Subfoveal Choroidal Blood Flow and Central Retinal Function in Retinitis Pigmentosa

Benedetto Falsini,[1] *Gian Mario Anselmi,*[1] *Dario Marangoni,*[1] *Fabiana D'Esposito,*[2,3] *Antonello Fadda,*[4] *Antonio Di Renzo,*[5] *Emilio C. Campos,*[6] *and Charles E. Riva*[6]

Purpose. To determine whether subfoveal choroidal blood flow is altered in retinitis pigmentosa (RP) and whether this alteration is associated with central cone–mediated dysfunction.

Methods. In 31 RP patients (age range, 15–72 years) with preserved visual acuity (range: 20/30–20/20), subfoveal choroidal blood flow was measured by real-time, confocal laser Doppler flowmetry, and focal macular (18°) electroretinograms (FERGs) were elicited by 41 Hz flickering stimuli. Twenty normal subjects served as controls. The following average blood flow parameters were determined based on three 60-second recordings: volume (*ChBVol*), velocity (*ChBVel*), and flow (*ChBF*), the last being proportional to blood flow if the hematocrit remains constant. The amplitude and phase of the FERG first harmonic component were measured.

Results. On average, *ChBF* and *ChBVel* were reduced by 26% ($P \leq 0.02$) in RP patients compared to controls, whereas *ChBVol* was similar in the two groups. FERG amplitudes were reduced by 60% ($P < 0.01$) in patients compared with controls. FERG phases of patients tended to be delayed ($P < 0.08$) compared with their values in the controls. In patients, FERG phase delays were correlated ($r = 0.50$, $P < 0.01$) with *ChBF* and *ChBVel* values. FERG amplitudes were correlated ($r = 0.49$, $P < 0.01$) with *ChBVol* values.

Conclusions. These data indicate significant alterations of subfoveal choroidal hemodynamic in RP and suggest a link between the alteration of *ChBF* and the RP-associated central cone–mediated dysfunction as assessed by the FERG. (*Invest Ophthalmol Vis Sci.* 2011;52:1064–1069) DOI:10.1167/iovs.10-5964

Retinitis pigmentosa (RP) comprises a group of inherited disorders characterized by the progressive deterioration and death of retinal photoreceptors. RP is a major cause of blindness in the world and the most commonly inherited form of severe retinal degeneration, with a frequency of approximately 1 in 3500 births. At least 49 different genes and several loci have been identified so far as responsible for nonsyndromic forms of RP and the underlying genetic defect identifiable in approximately 50% of the cases. Genes encoding components of the phototransduction cascade, with rhodopsin playing a major role, proteins involved in retinol metabolism and cell–cell interaction, photoreceptor structural proteins and transcription factors, intracellular transport proteins, and splicing factors, have all been implicated in the pathogenesis of RP (RetNet, provided in the public domain at http://www.sph.uth.tmc.edu/RetNet/). In animal models of RP that have been tested biochemically, photoreceptor degeneration was proven to occur by apoptosis usually involving rods first and cones at a later stage.[1–3]

Numerous experimental and clinical studies indicate that ocular blood circulation is altered in RP,[4–6] adding to the complexity of disease pathophysiology and its full understanding. Retinal blood flow assessed by laser Doppler velocimetry[4] is significantly reduced in RP patients compared with its value in control subjects. In regard to choroidal circulation, studies in animal models of RP (Abyssinian cats with hereditary rod-cone degeneration,[5] RCS rats,[7] rd mice[8]) show loss of capillaries in the choroid. However, in Abyssinian cats this loss involved only a limited part of the choroid, that is, the region of the ocular fundus not covered by the tapetum lucidum (but containing the area centralis), a formation of densely packed cells within the inner part of the cat choroid. In RP patients, the ocular pulse amplitude (POBF), an indirect measure of pulsatile choroidal perfusion, was found to be reduced significantly at a relatively advanced disease stage.[6,9] Decreased POBF was correlated in some patients with the corresponding loss of differential light sensitivity.[9] These results suggest that alterations of the choroid circulation may either be associated with the disease or play a role in its pathogenesis, or both. It is unknown, however, whether or not the choroid circulation is spared in the posterior pole of the fundus, that is, the region where retinal cells underlying central visual function (mostly belonging to the macular cone system) are preserved until the later stage of the disease. In addition, it is unknown how a possible loss of choroidal blood vessels impacts the corresponding retinal function in individual patients. These questions are relevant both for a better understanding of the disease pathophysiology and for a therapeutic perspective, because any attempt of photoreceptor rescue or replacement may be counteracted by a loss in the major nutrients, which are indeed provided by the superficial choroid circulation.

In the present study, we evaluated in RP patients subfoveal choroidal blood flow by laser Doppler flowmetry (LDF) and central retinal function by macular focal electroretinograms (focal ERGs, FERGs), to determine whether choroidal blood flow is altered in RP, and whether this alteration is associated with central cone–mediated dysfunction. Both techniques have been already largely used to assess choroidal circulation and macular function, respectively, in normal and diseased retinas.[10–13] The LDF signal of the subfoveal choroid originates

From the [1]Department of Ophthalmology and Otolaryngology, Catholic University, Rome, Italy; [2]Department of Ophthalmology, University Federico II, Naples, Italy; [4]Health and Technology Department, Istituto Superiore di Sanitá, Rome, Italy; [5]Fondazione GB Bietti per l'Oftalmologia, IRCCS, Rome, Italy; [6]Department of Ophthalmology, University of Bologna, Bologna, Italy; and [3]CEINGE–Biotecnologie Avanzate, Naples, Italy.

Supported by a grant from Fondazione Cassa di Risparmio in Bologna, Italy, to the Department of Ophthalmology (ECC and CER) and a grant from Ministero della Ricerca, Fondi di Ateneo ex 60% (BF).

Submitted for publication May 27, 2010; revised July 14, 2010; accepted August 25, 2010.

Disclosure: **B. Falsini**, None; **G.M. Anselmi**, None; **D. Marangoni**, None; **F. D'Esposito**, None; **A. Fadda**, None; **A. Di Renzo**, None; **E.C. Campos**, None; **C.E. Riva**, None

Corresponding author: Benedetto Falsini, Department of Ophthalmology and Otolaryngology, Università Cattolica S. Cuore, Lgo F. Vito 1, 00198 Rome, Italy; bfalsini@rm.unicatt.it.

predominantly from the choriocapillaris (see Refs. 10, 11). The macular FERG reflects mainly the activity of retinal generators located in the middle and outer retinal layers.[14] By combining both techniques in the same patients, we sought to determine whether functional macular changes were associated with corresponding deficits in subfoveal superficial choroid circulation.

Materials and Methods

Thirty-one patients (15 males and 16 females; mean age, 42.5 years; age range, 15–72 years) with a diagnosis of typical RP were included in the study. Each patient underwent a complete general and ophthalmologic examination, and a detailed family and medical history was also obtained. Thirteen patients were categorized as having a sporadic (isolated) form of RP, six had a family history suggesting a dominant mode of transmission, eight were categorized as the autosomal recessive type, and four had X-linked RP. Each patient had severely reduced or nondetectable standard full-field electroretinograms (both scotopic and photopic recorded following the ISCEV [International Society for Clinical Electrophysiology of Vision] standard protocol), a central kinetic visual field of at least 20° (measured by Goldmann perimetry with a II/4e white target), and a Snellen acuity between 20/20 and 20/30. Kinetic visual field was staged according to a method similar to that used by Schmidt et al.[6] using the Goldmann target size III/4e. Based on the percentage loss from the normal visual field area, patients' results were divided into three groups: I, 10%–25% loss ($n = 11$); II, 26%–50% loss ($n = 14$); III, 51%–70% loss ($n = 6$). On fluorescein angiography, none of the patients had evidence of cystoid macular edema. All patients included in the study had stable central fixation (as evaluated by a Visuskope; Heine, Germany), clear optical media, and no concomitant ophthalmic diseases. The demographic and clinical data of patients are summarized in Table 1. Twenty normal subjects (9 males and 11 females; mean age, 35 years; age range, 12–72 years) provided normative subfoveal choroidal blood flow and FERG data. None of the subjects had a history of ophthalmic or neurologic disease. All subjects had normal general and ophthalmic examinations. Informed consent to participate in the study was obtained from all subjects; the research followed the tenets of the Declaration of Helsinki and was approved by the Institutional Ethics Committee.

Table 1. Summary of Demographic and Clinical Characteristics of the Study Population*

Mean age, y (range)	42.5 ± 18 (15–72)
Median visual acuity (range)	20/25 (20/30–20/20)
Goldmann visual field (range)	(V/4e target): mean field (major diameter), 45° (30°–55°)
Fundus	Typical RP appearance with waxy pale disc, attenuated retinal vessels and bone spicule pigmentation in the midperiphery
Ganzfeld ERG (ISCEV standard)	
Rod-mediated response	Nonrecordable
Maximal response	Severely reduced amplitude (<35% of the normal mean)
Single-flash cone-mediated response	Severely reduced amplitude (<40% of the normal mean) and delayed implicit time (3–8 ms slower than the normal mean)
Flicker response	Severely reduced amplitude (<40% of the normal mean)
Inheritance mode, *n*	
Dominant	6
Recessive	8
Isolated	13
X-linked	4

* $n = 31$.

Measurement of Subfoveal Choroidal Blood Flow

The LDF measurement of subfoveal choroidal blood flow has been previously described in detail,[10,12] and so only a brief summary will be given here. In the present study, a confocal LDF system was used with a probing beam in the near-infrared (laser diode, 785 nm).[10] Diameter and power of the beam at the cornea were 1.3 mm and 90 μW, respectively. This power conforms to the ANSI Z 136.1 standards for laser safety. The light scattered by the red blood cells (RBCs) in the volume sampled by the laser light was collected by a bundle of six optical fibers (core diameter of 110 μm each) and guided to an avalanche photodiode. These fibers were arranged on a circle with a diameter of 180 μm, which was imaged at the retina so that the center of the circle coincided with the focus of the incident beam.

The output current of the photodiode was sampled at a frequency of 44 kHz (bandwith of 22 kHz) and processed with a software implemented on a PC to determine the choroidal blood flow LDF parameters in real time at a rate of 21.5 Hz, using an algorithm based on a photon diffusion theory. These parameters are the velocity, *ChBVel* (kHz), the number, *ChBVol* (arbitrary units [a.u.]), and the relative flux, *ChBF* (a.u.) of RBCs within the sampled tissue region. This flux is proportional to blood flow if the hematocrit remains constant during the recordings. These parameters are related to each other through the relationship $ChBF = k \times ChBVel \times ChBVol$, where k represents an instrumental constant. The software automatically excludes the Doppler signal during blinks and artifacts caused by rapid eye movements. Only those recordings were kept where the direct current (DC), a measure of the intensity of the light scattered by both the tissue and RBCs in the illuminated volume and reaching the detector, did not fluctuate by >10% during the recording duration (approximately 1 minute). Subsequent data analysis and computation included average and SD of the flow parameters over the recording time.

The LDF flowmeter was mounted on a slit-lamp support equipped with a chin rest and head holder. Subjects, seated, looked into the instrument, directly at the laser beam. The instrument was aligned by the operator in such a way that the DC signal, continuously displayed on the computer screen, was maximum. Recordings were obtained after pupil dilation with tropicamide 1%. In both normal subjects and patients, pupil size was at least 8 mm, and no differences in mean pupil size were found between groups.

For each patient and control subject, intraocular pressure, brachial blood systolic, and diastolic pressures were determined at the end of each recording session. Mean arterial blood pressure (MAP) and mean ocular perfusion pressure (PPm) were determined according to published formulas.[12]

Electrophysiological Measurements

The FERG technique used in this study is similar to that published in previous studies.[13] Briefly, stimuli consisted of flickering uniform fields generated by an array of eight red LEDs (max lambda, 660 nm; half-height bandwidth, 25 nm; mean luminance, 80 cd/m^2) sinusoidally driven by a custom-made digital frequency generator and presented on the rear of a ganzfeld bowl illuminated at the same mean luminance as the stimulus. A diffusing filter placed in front of the LED array made it appear as a circle of uniform red light. A steady DC signal maintained constant the stimulus mean luminance. FERGs were recorded in response to the sinusoidal luminance modulation (92% modulation depth) of a homogeneous circular field, whose diameter subtended 18° of visual angle, presented to the macular region. A small central fixation mark allowed the field to be centered on the fovea. Patients and normal subjects always fixated monocularly at the fixation mark, from a distance of 30 cm. Stimulus temporal frequency was 41 Hz. In both controls and patients, pupils were pharmacologically (tropicamide 1%) dilated to at least 8 mm.

FERGs were recorded by an Ag-AgCl electrode taped on the skin over the lower eyelid. A similar electrode, placed over the eyelid of the contralateral patched eye, was used as reference (interocular ERG).[15] The use of skin electrodes with an interocular recording is an accepted

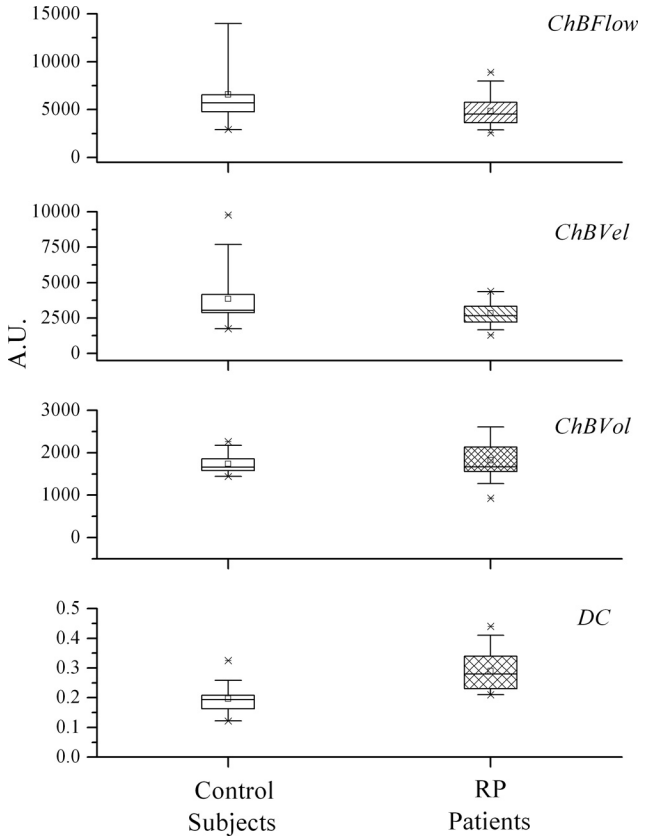

FIGURE 1. Box plots of subfoveal choroidal blood flow parameters in control subjects and RP patients. In each plot, the symbol (*open square*) is the mean, the *box* indicates the median and interquartile range, and the *bars* indicate the 99 percentiles.

technique,[10,16] and the extensive protocol of this study justified its use. FERG signals were amplified (100,000-fold), band pass filtered between 1 and 250 Hz (6 dB/oct), and averaged (12 bit resolution, 2 kHz sampling rate, 1600 repetitions in eight blocks). The averaging time (i.e., the sweep duration) corresponded to the stimulus period (24.4 ms). Single sweeps exceeding a threshold voltage (5 μV) were rejected to minimize noise coming from blinks or eye movements. A discrete Fourier analysis was performed offline to isolate the FERG fundamental harmonic (1F), whose peak-to-peak amplitude (in μV) and phase (in π radians) were measured. Averaging and Fourier analysis were also performed on signals sampled asynchronously at 1.1 times the temporal frequency of the stimulus to give an estimate of the background noise at the stimulus frequency. Under the present experimental conditions, the FERGs recorded individually from both control subjects and RP patients were above the noise level (noise amplitude ≤ 0.08 μV in all cases) and sufficiently reliable (the variation coefficient in amplitude was typically 20%; the phase SD was ± 0.11 π radians).

Statistical Analysis

Sample size estimates for this study were based on previous investigations[11,13] where the between- and within-subjects variability (expressed as data SD) of *ChBF* and FERG parameters were determined. The sample sizes of patients and controls provided a power of 80%, at $\alpha = 0.05$, for detecting a between-group difference of 0.2 log μV (SD, 0.1) and 30° (SD, 20) in FERG amplitude and phase, respectively, and a between-group difference of 20% in *ChBF* parameters.

Although the data from both eyes of each patient and control subject were analyzed, the main statistical comparisons and correlations were performed on the results from the right eyes. Interocular analysis was performed for both LDF and FERG by comparing the differences between right and left eye measurements, expressed as percentage of right eye values (FERG amplitude and LDF parameters) or as difference between absolute values (for FERG phase).

Each LDF parameter obtained from the RP and control eyes was evaluated by an unpaired *t*-test assuming a normal distribution of these parameters. These parameters were also compared between right and left eyes by paired *t*-tests. The IOP, MAP, and PPm were compared between patients and control eyes by unpaired *t*-tests. FERG 1F amplitudes from normal subjects and RP patients were statistically compared by analysis of variance (ANOVA), with group (normal subjects versus patients) as a between-subjects factor. Response amplitudes also underwent logarithmic transformation to better approximate a normal distribution. FERG phase values were averaged, and corresponding variances (and circular SDs) were estimated using a method[17] that takes into account the circular distribution of phase space, after measuring cosine and sine values from Fourier analysis. Because the Fourier analysis gives only the response phases in a 360° range, and the actual phase values can be in theory integer multiples of 360°, several assumptions were made to determine the exact response phases in both controls and patients.[13] First, it was assumed that the phase of the various FERG responses is mostly determined by a time delay, which is comparable to the implicit time of the standard cone flicker ERG. Second, it was assumed that, although in normal subjects the FERG response timing would be between 25 and 40 ms, this timing may be largely increased in RP patients compared with normal values, as shown for the photopic flicker ERG or for the fundamental component of the flicker ERG in the range 14–52 Hz (up to a 33 ms increase from normal mean values).[18] Therefore, timing of our FERG data of normal subjects and patients was expected to be between 25 and 73 ms. Correlations between the individual flow parameters and FERG amplitude and phase data were evaluated by Pearson's correlation and linear

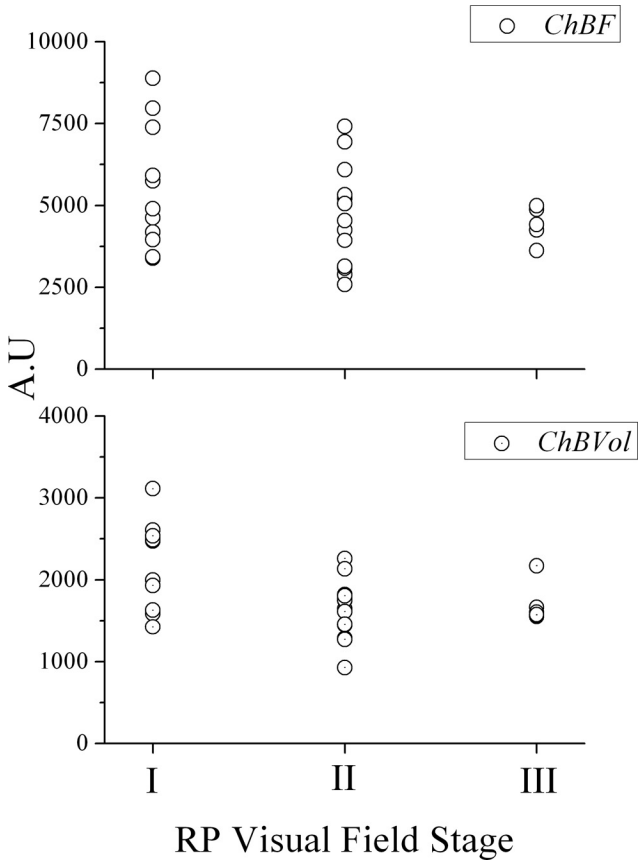

FIGURE 2. Choroidal blood volume and choroidal blood flow values plotted as a function of Goldmann visual field stage (I, 10%–25% loss; II, 26%–50% loss; III, 51%–70% loss).

regression analysis. P values < 0.05 were considered statistically significant.

Results

For all LDF parameters within-subject intratest variability, expressed as the SD of the three measurements obtained from each eye of RP patients and controls, was <10%. Figure 1 shows box plots of DC, *ChBVol*, *ChBVel,* and *ChBF* (depicting the mean, median, interquartile, and 99% percentile range), obtained from RP and control eyes. Mean *ChBVol* was not significantly different between control and patient groups. However, *ChBVel* and *ChBF* were significantly reduced (by 26%) in patients compared with controls (*ChBVel*, $t = 2.68$, $P = 0.01$; *ChBF,* $t = 2.33$, $P = 0.02$). The DC value showed on average a significant increase (approximately 25%, $t = 5.03$, $P < 0.01$) in patients compared with controls. No significant correlation was found between DC and *ChBVel* and *ChBF* values. No significant differences were found in IOP, MAP, and PPm between RP patients and control subjects. *ChBVol* and *ChBF* tended to be more decreased in patients with more advanced Goldmann visual field loss compared with those with relatively preserved fields. This relationship is shown in Figure 2, where the individual hemodynamic values are plotted as a function of RP visual field stage. The association reached the statistical significance for the *ChBVol* ($r = -0.42$, $P = 0.02$) only.

In Figure 3, mean 1F FERG amplitudes and phases are shown as box plots for RP patients and control subjects. In each plot, the symbol is the mean, the box indicates the median and interquartile range, and the bars indicate the 99 percentiles. ANOVA showed a significant difference between groups (multivariate Hotelling's statistics, $F = 3.51$, $P < 0.01$). Mean amplitude was significantly reduced in patients compared with controls ($P < 0.01$). FERG phases tended to be delayed in patients compared with controls, although the univariate F-test did not reach statistical significance ($P < 0.08$). As observed in other studies[13,19] FERG amplitude losses and delays tended to be more severe in patients with lower visual acuity (20/30) compared with those with more preserved acuity.

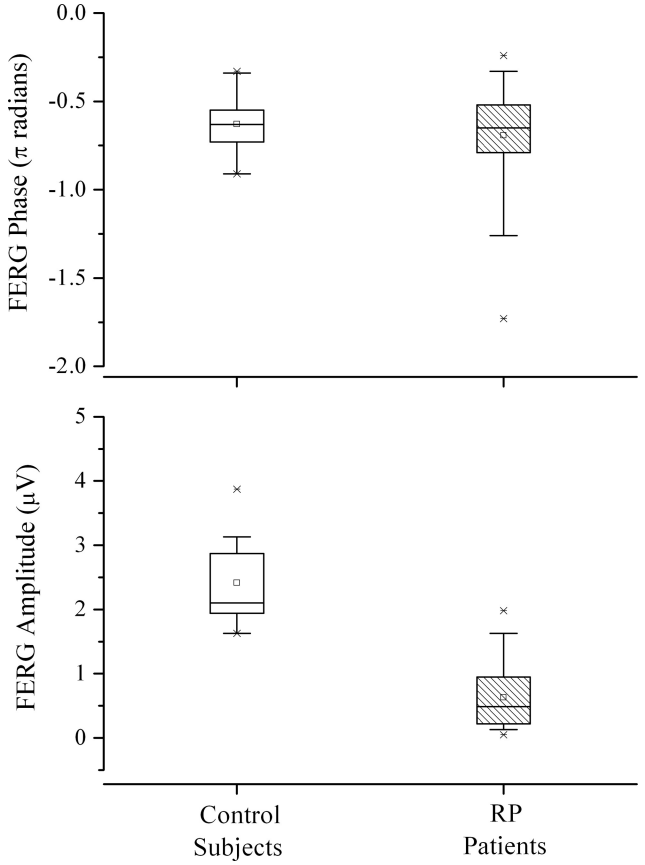

Figure 3. Box plots of the FERG parameters in control subjects and RP patients. In each plot, the symbol (*open square*) is the mean, the *box* indicates the median and interquartile range, and the *bars* indicate the 99 percentiles.

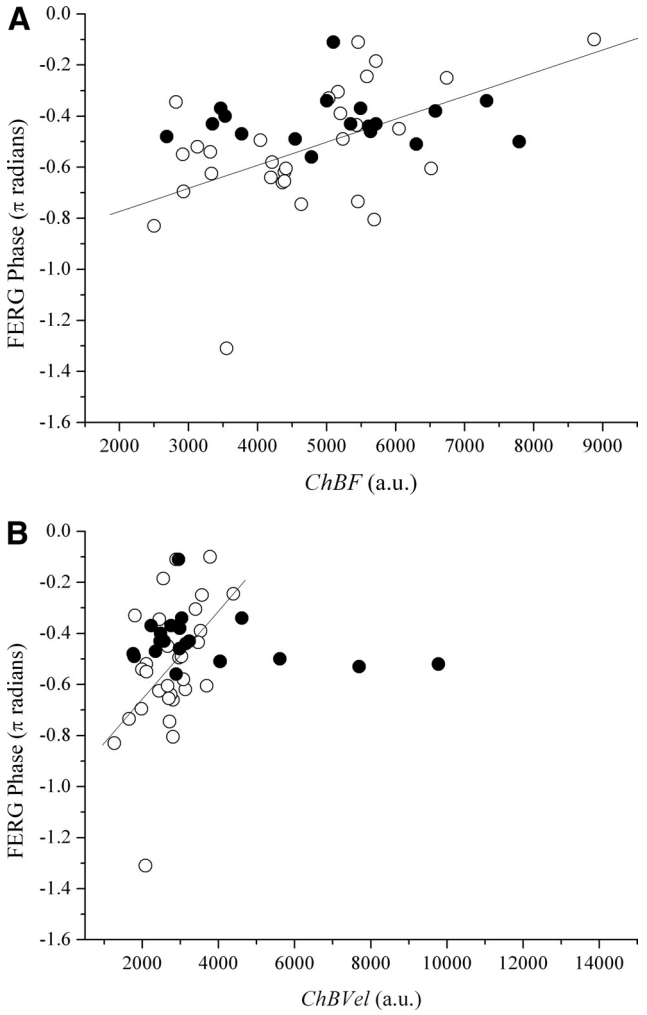

Figure 4. Scatterplots of FERG phase data, recorded in individual patients (*open circles*) and age-matched controls (*filled circles*), as a function of corresponding values of choroidal blood flow and choroidal blood velocity.

Correlations between the LDF and FERG data of the right eyes of individual RP patients are shown in the plots of Figures 4 and 5. In the same plots, the individual data of control subjects are also reported for comparison. It can be seen that FERG phase delays in patients were positively correlated ($r = 0.50$, $P < 0.01$) with corresponding *ChBF* and *ChBVel* values, respectively (Fig. 4). The same correlations were observed for the left eyes. FERG amplitudes of patients were positively correlated ($r = 0.49$, $P < 0.01$) with corresponding *ChBVol* values (Fig. 5). Although the latter correlation appeared to rely mainly on one or two data points having both high volume and amplitude values (i.e., potential outliers), it was significant and

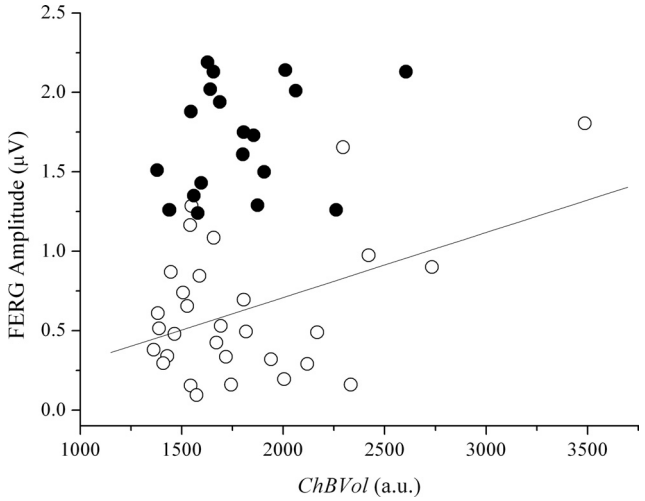

FIGURE 5. Scatterplot of FERG amplitude data, recorded in individual patients (*open circles*) and age-matched controls (*filled circles*), as a function of corresponding values of choroidal blood flow volume.

reproducible also when the data from the left eyes of patients were analyzed (not shown).

In both control subjects and patients, FERG and LDF values were not significantly different between right and left eyes. In Figure 6, the individual interocular differences in FERG phase recorded in RP patients are plotted as a function of the corresponding interocular differences in *ChBVel*. The interocular differences in FERG phase were positively correlated ($r = 0.45$, $P = 0.03$) with the corresponding differences in *ChBVel*, indicating that, in individual patients, the eye with the smaller *ChBVel* value also tended to have the more delayed FERG phase value.

No significant correlations were found between each of the LDF parameters and intraocular pressure, brachial blood systolic and diastolic pressures, MAP, and PPm, measured in RP patients and in controls.

DISCUSSION

This study was designed to evaluate whether subfoveal choroid blood flow is altered in RP patients with relatively preserved visual acuity, and whether there is an association between this flow abnormality and the losses in central cone–mediated function, the latter assessed objectively by FERG. The results showed that subfoveal *ChBF* was significantly altered in RP patients, mainly because of a reduction of *ChBVel*. *ChBF* and *ChBVol* tended to be more reduced in patients with more advanced total visual field loss. The DC values in RP patients were significantly increased on average compared with control values. This is consistent with a general increase in the reflectance of the photoreceptors/retinal pigment epithelium/choriocapillaris complex, an effect that is probably due to the microanatomic retinal changes previously described in human RP retinas.[20,21] The increase in DC values found in RP patients is not expected to affect the flowmetric results; however, because no correlation was found between DC and *ChBVel* and *ChBF* values. *ChBVel* and *ChBF* values in patients were significantly correlated with the corresponding FERG phases and *ChBVol* with FERG amplitudes, which suggests that retinal functional deficits are associated with an abnormal circulation in the superficial layers of the choroid.

Although previous studies (see the introduction) have suggested that choroid hemodynamic is altered in RP, none of these studies investigated subfoveal choroidal blood flow. In this study, we used confocal LDF to assess this flow.[12] This technique has shown adequate reproducibility[22] and has been successfully used in several physiological[23,24] and clinical studies (i.e., age-related macular degeneration[25–27] and glaucoma[28,29]). In our patients, confocal LDF provided reliable results, as shown by the good intratest reproducibility. *ChBVol* was found to be similar to control values, whereas *ChBVel* showed a significant reduction. This finding may be the consequence of the well-described histopathologic changes involving the choroid both in animal models of RP (see the introduction) and in human donor RP eyes,[20,21] consisting mainly of a reduction in the number of capillaries. In addition, given the tight relationship between retinal pigment epithelium and choriocapillaris,[30,31] even a dysfunction of retinal pigment epithelial cells might result in anatomic and physiological changes of choriocapillaris. Indeed, the selection of our RP patients with relatively preserved visual acuity does not necessarily means selection of patients with healthy subfoveal retinal pigment epithelium. It may be that, in our patients, the small vessels of the subfoveal choriocapillaris were either reduced in number or significantly attenuated in their lumen. It should be stressed, however, that RP has a complex pathophysiology. The causal genes of some patients are specific for retinal pigment epithelium, while in some others photoreceptor apoptosis is caused by oxidative stress. It may well be that the gene-specific disease mechanisms influence the role of choroidal hemodynamic in the sequence of events leading to central retina dysfunction and degeneration. In the present study, choroid hemodynamic was assessed in relation to cone-mediated function. It is unknown whether hemodynamic changes are even better correlated with rod-mediated function. None of the patients included in our study population had detectable rod function in the central retina. To address these issues, we are planning to test selected patients with known genotypes and detectable central and pericentral rod function.

It was important in our study to attempt to establish how the observed circulatory changes were related to the abnormalities of central retinal function in RP patients. In this regard, a brief reappraisal of the physiological abnormalities of central retinal function in RP, as detected by FERG, may help in the interpretation of the present findings. Since the original work by Seiple et al.,[19] abnormalities of the FERG in RP patients have been reported by several studies (see, e.g., Refs. 13, 18, 32 for a review). FERG has been used to quantify temporal responsiveness of the central retina in various disease stages. It has been shown that FERG measurements reliably reflect the loss in the number and sensitivity of photoreceptors and bipolar cells,

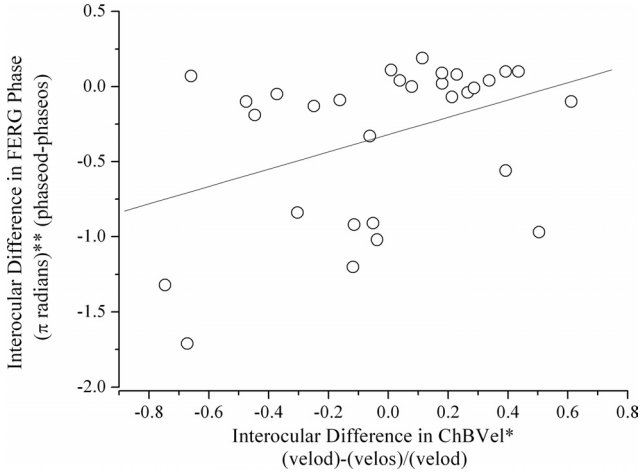

FIGURE 6. Scatterplot of interocular differences in FERG phase recorded in RP patients as a function of corresponding interocular differences in choroidal blood flow velocity.

which are the main generators underlying the responses.[14] The latter are well correlated with perimetric central and paracentral sensitivity, assessed by Humphrey automated perimetry,[13] and show different degrees of abnormality according to the severity of visual acuity loss.[13]

A well-described FERG abnormality in RP is represented by the FERG response delay.[13,16,18,19,33,34] Although in our patients the FERG phase was not, on average, significantly different from that of controls, in some patients the FERG phase tended to be delayed or showed substantial delays beyond the normal 95% confidence limits (see Fig. 2). Similar to the delay found for the Ganzfeld full-field ERGs (both flicker and single flash),[35] FERG phase delay may not be fully accounted for by a loss in sensitivity of photoreceptors, but it may reflect an abnormality at or beyond the synapse of photoreceptors with bipolar cells. Therefore, FERG delays in RP are thought to reflect either photoreceptor (sensitivity loss) or post-photoreceptoral abnormalities (synaptic malfunction/inner retinal abnormalities). Based on these considerations, it is reasonable to suggest that the correlations between *ChBVel* and FERG phase delays found in the present study reflect a pathologic process where a deficit in the choroidal circulation, in addition to the well-described intraretinal vascular changes,[20] results in a malfunction at the level of cone/bipolar synapse, leading to a severe delay in the FERG response.

The prognostic value of the current findings is unclear. It may be worthwhile to investigate, in a longitudinal study, whether alterations of the choroidal circulation, which appear to be correlated with those in the macular FERG, are predictive of a faster deterioration of the clinical picture, or, alternatively, their relative sparing can predict a better long-term prognosis for visual function. Future longitudinal studies, employing clinical, electrophysiologic, and flowmetric measurements, will address these relevant questions.

References

1. Portera-Cailliau C, Sung CH, Nathans J, Adler R. Apoptotic photoreceptor cell death in mouse models of retinitis pigmentosa. *Proc Natl Acad Sci U S A.* 1994;91:974–978.
2. Remé CE, Grimm C, Hafezi F, Marti A, Wenzel A. Apoptotic cell death in retinal degenerations. *Prog Retin Eye Res.* 1998;17:443–464.
3. Marigo V. Programmed cell death in retinal degeneration: targeting apoptosis in photoreceptors as potential therapy for retinal degeneration. *Cell Cycle.* 2007;6:652–655.
4. Grunwald JE, Maguire AM, Dupont J. Retinal hemodynamics in retinitis pigmentosa. *Am J Ophthalmol.* 1996;122:502–508.
5. May CA, Narfström K. Choroidal microcirculation in Abyssinian cats with hereditary rod-cone degeneration. *Exp Eye Res.* 2008;86:537–540.
6. Schmidt KG, Pillunat LE, Kohler K, Flammer J. Ocular pulse amplitude is reduced in patients with advanced retinitis pigmentosa. *Br J Ophthalmol.* 2001;85:678–682.
7. May CA, Horneber M, Lütjen-Drecoll E. Quantitative and morphological changes of the choroid vasculature in RCS rats and their congenic controls. *Exp Eye Res.* 1996;63:75–84.
8. Neuhardt T, May CA, Wilsch C, Eichhorn M, Lütjen-Drecoll E. Morphological changes of retinal pigment epithelium and choroid in rd-mice. *Exp Eye Res.* 1999;68:75–83.
9. Langham ME, Kramer T. Decreased choroidal blood flow associated with retinitis pigmentosa. *Eye.* 1990;4:374–381.
10. Riva CE, Cranstoun SD, Grunwald JE, Petrig BL. Choroidal blood flow in the foveal region of the human ocular fundus. *Invest Ophthalmol Vis Sci.* 1994;35:4273–4281.
11. Riva CE. Sub-foveal choroidal blood flow by LDF: measurement and applications to the physiology and pathology of the choroidal circulation. *Bull Soc Belg Ophthalmol.* 2006;302:182–194.
12. Lovasik JV, Kergoat H, Riva CE, Petrig BL, Geiser M. Choroidal blood flow during exercise-induced changes in the ocular perfusion pressure. *Invest Ophthalmol Vis Sci.* 2003;44:2126–2132.
13. Iarossi G, Falsini B, Piccardi M. Regional cone dysfunction in retinitis pigmentosa evaluated by flicker ERGs: relationship with perimetric sensitivity losses. *Invest Ophthalmol Vis Sci.* 2003;44:866–874.
14. Kondo M, Sieving PA. Post-photoreceptoral activity dominates primate photopic 32-Hz ERG for sine-, square-, and pulsed stimuli. *Invest Ophthalmol Vis Sci.* 2002;43:2500–2507.
15. Fiorentini A, Maffei L, Pirchio M, et al. The ERG in response to alternating gratings in patients with diseases of the peripheral visual pathway. *Invest Ophthalmol Vis Sci.* 1981;21:490–493.
16. Falsini B, Iarossi G, Porciatti V, et al. Postreceptoral contribution to macular dysfunction in retinitis pigmentosa. *Invest Ophthalmol Vis Sci.* 1994;35:4282–4290.
17. Zar JH. Circular distributions. In: Zar JH, ed. *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice Hall; 1974:310–328.
18. Falsini B, Iarossi G, Fadda A, et al. The fundamental and second harmonic of the photopic flicker electroretinogram: temporal frequency-dependent abnormalities in retinitis pigmentosa. *Clin Neurophysiol.* 1999;110:1554–1562.
19. Seiple WH, Siegel IM, Carr RE, Mayron C. Evaluating macular function using the focal ERG. *Invest Ophthalmol Vis Sci.* 1986;27:1123–1130.
20. Milam AH, Li ZY, Fariss RN. Histopathology of the human retina in retinitis pigmentosa. *Prog Retinal Eye Res.* 1998;17:175–205.
21. Li ZY, Possin DE, Milam AH. Histopathology of bone spicule pigmentation in retinitis pigmentosa. *Ophthalmology.* 1995;102:805–816.
22. Polska E, Polak K, Luksch A, et al. Twelve hour reproducibility of choroidal blood flow parameters in healthy subjects. *Br J Ophthalmol.* 2004;88:533–537.
23. Longo A, Geiser M, Riva CE. Subfoveal choroidal blood flow in response to light-dark exposure. *Invest Ophthalmol Vis Sci.* 2000;41:2678–2683.
24. Fuchsjäger-Mayrl G, Polska E, Malec M, Schmetterer L. Unilateral light-dark transitions affect choroidal blood flow in both eyes. *Vision Res.* 2001;41:2919–2924.
25. Grunwald JE, Hariprasad SM, DuPont J, et al. Foveolar choroidal blood flow in age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 1998;39:385–390.
26. Lutty G, Grunwald J, Majji AB, Uyama M, Yoneya S. Changes in choriocapillaris and retinal pigment epithelium in age-related macular degeneration. *Mol Vis.* 1999;5:35.
27. Metelitsina TI, Grunwald JE, DuPont JC, Ying GS. Effect of systemic hypertension on foveolar choroidal blood flow in age related macular degeneration. *Br J Ophthalmol.* 2006;90:342–346.
28. Grunwald JE, Piltz J, Hariprasad SM, DuPont J. Optic nerve and choroidal circulation in glaucoma. *Invest Ophthalmol Vis Sci.* 1998;39:2329–2336.
29. Emre M, Orgül S, Gugleta K, Flammer J. Ocular blood flow alteration in glaucoma is related to systemic vascular dysregulation. *Br J Ophthalmol.* 2004;88:662–666.
30. Henkind P, Gartner S. The relationship between retinal pigment epithelium and the choriocapillaris. *Trans Ophthalmol Soc UK.* 1983;103(pt 4):444–447.
31. McLeod DS, Grebe R, Bhutto I, Merges C, Baba T, Lutty GA. Relationship between RPE and choriocapillaris in age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2009;50:4982–4991.
32. Parisi V, Falsini B. Electrophysiological evaluation of the macular cone system: focal electroretinography and visual evoked potentials after photostress. *Semin Ophthalmol.* 1998;13:178–188.
33. Sandberg MA, Effron MH, Berson EL. Focal cone electroretinograms in dominant retinitis pigmentosa with reduced penetrance. *Invest Ophthalmol Vis Sci.* 1978;17:1096–1101.
34. Berson EL. Retinitis pigmentosa: the Friedenwald lecture. *Invest Ophthalmol Vis Sci.* 1993;34:1659–1676.
35. Hood DC, Birch DG. Abnormalities of the retinal cone system in retinitis pigmentosa. *Vision Res.* 1996;36(11):1699–1709.