

# Macular Features in Retinitis Pigmentosa: Correlations Among Ganglion Cell Complex Thickness, Capillary Density, and Macular Function

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**PURPOSE.** To investigate correlations among macular ganglion cell complex (GCC) layer thickness, macular capillary density, and macular function in patients affected by retinitis pigmentosa (RP).

**METHODS.** Fourteen patients (28 eyes) with previous diagnosis of RP were enrolled. The diagnosis of these conditions was made based on both clinical features and electrophysiological examination. All patients underwent a complete ophthalmologic examination, including optical coherence tomography angiography (OCTA) and multifocal electroretinogram (mfERG). Main outcome measures were macular GCC layer thickness; superficial capillary plexus (SCP) vessel density; deep capillary plexus (DCP) vessel density; choriocapillaris plexus (CCP) vessel density; and amplitudes of mfERG N1 and P1 waves.

**RESULTS.** Average GCC thickness was significantly thinner in RP patients ( $76.0 \pm 25.1$  and  $109.2 \pm 17.5 \mu\text{m}$ ,  $P = 0.028$ ). Superficial capillary plexus density was  $42.2 \pm 3.4\%$  in the RP group and  $51.4 \pm 2.3\%$  in the control group ( $P < 0.001$ ). Deep capillary plexus density was reduced in RP patients ( $42.7 \pm 6.2\%$ ) after the comparison with healthy subjects ( $56.6 \pm 2.2\%$ ,  $P < 0.001$ ). Choriocapillaris plexus density was significantly less in RP patients than in the control group ( $65.3 \pm 2.7\%$  and  $67.2 \pm 1.4\%$ ,  $P = 0.024$ ). Superficial capillary plexus and DCP density were significantly correlated with both mfERG values and GCC thickness.

**CONCLUSIONS.** We showed that both choroid and retinal vessels were modified in RP patients after comparison with healthy subjects. Moreover, we demonstrated that the SCP and DCP vessel densities are correlated with the macular function, as well as with the GCC thickness.

Keywords: retinitis pigmentosa, optical coherence tomography angiography, optical coherence tomography, electrophysiology

Retinitis pigmentosa (RP) is the name used for a group of inherited retinal disorders that are characterized by progressive retinal dysfunction, loss of the outer retina cells, and eventually atrophy of the retinal tissue.<sup>1–5</sup> The occurrence of RP in the general population is estimated to be 1 in 4000 individuals.<sup>5,6</sup>

Many factors have been implicated in RP pathogenesis and progression. Retinitis pigmentosa can be considered a photoreceptor disease; however, there is increasing evidence that the inner retina becomes disorganized, with retinal ganglion cell (RGC) death, as the outer retina can be affected.<sup>7–9</sup> Furthermore, there is abundant evidence showing that vascular changes (e.g., perivascular cuffing, arteriolar attenuation, and reduced ocular blood flow) featured in RP and were hypothesized to be part of the pathogenic process.<sup>10</sup>

Clinically, RP manifests initially as night blindness (nyctopia), eventually followed by clear visual field loss.<sup>1,4,5</sup> Moreover, the age of onset of visual impairment ranges from infancy to late adulthood.<sup>1,4,5</sup> Several previous studies used the best-

corrected visual acuity (BCVA) to evaluate macular function in RP patients. Nevertheless, the introduction of the multifocal electroretinogram (mfERG)<sup>11–14</sup> has allowed clinicians to test the macular function in the whole macular area and to study morphofunctional correlations in eyes affected by RP.

Optical coherence tomography angiography (OCTA) has recently been developed to study retinal and choroidal microvasculature without needing a dye injection and has allowed us to examine both the superficial and the deep retinal capillary plexuses (SCP and DCP, respectively), as well as the choriocapillaris plexus (CCP).<sup>15</sup> Battaglia Parodi et al.<sup>16</sup> recently showed vascular alterations in RP by means of OCTA. However, they did not evaluate a possible correlation between vascular perfusion and retinal function.

In this cross-sectional study, we investigated the vascular changes in eyes affected by RP, and we assessed the relationships between inner retina, vascular structure, and functional data; the latter were obtained using mfERG.



## METHODS

### Study Participants

Fourteen patients (28 eyes) with a previous diagnosis of either mid- or late-stage RP<sup>17</sup> were enrolled at the Retina Service of the Ophthalmology Clinic, University G. d'Annunzio of Chieti-Pescara, Italy. University Gabriele D'Annunzio Ethics Committee approved this study and all patients gave informed consent to the use of their data. The study adhered to the tenets of the Declaration of Helsinki. The diagnosis of these conditions was made based on both the clinical features and the electrophysiological examination. Patients were selected with a BCVA of at least 0.8 logMAR to ensure proper execution of the examinations.

Exclusion criteria were (1) age younger than 18 years; (2) evidence of an advanced form of RP (either undetectable ERG or extended macular atrophy)<sup>17</sup>; (3) any retinal dystrophy affecting the patient other than RP; (4) any history of either maculopathy or ocular vascular disease; (5) any optic neuropathy, including glaucoma, or any condition increasing the risk of secondary glaucoma (e.g., pigment dispersion syndrome or pseudoexfoliation syndrome); and (6) intraocular pressure (IOP) greater than 21 mm Hg.

All patients had undergone a complete ophthalmologic examination, which included the measurement of BCVA, IOP, fundus examination, color fundus photography, OCTA, and mfERG.

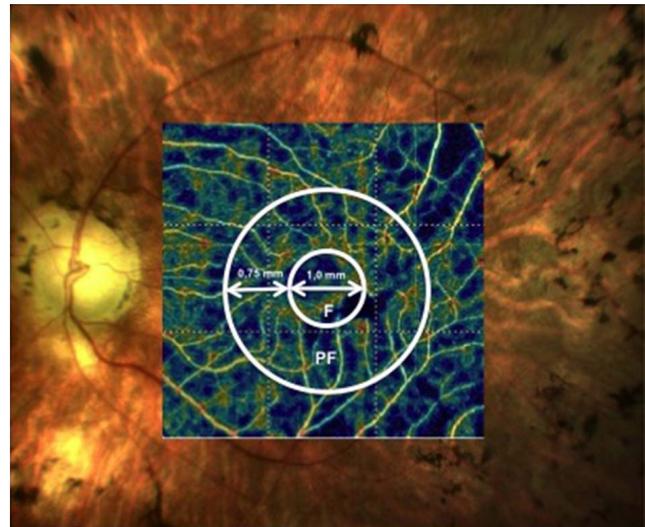
A control group homogeneous for age and sex was also included in the current analysis. All control subjects also underwent a complete ophthalmologic examination, including BCVA, IOP, fundus examination, mfERG, and OCTA.

### Procedures

**Spectral-Domain Optical Coherence Tomography Angiography With XR Avanti.** XR Avanti AngioVue OCTA (Optovue, Inc., Fremont, CA, USA) is a device with a high speed of 70,000 axial scans per second using a light source of 840 nm and an axial resolution of 5  $\mu$ m. The AngioVue OCTA system, which is based on the split-spectrum amplitude-decorrelation angiography (SSADA) algorithm (Version: 2016.100.0.45), uses blood flow as an intrinsic contrast. Indeed, the flow is detected as a variation over time in the speckle pattern formed by the interference of light scattered from red blood cells and adjacent tissue structure.<sup>18</sup> In addition, the spectral-domain optical coherence tomography (SD-OCT) tool was skilled in acquiring the standard structural OCT scans, including ganglion cell complex (GCC) thickness evaluation, which is typically used by commercially available devices.

Before imaging, each subject's pupils were dilated with a combination of 0.5% tropicamide and 10% phenylephrine. Study participants underwent SD-OCT imaging following a protocol that included the AngioVue OCT 3D volume set of 6  $\times$  6 mm. An internal fixation light was used to center the scanning area. The OCT signal position and quality were optimized by means of the Auto All function, which performs in sequence with (1) the Auto Z function to find the best position for obtaining the retina OCT image; (2) the Auto F function to find the best focus for the particular subject's refraction; and (3) the Auto P function to find the best polarization match for the particular subject's ocular polarization.

One FastX (horizontal raster) set and one FastY (vertical raster) set were performed for each acquisition scan. Each set took approximately 3 seconds to complete. The software then performed the motion correction technology to remove saccades and minor loss of fixation. Scans with low quality



**FIGURE 1.** A picture of the fundus in a patient with retinitis pigmentosa is overlaid with optical coherence tomography angiography (OCTA) macula 6  $\times$  6 scan showing the superficial vascular plexus. The vessel density was defined as the percentage area occupied by vessels in a circle region of interest (ROI) centered on the center of the foveal avascular zone and with a diameter of 2.5 mm. The AngioVue software automatically splatted the ROI, as well as the vessel density evaluation, into two fields: the foveal area (F), a central circle with a diameter of 1 mm; and the parafoveal area (PF) that constitutes the remaining part inside the ROI. Finally, the vessel density of the entire 6  $\times$  6 area was collected for the analysis.

(i.e., if the subject blinked or if there were many motion artifacts in the data set) were excluded and repeated until good quality was achieved. Three scans for each patient were captured; then the best one for quality (without significant motion artifacts and with a signal strength index > 60) was considered for the analysis.

**Vascular Layer Segmentation.** Vascular retinal layers were visualized and segmented as previously described.<sup>18–21</sup> To evaluate the superficial retinal plexus, we used a layer thickness of 60  $\mu$ m from the inner limiting membrane in order to include all the vessels of this plexus. To visualize the deep retinal plexus, we used a 30- $\mu$ m-thick layer from the inner plexiform layer for the purpose of visualizing the plexus in its entirety. The CCP was imaged by means of a 30- $\mu$ m-thick layer from the retinal pigment epithelium. Two investigators checked the segmentation quality before testing the vessel density.

**Vessel Density Analysis.** Objective quantification of vessel density was evaluated for each eye using the SSADA software. Quantitative analysis was performed on the OCTA en face images for each eye using the AngioVue software. The vessel density was defined as the percentage area occupied by vessels in a 3  $\times$  3-mm-square region of interest (ROI) concentrating on the center of the foveal avascular zone. (AngioVue software automatically outputs the flow area value within the ROI.)

The vessel density was calculated using the formula previously described,<sup>22</sup> as follows:

$$\text{Vessel Density} = \frac{\int V \cdot dA}{\int dA},$$

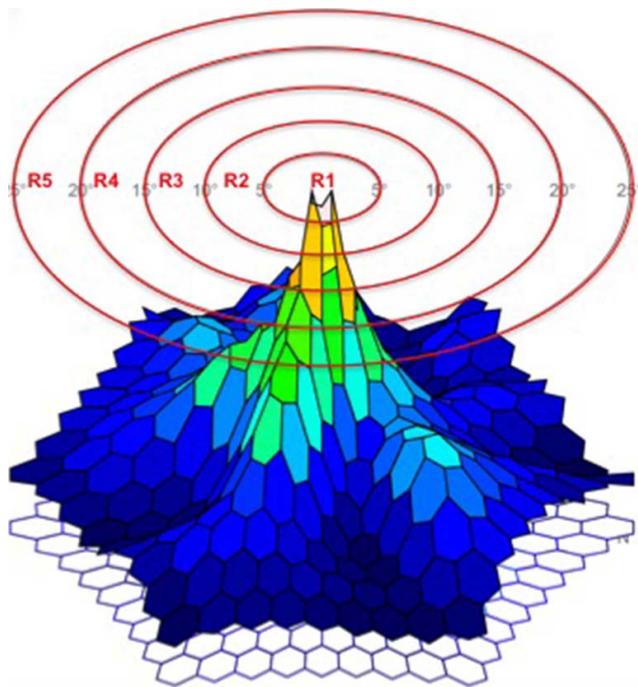


FIGURE 2. Multifocal electroretinogram of a patient affected by retinitis pigmentosa. Amplitudes of N1 (first negative component) and P1 (first positive component) of the first-order kernel were analyzed for five regional ring groups (R1, R2, R3, R4, and R5).

where  $V$  is 1 when the OCTA value is above a background threshold and 0 is otherwise.  $A$  is the area of interest. The three capillary systems were evaluated in the following areas: foveal, parafoveal, and  $3 \times 3$  whole area (Fig. 1).

### Multifocal Electroretinogram

Multifocal ERG (Retimax CSO, Florence, Italy) was recorded for each patient, according to the International Society for Clinical Electrophysiology of Vision (ISCEV) protocols that were updated in 2012 and 2011.<sup>23,24</sup> During the mfERG examination, the ocular fundus was segmented by an array of 61 hexagons. The amplitudes of N1 (first negative component) and P1 (first positive component) of the first-order kernel were calculated for five regional ring groups (R1, R2, R3, R4, and R5) (Fig. 2).

### Statistical Analysis

All quantitative variables were summarized as mean and standard deviation, and qualitative variables as frequency and percentage. To detect departures from normality distribution, Shapiro-Wilk's test was performed for all quantitative variables.

Statistical significance of the differences between groups (RP versus control) for qualitative variables was assessed using Fischer's exact test. The Mann-Whitney  $U$  test was applied for assessing the comparison of the quantitative variables between the two groups.

Analysis of variance (ANOVA), with nested design, was performed to assess the differences between groups for macular thickness, vessel density, and mfERG parameters. The nested ANOVA was used to account for using both eyes from RP patients in the same sample. The false discovery rate correction (FDR) was used to control the family-wise type I error rate, and an FDR adjusted  $P$  value  $< 0.05$  was determined to be statistically significant.

TABLE 1. Characteristics of the Enrolled Patients

Characteristic	Retinitis Pigmentosa Group, $n = 14$	Control Group, $n = 24$	$P$ Value
Number of eyes	26	24	0.290*
Age, y, mean $\pm$ SD	40.1 $\pm$ 7.3	42.2 $\pm$ 6.5	1.000†
Sex, $n$			
Male	8	14	
Female	6	10	
BCVA, logMAR, mean $\pm$ SD	0.5 $\pm$ 0.2	0.0 $\pm$ 0.0	$< 0.001^*$

$n$ , number of patients; SD, standard deviation.

\* Mann-Whitney  $U$  test.

† Fischer's Exact test.

Spearman's correlation coefficient was tested to evaluate the linear correlation among variables in RP patients. To adjust the  $P$  value of multiple correlation coefficients, a permutation test was conducted and a 95% confidence interval (95%CI) was reported. Finally, the parameters were correlated considering the macular field tested.

All statistical analyses were performed using Statistical Package for Social Sciences (version 20.0; SPSS, Inc., Chicago, IL, USA).

### RESULTS

Two eyes in the patient group had to be excluded from the analysis because of the presence of extended macular atrophy. Patients' and healthy subjects' demographic data and visual acuities are reported in Table 1. All the subjects enrolled were Caucasian and not affected by diabetes. Furthermore, there were no significant differences in systemic hypertension or the use of systemic antihypertensive medications between the groups.

### mfERG Analysis

Table 2 shows mfERG values of the enrolled RP patients and the healthy subjects. P1 and N1 amplitudes were significantly reduced in the RP group in all of the five rings (except for the N1R1 and N1R3 amplitude values).

TABLE 2. Multifocal Electroretinogram Values of Retinitis Pigmentosa Patients and Healthy Controls

mfERG	Retinitis Pigmentosa Group	Control Group	$P$ Value*
P1R1 amplitude, $\mu$ V	0.42 $\pm$ 0.38	0.90 $\pm$ 0.33	<b>0.005</b>
N1R1 amplitude, $\mu$ V	-0.32 $\pm$ 0.44	-0.66 $\pm$ 0.34	0.321
P1R2 amplitude, $\mu$ V	0.20 $\pm$ 0.17	0.61 $\pm$ 0.18	<b>&lt; 0.001</b>
N1R2 amplitude, $\mu$ V	-0.16 $\pm$ 0.12	-0.33 $\pm$ 0.15	<b>0.016</b>
P1R3 amplitude, $\mu$ V	0.15 $\pm$ 0.14	0.60 $\pm$ 0.15	<b>&lt; 0.001</b>
N1R3 amplitude, $\mu$ V	-0.13 $\pm$ 0.11	-0.33 $\pm$ 0.22	0.086
P1R4 amplitude, $\mu$ V	0.14 $\pm$ 0.11	0.48 $\pm$ 0.17	<b>0.003</b>
N1R4 amplitude, $\mu$ V	-0.09 $\pm$ 0.10	-0.33 $\pm$ 0.16	<b>0.007</b>
P1R5 amplitude, $\mu$ V	0.14 $\pm$ 0.13	0.49 $\pm$ 0.11	<b>&lt; 0.001</b>
N1R5 amplitude, $\mu$ V	-0.09 $\pm$ 0.10	-0.36 $\pm$ 0.18	<b>0.003</b>

Data are expressed as mean  $\pm$  standard deviation.

\*  $P$  values are relative to group effect in the nested analysis of variance (ANOVA). Bold indicates significant values.

**TABLE 3.** Macular Thickness and Vessel Density in Retinitis Pigmentosa Patients and Controls

Parameter	Retinitis Pigmentosa Group	Control Group	P Value*
Foveal macular thickness, $\mu\text{m}$	240.4 $\pm$ 74.5	249.6 $\pm$ 56.2	0.942
Full parafoveal macular thickness, $\mu\text{m}$	280.0 $\pm$ 48.2	324.5 $\pm$ 17.4	<b>0.030</b>
Average GCC thickness, $\mu\text{m}$	76.0 $\pm$ 25.1	109.2 $\pm$ 17.5	<b>0.028</b>
Whole superficial vessel density, %	42.2 $\pm$ 3.4	51.4 $\pm$ 2.3	< <b>0.001</b>
Foveal superficial vessel density, %	32.1 $\pm$ 9.2	31.4 $\pm$ 5.4	0.794
Parafoveal superficial vessel density, %	42.4 $\pm$ 4.1	52.5 $\pm$ 2.9	< <b>0.001</b>
Whole deep vessel density, %	42.7 $\pm$ 6.2	56.6 $\pm$ 2.2	< <b>0.001</b>
Foveal deep vessel density, %	28.2 $\pm$ 8.9	29.7 $\pm$ 7.4	0.884
Parafoveal deep vessel density, %	48.1 $\pm$ 4.7	59.1 $\pm$ 2.8	< <b>0.001</b>
Whole choriocapillaris vessel density, %	65.3 $\pm$ 2.7	67.2 $\pm$ 1.4	<b>0.024</b>
Foveal choriocapillaris vessel density, %	64.3 $\pm$ 5.5	67.5 $\pm$ 5.4	< <b>0.001</b>
Parafoveal choriocapillaris vessel density, %	64.6 $\pm$ 2.8	67.1 $\pm$ 1.4	<b>0.006</b>

*n*, number of eyes examined; data are expressed as mean  $\pm$  standard deviation.

\* *P* values are relative to group effect in the nested analysis of variance (ANOVA). Bold indicates significant values.

### Macular Thickness Analysis

Foveal macular thickness was 240.4  $\pm$  74.5  $\mu\text{m}$  in RP patients and 249.6  $\pm$  56.2  $\mu\text{m}$  in healthy subjects ( $P = 0.942$ ). Parafoveal macular thickness was 280.0  $\pm$  48.2  $\mu\text{m}$  in the RP group and 324.5  $\pm$  17.4  $\mu\text{m}$  in the normal group ( $P = 0.030$ ).

Average GCC thickness was significantly thinner in RP patients (76.0  $\pm$  25.1  $\mu\text{m}$  and 109.2  $\pm$  17.5  $\mu\text{m}$ ;  $P = 0.028$ ) (Table 3).

### Vessel Density Analysis

Whole SCP vessel density was 42.2  $\pm$  3.4% in the RP group and 51.4  $\pm$  2.3% in the control group ( $P < 0.001$ ). Moreover, the parafoveal SCP vessel density was reduced in RP patients (42.4  $\pm$  4.1% and 52.5  $\pm$  2.9%;  $P < 0.001$ ).

Whole DCP vessel density was reduced in RP patients (42.7  $\pm$  6.2%) after the comparison with healthy subjects (56.6  $\pm$

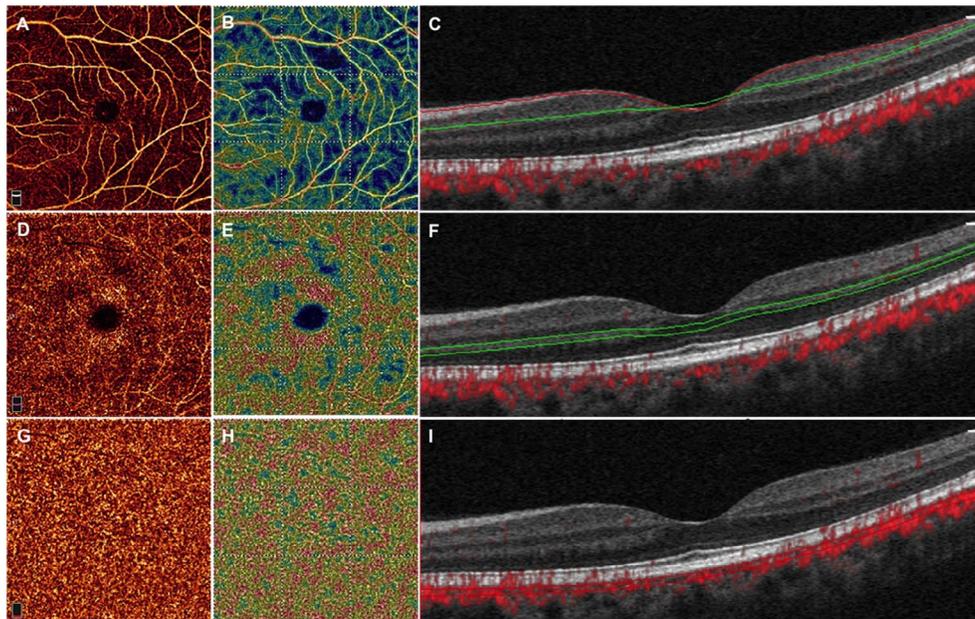
2.2%;  $P < 0.001$ ). Moreover, also considering the parafoveal area, the DCP vessel density was reduced (48.1  $\pm$  4.7% and 59.1  $\pm$  2.8%;  $P < 0.001$ ).

Whole CCP vessel density was significantly less in RP patients than in the control group (65.3  $\pm$  2.7% and 67.2  $\pm$  1.4%;  $P = 0.024$ ). Furthermore, the choriocapillaris vessel density was reduced in both the foveal area (64.3  $\pm$  5.5% and 67.5  $\pm$  5.4%;  $P < 0.001$ ) and the parafoveal area (64.6  $\pm$  2.8% and 67.1  $\pm$  1.4%,  $P = 0.006$ ) (Table 3; Fig. 3).

### Correlation Analysis

Table 4 and Table 5 show the results of the correlation analysis.

Parafoveal SCP and DCP vessel densities were significantly correlated with mfERG values. Parafoveal CCP vessel density was directly correlated with the P1R2 amplitude. Moreover, the average GCC thickness results correlated with mfERG



**FIGURE 3.** Optical coherence tomography angiography (OCTA) from an enrolled retinitis pigmentosa patient. (A) OCTA macula 6  $\times$  6 scan showing the superficial vascular plexus; (B) corresponding color-coded flow density map of the superficial vascular plexus flow density (the warmer the color, the greater the flow); (C) OCT B-scan showing the slab set to evaluate the superficial retinal plexus; (D) OCTA macula 6  $\times$  6 scan showing the deep vascular plexus; (E) corresponding color-coded flow density map of the deep vascular plexus flow density; (F) OCT B-scan showing the slab set to evaluate the deep retinal plexus; (G) OCTA macula 6  $\times$  6 scan showing the deep vascular plexus; (H) corresponding color-coded flow density map of the deep vascular plexus flow density; (I) OCT B-scan showing the slab set to evaluate the deep retinal plexus.

TABLE 4. Correlations Among Morphologic and Functional Parameters in Retinitis Pigmentosa Patients

Parameter	mfERG					
	P1R1	N1R1	P1R2	N1R2	P1R3	N1R3
Foveal superficial vessel density						
rho (95%CI)	-0.167 (-0.577; 0.297)	0.005 (-0.382; 0.392)	-	-	-	-
P value	0.405	0.981	-	-	-	-
Parafoveal superficial vessel density						
rho (95%CI)	-	-	0.623 (0.327; 0.798)	-0.544 (-0.765; -0.222)	0.718 (0.465; 0.860)	-0.499 (-0.798; -0.071)
P value	-	-	<b>0.001</b>	<b>0.003</b>	<b>&lt;0.001</b>	<b>0.008</b>
Foveal deep vessel density						
rho (95%CI)	-0.318 (-0.504; 0.503)	-0.379 (-0.517; 0.182)	-	-	-	-
P value	<b>0.047</b>	0.050	-	-	-	-
Parafoveal deep vessel density						
rho (95%CI)	-	-	0.804 (0.609; 0.909)	-0.580 (-0.799; -0.217)	0.756 (0.548; 0.856)	-0.528 (0.0760; -0.154)
P value	-	-	<b>&lt;0.001</b>	<b>0.002</b>	<b>&lt;0.001</b>	<b>0.005</b>
Foveal choriocapillaris vessel density						
rho (95%CI)	-0.474 (-0.723; -0.119)	0.197 (-0.214; 0.564)	-	-	-	-
P value	<b>0.012</b>	0.324	-	-	-	-
Parafoveal choriocapillaris vessel density						
rho (95%CI)	-	-	0.598 (0.263; 0.811)	-0.148 (-0.485; 0.238)	0.374 (0.005; 0.690)	-0.238 (-0.584; 0.216)
P value	-	-	<b>0.001</b>	0.461	0.055	0.232
Average GCC thickness						
rho (95%CI)	-	-	0.286 (-0.146; 0.657)	-0.453 (-0.694; -0.144)	0.430 (0.029; 0.739)	-0.334 (-0.656; 0.063)
P value	-	-	0.148	<b>0.018</b>	<b>0.025</b>	0.089

Spearman's rho correlation coefficient was performed to evaluate the linear correlation among variables. Bold indicates significant values.

**TABLE 5.** Correlations Between Ganglion Cell Complex Thickness and Vessel Density in Retinitis Pigmentosa Patients

Parameter	Average GCC Thickness
Parafoveal superficial vessel density	
rho (95%CI)	0.488 (0.063; 0.754)
P value	<b>0.005</b>
Parafoveal deep vessel density	
rho (95%CI)	0.415 (0.035; 0.734)
P value	<b>0.010</b>
Parafoveal choriocapillaris vessel density	
rho (95%CI)	0.199 (−0.161; 0.538)
P value	0.230

Spearman's rho correlation coefficient was performed to evaluate the linear correlation among variables. Bold indicates significant values.

values (Table 4). (Only the correlation with NIR3 amplitude did not reach statistical significance.)

A direct correlation was found between parafoveal SCP and DCP vessel densities and the average GCC thickness (Table 5).

## DISCUSSION

In this cross-sectional study, we investigated the GCC layer thickness, vessel changes, and macular function in patients affected by RP. Overall, we found that RP patients were characterized by both retinal and choroidal vessel changes, and that these alterations were associated with the macular function.

In recent years, there has been a debate on the efficacy of photoreceptor restorative therapies. Nevertheless, restoration of vision is possible only if RGCs are preserved.<sup>25</sup> It is well known that after photoreceptor damage, some changes affect inner retinal neurons, including RGCs. Indeed, although several studies have shown comparable GCC and retinal nerve fiber layer thicknesses in RP patients compared with healthy subjects,<sup>26,27</sup> a reduced number of RGCs has been demonstrated in those RP patients affected by decreased macular function.<sup>28</sup> Some authors have attributed this loss to the reduced transsynaptic signal because of photoreceptor cell degeneration.<sup>8,9</sup> Nevertheless, other authors have implicated diminished blood flow to the inner retinal layers.<sup>29</sup> The GCC layer was reduced in our study RP patients, and this result could be explained by the reduced macular function affecting them. Moreover, we showed that a direct correlation was present between the GCC thickness and the macular function; the latter was measured by means of mfERG. Indeed, despite the fact that mfERG responses were mainly attributable to both bipolar cell and photoreceptor functions, several studies support the hypothesis that ganglion cells contribute to mfERG responses.<sup>30,31</sup>

Alterations in the ocular blood flow, as well as in the retinal vessels, have been fully described as being part of the RP pathogenesis. Wolf et al.<sup>32</sup> showed that increased arteriovenous transit time and reduced blood flow velocity are early hemodynamic findings of RP patients without any clinically detectable ocular pathology. Furthermore, reduction of the retinal blood vessels, with perivascular pigment deposits, is a fundusoscopic feature of patients affected by RP.<sup>4</sup>

The recent advent of OCTA has made it possible to separately image both the superficial and the deep retinal capillary plexuses in vivo in humans. The two capillary plexuses have different patterns: the SCP is composed of capillaries developing an interconnected vascular network between arterioles and venules; the DCP is compounded by

polygonal units in which the capillaries converge radially toward an epicenter, called the capillary vortex.<sup>15,33</sup>

To the best of our knowledge, no study exists that evaluates both vascular changes and macular function in patients affected by RP.

We have demonstrated that both the SCP and the DCP vessel densities are reduced in patients affected by RP. Interestingly, we found a significant correlation between SCP and DCP densities and GCC thickness. The correlation between GCC thinning and the reduction of blood flow in the SCP and DCP might be explained by a reduction of metabolic demand and a subsequent reduction in blood flow.

A novel and tentative hypothesis is that vascular depletion, as demonstrated by SCP and the DCP vessel density reduction, could be an early event in the disease, which eventually causes ischemia and tissue loss. There is a body of evidence indicating that the reduced ocular blood flow is implicated in RP. Indeed, Konieczka et al.<sup>10</sup> demonstrated that typical symptoms of the primary vascular dysregulation (PVD) syndrome—an illness characterized by vessel predisposition that reacts differently to a number of stimuli—occur more frequently in RP patients; they then speculated that the PVD is cause of the reduced ocular blood flow. Moreover, several histopathologic studies have confirmed that the reduced ocular blood flow is a primary event due to retinal vessel damage. In fact, vessel narrowing and sclerosis, followed by thickening of the blood vessel wall and consequently lumen occlusion, are features of RP.<sup>4,29</sup> In the assumption that vessel damage is an early event, the damage involving both the SCP and DCP could contribute to ganglion cell death by inducing ischemia to the inner retinal layers, as specified above. The cross-sectional nature of this study cannot allow us to clarify the cause-and-effect relationship between vessel damage and other alterations.

Superficial capillary plexus and DCP are significantly correlated to macular function. The latter correlation demonstrated that the vessel damage affects the macular function. It could be worthwhile to investigate, in a longitudinal study, whether alterations of the SCP and DCP plexuses, which appear to be correlated with those in the mfERG, are predictive of a faster clinical deterioration; alternatively, whether retinal capillary plexuses' relative sparing can predict a better visual prognosis. Future longitudinal studies will address these relevant questions.

With regard to choroidal circulation, several studies demonstrated its alteration in RP patients. The ocular pulse amplitude, an indirect measure of the choroidal perfusion, was found to be significantly reduced in RP. Furthermore, several studies demonstrated that choroidal thickness is significantly reduced in patients with RP.<sup>34,35</sup> In contrast to our results, Battaglia Parodi et al.<sup>16</sup> recently showed no difference in CCP vessel density between RP patients and healthy subjects, which were tested by means of OCTA. Nevertheless, this is probably secondary to the different OCTA types used (Optovue in our study and Topcon in the Battaglia Parodi et al. study), or to the different method of analysis (AngioVue software versus ImageJ software), or to different disease stages and genetic features of the groups enrolled (considering there is a poor genetic characterization in both studies).

Our study has several limitations. The series presented here is relatively small. However, one should look at the current series in consideration of (1) the rarity of RP disease and (2) the strict inclusion criteria for patients and the control group, as well as the similarity of groups with respect to meaningful characteristics, such as age. Another major limitation is that the patients had poor genetic characterization; therefore, the results can be interpreted only as generic for RP.

In conclusion, we have provided a fully integrated study of retinal and choroidal vessels in RP patients, and shown that

both the choroid and retinal vessels were modified in these patients after comparison with healthy subjects. Moreover, we demonstrated that the DCP vessel density correlates with the macular function. A prospective longitudinal evaluation of retinal vessels in RP patients, and correlation with retinal architecture and function, will help shed further light on the role of the retinal vessels in RP. Vessel measures may become a new useful tool to monitor disease activity and efficacy of new therapeutic approaches. Finally, the influence of the vascular status on the efficacy of the photoreceptor restorative therapies should be evaluated.

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