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. Riva6

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 ($ChBF_{Vol}$), velocity ($ChBF_{Vel}$), and flow ($ChBF_r$), 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 $ChBF_{Vol}$ were reduced by 26% ($P \leq 0.02$) in RP patients compared to controls, whereas $ChBF_{Vol}$ was similar in the two groups. FERG amplitudes were reduced by 60% ($P < 0.01$) in patients compared with controls. FERG phase delays 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 $ChBF_{Vol}$ values. FERG amplitudes were correlated ($r = 0.49, P < 0.01$) with $ChBF_{Vol}$ values.

CONCLUSIONS. These data indicate significant alterations of subfoveal choroidal hemodynamics 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 Ophthal Vis Sci. 2011;52:1064–1069) DOI:10.1167/iovs.10-5964

From the 1Department of Ophthalmology and Otolaryngology, Catholic University, Rome, Italy; 2Department of Ophthalmology, University Federico II, Naples, Italy; 3Health and Technology Department, Istituto Superiore di Sanità, Rome, Italy; 4Fondazione GB Bietti per l’Oftalmologia, IRCCS, Rome, Italy; 5Department of Ophthalmology, University of Bologna, Bologna, Italy; and 6CEINGE–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, Igo F. Vito 1, 00198 Rome, Italy; bfalsini@rm.unicatt.it

Copyright 2011 The Association for Research in Vision and Ophthalmology, Inc.
participate in the study was obtained from all subjects; the research had normal general and ophthalmic examinations. Informed consent to subjects had a history of ophthalmic or neurologic disease. All subjects (11 females; mean age, 35 years; age range, 12–72 years) provided intent ophthalmic diseases. The demographic and clinical data of patients included in the study had stable central fixation (as evaluated by a Visuskope; Heine, Germany), clear optical media, and no concomitant ERG (ISCEV Fundus Typical RP appearance with waxy pigmentation in the midperiphery). Ganzfeld ERG (ISCEV standard protocol), a central kinetic visual field of at least 20º (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. Using the Goldmann target size III/4c. 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), 45º (30º–55º) |
| 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.
technique, 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 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 radians).

Statistical Analysis

Sample size estimates for this study were based on previous investigations, 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 α = 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. Intercorrelation 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 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. 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). 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...
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.

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
technique has shown adequate reproducibility and has been successfully used in several physiological and clinical studies (i.e., age-related macular degeneration and glaucoma). In our patients, confocal LDF provided reliable results, as shown by the good intratest reproducibility. CbBVel 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, consisting mainly of a reduction in the number of capillaries. In addition, given the tight relationship between retinal pigment epithelium and choriocapillaris, 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 mean 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., 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,
which are the main generators underlying the responses. The latter are well correlated with perimetric central and paracentral sensitivity, assessed by Humphrey automated perimetry, and show different degrees of abnormality according to the severity of visual acuity loss.

A well-described FERG abnormality in RP is represented by the FERG response delay. 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), 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-photoreceptor abnormalities (synaptic malfunction/inner retinal abnormalities). Based on these considerations, it is reasonable to suggest that the correlations between OcbViel 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, results in a mal-function 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