MORPHOFUNCTIONAL EVALUATION OF MACULAR-FOVEAL CAPILLARIES

A Comparative Optical Coherence Tomography Angiography and Microperimetry Study

ELISABETTA PILOTTO, MD, FEBO,* FRANCESCA LEONARDI, MD, FEBO,* DAVIDE DEGANELLO, MD,* ENRICA CONVENTO, MSc,* EDOARDO MIDENA, MD, PhD, FEBO, FARVO,*† LUISA FRIZZIERO, MD, FEBO†

Purpose: To analyze the macular function of eyes with macular-foveal capillaries (MFC), a condition characterized by the absence of the foveal avascular zone (FAZ), identified by optical coherence tomography angiography.

Methods: Eight eyes with MFC at optical coherence tomography angiography and normal visual acuity were consecutively recruited. Eight eyes of healthy subjects were enrolled as healthy controls. All eyes underwent optical coherence tomography, optical coherence tomography angiography, best-correct visual acuity, low-luminance visual acuity, contrast sensitivity measurement, colour vision tests, and both mesopic and scotopic microperimetry.

Results: Best-corrected visual acuity, low-luminance visual acuity, contrast sensitivity, and colour vision tests did not differ between the two groups. At mesopic microperimetry, both foveal retinal sensitivity and mean mesopic retinal sensitivity of the central 1° were statistically inferior in MFC versus control eyes ($P < 0.0001$ and $P < 0.0001$, respectively). At scotopic microperimetry, a dense foveal scotoma, normally present in control eyes, was completely lacking in MFC eyes. Scotopic foveal retinal sensitivity was statistically superior in MFC versus control eyes ($P = 0.009$).

Conclusion: The absence of the foveal dense scotoma in scotopic conditions underlines that the foveal rod-free zone is not present when capillaries are present in this area. An anomalous foveal distribution of photoreceptors, with both rods and cones present in this area, may be postulated in MFC eyes.

RETINA 00:1–7, 2019

Fovea centralis, also known as fovea, is characterized by a slope of the inner retina layers (foveal pit), as a result of the outward displacement of ganglion, bipolar, and amacrine cells during development, at the center of the foveola.[1,2] In the foveola, cone photoreceptors and Müller cells are the only cellular subtypes, with full lack of both inner retinal layers and rods (rod-free zone).[1–3] The foveola is centered in a small region where retinal vasculature is completely absent: the foveal avascular zone (FAZ). But, FAZ may be sometimes absent: a peculiar anatomical condition referred as macular-foveal capillaries (MFC). The term MFC, proposed by Yeung et al[4] in 1973 and more recently also used by Cicinelli et al,[5] describes the complete or partial absence of the physiologic FAZ, which is instead crossed by intraretinal vascular nets that communicate with the surrounding retinal capillary networks. Macular-foveal capillaries may be observed even in subjects without macular dystrophies and normal visual acuity.[5,6] However, visual acuity quantification (which is a high contrast visual function) has many drawbacks and cannot be

From the *Department of Ophthalmology, University of Padova, Padova, Italy; and †IRCCS-Fondazione Bietti, Rome, Italy.

The research contribution by IRCCS Fondazione Bietti was supported by Fondazione Roma and Ministry of Health.

None of the authors has any financial/conflicting interests to disclose.

E. Midena and E. Pilotto had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Reprint requests: Edoardo Midena, MD, PhD, FEBO, FARVO, Department of Ophthalmology, University of Padova, Via Giustiniani 2, 35128 Padova, Italy; e-mail: edoardo.midena@unipd.it

considered a complete visual function test. Microperimetry is a noninvasive method used to analyze retinal fixation and macular sensitivity in a topographic-related manner. The introduction of mesopic and more recently scotopic microperimetry, in research and clinical practice, allows to better investigate macular cone and rod function respectively, strictly related to macular morphology.[7,8]

The aim of this study was to extensively investigate foveal function in eyes with MFC detected at optical coherence tomography angiography (OCTA) and with normal best-corrected visual acuity (BCVA), using low-luminance visual acuity (LLVA), contrast sensitivity and colour tests, and both mesopic and scotopic microperimetry.

Material and Methods

This study was compliant with the tenets of the Declaration of Helsinki and approved by an institutional review board. Informed consent was obtained from each patient. Patients with an incidental finding of MFC at OCTA were consecutively recruited between January 2017 and April 2017. Inclusion criteria were as follows: presence of MFC at the full retina OCTA enface image, refraction between −6 and +6 diopters, adequate optical coherence tomography (OCT) linear scans, adequate OCTA enface images (signal strength index more than 7), and normal BCVA. Exclusion criteria were as follows: history of any ophthalmologic disease affecting visual function or impairing fixation and any alteration of retina or choroid that could modify OCT analysis. Healthy eyes without MFC and normal FAZ appearance were enrolled as healthy controls.

At enrollment, all included eyes underwent full ophthalmic examination, including BCVA and LLVA measurement, Pelli Robson contrast sensitivity test and color Farnsworth–Munsell 100-hue color tests, and both mesopic and scotopic microperimetry. Best-corrected visual acuity and LLVA measurement, both expressed as Snellen visual acuity ratios and the logarithm of minimal angle of resolution, were performed using standard Early Treatment Diabetic Retinopathy Study (ETDRS) charts using a standard protocol for refraction and measurement of visual acuity. A neutral density filter (Kodak Wratten Filter, Rochester, NY, causing a reduction of 2.0 log unit in luminance) was placed in front of the tested eye to perform LLVA.

Optical Coherence Tomography and Optical Coherence Tomography Angiography

Optical coherence tomography and OCTA were performed using Spectralis HRA + OCTA (Heidelberg Engineering, Heidelberg, Germany). The scan protocol included the following: a single horizontal scan (180° line scan, 9-mm length, automated real time set at 100 frames) and an OCTA scan pattern of $10° \times 10°$ (3.0×3.0 mm; 512 B-scans separated by 6 μm) both centered onto the fovea. The inbuilt software automatically generated the enface OCTA images of the full retina (extending from the inner limiting membrane to the Bruch membrane) and of the superficial vascular complex (SVC) and deep vascular complex (DVC), as automatically segmented by the device: the SVC from the inner limiting membrane to the inner plexiform layer and the DVC from the inner plexiform layer to the outer plexiform layer.

Microperimetry

Mesopic microperimetry was performed using MP1 Microperimeter (MP1; Nidek Technologies, Gamagori, Japan) using the following parameters: a red ring fixation target, 2° in diameter, with a white monochromatic background at 4 abs; stimulus size Goldman III with projection time of 200 ms. A 4-2 double-staircase strategy and a customized grid of 57 points covering the central 20° centered on the anatomical fovea was used with an automatic eye tracker.

Scotopic microperimetry, which evaluates rod function, was performed using MP1S Microperimeter (MP1 scotopic Nidek Technologies), a modified version of the MP1 Microperimeter. This technique has been previously described in details.[7] Scotopic microperimetry was performed after dark adaptation for 30 minutes in a dark room (−0.1 lux). For the purpose of this study, the following parameters were used: a 1.0-log unit neutral density filter; a fixation target consisting of a white circle, 3° in radius with "fade-out protection mode" activated; background luminance of 0.0025 cd/m^2; stimulus size Goldman IV with projection time of 200 ms; and 4-2 double-staircase strategy and a customized grid of 57 points covering the central 20°centered on the anatomical fovea.[7] In both mesopic and scotopic tests, dense scotomas were defined as tested loci that elicited no response even at the highest tested stimulus (0 dB). The following parameters were quantified: foveal retinal sensitivity (RS) (consistent with the stimulus located in the center of the grid), and mean RS of

central 1° (containing the remaining stimuli located in the central 1° area). Moreover, at scotopic microperimetry, the presence of dense scotopic foveal scotoma normally present (consistent with the rod-free zone) was also evaluated.

Statistical Analysis

Parameters have been summarized according to the usual methods for descriptive statistics: The mean, SD, and range (minimum and maximum values) have been computed.

Age of the two groups has been compared by the Wilcoxon–Mann–Whitney test.

Because of replication of measurement on both eyes of all patients and controls, comparisons between groups (Patients vs. Controls) have been made by two-way analysis of variance mixed-effect model (PROC MIXED) for repeated measures, with the group and eye as "between" and "within" factors, respectively, and the compound symmetry covariance structure for the measures in the two eyes.

For all the analyses, statistical software SAS 9.3 has been used. All statistical tests have been interpreted as significant when $P < 0.05$.

Results

Eight eyes of five patients (three women and two men) with MFC and eight control eyes of four subjects (two women and two men) were studied. The mean age of MFC and controls was 45.2 ± 19.5 years and 25 ± 1.63 years, respectively ($P = 0.1761$). In all eyes, FAZ was not present both in the full retina and in the DVC. In four eyes of two patients, FAZ was detectable in the SVC but not in the DVC. No history of prematurity or other ophthalmological conditions that can be associated with foveal hypoplasia and lack of FAZ (albinism, achromatopsia, aniridia, nanophthalmos, and incontinentia pigmenti) was found. A shallow foveal pit incursion of inner retinal layers, outer nuclear layer widening, and outer segments lengthening, consistent with Grade 1 foveal hypoplasia, according to the OCT-based grading system, was detected in all MFC eyes[9] (Figure 1). A normal foveal pit was present in all control eyes. The central subfield thickness (CST, the average thickness of the macula in the central 1-mm ETDRS grid) was significantly higher in MFC eyes compared with controls, as expected, because of the foveal pit feature.

Both BCVA and LLVA did not differ between the studied groups ($P = 0.3246$ and $P = 0.3923$, respectively). At mesopic microperimetry, both foveal RS and mean mesopic RS of the central 1° were statistically different between the two groups (16.71 ± 0.55 dB vs. 19.87 ± 0.35 dB, $P < 0.0001$ and 16.01 ± 1.29 dB vs. 19.54 ± 0.71 dB $P < 0.0001$, in MFC vs. control eyes, respectively). Retinal fixation was central and stable in all studied eyes. At scotopic microperimetry, the physiological dense foveal scotoma (corresponding to the rod-free zone) was normally present in control eyes. Conversely, it was undetectable in all MFC eyes, and a rod response was recordable at the fovea (Figure 2). Scotopic foveal RS was statistically different between the groups (5.13 ± 3.04 dB in MFC vs. 0.0 ± 0.0 dB in control eyes, $P = 0.009$). Scotopic RS in the remaining central 1° was slightly superior in the MFC eyes than in controls (8.01 ± 3.82 dB vs. 4.59 ± 4.3 dB, $P = 0.163$) (Table 1). Colour vision and contrast sensitivity were normal in both groups.

Discussion

Macular-foveal capillaries, an anatomical foveal condition characterized by the absence of FAZ due to the presence of foveal capillaries, have been reported not only in individuals with a multitude of ocular diseases (including albinism, aniridia, achromatopsia, nanophthalmos, Prader–Willi syndrome, and retinopathy of prematurity) but also in subjects with normal visual acuity.[10–14] In this study, using mesopic and scotopic microperimetry, we documented the presence of an abnormal foveal mesopic and scotopic function in eyes with MFC and normal visual acuity without any associated ocular diseases. No other visual function test differed between MFC and control eyes. Moreover, in all MFC cases, Grade 1 foveal hypoplasia was detected. Recently, Yokoyama et al[6] found MFC in 1.5% of 267 normal eyes with normal visual acuity. A low grade of foveal hypoplasia was present in all their cases.[6] The presence of the FAZ is an essential condition for the normal foveal development.[1,2] In animal models, during development, the FAZ is defined before the foveal pit formation.[15] In the human eye, the foveal area is never vascularized throughout its development, and a complete circle of vessels, encompassing the mature FAZ, is present at 37-week gestation (WG).[2] It has been hypothesized that Müller cells and/or local antiangiogenetic factors (mainly expressed by ganglion cells of the incipient fovea) may early prevent the formation of blood vessels in this area.[2,16,17] Foveal pit formation is directly linked to

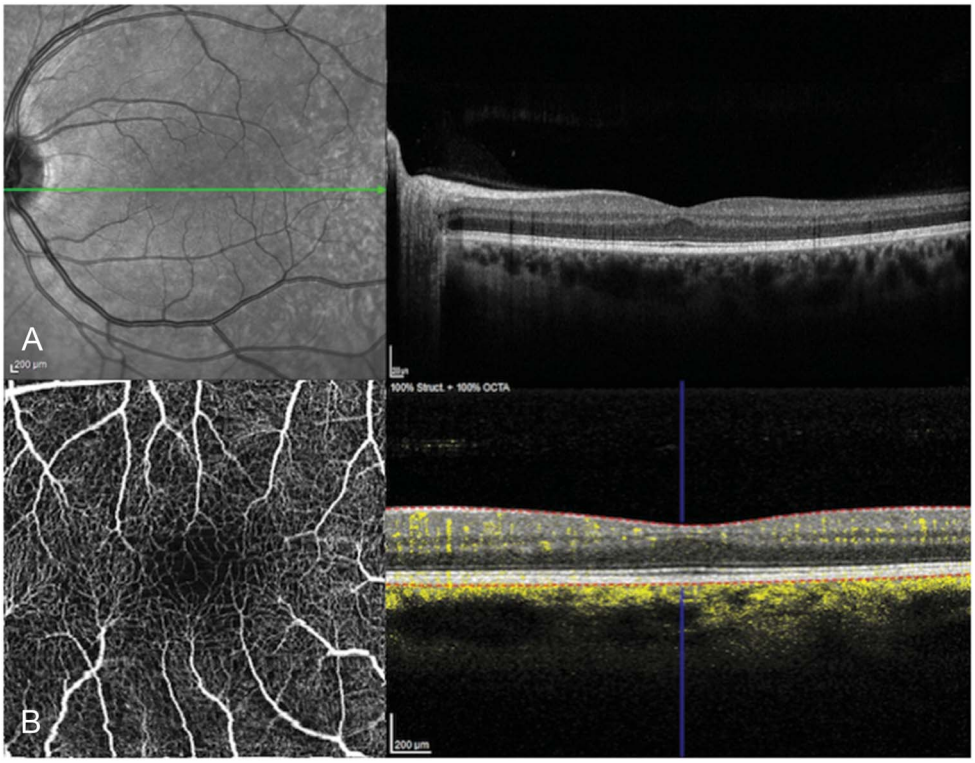

Fig. 1. A and **B.** Optical coherence tomography and OCTA in a MFC eye. The linear OCT scan, centered onto the fovea, shows a Grade 1 of foveal hypoplasia (**A**). The en-face OCTA of the full retina (extending from the inner limiting membrane to the Bruch membrane) of the same eye shows the absence of the FAZ (**B**).

the presence of a normal FAZ; a foveal depression does not develop when retinal vessels are present.[17] Pit formation begins shortly after 24 to 25 WG with outward displacement of ganglion cells and bipolar and amacrine cells in the inner retina. By 13 months to 15 months of age, pit formation is completed with a single layer of photoreceptors in the pit center.[18] Changes in the outer retina mainly occur after birth, with a centripetal displacement of photoreceptors, packing, and elongation of both inner- and outer-cone segments.[2,17,18]

Histologic studies report that at 20 to 27 WG, rods are absent across the central 1,500 μm to 1,800 μm, forming the rod-free zone. At 28 to 37 WG, when a distinct foveal pit with an outward displacement of the inner retinal layers is detectable, the rod-free zone is narrower, with rods present outside 600 μm from the pit center.[18,19] Immunolabeled studies suggest that FAZ is centered onto the rod-free zone throughout development.[2] A rod-free zone is a precondition for the development of a foveal pit, and it is hypothesized to be produced by inhibition of the rod development from late retinal progenitor cells.[1,17] Several soluble factors, both stimulatory and inhibitory, seem to be involved in rods differentiation.[20] Owing to the absence of rods in the foveal region, a scotopic response is not usually detectable in this area. In this study, a rod response in the foveal area (analyzing rod function) was detected

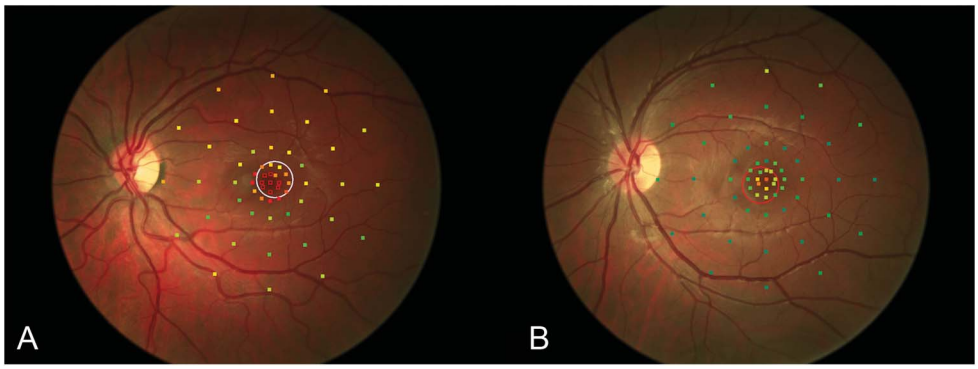

Fig. 2. A and **B.** Scotopic microperimetry in an eye with normal FAZ appearance (**A**) and in a MFC eye (**B**). The tested stimuli on the retina are represented in different colors according to each scotopic sensitivity value. A dense foveal scotoma (empty red squares), normally present in the normal eye, is not detectable in the MFC eye.

Table 1. Macular Function in Macular-Foveal Capillaries Eyes and Control Eyes

			Visual Acuity		Mesopic Microperimetry (Retinal Sensitivity)		Scotopic Microperimetry (Retinal Sensitivity)		OCT and OCTA Analysis				
											FAZ Identification		
Patient	Age	Eye	BCVA Snellen (Letters)	LLVA Snellen (Letters)	Foveal (Decibel)	Central 1° (Decibel)	Foveal (Decibel)	Central 1° (Decibel)	CST	Foveal Hypoplasia Grade	Full Retina	SVC	DVC
1	26	RE	20/16 (89)	20/25 (82)	17	16.8	9	12.1	352	1	No	Yes	No
		LE	20/16 (90)	20/25 (83)	16	13	7	12.4	348	1	No	Yes	No
2	69	RE	20/16 (90)	20/25 (79)	17.2	16.7	2	1.5	299	1	No	No	No
3	52	RE	20/20 (84)	20/25 (82)	17	16.7	4	4.5	311	1	No	No	No
4	24	RE	20/16 (93)	20/16 (89)	16.2	16	6	9.3	319	1	No	Yes	No
		LE	20/13 (95)	20/16 (9) 0	17	16.8	2	10.2	321	1	No	Yes	No
5	55	RE	20/25 (80)	20/32 (78)	17.3	16.5	9	8.3	311	1	No	No	No
		LE	20/25 (81)	20/25 (80)	16	15.6	2	5.8	313	1	No	No	No
MFC eyes (mean ± SD)	45.2 ± 19.5		20/20 (87.8 ± 5.5)	20/25 (82.9 ± 4.4)	16.71 ± 0.55	16.01 ± 1.29	5.13 ± 3.04	8.01 ± 3.82	321.75 ± 18.66				
1	27	RE	20/13 (95)	20/25 (82)	20	18.1	0	2	283	NA	Yes	Yes	Yes
		LE	20/13 (94)	20/16 (89)	20	20	0	0.6	284	NA	Yes	Yes	Yes
2	25	RE	20/16 (89)	20/20 (85)	20	20	0	2.1	244	NA	Yes	Yes	Yes
		LE	20/16 (89)	20/20 (85)	20	19.2	0	14.3	252	NA	Yes	Yes	Yes
3	25	RE	20/16 (90)	20/20 (84)	20	20	0	2.9	262	NA	Yes	Yes	Yes
		LE	20/16 (89)	20/20 (84)	19	19	0	4	264	NA	Yes	Yes	Yes
4	23	RE	20/16 (90)	20/25 (82)	20	20	0	6.3	253	NA	Yes	Yes	Yes
		LE	20/16 (89)	20/25 (83)	20	20	0	4.5	254	NA	Yes	Yes	Yes
Control eyes (mean ± SD)	25 ± 1.63		20/16 (90.6 ± 2.2)	20/20 (84.3 ± 2.3)	19.87 ± 0.35	19.54 ± 0.71	0 ± 0	4.59 ± 4.3	262 ± 14.63				
P	0.1761		ns	ns	<0.0001	<0.0001	0.009	0.163	0.0019				

DVC, deep vascular plexus; ns, not significant; SVC, superficial vascular plexus.

in vivo MFC eyes, by means of scotopic microperimetry. Scotopic microperimetry is a new visual function technique that allows for the quantification of rod sensitivity in dark-adapted eyes in a dark environment, correlating it to the fundus features. Its usefulness has been recently documented in both acquired and congenital maculopathies.[21,22] The lack of FAZ, foveal pit, and rod-free zone has been described, using light and electron microscopy, in a case of oculocutaneous albinism.[23] In this case, both photoreceptors were present in the macular region, with cones equal or slightly superior in number to rods.[23] Therefore, we hypothesize an anomalous foveal distribution of photoreceptors in MFC eyes. However, it is believed that the development of the foveal outer retina is partially independent from that of the inner retina; our results suggest that a possible link may exist between foveal capillaries and photoreceptor differentiation in the fovea.[17] Moreover, when SVC and DVC were analyzed separately, FAZ was absent in DVC in all MFC eyes but was detectable in the SVC of just two patients (Figure 3). These data suggest that the presence of central foveal retinal capillaries in the DVC may be relevant (compared to SVC) to foveal development and, therefore, to physiologic foveal function.

Prematurity was excluded in all our patients. Several studies reported a reduced or absent FAZ with the persistence of inner retinal layers in the foveal region of premature infants. Although the photoreceptor topographic layout starts very early, with a complete rod-free zone visible since 26 WG, centrifugal migration of the inner retinal layers occurs only after vascular maturation (37–40 WG). Therefore, preterm birth and the accompanying change in retinal oxygen tension may influence only FAZ formation and subsequent displacement of inner retinal layers away from the incipient fovea, but not the topographic distribution of photoreceptors, which already occurred.[16,24,25] Even if no apparent paucity of foveal cones has been detected in adults with mild ROP by means of an adaptive optics Fourier-domain OCT, according to our results, further studies aimed at investigating the function of both photoreceptors (rods and cones) may be useful to better identify the distribution of photoreceptors in this area.[25]

The small sample size is the main limitation of this study, but MFC is an unusual finding in healthy population (1.5% of normal eyes).[6]

In conclusion, this study shows the absence of the normal scotopic scotoma at the fovea of patients with congenital MFC and normal visual acuity, suggesting that the foveal rod-free zone may be absent when capillaries develop in this area. Additional studies may be useful to better understand the exact correlation between the lack of the FAZ, foveal pit, and photoreceptors differentiation during development.

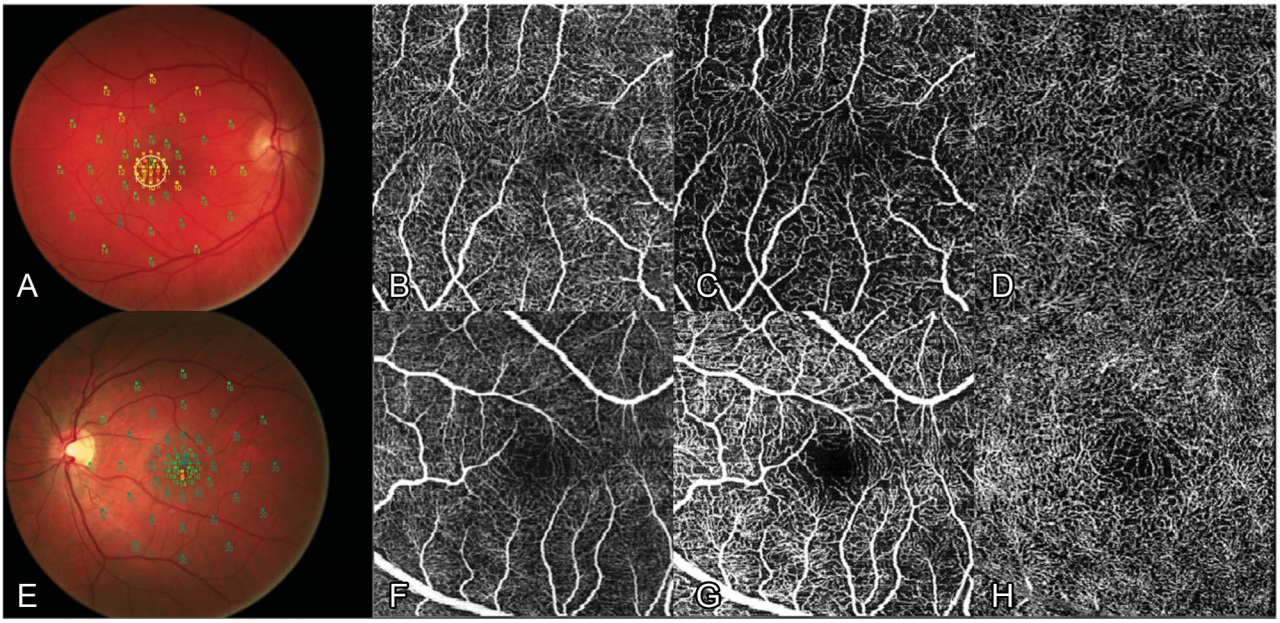

Fig. 3. A–H. Scotopic microperimetry (**A** and **E**) and OCTA (**B–D**, **F–H**) in two MFC eyes of two patients. A dense foveal scotoma (empty red squares), normally present in the normal eye, is not detectable in both MFC eyes (**A** and **E**). The enface OCTA of the full retina (extending from the inner limiting membrane to the Bruch membrane, **B**, **F**) and of the deep vascular complex (extending from the inner plexiform layer to the outer plexiform layer, **D**, **H**) of both eyes shows the absence of the FAZ. Conversely, the enface OCTA of the superficial vascular complex (extending from the inner limiting membrane to the inner plexiform layer, **C**, **G**) shows the absence of FAZ in the first eye (**C**) and its presence in the second one (**G**).

Key words: macular-foveal capillaries, foveal hypoplasia, foveal function, mesopic microperimetry, scotopic microperimetry, optical coherence tomography angiography, foveal development.

References

1. Provis JM, Dubis AM, Maddess T, Carroll J. Adaptation of the central retina for high acuity vision: cones, the fovea and the avascular zone. Prog Retin Eye Res 2013;35:63–81.
2. Provis JM, Hendrickson AE. The foveal avascular region of developing human retina. Arch Ophthalmol 2008;126:507–511.
3. Dubis AM, Costakos DM, Subramaniam CD, et al. Evaluation of normal human foveal development using optical coherence tomography and histologic examination. Arch Ophthalmol 2012;130:1291–1300.
4. Yeung J, Crock G, Cairns J, et al. Macular-foveal capillaries in human retina. Aust J Ophthalmol 1973;1:17–23.
5. Cicinelli MV, Carnevali A, Rabiolo A, et al. Clinical spectrum of macular-foveal capillaries evaluated with optical coherence tomography angiography. Retina 2017;37:436–443.
6. Yokoyama T, Maruko I, Koizumi H, et al. Unmeasurable small size of foveal avascular zone without visual impairment in optical coherence tomography angiography. Eye (Lond) 2018;32:1062–1066.
7. Longhin E, Tormene AP, Olivato E, et al. Rod function in diabetic patients without and with early diabetic retinopathy. Eur J Ophthalmol 2016;26:418–424.
8. Midena E, Pilotto E. Microperimetry in age: related macular degeneration. Eye (Lond) 2017;31:985–994.
9. Thomas MG, Kumar A, Mohammad S, et al. Structural grading of foveal hypoplasia using spectral domain optical coherence tomography a predictor of visual acuity? Ophthalmology 2011; 118:1653–1660.
10. Charles SJ, Green JS, Grant JW, et al. Clinical features of affected males with X linked ocular albinism. Br J Ophthalmol 1993;77:222–227.
11. Walsh MK, Goldberg MF. Abnormal foveal avascular zone in nanophthalmos. Am J Ophthalmol 2007;143:1067–1068.
12. Martinez-Castellanos MA, Velez-Montoya R, Price K, et al. Vascular changes on fluorescein angiography of premature infants with low risk of retinopathy of prematurity after high oxygen exposure. Int J Retina Vitreous 2017;3:2.
13. Recchia FM, Recchia CC. Foveal dysplasia evident by optical coherence tomography in patients with a history of retinopathy of prematurity. Retina 2007;27:1221–1226.
14. Hamid MA, Mehta MC, Kuppermann BD. Multimodal imaging in a patient with Prader-Willi syndrome. Int J Retina Vitreous 2018;4:45.
15. Hendrickson A, Troilo D, Possin D, Springer A. Development of the neural retina and its vasculature in the marmoset Callithrix jacchus. J Comp Neurol 2006;497:270–286.
16. Lutty GA, McLeod DS. Development of the hyaloid, choroidal and retinal vasculature in the fetal human eye. Prog Ret Eye Res 2018;62:58–76.
17. Bringmann A, Syrbe S, Görner K, et al. The primate fovea: structure, function and development. Prog Retin Eye Res 2018; 66:49–84.
18. Hendrickson A, Possin D, Vajzovic L, Toth CA. Histologic development of the human fovea from midgestation to maturity. Am J Ophthalmol 2012;154:767–e2.
19. Diaz-Araya C, Provis JM. Evidence of photoreceptor migration during early foveal development: a quantitative analysis of human fetal retinae. Vis Neurosci 1992;8:505–514.
20. Levine EM, Fuhrmann S, Reh TA. Soluble factors and the development of rod photoreceptors. Cell Mol Life Sci 2000; 57:224–234. Review.
21. Strauss RW, Kong X, Bittencourt MG, et al; for the SMART Study Group. Scotopic microperimetric assessment of rod function in stargardt disease (SMART) study: design and baseline characteristics (report No. 1). Ophthalmic Res 2019;61: 36–43.
22. Steinberg JS, Fitzke FW, Fimmers R, et al. Scotopic and photopic microperimetry in patients with reticular drusen and age-related macular degeneration. JAMA Ophthalmol 2015;133: 690–697.
23. Fulton AB, Albert DM, Craft JL. Human albinism. Light and electron microscopy study. Arch Ophthalmol 1978;96:305–310.
24. Yanni SE, Wang J, Chan M, et al. Foveal avascular zone and foveal pit formation after preterm birth. Br J Ophthalmol 2012; 96:961–966.
25. Hammer DX, Iftimia NV, Ferguson RD, et al. Foveal fine structure in retinopathy of prematurity: an adaptive optics Fourier domain optical coherence tomography study. Invest Ophthalmol Vis Sci 2008;49:2061–2070.