Progressive Loss of Retinal Ganglion Cell Function Precedes Structural Loss by Several Years in Glaucoma Suspects

Michael R. Banitt, Lori M. Ventura, William J. Feuer, Eleonore Savatovsky, Gabriel Luna, Olga Shif, Brandon Bosse, and Vittorio Porciatti

Purpose. We determined the time lag between loss of retinal ganglion cell function and retinal nerve fiber layer (RNFL) thickness.

Methods. Glaucoma suspects were followed for at least four years. Patients underwent pattern electroretinography (PERG), optical coherence tomography (OCT) of the RNFL, and standard automated perimetry testing at 6-month intervals. Comparisons were made between changes in all testing modalities. To compare PERG and OCT measurements on a normalized scale, we calculated the dynamic range of PERG amplitude and RNFL thickness. The time lag between function and structure was defined as the difference in time-to-criterion loss between PERG amplitude and RNFL thickness.

Results. For PERG ($P < 0.001$) and RNFL ($P = 0.030$), there was a statistically significant difference between the slopes corresponding to the lowest baseline PERG amplitude stratum ($\leq$50%) and the reference stratum (>90%). Post hoc comparisons demonstrated highly significant differences between RNFL thicknesses of eyes in the stratum with most severely affected PERG ($\leq$50%) and the two strata with least affected PERG (>70%). Estimates suggested that the PERG amplitude takes 1.9 to 2.5 years to lose 10% of its initial amplitude, whereas the RNFL thickness takes 9.9 to 10.4 years to lose 10% of its initial thickness. Thus, the time lag between PERG amplitude and RNFL thickness to lose 10% of their initial values is on the order of 8 years.

Conclusions. In patients who are glaucoma suspects, PERG signal anticipates an equivalent loss of OCT signal by several years. (*Invest Ophthalmol Vis Sci.* 2013;54:2346–2352) DOI: 10.1167/iovs.12-11026

An issue central to the treatment of glaucoma is determining the onset of the disease. The current understanding is that early signs of glaucoma often manifest as permanent atrophic changes in the optic nerve, which are detected by characteristic visual field defects. Structural changes can be observed directly by examining the optic nerve, but also by measuring the optic nerve and retinal nerve fiber layer (RNFL) thickness with imaging devices. It is likely that these clinically manifest structural–functional changes are preceded by subclinical stages, at which retinal ganglion cells (RGC) have lost their autoregulatory ability in response to a chronically stressful biomechanical, vascular, or molecular environment, and become increasingly dysfunctional over time until they die and are eliminated from the neuronal pool.[1] The transition between normal and abnormal homeostasis may be considered the true time of disease onset, whereas the stage of RGC dysfunction preceding death represents the ideal stage during which therapeutic strategies to prevent cell death and visual loss should be initiated.

The electrical responsiveness of RGC to contrast-reversing visual stimuli can be monitored noninvasively in human and experimental models of glaucoma with the pattern electroretinogram (PERG).[2–7] Recent studies in human and mouse models of glaucoma have shown that in the early stages of the disease the magnitude of loss of PERG signal exceeds that expected from loss of RGC axons.[5,6,8] This implies the presence of a population of viable, but dysfunctional RGC. In human and mouse models of glaucoma, loss of PERG signal can be at least partly restored after IOP lowering.[9–13] Altogether, these results support the idea of a stage of reversible RGC dysfunction that precedes loss of retinal ganglion cell axons.

Our study attempts to determine the time lag between loss of RGC function and loss of RNFL thickness by comparing PERG and optical coherence tomography (OCT) over time in a population of glaucoma suspects (GS). GS do not have definite optic nerve damage or vision loss, but have one or more risk factors for glaucoma development or may have the disease at a level undetectable by standard diagnostic tests. It will be shown that in these patients a given loss of PERG signal anticipates the equivalent loss of OCT signal by several years.

Methods

Subjects

Study subjects were part of a longitudinal cohort of patients enrolled as glaucoma suspects at their initial visit based on detailed medical and ocular history, and a comprehensive eye examination as described previously.[14] Inclusion criteria were refractive errors within −5 to +3 diopters, best corrected visual acuity (BCVA) better than or equal to 20/20 (Snellen), normal standard automated perimetry (SAP) according to the Ocular Hypertension Treatment Study (OHTS) criteria[15] (reliability < 15% on all indices, normality > 5% on all global indices in two consecutive sessions 6 months apart), and glaucomatous optic

From the Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, Florida.

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Corresponding author: Michael R. Banitt, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, 900 NW 17th Street, Miami, FL 33136; mbanitt@med.miami.edu.

disc appearance (vertical cup-to-disc ratio [C/D] $\geq$ 0.5, C/D or asymmetry $\geq$ 0.2, localized thinning of the disc, splinter hemorrhage) or moderately increased IOP (>21 to <28 mm Hg). To qualify for this study, patients had to be followed with PERG and OCT for at least four years with no less than six visits during this period. Some eyes received treatment at some point during follow-up. Initiation of therapy in these patients took into account a constellation of risk factors, including optic disc cupping, corneal thickness, family history, race, life expectancy, and psychosocial issues.[16]

Treatment consisted of either prostaglandin analogs or beta-blockers, medications that are known not to cause changes in pupil size (American Academy of Ophthalmology Preferred Practice Pattern, Glaucoma Panel, Hoskins Center for Quality Eye Care, available in the public domain at www.aao.org/ppp). The study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Miami. Informed written consent was obtained from all subjects after the nature of the test and possible risks were explained in detail.

PERG Recording

We used a recording paradigm optimized for glaucoma detection (PERGLA) whose details have been described previously.[17] The paradigm yields steady-state responses reported to have relatively low test–retest and operator-dependent variability.[10,17–21] In brief, retinal signals were recorded simultaneously from both eyes by means of standard 10 mm Grass gold surface electrodes taped on the lower eyelids. Similar electrodes were placed over the ipsilateral temples (reference) and central forehead (common ground). Subjects were fitted with the appropriate lens correction to reach J1+ visual acuity for viewing a pattern stimulus placed at 30 cm, and were instructed to fixate on a target at the center of it. Subjects did not receive dilating drops, and were allowed to blink freely. The pattern stimulus consisted of horizontal gratings (1.7 cy/deg, 25° diameter circular field, 98% contrast, 40 cd/m^2 mean luminance), reversing 16.28 times per second. Electrical signals were conventionally band-pass filtered (1–30 Hz), amplified (100,000-fold), and averaged in synchrony to the reversal period. During signal acquisition, sweeps contaminated by eye blinks or gross eye movements were rejected automatically over a threshold voltage of 25 μV. Two successive responses of 300 artifact-free sweeps each were recorded, separated by a brief pause. The first 30 sweeps of each response were rejected to allow steady-state conditions. The software (GLAID; Lace Elettronica, Rome, Italy) allowed visual inspection of two consecutive responses superimposed to check for consistency, and then computed the final PERG waveform (600 artifact-free sweeps). The PERGLA instrument provides a measure of within-test amplitude variability that was below 30% in all subjects.[17,22] Since the PERG was recorded in response to relatively fast alternating gratings, the response waveforms typically were sinusoidal-like, with a temporal period corresponding to the reversal rate (examples reported by Ventura et al.[14]). PERG waveforms were analyzed automatically in the frequency domain by discrete Fourier transform (DFT) to isolate the frequency component at the contrast-reversal rate (16.28 Hz), and compute its amplitude in μV. The stimulus luminance/contrast did not change over the observation period as measured with a photometer (DR-2000-1; Gamma Scientific, San Diego, CA).

OCT Recording

Peripapillary mean RNFL thickness was evaluated using the fast RNFL program of the Stratus OCT (Carl Zeiss Meditec, Dublin, CA), and analyzed using software version 3.0 (Carl Zeiss Meditec). OCT measurements were performed with signal strength of 7 or higher. RNFL thickness was determined at 256 points of a circular scan (diameter 3.4 mm) centered around the center of the optic disc that is repeated 3 consecutive times. For each eye, RNFL scans were repeated 4 times at each visit and exported on an electronic worksheet, and an average scan was computed. Mean RNFL thickness was evaluated from the average scan as described previously.[8] In a previous cross-sectional study,[8] PERG changes were compared with RNFL changes at congruent locations along the temporal wedge of the optic disk.[23] In our study, RNFL changes over time were expected to be small and the variability of sectorial RNFL thicknesses was expected to be high compared to the mean RNFL.[24] To reduce variability of estimates, we compared PERG changes with changes of the mean RNFL thickness. The mean RNFL thickness was correlated significantly with the RNFL thickness of the temporal wedge ($r = 0.54$, $P < 0.001$). Correlations were similar across different baseline PERG strata (see below): $\leq$50% of normal $r = 0.68$, >50% to 70% of normal $r = 0.63$, 70% to 90% of normal $r = 0.61$, and >90% of normal $r = 0.41$ (all $P < 0.001$).

To compare PERG and OCT measurements on a normalized scale, we calculated the dynamic range of PERG amplitude and RNFL thickness. The dynamic range was defined as the difference between the mean baseline PERG amplitude/RNFL thickness (Table 1) and the PERG amplitudes/RNFL thicknesses that are measurable in advanced stages of the disease (floor). The floor of PERG amplitude was calculated from previous unpublished measurements obtained in eyes with advanced glaucoma (SAP-mean deviation [MD] −18.26 ± 2.99 decibels [dB], PERG amplitude 0.29 μV ± 0.087, $n = 8$). An estimate of the floor of RNFL thickness was obtained from a previous report from our group on RNFL thickness in advanced glaucoma using the Stratus OCT (45 μm).[25] Progressive PERG and OCT changes were expressed as percent of their dynamic range[8] according to the formula (normalized loss = value/[mean baseline − floor] × 100). Finally, we calculated the number of years required for PERG and RNFL to reach a criterion percent loss of their dynamic range. The time lag between function and structure was defined as the difference in time-to-criterion loss between PERG amplitude and RNFL thickness.

The distributions of variables were summarized with means and SDs. Rates of change of PERG amplitude and RNFL thickness were estimated as pooled within eye slopes using mixed linear models to account for the inclusion of data from both eyes of patients in the analysis (see below). Eyes were divided into baseline PERG amplitude percent normal strata of $\leq$50% normal, >50% to 70% normal, >70% to 90% normal, and >90% of normal.[8] The rationale for 50% being the upper limit of the bottom stratum was based on PERG measurements made of eyes affected severely with glaucoma. We chose 90% as the lower limit of the top stratum because 100% seemed too stringent a criterion. This gave us a sample size of 13 eyes in the most severely affected stratum ($\leq$50%) and 89 eyes in the least affected stratum. The sample size of eyes with PERG amplitude between 50% and 90% baseline, $n = 99$, split neatly when we divided this category in half.

Data Transformation and Analyses

To establish baseline PERG amplitude and RNFL thickness, the first three longitudinal measurements of each eye were averaged,[24] which reduced the amplitude test–retest variance component to approximately 20% of total variance. The statistical significance of differences in rates of change between percent normal strata were assessed with a test of slope × stratum interaction in the mixed linear model analysis.

This study includes three sets of analyses: comparison of mean RNFL thickness between baseline PERG amplitude strata (Fig. 1 and Table 2), calculation and comparison of slopes of PERG amplitude and RNFL thickness loss during follow-up among the baseline amplitude strata (Fig. 2 and Table 3), and estimation of time required for a clinically meaningful decrease in PERG amplitude versus RNFL thickness by baseline amplitude strata.

To create the baseline PERG amplitude strata, PERG amplitude was expressed as a percentage of normal, but these age-normalized values were not used for analyses. Age-normalization for PERG amplitude was necessary due to the substantial effect of age on PERG.[8] Over two decades, in the absence of disease, a 50-year-old's PERG amplitude decreases from 0.93 to 0.82 μV (12%). There was no significant or substantial difference in mean age among the four baseline amplitude

Table 1. Summary of Patient Characteristics

107 Patients, 201 Eyes	Mean ± SD
Age, y	56.1 ± 10.1
Baseline SAP-MD, dB	−0.83 ± 1.41
Baseline C/D, vertical	0.51 ± 0.16
Baseline IOP, mm Hg	15.9 ± 3.9
Central corneal thickness, um	538.5 ± 35.3
Follow-up, y	5.2 ± 0.7
N PERG tests	9.5 ± 1.9
N OCT tests	8.9 ± 1.9
Baseline OCT mean thickness, μm	100.9 ± 11.0
Baseline PERG amplitude, μV	0.80 ± 0.29

strata: >90%, 55.7 ± 8.7; 70% to 90%, 53.9 ± 8.8; 50% to 70%, 56.2 ± 10.5; and ≤50%, 61.0 ± 8.7 ($P = 0.15$, one way ANOVA). There also is a well-known dependency of Stratus OCT on age,[8] but the magnitude is smaller, approximately 4% and the lack of a difference in age between the amplitude groups convinced us it was not necessary to transform RNFL measurements into percent normal mean in Analysis 1 presented in Figure 1. No transformations were used in the calculation or comparison of slopes in Analysis 2, the follow-up period being too short to merit percent normal transformation. Analysis 3, the comparison of loss rates between PERG amplitude and Stratus mean RNFL thickness, was done as a percent of dynamic range.

We used a standard method for including two correlated eyes of subjects[26] in Analyses 1 and 2. The results of Analysis 2 then were used in Analysis 3. Specifically, we nested eyes (included them as a "random effect") within subject in the ANOVA model. This generated between eye variance component estimates that were extracted from the analysis so that they did not contribute to an inflated estimate of (in our case) the difference between strata or the slopes.

Results

Patient characteristics are summarized in Table 1. The raw PERG amplitudes for all eyes of patients were converted to percent deviation from age-expected normal values.[8] For all eyes, the baseline PERG amplitude deviation was defined as the average of the first three measurements of the longitudinal series. Baseline PERG amplitude deviations then were stratified into four levels of abnormality, whose characteristics are summarized (Table 2). The mean of the first three PERG amplitudes was used to improve the reliability of the measurement. Similarly, this was done for the baseline OCT RNFL thickness. The mean follow-up for this longitudinal series was 5.2 ± 0.7 years.

Figure 1 demonstrates the relationship between baseline PERG amplitude levels and corresponding baseline RNFL thickness (average of first three measurements of the longitudinal series for PERG and OCT). There was a statistically significant difference between average RNFL thickness ($P = 0.049$, mixed model ANOVA accounting for correlation between eyes of patients). Post hoc least significant difference (LSD) comparisons demonstrated highly significant differences between RNFL thicknesses of eyes in the level with the most severely affected PERG (≤50%) and the two with the least affected PERG (>70%). This would mean that the RNFL thickness did not decrease until the PERG amplitude had lost at least 50% of its age-expected normal value.

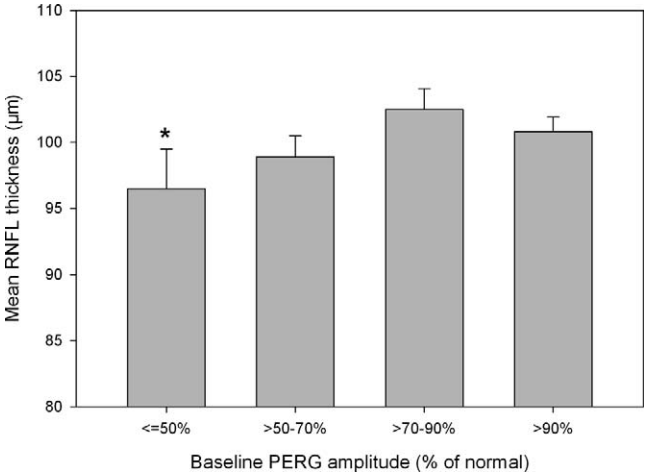

Figure 1. Baseline mean RNFL thickness for four strata of baseline PERG abnormality, based on the magnitude of percentage deviation from normal. For PERG and RNFL, baseline was calculated as the average of first 3 measurements of the longitudinal series. *Error bars* represent the SEM. The *asterisk above the bar* represents the level of statistical significance ($P < 0.05$).

In individual eyes, longitudinal PERG amplitudes and RNFL thicknesses were fit with linear regressions, and corresponding slopes calculated. Slopes then were pooled for each stratum of PERG abnormality (Table 3).

Figure 2 displays the rate of decrease of PERG amplitude (pooled slopes, μV/y) and RNFL thickness (pooled slopes, μm/y) for different levels of baseline PERG amplitude. For PERG and RNFL, there was a statistically significant difference between the slopes corresponding to the lowest baseline PERG amplitude stratum (≤50%) and the reference stratum (>90%)—mixed model slope/stratum interaction test, $P = 0.026$. A second set of analyses accounting for use of medical therapy gave very similar estimates. It is readily apparent from Figure 2 that PERG and RNFL slopes drastically differ in the way they change with increasing abnormality of baseline PERG. The RNFL slope is approximately constant in the range > 50 to >90%, but its steepness doubles for baseline PERG

Table 2. Baseline PERG Amplitude (Percent Deviation from Normal) Stratified in Four Levels of Abnormality, and Corresponding Mean PERG Amplitudes and RNFL Thicknesses

		PERG Amplitude, μV			RNFL Thickness, μm	
Baseline PERG, % of Normal	*N* Eyes	Mean	SD	*N* Eyes	Mean	SD
≤50%	13	0.4	0.05	12	96.5	10.4
>50 to 70%	49	0.55	0.06	48	98.9	11.0
>70 to 90%	50	0.73	0.07	50	102.5	11.1
>90%	89	1.03	0.19	89	100.8	10.5
Total	201			199		

Baseline PERG amplitudes and RNFL thicknesses represent the average of the first 3 measurements of the longitudinal series.

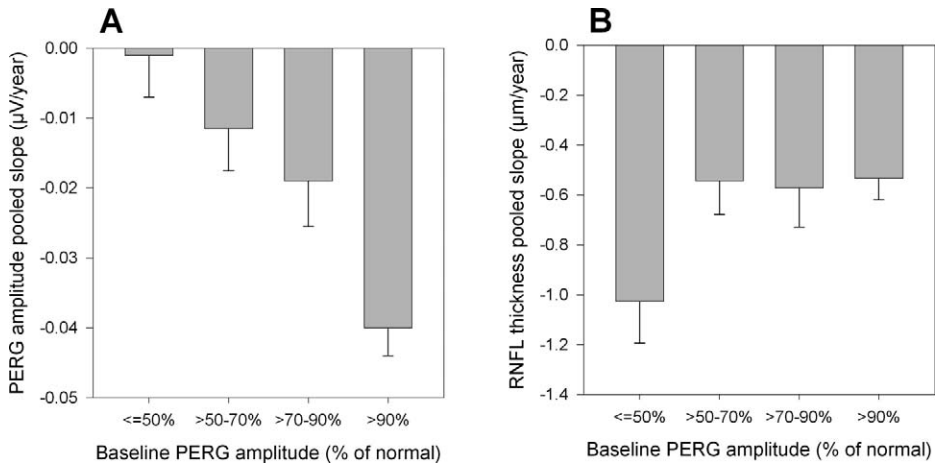

Figure 2. Pooled slopes of PERG amplitude (**A**) and RNFL thickness (**B**) for the four strata of baseline PERG abnormality. *Error bars* represent the SEM.

amplitudes loss of 50% or more. In contrast, the PERG slope becomes progressively less steep with increasing severity of baseline PERG. The PERG behavior, although apparently odd, is not unexpected. It is known that the PERG signal may become reduced early in glaucoma.[2,3] As it approaches the bottom of its dynamic range (see Methods), there is progressively less room left for further reduction. Therefore, the slope of PERG amplitude progression will decrease with decreasing baseline amplitude. This reasoning is less relevant for RNFL thickness, as for all strata baseline values are far from the floor (Fig. 3).

Figure 3 shows the baseline PERG amplitude/RNFL thickness for the four different levels compared to their respective floors. Baseline PERG/RNFL data shown in Table 2 were recast by expressing them as percent loss compared to the reference level (PERG > 90% of normal). Note that the PERG drops rapidly to the bottom of its dynamic range with increasing severity of baseline PERG amplitude, whereas the RNFL thickness shows relatively lesser changes.

We asked the question of whether there was a measurable time lag between equivalent losses of PERG amplitude and RNFL thickness over time. To determine this, we compared PERG and RNFL slopes for different strata on a normalized scale. Normalization was performed on the residual dynamic range of PERG and RNFL. That is, for each stratum we calculated the difference between the mean PERG/RNFL values shown in Table 1 and the respective floors). Then, based on the pooled slopes shown in Table 3, we calculated the number of years required to lose 10% of these dynamic ranges (delta). The estimates provided in Table 4 suggested that in the strata > 50% to >90% the PERG amplitude takes 1.9 to 2.5 years to lose 10% of its initial amplitude, whereas the RNFL thickness takes 9.9 to 10.4 years to loss 10% of its initial thickness. Thus, the time lag between PERG amplitude and RNFL thickness to lose 10% of their initial values is on the order of 8 years. The level ≤ 50% is too close to the PERG floor to yield a significant PERG slope. For this level, the time needed for the RNFL thickness to lose 10% of its initial value is 5 years, which is twice as steep as the RNFL thinning of stratum > 50%.

Effect of IOP Treatment

Substantial directional shifts in IOP over follow-up could have led to overestimation or underestimation of PERG loss rates.[13] A total of 63 eyes (31.3%) of 56 patients received pressure-lowering medicines at some point during the follow-up period, 48 eyes (23.9%) received them for more than 50% of their follow-up visits and 19 (9%) received them for all of their follow-up visits. Of these 63 eyes, the average time from the first study visit to the initiation of treatment was 1.2 ± 1.2 years. Table 5 provides baseline IOPs and final IOPs for the four baseline amplitude strata, and also compares the PERG amplitude slopes with or without accounting for IOP changes over the observation period. There was no effect of medication use on the difference in PERG amplitude loss rates between percent normal groups (*P* value slope × medication use interaction = 0.87).

Effect of Regression to the Mean

To explore how much regression to the mean might be affecting the PERG slopes, we calculated for each stratum the mean numbers of visits for which PERG amplitudes either were greater or smaller than the baseline. If regression to the mean were operating, one would expect a majority of visits with PERG amplitudes smaller than baseline in the >90% stratum as

Table 3. Pooled Slopes of PERG and RNFL Thickness for the Four Levels of Baseline PERG Abnormality

	PERG Amplitude				RNFL Thickness			
Baseline PERG, % of Normal	***N* Eyes**	**Pooled Slope, µV/y**	**SE**	***P***	***N* Eyes**	**Pooled Slope, µm/y**	**SE**	***P***
≤50%	13	−0.001	0.006	0.91	13	−1.026	0.168	<0.001
>50 to 70%	49	−0.012	0.004	0.005	49	−0.543	0.095	<0.001
>70 to 90%	50	−0.018	0.004	<0.001	50	−0.55	0.112	<0.001
>90%	89	−0.04	0.004	<0.001	89	−0.533	0.086	<0.001
Total	201				201			

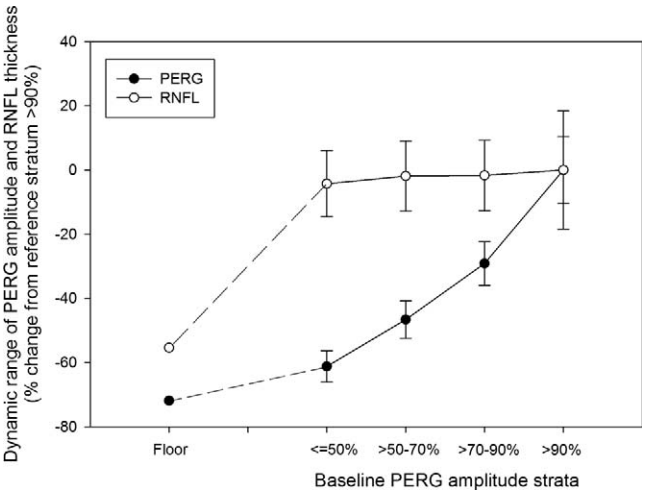

Figure 3. Dynamic ranges of PERG amplitude and RNFL thickness. Data are expressed as percent deviation from the reference stratum with highest PERG amplitude (>90% of normal). For PERG, the floor represents the mean amplitude (0.29 μV) recorded in a sample of patients with advanced glaucoma. For RNFL, the floor represents the minimum average RNFL thickness (45 μm) measured in patients with severe glaucoma using the same OCT instrument. *Error bars* represent the SD.

opposed to a minority of visits in the ≤50% stratum. Analysis showed that all strata had a majority of visits with PERG amplitudes smaller than baseline: ≤50% stratum had 5.37 greater than baseline versus 4.20 smaller than baseline, >50% to 70% stratum had 4.79 greater than baseline versus 4.67 smaller than baseline, >70% to 90% stratum had 4.88 greater than baseline versus 4.60 smaller than baseline; and >90% stratum had 5.57 greater than baseline versus 3.86 smaller than baseline (difference among strata, $P > 0.05$).

Discussion

The PERG has been thought of and used as an important indicator of retinal ganglion cell function in glaucoma. In recent publications, PERG has been shown to be reduced in glaucoma suspects, and ocular hypertensive and glaucoma patients.[27–29] Many attempts have been made to correlate the structure and function of the optic nerve in glaucoma.[30–36] Structural measurements have been made in the past measuring the size of the optic nerve, and the thickness of the optic nerve and RNFL. The function of the optic nerve has been assessed with standard automated perimetry. In general, there is some amount of structural abnormality before any functional abnormality is observed. PERG is thought to be abnormal before any structural abnormality is observed.[2,3] It has been proposed that abnormality in the PERG indicates dysfunction predating cell death and, thus, would signal a suitable time for intervention.

We found that patients with significantly reduced baseline PERG amplitudes had lower baseline RNFL thicknesses. Importantly, the highest rate of RNFL thinning occurred in the subgroup with the most reduced baseline PERG amplitudes. These results demonstrated that PERG amplitude, when reduced significantly, indicates that a glaucoma suspect needs closer monitoring or treatment as he or she will have a higher rate of RNFL thinning.

The time lag between changes in the PERG, and structural abnormality, and functional abnormality is not well known. In our study we were able to make an estimation of the time lag between the PERG and structural abnormality as measured by time domain OCT. We demonstrated that in our study population of glaucoma suspects, it takes an average of approximately 2 years for a 10% change in PERG and 10 years to see a 10% change in RNFL. This indicates that there is a time lag of approximately 8 years between changes in PERG and RNFL. To our knowledge, this is the first estimation of a time lag between potentially reversible ganglion cell dysfunction

Table 4. Residual Dynamic Range of PERG Amplitude and Mean RNFL for the Four Levels of Baseline PERG Abnormality

	PERG Amplitude			RNFL Thickness		
Baseline PERG, % of Normal	**Dynamic Range, μV**	**Delta, μV**	**Y**	**Dynamic Range, μm**	**Delta, μm**	**Y**
≤50%, $n = 13$	0.11	0.011	N.S.	51.5	5.15	5.02
>50 to 70%, $n = 49$	0.26	0.026	2.2	53.9	5.39	9.9
>70 to 90%, $n = 50$	0.44	0.044	2.4	57.5	5.75	10.45
>90%, $n = 89$	0.74	0.0748	1.9	55.8	5.58	10.47
Average > 50%			2.2			10.3

For each stratum, the PERG/RNFL dynamic ranges are calculated as differences between the mean amplitudes/thicknesses shown in Table 1 and the corresponding PERG/RNFL floor, defined as the average amplitude/thickness measurable in advanced stages of glaucoma (0.29 μV/45 μm). Delta, 10% loss of the residual dynamic range; Y, the number of years needed to lose 10% of the residual dynamic range; N.S., not significantly different from zero.

Table 5. Effect of IOP Changes on PERG Amplitude Slope

			PERG Amplitude Slope, μV/y	
Stratum	**Baseline IOP, mm Hg ± SD**	**Final IOP, mm Hg ± SD**	**w/o IOP**	**w IOP**
>90%	15.9 ± 3.5	16.2 ± 5.3	−0.04	−0.039
70 to 90%	16.2 ± 4.3	15.4 ± 2.7	−0.018	−0.017
50 to 70%	16.0 ± 4.2	15.2 ± 2.8	−0.012	−0.011
≤50%	15.9 ± 3.5	14.5 ± 3.6	−0.001	−0.002
P value	0.88	0.38	<0.001	<0.001

Baseline IOP, final IOPs, and PERG amplitude slopes for the four baseline amplitude strata without (w/o) or with (w) accounting for IOP changes over the observation period.

and permanent structural loss of ganglion cell axons. Others have studied the structural/functional correlation between RNFL and perimetry. Ajtony et al. demonstrated that an RNFL of less than 70 μm was correlated with visual field abnormality when measured by time domain OCT and standard automated perimetry.[37] Wollstein et al. used spectral domain OCT and standard automated perimetry, and demonstrated that a structural loss of 17% was necessary to be able to detect a functional loss of vision.[35] Both studies measured structural loss compared to permanent visual loss in perimetry. Hood and Kardon, in a review of cross-sectional data in subjects with manifest glaucoma, found evidence for a linear relationship between peripapillary thinning and loss of field sensitivity when both are expressed in linear units.[38] Their model does not exclude a time lag between RNFL loss and visual field loss. They argue that which test is detected first will depend upon the initial level of sensitivity and RNFL thickness when the eye is healthy, and on the relative standard deviation of the measures. In our longitudinal study of glaucoma suspects the initial level of sensitivity (PERG amplitude) and RNFL thickness did not influence the model. The relevant measure was the slope of change with time (expressed in linear units for PERG and OCT), and the variability of the estimate that was accounted for in the analysis of pooled data. Previous longitudinal studies of our group[27] and others[28] have reported progressive PERG changes in glaucoma suspects that were not associated with significant visual field changes.

Our study measured RGC dysfunction with permanent structural change. We assumed that the structural change measured by OCT is a permanent irreversible change. It is possible that there is some amount of reversibility or even cell shrinkage before cell death.

With respect to the effect of medications, Ventura et al.[13] have demonstrated that in some eyes the initiation of IOP lowering medications alters the slope of PERG amplitude losses, but this effect was not found in our analysis. There are two likely causes for this. A total of 78% of eyes followed in our study either received no medications or were taking them at all follow-up visits. Also, this effect did not extend to all eyes in the previous publication. It perhaps is not surprising that this heterogeneous effect would not be observed in a study group featuring relatively few eyes with pre- and postmedication onset experience.

Limitations to our study included that some of the patients received treatment. This potentially could confound the results, since if RGC dysfunction is reversible, directional shifts in IOP over follow-up could lead to over- or underestimation of PERG loss rates, but have no effect on the OCT. However, analysis with or without accounting for IOP changes over the observation period yielded very similar PERG amplitude slopes. We cannot rule out the possibility of regression to the mean influencing the slopes or PERG losses. However, a strong effect of regression to the mean seemed unlikely for the following reasons: We computed PERG baselines using three consecutive measurements made over a 12-month interval, which minimized the regression to the mean. If regression to the mean were operating, one would expect a negative slope in the >90% stratum (which was the case) and a positive slope in the ≤50% stratum (which, instead, had a slope close to zero). Further, one would expect a majority of visits with PERG amplitudes smaller than baseline in the >90% stratum as opposed to a minority of visits in the ≤50% stratum. Instead, all strata had a majority of visits with PERG amplitudes smaller than baseline.

Based on the results, we demonstrated it would take approximately 10 years to observe a 10% loss in RNFL in this study population. The study was performed only for 5 years on average, which is approximately half as long as the expected time needed to see a change in OCT. It would be more accurate to continue following the study population for longer to confirm the changes, but this would require much more follow-up.

In summary, we observed that when PERG amplitude is reduced to 50% of its age-adjusted norm, the rate of decrease in OCT RNFL is greatest. We also estimated that there is an 8-year time delay between seeing a 10% reduction in potentially reversible PERG amplitude to seeing a 10% irreversible structural RNFL reduction. This represents a substantial window for intervention before permanent loss of structure from glaucoma.

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