Changes in Relative Position of Choroidal Versus Retinal Vessels in Preterm Infants

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See the appendix for the members of the Imaging and Informatics in Retinopathy of Prematurity (i-ROP) Research Consortium.

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Purpose. The purpose of this study was to characterize a novel finding that relative positions of choroidal and retinal vessels change over time in preterm infants and to identify factors associated with this finding using quantitative analysis.

Methods. Fundus images were obtained prospectively through a retinopathy of prematurity (ROP) cohort study. Images were excluded if choroidal vessels could not be identified. Changes in relative position of characteristic choroidal landmarks with respect to retinal vessels between two time points 5 to 7 weeks apart were measured. Univariate and multivariate regression analyses were performed to identify associated factors with the amount of change.

Results. The discovery and replication cohorts included 45 and 58 patients, respectively. Ninety-two of them (89%) were non-Hispanic Caucasians. Changes in relative position of choroidal versus retinal vessels were detected in all eyes of the discovery and replication cohorts (mean amount = 0.42 ± 0.12 and 0.35 ± 0.12 mm, respectively). On combined multiple regression analysis of the two cohorts, type 1 ROP, higher postmenstral age at the first time point, and shorter distance from optic disc to choroidal landmark were significantly associated with less change in relative position.

Conclusions. Choroidal vessels grow anteriorly with respect to retinal vessels at posterior pole in preterm infants, suggesting relatively faster peripheral growth of choroidal versus retinal vessels. Eyes with severe ROP showed less difference in growth, which might represent alterations in choroidal development due to advanced ROP. These findings may contribute to better understanding about the physiology of choroidal development and involvement in ROP.

Keywords: choroid, development, premature infant, retinopathy of prematurity

Choroidal development starts as early as the fourth week after conception.[1] After choroidal vessels are connected to posterior ciliary arteries and vortex veins, choroidal arteries and veins become observable after the fifth month of gestation.[1,2] After that, maturation including pigmentation occurs.[2] The area of the choroid increases as the globe size increases during development.

However, little is known about the regional or topographic differences in choroidal vascular development. Several studies have investigated regional differences in retinal development and showed proportionally less growth in the macular region within the rod ring.[3–6] However, regional differences in choroidal development with respect to retinal development are not known. In addition, only a few studies have investigated choroidal development in preterm infants with or without retinopathy of prematurity (ROP). Previous optical coherence tomography (OCT) studies reported choroidal thinning in preterm infants with ROP, and an animal model of ROP has shown central choroidal involution, suggesting possible choroidal involvement in ROP.[7–9]

From serial wide-field fundus images for ROP screening, we observed that the relative positions of choroidal and retinal vessels change over time in preterm infants. In the representative figure, choroidal branch points move outward with respect to retinal vessels during the screening period for ROP (Fig. 1). This study aims to describe and characterize this novel finding and to identify factors associated with the finding using quantitative analysis.

Methods

This study is a secondary analysis of prospectively collected data as part of the Imaging and Informatics for ROP (i-ROP) study. This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional

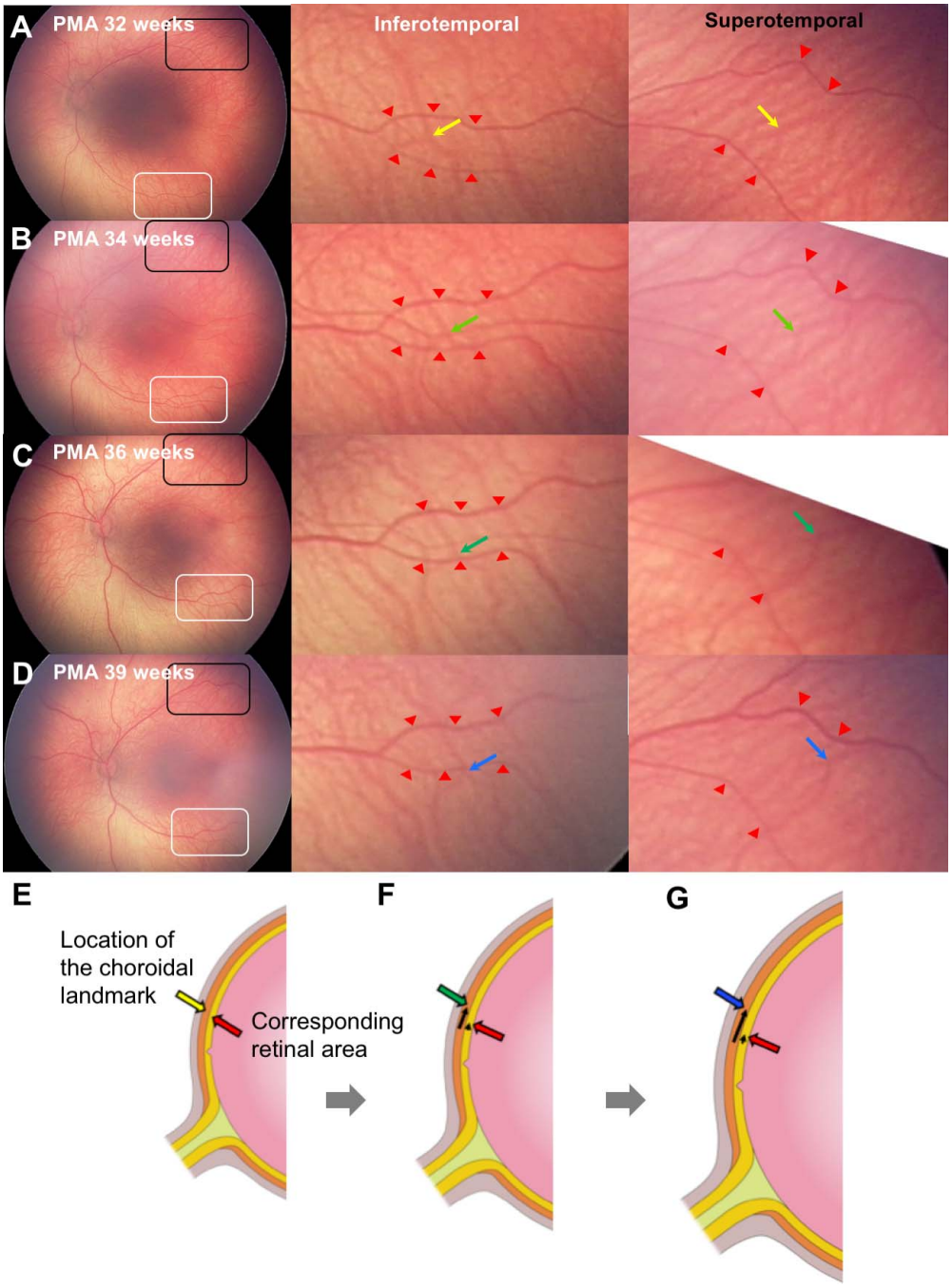

Figure 1. (**A–D**) Representative serial fundus photographs showing that relative positions of the choroidal (*yellow, green*, and *blue arrows*) and retinal vessels (*red arrowheads*) change over time in a preterm infant. Note that the locations of both inferotemporal and superotemporal landmarks move toward periphery with respect to retinal vessels. (**E–G**) Illustrated cross-sectional images of eyeballs showing changes in relative positions of the choroidal landmark (*yellow, green*, and *blue arrows*) and retinal vessels (*red arrows*) in growing eyes of preterm infants.

Review Board at the coordinating center (Oregon Health & Science University) and at each of study centers. Written informed consent for the study was obtained from parents of infants.

Image Capture and Selection

We included consecutive patients with wide-angle fundus images (RetCam; Natus, Pleasanton, CA, USA) using a 130° field-of-view lens obtained for ROP screening from July 1, 2011 to December 31, 2016. Preterm infants in the discovery cohort were enrolled from Oregon Health & Science University, and those in the replication cohort were from William Beaumont Hospital, Columbia University, and Weill Cornell Medical College. Inclusion criteria were as follows: eyes with fundus images from two sessions 5 to 7 weeks apart and fundus images with quality sufficient to enable visualization of choroidal and retinal vessels. If images from both eyes of an infant were eligible, only images of right eyes were included to minimize bias. Data on demographic characteristics including sex, race/ethnicity, gestational age (GA), and birth weight (BW) and classification of ROP were collected from included

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subjects. ROP was classified according to the revised International Classification of Retinopathy of Prematurity.[10] The screening for ROP followed the recommendations proposed by the American Academy of Ophthalmology, American Association for Pediatric Ophthalmology and Strabismus, and the American Academy of Pediatrics.[11,12] A reference standard diagnosis was assigned to each of the images based on the consensus image-based diagnosis of three independent trained graders and the clinical diagnosis at each study center as previously described.[13] Data on classification of ROP included highest stage, lowest zone, plus disease (plus, pre-plus, or neither), and most severe category based on the Early Treatment for Retinopathy of Prematurity trial (type 1 or worse ROP, type 2 ROP or pre-plus disease, mild ROP defined as ROP less than type 2 ROP, and no ROP).[14]

Image Analysis

In each eye, two posterior retinal images from different time points 5 to 7 weeks apart were selected and analyzed (Fig. 2). On the image from the first time point, a characteristic landmark of choroidal vessels (e.g., vascular branch point) was identified and established for comparison with the follow-up examination. This landmark was selected by one author (SJK) to be near the inferotemporal or superotemporal major vascular arcades, where choroidal vessels were typically the most clearly visible. Qualitative assessment was performed comparing the relative positions of choroidal and retinal vessels between the images from the first time point and the second time point to assess whether the relative positions have changed (Fig. 1). Quantitative analysis was also performed to measure the distance of relative movement of the choroidal landmark between the two images (Fig. 2). First, after making the second image partially transparent, the two images were overlapped using image manipulation software (PowerPoint 2013; Microsoft, Redmond, WA, USA). Then, the location of the selected landmark of the choroidal vessels was marked on both images. A line was drawn between the corresponding locations from the two time points, and the distance was measured in pixels. The pixels were converted to millimeters with a conversion ratio of 0.023 mm per pixel. This conversion ratio from pixel to millimeters was calculated as follows: the mean vertical optic disc diameter on RetCam photographs at the first time point in all 45 study eyes of the discovery cohort measured with ImageJ (National Institutes of Health, Bethesda, MD, USA) was 53.9 ± 5.85 pixels. In an OCT study in preterm infants, the mean vertical disc diameter was 1.254 ± 0.193 mm at postmenstrual age (PMA) of 31 to 36 weeks.[15] Thus, the conversion ratio was estimated to be 0.023 mm/pixel (=1.254/53.9).

Data Analysis

The categorical data were analyzed using the χ^2 test or Fisher's exact test; *t*-tests were used to compare the mean distances of the two groups, and Pearson correlation tests were performed to examine the relationships of independent variables with the distance of relative movement of the choroidal landmarks. Specific independent variables examined were BW, GA, PMA at the first time point, and distance from optic disc to choroidal landmark. Univariate and multivariate analyses were performed to identify factors associated with the amount of change in relative vascular positions in the discovery and replication cohort and combined dataset of the two cohorts. Specific factors examined were BW, GA, plus disease, lowest zone, highest stage, ROP severity category, type 1 ROP, PMA at the first time point, interval between the two time points, and distance from optic disc to choroidal landmark. Data analysis was performed using IBM SPSS statistics, Version 24 (IBM, Armonk, NY, USA).

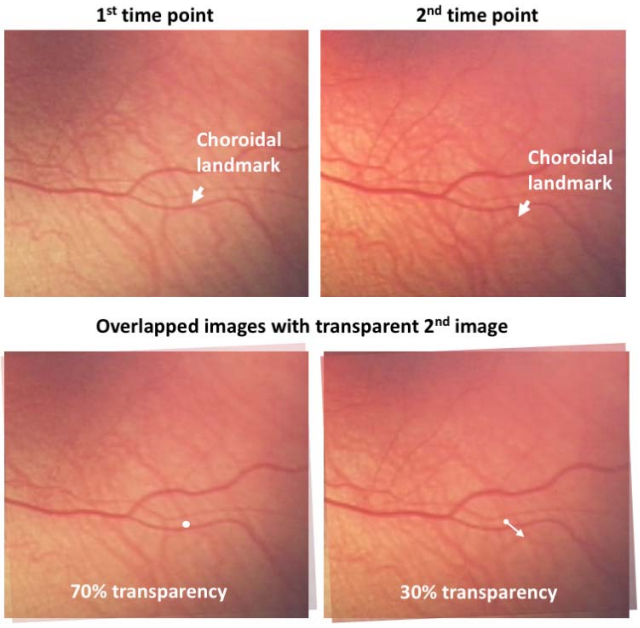

Figure 2. Quantitative analysis to measure the distance of relative movement of the choroidal landmark (branch point) between the two images from sessions about 6 weeks apart. After making the second image partially transparent and overlapping the two images using image processing software, a line was drawn between the two locations of the choroidal landmark and the distance was measured as pixels.

Results

Discovery Cohort

In the discovery cohort, 45 eyes from 45 preterm infants were analyzed. Demographic and ocular characteristics are shown in Table 1. The mean GA of infants included in this study was 26.2 ± 1.4 weeks, ranging from 24 to 30 weeks. Nine of the 45 infants (20%) showed zone I disease, and 13 of 45 infants (29%) were diagnosed as having type 1 ROP.

In all 45 eyes, changes in relative position of the choroidal vessels versus the retinal vessels were observed when comparing two serial images, of which the mean interval between the capture of the two images was 6.1 ± 0.6 weeks (Table 2). The representative retinal images are shown in Figure 1 (type 2 ROP) and Supplementary Figures S1 (mild ROP) and S2 (type 1 ROP). The distance of relative movement of the choroidal landmarks between the two time points was 0.42 ± 0.12 mm, and the choroidal vessels moved further than the retinal vessels in each case. The choroidal landmarks for measurement of the distance were located near the inferotemporal (58%) or superotemporal (42%) major vascular arcades (Table 2). Eyes with type 1 ROP showed a lower distance of relative movement than those that had less than type 1 ROP ($P = 0.016$ by *t*-test). The PMA at the first time point showed a moderate negative correlation with the distance of relative movement ($R = -0.49$, $P = 0.001$ by Pearson correlation test; Fig. 3). However, BW, GA, and distance from optic disc to choroidal landmark on the initial image were not significantly associated with the distance of relative movement ($P = 0.409$, 0.498, and 0.059, respectively, by Pearson correlation test; Fig. 3).

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Table 1. Demographic and Clinical Characteristics of Preterm Infants Undergoing Screening Examinations for ROP in the Discovery and Replication Cohort

Parameters	Discovery Cohort	Replication Cohort	*P* Value*
No. of infants	45	58	NA
Gestational age (wk), mean ± SD (range)	26.2 ± 1.4 (24.0 to 30.0)	26.2 ± 2.0 (23.0 to 32.6)	0.857
Birth weight (g), mean ± SD (range)	854.4 ± 261.2 (350 to 1586)	785.5 ± 197.3 (398 to 1307)	0.145
Sex, male (%)	24 (53%)	27 (47%)	0.495
Race/ethnicity (%)			0.340
Non-Hispanic white	42 (93%)	50 (86%)	
Other	3 (7%)	8 (14%)	
Lowest zone, no. of eyes (%)			0.270
Zone I	9 (20%)	7 (12%)	
Zone II or III	36 (80%)	51 (88%)	
Highest stage of ROP, no. of eyes (%)			0.081
Stage 0	4 (9%)	14 (24%)	
Stage 1	11 (24%)	8 (14%)	
Stage 2	19 (42%)	17 (29%)	
Stage 3	11 (24%)	19 (33%)	
Plus disease, no. of eyes (%)			
Plus	13 (29%)	15 (26%)	0.732
No plus	32 (71%)	43 (74%)	
Pre-plus	7 (16%)	17 (29%)	
Normal	25 (56%)	26 (45%)	
Severity category, no. of eyes (%)			
Type 1	13 (29%)	15 (26%)	0.732
Non–type 1	32 (71%)	43 (74%)	
Type 2 or pre-plus	9 (20%)	19 (33%)	
Mild (stage 1 or 2)	19 (42%)	11 (19%)	
No ROP	4 (9%)	13 (22%)	

* Categorical data were analyzed using the χ^2 test or Fisher's exact test, and numerical data were analyzed using *t*-test. NA, not applicable.

Replication Cohort

In the replication cohort, 58 eyes from 58 preterm infants were analyzed. Demographic and ocular parameters were not significantly different from those of discovery cohort (Table 1).

Changes in relative position of the choroidal vessels versus the retinal vessels were observed in all 58 eyes (Table 2). The time of measurements was significantly later and the distance from optic disc to choroidal landmark was significantly shorter than those of discovery cohort (Table 2). The distance of relative movement of the choroidal landmarks between the two time points was significantly shorter than that of discovery cohort (0.42 ± 0.17 versus 0.35 ± 0.12 mm, $P = 0.005$ by *t*-test; Table 2).

Eyes with type 1 ROP showed a lower distance of relative movement than those that had less than type 1 ROP ($P < 0.001$ by *t*-test; Supplementary Fig. S3). BW, GA, and distance from optic disc to choroidal landmark showed a weak positive correlation with the distance ($R = 0.39$, 0.43, and 0.32, respectively; *P* value by Pearson correlation test = 0.003, 0.001, and 0.014, respectively; Supplementary Fig. S3). PMA at the first time point showed a weak negative correlation with the distance ($R = -0.36$, $P = 0.005$ by Pearson correlation test; Supplementary Fig. S3).

Regression Analysis

Univariate linear regression analysis showed that plus disease, severity of the category of ROP, type 1 ROP, and PMA at the first

Table 2. Quantification of Changes in Relative Position of Choroidal Versus Retinal Vessels in Preterm Infants of the Discovery and Replication Cohort

Parameters	Discovery Cohort (45 Eyes)	Replication Cohort (58 Eyes)	*P* Value*
Eyes showing changes in relative position of choroidal versus retinal vessels (%)	45 (100%)	58 (100%)	NA
Location of choroidal landmark, superotemporal versus inferotemporal (%)	19 (42%) vs. 26 (58%)†	26 (45%) vs. 32 (55%)†	0.791
PMA at the first time point (wk), mean ± SD	32.6 ± 1.4	33.7 ± 2.2	0.002‡
PMA at the second time point (wk), mean ± SD	38.7 ± 1.4	39.6 ± 2.4	0.022‡
Interval between the two time points (wk), mean ± SD	6.1 ± 0.6	5.8 ± 0.7	0.052
Distance from optic disc to choroidal landmark (mm), mean ± SD	5.81 ± 1.20	5.12 ± 1.27	<0.001‡
Distance of relative movement of the choroidal landmark (mm), mean ± SD (range)	0.42 ± 0.17 (0.18 to 0.62)	0.35 ± 0.12 (0.13 to 0.65)	0.005‡

NA, not applicable.
* The categorical data were analyzed using the χ^2 test, and numerical data were analyzed using *t*-test.
† The proportion of superotemporal versus inferotemporal location was not statistically different.
‡ Statistically significant ($P < 0.05$) between the two cohorts by *t*-test.

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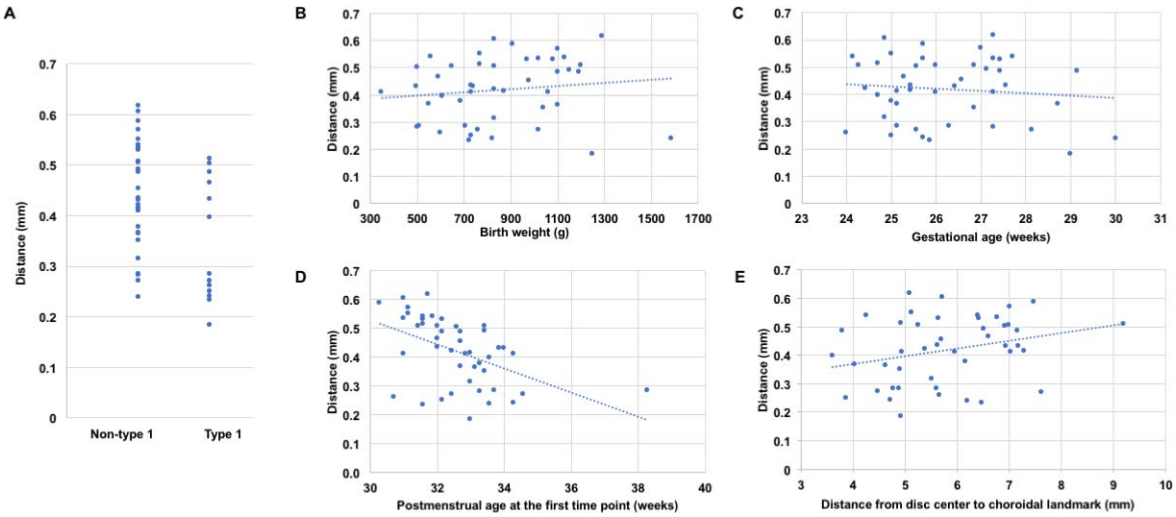

Figure 3. Relative moving distance according to type 1 ROP (**A**), birth weight (**B**), gestational age (**C**), PMA at the first time point (**D**), and distance from optic disc to choroidal landmark (**E**) in discovery cohort. Compared with eyes with non-type 1 ROP, eyes with type 1 ROP showed lower distance ($P = 0.016$ by *t*-test), and PMA at the first time point showed a moderate negative correlation with the distance ($R = -0.49$, $P = 0.001$ by Pearson correlation test).

time point were significantly associated with the distance of relative movement of the choroidal landmarks in the discovery cohort (Table 3). In the replication cohort and combined dataset of the two cohorts, GA, BW, lowest zone, and disc to choroidal landmark distance were also significantly associated with the distance in addition to the significantly associated variables in discovery cohort (Table 3).

Multiple linear regression analysis showed that type 1 ROP and higher PMA at the first time point were significantly associated with a smaller distance in the discovery cohort (Table 4). In the replication cohort, type 1 ROP and shorter disc to choroidal landmark distance were significantly associated with a smaller distance, and higher PMA at the first time point was associated with a smaller distance with borderline significance ($P = 0.053$; Table 4). On combined multiple regression analysis of the two cohorts, type 1 ROP, higher PMA at the first time point, and shorter disc to choroidal landmark distance were significantly associated with a smaller distance.

Discussion

This study aimed to determine whether the relative position of the choroidal vessels and retinal vessels change over time in preterm infants and to identify factors associated with any changes by using quantitative analysis. The key findings from this study are as follows: (1) longitudinal changes in the relative position of the choroidal vessels and retinal vessels were observed at the posterior pole of preterm infants, suggesting relatively faster peripheral growth of choroidal vessels with respect to retinal vessels at the posterior pole in these infants; (2) a negative correlation between changes in the relative position of the choroidal vessels versus the retinal vessels and earlier PMA was found, suggesting that the rate of anterior choroidal growth in preterm infants appears to slow over time; and (3) eyes with type 1 ROP showed significantly less change in the relative position of the choroidal vessels, which may represent compromised or delayed choroidal development in eyes with severe ROP.

Table 3. Univariate Linear Regression Analysis for the Distance of Relative Movement of the Choroidal Landmark in the Discovery and Replication Cohort of Preterm Infants Undergoing Screening Examinations for ROP

	Discovery Cohort (45 Eyes)			Replication Cohort (58 Eyes)			Combined Data (103 Eyes)		
Independent Variables	**Coefficient**	**95% CI**	***P***	**Coefficient**	**95% CI**	***P***	**Coefficient**	**95% CI**	***P***
Gestational age	−0.104	−0.033, 0.016	0.498	0.430	0.011, 0.040	0.001*	0.241	0.003, 0.030	0.014*
Birth weight	0.000	−0.000, 0.000	0.409	0.388	0.000, 0.000	0.003*	0.284	0.000, 0.000	0.004*
Plus disease	−0.048	−0.085, −0.011	0.013*	−0.554	−0.114, −0.049	<0.001*	−0.464	−0.093, −0.042	<0.001*
Lowest zone	0.045	−0.043, 0.132	0.308	0.413	0.063, 0.243	0.001*	0.245	0.018, 0.148	0.012*
Highest stage	−0.038	−0.075, 0.000	0.049*	−0.354	−0.063, −0.011	0.006*	−0.303	−0.057, −0.013	0.002*
ROP severity category	−0.042	−0.075, −0.008	0.016*	−0.528	−0.083, −0.033	<0.001*	−0.432	−0.072, −0.030	<0.001*
Type 1 ROP	−0.100	−0.172, −0.029	0.007*	−0.536	−0.210, −0.085	<0.001*	−0.447	−0.172, −0.075	<0.001*
PMA at the first time point	−0.042	−0.065, −0.019	0.001*	−0.361	−0.034, −0.006	0.005*	−0.450	−0.041, −0.018	<0.001*
Interval between the two time points	−0.008	−0.069, 0.053	0.791	−0.094	−0.059, 0.028	0.482	−0.018	−0.039, 0.032	0.855
Disc to choroidal landmark distance	0.284	−0.001, 0.056	0.059	0.321	0.007, 0.055	0.014*	0.358	0.017, 0.052	<0.001*

CI, confidence interval.
* Statistically significant ($P < 0.05$).

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Table 4. Multiple Linear Regression Analysis for the Distance of Relative Movement of the Choroidal Landmark in the Discovery and Replication Cohort of Preterm Infants Undergoing Screening Examinations for ROP

	Discovery Cohort (45 Eyes)			Replication Cohort (58 Eyes)			Combined Data (103 Eyes)		
Independent Variables	**Coefficient**	**95% CI**	***P***	**Coefficient**	**95% CI**	***P***	**Coefficient**	**95% CI**	***P***
Gestational age	−0.287	−0.053, 0.006	0.119	0.192	−0.010, 0.033	0.281	0.071	−0.011, 0.021	0.539
Birth weight	0.171	0.000, 0.000	0.352	−0.044	0.000, 0.000	0.783	−0.019	0.000, 0.000	0.866
Type 1 ROP	−0.392	−0.163, −0.036	0.003*	−0.385	−0.185, −0.027	0.009*	−0.373	−0.152, −0.054	<0.001*
PMA at the first time point	−0.431	−0.059, −0.015	0.002*	−0.253	−0.029, 0.000	0.053	−0.357	−0.034, −0.012	<0.001*
Interval between the two time points	0.003	−0.052, 0.053	0.981	−0.054	−0.047, 0.030	0.647	0.013	−0.026, 0.031	0.764
Disc to choroidal landmark distance	0.190	−0.007, 0.044	0.153	0.283	0.004, 0.050	0.021*	0.259	0.010, 0.041	0.002*

* Statistically significant ($P < 0.05$).

The first key finding is that in the posterior pole, the choroidal vessels move anteriorly more rapidly with respect to the retinal vessels over time. The retina is known to show proportionally less growth at the macular region.[3–6,16,17] Previous studies have shown that the posterior pole of the retina is dimensionally stable during the developmental period and peripheral retinal expansion accounts for most of the retinal growth.[3–6,16,17] A histologic study on human fetal eyes showed that the distance from optic nerve to fovea is stable after fetal week 15.[6] Therefore, findings from this study suggest that changes in relative position of choroidal and retinal vessels are caused by anterior growth of choroidal vasculature. This should be interpreted with caution, because anterior shift of large choroidal vessels shown in this study do not necessarily mean all choroidal tissues such as choriocapillaris and Bruch's membrane move toward periphery. The exact histologic location of relative movement of large choroidal vessels and retinal vessels cannot be determined from this study. However, it is unlikely that the anterior shift occurs between RPE and photoreceptors because RPE moves centripetally in the developmental stage.[3]

The second key finding in our study is that the earlier the measurement was done, the more change in the relative position of the choroidal vessels versus the retinal vessels was observed. This suggests the rate of anterior choroidal growth appears to slow over time in preterm infants at least from approximately 32 to 40 weeks PMA. During this period, axial length is known to continue to increase with stable rate.[18–20] Therefore, our finding also suggests that choroidal growth is not in parallel with eyeball growth during this period.

The third key finding is that eyes with type 1 ROP showed significantly less difference in growth between the choroidal and retinal vasculature. This may indicate less anterior growth of choroidal vasculature in eyes with more severe ROP. Little is known about choroidal development in preterm infants and the effect of ROP. Animal model studies have revealed choroidal vascular involution during the development of ROP, suggesting that ROP does not only affect the inner retina, but also involves the choroid.[9,21] Previous OCT studies have also shown that the choroid is thinner in infants with acute stages of ROP and in older children and young adults with a history of ROP.[7,8,22–24] Together with these findings, there seems to be compromised or delayed choroidal development in severe ROP. ROP is a neurovascular disease accompanying compromised photoreceptor dysfunction.[25] Choroid supplies oxygen and nutrients to the photoreceptors, and dysfunctional choroidal supplies may result in damages on the photoreceptors.[26] Therefore, compromised or delayed choroidal development in eyes with severe ROP might be one of the mechanisms of photoreceptor dysfunction in ROP. This finding also suggests that the movement of choroidal vasculature might be used as a potential prognostic feature for screening ROP. Further studies are warranted to investigate the prognostic value of this finding, the long-term effect of choroidal changes in ROP on visual function, and the mechanisms underlying choroidal growth and development.

The demographic and ocular characteristics were not significantly different between the discovery and replication cohorts. However, there was a difference in the change in the relative position of the choroidal vessels between the discovery and replication cohorts. Less change in relative position in replication cohort may be explained by higher PMA at the measurements and shorter distance from optic disc to choroidal landmark in the replication study. These two parameters were significantly associated with less change in relative position from the multiple regression analysis.

There are several limitations to our study. First, measurement on fundus images may not be an accurate method. There may be quantification errors from differences in axial length, refractive error, and distance between the lens of the camera and cornea. Also, quantification of distance on fundus images in growing eyes is more challenging. Further studies with en face OCT angiography of the choroidal layer may produce more precise measurements for analysis. Second, there may be a selection bias, because only lighter pigmented eyes with high image quality among the eyes enrolled for the i-ROP study were included for this study. Thus, we might not be able to apply the results of this study to a different patient population. Third, the quantitative analysis was performed without masking. The examiner knew the time order of the two images and could diagnose the severity of ROP, which could induce bias. Fourth, axial lengths of enrolled infants were not measured. Although several previous studies have shown changes in axial length in preterm infants with and without ROP, no direct comparison or correlation analysis was possible in our study. Fifth, the moving distance of a choroidal landmark was measured in only one choroidal landmark per eye and all the analyses were done in the temporal posterior pole. This is because we could find only one or two distinct choroidal landmarks at posterior per eye. Selection and segmentation of choroidal vessels on fundus images with longitudinal analysis of choroidal vascular morphology or en face OCT angiography studies may be better to present our findings in the near future.

In conclusion, we found that choroidal vessels grow anteriorly with respect to retinal vessels at posterior pole in preterm infants. The amount of movement showed a negative

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correlation with PMA. In addition, type 1 ROP was associated with less choroidal growth, suggesting compromised or delayed development of choroidal vasculature in eyes with severe ROP. These findings may help us to understand the physiology of choroidal development during the early period in life and the pathophysiology of choroidal involvement in ROP. Further studies are warranted to investigate relationships between these findings and the effect of treatment for ROP, long-term visual and anatomical prognosis and the possible role of evaluating choroidal vasculature as a prognostic biomarker for monitoring ROP.

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References

1. Sellheyer K. Development of the choroid and related structures. *Eye (Lond)*. 1990;4:255–261.
2. Saint-Geniez M, D'Amore PA. Development and pathology of the hyaloid, choroidal and retinal vasculature. *Int J Dev Biol*. 2004;48:1045–1058.
3. Robb RM. Regional changes in retinal pigment epithelial cell density during ocular development. *Invest Ophthalmol Vis Sci*. 1985;26:614–620.
4. De Silva DJ, Cocker KD, Lau G, Clay ST, Fielder AR, Moseley MJ. Optic disk size and optic disk-to-fovea distance in preterm and full-term infants. *Invest Ophthalmol Vis Sci*. 2006;47:4683–4686.
5. Packer O, Hendrickson AE, Curcio CA. Development redistribution of photoreceptors across the *Macaca nemestrina* (pigtail macaque) retina. *J Comp Neurol*. 1990;298:472–493.
6. Hendrickson A. Development of retinal layers in prenatal human retina. *Am J Ophthalmol*. 2016;161:29–35.
7. Moreno TA, O'Connell RV, Chiu SJ, et al. Choroid development and feasibility of choroidal imaging in the preterm and term infants utilizing SD-OCT. *Invest Ophthalmol Vis Sci*. 2013;54:4140–4147.
8. Erol MK, Coban DT, Ozdemir O, Dogan B, Tunay ZO, Bulut M. Choroidal thickness in infants with retinopathy of prematurity. *Retina*. 2016;36:1191–1198.
9. Shao Z, Dorfman AL, Seshadri S, et al. Choroidal involution is a key component of oxygen-induced retinopathy. *Invest Ophthalmol Vis Sci*. 2011;52:6238–6248.
10. International Committee for the Classification of Retinopathy of Prematurity. The International Classification of Retinopathy of Prematurity revisited. *Arch Ophthalmol*. 2005;123:991–999.
11. Fierson WM; American Academy of Pediatrics Section on Ophthalmology; American Academy of Ophthalmology; American Association for Pediatric Ophthalmology and Strabismus; American Association of Certified Orthoptists. Screening examination of premature infants for retinopathy of prematurity. *Pediatrics*. 2013;131:189–195.
12. Section on Ophthalmology American Academy of Pediatrics; American Academy of Ophthalmology; American Association for Pediatric Ophthalmology and Strabismus. Screening examination of premature infants for retinopathy of prematurity. *Pediatrics*. 2006;117:572–576.
13. Ryan MC, Ostmo S, Jonas K, et al. Development and evaluation of reference standards for image-based telemedicine diagnosis and clinical research studies in ophthalmology. *AMIA Annu Symp Proc*. 2014;2014:1902–1910.
14. Early Treatment for Retinopathy of Prematurity Cooperative Group. Revised indications for the treatment of retinopathy of prematurity: results of the early treatment for retinopathy of prematurity randomized trial. *Arch Ophthalmol*. 2003;121:1684–1694.
15. Tong AY, El-Dairi M, Maldonado RS, et al. Evaluation of optic nerve development in preterm and term infants using handheld spectral-domain optical coherence tomography. *Ophthalmology*. 2014;121:1818–1826.
16. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. *J Comp Neurol*. 1990;292:497–523.
17. Hendrickson A, Troilo D, Possin D, Springer A. Development of the neural retina and its vasculature in the marmoset *Callithrix jacchus*. *J Comp Neurol*. 2006;497:270–286.
18. Laws DE, Haslett R, Ashby D, O'Brien C, Clark D. Axial length biometry in infants with retinopathy of prematurity. *Eye (Lond)*. 1994;8:427–430.
19. 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.
20. Ozdemir O, Tunay ZO, Acar DE, Erol MK, Sener E, Acar U. The relationship of birth weight, gestational age, and postmenstrual age with ocular biometry parameters in premature infants. *Arq Bras Oftalmol*. 2015;78:146–149.
21. Zhou TE, Rivera JC, Bhosle VK, et al. Choroidal involution is associated with a progressive degeneration of the outer retinal function in a model of retinopathy of prematurity: early role for IL-1β. *Am J Pathol*. 2016;186:3100–3116.
22. Wu WC, Shih CP, Wang NK, et al. Choroidal thickness in patients with a history of retinopathy of prematurity. *JAMA Ophthalmol*. 2013;131:1451–1458.
23. Park KA, Oh SY. Analysis of spectral-domain optical coherence tomography in preterm children: retinal layer thickness and choroidal thickness profiles. *Invest Ophthalmol Vis Sci*. 2012;53:7201–7207.
24. Anderson MF, Ramasamy B, Lythgoe DT, Clark D. Choroidal thickness in regressed retinopathy of prematurity. *Eye (Lond)*. 2014;28:1461–1468.
25. Hansen RM, Moskowitz A, Akula JD, Fulton AB. The neural retina in retinopathy of prematurity. *Prog Retin Eye Res*. 2017;56:32–57.
26. Nickla DL, Wallman J. The multifunctional choroid. *Prog Retin Eye Res*. 2010;29:144–168.

Appendix

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