

Peripheral Vascular Alterations in *USH2A*-Retinitis Pigmentosa and Relationship with Macular Involvement: A Cross-Sectional Study

Alessandro Arrigo, MD, PhD,^{1,2} Giovanni Forte, MD,¹ Emanuela Aragona, MD, PhD,¹ Virginia Pini, MD,³ Camilla Petruzzello, MD,³ Ottavio Cremona, MD,^{3,4} Filippo Casoni, MD,^{3,4} Alessio Antropoli, MD,¹ Lorenzo Bianco, MD,¹ Maurizio Battaglia Parodi, MD,^{1,3} Francesco Bandello, MD, FEBO^{1,3}

Objective: To characterize peripheral retinal vascular alterations in *USH2A*-related retinitis pigmentosa (RP) using widefield OCT angiography (WF-OCTA) and to investigate their relationship with macular changes assessed by multimodal retinal imaging.

Design: Cross-sectional observational study.

Participants: Thirty eyes of 30 patients with genetically confirmed *USH2A*-related RP and 30 age- and gender-matched healthy controls.

Methods: We performed complete ophthalmologic examination and multimodal retinal imaging, including fundus autofluorescence (FAF), OCT, OCTA, AF-based macular pigment optical density, and 130° WF-OCTA. A quantitative WF-OCTA analysis pipeline was developed to assess peripheral perfusion through concentric fovea-centered retinal rings. The Concentric Peripheral Perfusion Index, peripheral perfusion loss gradient, and perfusion profile slope were calculated. Associations between peripheral vascular status and central retinal imaging biomarkers were analyzed.

Main Outcome Measures: WF-OCTA perfusion quantitative metrics

Results: Compared with controls, RP eyes showed significantly lower WF-OCTA perfusion indices across all eccentricity rings (all $P < 0.05$), with more pronounced perfusion loss in the outer retina and a steeper perfusion decline toward the periphery. Peripheral vascular impairment significantly correlated with visual field indices, preserved FAF area, retinal layer thicknesses, and macular vascular density, particularly at the intermediate capillary plexus. Eyes with bone spicule pigmentation or retinal vascular attenuation showed more severe peripheral vascular loss, whereas eyes with hyperautofluorescent ring demonstrated more preserved perfusion profiles. Autofluorescence-based macular pigment optical density values were reduced in RP and significantly associated with central vascular and structural parameters. Hyperreflective ganglion cell band was associated with more severe peripheral vascular impairment and worse macular vascular status.

Conclusions: *USH2A*-related RP is characterized by marked peripheral retinal vascular rarefaction, more evident at increasing eccentricities and closely associated with central retinal structural, vascular, and pigmentary alterations. These findings support the concept of RP as a diffuse neurovascular retinal disorder extending beyond the outer retina. Quantitative WF-OCTA may provide clinically meaningful biomarkers for disease characterization and monitoring.

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Retinitis pigmentosa (RP) is the most common inherited retinal dystrophy, characterized by a rod–cone degeneration.¹ *USH2A* is the most prevalent gene for autosomal-recessive RP, among all the known pathogenic variants.^{2,3} This gene encodes the Usher protein, which is involved in photoreceptor cell maintenance and organization, structural support at the connecting cilium of photoreceptors, and synaptic function.⁴

Retinitis pigmentosa shows highly heterogeneous phenotypes, considering both peripheral alterations (e.g., bone spicule and atrophy) and central retinal changes. In the latter case, two clinically relevant alterations are represented by cystoid maculopathy and hyperreflective ganglion cell layer band (HGB), deeply investigated by OCT and OCT angiography (OCTA).^{5–9} These findings have highlighted wide inner retinal involvement and how it may

contribute to the morphological and functional deterioration of the retina.^{5–10} Moreover, it is important to mention the contribution of fundus autofluorescence (FAF) technique, first of all considering the relevance of the hyperautofluorescent ring detection.¹¹ Moreover, FAF is also relevant to assess macular pigment changes. These can be tested by using macular pigment optical density (MPOD), a noninvasive approach to quantify, *in vivo*, macular pigments. Macular pigment optical density may be computed using different approaches, including fundus reflectometry,^{12,13} FAF,¹⁴ and Raman spectroscopy.¹⁵ Among these techniques, FAF-based MPOD has been found to provide consistent measures of macular pigmentary changes.^{14,16,17}

The high heterogeneity of RP phenotypes can be found also in *USH2A*-related phenotypes. A current clinical challenge is to assess the clinical role of peripheral vascular changes in the morphological and functional deterioration of the retina. If peripheral pigment changes have been already tested by ultra-widefield (UWF) fundus approaches, vascular alterations remain to be explored. About this point, a meaningful contribution might come from the more recently introduced widefield OCTA (WF-OCTA).

In this study, we aimed to test the peripheral vascular changes in a cohort of RP eyes affected by *USH2A* pathogenic variants and to assess the relationship between peripheral retinal pigment changes and macular involvement. In addition, we included the analysis of the possible effect of cystoid maculopathy or HGB.

Methods

The study was designed as across-sectional, observational study. We included patients with RP with a genetically confirmed diagnosis of *USH2A* pathogenic variants and a control group matched for age and gender, recruited from January 2024 to February 2025. All the patients with RP were recruited at the Inherited Retinal Dystrophy Unit of Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Scientific Institute in Milan. Healthy controls were recruited on a voluntary basis among individuals undergoing routine ophthalmologic evaluation at the General Ophthalmology Unit of the same institution. All the included patients and controls completed the clinical and imaging assessment. Signed informed consent was obtained before inclusion. The study was performed in accordance with the Declaration of Helsinki and good clinical practice, under the local ethical committee approval (ethical committee of IRCCS San Raffaele Scientific Institute in Milan; protocol ID: PNRR-MR1-2023-12378435).

The exclusion criteria were a positive history of other retinal diseases (including posterior uveitis, infections or inflammatory retinopathies, glaucoma, or other ocular diseases), dry eye or anterior segment diseases, and uncontrolled systemic conditions potentially interfering with the study. Glaucoma was excluded based on the absence of a previous diagnosis or intraocular pressure (IOP)-lowering treatment, normal IOP at the study visit, absence of glaucomatous optic nerve head changes on dilated examination, and absence of visual field defects specifically suggestive of glaucoma on 30-2 testing. Peripapillary nerve fiber measurements were not used as a primary exclusion criterion because of their potentially confounded interpretation in RP. In addition, it was mandatory to have clear media, no history of smoking, no history of lutein and zeaxanthin nutraceuticals intake, no clinically relevant vitreoretinal

interface alterations, and high myopia (we considered a myopic spherical equivalent no greater than -3.00 D), both for patients with RP and healthy controls. Looking at systemic diseases, these included uncontrolled arterial hypertension or diabetes mellitus; active systemic inflammatory, autoimmune, or infectious diseases; recent major cardiovascular or cerebrovascular events; and advanced renal or hepatic disease. Systemic eligibility was assessed on the basis of medical history, available clinical records, and current clinical and therapeutic status. These criteria were fundamental since their presence is known to potentially affect imaging results. To further reduce potential bias, we included only *USH2A* pathogenic variants with no clinical signs of hearing loss, verified on patients' medical history. Clinical staging of RP severity was performed according to Iftikhar et al.¹⁸

Complete ophthalmological assessment of anterior and posterior segments, best-corrected visual acuity (BCVA) by standard ETDRS chart, and 30.2 Humphrey visual field test were performed.

Multimodal retinal imaging data were obtained after pharmacologic pupil dilation. Imaging protocol included OCT (high-resolution radial, raster, and dense scans and automatic real-time $>25^\circ$), OCTA ($15^\circ \times 15^\circ$, high resolution 125 kHz scans), blue-light FAF, and MPOD (HRA2+OCT Spectralis Heidelberg, Heidelberg Eng.). For confocal imaging, we carefully ensured accurate focus settings prior to acquisition. We ensured optimal alignment of the patient's head and eye with the device, following the Heidelberg Spectralis manual. The focus was initially adjusted using the infrared image. Subsequently, for each confocal modality, the focus was fine-tuned by approximately -0.5 diopters (D) relative to the infrared image. No further focus adjustments were made during acquisition.

From confocal blue FAF images, we extracted the following metrics: presence of a hyperautofluorescent ring and area of central preserved FAF. The latter was measured by two independent researchers using manufacturer segmentation tool.

Ultra-widefield fundus images were acquired using an Optos California device (Optos). From these fundus images, we assessed the presence of bone spicule pigmentations and retinal vascular attenuation. Bone spicule pigmentations were defined as clearly visible intraretinal pigment clumps distributed in a perivascular bone spicule-like configuration in the midperipheral retina. Retinal vascular attenuation, also named hyalinized retinal vessels or sclerotic retinal vessels, was defined as retinal vessels exhibiting narrowing together with increased wall reflectivity or whitish sheathing, consistent with retinal vascular sclerosis.

Widefield OCTA images were acquired using a DREAM OCT Intalight device (Intalight Inc.). We specifically refer to WF-OCTA rather than UWF-OCTA, as only single-scan WF-OCTA images with a 130° field of view (26×21 mm) were considered for the analysis. Only high-quality data were included by using manufacturer-recommended quality control tools. For all the independent measures, we tested intergrader agreement by Cohen's k coefficient (with 95% bootstrap confidence intervals).

OCT and OCTA Analyses

Retinal thickness measurements were automatically obtained from OCT scans using the ETDRS grid available in the HEYEX software. Retinal layer segmentation was automatically generated through the device's integrated software. All segmentation results were systematically reviewed by an experienced grader to confirm accurate identification of retinal boundaries. No manual adjustments were made to preserve consistency and minimize operator-dependent variability among subjects. Eyes were included only when segmentation lines were clearly consistent with anatomical boundaries within the central 6° region. Scans showing evident segmentation errors or substantial boundary misidentification were

excluded from the analysis. Minor focal deviations, commonly inherent to OCT-based automated segmentation in most studies, were considered negligible and unlikely to influence the overall quantitative findings. For each eye, the following parameters were considered: central macular thickness, retinal pigment epithelium (RPE), outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, and retinal nerve fiber layer. For all measurements, the mean value across the inner ETDRS subfields within 6.0° eccentricity was used to ensure precise spatial correspondence with MPOD and macular OCTA measurements. In addition, ellipsoid zone (EZ) width was manually assessed on both horizontal and vertical fovea-centered OCT scans. These measurements were independently performed by two expert graders. Intergrader agreement was evaluated by calculating the intraclass correlation coefficient.

Additionally, the following OCT metrics were recorded: cystoid maculopathy, HGB, and vitreoretinal interface anomaly, defined as the presence of a hyperreflective inner limiting membrane and/or partial epiretinal membrane (> grade 1 according to OCT classification)¹⁹, in the absence of a continuous epiretinal membrane, retinal profile distortion, and vitreomacular traction.

Regarding OCTA, 15° × 15° high-resolution scans were acquired (HRA2+OCT Spectralis Heidelberg, Heidelberg Eng.), and the following segmentations were automatically extracted: superficial capillary plexus (SCP), intermediate capillary plexus (ICP), deep capillary plexus (DCP), and choriocapillaris. A full-retina segmentation, extending from the RPE to the inner limiting membrane, was also included to account for possible segmentation artifacts related to RP-associated macular alterations. Furthermore, the same two expert graders manually measured the foveal avascular zone (FAZ) area on the full-retina segmentation. Images were exported to ImageJ software.²⁰ OCT angiography reconstructions were then binarized using the following workflow: Import.tiff image → Adjust → Threshold → Automatic threshold → Mean thresholding → Export binarized image. Vessel density (VD) was calculated as the ratio of white to black pixels, with the manually segmented FAZ included among the exclusion criteria.

Autofluorescence-Based MPOD Analysis

For autofluorescence-based MPOD (AF-MPOD) analysis, we used the MPOD module integrated into the Heidelberg Spectralis system.^{16,17} This method is based on the combined acquisition of green autofluorescence (AF) and blue AF images. The module estimates MPOD by assessing the relative attenuation of the lipofuscin AF signal, primarily arising from the RPE, induced by the presence of macular pigments. In accordance with the Heidelberg Spectralis manual recommendations, particular attention was paid to achieving optimal alignment of the patient's head and eye with the device. Focusing was initially adjusted on the infrared image. Subsequently, imaging was switched to confocal AF mode, with the focus refined by approximately −0.5 D relative to the initial infrared image. No further focus adjustments were performed during acquisition. Care was taken to identify potential artifacts related to floaters, overexposure, reduced contrast, suboptimal focus, or inadequate foveal centration. Images considered unsuitable for analysis were excluded. A single AF-MPOD scan was acquired for each eye.

Autofluorescence-based MPOD maps were generated by digitally subtracting the logarithmic AF intensities obtained from the green FAF and blue FAF images. The resulting attenuation maps were spatially aligned with concentric rings centered on the fovea, corresponding to standard retinal eccentricities. The following standard AF-MPOD parameters were considered: 1.0° eccentricity, 2.0° eccentricity, interval 1.0°–2.0° eccentricity, and plateau

6.0° eccentricity. This configuration enabled spatial profiling of macular pigment distribution across the central macular region.

WF-OCTA Analysis

We developed and proposed the following quantitative pipeline to assess peripheral perfusion changes based on WF-OCTA. As a preliminary step, we exported all the 130° OCTA reconstructions considering full-retina segmentation (the volume included between the inner limiting membrane and RPE), and we carefully addressed the presence of artifacts.

The effective analyzable retinal area (A_T) was defined as all retinal tissue visible within the 130° WF-OCTA field after exclusion of nonretinal and artifactual regions. These were evaluated by an independent, blinded expert researcher (GF).

Excluded regions included: background outside retinal tissue, image borders, peripheral truncation, masking artifacts, motion artifacts, and signal loss unrelated to retinal perfusion (e.g., segmentation artifacts). This area represented the denominator for all quantitative measurements. Widefield OCTA images were considered eligible when they were adequately centered, provided sufficient vascular signal for reliable perfusion classification, and contained no diffuse or noncorrectable artifacts affecting the analytical regions. Localized artifacts did not automatically lead to image exclusion when they were clearly identifiable and could be reliably masked without compromising quantitative assessment of the remaining retinal area.

To assess topographic perfusion across retinal eccentricity, each WF-OCTA image was subdivided into the following four concentric fovea-centered retinal regions:

- R0: central foveal/macular region (0–30°)
- R1: inner parafoveal/perifoveal retina (30°–60°)
- R2: midperipheral retina (60°–90°)
- R3: peripheral retina extending to the outer boundary of the 130° field (90°–130°)

Because the nominal horizontal field of view of 130° corresponds to a rectangular 26- × 21-mm OCTA acquisition, the outermost region (R3) does not constitute a complete ring, as the vertical extent of the scan is smaller than its horizontal extent. Accordingly, R3 measurements were calculated over the entire valid analyzable retinal area captured within the acquisition footprint, excluding portions of the nominal annulus extending beyond the scan boundaries.

The total analyzable retinal area was therefore defined as:

$$A_T = A_{R0} + A_{R1} + A_{R2} + A_{R3}$$

where,

- A_{R0} = analyzable area in R0
- A_{R1} = analyzable area in R1
- A_{R2} = analyzable area in R2
- A_{R3} = analyzable area in R3

Together, R0–R3 covered the entire analyzable 130° retinal field.

Perfused retina was defined as retinal tissue demonstrating visible OCTA flow signal corresponding to retinal vascular structures or capillary plexus.

Perfusion classification was based on: (I) grayscale vessel signal extraction, (II) topographic continuity of vascular structures, and (III) manual review to exclude artifacts.

The total perfused retinal area was denoted A_P .

Perfused retinal area percentage was calculated as:

$$PRA(\%) = \frac{A_P}{A_T} \times 100$$

Ring-specific perfusion was calculated for each region:

$$PRA_{R_i}(\%) = \frac{A_{P,R_i}}{A_{R_i}} \times 100$$

for

$$i \in \{0, 1, 2, 3\}$$

where,

- A_{P,R_i} = perfused retinal area within ring R_i

Nonperfused retina was defined as analyzable retinal tissue without detectable OCTA vascular flow signal and not attributable to artifact.

A region was classified as nonperfused only when: (I) vascular flow signal was absent, (II) adjacent vascular continuity did not explain signal loss, (III) no masking artifact was present, and (IV) the finding was anatomically plausible.

The total nonperfused retinal area was denoted A_{NP} .

Nonperfused retinal area percentage was calculated as:

$$NPA(\%) = \frac{A_{NP}}{A_T} \times 100$$

Ring-specific non-perfusion was calculated as:

$$NPA_{R_i}(\%) = \frac{A_{NP,R_i}}{A_{R_i}} \times 100$$

By definition:

$$A_P + A_{NP} = A_T$$

and

$$PRA + NPA = 100\%$$

Similarly, within each ring:

$$A_{P,R_i} + A_{NP,R_i} = A_{R_i}$$

and

$$PRA_{R_i} + NPA_{R_i} = 100\%$$

To quantify the proportion of perfused versus non-perfused retina at each eccentricity, a Concentric Peripheral Perfusion Index (CPPI) was calculated for each concentric retinal region.

For each ring R_i ($i = 0, 1, 2, 3$), CPPI was defined as the ratio between perfused and nonperfused retinal area:

$$CPPI_{R_i} = \frac{A_{P,R_i}}{A_{NP,R_i}}$$

where,

- A_{P,R_i} = perfused retinal area in ring R_i
- A_{NP,R_i} = nonperfused retinal area in ring R_i

Interpretation:

$$CPPI_{R_i} > 1$$

indicates predominance of perfused retina within that ring.

$$CPPI_{R_i} = 1$$

indicates equal perfused and nonperfused retinal area.

$$CPPI_{R_i} < 1$$

indicates predominance of nonperfused retina.

A global CPPI for the entire 130° field was also calculated as:

$$CPPI_{global} = \frac{A_P}{A_{NP}}$$

This metric directly quantifies the balance between preserved and not perfused retina across the field of view.

To quantify perfusion reduction from the central retina toward the peripheral retina, a peripheral perfusion loss gradient (PPLG) was calculated.

Peripheral perfusion loss gradient was defined as the absolute difference between CPPI in the innermost region (R0) and the outermost peripheral region (R3):

$$PPLG = CPPI_{R0} - CPPI_{R3}$$

Interpretation: higher PPLG values indicate a steeper reduction in retinal perfusion with increasing eccentricity.

A value close to zero indicates relatively homogeneous perfusion distribution between center and periphery.

A large positive value indicates marked peripheral perfusion loss relative to the central retina.

To characterize the overall rate of perfusion decline across retinal eccentricities, a perfusion profile slope (PPS) was calculated.

For each eye, CPPI values from:

$$R0, R1, R2, R3$$

were plotted against retinal eccentricity.

A first-order linear regression model was then fitted:

$$CPPI_{R_i} = \beta_0 + \beta_1 E_i$$

where,

- E_i = eccentricity corresponding to ring R_i
- β_0 = intercept
- β_1 = slope

The slope term was defined as PPS:

$$PPS = \beta_1$$

The interpretation is:

$$PPS < 0$$

means progressive decline in perfusion toward the periphery

$$PPS \approx 0$$

means a relatively stable perfusion profile.

Hence, more negative PPS values indicate steeper peripheral perfusion loss.

The proposed quantitative pipeline is shown in [Figure 1](#).

Statistical Analyses

All imaging data underwent strict quality assessment before inclusion. To avoid intereye correlation and ensure independence of observations, only one eye from each participant was included in the analysis. The distribution of continuous variables was evaluated through quantile–quantile plots and visual inspection of histograms. Age and gender were included as fixed covariates in all inferential analyses. Group differences between RP and control eyes were assessed for all clinical and imaging parameters. Demographic and clinical characteristics were summarized separately for RP and healthy control eyes. Continuous variables were reported as mean $\pm$ standard deviation. Between-group comparisons for age were performed using an independent samples *t*-test. A two-sided *P* value < 0.05 was considered statistically significant.

The primary outcomes were WF-OCTA parameters (CPPI, PPLG, and PPS). Given the biological and statistical correlation among these variables, a multivariate analysis of covariance (MANCOVA) was used to assess overall between-group differences while adjusting for age. Following a significant multivariate effect, separate univariate analysis of covariance (ANCOVA) were performed for each parameter. Multiple comparisons were

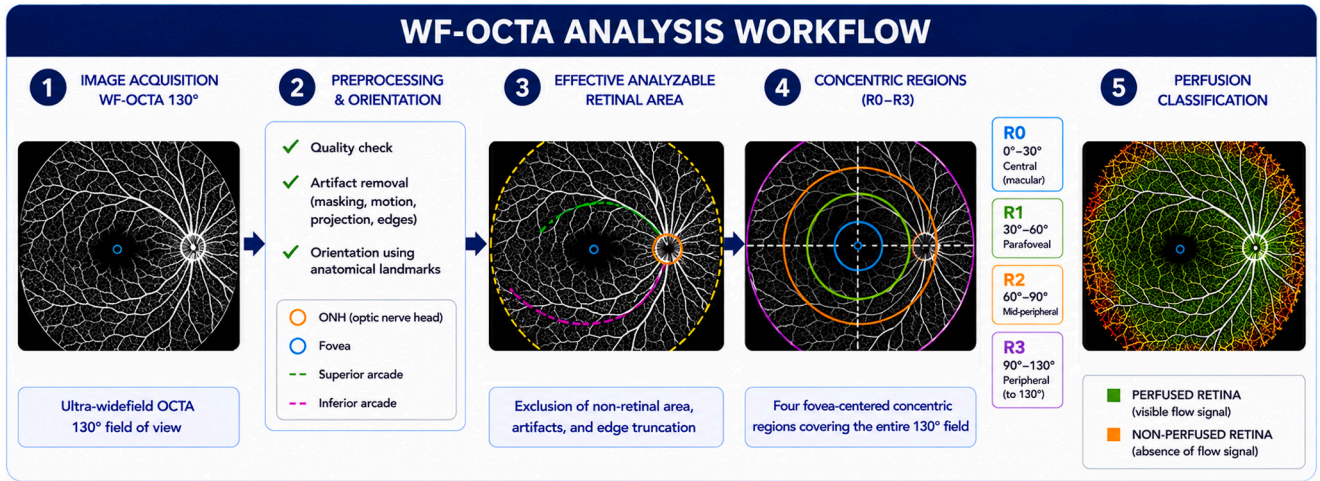


Figure 1. Quantitative WF-OCTA pipeline. WF-OCTA = widefield OCT angiography.

controlled using the Holm–Bonferroni correction. Effect sizes were reported as partial eta squared.

The secondary structural retinal outcomes included MPOD values at each eccentricity sector, OCT-derived central macular thickness, individual retinal layer thicknesses, EZ length measured on both horizontal and vertical scans, and OCTA metrics. When the multivariate model was significant, separate ANCOVA models were performed for each parameter. Multiple testing was

controlled using the Holm–Bonferroni correction. Effect sizes were expressed as partial eta squared.

Sensitivity analyses were conducted to evaluate the robustness of the main findings. First, all between-group comparisons were repeated using models adjusted for age only. Second, nonparametric Mann–Whitney *U* tests were performed to confirm unadjusted between-group differences. Third, bootstrap-derived 95% confidence intervals were calculated for the main effect estimates. Finally, regression diagnostics were used to identify influential observations based on leverage values and Cook’s distance.

Within the RP group, associations between clinical and imaging variables were assessed using Spearman rank correlation coefficient. Separate correlation analyses were conducted for WF-OCTA, MPOD, OCT structural parameters, OCTA vascular parameters, and visual field metrics. Correlation strength was interpreted according to standard criteria. *P* values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate procedure.

We additionally investigated the relationship between peripheral and central vascular alterations and clinically relevant features, including the presence of a central hyperautofluorescent ring, cystoid maculopathy, HGB, bone spicule pigmentation, and retinal vascular attenuation. Statistical analyses were performed using the SPSS software package (IBM Corp.).

Results

Clinical and Imaging Findings

We included 30 RP eyes (30 USH2A-RP Caucasian patients; 12 males; age 38 ± 9 years) and 30 healthy eyes (30 Caucasian controls; 15 males; age 37 ± 8 years) ($P > 0.05$). Pathogenic variants are shown in Table 1. Five additional RP patients have been excluded because of artifacts and low-quality images. All the eyes were phakic, with clear media confirmed at the slit lamp examination. According to the RP staging criteria, 4 eyes were classified as grade 1, 22 eyes as grade 2, and 4 eyes as grade 3.

All clinical and imaging data are shown in Table 2. Best-corrected visual acuity was significantly lower in RP eyes compared with healthy controls ($P = 0.001$). Cystoid

Table 1. USH2A Pathogenic Variants

Patient	Variant 1	Variant 2
1	c.2276G>T	c.1841-2A>G
2	c.3938delA	c.10702A>T
3	c.2299del	c.8232G>A
4	c.2296T>C	c.2276G>T
5	c.908G>A	c.2296T>C
6	c.908G>A	c.2276G>T
7	c.2276G>T	c.2330G>A
8	c.2276G>T	c.5858C>G
9	c.2276G>T	c.2296T>C
10	c.12559C>T	c.7339A>C
11	c.3332T>G	c.10437G>T
12	c.13392G>A	c.2276G>T
13	c.2276G>T	c.13477C>T
14	c.2276G>T	c.9424G>T
15	c.10436G>A	c.5776+1G>A
16	c.2710_2720dup	c.1055C>T
17	c.2276G>T	c.2296T>C
18	c.2296T>C	c.10712C>T
19	c.2276G>T	c.1841-2A>G
20	c.3332T>G	c.908G>A
21	c.2299del	c.2276G>T
22	c.2276G>T	c.2299del
23	c.15089C>A	c.2296T>C
24	c.2276G>T	c.949C>A
25	c.2299del	c.9424G>T
26	c.653T>A	c.2276G>T
27	c.15020C>T	c.2071T>C
28	c.2276G>T	c.10712C>T
29	c.1036A>G	c.2296T>C
30	c.10712C>T	c.9424G>T

Table 2. Clinical and Imaging Findings in RP and Healthy Cohorts

Parameter	Clinical and Imaging Data		Adjusted P Value
	RP Eyes	Controls	
Clinical data			
N	30	30	
Gender (M/F)	12/18	15/15	
Age (years old)	38 ± 9	37 ± 8	>0.05
ETDRS BCVA (letters)	81 ± 6	85 ± 2	0.001
Refractive error (diopters)	−1.00 ± 0.50	−0.75 ± 0.50	>0.05
OCT (μm)			
CMT	320 ± 88	351 ± 16	0.062
RPE	14 ± 1	15 ± 1	<0.001
ONL	76 ± 55	77 ± 6	0.859
OPL	43 ± 7	38 ± 6	0.002
INL	51 ± 6	39 ± 4	<0.001
IPL	28 ± 9	44 ± 1	<0.001
GCL	26 ± 11	52 ± 2	<0.001
RNFL	21 ± 21	23 ± 2	0.569
OCTA vessel density			
SCP VD	0.19 ± 0.06	0.47 ± 0.03	<0.001
ICP VD	0.20 ± 0.06	0.41 ± 0.03	<0.001
DCP VD	0.26 ± 0.05	0.44 ± 0.03	<0.001
CC VD	0.46 ± 0.04	0.50 ± 0.01	<0.001
Full-retina VD	0.18 ± 0.06	0.44 ± 0.03	<0.001
FAZ area (mm ²)	0.33 ± 0.12	0.33 ± 0.09	0.753
AF-MPOD (density units [DU])			
1.0° eccentricity	0.40 ± 0.14	0.57 ± 0.02	<0.001
2.0° eccentricity	0.26 ± 0.09	0.43 ± 0.04	<0.001
Interval 1.0°-2.0° eccentricity	0.21 ± 0.08	0.34 ± 0.04	<0.001
6.0° eccentricity	0.10 ± 0.03	0.11 ± 0.01	0.082
Visual field 30.2			
Fovea dB	33 ± 9		
VFI %	41 ± 26		
MD 30-2 dB	−21 ± 11		
PSD 30-2 dB	5 ± 10		
Other OCT metrics			
Cystoid maculopathy (%)	6/30 (20%)		
HGB (%)	16/30 (53%)		
VRI anomalies (%)	10/30 (35%)		
Horizontal EZ length (μm)	2148 ± 1328		
Vertical EZ length (μm)	1767 ± 1199		
FAF metrics			
Hyperautofluorescent ring (%)	19/30 (63%)		
Preserved FAF area (mm ²)	87 ± 61		
UWF fundus metrics			
Bone spicule (%)	22/30 (73%)		
Retinal vascular attenuation (%)	12/30 (40%)		
WF OCTA metrics			
Perfused retina R0 (%)	85 ± 11	96 ± 3	<0.001
Perfused retina R1 (%)	76 ± 15	96 ± 4	<0.001
Perfused retina R2 (%)	54 ± 27	93 ± 4	<0.001
Perfused retina R3 (%)	38 ± 29	87 ± 3	<0.001
CPPI R0	16 ± 20	36 ± 24	<0.001
CPPI R1	7 ± 8	39 ± 20	<0.001
CPPI R2	5 ± 11	16 ± 9	<0.001
CPPI R3	2 ± 5	7 ± 2	<0.001
PPLG	46 ± 20	4 ± 1	0.005
PPS (%/grade)	−0.58 ± 0.29	−0.05 ± 0.02	<0.001

AF-MPOD = autofluorescence-based macular pigment optical density; BCVA = best-corrected visual acuity; CC = choriocapillaris; CMT: central macular thickness; CPPI = Concentric Peripheral Perfusion Index; DCP = deep capillary plexus; EZ = ellipsoid zone; FAZ = foveal avascular zone; GCL = ganglion cell layer; HGB = hyperreflective ganglion cell band; ICP = intermediate capillary plexus; INL = inner nuclear layer; IPL = inner plexiform layer; MD = mean deviation; ONL = outer nuclear layer; OPL = outer plexiform layer; PPLG = peripheral perfusion loss gradient; PPS = perfusion profile slope; PSD = pattern standard deviation; RNFL = retinal nerve fiber layer; RP = retinitis pigmentosa; RPE = retinal pigment epithelium; SCP = superficial capillary plexus; UWF = ultra-widefield; VD = vessel density; VFI = visual field index; VRI = vitreoretinal interface.

maculopathy was found in 6/30 eyes (20%), whereas HGB was detected in 16/30 eyes (53%). Vitreoretinal interface anomalies were found in 10/30 eyes (35%). The absence of a defined epiretinal membrane and tractional component was confirmed in 30/30 eyes (100% of cases). Horizontal EZ length was $2148 \pm 1328 \mu\text{m}$, whereas vertical EZ length was $1767 \pm 1199 \mu\text{m}$. Hyperautofluorescent ring was found in 19/30 eyes (63%). Preserved FAF area was $87 \pm 61 \text{ mm}^2$. Looking at UWF fundus images, bone spicules were found in 22/30 eyes (73%), whereas retinal vascular attenuations were found in 12/30 eyes (40%). Based on the analysis of WF-OCTA images, we calculated CPPI for R0, R1, R2, and R3 sectors and PPLG for all RP eyes (reported in Table 2). Moreover, PPS was $-0.58 \pm 0.29\%/grade$.

Testing peripheral perfusion metrics obtained from WF-OCTA, MANCOVA showed a significant overall effect of group on CPPI and PPLG parameters after adjustment for age and BCVA (Wilks' $\lambda = 0.102$, $F = 28.313$, $P < 0.001$).

Subsequent ANCOVA analyses showed significantly lower CPPI values in RP eyes compared with healthy controls across all R sectors (from R0 to R3). Moreover, RP eyes showed significantly lower PPLG values than controls. These findings remained significant after Holm–Bonferroni correction. The calculated PPS was $-0.58 \pm 0.29\%/grade$ for RP eyes vs. $-0.05 \pm 0.02\%/grade$ for healthy controls ($P < 0.001$) (Fig 2).

Looking at OCT metrics, MANCOVA showed a significant overall effect of group on macular structural parameters after adjustment for age and BCVA (Wilks' $\lambda = 0.108$, $F = 51.419$, $P < 0.001$).

Post hoc ANCOVA analyses reported significant thinning of several retinal layers in RP eyes compared with healthy controls. In particular, RP eyes showed reduced RPE, inner plexiform layer, and ganglion cell layer. Moreover, the outer plexiform layer and inner nuclear layer were significantly thicker in RP eyes than in controls. These findings remained significant after Holm–Bonferroni correction.

Multivariate analysis of covariance also showed a significant overall effect of group on macular OCTA vascular parameters after adjustment for age and BCVA (Wilks' $\lambda = 0.075$, $F = 107.505$, $P < 0.001$).

Post hoc ANCOVA analyses demonstrated significantly lower full retina, SCP, ICP, DCP, and choriocapillaris VD values in RP eyes compared with healthy controls. These findings remained significant after Holm–Bonferroni correction. No significant differences were observed for FAZ area after adjustment for age, BCVA, and multiple comparisons.

Looking at macular pigment values, MANCOVA showed a significant overall effect of group on AF-MPOD parameters after adjustment for age and BCVA (Wilks' $\lambda = 0.305$, $F = 30.750$, $P < 0.001$).

Subsequent ANCOVA analyses showed significantly lower AF-MPOD values in RP eyes compared with healthy controls at 1.0° eccentricity, 2.0° eccentricity, and interval 1.0° – 2.0° eccentricity. These findings remained significant

after Holm–Bonferroni correction. No significant difference was found at the 6.0° eccentricity sector.

Sensitivity analyses confirmed the robustness of the main findings. Significant between-group differences in WF-OCTA, AF-MPOD, structural OCT, and OCTA parameters remained consistent after adjustment for age alone, nonparametric testing, bootstrap resampling, and exclusion of influential observations. These analyses support the stability of the observed associations and reduce the likelihood that results were driven by model specification, distributional assumptions, or outlier effects.

Correlation Analysis Findings

In RP eyes, higher CPPI values showed positive correlations with visual field metrics (visual field index % and central visual field 6° (sum/12) dB), preserved FAF area, retinal layer thickness, and ICP VD, considering R0, R1, R2, and R3 values. In addition, CPPI values at R0 and R1 showed negative correlation with the presence of HGB, whereas CPPI values at R2 and R3 showed negative correlation with the presence of retinal vascular attenuation.

Peripheral perfusion loss gradient values showed negative correlations with preserved FAF area, central macular thickness, outer nuclear layer, outer plexiform layer, inner plexiform layer, and ICP VD.

We also tested the presence of other significant correlations. Here, we wanted to highlight the most relevant ones, including negative correlations between the presence of bone spicule and AF-MPOD values collected at all eccentricity sectors. The presence of bone spicule negatively correlated with EZ length as well.

Moreover, the presence of cystoid maculopathy positively correlated with EZ length and full-retina VD values.

All significant data are shown in Table 3.

The Relationship between Retinal Vascular Attenuation and Peripheral and Central Vascular Status

The presence of retinal vascular attenuation was significantly associated with CPPI after adjustment for age and BCVA on MANCOVA analysis (Wilks' $\lambda = 0.352$, $F = 5.968$, $P < 0.001$). Post hoc ANCOVA analyses reported significantly lower CPPI values in RP eyes with retinal vascular attenuation at R0 (11 ± 15 vs. 20 ± 22 ; $F = 10.344$, $P < 0.001$), R1 (4 ± 4 vs. 9 ± 9 ; $F = 8.609$, $P < 0.001$), R2 (1 ± 1 vs. 7 ± 14 ; $F = 13.782$, $P < 0.001$), and R3 (1 ± 1 vs. 3 ± 6 ; $F = 58.487$, $P < 0.001$). In addition, RP eyes with retinal vascular attenuation were characterized by significantly higher PPLG than RP eyes without retinal vascular attenuation (54 ± 17 vs. 40 ± 21 ; $F = 4.036$, $P = 0.005$). The calculated PPS was $-0.83 \pm 0.02\%/grade$ for RP eyes with retinal vascular attenuation vs. $-0.49 \pm 0.29\%/grade$ for RP eyes without retinal vascular attenuation ($P = 0.002$). In addition, RP eyes with retinal vascular attenuation showed significantly lower macular VD values for ICP (0.18 ± 0.06 vs. 0.22 ± 0.07 ; $F = 3.306$, $P = 0.01$) and DCP (0.25 ± 0.04

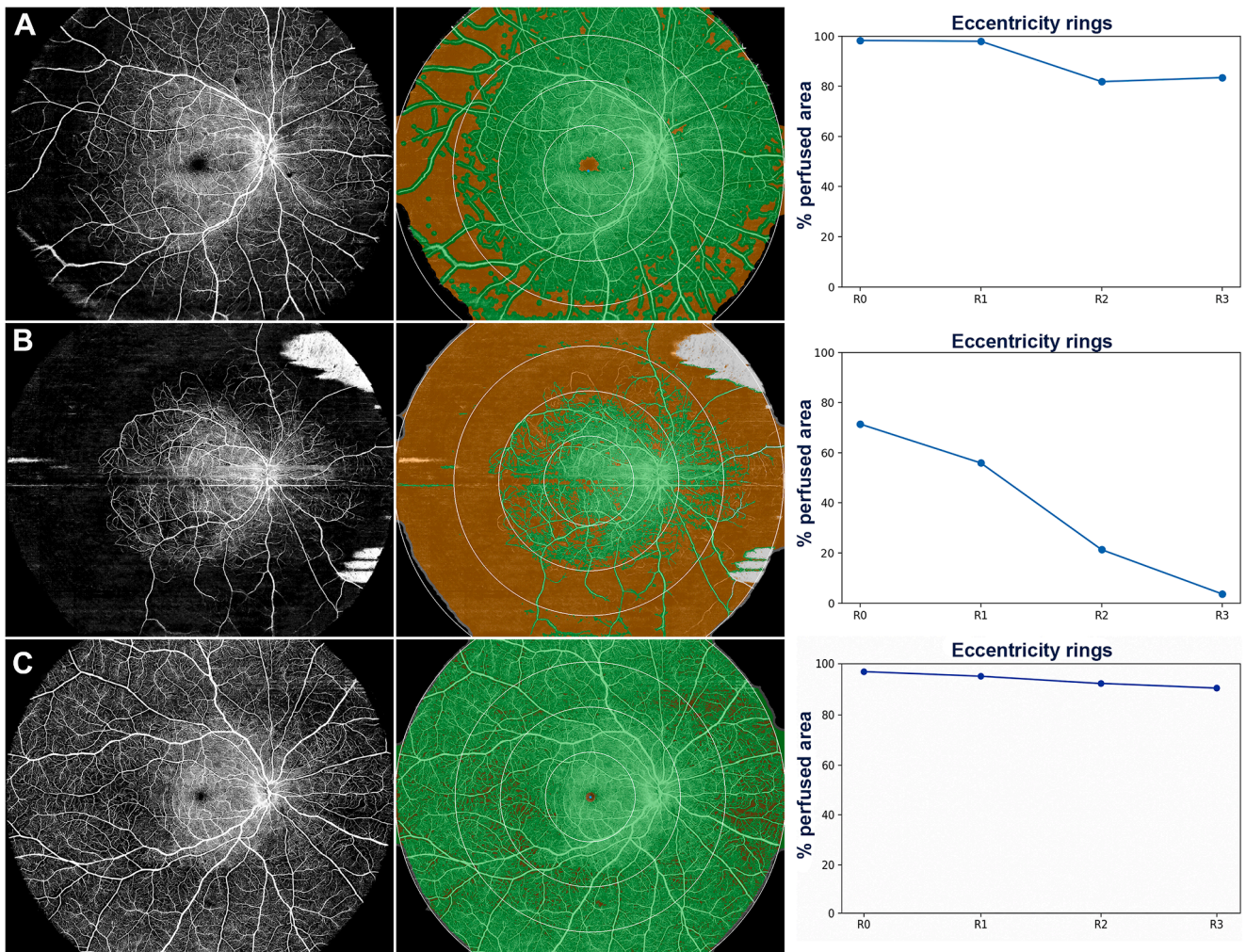


Figure 2. Widefield OCT angiography peripheral perfusion changes in RP. For all cases, we show the original WF-OCTA reconstruction, the green map representing the perfused retina, the orange map showing the nonperfused retina, and the profile of peripheral retinal perfusion. The first case (A) is characterized by mild central involvement. Peripheral perfusion decreases toward the more external rings. This is clearly shown in the graph plot. The second case (B) is characterized by both central and peripheral involvement, with severe perfusion loss in the outer rings, as shown by the graph plot. The third case (C) is a healthy control, to be considered as a reference. In panel B, localized nasal segmentation loss is visible. These regions were identified during quality assessment, masked, and excluded from the analysis. RP = retinitis pigmentosa; WF-OCTA = widefield OCT angiography.

vs. 0.27 ± 0.05 ; $F = 2.534$, $P = 0.03$) than eyes without retinal vascular attenuation. No other significant differences were found.

In addition, qualitative comparison between UWF pseudocolor fundus imaging and WF-OCTA provided complementary insights into peripheral retinal vascular involvement. While UWF pseudocolor allowed broader visualization of the far retinal periphery, WF-OCTA enabled a more detailed assessment of the peripheral microvasculature by directly detecting capillary flow. Importantly, WF-OCTA showed that some retinal vessels appearing attenuated on pseudocolor imaging still exhibited preserved perfusion signal (Fig 3). These findings underscore the ability of WF-OCTA to differentiate structural vascular attenuation from true nonperfusion, refining the characterization of peripheral retinal vascular attenuation in RP.

The Relationship between Bone Spicule and Peripheral and Central Vascular Status

The presence of bone spicule was significantly associated with CPPI after adjustment for age and BCVA on MANCOVA analysis (Wilks' $\lambda = 0.316$, $F = 7.143$, $P < 0.001$). Post hoc ANCOVA analyses reported significantly lower CPPI values in RP eyes with bone spicule at R0 (16 ± 17 vs. 19 ± 28 ; $F = 9.682$, $P < 0.001$), R1 (6 ± 6 vs. 9 ± 12 ; $F = 8.063$, $P < 0.001$), R2 (2 ± 3 vs. 11 ± 20 ; $F = 12.322$, $P < 0.001$), and R3 (1 ± 2 vs. 5 ± 9 ; $F = 47.630$, $P < 0.001$). Retinitis pigmentosa eyes with bone spicule were also characterized by significantly higher PPLG than RP eyes without bone spicule (47 ± 20 vs. 42 ± 24 ; $F = 3.939$, $P = 0.006$). The calculated PPS was $-0.73 \pm 0.12\%/grade$ for RP eyes with bone spicule vs. $-0.19 \pm 0.24\%/grade$ for RP eyes without bone spicule ($P < 0.001$). In addition, RP

Table 3. Correlation Analysis

Correlation Analysis		
Variable	Spearman ρ	Adjusted P Value
CPPI R0 vs. VFI %	0.522	0.003
CPPI R0 vs. CV 6° (sum/12) dB	0.512	0.004
CPPI R0 vs. preserved FAF area	0.407	0.02
CPPI R0 vs. HGB	−0.436	0.01
CPPI R0 vs. CMT	0.573	0.001
CPPI R0 vs. RPE	0.426	0.02
CPPI R0 vs. ONL	0.501	0.005
CPPI R0 vs. OPL	0.521	0.003
CPPI R0 vs. IPL	0.483	0.007
CPPI R0 vs. GCL	0.401	0.02
CPPI R0 vs. ICP VD	0.473	0.008
CPPI R1 vs. VFI %	0.401	0.02
CPPI R1 vs. CV 6° (sum/12) dB	0.426	0.01
CPPI R1 vs. preserved FAF area	0.414	0.02
CPPI R1 vs. HGB	−0.371	0.03
CPPI R1 vs. CMT	0.582	0.001
CPPI R1 vs. RPE	0.446	0.01
CPPI R1 vs. ONL	0.504	0.004
CPPI R1 vs. OPL	0.530	0.003
CPPI R1 vs. IPL	0.499	0.005
CPPI R1 vs. ICP VD	0.493	0.006
CPPI R2 vs. VFI %	0.421	0.02
CPPI R2 vs. CV 6° (sum/12) dB	0.457	0.01
CPPI R2 vs. preserved FAF area	0.435	0.01
CPPI R2 vs. retinal vascular attenuation	−0.369	0.03
CPPI R2 vs. CMT	0.592	0.001
CPPI R2 vs. RPE	0.403	0.03
CPPI R2 vs. ONL	0.535	0.002
CPPI R2 vs. OPL	0.520	0.003
CPPI R2 vs. IPL	0.491	0.006
CPPI R2 vs. ICP VD	0.475	0.008
CPPI R3 vs. VFI %	0.432	0.02
CPPI R3 vs. CV 6° (sum/12) dB	0.480	0.007
CPPI R3 vs. preserved FAF area	0.471	0.009
CPPI R3 vs. retinal vascular attenuation	−0.417	0.02
CPPI R3 vs. CMT	0.556	0.001
CPPI R3 vs. RPE	0.404	0.03
CPPI R3 vs. ONL	0.491	0.006
CPPI R3 vs. OPL	0.499	0.005
CPPI R3 vs. IPL	0.483	0.007
CPPI R3 vs. ICP VD	0.472	0.008
PPLG vs. preserved FAF area	−0.464	0.01
PPLG vs. CMT	−0.498	0.005
PPLG vs. ONL	−0.423	0.02
PPLG vs. OPL	−0.443	0.01
PPLG vs. IPL	−0.397	0.03
PPLG vs. ICP VD	−0.465	0.01
1.0° eccentricity AF-MPOD vs. BCVA	0.398	0.01
1.0° eccentricity AF-MPOD vs. full-retina VD	0.460	0.01
1.0° eccentricity AF-MPOD vs. FAZ Area	−0.592	0.001
1.0° eccentricity AF-MPOD vs. preserved FAF area	0.494	0.005
1.0° eccentricity AF-MPOD vs. hyperautofluorescent ring	0.488	0.006
1.0° eccentricity AF-MPOD vs. bone spicule	−0.422	0.02
2.0° eccentricity AF-MPOD vs. full-retina VD	0.404	0.02
2.0° eccentricity AF-MPOD vs. preserved FAF area	0.363	0.04
2.0° eccentricity AF-MPOD vs. bone spicule	−0.406	0.02
Interval 1.0°-2.0° eccentricity AF-MPOD vs. bone spicule	−0.375	0.04
6.0° eccentricity AF-MPOD vs. full-retina VD	0.527	0.003
6.0° eccentricity AF-MPOD vs. preserved FAF area	0.402	0.03
6.0° eccentricity AF-MPOD vs. bone spicule	−0.466	0.009
EZ horizontal vs. cystoid maculopathy	0.693	<0.001
EZ horizontal vs. CMT	0.470	0.009

(Continued)

Table 3. (Continued.)

Variable	Correlation Analysis	
	Spearman ρ	Adjusted P Value
EZ horizontal vs. bone spicule	−0.383	0.04
EZ vertical vs. cystoid maculopathy	0.645	<0.001
EZ vertical vs. CMT	0.455	0.01
EZ vertical vs. bone spicule	−0.444	0.02
Full-retina VD vs. cystoid maculopathy	0.612	<0.001
Full-retina VD vs. HGB	−0.412	0.03

AF-MPOD = autofluorescence-based macular pigment optical density; BCVA = best-corrected visual acuity; CMT: central macular thickness; CPPI = Concentric Peripheral Perfusion Index; CV = central visual field; EZ = ellipsoid zone; FAF = fundus autofluorescence; FAZ = foveal avascular zone; GCL = ganglion cell layer; HGB = hyperreflective ganglion cell band; HGB = hyperreflective ganglion cell band; ICP = intermediate capillary plexus; IPL = inner plexiform layer; ONL = outer nuclear layer; OPL = outer plexiform layer; RPE = retinal pigment epithelium; SCP = superficial capillary plexus; VD = vessel density; VFI = visual field index

eyes with bone spicule showed significantly lower macular VD values for ICP (0.20 ± 0.06 vs. 0.21 ± 0.09 ; $F = 2.601$, $P = 0.04$) and DCP (0.25 ± 0.05 vs. 0.28 ± 0.05 ; $F = 3.85$, $P = 0.02$) than eyes without bone spicule. No other significant differences were found.

The Relationship between Hyperautofluorescent Ring and Peripheral and Central Vascular Status

The presence of hyperautofluorescent ring was significantly associated with CPPI after adjustment for age and BCVA on MANCOVA analysis (Wilks' $\lambda = 0.334$, $F = 7.067$, $P < 0.001$). Post hoc ANCOVA analyses reported significantly higher CPPI values in RP eyes with hyperautofluorescent ring at R0 (20 ± 22 vs. 11 ± 15 ; $F = 10.440$, $P < 0.001$), R1 (9 ± 9 vs. 4 ± 4 ; $F = 9.122$, $P < 0.001$), R2 (7 ± 13 vs. 1 ± 1 ; $F = 13.851$, $P < 0.001$), and R3 (3 ± 6 vs. 1 ± 1 ; $F = 58.723$, $P < 0.001$). Peripheral perfusion loss gradient was significantly lower in RP eyes with hyperautofluorescent ring (41 ± 22 vs. 55 ± 15 ; $F = 5.271$, $P = 0.001$). The calculated PPS was $-0.51 \pm 0.34\%$ /grade for RP eyes with hyperautofluorescent ring vs. $-0.71 \pm 0.14\%$ /grade for RP eyes without hyperautofluorescent ring ($P < 0.001$). Moreover, RP eyes with hyperautofluorescent ring showed significantly higher macular VD values for ICP (0.22 ± 0.06 vs. 0.17 ± 0.07 ; $F = 6.624$, $P < 0.001$) and DCP (0.28 ± 0.05 vs. 0.24 ± 0.05 ; $F = 9.060$, $P < 0.001$), as well as significantly lower FAZ area (0.28 ± 0.11 mm² vs. 0.41 ± 0.10 mm²; $F = 5.537$, $P < 0.001$) than RP eyes without hyperautofluorescent ring. No other significant differences were found.

The Relationship between HGB and Peripheral and Central Vascular Status

The presence of HGB was significantly associated with CPPI after adjustment for age and BCVA on MANCOVA analysis (Wilks' $\lambda = 0.315$, $F = 7.576$, $P < 0.001$). Post hoc ANCOVA analyses reported significantly lower CPPI values in RP eyes with HGB at R0 (8 ± 9 vs. 27 ± 24 ;

$F = 7.004$, $P < 0.001$), R1 (4 ± 4 vs. 11 ± 10 ; $F = 6.339$, $P < 0.001$), R2 (1 ± 2 vs. 8 ± 15 ; $F = 12.797$, $P < 0.001$), and R3 (1 ± 1 vs. 4 ± 7 ; $F = 48.771$, $P < 0.001$). Peripheral perfusion loss gradient was significantly higher in RP eyes with HGB (53 ± 16 vs. 38 ± 23 ; $F = 3.311$, $P = 0.013$). The calculated PPS was $-0.67 \pm 0.14\%$ /grade for RP eyes with HGB vs. $-0.44 \pm 0.45\%$ /grade for RP eyes without HGB ($P < 0.001$). Moreover, RP eyes with HGB showed significantly lower macular VD values for ICP (0.16 ± 0.04 vs. 0.25 ± 0.07 ; $F = 15.137$, $P < 0.001$) and DCP (0.24 ± 0.03 vs. 0.29 ± 0.05 ; $F = 4.051$, $P = 0.005$), as well as significantly higher FAZ area (0.34 ± 0.13 mm² vs. 0.32 ± 0.11 mm²; $F = 4.867$, $P = 0.002$) than RP eyes without HGB. No other significant differences were found.

Discussion

In this study, we described peripheral vascular alterations characterizing *USH2A*-RP eyes. Our data revealed heterogeneous patterns of peripheral vascular nonperfusion, as quantified through our proposed WF-OCTA analysis pipeline. Specifically, we introduced the CPPI as a quantitative measure of peripheral retinal perfusion, defined as the ratio between perfused and non-perfused retinal areas. To ensure reproducibility and standardization, the analysis was based on fixed eccentricity rings spanning the entire 130° field of view of WF-OCTA. In addition, we introduced the PPLG as a quantitative metric reflecting the severity of perfusion reduction from the innermost retinal rings (macular region) toward the far periphery. This was further complemented by the PPS, which represents the overall rate of perfusion decline across retinal eccentricities and provides an estimate of the stability of the perfusion profile throughout the entire 130° retinal field. Accordingly, while CPPI may be considered a peripheral surrogate of the conventional OCTA VD metric, PPLG specifically quantifies the extent to which retinal perfusion progressively decreases with increasing eccentricity. In parallel, PPS captures the global steepness of this decline, thereby summarizing the overall distribution of retinal perfusion across the widefield scan.

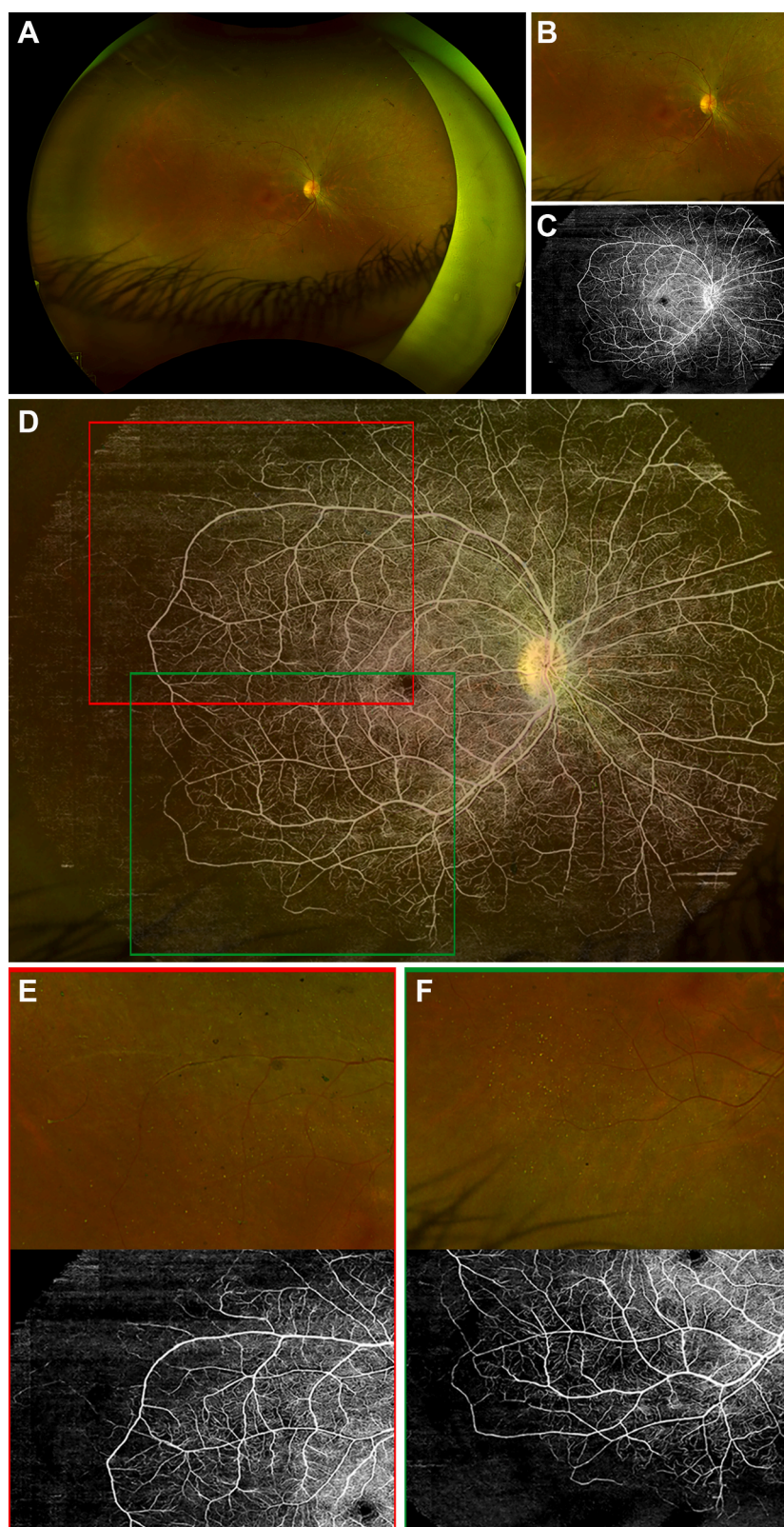


Figure 3. Qualitative comparison between UWF pseudocolor fundus imaging and WF-OCTA in RP for detecting retinal vascular attenuation. (A) Representative UWF pseudocolor fundus image provides retinal visualization extending up to the extreme periphery. (B) Magnified pseudocolor view of the central 130° field of view. (C) Corresponding 130° WF-OCTA image of the same eye, allowing direct visualization of the retinal vascular network and capillary perfusion. (D) Overlay between UWF pseudocolor and WF-OCTA demonstrating the complementary information provided by the two imaging

Eyes with RP showed significantly lower perfusion indices compared with healthy controls on WF-OCTA. This difference was more pronounced in the outer eccentricity rings, consistent with the known rod-to-cone centripetal pattern of retinal degeneration in RP. As expected, both CPPI and PPLG demonstrated significant correlations with visual field parameters and markers of macular involvement, supporting the concept that more severe peripheral retinal impairment is associated with greater macular structural and functional deterioration. Moreover, CPPI and PPLG significantly correlated also with morphological integrity of the central retina, as represented by retinal layer thicknesses. In RP, the primary damage is to photoreceptors, initially more peripheral and rod-dominant. Loss of photoreceptors reduces local metabolic demand, alters neurovascular coupling, and is associated with narrowing/rarefaction of the retinal vascular bed. For this reason, a very nonperfused periphery may represent a topographic marker of the global severity of degeneration. This was quantitatively assessed by WF-OCTA and our proposed metrics.

Previous OCTA studies support the presence of diffuse retinal vascular involvement in RP, although differences in acquisition field, device, segmentation strategy, genetic composition, and quantitative methodology limit direct comparisons.²¹ Rezaei et al²² used an OCT-based micro-angiography algorithm and a montage acquisition of approximately 6.7×6.7 mm, demonstrating abnormalities of the deep retinal and choroidal microvasculature associated with outer retinal structural damage. In a genetically characterized cohort, Hagag et al²³ identified macular microvascular abnormalities in patients carrying biallelic USH2A or MYO7A variants and reported associations between retinal vascular impairment, EZ preservation, and macular sensitivity. Widefield swept-source OCTA showed reduced perfusion density and vessel length density in both central and near/midperipheral retinal regions in RP eyes compared with healthy controls.²⁴ More recently, widespread structural and vascular abnormalities involving both the central and peripheral retina and choroid using UWF swept-source OCTA have been reported.²⁵

Our findings are consistent with this overall evidence of central and peripheral vascular impairment but extend previous observations by providing a standardized assessment of the entire analyzable retinal area captured within a single $26^\circ \times 21^\circ$ acquisition. The proposed CPPI, PPLG, and PPS metrics characterize not only the amount of preserved retinal perfusion but also its spatial decline from the posterior pole toward the periphery. Moreover, the restriction of the cohort to genetically confirmed USH2A-related RP reduces variability related to genetic

heterogeneity and enables peripheral perfusion profiles to be associated with macular structural, vascular, pigmentary, and functional biomarkers.

Consistent with this previous evidence, both CPPI and PPLG showed significant correlations with ICP status. This finding is biologically plausible and may be interpreted through potentially complementary mechanisms. Together with the DCP, the ICP belongs to the deep vascular complex, which represents the retinal vascular network most closely associated with the synaptic and nuclear layers involved in visual signal transmission from photoreceptors to inner retinal neurons.²⁶ The DCP is known to operate under relatively low perfusion pressure and is therefore often among the earliest plexuses to show vascular impairment in retinal disease. By contrast, the ICP receives a greater perfusion contribution from the SCP, which may render it relatively more resistant to mild vascular disturbances. Within this framework, the observed association between peripheral perfusion loss and ICP impairment in RP may reflect a broader pattern of retinal neurovascular remodeling. Progressive photoreceptor degeneration reduces metabolic demand and alters neurovascular coupling, with downstream effects extending from the outer retina to the inner retinal vascular networks. In this context, the ICP may represent a particularly sensitive compartment for detecting secondary microvascular changes related to photoreceptor loss, altered metabolic requirements, and impaired neurovascular regulation, thereby mirroring the extent of peripheral retinal involvement. Foveal avascular zone area did not differ significantly between RP and control eyes. Although some studies have reported FAZ enlargement in RP, particularly at the DCP, the available literature remains heterogeneous. Longitudinal data indicate that FAZ area may progressively increase during the course of RP, with potentially greater changes in the deeper vascular plexa.²⁷ However, this does not necessarily imply that FAZ enlargement is detectable in every cross-sectional cohort. Other case-control studies have reported no significant differences in superficial or deep FAZ area, or differences confined to patients with more impaired central visual function.²⁸

These discrepancies may reflect differences in disease stage, genotype, preservation of the central retina, OCTA platform, scan protocol, segmentation boundaries, and FAZ measurement technique, together with the substantial physiological interindividual variability of this parameter. In the present study, FAZ area was measured on the full-retina slab and therefore represented a composite measure integrating flow signals from the different retinal vascular plexuses. This approach may have reduced sensitivity to alterations confined to the ICP or DCP. Importantly,

modalities. In this way, it is possible to qualitatively evaluate the WF-OCTA signal corresponding to retinal vascular attenuation detected on UWF pseudocolor fundus image. Colored boxes identify representative peripheral regions selected for detailed comparison. (E–F) Magnified comparison of peripheral retinal areas highlighted in panel D. The upper panels show UWF pseudocolor images, and the lower panels show the corresponding WF-OCTA scans. In both examples, retinal vessels appearing markedly attenuated on pseudocolor imaging retain detectable flow signal on WF-OCTA, indicating partially preserved perfusion despite structural vascular narrowing. These findings highlight the ability of WF-OCTA to distinguish retinal vascular attenuation from true capillary nonperfusion and to refine the characterization of peripheral retinal vascular involvement in RP. RP = retinitis pigmentosa; UWF = ultra-widefield; WF-OCTA = widefield OCT angiography.

unchanged FAZ area coexisted with significantly reduced macular VD across multiple capillary plexuses, indicating that parafoveal capillary rarefaction may occur without measurable enlargement of the central avascular boundary. Future investigations should assess FAZ status separately at each retinal capillary plexus using standardized segmentation and acquisition protocols.

We also evaluated the potential contribution of additional imaging biomarkers. In particular, the presence of retinal vascular attenuation was associated with more severe peripheral perfusion profiles in our cohort. Retinal vascular attenuation, also referred to as hyalinized retinal vessels, represents a characteristic fundus sign of progressive peripheral vascular impairment and are widely recognized as a hallmark of disease progression in RP.²⁹ Consistently, a recent study reported significant associations between the extent of retinal vascular attenuation, reflecting progressive loss of peripheral retinal vasculature, and both morphological and functional impairment in RP.³⁰ However, the quantitative assessment of retinal vascular attenuation extension remains challenging for several reasons, including (I) interference from peripheral bone spicule pigmentation, which may obscure vessel margins; (II) variability in the quality and contrast of fundus images; and (III) imaging artifacts related to confocal pseudocolor acquisition, which may affect accurate vessel delineation. These limitations may reduce reproducibility and hinder precise quantification, further supporting the potential value of WF-OCTA-based perfusion metrics as objective biomarkers of peripheral vascular involvement in RP. For this reason, in this study, we decided to include only the presence of retinal vascular attenuation, without introducing poorly standardizable quantification. As expected, the significant association involved more peripheral WF-OCTA rings (R2 and R3).

Widefield OCTA metrics also showed significant relationships with central and peripheral pigments changes. Central macular pigments were significantly lower in RP eyes than in healthy controls at all assessed eccentricities except 6.0°. The absence of a significant difference at 6.0° may reflect the physiologically low macular pigment density at this eccentricity, which reduces the magnitude and detectability of between-group differences. The presence of bone spicule was associated with lower CPPI values and higher PPLG values. Interestingly, RP eyes with hyperautofluorescent ring showed higher CPPI values and lower PPLG values. The presence of bone spicule and the absence of the hyperautofluorescent ring were overall associated with a worse vascular and morphological status of the macula, considering OCT, OCTA, and AF-MPOD assessments. This can explain the significant association between WF-OCTA metrics and the extension of the central preserved FAF area found in the present study.

The inverse association between macular pigment levels and bone spicules may reflect a more advanced and widespread stage of retinal degeneration in RP. Bone spicules represent intraretinal migration of RPE cells secondary to progressive photoreceptor loss and are considered a hallmark of advanced peripheral retinal remodeling.³¹ Their presence may therefore identify RP eyes with more

diffuse retinal involvement, in which secondary macular structural and metabolic alterations reduce the accumulation and maintenance of macular carotenoids. In this context, decreased macular pigments and peripheral bone spicule pigmentation may represent parallel manifestations of a common degenerative process involving photoreceptors, RPE dysfunction, retinal remodeling, and impaired retinal homeostasis.

At the same time, the positive association between macular pigment levels and the presence of a hyperautofluorescent ring is biologically plausible and may reflect better preservation of central retinal architecture in RP eyes showing this phenotype. The hyperautofluorescent ring is widely considered a topographic marker delineating the transition between relatively preserved central retina and more advanced peripheral photoreceptor degeneration.¹¹ Eyes with a visible ring generally show greater preservation of the EZ and outer retinal structure within the central macula.¹¹ Because macular pigments are concentrated within the foveal region and depend on the integrity of retinal neuronal and Müller cell architecture,³² their higher levels in RP eyes with hyperautofluorescent ring may reflect better maintenance of central retinal homeostasis. In this context, the hyperautofluorescent ring and increased macular pigments may represent parallel markers of relative foveal preservation and a less advanced stage of central retinal involvement in RP.

In addition, we found a significant negative association between WF-OCTA peripheral vascular status and the presence of HGB. Hyperreflective ganglion cell band is a recently described OCT biomarker in RP and has been associated with SCP-related gliotic changes, as well as with more pronounced impairment of the macular intraretinal vascular network showing a centripetal distribution.^{8,9} Therefore, its association with more severe peripheral vascular impairment appears biologically plausible. In RP, progressive photoreceptor degeneration is known to trigger secondary retinal remodeling involving not only the outer retina but also the inner retinal layers, including Müller cell activation, gliosis, and progressive microvascular alterations.^{9,32} Within this framework, HGB may represent a structural marker of inner retinal remodeling linked to these neurovascular changes. Its association with worse peripheral perfusion suggests that HGB may reflect a more diffuse disease phenotype, in which peripheral vascular rarefaction parallels macular neurovascular and glial involvement. These findings further support the potential clinical relevance of HGB in RP, expanding its role beyond a localized macular OCT feature and suggesting that it may serve as an indicator of broader retinal disease severity, integrating both central and peripheral manifestations of retinal degeneration.

This study has several limitations that should be acknowledged. First, its cross-sectional design limits the ability to draw conclusions regarding temporal relationships or causality between peripheral vascular changes, macular alterations, and functional impairment in RP. Longitudinal studies will be necessary to better define the sequence and progression of these retinal changes over time. A further limitation relates to image acquisition. Retinal imaging

measurements may be influenced by several technical factors, including suboptimal focus, signal attenuation, and media opacities.³¹ More broadly, all imaging modalities used in multimodal retinal assessment remain susceptible to acquisition- and interpretation-related artifacts.³³ To reduce these confounding effects, we included only patients with clear ocular media and adequate image quality. Nevertheless, because of the exploratory nature of the study and to minimize patient burden during a multimodal imaging protocol, only a single acquisition was obtained for each imaging modality. As a result, intrasession and intersession repeatability could not be formally assessed. We also included only one eye per participant to avoid statistical bias related to intereye correlation. Although methodologically appropriate, this approach may introduce selection bias, particularly in inherited retinal dystrophies, where intereye asymmetry may occasionally occur. Future studies including bilateral imaging or mixed-effects statistical modeling may help determine whether eye selection influences quantitative retinal imaging outcomes. Another limitation concerns the distribution of disease severity within the cohort. Although RP stage was reported descriptively, the relatively small number of eyes in the earliest (grade 1) and most advanced (grade 3) stages limited the possibility of meaningful stage-specific subgroup analyses. Larger cohorts with a more balanced representation across disease stages will be important to clarify the relationship between disease severity and the imaging biomarkers investigated here.

Cystoid maculopathy was also included among the imaging variables analyzed. However, because only a limited number of RP eyes presented this feature (6/40 eyes), dedicated subgroup analyses comparable to those performed for other parameters, such as HGB, were not feasible. Prospective studies with larger cohorts will be required to better define the relationship between peripheral vascular impairment and cystoid maculopathy in RP.

An additional consideration concerns the multifactorial determinants of retinal pigment variability. Macular pigment density and distribution may be influenced by a wide range of systemic and local factors, including oxidative stress, inflammatory pathways, metabolic syndrome, obesity, and dyslipidemia.³⁴ Therefore, the retinal changes observed through multimodal imaging likely represent the combined effect of several biological mechanisms, and imaging alone cannot fully disentangle the relative contribution of each underlying pathway.

Regarding WF-OCTA, our analysis was limited to the 130° field of view. We acknowledge that DREAM OCT Intalight device enables a wider field of view through montage acquisition. However, montage images provide limited segmentation options, with no possibility for manual correction. Moreover, given the pronounced peripheral retinal involvement typically observed in RP, the use of montage UWF-OCTA images could increase the impact of segmentation artifacts, thereby reducing the reliability of the analysis. In addition, montage UWF-OCTA images do not provide a standardized retinal field of view, as the final extension largely depends on patient cooperation during acquisition. By contrast, restricting the

analysis to single-scan WF-OCTA images with a 130° field of view minimized imaging artifacts while ensuring a standardized field of view across all included eyes. Future studies should investigate peripheral vascular alterations using larger retinal fields whenever technically feasible. The use of full-retina WF-OCTA reconstructions may produce partial merging of vascular signals within the posterior pole because of the superimposition of multiple retinal capillary plexuses and the representation of a large retinal field within a single enface image. This may reduce the precision of individual vessel boundaries and influence absolute central perfusion estimates. Although the use of the same acquisition and processing protocol in all participants limits its impact on between-group comparisons, direct comparison of absolute values across different OCTA platforms should be performed cautiously. At the same time, current limitations of montage-based UWF-OCTA, including potential misalignment, variable peripheral signal quality, and stitching artifacts, should be carefully considered when interpreting broader peripheral vascular measurements. An additional limitation concerns FAZ assessment. Although vascular alterations in RP may preferentially involve the intermediate and deep capillary plexa, FAZ area was measured only on the full-retina slab. This approach provided a composite estimate of the macular avascular region but did not permit the evaluation of plexus-specific FAZ changes. We selected the full-retina segmentation because FAZ delineation within the deeper vascular slabs may be affected by less distinct vascular boundaries, segmentation inaccuracies, projection-related effects, RP-associated retinal thinning, and substantial physiological interindividual variability. Nevertheless, this methodological choice may have reduced sensitivity to early or layer-specific microvascular abnormalities. Future studies should investigate the relationship between peripheral perfusion impairment and FAZ area, separately at the superficial, intermediate, and deep capillary plexa.

Finally, this study focused exclusively on USH2A-related RP. Further investigations across additional genetic subtypes of RP, including both syndromic and nonsyndromic forms, will be necessary to determine whether the imaging patterns identified in this cohort reflect shared mechanisms of retinal degeneration or represent genotype-specific phenotypes.

In conclusion, this study provides a multimodal characterization of peripheral vascular alterations in RP, integrating WF-OCTA, UWF fundus imaging, FAF, OCT, OCTA, and AF-MPOD. In particular, for WF-OCTA, we developed a quantitative analysis pipeline aimed at standardizing the assessment of peripheral retinal perfusion abnormalities in retinal diseases. Our findings highlight the close relationship between peripheral and central retinal alterations in RP, supporting the concept that retinal degeneration in this condition represents a widespread process involving not only the outer retina, but also broader metabolic, vascular, anatomical, and functional retinal networks.

Taken together, these results further expand the current understanding of RP pathophysiology and suggest that WF-OCTA-derived peripheral perfusion metrics may provide clinically meaningful biomarkers of disease

burden and retinal involvement. Future prospective longitudinal studies are warranted to further define the diagnostic and monitoring value of WF-OCTA and to clarify

its potential role in the clinical assessment and follow-up of patients with RP.

Footnotes and Disclosures

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¹ Ophthalmology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy.

² Eye Repair Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy.

³ Medical School, University Vita-Salute San Raffaele, Milan, Italy.

⁴ Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy.

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HUMAN SUBJECTS: Human subjects were included in this study. Signed informed consent was obtained before inclusion. The study was performed in accordance with the Declaration of Helsinki and good clinical practice, under the local ethical committee approval (ethical committee of IRCCS San Raffaele Scientific Institute in Milan; protocol ID: PNRR-MR1-2023-12378435).

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Author Contributions:

Conception and design: Arrigo, Aragona, Antropoli, Cremona, Casoni

Analysis and interpretation: Arrigo, Aragona, Battaglia Parodi, Bandello

Data collection: Arrigo, Forte, Aragona, Pini, Petruzzello, Cremona, Casoni, Antropoli, Bianco, Battaglia Parodi, Bandello

Obtained funding: Battaglia Parodi

Overall responsibility: Arrigo, Forte, Aragona, Pini, Petruzzello, Cremona, Casoni, Antropoli, Bianco, Battaglia Parodi, Bandello

Abbreviations and Acronyms:

AF = autofluorescence; **AF-MPOD** = autofluorescence-based MPOD; **ANCOVA** = analysis of covariance; **BCVA** = best-corrected visual acuity; **CPPI** = Concentric Peripheral Perfusion Index; **DCP** = deep capillary plexus; **EZ** = ellipsoid zone; **FAF** = fundus autofluorescence; **FAZ** = foveal avascular zone; **HGB** = hyperreflective ganglion cell band; **ICP** = intermediate capillary plexus; **IOP** = intraocular pressure; **MANCOVA** = multivariate analysis of covariance; **MPOD** = macular pigment optical density; **OCTA** = OCT angiography; **PPLG** = peripheral perfusion loss gradient; **PPS** = perfusion profile slope; **RP** = retinitis pigmentosa; **RPE** = retinal pigment epithelium; **SCP** = superficial capillary plexus; **UWF** = ultra-widefield; **VD** = vessel density; **WF-OCTA** = widefield OCT angiography.

Keywords:

Widefield, OCTA, Retinitis pigmentosa, OCT, Ultra-widefield.

Correspondence:

Alessandro Arrigo, MD, PhD, Department of Ophthalmology, IRCCS San Raffaele Scientific Institute, via Olgettina 60, Milan, 20132, Italy. E-mail: alessandro.arrigo@hotmail.com.

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