Clinical and Epidemiologic Research

Subclinical Decrease in Central Inner Retinal Activity Is Associated With Myopia Development in Children

Serena Zhe-Chuang Li, Wing-Yan Yu, Kai-Yip Choi, Christie Hang-I Lam, Yamunadevi Lakshmanan, Francisca Siu-Yin Wong, and Henry Ho-Lung Chan

Laboratory of Experimental Optometry (Neuroscience), School of Optometry, The Hong Kong Polytechnic University, Hong Kong Special Administrative Region

Correspondence: Henry H.-L. Chan, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong Special Administrative Region; henryhl.chan@polyu.edu.hk.
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Purpose. To investigate the characteristics of retinal electrophysiological activity in relation to early myopia development in children.

Methods. Fifty-six children aged 6 to 9 years with emmetropic refractive error (defined as ≥−0.5 diopter [D] and ≤+0.5 D) were recruited. Cycloplegic refraction, axial length, and global flash multifocal electroretinogram (MOFO mERG) at 49% and 96% contrast levels were recorded in all children at their first visit. The refraction and axial length measurements were repeated after 1 year. The amplitudes and implicit times of the direct component (DC) and the induced component (IC) of the MOFO mERG obtained at the initial visit were analyzed. Correlations between the MOFO mERG parameters and changes in refractive error and axial length were investigated.

Results. The mean spherical equivalent refractive error and axial length of the eyes of the children at the first visit were +0.19 ± 0.35 D and 23.14 ± 0.6 mm, respectively. After 1 year, the mean refractive error increased by −0.55 ± 0.53 D, whereas axial length increased by 0.37 ± 0.22 mm. The changes in refractive error and axial length were significantly correlated with the central IC amplitudes at 49% contrast level measured at the initial visit (ρ = 0.46, P < 0.001 and ρ = −0.34, P = 0.01, respectively).

Conclusions. The prospective changes we have shown are believed to derive from central inner retina. These changes appear to precede myopia and could be a potential reference for juvenile myopia development.

Keywords: myopia development, multifocal electroretinogram, children vision, inner retina

Excessive eyeball elongation causes myopia. In severe cases, it may result in retinal stretching, thinning, and changes in retinal cell morphology and pathology. Application of the ERG technique has provided ample evidence to confirm that myopia results in impaired retinal function. It has been reported that myopia in adults was associated with decreased nonlinear components of ERG responses, multifocal ERG (mERG) responses, retinal adaptation response, and inner retinal function. Axial length was shown to be linearly related to ERG amplitudes and the first slice of second-order kernel of mERG responses. The reduction in mERG responses in myopic adults is believed to be due to the deterioration in retina function associated with long-standing myopia. However, this explanation cannot be applied to myopic children, and discrepancies of ERG characteristics have been noted between myopic adults and children. Luu and his colleagues conducted a cross-sectional study of mERG measurement in 104 children and 31 adults with a range of refractive errors. They found a significant correlation between refractive error and mERG response in adults, but this correlation was not observed in children. Ho et al. also demonstrated different characteristics of retinal electrophysiological activities in adults and children in terms of retinal regions and mERG components.

To the best of our knowledge, no study has previously investigated retinal function in young children with emmetropic refractive status, nor the correlation between retinal function and subsequent myopic change. The current study sought to use global flash mERG (MOFO mERG) parameters to predict early myopic development in children. We hypothesized that emmetropic children with decreased retinal response measured by the global flash mERG would subsequently develop myopia.

Methods

Subjects

Fifty-six children (29 girls and 27 boys) aged 6 to 9 years (mean of 7.63 years) with emmetropic refractive error were recruited for the study conducted at the Optometry Clinic of The Hong Kong Polytechnic University. All subjects had a comprehensive eye examination including cycloplegic refraction, axial length measurement, and ocular health assessment by an optometrist (SZL). One drop of 0.4% oxybuprocaine (Agepha Pharmaceuticals, Wien, Austria) and two drops of 1% Tropicamide (Alcon Laboratories, Inc., Fort Worth, TX, USA) were instilled at 5-minute intervals into both eyes 30 minutes before subjective refraction. Two drops of tropicamide have been proven to be equivalent to cyclopentolate in terms of the cycloplegic effect. The resolution of refraction was 0.25 diopter (D). Visual acuity was tested with a Thomson
computerized chart. Axial length measurement was conducted with an IOL master (V-4.08; Carl Zeiss Meditec, Inc., Dublin, CA, USA). Five readings with a range of less than 0.10 mm were averaged. Color vision was assessed with the 24-plate version of Ishihara color vision test under standard illuminant C, and the passing criterion was defined as correct recognition of all 24 plates. The inclusion criteria were best-corrected logMAR visual acuity of 0.00 or better, normal color vision, cycloplegic refractive error (defined as spherical equivalent $\geq -0.5 \, D$ and $\leq +0.5 \, D$, and astigmatism $\leq 1.0 \, D$), and normal ocular health in both eyes. Subjects with a family history of inherited ocular diseases, clinically significant retinal degeneration, or systemic diseases were excluded from the study.

Subjects underwent cycloplegic refraction and axial length measurements for both eyes; only the right eye was tested for MOFO mfERG measurements at the initial baseline visit. All subjects were followed up for cycloplegic refraction and axial length measurement after 1 year. Only the data for the right eye of the subjects were included in the statistical analysis. The study was approved by the Human Ethics Committee of The Hong Kong Polytechnic University and adhered to the tenets of the Declaration of Helsinki. The parents of the subjects gave consent for their children to participate in this study.

MOFO mfERG Recording

The MOFO mfERG was recorded with Dawson-Trick-Litzkow fiber acting as the active electrode (located on the cornea of the right eye) and gold-cup surface electrodes as reference (located at the outer canthus of the right eye) and ground (located at the forehead). The recordings were commenced after the pupil of the subject’s right eye was dilated to at least 7 mm diameter. The stimulus pattern was generated by the Visual Evoked Response Imaging System (VERIS Science 6.0.6d19; EDI, San Mateo, CA, USA) and displayed on a 22-inch LED monitor (model VG2239M-LED; ViewSonic, Walnut, CA, USA). The stimulus pattern consisted of 61 hexagons subtending 37 degrees horizontally and 33 degrees vertically at a working distance of 40 cm. Full correction was provided to compensate for a subject’s spherocylindrical refractive error and working distance.

The global flash paradigm was composed of four video frames as shown in Figure 1A: starting with a frame of multifocal flashes, followed by a dark frame, a full-screen flash frame and a second dark frame in each slice of the pseudorandom binary m-sequence (2^7–1). Frame frequency of the monitor was set at 75 Hz. The luminance of the multifocal flash stimulus in light and dark states was 185 and 4 cd/m², respectively, for 96% contrast level, and at 140 and 4 cd/m², respectively, for 49% contrast level. The mean luminance of the multifocal flashes and the background was approximately 94 cd/m² for both contrast levels. The recording time of 4 minutes for each contrast level was divided into 16 segments to allow the subject to rest between runs. A central cross was used for fixation. The signal was monitored using the real-time response provided by the VERIS program and any segment contaminated by blinks or fixation loss was re-recorded. An amplifier (model 15A54, Physiodata Amplifier System; Grass Technologies, Astro-Med, Inc., West Warwick, RI, USA) was used with a signal gain of 100,000 times and the band pass filter between 10 and 300 Hz.

Analysis

Groups of responses from the MOFO mfERG trace arrays were averaged to five successive rings from the center to the periphery as shown in Figure 1B. The peak-to-peak amplitudes of the direct component (DC) and the induced component (IC) responses were calculated. The implicit times of DC and IC response were counted from the onset of multifocal flash and global flash, respectively, to the peak of the response (Fig. 1C). Refractive error and axial length changes were calculated by subtracting the results of the initial visit from that of the follow-up visit.

The normality of the variables was determined by the Shapiro-Wilk Test (SPSS 23.0; IBM Corporation, Chicago, IL, USA). As the data of the changes in refractive error and axial length were not normally distributed, nonparametric tests were used for the statistical analysis. Wilcoxon signed ranks test was used to compare the refractive error and axial length between the two visits and to compare the amplitudes and implicit times between the DC and IC of mfERG response within the same subject. The correlation between refractive error change and axial length change was tested by the Spearman test. Spearman’s rank correlation was also used to analyze the relationship between the MOFO mfERG responses and the myopic development in terms of changes in refractive error as axial length. Bonferroni adjustment was applied, as there were five retinal regions within each subject’s right eye for comparison, and thus the adjusted significance level was set to 0.01.

Intra-sessional measurement variability of MOFO mfERG responses at 49% contrast level was tested on 11 children (7 girls and 4 boys) aged from 8 to 11 years. The method of Bland and Altman was used to calculate the coefficient of repeatability (COR), defined as 1.96 times the SD of the differences between the paired measurements. The confidence interval was 95%. The COR results and Bland-Altman plots are shown in the Appendix (Fig. A1; Table A1).

Results

As shown in Table 1, all subjects were emmetropic with a mean refractive error of $+0.19 \, D$ and axial length of $23.14 \, mm$ at their initial visit. At the follow-up visit, 43 of the 56 subjects showed myopic changes in spherical equivalent refractive error, whereas the refractive errors remained the same for the other 13 subjects. The mean follow-up time was 0.99 year with a SD of 0.05 year. There was an average change in refractive error of $-0.55 \, D$ and in axial elongation of $0.37 \, mm$, and the changes of refractive error and axial length were statistically significantly different between the two visits (refractive error $P < 0.001$; axial length $P < 0.001$, Wilcoxon signed ranks test). The correlation between the baseline refractive error and the change in refractive error was significant ($\rho = 0.34$, $P = 0.015$, Spearman’s Rho), whereas the correlation between the baseline axial length and the change in axial length was not significant ($\rho = 0.19$, $P = 0.17$, Spearman’s Rho). The change of refractive error was highly correlated with the axial elongation ($\rho = -0.84$, $P < 0.001$, Spearman’s Rho) (Fig. 2), which indicated that the increasing myopia is mostly axial in nature.

To understand the normal ocular growth in young children, 1-year axial length changes of another group of subjects, who had no myopic development, were calculated retrospectively. Data were selected from 18 children (age from 6 to 9 years) with similar range of cycloplegic refractive error ($+0.21 \pm 0.30 \, D$) and axial length ($23.11 \pm 0.47 \, mm$) at the baseline. It was found that 1-year normal ocular growth for this age group of children was $0.16 \pm 0.06 \, mm$. The parameters of MOFO mfERG responses of the emmetropic children are shown in Figures 3 and 4. The response amplitude decreased dramatically with increased retinal eccentricity, and the response
implicit time slightly shortened with augmentation of eccentricity. Under high-contrast (96%) mfERG stimulation, the IC amplitude was significantly larger than the DC amplitude at all regions (all \( P < 0.001 \), Wilcoxon signed ranks test). However, under low-contrast (49%) mfERG stimulation, no differences between DC and IC amplitudes were found from central ring 1 to ring 3 (\( P = 0.795 \) for ring 1; \( P = 0.714 \) for ring 2; \( P = 0.077 \); Wilcoxon signed ranks test). From ring 4 to ring 5, the differences between DC and IC amplitudes reached a significance level of 0.05, but not the adjusted significance level of 0.01 after Bonferroni correction (\( P = 0.02 \) for ring 4; \( P = 0.028 \) for ring 5; Wilcoxon signed ranks test). For the implicit time, significant difference between DC and IC responses was present only at ring 1 for both high- and low-contrast stimulation conditions (both \( P < 0.001 \), Wilcoxon signed ranks test). The mean central IC response was delayed by 1.61

**FIGURE 1.** (A) The global flash paradigm was composed of four video frames: starting with a frame of multifocal flashes, followed by a dark frame, a full-screen flash frame, and a second dark frame in each slice of the pseudorandom binary m-sequence (212). Frame frequency of the monitor was set at 75 Hz, and thus the frame interval was 13.3 ms. (B) The 61 response trace arrays were averaged to five successive rings from center to periphery. (C) A typical waveform of the MOFO mfERG response and its components.
ms compared with the mean central DC response with high-contrast stimulation, whereas the IC implicit time was delayed by 1.10 ms compared with the DC implicit time with low-contrast stimulation. The implicit time differences between the DC and IC responses for all other regions under both high- and low-contrast level conditions did not reach the adjusted significance level.

The Spearman’s rank correlation coefficients between the MOFO mfERG amplitudes and changes of refractive error and axial length are summarized in Table 2. Among the parameters recorded at the two contrast levels and from five retinal eccentricities, ring 1 IC amplitude recorded at 49% contrast level at the initial visit was found to be significantly correlated with the subsequent change in refractive error \((\rho = 0.46, P < 0.001, \text{Spearman’s Rho})\). It was also significantly correlated to the axial elongation \((\rho = -0.54, P = 0.01, \text{Spearman’s Rho})\). Figures 5 and 6 show the trends of the correlation between the ring 1 IC amplitude toward the change in refractive error and axial length, respectively. Table 3 lists the correlation coefficients between the MOFO mfERG implicit times and changes of refractive error and axial length. Baseline implicit times of DC and IC responses, however, were found to correlate with neither the changes in refractive error nor in axial length.

### DISCUSSION

The baseline IC amplitude from ring 1 measured at 49% contrast level was found to be significantly correlated with the subsequent refractive error change. This mfERG parameter was measured in young emmetropic children with normal visual acuity and good ocular health, which indicates the variance of this mfERG parameter could not be attributed to long-standing myopia or any other pathological effects. The MOFO mfERG was developed by Sutter et al.\(^1\) to enhance inner retinal response contributions. Shimada et al.\(^2\) suggested that IC was derived from the difference in the global flash response in the presence and absence of the preceding focal flash. Previous studies have shown that IC represents predominantly inner retinal function.\(^3\)–\(^5\) Based on this evidence, emmetropic children with subclinical decreased inner retinal function in the central region may be more likely to subsequently develop myopia.

Among variant parameters of MOFO mfERG response, only one parameter, which mainly represents central inner retina response, was found to be significantly related to myopia development. This finding concurs with those from previous studies. Luu and his coworkers followed the changes of

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**TABLE 1.** Refractive Error and Axial Length of the Subjects at the Initial and Follow-up Visits

<table>
<thead>
<tr>
<th>Range</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
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<tr>
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<td>+0.50</td>
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<tr>
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<td>24.71</td>
<td>23.14</td>
<td>0.60</td>
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<tr>
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<tr>
<td>Changes, = follow-up − initial</td>
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<td>0.93</td>
<td>0.37</td>
<td>0.22</td>
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**FIGURE 2.** Correlation between axial elongation and refractive error change \((n = 56)\).
refractive error in 81 children for 2 years and divided these children into three subgroups according to their myopic progression rate. They found that the fast myopic progression subgroup had decreased central mfERG amplitudes at the initial visit. A significant correlation was also found between the central mfERG amplitude and the change in vitreous chamber length, but not with the change of refractive errors. They suggested the central retinal function could be a predictor of children’s myopia progression rate. However, the subjects recruited in their study were all already myopic, ranging from \(-1.00\) D to \(-5.88\) D at the initial visit. Previous studies have also shown that highly myopic children have more changes in electro-retinal activity than those with less myopia. Luu and colleagues recorded conventional mfERG responses from 104 children with various refractive errors and P1 implicit time was found to be highly correlated with the severity of myopia. Ho et al. also conducted a cross-sectional mfERG study in 52 children with refractive errors ranging from plano to \(-5.50\) D using the protocol of MOFO with two different contrast stimulations. The combined effect of refractive error and axial length accounted for approximately 18% reduction of ring 1 log-DC amplitude at high-contrast level. Thus, the myopic eye is highly predisposed to have an adverse effect on retinal function. In our current study, all the subjects were emmetropic, which minimized this possible confounding factor. This may explain why we obtained the significant correlation between mfERG response and changes in refractive errors. A recent longitudinal study involving a total of 26 myopic children followed for 1 year investigated the changes of global flash mfERG responses with myopia progression. Under low-contrast level stimulation, central DC and IC amplitudes significantly reduced after a year and such reductions of mfERG responses were correlated with changes in myopic refractive error. It was hence suggested that central inner retinal function attenuates with myopic progression. Collectively, we speculate that the central inner retina may play a role in the manipulation of myopia development. Subclinical decreased central inner retinal function in young children could be a forewarning of myopia development.

Ample evidence has shown a significant correlation between ERG amplitude and increased axial length in myopic adults. Researchers have suggested that the reduced ERG amplitude could result from an increase in the subretinal space, reduced image size, decreased retinal illumination, and the reduced retinal cell density in the elongated eyeball. However, none of these factors could explain the findings in children. Our results demonstrated that reduced central ERG response appeared before myopia development, and thus it is more likely to be an inducement to myopia rather than a secondary effect. We suggest that children with a

### Table 2

<table>
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<th>Component</th>
<th>Contrast</th>
<th>Region</th>
<th>Parameter</th>
<th>DC</th>
<th>IC</th>
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<td>(P) Value</td>
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<td>(P) Value</td>
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<td></td>
<td>AL</td>
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<td>0.30</td>
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<td></td>
<td></td>
<td>Ring 4</td>
<td>RE</td>
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<td>RE</td>
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<td>-0.04</td>
<td>0.78</td>
<td>-0.07</td>
<td>0.63</td>
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* Denotes \(P \leq 0.01\), Bonferroni-adjusted statistical significance cutoff value.

**FIGURE 5.** Correlation between MOFO mfERG IC amplitude of ring 1 at 49% contrast level and change in refractive error.

**FIGURE 6.** Correlation between MOFO mfERG IC amplitude of ring 1 at 49% contrast level and change in axial length.
decreased central retinal function are prone to eyeball elongation and myopic development. The decrement in retinal function seems to be inversely proportional to the rate of myopic development. Over years of myopic progression as children grow into adults, the reduced ERG amplitude may be significantly associated with the severity of myopia. We believe this decreased retinal function may have an interactive effect with other myopigenic mechanisms and that the level of the reduction is mutable. As Ho and coworkers\textsuperscript{17} have shown that myopic progression in children caused the reduction of central inner retinal function, together with the findings of our study, we further propose that myopic children with normal and stable central inner retinal function would have little or no myopic development. This hypothesis needs to be tested by future longitudinal study.

Interestingly, we found that the correlation between reduced retinal function and changes in refractive error was stronger than that with axial length. This finding is consistent with the finding of Chen et al.\textsuperscript{22} that refractive error accounted for a greater proportion of the variability than axial length in mfERG responses in myopic adults. The studies of Ho et al.\textsuperscript{5,6} used a hierarchical regression model that evaluates the individual effect of both variables and these researchers also showed that refractive error contributed most of the reduction in ERG response in adults. In our study, we calculated the 1-year changes in refractive error (−0.55 ± 0.53 D) and axial length (0.37 ± 0.22 mm) of young children. The refractive error change indicated the change of optical status of the eyeball, whereas the axial elongation represented the consequences of both normal eye growth and extra eye growth due to myopic change. We found normal eye growth was 0.16 ± 0.06 mm for this age group of children; it was estimated to contribute approximately 40% of the total axial length change while the remaining 60% corresponded to the myopic change. As the reduction of central retinal function is hypothesized to correlate with myopic development, it is not surprising to find that the correlation between axial elongation and the reduction of retinal response is weaker, as the relationship is masked by the effect of normal eyeball growth in children.

Myopia is reaching epidemic proportion worldwide. A recent systematic review and meta-analysis predicted that the prevalence of myopia and high myopia in the world population by 2050 will be 49.8% and 9.8%, respectively.\textsuperscript{23} With the known ocular complications of high myopia, such as retinal detachment, glaucoma,\textsuperscript{24} maculopathy,\textsuperscript{25} and cataracts,\textsuperscript{26} it is particularly urgent to identify children who are at high risk of myopic development and to provide them with early intervention of myopia control. The common myopia control methods, especially in Asia, include orthokeratology and atropine.\textsuperscript{27,28} However, it is difficult for clinicians to identify children who are prone to myopia development and need early myopia intervention. The present study recognized the retinal electrophysiological characteristics of young emmetropic children with subsequent myopia development. Our findings indicate that myopia development in children could be predicted through assessing central inner retinal function by the measurement of the MOFO mfERG. For children with subclinical decreased IC amplitudes under low-contrast stimulation, early myopia control interventions should be considered. It would be beneficial for those children to prevent future myopia-related morbidity, such as high myopia, ocular complications, and degenerations; however, to further validate this method of myopic development prediction, studies with larger sample sizes and longer follow-up time would be necessary. In addition, the current MOFO mfERG recording paradigm is laborious and, as only central IC amplitude among variant mfERG parameters was found to be significantly related to myopia development, simplifying the protocol could allow direct and efficient measurement of central inner retina function in children.

In conclusion, the central IC amplitude obtained using MOFO mfERG under 49% contrast stimulation was significantly correlated with later changes of refractive error in young children. This finding indicates that subclinical reduction of the central inner retinal function in emmetropic children could be a myopigenic factor and this retinal response might be a potential reference for juvenile myopia development.

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References


Figure A1. Bland-Altman analysis of repeatability for (A) DC implicit time; (B) IC implicit time; (C) DC amplitude; and (D) IC amplitude.

Table A1. Measurement Variability of MOFO mfERG

<table>
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<th>Measurement</th>
<th>Units</th>
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<th>SD of Differences</th>
<th>COR</th>
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<td>4.76</td>
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<tr>
<td>IC implicit time</td>
<td>ms</td>
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<td>nV/deg²</td>
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