assessment microperimetry; NA, not applicable; OCT, optical coherence tomography; SCP, superficial capillary plexus.
Hagag AM, et al. Br J Ophthalmol 2020;104:480–486. doi:10.1136/bjophthalmol-2019-314243

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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 SCP and DCP between healthy participants, USH2A and MYO7A patients. Significant reductions in vessel density were found in all plexuses between patients and healthy participants. However, no significant differences were observed between USH2A and MYO7A groups. (B,C) Scatter plots illustrating the positive correlation between macular perfusion at the level of the DCP and macular sensitivity and the width of EZ, respectively. DCP, deep capillary plexus; EZ, ellipsoid zone; MAIA, macular integrity assessment microperimetry; OCT, optical coherence tomography; Rho, Spearman’s rank correlation coefficient; SCP, superficial capillary plexus.

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 CC.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,30–32,41–42 while 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,46 colour Doppler ultrasonography,47 functional MRI,48,49 bidirectional laser Doppler velocimetry,50 laser speckle flowgraphy32 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.42,43,52 Moreover, Toto31 and Koyanagi32 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 patients with RP. 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 characteristics of patients with RP.
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. 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. Thus, understanding of OCTA image artefacts is essential for appropriate interpretation and processing of the angiograms as well as developing advanced image analysis tools.

In conclusion, OCTA was able to characterise microvascular changes in retinal and choroidal circulations in the macula of patients with USH. Decreased VD was detected in the SCP and DCP of patients with MYO7A and USH2A mutations. CC defects were detected earlier and more commonly in MYO7A than USH2A mutations. 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 RP as well as the associations between vascular abnormalities and specific gene mutations.

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

Funding This work was supported by Wellcome Trust Career Development grant number 205174/2/16/2, National Institute for Health Research (NIHR) Rare Diseases Translational Research Collaboration Award as well as the NIHR Research Centre at Moorfields and UCL Institute of Ophthalmology.

Disclaimer Funders had no role in study design, collection, analysis and interpretation of the data, writing the article or in the decision to submit the paper for publication.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on request.

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