

Corneal Endothelial Morphology and Ocular Biometric Indexes in Premature Children With and Without Retinopathy of Prematurity

Hung-Chi Chen,^{1,3} Shun-Fa Yang,^{4,5} Chia-Yi Lee,^{4,6,7} Jing-Yang Huang,⁵ Yi-Jen Hsueh,^{1,2} Ming-Hui Sun,^{1,3} Ming-Chou Chiang,^{3,8} Yu-Shu Huang,^{3,9} Shih-Ming Chu,^{3,8} Jen-Fu Hsu,^{3,8} Chun-Hsiu Liu,^{1,3} Chao-Kai Chang,^{6,10} Kuan-Jen Chen,^{1,3} Yih-Shiou Hwang,^{1,3} Chi-Chun Lai,^{1,3} Chung-Ying Huang,^{1,3} and Wei-Chi Wu^{1,3}

¹Department of Ophthalmology, Chang Gung Memorial Hospital, Linkou, Taiwan

²Center for Tissue Engineering, Chang Gung Memorial Hospital, Linkou, Taiwan

³Department of Medicine, Chang Gung University College of Medicine, Taoyuan, Taiwan

⁴Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan

⁵Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan

⁶Nobel Eye Institute, Taipei, Taiwan

⁷Department of Ophthalmology, Jen-Ai Hospital Dali Branch, Taichung, Taiwan

⁸Department of Pediatrics, Chang Gung Memorial Hospital, Linkou, Taiwan

⁹Department of Psychiatry, Chang Gung Memorial Hospital, Linkou, Taiwan

¹⁰Department of Optometry, Da-Yeh University, Chunghua, Taiwan

Correspondence: Wei-Chi Wu, Department of Ophthalmology, Chang Gung Memorial Hospital, 5 Fuxing Street, Guishan District, Taoyuan 33305, Taiwan; weichi666@gmail.com.

HCC and SFY contributed equally to this work and share the first authorship.

Received: November 16, 2023

Accepted: March 6, 2024

Published: May 23, 2024

Citation: Chen HC, Yang SF, Lee CY, et al. Corneal endothelial morphology and ocular biometric indexes in premature children with and without retinopathy of prematurity. *Invest Ophthalmol Vis Sci*. 2024;65(5):37. <https://doi.org/10.1167/iov.65.5.37>

PURPOSE. The purpose of this study was to analyze human corneal endothelial cells (HCECs) morphology and ocular biometrics in premature (PM) children with or without retinopathy of prematurity (ROP).

METHODS. Retrospective data on patient demographics, HCECs status, and ocular biometrics with at least 2 visits between 2016 and 2021 were reviewed. The main outcomes were endothelial cell density (ECD), coefficient of variation (CV), hexagonal cell ratio (HEX), central corneal thickness (CCT), axial length, anterior chamber depth, keratometry, corneal diameter, pupil diameter, and refraction status. Generalized estimating equation was used to evaluate the differences between PM no-ROP and ROP groups. We also analyzed the trend of ECD, CV, HEX, and CCT change with age between groups.

RESULTS. The study included 173 PM patients without ROP and 139 patients with ROP. A total of 666 and 544 measurements were recorded in the PM no-ROP and ROP groups, respectively. The ROP group had higher spherical power, myopic spherical equivalent (SE), and steeper steep keratometry (K; $P < 0.05$). The ROP group had higher CV ($P = 0.0144$), lower HEX ($P = 0.0012$) and thicker CCT ($P = 0.0035$). In the HCECs parameters, the ROP group had slower ECD decrement ($P < 0.0001$), faster CV decrement ($P = 0.0060$), and faster HEX increment ($P = 0.0001$). A difference in corneal morphology changes between the ROP and PM no-ROP groups were prominent in patients with lower gestational age (GA) in the subgroup analysis.

CONCLUSIONS. Worse HCECs morphology and higher myopic status were initially observed in patients with prior ROP but not in PM patients with no-ROP. ECD and HCECs morphology improved with age, especially in patients with low GA.

Keywords: retinopathy of prematurity (ROP), human corneal endothelial cells (HCECs), endothelial cell density (ECD), morphology, anterior segment

Retinopathy of prematurity (ROP) is a vasoproliferative retinal disease that occurs in premature (PM) newborns, especially in those with low gestational age (GA) and birth weight (BW).¹ The main pathophysiology of ROP is retinal hypoxia, ischemia, and consequent neovascularization because of excessive oxidative stress on the preterm fetal retina.^{2,3} ROP is a two-phased disease.¹ In the first phase, oxygen treatment in the preterm infant

ceases the retinal vascular development and retinal vascular obliteration.^{2,3} After the end of the oxygen supply, babies under normoxia (relative hypoxia) have insufficient retinal oxygenation which could contribute to retinal ischemia.^{2,3} In the second phase, the ischemic retina stimulates the secretion of vascular endothelial growth factor inducing subsequent pathological retinal angiogenesis and possible neovascularization.⁴ In those with advanced ROP, total trac-

tional retinal detachment may develop and lead to severe visual impairment and blindness.⁴ Epidemiological studies have shown that ROP occurs in approximately one third of surviving infants necessitating treatment, and it is the leading cause of preventable blindness in children along with cataract and corneal opacity.⁵ Other than retinal complications, ROP influences the anterior segment contributing to a steeper cornea, shallower anterior chamber, and greater crystalline lens thickness.⁶

There is a paucity of research evaluating the HCECs status in PM patients. One recent study on full-term children showed a rapid decrement in endothelial cell density (ECD) during the first 2 years, after which the ECD decline rate resumed to a level similar to that in adults.⁷ However, only the general population of toddlers and preschoolers without ROP were surveyed in that research.⁷ Another earlier research evaluated the corneal endothelial status in patients with cicatricial ROP and found that lower BW was associated with smaller mean cell area and poorer HCECs development.⁸ Still, the research only surveyed the corneal morphology among different ROP populations with relatively fewer case numbers.⁸ Although ROP may influence the anterior segment and loss of HCECs may lead to visual impairment,^{6,9} it remains unclear whether HCECs are altered in patients with ROP. Additionally, although one previous study showed the effect of ROP on ocular biometrics,⁶ the PM infants without ROP can have a different profile of ocular biometrics. We speculate that the presence of ROP after birth can alter the following growth of the globe as well as the anterior segment structure,^{10,11} and that myopia in patients with ROP would progress later in life.¹² More recently, we have demonstrated that PM babies may be less suited for the refractive surgery due to their corneal topographical condition,¹³ which could influence refractive prognosis. Collectively, whether the effect on ROP in early stages may result in permanent and significant change of corneal morphology still needs further investigation.

Our study aimed to investigate the HCEC morphology and other ocular biometric values between PM children with and without ROP. We also evaluated the trend of corneal endothelial and biometric parameters over time.

MATERIALS AND METHODS

Ethical Statement

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Review Board at the Chang Gung Medical Foundation, Taiwan (project code: 202201840B0). Informed consents were obtained from parents of patients after a full explanation of the study. Patients and parents could choose to discontinue participation in the study at any time without giving any reason.

Patient Selection

A retrospective study on a cohort with longitudinal follow-up was conducted at the Chang Gung Memorial Hospital from January 2016 to July 2021. We enrolled PM patients without a history of ROP and aged <10 years at initial presentation as the PM no-ROP group. For comparison, PM children with prior ROP but without treatment who aged <10 years at initial enrollment were grouped as the ROP group. Patients were excluded if the following

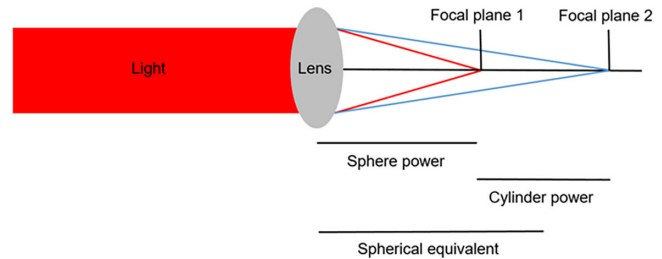


FIGURE 1. Illustration of spherical power, cylinder power, and spherical equivalent.

conditions occurred: (1) hydrocephalus, (2) congenital glaucoma, (3) congenital cataract, (4) persistent fetal vasculature, (5) congenital ocular infection including rubella and gonorrhea, (6) total retinal detachment at initial examination, (7) progressive ROP warranting pars plana vitrectomy, (8) death of the infant before 2 years old, (9) undetermined parents even being well-informed, (10) refusal of the parents to join the study, (11) a follow-up period shorter than 1 year, and (12) received any ROP-related treatment including but not limiting to antivascular endothelial growth factor injection, argon laser treatment, or xenon laser treatment. Patients were regularly followed up every year, and data up to 4 years of follow-up were analyzed.

Data Collection and Main Outcome Measurements

The demographic data of patients, including initial age, gender, type of parturition, GA, BW, and Apgar score at 1 and 5 minutes were collected from the medical documents. After getting enrolled in the current study, children were assessed for the corneal endothelial and ocular biometric examinations at the initial visit and at each following visit with an interval of approximately 1 year (from 10 months to 14 months) between each visit. Only the data in the first 4 years were collected to standardize the follow-up period. The following parameters were obtained at each visit: ECD, coefficient of variation (CV), hexagonal cell ratio (HEX) by a non-contact in vivo specular microscope (CEM-530; Nidek, Gamagori, Japan), central corneal thickness (CCT), axial length (AXL), anterior chamber depth (ACD), flat as well as steep keratometry (K), corneal diameter (CD) and pupil diameter by the IOL Master (Carl Zeiss Meditec, Inc., Dublin, CA, USA). In addition, the spherical power, cylinder power, and spherical equivalent (SE) were obtained via an auto-refractor (Nikon NRK 8000; Nikon Inc., Tokyo, Japan). The illustration image of spherical power, cylinder power, and spherical equivalent is shown in Figure 1. All measurements were taken three times at each visit and the average values were used for the subsequent statistical analyses.

Statistical Analysis

SAS version 9.4 (SAS Institute Inc, Cary, NC, USA) was used for statistical analyses. The Shapiro-Wilk test was applied to decide whether the study population is normal distribution before further analyses and the results indicated that both the PM no-ROP group and the ROP group followed a normal distribution ($P > 0.05$). For variables that were measured once, including gender, type of delivery, GA, BW, and Apgar scores, independent *t*-test and Chi-square test were used to compare data between the groups, where appropriate. For

variables that were repeatedly measured over time and for both the eyes including sphere, cylinder, SE, ECD, CV, HEX, CCT, AXL, ACD, and CD, the generalized estimating equation (GEE) was used with autoregressive within-subject covariance structure to determine differences between the groups. The effect of variables, such as initial age, gender, and GA, were included in the GEE model. We compared the differences in each index between the groups based on the time of the visits. We plotted a scatter plot with fit curve to demonstrate the distribution of each HCEC parameter between the two groups based on age, and the GEE was used to evaluate the trend of each parameter between the two groups after considering the effect of gender and GA. Then, we stratified the study population into those with GA of 23 to 28 weeks, GA of 28 to 31 weeks, GA of 31 to 33 weeks, and GA more than 33 weeks, and the GEE was applied to evaluate the trend of each corneal parameter between the 2 groups with different GA. Similarly, the correlation of other parameters and the corneal morphology in the ROP group were analyzed by GEE. A P value < 0.05 was considered statistically significant.

RESULTS

A total of 312 patients (173 and 139 patients in the PM no-ROP group and the ROP group, respectively) were included. The mean initial presenting age was 5.80 ± 2.30 years in the PM no-ROP group and 6.25 ± 2.27 years in the ROP group ($P = 0.0857$). The gender distribution and parturition types were similar between the two groups (both $P > 0.05$). However, a significantly lower GA, BW, Apgar score at 1 minute, and Apgar score at 5 minutes were noted in the ROP population ($P < 0.0001$; Table 1).

For the longitudinal visit, the spherical power, SE, AXL, ACD, Ks, pupil diameter, and ECD showed significant change by time in the whole study population ($P < 0.05$; Table 2). Analysis of the trend of each HCEC parameter between the 2 groups showed that the ECD decreased significantly slowly in the ROP group compared to the PM no-ROP group from

age 3 to 12 years ($P < 0.0001$; Fig. 2). The ROP group had a significant decrement of CV ($P = 0.0060$) and increment of HEX ($P = 0.0001$) as the patients grew older compared to the PM no-ROP population (Fig. 3, Fig. 4). The CCT change demonstrated a similar trend between the two groups ($P = 0.3951$; Fig. 5).

In the subgroup analysis stratified by GA, the ROP groups showed a slower ECD decrement in those with GA of 23 to 28, 28 to 31, and more than 33 weeks, faster CV decrement in those with GA of 28 to 31, 31 to 33, and more than 33 weeks, faster HEX increment in those with GA of 23 to 28 and 28 to 31 weeks, and a faster CCT decrement in those with GA from 23 to 28 weeks (Table 3). About the association between BW and corneal morphology in the ROP group, lower BW was correlated with higher ECD ($P = 0.0162$) but not with abnormal values of CV, HEX, and CCT ($P > 0.05$). In terms of the correlation between other parameters and corneal morphology in the ROP population, the lower GA, higher steep K, and smaller CD correlated to higher ECD ($P < 0.05$), whereas lower GA and smaller CD correlated to lower HEX ($P < 0.05$). None of the variables were significantly associated with CV or CCT ($P > 0.05$).

DISCUSSION

The current study revealed a more myopic status and steeper K in the ROP group compared to the PM no-ROP group. Besides, the HCEC evaluation showed worse CV and HEX in the ROP groups during the 4 years of follow-up from a mean age of 6 years. On the contrary, the trend analyses illustrated slower ECD decrement, better CV, and HEX recovery in the ROP group especially in the patients with lower GA.

Derived from the neural crest embryologically,¹⁴ the human corneal endothelium consists of a monolayer of HCECs with ECD reaching a peak of 6000 cells/mm² immediately after birth,¹⁵ and gradually declines to 3500 cells/mm² by the age of 5 years.¹⁶ HCECs do not actively proliferate in vivo for unknown reasons with the cell cycle arrested at the G1 phase due to contact inhibition mediated by intercellu-

TABLE 1. Birth Characteristics of the Study Population

Characteristics	PM No-ROP (N = 173)	ROP (N = 139)	P Value
Age, # y mean \pm SD	5.80 \pm 2.30	6.25 \pm 2.27	0.0857
Gender N (%)			0.2457
M	88 (50.9)	80 (57.6)	
F	85 (49.1)	58 (41.7)	
Parturition			0.2426
NSD	42 (24.3%)	42 (30.2%)	
CS	129 (74.6%)	93 (66.9%)	
GA, wk, mean \pm SD	32.06 \pm 2.99	27.13 \pm 2.48	<0.0001*
<28	18 (10.4%)	92 (66.2%)	
28 - < 37	152 (87.9%)	47 (33.8%)	
≥ 37	3 (1.7%)	0 (0.0%)	
BW, g, mean \pm SD	1664.92 \pm 589.65	933.71 \pm 314.89	< 0.0001*
<1500	74 (42.8%)	128 (92.1%)	
1500 - < 2500	83 (48.0%)	11 (7.9%)	
2500 - < 3500	15 (8.7%)	0 (0.0%)	
≥ 3500	1 (0.6%)	0 (0.0%)	
Apgar score at 1 min	7.35 \pm 1.64	4.96 \pm 2.15	<0.0001*
Apgar score at 5 min	8.86 \pm 1.22	7.12 \pm 1.79	<0.0001*

BW, birth weight; CS, Cesarean section; GA, gestational age; N, number; NSD, normal spontaneous delivery; PM, premature; ROP, retinopathy of prematurity; SD, standard deviation.

Age at baseline (initial presentation) of the ophthalmic department.

* Denotes significant differences between the groups.

TABLE 2. Corneal Endothelial and Ocular Biometric Parameters of the PM No-ROP and ROP Groups During Follow-Up

Parameters	Mean \pm SD of Value					P Value
	Baseline	1 Y	2 Y	3 Y	4 Y	
Spherical power						<0.0001*
PM no-ROP	1.32 \pm 1.44	1.27 \pm 1.49	1.13 \pm 1.70	1.12 \pm 1.66	0.73 \pm 1.23	
ROP	0.57 \pm 3.04	0.45 \pm 3.09	0.26 \pm 3.13	0.44 \pm 2.87	−0.30 \pm 3.23	
Cylinder power						0.6269
PM no-ROP	−1.11 \pm 1.10	−1.11 \pm 1.11	−1.08 \pm 1.12	−1.10 \pm 1.21	−1.12 \pm 1.25	
ROP	−1.36 \pm 1.04	−0.23 \pm 13.79	−1.39 \pm 1.07	−1.36 \pm 1.06	−1.45 \pm 1.15	
SE						<0.0001*
PM no-ROP	0.81 \pm 1.49	0.76 \pm 1.50	0.64 \pm 1.78	0.61 \pm 1.71	0.23 \pm 1.36	
ROP	−0.08 \pm 3.19	−0.26 \pm 3.29	−0.33 \pm 3.40	−0.29 \pm 2.98	−0.99 \pm 3.47	
AXL, mm						<0.0001*
PM no-ROP	22.31 \pm 1.00	22.39 \pm 0.98	22.64 \pm 1.16	22.72 \pm 1.08	22.99 \pm 1.05	
ROP	22.24 \pm 1.26	22.37 \pm 1.27	22.51 \pm 1.35	22.61 \pm 1.29	22.86 \pm 1.28	
ACD, mm						<0.0001*
PM no-ROP	3.17 \pm 0.59	3.25 \pm 0.45	3.26 \pm 0.42	3.37 \pm 0.33	3.45 \pm 0.27	
ROP	3.14 \pm 0.40	3.15 \pm 0.51	3.19 \pm 0.40	3.30 \pm 0.42	3.38 \pm 0.36	
Flat K						0.0398*
PM no-ROP	43.34 \pm 1.67	43.33 \pm 1.64	43.35 \pm 1.67	43.32 \pm 1.59	43.69 \pm 1.66	
ROP	43.77 \pm 2.14	43.95 \pm 1.83	44.03 \pm 1.78	43.87 \pm 1.73	44.38 \pm 1.63	
Steep K						<0.0001*
PM no-ROP	44.74 \pm 3.24	45.06 \pm 2.03	44.88 \pm 1.83	44.69 \pm 1.95	44.59 \pm 1.79	
ROP	45.69 \pm 2.14	45.81 \pm 2.14	45.90 \pm 2.20	45.80 \pm 2.17	45.39 \pm 2.29	
CD, mm						0.5032
PM no-ROP	12.01 \pm 0.48	11.99 \pm 0.49	11.95 \pm 1.01	11.82 \pm 2.48	12.06 \pm 0.45	
ROP	11.79 \pm 0.49	11.78 \pm 0.50	11.82 \pm 0.59	11.55 \pm 2.74	11.82 \pm 0.52	
Pupil diameter, mm						<0.0001*
PM no-ROP	3.72 \pm 1.48	3.74 \pm 1.45	4.13 \pm 1.69	4.19 \pm 1.75	4.00 \pm 1.72	
ROP	4.04 \pm 1.75	3.58 \pm 1.45	3.90 \pm 1.62	4.18 \pm 1.76	4.84 \pm 1.84	
ECD, cells/mm ²						<0.0001*
PM no-ROP	3257.89 \pm 265.58	3250.71 \pm 282.49	3219.39 \pm 266.02	3190.78 \pm 274.57	3146.26 \pm 267.24	
ROP	3303.18 \pm 317.42	3310.77 \pm 290.35	3261.38 \pm 286.59	3203.48 \pm 270.61	3177.37 \pm 273.88	
CV, %						0.7419
PM no-ROP	22.86 \pm 5.44	23.64 \pm 6.03	23.09 \pm 4.98	23.06 \pm 6.15	23.95 \pm 4.81	
ROP	25.54 \pm 9.69	24.71 \pm 6.12	24.16 \pm 5.68	24.70 \pm 6.52	23.95 \pm 6.07	
HEX, %						0.1110
PM no-ROP	69.48 \pm 6.05	68.70 \pm 8.16	69.09 \pm 6.63	69.35 \pm 5.22	68.56 \pm 4.85	
ROP	65.92 \pm 10.03	66.12 \pm 10.54	68.62 \pm 6.85	67.18 \pm 10.21	68.33 \pm 6.09	
CCT, μ m						0.2171
PM no-ROP	551.02 \pm 43.35	550.40 \pm 38.09	551.76 \pm 41.26	550.35 \pm 37.84	541.95 \pm 32.64	
ROP	563.67 \pm 43.20	566.32 \pm 39.92	560.06 \pm 37.13	558.51 \pm 35.73	556.41 \pm 30.02	

ACD, anterior chamber depth; AXL, axial length; CCT, central corneal thickness; CD, corneal diameter; CV, coefficient of variant; ECD, endothelial cell density; HEX, hexagonality; K, keratometry; PM, premature; ROP, retinopathy of prematurity; SD, standard deviation; SE, spherical equivalent.

* Denotes significant differences in the total study population at different visits.

lar junctions.¹⁷ Central ECD decreases at an annual rate of about 0.6% during a lifetime,¹⁸ so that the mean ECD drops from approximately 3300 cells/mm² at adolescence to 2300 cells/mm² at age 85 years.^{19,20} Besides, racial or geographic differences have been reported, with higher ECD found in Japanese than American individuals.¹⁹ In a previous study, ROP in the posterior segment was shown to influence the cornea in the anterior segment.²¹ In addition, the corneal curvature and anterior chamber depth demonstrated a significant change in patients with ROP during the early ages.^{21,22} Furthermore, the anterior segment abnormality in patients with ROP with or without treatment had been reported in earlier literature.^{23,24} Consequently, the presence of ROP at birth may cause subsequent change of corneal morphology in later years. The exact reason why ROP in the posterior segment can influence the development of the anterior segment remains unknown. We hypothesized that there is

an “anterior chamber growth factor X” which is secreted by the retina, and which could be disturbed or impaired by retinopathy. This is supported by prior experiments showing that the peripheral retina is vital for the emmetropization of the eye and the damaged peripheral retina could result in high myopia in the eye.^{25–27} Peripheral retinal destruction, such as laser or cryotherapy, could result in steeper cornea, shallower anterior chamber, and thicker lens, and cause higher degree of refractive errors.^{10,11} Destruction to the peripheral retina, such as in cryotherapy, could result in a higher degree of refractive errors than the use of therapy that has less retinal destruction like laser photocoagulation or antivascular endothelial growth factor. It is vital to identify such factors to verify this hypothesis in the future. It is possible that mediation or restoration of such factors could result in better anterior segment growth in these patients. The concept was supported by the results of our study.

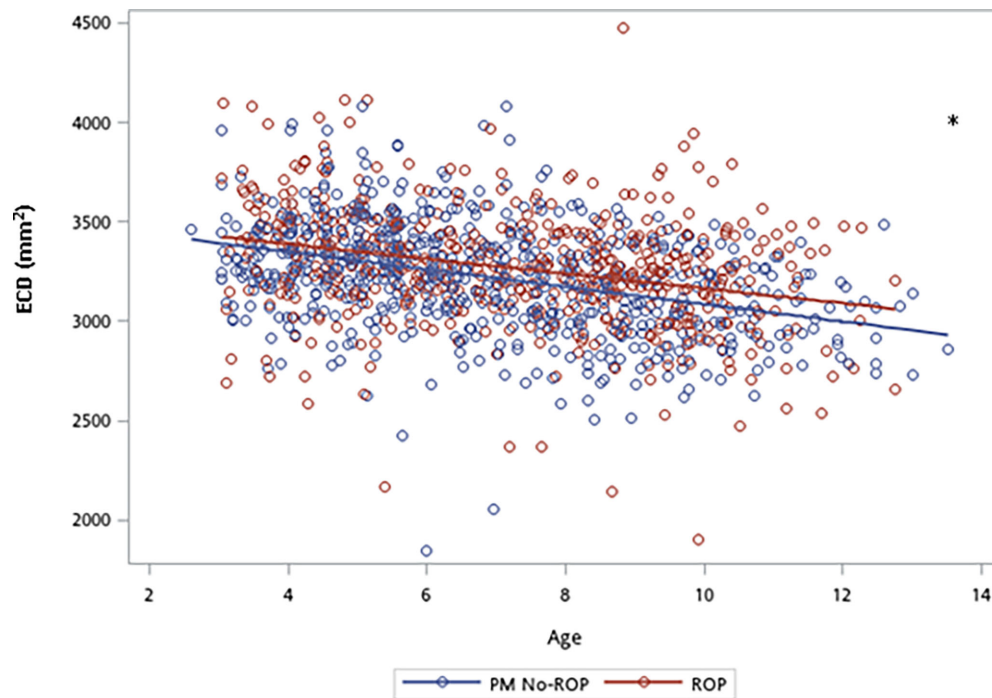


FIGURE 2. The trend of endothelial cell density via age between the two groups. ECD, endothelial cell density. * Denotes significant trend differences between the two groups.

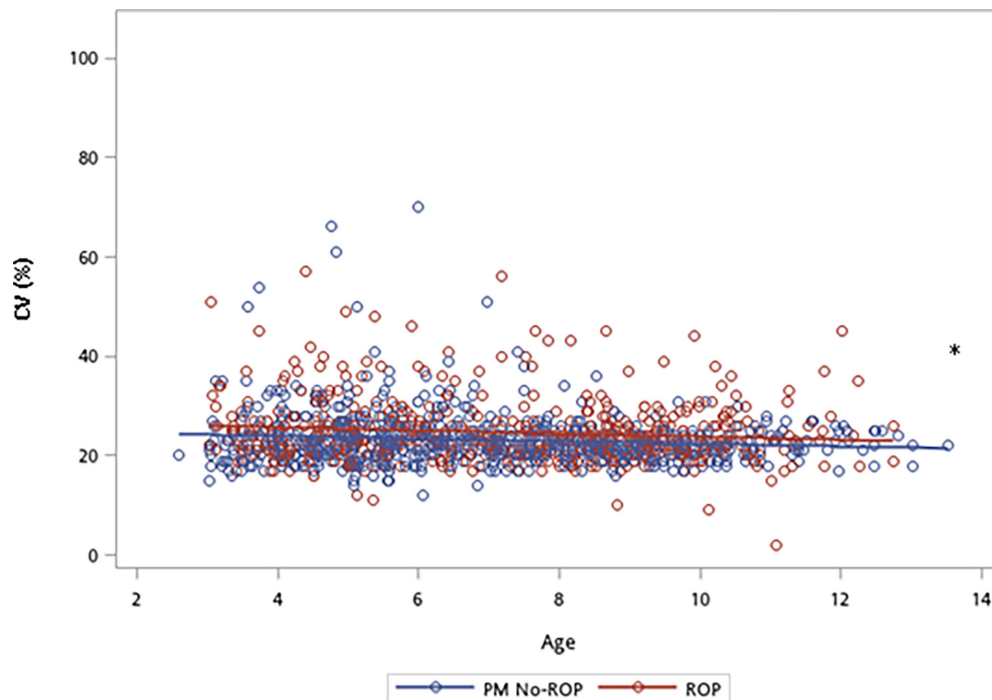


FIGURE 3. The trend of coefficient of variant via age between the two groups. CV, coefficient of variant. * Denotes significant trend differences between the two groups.

In the current study, the presence of ROP did not negatively affect HCECs because the ROP group presented a similar ECD value throughout the 4 years of follow-up after adjusting for several possible risk factors in the multivariable model. A similar ECD in the ROP group was not compatible

with the hypothesis that excessive oxidative stress in patients with ROP leads to corneal endothelial damage.²⁸ One possible explanation for the numerically higher (but not statistically significant) ECD in the patients with ROP is that the CD in the ROP group was also numerically lower than the partic-

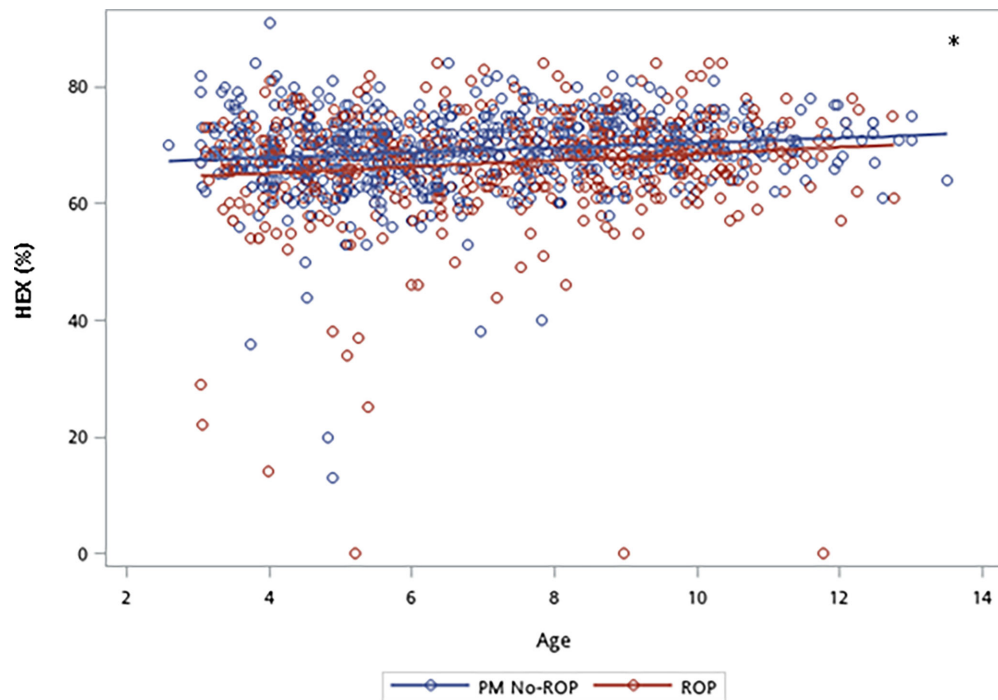


FIGURE 4. The trend of hexagonality via age between the two groups. HEX, hexagonality. * Denotes significant trend differences between the two groups.

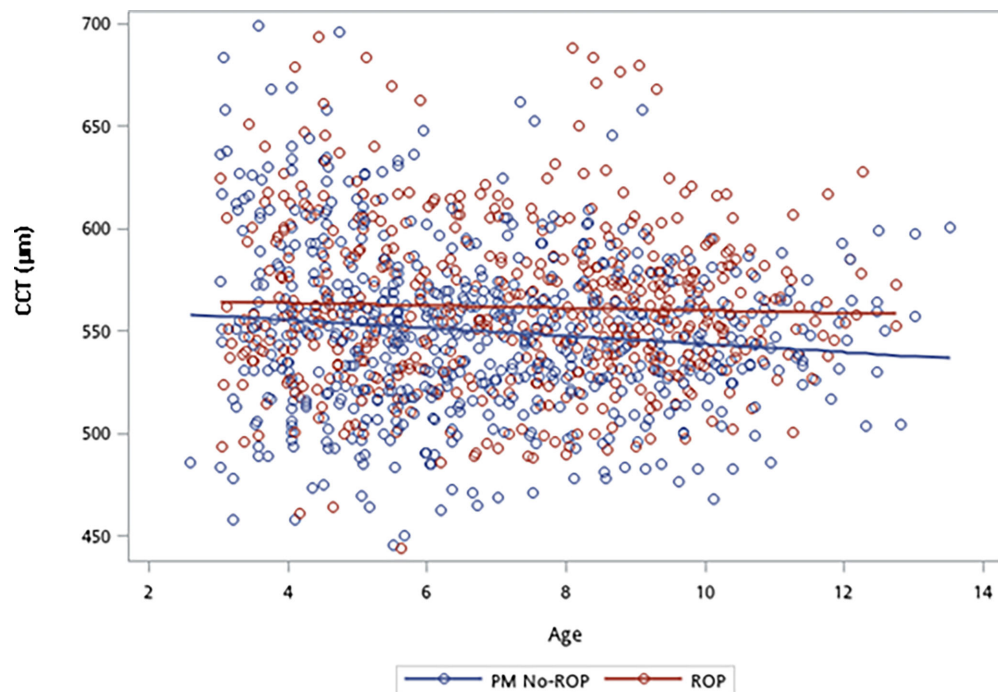


FIGURE 5. Trend of central corneal thickness based on age between the two groups. CCT, central corneal thickness.

ipants in the PM no-ROP group and resulted in a higher ECD value according to a previous study.⁷ Paradoxically, although the growth of HCEC ceases soon after birth due to contact inhibition and TGF- β 2,¹⁷ clusters of small cells with mitosis were observed by specular microscopy in both infant²⁹ and adult³⁰ corneas. It is possible that because the GA of

the ROP group was significantly younger, the HCECs in that group might retain a certain ability of proliferation even after birth due to the more primitive status of the HCECs, resulting in the numerically higher ECD. But the exact mechanism needs further evaluation. About the relation between BW and corneal morphology, a previous study revealed smaller

TABLE 3. Subgroup Analysis Stratified by Gestational Age for the Trend of Corneal Morphology Change Between the Two Groups

Parameters	GA 23 to 28		GA 28 to 31		GA 31 to 33		GA More Than 33	
	Beta ^a	P Value	Beta	P Value	Beta	P Value	Beta	P Value
ECD	−6.58	0.0031 [*]	−8.42	0.0026 [*]	−3.58	0.0826	−14.35	0.0011 [*]
CV	−0.46	0.2715	3.73	0.0180 [*]	8.25	0.0019 [*]	1.86	0.3137
HEX	−3.47	0.0349 [*]	−6.51	0.0024 [*]	0.38	0.0146	1.37	0.4560
CCT	−5.07	0.0375 [*]	1.66	0.4893	−1.59	0.2451	1.01	0.4585

CCT, central corneal thickness; CV, coefficient of variant; ECD, endothelial cell density; GA, gestational age; HEX, hexagonality.

^a The ROP group against the PM no-ROP group, negative value indicated slower decrement or faster increment of parameter in the ROP group than in the PM no-ROP group.

^{*} Denotes significant differences between the two groups.

mean cell area in patients with low BW.⁸ Likewise, our study revealed significant correlation between lower BW and high ECD. The two studies are seemingly compatible because the smaller cell area means more cells could be compacted into a defined area, rendering a higher ECD.⁸

With regard to the trend analyses on the change in ECD over time between the two groups, the ROP group showed a significantly lower decrement rate compared to PM no-ROP population. When we analyzed the ECD change between the two groups, the ECDs of the ROP population were higher in all the visits than the patients in the PM no-ROP, and the initial age in the patients with ROP were significantly older suggesting that patients with ROP had a higher value of ECD compared to the patients in the PM no-ROP group even at an older age. It might also be attributed to the relatively poor proliferation ability and similar oxidative stress in the patients with PM no-ROP compared to the younger patients with ROP, and thus the ECD in the PM no-ROP group decreased at a faster rate throughout the study period. According to the subgroup analysis, all the GA subgroups had a slower ECD decrement in the ROP group, which indicated that the ROP was still independently associated with lower ECD reduction rate after the adjustment of GA. The patients with ROP with GA of 31 to 33 weeks presented an insignificant ECD reduction rate compared to the PM no-ROP group, and the trend of ECD decrement was still numerically lesser in the ROP population than the PM no-ROP population. On the other hand, the parameters that were associated with higher ECD in the ROP group implied a preterm status,^{21,31} which might indicate that the relationship between ROP and higher ECD value was more significant in this population. The ECD decline was fastest within the first 2 years after birth with an annual decrement of 17.73% in full term children.⁷ Children in the current study experienced a milder ECD loss with about 1% to 2% of annual decrement rate because the youngest case in the current study was more than 2 years old. Whether the milder ECD loss in the ROP group simply results from the potential proliferative capability of HCECs or other molecular pathways, such as ROCK, PI3/Akt, Smad2 activation, or interleukin-1 β secretion,^{32–35} needs further investigation.

The other HCEC-associated parameters, including HEX, CV, and CCT, revealed better value in the PM no-ROP group. The HEX was statistically significantly higher in the patients with PM compared with the patients with ROP. One possible explanation for the significantly higher HEX in the PM group is that the arrangement of HCECs in patients with PM might be influenced by the prominently decreasing GA and preterm status like higher rate of myopic status and more than two-folds of strabismus possibility in patients

with ROP compared to the PM-only counterparts.^{22,31} Thus, in our study, the arrangement of HCECs was better in the PM no-ROP group compared to the ROP group. The CV, which indicates polymegathism of HCECs, showed a higher value in the ROP population. This could be suggestive of similar mechanisms mentioned above due to worse HCEC status in patients with ROP. Nevertheless, the trend analyses revealed that the HEX and CV improved with age in the ROP group than the PM no-ROP group, implying the HCEC morphology of patients with ROP refines by time. In addition, the CCT showed a significantly higher value in the ROP group than PM no-ROP group. This pattern is comparable to the previous finding that the CCT was thicker in PM infants with lower GA.³⁶ The change of CCT between different visits was not significant in all patients, which is contradictory to the previous study and might be due to the duration of follow-up.³⁷ The trends of CV, HEX, and CCT change in the ROP group compared to the PM no-ROP group were more significant in the patients with low GA according to the subgroup analysis, and the lower GA and smaller CD was correlated to lower HEX. The above results suggest that ROP might affect the morphology of CV, HEX, and CCT more commonly in preterm patients.

The ocular biometric parameters, including spherical power, SE, and steep K value, demonstrated more myopic status in the children with prior ROP compared to the PM no-ROP group. The myopic shift of the patients with ROP compared to the patients in the PM no-ROP group was similar to that in the previous study that the presence of ROP is an important risk factor for the myopia development.^{22,31,38} Recently, we showed that the ACD and AXL in patients with ROP were shallower and shorter compared to those without ROP.⁶ These findings are similar to our current study results. Previous research also speculated that the ROP can affect the site of ocular development and that impairment of this site can result in abnormality of anterior segment formation.³⁹ With regard to ocular growth, the AXL became significantly longer by time in the whole study population, which is reasonable because the AXL elongates with advancing age.⁴⁰ Similarly, the ACD in the entire study population showed significant increment over time which may due to the normal growth of the globe. However, CD was not positively associated with age, ACD, and AXL in the current study, which was conflicting to previous findings on full term (FT) or PM children.^{41–43} It is possible that the follow-up period in the current study was not long enough to see significant enlargement of CD compared to a previous study with a follow-up of more than 10 years.⁴¹ These findings indicate that the presence of ROP not only influences the retina but also affects the normal ocular

structural development,²¹ thus leading to different biometric parameters.

There are some limitations in our study. First, the different time points of ophthalmic examination at different ages for each patient might decrease the homogeneity of the study population, even though we adjusted for the effect of initial age in the GEE model. Second, the distribution of age was not identical among the study groups and this could influence the intergroup analysis because the ECD is negatively associated with age.⁷ Moreover, the absence of early corneal/biometric parameter measurements during the birth in our patients prevented us from analyzing the early corneal morphology when ROP was active with the difference of corneal morphology between the PM no-ROP and the ROP groups could be more apparent. Last, we did not perform gender matching, thus a slightly higher ratio of male population was present in the current study. However, because the prevalence of ROP is higher in the male subjects,^{44,45} we attributed this gender imbalance to natural distribution of ROP.

In conclusion, the patients with prior ROP illustrate an initially less intact HCECs morphology compared to the patients with PM no-ROP, but the ECD preservation and HCEC morphology improvement were better in these patients, and specifically in the low-GA ROP population. More prominent myopic shifts with steeper K value are observed in the ROP children. Further prospective studies to clarify the impact of prematurity, ROP, and various treatments on the development of corneal and anterior segment disorders is needed.

Acknowledgments

Supported by grants from Chang Gung Memorial Hospital (CMRPG3G0581-3, CMRPG3I0071-3, and CLRPG3D0046) and the Ministry of Science and Technology (MOST 106-2314-B-182A-040-MY3).

Disclosure: **H.-C. Chen**, None; **S.-F. Yang**, None; **C.-Y. Lee**, None; **J.-Y. Huang**, None; **Y.-J. Hsueh**, None; **M.-H. Sun**, None; **M.-C. Chiang**, None; **Y.-S. Huang**, None; **S.-M. Chu**, None; **J.-F. Hsu**, None; **C.-H. Liu**, None; **C.-K. Chang**, None; **K.-J. Chen**, None; **Y.-S. Hwang**, None; **C.-C. Lai**, None; **C.-Y. Huang**, None; **W.-C. Wu**, None

References

- Kim SJ, Port AD, Swan R, Campbell JP, Chan RVP, Chiang MF. Retinopathy of prematurity: a review of risk factors and their clinical significance. *Surv Ophthalmol*. 2018;63:618–637.
- Hartnett ME, Penn JS. Mechanisms and management of retinopathy of prematurity. *N Engl J Med*. 2012;367:2515–2526.
- Tsai AS, Chou HD, Ling XC, et al. Assessment and management of retinopathy of prematurity in the era of anti-vascular endothelial growth factor (VEGF). *Prog Retin Eye Res*. 2022;88:101018.
- Hartnett ME. Pathophysiology and mechanisms of severe retinopathy of prematurity. *Ophthalmology*. 2015;122:200–210.
- Solebo AL, Teoh L, Rahi J. Epidemiology of blindness in children. *Arch Dis Child*. 2017;102:853–857.
- Chang SHL, Lee YS, Wu SC, et al. Anterior chamber angle and anterior segment structure of eyes in children with early stages of retinopathy of prematurity. *Am J Ophthalmol*. 2017;179:46–54.
- Elbaz U, Mireskandari K, Tehrani N, et al. Corneal endothelial cell density in children: normative data from birth to 5 years old. *Am J Ophthalmol*. 2017;173:134–138.
- Kagotani Y, Nomura K, Yamamoto M. Corneal endothelial cells in cicatricial retinopathy of prematurity. *Nippon Ganka Gakkai Zasshi*. 1995;99:178–185.
- Oie Y, Watanabe S, Nishida K. Evaluation of visual quality in patients with Fuchs endothelial corneal dystrophy. *Cornea*. 2016;35(Suppl 1):S55–S58.
- Chou YB, Wang AG, Yang HY, Chen KJ, Yang CS. Refractive status, biometric components, and functional outcomes of patients with threshold retinopathy of prematurity: systemic review and a 17-year longitudinal study. *Graefes Arch Clin Exp Ophthalmol*. 2022;260:3809–3816.
- Özdemir HB, Özdek S. Late sequelae of retinopathy of prematurity in adolescence and adulthood. *Saudi J Ophthalmol*. 2022;36:270–277.
- Choi MY, Park IK, Yu YS. Long term refractive outcome in eyes of preterm infants with and without retinopathy of prematurity: comparison of keratometric value, axial length, anterior chamber depth, and lens thickness. *Br J Ophthalmol*. 2000;84:138–143.
- Wu PY, Chen HC, Hsueh YJ, et al. Corneal topography in preterm children aged 2 years to 12 years with or without retinopathy of prematurity. *Eye (Lond)*. 2023;37:2565–2572.
- Bahn CF, Falls HF, Varley GA, Meyer RF, Edelhauser HF, Bourne WM. Classification of corneal endothelial disorders based on neural crest origin. *Ophthalmology*. 1984;91:558–563.
- Bahn CF, Glassman RM, MacCallum DK, et al. Postnatal development of corneal endothelium. *Invest Ophthalmol Vis Sci*. 1986;27:44–51.
- Nucci P, Brancato R, Mets MB, Shevell SK. Normal endothelial cell density range in childhood. *Arch Ophthalmol*. 1990;108:247–248.
- Joyce NC, Harris DL, Mello DM. Mechanisms of mitotic inhibition in corneal endothelium: contact inhibition and TGF-beta2. *Invest Ophthalmol Vis Sci*. 2002;43:2152–2159.
- Bourne WM, Nelson LR, Hodge DO. Central corneal endothelial cell changes over a ten-year period. *Invest Ophthalmol Vis Sci*. 1997;38:779–782.
- Bourne WM. Biology of the corneal endothelium in health and disease. *Eye (Lond)*. 2003;17:912–918.
- Yunliang S, Yuqiang H, Ying-Peng L, Ming-Zhi Z, Lam DS, Rao SK. Corneal endothelial cell density and morphology in healthy Chinese eyes. *Cornea*. 2007;26:130–132.
- Cook A, White S, Batterbury M, Clark D. Ocular growth and refractive error development in premature infants with or without retinopathy of prematurity. *Invest Ophthalmol Vis Sci*. 2008;49:5199–5207.
- Al Oum M, Donati S, Cerri L, Agosti M, Azzolini C. Ocular alignment and refraction in preterm children at 1 and 6 years old. *Clin Ophthalmol*. 2014;8:1263–1268.
- Fieß A, Nauen H, Mildenerberger E, et al. Ocular geometry in adults born extremely, very and moderately preterm with and without retinopathy of prematurity: results from the Gutenberg Prematurity Eye Study. *Br J Ophthalmol*. 2023;107:1125–1131.
- Gunay M, Sekeroglu MA, Celik G, Gunay BO, Unlu C, Ovali F. Anterior segment ischemia following diode laser photocoagulation for aggressive posterior retinopathy of prematurity. *Graefes Arch Clin Exp Ophthalmol*. 2015;253:845–848.
- Hartwig A, Charman WN, Radhakrishnan H. Accommodative response to peripheral stimuli in myopes and emmetropes. *Ophthalmic Physiol Opt*. 2011;31:91–99.
- Kaneko Y, Moriyama M, Hirahara S, Ogura Y, Ohno-Matsui K. Areas of nonperfusion in peripheral retina of eyes with pathologic myopia detected by ultra-widefield fluorescein angiography. *Invest Ophthalmol Vis Sci*. 2014;55:1432–1439.

27. Lin Z, Xi X, Wen L, et al. Relative myopic defocus in the superior retina as an indicator of myopia development in children. *Invest Ophthalmol Vis Sci*. 2023;64:16.
28. Čejková J, Čejka Č. The role of oxidative stress in corneal diseases and injuries. *Histol Histopathol*. 2015;30:893–900.
29. Speedwell L, Novakovic P, Sherrard ES, Taylor DS. The infant corneal endothelium. *Arch Ophthalmol*. 1988;106:771–775.
30. Laing RA, Neubauer L, Oak SS, Kayne HL, Leibowitz HM. Evidence for mitosis in the adult corneal endothelium. *Ophthalmology*. 1984;91:1129–1134.
31. Chou HD, Yao TC, Huang YS, et al. Myopia in school-aged children with preterm birth: the roles of time spent outdoors and serum vitamin D. *Br J Ophthalmol*. 2021;105:468–472.
32. Hsueh YJ, Chen HC, Wu SE, Wang TK, Chen JK, Ma DH. Lysophosphatidic acid induces YAP-promoted proliferation of human corneal endothelial cells via PI3K and ROCK pathways. *Mol Ther Methods Clin Dev*. 2015;2:15014.
33. Hsueh YJ, Meir YJ, Lai JY, et al. Lysophosphatidic acid improves corneal endothelial density in tissue culture by stimulating stromal secretion of interleukin-1 β . *J Cell Mol Med*. 2020;24:6596–6608.
34. Kim KW, Park SH, Lee SJ, Kim JC. Ribonuclease 5 facilitates corneal endothelial wound healing via activation of PI3-kinase/Akt pathway. *Sci Rep*. 2016;6:31162.
35. Sabater AL, Andreu EJ, García-Guzmán M, et al. Combined PI3K/Akt and Smad2 activation promotes corneal endothelial cell proliferation. *Invest Ophthalmol Vis Sci*. 2017;58:745–754.
36. Liu C, Wu H, Lao J, et al. Measurement and analysis of central corneal thickness at different postnatal stages in Chinese premature infants. *J Ophthalmol*. 2020;2020:7313909.
37. Mwanza JC, Tulenko SE, Budenz DL, et al. Longitudinal change in central corneal thickness in the Tema Eye Survey. *Am J Ophthalmol*. 2018;186:10–18.
38. Filippi L, Cavallaro G, Perciasepe L, et al. Refractive outcome in preterm newborns with ROP after propranolol treatment. A retrospective observational cohort study. *Front Pediatr*. 2019;7:479.
39. Fielder AR, Quinn GE. Myopia of prematurity: nature, nurture, or disease? *Br J Ophthalmol*. 1997;81:2–3.
40. Yin G, Wang YX, Zheng ZY, Yang H, Xu L, Jonas JB. Ocular axial length and its associations in Chinese: the Beijing Eye Study. *PLoS One*. 2012;7:e43172.
41. Jiang WJ, Wu H, Wu JF, et al. Corneal diameter and associated parameters in Chinese children: the Shandong Children Eye Study. *Clin Exp Ophthalmol*. 2017;45:112–119.
42. Ronneburger A, Basarab J, Howland HC. Growth of the cornea from infancy to adolescence. *Ophthalmic Physiol Opt*. 2006;26:80–87.
43. Sehrawat P, Beri S, Garg R, Datta V, Shandil A. Central corneal thickness and corneal diameter in preterm and term newborns and preterm neonates at term. *Indian J Ophthalmol*. 2019;67:1575–1578.
44. Thomas K, Shah PS, Canning R, Harrison A, Lee SK, Dow KE. Retinopathy of prematurity: risk factors and variability in Canadian neonatal intensive care units. *J Neonatal Perinatal Med*. 2015;8:207–214.
45. Ying GS, Quinn GE, Wade KC, Repka MX, Baumritter A, Daniel E. Predictors for the development of referral-warranted retinopathy of prematurity in the telemedicine approaches to evaluating acute-phase retinopathy of prematurity (e-ROP) study. *JAMA Ophthalmol*. 2015;133:304–311.