REFRACTIVE DEVELOPMENT IN CHILDREN WITH A HISTORY OF RETINOPATHY OF PREMATURITY (ROP)

A thesis presented to the graduate faculty of

New England College of Optometry in partial fulfillment

of the requirements for the degree of Master of Science

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May, 2023

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This manuscript has been read and accepted by the Thesis Committee in satisfaction of the thesis requirement for the degree of Master of Science

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Abstract

REFRACTIVE DEVELOPMENT IN CHILDREN WITH A HISTORY OF RETINOPATHY OF PREMATURITY (ROP)

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New England College of Optometry, 2023

Purpose: Significant refractive errors are common among former preterms, especially those who have had retinopathy of prematurity (ROP). The mechanisms by which ROP refractive errors arise remain to be determined. The retina is, however, a recognized controller of refractive development. In children with a history of preterm birth, we evaluated the course of refractive development for association with rod and rod mediated retinal dysfunction.

Methods: We retrieved the results of cycloplegic refractions from research and medical records of former preterms (n=26), the majority of whom had been monitored for more than a decade. The former preterms had a history of Mild ROP (n = 20) and others had no history of ROP (n=6). We evaluated the spherical equivalent (SE) as a function of age and compared these SEs in the former preterms to the spherical equivalent in normal development. Spherical equivalents within the 95% prediction interval (PI) for normal development were categorized as Normal; those outside the PI were categorized as Abnormal. Rod and rod mediated electroretinographic (ERG) responses had been previously studied in the same children, and in healthy term born controls. Based on the

ERG a-wave, the activation of rod phototransduction was represented by S_{rod} and deactivation by t_{50}. The companion ERG b-wave, representing activity in rod driven post receptor neurons, was represented by log σ (a sensitivity parameter), and V_{max} (the saturated amplitude parameter). Each of the ERG parameters was compared to that in age similar controls and based on this comparison were categorized as normal if within the range of controls, or abnormal if outside of the range of controls.

Results: Five of the former preterms developed Spherical Equivalent (SE) values outside the PI for normal refractive development; three of those eyes were more myopic, and two more hyperopic than normal. The most common ERG abnormality was b-wave log σ present in 4 of the 5 with abnormal refractive development. Nonetheless, log σ was abnormal in another four patients with Mild ROP who had a normal refractive development. All five had a history of Mild ROP. Among them, two subjects have abnormal t_{50} and other two subjects have abnormal S_{rod}. All five had b-wave V_{max} within the range found in controls. We evaluated the correlation of SE at school age for each ERG parameter.

Conclusions: Although abnormal rod and rod mediated post receptor retinal function is found in some patients with a history of Mild ROP, consideration of other retinal mechanisms underpinning abnormal refractive development in ROP is warranted, discussed in this study.

Table of Contents

Abstract

1 Introduction

1.1 Retinopathy of prematurity

1.2 Refractive development in children with a history of ROP

1.3 Rods and Phototransduction

1.4 Rods (a-wave) and post-receptor neurons (b-wave) with the electroretinography

1.5 Experimental goals

2 Methods

2.1 Subjects

2.2 Experiment Procedures

2.3 Statisitical Analyses

3 Results

3.1 Refractive Errors

3.2 ERG results

3.2a Activation of phototransduction

3.2b Deactivation of phototransduction

3.2c Post-receptor b-wave sensitivity parameter

3.2d Post-receptor b-wave saturated amplitude parameter

3.3 Evaluation of development of Spherical Equivalent for significant association with ERG parameters

4 Discussion

5 Conclusion

6 References

List of Figures and Tables

Figure 1. Activation Phototransduction in Rods……...……6

Figure 2. Deactivation Phototransduction in Rods……6

Table 1. Characteristics of Subjects with A History of Preterm Birth…….9

Figure 3. The International Classification of Retinopathy of Prematurity……...11

Figure 4. Sample Records of ERG a-wave and t_{50} Determination……...…….14

Figure 5. Spherical Equivalent Development with Age……..…….…….. ….16

Figure 6. Activation Parameter S_{rod} Values……...……...…….17

Figure 7. Deactivation Parameter t_{50} values……...…...…….18

Table 2a. Photoreceptor Parameters for Infants…….……18

Table 2b. Photoreceptor Parameters for Mature Subjects…….……..18

Figure 8. Post-receptor Parameter log σ values……...……...19

Figure 9. Post-receptor Parameter V_{max} values……...……...20

Table 3a. Post-receptor Parameters for Infants …….……...…..21

Table 3b. Post-receptor Parameters for Mature Subjects …….……..…21

Table 4a. Photoreceptor Parameters for infant preterms with outside predication SE …....23

Table 4b. Photoreceptor Parameters for mature preterms with outside predication SE..…23

Table 5a. Post-receptor Parameters for infant preterms with outside predication SE……..23

Table 5b. Post-receptor Parameters for mature preterms with outside predication SE ..…24

Figure 10. All preterms' ERG parameters vs SE ……...…….24

Figure 11. ERG parameters vs Δ Normal SE for all mature mild ROP patients ..……..…..25

1 Introduction

1.1 Retinopathy of prematurity (ROP)

ROP is a leading cause of pediatric blindness in the United States. ROP occurs in prematurely born infants. The onset of ROP is at preterm ages during which the retinal vasculature and neural retina are still developing (Fulton, Hansen, Moskowitz & Akula, 2009, Hansen, Moskowitz, Akula & Fulton, 2017). Fortunately, most cases of ROP are mild and resolve spontaneously (Steinkuller, Du, Gilbert, Foster, Collins & Coats, 1999), but more severe cases require treatment using laser ablation or intraocular injections of anti-VEGF drugs (Steinkuller et al., 1999, Wallace & Wu, 2013, Walz, Bemme, Pielen, Aisenbrey, Breuss, Alex, Wagenfeld, Schiedel, Krohne, Stahl & Retina.net, 2016).

Among individuals with a history of ROP, significant refractive errors are common (Wiecek, Akula, Vanderveen, Mantagos, Wu, Curran, De Bruyn, Peterson & Fulton, 2022). The retina is a known controller of refractive development (Troilo & Wallman, 1991). We evaluated rod and rod driven post-receptor function for association with refractive errors in children with a history of preterm birth and Mild ROP. Electroretinography (ERG) was used to evaluate these retinal functions.

The two main types of photoreceptors in the eye, the rods and the cones, the rods modulate scotopic vision, and are the last retinal cells to mature, except for the far less numerous foveal cones (Curcio, Sloan, Kalina & Hendrickson, 1990). In monkeys,

researchers found that two weeks before birth, cone density was higher, and rods were sparse in the central fovea. Growth of the mid-periphery of the retina increases with eccentricity up to 13 weeks postnatally, but later only the periphery of the retina persists. In the first year after birth, monkey cone density increases, rod-sparse area diameter decreases, and peripheral photoreceptor density decreases. Rod and cone density decrease with age due to retinal growth. However, the fovea photoreceptor density increases as differentiated photoreceptors migrate to the foveal center (Packer, Hendrickson & Curcio, 1990).

In early infancy, as rod photoreceptors develop, their outer segments elongate, and the rhodopsin content of the retina increases. These developmental processes coincide with the onset of ROP. Prior studies have shown that rod function is altered in eyes of former preterms (Fulton et al., 2009, Hansen et al., 2017). Remarkably, the alterations persist for years after acute phases of ROP have resolved and are most marked in those eyes with a history of severe ROP. The main purpose of our research is to determine if alterations in rod and rod mediated retinal function is associated with refractive development in former preterms with a history of ROP.

Rat models of ROP provide a comprehensive view of the role of the rods in ROP. ROP rod dysfunction is characterized as having low sensitivity, S_{rod}, in response to light and is associated with subtle structural alterations in the rods even though the rhodopsin content

in the ROP and control retinas did not differ significantly (Dodge, Fulton, Parker, Hansen & Williams, 1996, Fulton, Reynaud, Hansen, Lemere, Parker & Williams, 1999). This is likely the basis for the rod dysfunction that has also been demonstrated in infants and children with a history of ROP (Fulton et al., 2009, Fulton, Hansen, Petersen & Vanderveen, 2001). Phototransduction processes, at least as pertains to activation, become inefficient (Hansen et al., 2017). The rods' role in ROP pathogenesis is further supported by additional research in rat models. Early rod photoreceptor dysfunction predicts blood vessel abnormalities that are the clinical hallmark of ROP (Akula, Hansen, Martinez-Perez & Fulton, 2007). The study also found that by lowering rod energy consumption, ROP rats showed more robust rod and post-receptor responses, as well as improved retinal vascular status (Akula, Hansen, Tzekov, Favazza, Vyhovsky, Benador, Mocko, McGee, Kubota & Fulton, 2010). These results raise the interesting possibility of using the rods as a target for treating ROP.

1.2 Refractive development in children with a history of ROP

Earlier studies have shown high refractive error, as measured under cycloplegia, is the most common ocular sequelae in premature infants. High myopia, especially, is common among children with a history of ROP (Fielder & Quinn, 1997, Lue, Hansen, Reisner, Findl, Petersen & Fulton, 1995, Moskowitz, Hansen & Fulton, 2005). The retina is recognized as one of the controllers of eye growth and refractive development (Troilo & Wallman, 1991).

Nonetheless, the specific mechanisms of retinal control of eye growth and refractive development in ROP remain unclear.

Children with a history of ROP become more severely myopic more frequently than those with typically developing eyes without ROP (Munro, Fulton, Chui, Moskowitz, Ramamirtham, Hansen, Prabhu & Akula, 2015, Wiecek et al., 2022). In some ROP infants, the low rod photoreceptor sensitivity, S_{rod}, was associated with development of myopia (Moskowitz et al., 2005). In a recent psychophysical study of rod-mediated visual thresholds, peripheral, but not posterior, threshold was significantly associated with refractive error in childhood (De Bruyn, Mergia, Selian, Hansen, Akula & Fulton, 2021). Results of some previous studies suggesting that rod dysfunction may have a role in refractive development, which raised the interest in further research.

1.3 Rods and Phototransduction

Photoreceptors, rods, and cones, transduce light into electrical signals. Photoreceptors have an outer segment, an inner segment, a nuclear region, and a synaptic terminal. The rod outer segments have regularly spaced discs containing rhodopsin, the visual pigment that absorbs photons.

A cilium connects the outer segment to the inner segment. The inner segment is dense with mitochondria, generating energy needed for phototransduction and other

photoreceptor processes. Light signals from the photoreceptors are transmitted to the post-receptor, including the bipolar cells. These post-receptor cells are designated “second order retinal neurons”.

Activation of phototransduction begins with the capture of photons by rhodopsin in the disc’s membrane of the rod outer segment (Figure 1). Rhodopsin diffuses in the disc membrane, and the other molecular components of the transduction cascade, transducin, G*, and phosphodiesterase, PDE*, are activated leading to the closure of cyclic GMP-gated channels in the photoreceptor membrane.

Recovery from activation, that is deactivation of phototransduction, is critical to ensuring the photoreceptors are ready to respond to next photons. Deactivation of mammalian rods (Figure 2) occurs rapidly, beginning less than 40 ms after activation and continues simultaneously with the activation (Burns & Pugh, 2010, Gross, Pugh & Burns, 2012). This recovery process involves stepwise inactivation of light activated rhodopsin (R*), G-protein (G*), and phosphodiesterase (PDE*). By these steps, rhodopsin is regenerated and cyclic GMP is restored, readying the photoreceptors for their next response to light.

Figure 1. Activation Phototransduction in Rods

[Figure 1&2 are reproduced based on (Chalupa, 2004, Fu, 1995, Rodieck, 1998)]

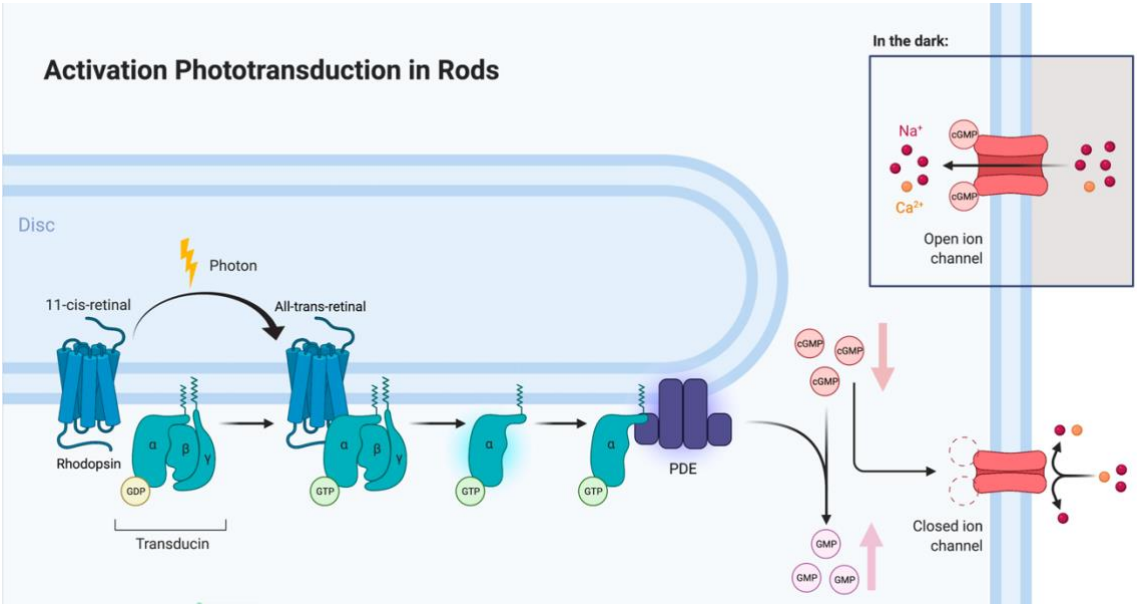
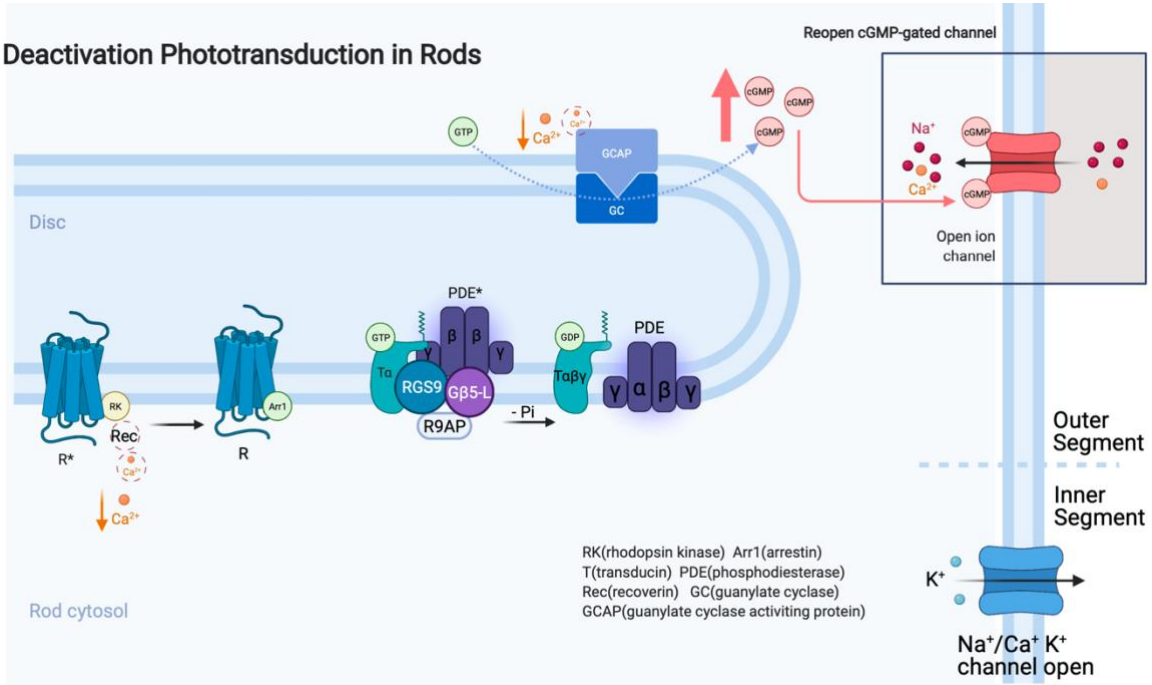


Figure 2. Deactivation Phototransduction in Rods



The kinetics of activation are slower in ROP (Fulton et al., 2009). Preliminary data show that deactivation of ROP rods may also be slow, but not necessarily correlated with the slow kinetics of activation (Hansen, Harris, Moskowitz & Fulton, 2010).

1.4 Rods (a-wave) and post-receptor neurons (b-wave) with the electroretinography

Activity in all retinal regions contributes to the ERG responses to full-field stimuli. Full-field ERG (ffERG) can be used to evaluate the phototransduction process, including the rod activation and deactivation.

ERG electrodes, positioned on the surface of the eye, are used to record the ERG responses to flashes of light. The main components of the ERG response to full field stimuli are the a-wave (originating from the photoreceptors) and the b-wave (originating from the post-receptor neurons) (Granit, 1933). According to the model (Hood & Birch, 1990, Lamb & Pugh, 1992) of activation of phototransduction, the a-wave depends on the concentrations and kinetics of the molecules in the rod outer segment disks.

Deactivation processes in the rod can also be derived from ERG. This is done using a paired flash paradigm. In the paired flash procedure, a conditioning flash (first flash) is delivered. Then at various times after the conditioning flash, a probe flash (second flash) with the same stimulus strength as first flash is delivered. Responses to this series of pairs of flashes trace out the course of recovery of the rod (deactivation) over the various

interstimulus intervals (ISIs) (Birch, Hood, Nusinowitz & Pepperberg, 1995, Pepperberg, Birch & Hood, 1997). As we will show below (Methods), the kinetics of deactivation are specified by t_{50}. At each interval, the amplitude (R) at 8ms was compared with maximal amplitude (R_{max}) evoked by the conditioning flash. By interpolation, the time at which the response to the probe is half its R_{max} amplitude is determined t_{50} (Akula et al., 2010, Hansen et al., 2010).

1.5 Experimental goals

This study focuses on the development of refractive error in former preterms. The aim is to determine if refractive development is significantly associated with rod and rod mediated post receptor activity as determined by ffERG. We investigate the rod activation parameter (S_{rod}) and deactivation parameter (t_{50}) of phototransduction, derived from the ERG a-wave, and the post-receptor ERG b-wave response parameters log σ (a sensitivity parameter) and V_{max} (saturated amplitude parameter) for significant association with spherical equivalent in former preterms including those with Mild ROP.

2 Methods

2.1 Subjects

Subjects (Table 1) with a history of preterm birth (n = 26) were recruited for ERG testing. Two categories, No ROP or Mild ROP were studied. Inclusion criteria were gestational age at birth less than 32 weeks and birth weight for appropriate gestational age.

Exclusion criteria were partial or complete retinal detachment, structural anomalies of anterior or posterior segment, structural anomalies of the brain, family history of any heritable disorder including retinal degenerations and anomalies of retinal function. Each subject had been followed in the Newborn Intensive Care Nursery (NICU) with serial examinations using indirect ophthalmoscopy; the indications and schedule of examinations were similar to those used in the multicenter ROP treatment trials (Good & Hardy, 2001).

Healthy, term born, control subjects (n=21) were also studied. Inclusion criteria included gestational age at birth greater than 37 weeks and lack of ophthalmic and systemic diseases. The exclusion criteria was the same as for the preterm subjects.

Each subject's age at ERG test were either in infancy at corrected age 10 weeks post term which is known as infants, or in childhood or adolescence, ages at which ERG response parameters are known to be matures. As shown in Table 1 below, the median age at birth and birthweight of former preterms with and without ROP overlapped broadly, although the median birth weight and gestational age at birth was slightly lower in those who developed Mild ROP.

Table 1. Characteristics of Subjects with a history of preterm birth [Median(range)]

	Mild ROP (n=20)		**None ROP (n=6)**	
	Infant	Mature	Infant	Mature
N (Total n=26)	8	12	3	3
Age at ERG test	22.1 (19.0-25.4) weeks	14.3 (9.5-23.6) years	21.0 (20.7-26.9) weeks	14.1 (10.3-18.5) years
Gestational Age (week)	28 (26-31)	26 (23-28)	29 (25-30)	31 (26-32)
Birth Weights (g)	1020 (626-1170)	815 (550-1077)	1230 (740-1360)	1430 (820-1590)
Follow-up(year)	16.5 (0.3 ~ 34.3)		11.6 (7.2 ~ 22.0)	

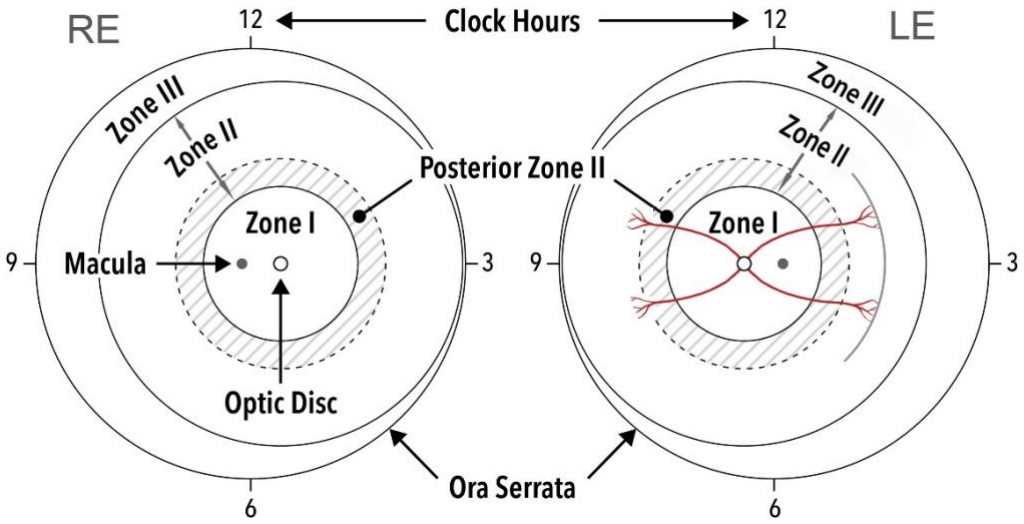
** Age at ERG = postnatal age. For the infants, preterms and controls, median age at test was 10 weeks post term.*

The International Classification of Retinopathy of Prematurity (ICROP) specifies the location of disease by three zones(I to III) concentric with the optic disc; the extent of disease is specified by 12 hours of the clock (Figure 3); the stage of disease to classify the level of vascular abnormality by 5 stages (1 to 5). The larger number the more severe vascular change (Chiang, Quinn, Fielder, Ostmo, Paul Chan, Berrocal, Binenbaum, Blair, Peter Campbell, Capone, Chen, Dai, Ells, Fleck, Good, Elizabeth Hartnett, Holmstrom, Kusaka, Kychenthal, Lepore, Lorenz, Martinez-Castellanos, Ozdek, Ademola-Popoola, Reynolds, Shah, Shapiro, Stahl, Toth, Vinekar, Visser, Wallace, Wu, Zhao & Zin, 2021).

We reviewed the medical records for details about ROP diagnosis in the former preterms. Results of serial examinations in the NICU indicated that 20 of the 26 subjects had Mild ROP that never reached criteria for treatment. Their ROP resolved clinically without any intervention. The mild ROP subjects had ROP in zone II and III, with stage 1, 2 or 3, without plus disease. The results of serial examinations showed no evidence of ROP in 6 former preterm subjects.

In former preterms with mild ROP, or without ROP, we had used the ERG to evaluate the phototransduction process, specifically rod activation and deactivation. A potential confounding factor is the possibility that laser treatment alone may interfere with retinal function. For the mild ROP, no laser treatment had been given, thus avoiding any confounding effects of the laser on eye growth or refractive development.

Figure 3. The International Classification of Retinopathy of Prematurity(Chiang et al., 2021)



**Scheme of retina of right eye (RE) and left eye (LE) showing zone borders and clock hours used to describe the location and extent of retinopathy of prematurity.*

This study conformed to the tenets of the Declaration of Helsinki and was approved by the Children's Hospital Committee on Clinical Investigation. For the ERG. informed consent had been obtained from the parents of the infants and children, assent from the older children, and consent from those 18 years and older.

2.2 Experiment procedures

Cycloplegic refractions were usually obtained using a streak retinoscope following cyclopentolate. In some older children, a Grand Seiko autorefractor (Model WR-5100K)

was used to measure refractive error while the child fixated at the distant target. The examiner took several readings of refractive errors.

In preparation for the ERG test, the pupil of one eye was dilated using tropicamide 1%. The patient, accompanied by a parent, stayed in a dark environment for 30 minutes for dark adaptation. Then under dim red light, following a drop of 0.5% proparacaine, a bipolar Burian-Allen electrode was placed on the cornea. A ground electrode were placed on the skin over the ipsilateral mastoid. Electroretinographic (ERG) responses recorded to assess rod photoreceptor and rod mediated post-receptor function using the system (Diagnosys, Lowell, MA) or using the Compact 4 system (Nicolet, Madison, WI). Data obtained using the two systems were combined due to the fact that there are no differences in the parameters between the two systems (Hansen et al., 2010). In both systems, the gain was 1,000 and the bandpass was 0.5 – 1,000 Hz. The digitization rate was 2,564 Hz and 2,000 Hz for the Nicolet and Espion system respectively.

From the dark adapted eye, responses to 5 or so stimuli, +2.1 to +3.3 log scotopic troland seconds were recorded. The activation parameter S_{rod} was derived from the a-wave component of these ERG responses. Activation responses ranging from +2.1 to +3.3 log scot td s were recorded to full-field, brief(< 3ms), blue stimuli. The rod activation parameter S_{rod} were calculated using the Hood and Birch formulation of the Lamb & Pugh model (Hood & Birch, 1990, Hood & Birch, 1992, Lamb & Pugh, 1992). The equation is

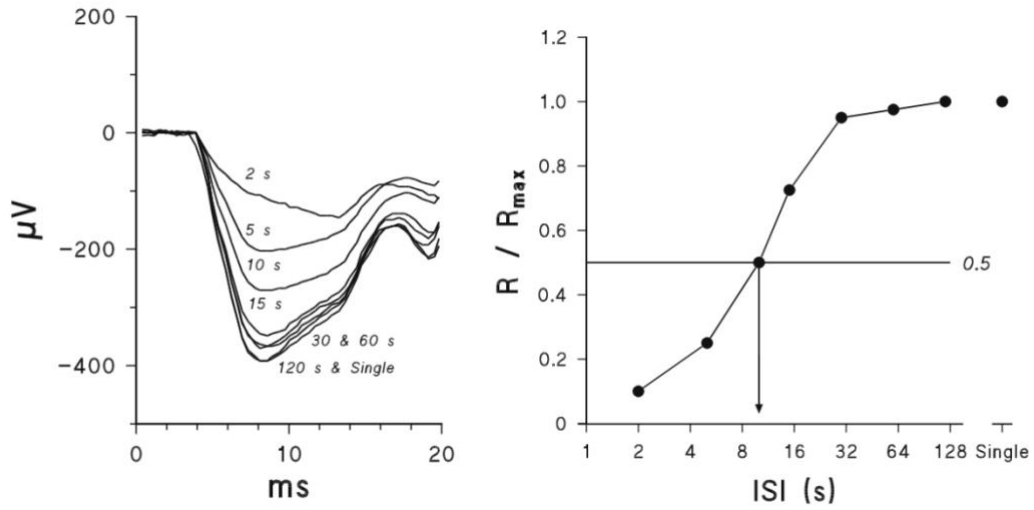
$$R(I,t) = [1 - \exp\{-0.5I\, S_{rod}(t - t_d)^2\}]R_{rod}$$

where I is the flash in isomerizations per rod per flash, S_{rod} is the sensitivity parameter $[(\text{scot td})^{-1}\text{s}^{-3}]$ that depends on the time constants of the steps in activation of phototransduction, R_{rod} the saturated response amplitude (uV), and t_d a brief delay (ms). All three parameters, (S_{rod}, R_{rod}, and t_d) were free to vary.

To study rod deactivation, the paired flash ERG procedure was used. A series of pairs of flashes, a conditioning that followed by a probe flash, were presented. In this work we used 7 pairs, with the interval between conditioning and probe flash ranging from 120 to 2 seconds. The amplitude of the response to the initial conditioning flash was Rmax.

A subject's results are shown by plotting R/R_{max} (y-axis) as a function of the interstimulus interval (ISI). If the ISI is large, the response amplitude(R) to the probe flash is large, approaching R_{max}. The ratio of R/R_{max} increases as intervals increase until reaching the maximum ratio of 1.0. By interpolation, the time which R/R_{max} is half-maximum, t_{50}, is used to summarize the kinetics of rod deactivation (Hansen et al., 2010). A sample plot is shown in Figure 4.

Figure 4. Sample Records of ERG a-wave and t_{50} Determination (Hansen et al., 2010)



(Figure 4. At each interval, the amplitude (R) at 8ms was compared with maximal amplitude (R_{max}) evoked by the conditioning flash (left plot). By interpolation (right plot), the time at which the response to the probe is half its R_{max} amplitude is determined t_{50})

Rod-driven b-waves are produced by ON-bipolar and other second and third-order retinal neurons. The scotopic b-wave amplitude was fit by this equation:

$$V = V_{max}[I/(I + \sigma)]$$

V is the b-wave amplitude produced by a flash of intensity I, V_{max} is the saturated b-wave amplitude, and σ the flash intensity producing a half maximum (semi-saturated) b-wave response (Fulton & Rushton, 1978) .

2.3 Statisitical Analyses

We categorized each preterm subject's four ERG parameters (S_{rod}, t_{50}, log σ, V_{max}) as normal or abnormal. The former preterms subjects ERG parameters that were within the range found in age similar controls were designated Normal, those outside of that range were designated Abnormal. The course of development of spherical equivalent of each subject was compared to that reported for healthy term born subjects, the preterms; those values within the 95% prediction limits (PL) for term born controls were designated Normal, those outside of the PL as abnormal. The data were examined for significant association of ERG parameters with SE.

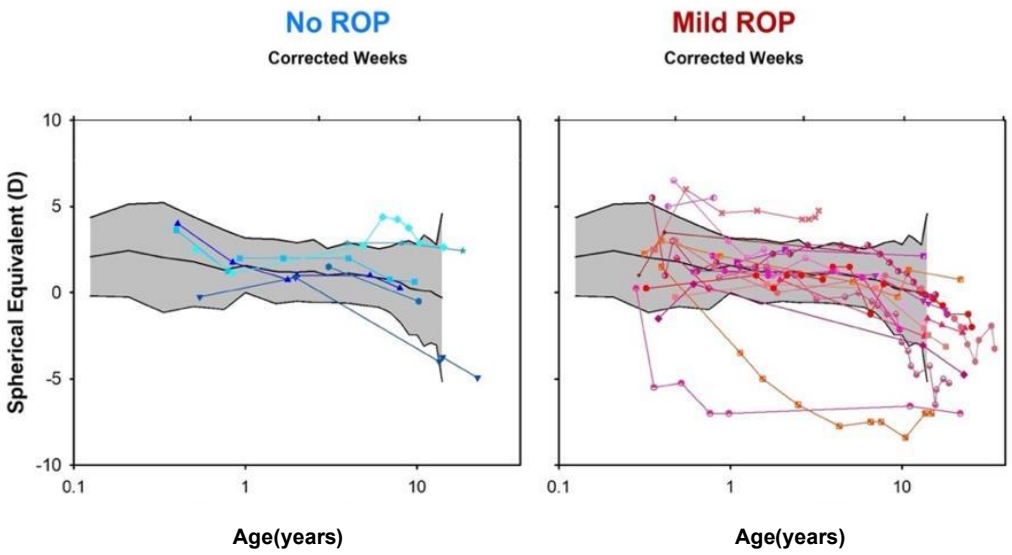
3 Results

3.1 Refractive Errors

In Figure 5, the spherical equivalent (SE, diopters) of the former preterms is shown as a function of age (years). Each individual preterm patient is represented by a particular symbol; longitudinal data of an individual are connected by line segments. The grey band shows the 95% prediction interval for normal development of SE reported in previous publications: for 1–48 months old (Mayer, Hansen, Moore, Kim & Fulton, 2001) and for school aged children (Zadnik, Manny, Yu, Mitchell, Cotter, Quiralte, Shipp, Friedman, Kleinstein, Walker, Jones, Moeschberger, Mutti, Collaborative Longitudinal Evaluation of & Refractive Error Study, 2003). Only one of the No ROP patients has points outside of normal limits. More of the Mild ROP patients have points outside of normal limits. Two

mild ROP patients have points below the grey band indicating increasing myopia and two patients have points above the band indicating high hyperopia.

Figure 5. Spherical Equivalent Development with Age



3.2 ERG results

3.2a Activtivation of phototransduction

In Figure 6, the activation parameter S_{rod} for the two age groups, infants and mature, is shown. Infants' S_{rod} values are on average slower than in mature subjects. Among the infants, three mild ROP patients have lower S_{rod} values than the controls. Among the mature subjects, five mild ROP patients have lower S_{rod} values than in the mature controls.

Figure 6. Activation Parameter S_{rod} Values



3.2b Deactivation of phototransduction

Figure 7 shows the deactivation parameter, t_{50} , for infants and mature subjects. For infants, the t_{50} values of former preterms and controls overlap broadly. Among the mature subjects, six mild ROP patients have longer t_{50} values than any of the controls.

Table 2 summarizes rod photoreceptor's two parameters S_{rod} and t_{50} for all preterms (n=26). Table 2a summarized all eleven infants preterms and Table 2b summarized all fifteen mature preterms. We have bolded S_{rod} and t_{50} values that are outside the range found in age similar controls.

Figure 7. Deactivation parameter t_{50} values

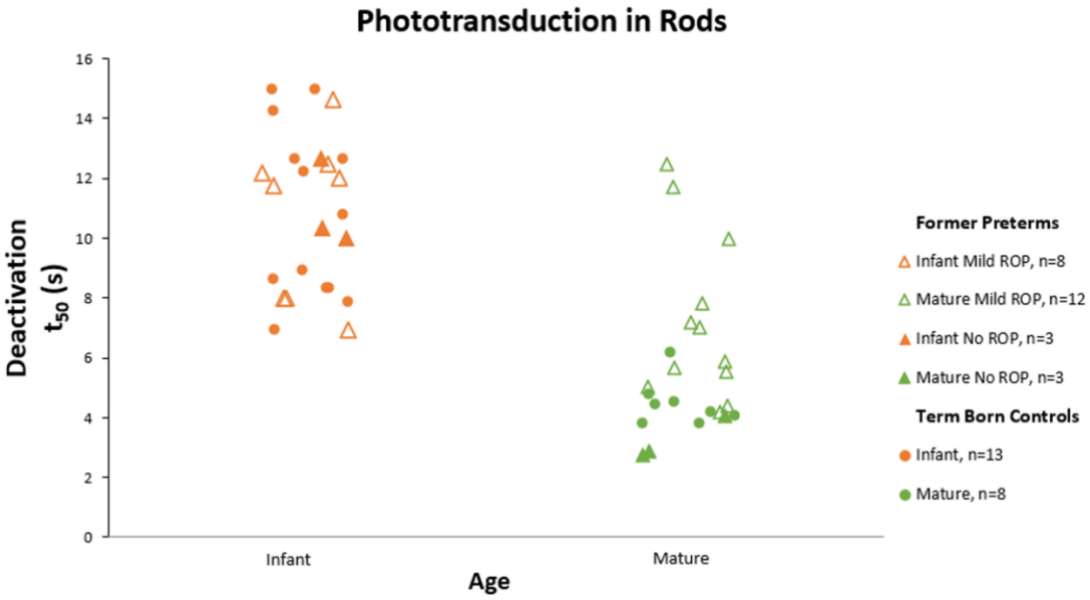


Table 2a. Photoreceptor Parameters for Infants

Infant	ERG a-wave Parameters	
Subject	S_{rod}	t_{50}
None ROP		
A	2017.05	12.68
B	1293.83	10.36
C	1522.08	10.00
Mild ROP		
D	686.73	12.00
E	483.69	6.93
F	2046.91	8.00
G	1265.63	12.46
H	1439.80	8.00
I	1054.97	12.20
J	1741.70	11.75
K	550.71	14.65

For infant control subjects, median S_{rod} 1146.05 (364.32 - 2841.46) and median t_{50} 10 (6.86 - 15.00)

Table 2b. Photoreceptor Parameters for Mature Subjects

Mature	ERG a-wave Parameters	
Subject	S_{rod}	t_{50}
None ROP		
L	**4857.66**	**2.76**
M	**5018.51**	4.20
N	**4664.64**	**2.89**
Mild ROP		
O	**1926.17**	**7.19**
P	3869.64	**12.46**
Q	**2654.02**	4.40
R	**2179.51**	**3.30**
S	3615.09	**15.00**
T	**3124.10**	5.86
U	**2389.42**	**13.06**
V	3751.82	**2.89**
W	4258.49	5.53
X	4013.20	**10.00**
Y	4435.43	5.65
Z	4355.00	**11.70**

For mature control subjects, median S_{rod} 4342.94 (3297.42 - 4624.42), median t_{50} 4.34 (3.86 - 6.20).

3.2c Post-receptor b-wave sensitivity parameter

The rod-driven b-wave sensitivity parameter, log σ , is plotted in Figure 8. Among

healthy control subjects, infants need higher flash intensity to produce a half maximum b-wave amplitude (log σ) than do mature subjects. Among the infants, the log σ values of former preterms and controls overlap. For the mature subjects, seven mild ROP patients have higher log σ values (less sensitive) than the controls. Some, but not all, of these seven mild ROP patients also have abnormal S_{rod} and t_{50} values.

Figure 8. Post-receptor parameter log σ values



3.2d Post-receptor b-wave saturated amplitude parameter

The b-wave saturated amplitude V_{max} is shown in Figure 9. The healthy control infants on average have lower V_{max} than mature control subjects. Among the infants, the V_{max} values of former preterms overlap broadly those in controls with one exception: one mild ROP patient at slightly higher value of V_{max}. For the mature subjects, only one mild

ROP patient has slightly lower V_{max} than controls; and two mild ROP patients have slightly higher V_{max}.

Table 4 summarizes the post-receptor's two parameters $\log \sigma$ and V_{max} for all preterms (n=26). Table 4a summarized all eleven infants preterms and Table 4b summarized all fifteen mature preterms. We have bolded the values that are outside the range found in age similar controls.

Figure 9. Post-receptor parameter V_{max} values



Table 3a. Post-receptor Parameters for Infants

Infant	ERG b-wave Parameters	
Subject	$\log \sigma$	V_{max}
None ROP		
A	-0.40	209
B	-0.29	177
C	-0.29	142
Mild ROP		
D	**0.33**	152
E	**0.32**	143
F	-0.10	197
G	-0.26	**348**
H	0.06	285
I	0.09	89
J	**-0.66**	134
K	-0.34	144

For infant control subjects, median $\log \sigma$ -0.26 (-0.59 – 0.30) and median V_{max} 217 (87 - 299)

Table 3b. Post-receptor Parameters for Mature Subjects

Mature	ERG b-wave Parameters	
Subject	$\log \sigma$	V_{max}
None ROP		
L	-1.00	433
M	-0.82	409
N	-0.86	372
Mild ROP		
O	**-0.63**	315
P	**-0.42**	323
Q	-0.80	426
R	-0.77	**627**
S	**-0.66**	355
T	**-0.17**	326
U	**-0.48**	**249**
V	-0.85	400
W	-1.04	361
X	**-0.37**	408
Y	-0.87	**542**
Z	**-0.43**	411

For mature control subjects, median $\log \sigma$ -0.88 (-1.04 – -0.70), median V_{max} 406.5 (303 - 494).

3.3 Evaluation of development of SE for significant association with ERG parameters

We categorized the former preterms' development of SE as normal or abnormal depending on whether within or outside of normal limits (Figure 5). For each ERG parameter, we categorized the former preterms' results as within the range of values for age similar healthy controls (normal) or outside the range (abnormal), as shown in Figures 6 through 10.

Table 4 shows the photoreceptor parameters of subjects who had outside limit spherical

equivalent values in the past. For the infant subjects (Table 4a), one None ROP infant had outside limit hyperopia before 10 years old, and then reached the normal range after 10 years old. This subject's birth weight is the lightest one among all None ROP subjects. The other two Mild ROP infants both with high hyperopia. For subject I, we only had two visit data points of refractive errors before 1 year old, hence we can't predict if this subject had fallen into the normal range of SE in school age. These three infant subjects have S_{rod} and t_{50} values distributed in the range of data from control subjects.

As for the mature selected subjects (Table 4b), they have moderate to high myopia. All of them have low birth weight, the one None ROP subject has the lightest weight in the None ROP mature group but similar S_{rod} and t_{50} values compared to controls. Two mild ROP patients have longer t_{50} and other two patients have lower S_{rod} values than controls. The abnormal values were bolded.

Similarly, Table 5 shows the data of post-receptor parameters $\log \sigma$ and V_{max} from former preterms with abnormal SE. One mild ROP infant had lower $\log \sigma$ and three mild ROP matures have higher $\log \sigma$ values (less sensitive) than the controls.

Besides the subjects mentioned in Table 4 and 5, some other preterms had normal SE and abnormal ERG response parameters. Additional information is shown in Figure 10.

Table 4a. Photoreceptor Parameters for infant preterms with outside predication SE

Infant	Subject	Eye	SE (diopter)	BW (g)	S_{rod} $(cd^{-1}s^{-3}m^2)$	t_{50} (s)
None ROP	C	OS	2.63	740	1522.08	10.0
Mild ROP	I	OD	5.50	1065	1054.97	12.2
Mild ROP	J	OS	4.75	1050	1741.70	11.8

**Infant controls: S_{rod} median 1555.92(1025.11 – 2841.46); t_{50} median 10.81(6.98 – 14.30)*

Table 4b. Photoreceptor Parameters for mature preterms with outside predication SE

Mature	Subject	Eye	SE (diopter)	BW (g)	S_{rod} $(cd^{-1}s^{-3}m^2)$	t_{50} (s)
None ROP	M	OS	-4.93	820	**5018.51**	4.20
Mild ROP	P	OS	-5.25	780	3869.64	**12.5**
Mild ROP	O	OS	-7.00	810	**1926.17**	**7.20**
Mild ROP	T	OD	-7.00	820	**3124.10**	5.90

**Mature controls:S_{rod} median 4342.94 (3297.42 - 4624.42); t_{50} median 4.34 (3.86 - 6.20)*

Table 5a. Post-receptor Parameters for infant preterms with outside predication SE

Infant	Subject	Eye	SE (diopter)	BW (g)	$\log \sigma$ $(log\ scot\ Td\ sec)$	V_{max} (μV)
None ROP	C	OS	2.63	740	-0.29	142
Mild ROP	I	OD	5.50	1065	0.09	89
Mild ROP	J	OS	4.75	1050	**-0.66**	134

**Infant controls: $\log \sigma$ median -0.26 (-0.59 – 0.30); V_{max} median 217 (87 – 299)*

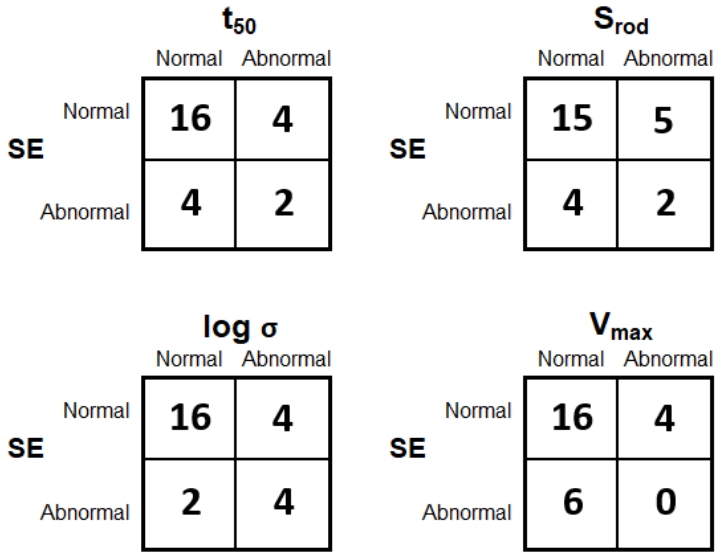
Table 5b. Post-receptor Parameters for mature preterms with outside predication SE

Mature	Subject	Eye	SE (diopter)	BW (g)	$\log \sigma$ ($log\ scot\ Td\ sec$)	V_{max} (μV)
None ROP	M	OS	-4.93	820	-0.82	409
Mild ROP	P	OS	-5.25	780	**-0.42**	323
Mild ROP	O	OS	-7.00	810	**-0.63**	315
Mild ROP	T	OD	-7.00	820	**-0.17**	326

**Mature controls: $\log \sigma$ median -0.88 (-1.04 – -0.70); V_{max} median 406.5 (303 – 494)*

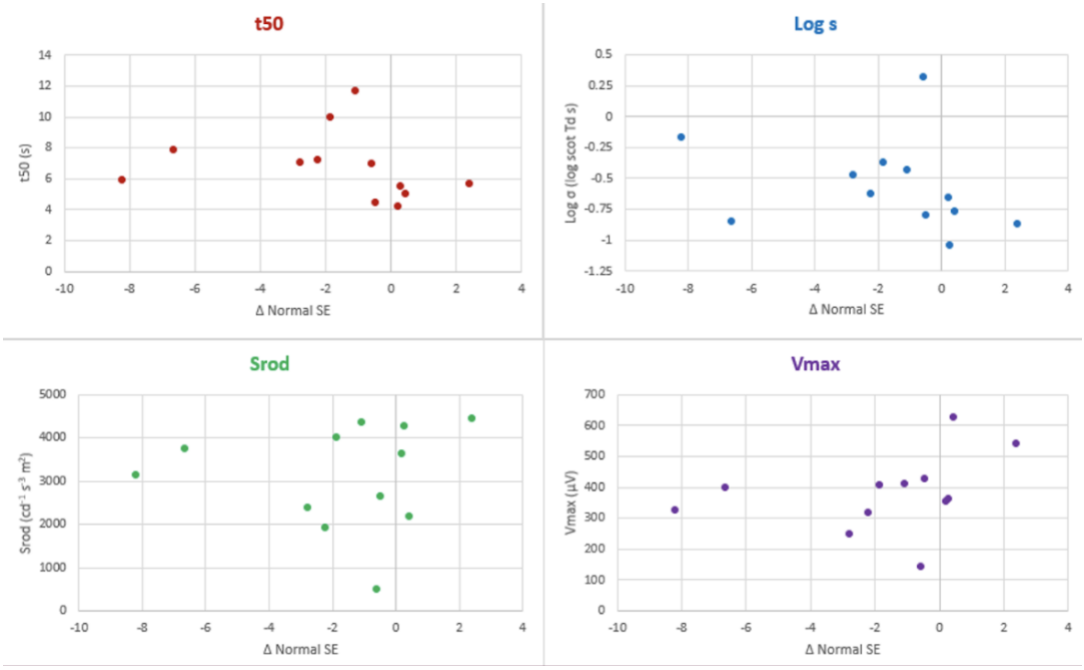
SE of every former preterms (n=26), categorized as normal or abnormal, is shown in Figure 10 for each ERG parameter (S_{rod}, t_{50}, log σ, V_{max}), as normal or abnormal. Six former preterms have abnormal SE in total. The abnormal SE is not necessarily associated with abnormal ERG parameters. Four preterms with abnormal SE have abnormal log σ, but there are four with normal SE who have normal log σ. Thus, we find no significant association of SE and log σ.

Figure 10. All preterms' ERG parameters vs SE



In addition, we examined the correlation between SE in childhood (age 7-13 years) and ERG parameters in mature mild ROP patients (Figure 11). Horizontal axis Δ Normal SE represents the difference between a patient's SE and the normal SE. Overall, we found no significant correlation.

Figure 11. ERG parameters vs Δ Normal SE for all mature mild ROP patients



4 Discussion

We find no significant association of spherical equivalent and rod mediated ERG responses in our sample of former preterms (Figure 10). Spherical equivalent was not correlated with any of the ERG parameters (Figure 11). According to previous reports, there is some evidence of rod photoreceptor sensitivity (S_{rod}) and rod mediated visual thresholds being associated with abnormal refractive error in former preterms (Moskowitz et al., 2005; De Bruyn et al., 2021). In the following section, we discuss several possible explanations for this apparent discrepancy between our results and those of previous studies.

In the present study, we have used the full field ERG. ERG responses to full field stimuli are mass responses registering activity of retinal cells in all retinal regions. The full field ERG test does not pick up regional variations in retinal function. Psychophysical study using selected small area stimuli can distinguish activity in different retinal regions. A psychophysical study of such rod mediated visual thresholds showed that activity in peripheral, but not posterior, retina is associated with refractive development in former preterms (De Bruyn et al., 2021). The full field ERG test results would not have captured such regional variations, and unless deficits in a particular retinal region, such as peripheral retina, were marked then would not have been captured by study of the full field ERG responses.

We have studied former preterms with No ROP or with Mild ROP. Perhaps more severe retinal disease would perturb the ERG parameters in a more marked fashion. Also, it is known that more severe ROP is associated with a higher frequency of significant refractive errors and the refractive errors are more marked the more severe the ROP. Therefore, although we avoided the possible confounding effect of laser treatment of ROP, our results on the No ROP and Mild ROP patients do not exclude the possibility that rod and rod mediated function are related to refractive development in ROP as previously reported (Moskowitz et al., 2005; De Bruyn et al., 2021).

5 Conclusion

Although abnormal rod and rod mediated post receptor retinal function is found in some patients with a history of Mild ROP, our study found no evidence of rod photoreceptors or post-receptor neurons playing a role in regulating the refractive development of humans with mild ROP history. Nonetheless, we found that 6 of 26 (23%) of our former preterms had abnormal spherical equivalent, a considerably higher prevalence than generally found in healthy infants and young children. Further study is warranted because at present the mechanisms leading to development of refraction errors in former preterms cannot be specified. Studies may include an evaluation of a larger group of former preterms, including those with a history of more severe ROP. Furthermore, there are additional ERG parameters that may reveal more of the processes in ROP eyes including those that develop high refractive errors at a young age.

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