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Oscillatory Potentials of X-Linked Carriers of Congenital Stationary Night Blindness

Rockefeller S. L. Young,* Alex Chaparro,* James Price,* and James Walters†

ERG oscillatory potentials (OPs) were recorded from obligate carriers of CSNB and from age-matched normal subjects. The OPs were recorded under four stimulus conditions and were analyzed in the time and frequency domains. The results, first of all, provide confirmation of the previous report that the OP amplitude is reduced in carriers. Second, the results show that, of four stimulus conditions examined, the best condition for discriminating the carriers and normal subjects was when the flash was blue and the eye was dark-adapted. Third, the results show that, in the frequency domain, optimal discrimination occurs when examining the power content of the OP at a center frequency of about 130 Hz using a 70 Hz bandwidth window. In the time domain, optimal discrimination occurs when examining the amplitude of the third peak of the response. Invest Ophthalmol Vis Sci 30:806–812, 1989

In a family with the X-linked recessive form of congenital stationary night blindness with myopia (CSNB), several members with the defective gene can be readily identified. One member is the affected male. He is diagnosed on the basis of: (1) a long history of night blindness; (2) unremarkable fundus appearance; (3) Schubert–Bornschein-type ERG; and (4) high myopia.[1–5] A second member, an obligate carrier, is identified either because she is a daughter of an affected male or because she has an affected son.[6] At the present time, however, there is no clinical method for ascertaining the genotype of the daughters of the obligate carriers (ie, prior to having an affected son).

Until recently, there had been little if any evidence that heterozygote carriers universally displayed a visual defect. In 1984, Miyake and Kawase[5] reported that the CSNB carriers were functionally normal in several—but not all—respects. The amplitudes of the scotopic and photopic b-waves were not significantly different from normal; and the final rod threshold of the dark adaptation curve was normal in all but one eye of a carrier. However, in their study of 12 obligate carriers, the OP amplitudes of the carriers were smaller than that of normals and the OP amplitudes for 17 out of 22 of the carrier eyes studied were below 2 standard deviations of the normal OP mean.

The aims of the current study were, first, to provide confirmation of Miyake et al's observation that the OP amplitudes are reduced in carriers; and, second, to explore methodological factors important to the development of a diagnostic test. In particular, the current study examines how well carriers can be discriminated from age-matched normal subjects for different stimulus conditions (light versus dark adaptation and red versus blue flash) and data reduction methods (time versus frequency domains analysis).

Materials and Methods

Selection of Subjects

Female carriers were recruited from four pedigrees known to have the X-linked inherited form of congenital stationary night blindness with myopia. Within each pedigree, females were identified as carriers either by their affected father or by their affected son. All carriers recruited were in good health at the time they were seen; specifically, they denied a history of diabetes and other systemic disorders. Carrier C4, however, had surgical removal of her pituitary gland about 1 year prior to our testing. She was in good health at the time of testing.

The control subjects consisted of healthy individuals who were of similar ages to the carriers and were in good health. Seven of the controls were either a friend or spouse of the carrier. As these subjects trav-

From the *Department of Ophthalmology and Visual Sciences, Texas Tech Health Sciences Center, Lubbock, Texas, and the †College of Optometry, University of Houston, Houston, Texas.

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Reprint requests: Professor R. S. L. Young, Department of Ophthalmology and Visual Sciences, Texas Tech Health Sciences Center, Lubbock, TX 79430-0001.

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elled with the carrier, both carrier and control were alternately tested on the same day. The other five control subjects (N4, N5, N7, N9, N12) were either graduate students or faculty. Informed consent was obtained from all subjects after the nature of the procedures had been explained fully.

Procedure

The subjects' eyes were dilated prior to the recordings. In the dark adaptation condition, the subjects remained in the dark for 30 min prior to testing. In the light adaptation condition, the subjects viewed the stimulus against a background of about 3 log scotopic trolands. The stimulus was a ganzfeld train of flashes (100 per sec for 200 msec). The rationale for using the relatively long exposure was to avoid confounding the early oscillatory potential wavelets with an off-response component.[7] An 18 sec interflash interval was used to minimize the light adaptation effects from the flashes themselves. The flashes were either red (ie, filtered with a Wratten #26 filter) or blue (Wratten #47). The red and blue flashes were approximately photopically balanced for the ERG a-wave, as described in a previous study.[8]

The ERG was recorded from one eye using a gold foil electrode placed under the lower eyelid. A reference electrode was placed on the forehead and a ground electrode was placed on the earlobe. Electrode impedance ranged from 2 to 10 KOhms (5 KOhms was typical). The ERGs were passed through an analog filter (80–1500 Hz). Each response was recorded over a 200 msec epoch with a sampling rate of 5120 Hz on a Nicolet (Madison, WI) Med-80 System. Responses were averaged over five flashes and the average response for each stimulus condition was replicated five times.

Data analysis was performed off-line on the Med-80 system. In analyzing the data in the frequency domain, eye blink artifacts were first removed from the records. The artifacts were removed by attenuating the waveform with an exponential decay function after the initial 57 msec (for the dark-adapted condition) or 40 msec (for the light-adapted condition). A fast Fourier Transform program was then applied to the data to derive the power spectrum. (Note: The computation of "power" is made with the assumption that the ERG voltages are measured across a hypothetical 1 Ohm resistor. Our program is based on the software written by Dr. Elmar Schmeisser and provided to us by Dr. William Dawson.)

Index of Diagnostic Performance

We used the approach described by Massof and Emmel[9] to evaluate how well carriers can be discriminated from normal subjects on the basis of the OP. Diagnostic efficiency is defined as the probability of correctly discriminating a carrier from a normal subject in a two-alternative forced-choice decision task. To compute the diagnostic efficiency in the current study, we examined 108 paired-comparisons (ie, each of the nine carrier data is compared with each of 12 normal data) and determined the frequency that the response from carriers is smaller than that from normal subjects. A diagnostic efficiency of 0.50 indicates that carriers and normals would be discriminated at a rate equivalent to chance performance. A diagnostic efficiency of 1.00 indicates that the carriers and normals would be discriminated 100% of the time. Somewhere between 0.50 and 1.00 efficiency is a critical value such that, for the number of subjects tested, values equal to or greater than the critical value would differ from 0.50 at the $P < 0.05$ level. The critical value was computed conservatively using equation 17 from Massof and Emmel's paper.[9]

The rationale for using the approach described above is two-fold. First, the statistics are parameter-

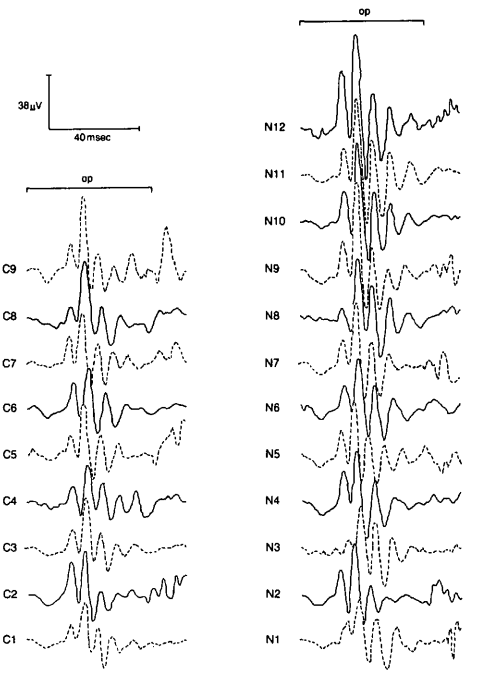

Fig. 1. OP responses for nine obligate carriers (left) and 12 age-matched normal subjects (right). The responses were elicited by a blue flash after the eye had been dark-adapted for 30 min. The OPs for this stimulus condition are composed of a series of five peaks over the initial 60 msec of the record.

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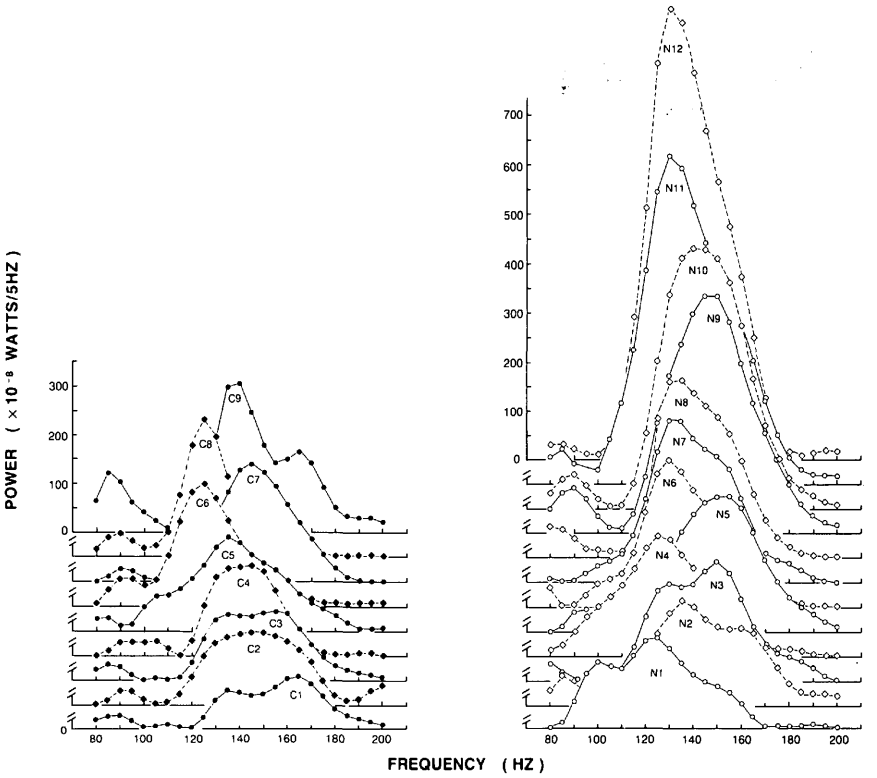

Fig. 2. Power spectra of the OP responses for the same nine carriers (left) and 12 normal subjects (right). Each power spectrum was computed from the corresponding OP responses shown in Figure 1.

independent. That is, the statistics can be used to compare the diagnostic performance from two tests even though the response parameters (eg, amplitude, power, time, etc.) used by the two tests are different. Second, the statistics are criterion-free. In our investigation, the benefit of a criterion-free approach is that the relative efficiencies for different stimulus conditions or data reduction methods can be evaluated without having to select, in advance, a "cutoff" value which defines normals and carriers.

Results

Differences in the OP Responses of Carriers and Normals

Figure 1 illustrates oscillatory potential (OP) responses displayed in the time domain for one of four stimulus conditions studied. The OP is composed of several wavelets occurring over the first 60 msec or so. These responses are qualitatively similar to those obtained under comparable conditions by previous investigators.[10,11] Figure 2 illustrates the same response displayed in the frequency domain. In the frequency domain, the OP is represented by a power spectrum showing the power content of the sine wave constituents. These responses are qualitatively similar to those reported previously.[12]

As a first order attempt to inquire how the OP responses of carriers and normal subjects differ, the average waveform across subjects was computed. The average waveforms for all four stimulus conditions are shown in Figures 3 and 4. As can be appreciated from Figure 3, the number and latency of the wavelets are similar for the carriers and normals. The main difference between the carriers and normals appears to lie in the amplitude of OPs. In every stimulus condition, the mean amplitude of the carrier responses is smaller than that of the normal responses. Figure 4 compares the average power spectrum for the carriers

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and normal subjects. The results suggest that if there is a difference in the frequency composition of the power spectra between carriers and normals, the difference must be small. For example, the peak frequency of the spectrum is nearly identical for the two groups in three out of the four stimulus conditions. The main difference between the carriers and the normals appears to lie in the magnitude of the power spectrum.

The power content of the frequency-domain response is less for the carriers than for the normals.

Analysis of the Diagnostic Performance of Different Methods of Data Reduction and Different Stimulus Conditions

This section considers how well carriers can be discriminated from normal subjects on the basis of the decision rule that: (1) OP amplitude of the carrier is less than that of the normal; or that (2) the power content of the OP for the carriers is less than that of the normal.

Discrimination based on OP amplitudes: The amplitude of the peaks was measured in the manner described previously.[5] As guided by the shape of the averaged waveforms in Figure 3, we identified five peaks for the responses elicited in the dark-adapted condition and three peaks for those in the light-adapted red flash condition. In the light-adapted blue flash condition, all subjects had the first two peaks. Some, however, had the suggestion of a third peak if allowances are made for large individual differences in peak implicit times. We therefore included in our analysis the possibility of a third peak with the rationale that if this peak contributes little information, our analysis will show this. Additionally, we also computed the sum of the peak amplitudes, an index that has proven useful not only in the study of the CSNB carriers[5] but also in studies of diabetic retinopathy.[13,14]

Figure 5 summarizes the results of our analysis of how well carriers and normal subjects can be discriminated on the basis of the OP amplitudes. For the number of carriers tested, the performance must reach or exceed 0.83 in order that it be significantly different from chance performance (at $P < 0.05$ level). The results show that carriers and normals can be discriminated when the analysis is based on OPs obtained from dark-adapted eyes that were stimulated with a blue flash. In particular, the diagnostic efficiencies for the amplitudes of the third and four peaks or for the total amplitude of all five peaks are 0.95, 0.84 and 0.94, respectively.

Discrimination based on the power content: Figure 6 summarizes how well carriers could be discriminated from normal subjects on the basis of the power

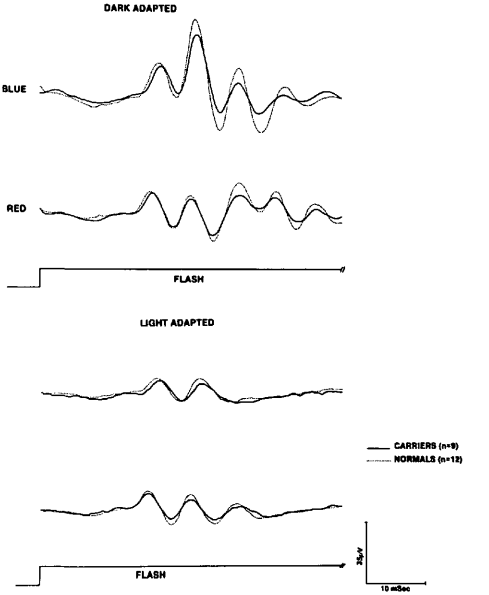

Fig. 3. Averaged response waveforms in the time domain for carriers (solid curve) and age-matched normal subjects (broken curve). The stimulus conditions, from top to bottom, are: (1) dark-adapted, blue flash; (2) dark-adapted, red flash; (3) light-adapted, blue flash; (4) light-adapted, red flash.

content of the OP. Because the power spectrum of the OP spans across a significant range of frequencies (Fig. 4), the power content was computed across of a "frequency window." The bandwidth and center frequency of the window were systematically varied to investigate whether specific portions of the power spectrum contained more diagnostic information than other portions. Figure 6 (top) shows the "highest diagnostic efficiency" obtained as the bandwidth of the window is varied. Figure 6 (bottom) shows how the diagnostic efficiency (for the optimal bandwidth of 70 Hz) varies with the center frequency of the window. (Note: For the data shown in the top of Figure 6, the diagnostic efficiencies were computed as a function of frequency at each bandwidth. The term "highest diagnostic efficiency" refers to the highest efficiency value obtained.)

Of the four stimulus conditions tested, the diagnostic performance of the dark-adapted, blue flash condition was superior. The diagnostic efficiency for this stimulus condition was significantly greater than chance and the efficiency value was greater than those of the other conditions. The optimal efficiency for the dark-adapted, blue flash condition occurs with a 70 Hz bandwidth and a 130 Hz center frequency.

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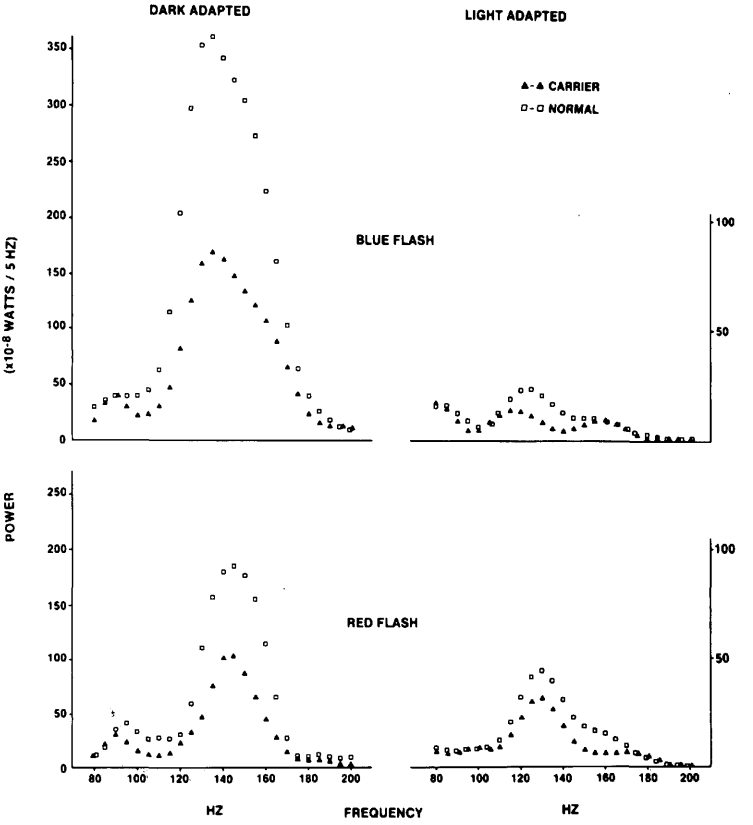

Fig. 4. Averaged response spectrum in the frequency domain for carriers (solid symbols) and of normal subjects (open symbols) for the four stimulus conditions.

Discussion

Since the study by Miyake and Kawase,[5] the attenuation of the OP in another female carrier of CSNB was reported.[15] The current results on nine CSNB carriers (from four X-linked recessive pedigrees) provide still additional support of Miyake's observation. Figure 3 shows that OP amplitudes of carriers are generally smaller than those of the age-matched normal subjects. The response difference between the groups is not robust when considering the variability among the subjects within each group (Figs. 1, 2). But our analysis documents that, for certain stimulus conditions, the carriers can be discriminated from normal subjects on the basis of these response differences (Figs. 5, 6).

In investigating the possibility that the OP responses—or any other candidate measure—might provide a diagnostic test for CSNB carriers, two issues should be distinguished.[9] The first deals with test efficiency. The issue is which test (among a variety of tests, testing conditions or testing methods) best discriminates the clinical condition from normal or from other clinical conditions? The second issue deals with the interpretation of the test results. After the best test has been chosen, a decision has to be made regarding the criterion or cutoff value that should be used to interpret when a test result is normal and when it is abnormal. In the development of a diagnostic test both issues eventually must be addressed. The aim of our study at this time was to address the first issue.

Little was known about the stimulus conditions that produce the OP defect in the carriers (or for that matter, in affected males). In previous studies of the carriers,[5,15] the OP defects were observed after the eyes had been dark-adapted and then stimulated with an intense white flash. In studies of affected males with well documented X-linked recessive transmission, OP defects were found under a dark-adapted

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condition with a red flash[3] or with a white flash,[5] and were found under a light-adapted condition for several flash wavelengths.[3]

The results of the current study show that the stimulus condition is an important factor in optimizing our ability to discriminate normal subjects from CSNB carriers. Of four stimulus conditions examined, the best condition for diagnosing carriers was found to include dark adapting and testing the eye with a blue flash (Figs. 5, 6). This stimulus condition is associated with the highest diagnostic efficiency, ranging from 0.95 to 0.98, depending on the method of data reduction, and is associated with the largest mean difference between the two groups (Figs. 3, 4).

The method of data reduction is another important consideration in finding the optimal methods for diagnosis. In this study, two methods were examined, the computation of OP amplitudes in the time domain and the computation of the power content in the frequency domain. The former is a conventional method used in clinical studies of the OPs[5]; the latter method is one which has received recent attention in the study of the duplex nature of the OP.[16] Our results show that, under the optimal stimulus conditions, the diagnostic efficiency for either data reduction method is excellent. The best efficiency value for the time domain analysis was 0.95 and that for the frequency domain analysis was 0.98. However, it became apparent in the course of the study that, within each data reduction method, the diagnostic efficiency could vary appreciably with the response parameter. In the case of the time domain analysis, for example, only the amplitudes of the third and fourth peaks or the total amplitudes provided statistically significant diagnostic information (dark-adapted, blue flash condition in Fig. 5). The amplitudes of the OPs may be important to note, as it is possible for each wavelet to differ in its origin in the retina.[17,18] In the case of the frequency domain analysis, the optimal efficiency is obtained with a bandwidth of 70 Hz and a center frequency of about 130 Hz.

Although the observation that an OP defect occurs in the female carriers is confirmed, the nature of this defect remains to be elucidated. Miyake and Kawase[5] militated against the trivial possibilities that the OP defect is associated with refractive errors or with incidental systemic diseases in the carriers. They suggested that the more likely explanation is that the OP defect in the carriers might reflect some aspect of the pathophysiology present in the afflicted male family members (whose OP responses are markedly attenuated). More formally, one might state the hypothesis that the OP defect is an expression of the gene that both female carriers and afflicted male members have. The results of the current study are not inconsistent with this hypothesis. Our finding that the OP defect in carriers is prominent when the eye is dark-adapted and tested with a blue flash parallels the fact

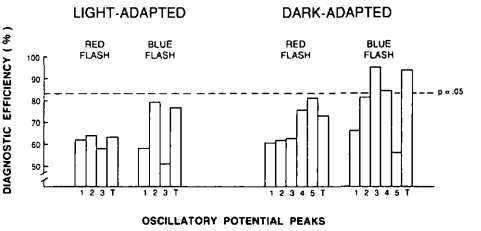

Fig. 5. Efficiency for discriminating CSNB carriers from normal subjects on the basis of the amplitude of individual OP peaks (1–5) or on the basis of the sum of the peak amplitudes (T). Diagnostic efficiency is computed from the data using the method described by Massof and Emmel.[9] For the number of subjects tested, the critical value at or above which the diagnostic efficiency is significantly different from chance ($P < 0.05$ level) is about 0.83.

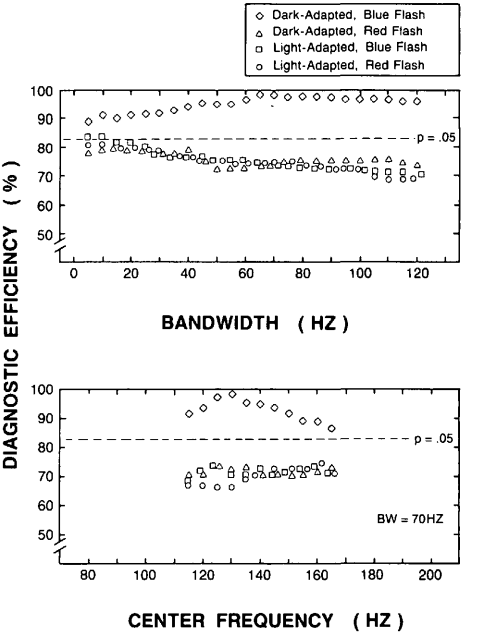

Fig. 6. Efficiency for discriminating CSNB carriers from normal subjects on the basis of the power content of the OP responses. In our application of Massof and Emmel[9] statistical method, we computed the highest diagnostic efficiency as the bandwidth of a measuring window is varied (top). For the optimal bandwidth (70 Hz), we also determined diagnostic efficiency as a function of the center frequency of the measuring window (bottom). The dash lines shows the critical value at or above which the diagnostic efficiency is significantly different from chance ($P < 0.05$ level).

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that the retinal defect observed in the afflicted men is primarily associated with night vision abnormalities.

In summary, the current results support the observation that there is an OP defect in female carriers of the X-linked recessive form of CSNB with myopia. Our results show that the stimulus condition is an important factor in optimizing diagnostic efficiency. Further studies are needed to refine the methods and then to investigate the sensitivity and specificity of the diagnostic test. Additionally, further studies are needed to elucidate the nature of the OP defect in both the female carriers and afflicted men.

Addendum

The ERG b-waves (either rod or cones) of the carriers were not examined in this study. Previous results,[5] however, found no significant difference between the carriers and normals.

Key words: oscillatory potential, X-linked carriers, night blindness, ERG, rods

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