Birth Weight Is a Significant Predictor of Retinal Nerve Fiber Layer Thickness at 36 Weeks Postmenstrual Age in Preterm Infants

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• PURPOSE: To assess retinal nerve fiber layer (RNFL) thickness in preterm infants.
• DESIGN: Prospective observational study.
• METHODS: We imaged 83 awake infants (159 eyes) at 36 ± 1 weeks postmenstrual age (defined as the time elapsed between the first day of the last maternal menstrual period and the time at imaging) using a handheld optical coherence tomography (OCT) system at the bedside. Blinded graders semi-automatically segmented RNFL in the papillomacular bundle (−15 to +15° relative to the fovea-optic nerve axis). We correlated RNFL thickness and 7 characteristics of interest (sex, race, ethnicity, gestational age, birth weight, stage of retinopathy at prematurity, and presence of pre-plus or plus disease) via univariable and multivariable regressions.
• RESULTS: RNFL was 3.4 μm thicker in the right eyes than in the left eyes ($P < .001$). Among 7 characteristics, birth weight was the only independent predictor of RNFL thickness ($P < .001$). A 250-g increase in birth weight was associated with 5.2 μm (95% confidence interval: 3.3-7.0) increase in RNFL thickness. Compared with very preterm infants, extremely preterm infants had thinner RNFL (58.0 ± 10.7 μm vs 63.4 ± 10.7 μm, $P = .03$), but the statistical significance disappeared after adjustment for birth weight ($P = .25$). RNFL thickness was 11.2 μm thinner in extremely low birth weight infants than in very low birth weight infants (55.5 ± 8.3 μm vs. 66.7 ± 10.2 μm; $P < .001$). The difference remained statistically significant after adjustment for gestational age.
• CONCLUSION: Birth weight is a significant independent predictor of RNFL thickness near birth, implying that the retinal ganglion cells reserve is affected by intrauterine processes that affect birth weight. (Am J Ophthalmol 2021;222:41–53. © 2020 Elsevier Inc. All rights reserved.)

EXTREMELY PRETERM (EPT; <28 WEEKS' GESTATIONAL age) and very preterm (VPT; between 28 and 32 weeks' gestational age) infants have an approximately 50% risk of major neurodevelopmental disabilities, including impaired cognitive and motor functions, cerebral palsy, hearing loss, and blindness in later life.[1,2] Ocular sequelae of EPT and VPT births include retinopathy of prematurity (ROP), optic atrophy, refractive errors, strabismus, amblyopia, and visual field deficit.[3–8] Compared with VPT infants, EPT infants have an even higher risk of neurodevelopmental and visual impairments.[1,2,6] Early identification of infants with insults to the central nervous system may allow for timely evaluation and interventions.

Quantification of the thickness of the retinal nerve fiber layer (RNFL) based on optical coherence tomography (OCT) provides a noninvasive measure of the axonal mass in the anterior visual pathway. RNFL thickness serves as a biomarker for optic nerve integrity and changes due to congenital or acquired optic neuropathies, including glaucoma, optic neuritis, or others.[9–12] RNFL thickness has also been associated with intraocular factors and diseases (eg, axial length, refractive error, and retinal ischemia),[13–15] intracranial diseases (eg, multiple sclerosis, Alzheimer disease, and Parkinson disease),[16–18] and systemic diseases (eg, type 1 diabetes, Marfan syndrome, and attention deficit hyperactivity disorder in children).[19–21] These findings have demonstrated the potential value of RNFL thickness in the management of adult and pediatric diseases. Recently, several studies found correlations between birth parameters and RNFL thickness in school-age children.[22–31] However, the results of these studies raised significant controversy regarding the correlations. For example, some studies demonstrated significantly thinner RNFL in children born at a younger gestational age,[24,29] whereas other studies proposed that gestational age was not an independent predictor of RNFL thickness.[22,27,28,30,31] Instead, the latter attributed thinner RNFL thickness in infants of younger gestational age to their

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lower birth weight[30,31] or to a history of severe ROP stage.[22,27,28] Because these studies included only school-age children, it is unknown whether the previous findings were confounded by later neurodevelopment. As the eye of a child grows, the measured RNFL thickness may change as well.[32] In addition, other factors (eg, sex, race, ethnicity, eye laterality) may also be related to RNFL thickness based on studies in adults.[33–37] Therefore, a direct measurement of RNFL thickness in infants is essential to better understand the variation in RNFL thickness across individuals, which may assist in the establishment of RNFL thickness as a biomarker for various diseases. This knowledge may also shed light on the pathophysiology of retinal conditions and neurodevelopment in preterm infants.

The recent advancement of portable, handheld OCT systems allows for noncontact, bedside imaging of RNFL in awake infants, which allowed studies on RNFL thickness in premature infants.[32,38–40] In our pilot study, which compared VPT with term infants, OCT imaging at term-equivalent age identified thinner RNFL in the papillomacular bundle (PMB) and temporal quadrant in VPT infants.[38] We also found that the thinner RNFL in these infants was associated with lower scores of cognitive and motor functions at 18-24 months corrected age.[38] Patel and associates also demonstrated the feasibility of handheld OCT imaging of the optic nerve head in term infants and children between 1 day and 13 years.[32] However, both previous studies only included a small number of infants, and these infants were imaged within a wide age window. Also, to the best of our knowledge, no previous studies reported RNFL thickness in EPT or extremely low birth weight (ELBW) infants who are the most vulnerable cohort for neurodevelopmental and visual impairments.[1,2,6]

To investigate the retinal microanatomy in preterm infants, we initiated the prospective observational Study of Eye Imaging in Premature Infants (BabySTEPS) using bedside swept-source OCT systems with custom-built, ultra-compact imaging probes. We report our findings from a cross-sectional analysis of the RNFL thickness in preterm infants imaged at 36 weeks postmenstrual age (PMA, defined as the time elapsed between the first day of the last maternal menstrual period and the time at imaging) before any treatment for ROP. Specifically, we tested the hypothesis that intrauterine processes affect RNFL thickness (and these could be an abnormality of development or injury before birth). We investigated the relationship between RNFL thickness and demographic factors (sex, race, and ethnicity), birth parameters (gestational age and birth weight), and ocular features (ROP stage and the presence of plus disease) in preterm infants. A finding of birth weight as an independent predictor of RNFL thickness would support our hypothesis. In contrast, if birth parameters were not associated with RNFL thickness, the variation in RNFL thickness among preterm infants would likely result from other processes (eg, genetics, postnatal processes, and intraocular factors). We also reported and compared RNFL thickness among EPT, VPT, ELBW, very low birth weight (VLBW), small for gestational age, and not small for gestational age infants.

METHODS

• **STUDY DESIGN AND PARTICIPANTS:** The BabySTEPS (ClinicalTrials.gov identifier: NCT02887157) study was a prospective, single-center, observational study that was prospectively approved by the Duke University Health System Institutional Review Board. We enrolled infants if they were eligible for ROP screening based on the guidelines by American Association of Pediatrics,[41] and the parent or legal guardian provided informed consent to participate in the study. The exclusion criteria were: 1) infants were unable to undergo an eye examination or imaging due to a health or eye condition; and 2) infants had a health condition (other than prematurity) that had a profound impact on brain development (eg, anencephaly, Aicardi syndrome, hydranencephaly, leukodystrophy, and Septo optic dysplasia). Brain hemorrhage or periventricular leukomalacia (PVL) was not considered as an exclusion criterion. Enrollment extended from August 2016 through November 2019. The study adhered to the tenets of the Declaration of Helsinki, Good Clinical Practice, and the Health Insurance Portability and Accountability Act.

The present cross-sectional analysis included infants who had sufficient OCT imaging for the measurement of peripapillary RNFL thickness in the PMB at 36 ± 1 weeks PMA before any ROP treatment (Supplemental Figure 1). We chose the age window to include the maximum number of infants with at least 1 session of OCT imaging before ROP treatment. For infants with multiple OCT imaging sessions during the 2-week window, we selected the session closest to 36 weeks PMA. For infants who had imaging sessions at equal intervals from 36 weeks PMA, we randomly selected 1 session. Among 118 infants enrolled in the BabySTEPS study, 85 infants (169 eyes) had at least 1 OCT imaging session within the PMA window. We reported the macular findings of the 85 infants at the selected sessions within 36 ± 1 weeks PMA in a separate paper,[42] and we used the same selected sessions in the present analysis. One of the 85 infants underwent OCT imaging 1 day outside of our chosen window. Of the 85 infants, 10 eyes from 8 infants did not have a measurement of PMB RNFL thickness at the selected sessions due to poor image quality (9 eyes) or a missing scan in the PMB (1 eye); 1 eye of 1 infant was excluded due to treatment for ROP administered before the selected session. Therefore, the present report included a total of 159 eyes from 83 infants with RNFL thickness measured within 36 ± 1 weeks PMA (5 infants with RNFL thickness only in the right eye and 2 infants with RNFL thickness only in the left eye).

• **STUDY PROCEDURES AND IMAGE ANALYSIS:** Infant health and medical outcome data, including demographics,

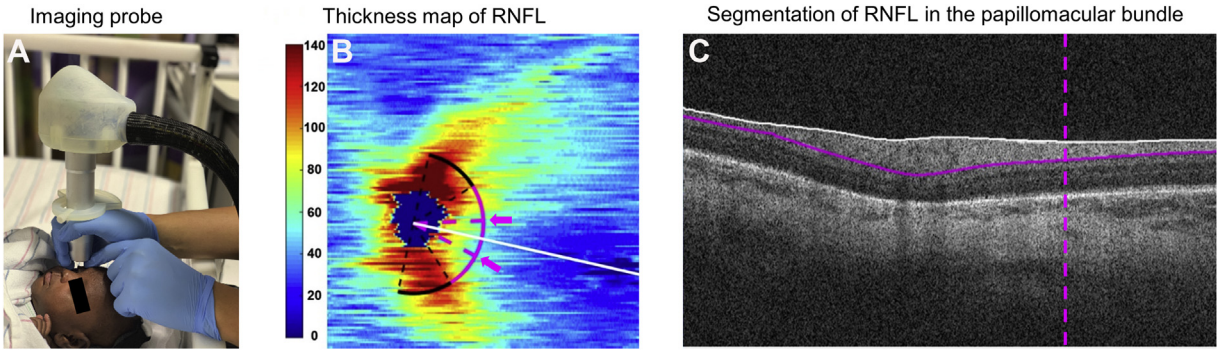

FIGURE 1. Demonstration of the optical coherence tomography imaging probe and segmentation of retinal nerve fiber layer (RNFL). (A) The ultracompact, noncontact, handheld imaging probe used to image an infant at the bedside in the Duke intensive care nursery. (B) Thickness map (in μm) of peripapillary RNFL derived from swept-source optical coherence tomography volumes of an eye of a preterm infant in our cohort. The white line represents the organizing axis from the optic nerve center to the fovea. The pink arc represents both temporal quadrants (arc from −45 to +45° relative to the organizing axis) at 1.5 mm from the optic nerve head center. The arc between 2 dashed pink lines and arrows represents the papillomacular bundle (arc from −15 to +15° relative to the organizing axis). (C) Segmentation of RNFL (between the white and pink solid lines) in the papillomacular bundle (vertical dashed pink line) in an optical coherence tomography b-scan of the same eye.

was extracted from the medical record consistent with data collected for the Generic Database, a registry of clinical information of VLBW infants born alive in Eunice Kennedy Shriver NICHD Neonatal Research Network centers (Generic Database of Very Low Birth Weight Infants [GDB]; ClinicalTrials.gov: NCT00063063). Clinical research coordinators collected the eye examination results of the infants directly from the pediatric ophthalmologists during clinical examinations for ROP. Then, the coordinators entered the eye examination data into the Research Electronic Data Capture software platform.[43,44]

The details of the OCT system and imaging procedure in this study were described in a separate paper.[42] In brief, certified imagers imaged awake infants without a lid speculum using an investigational noncontact, handheld 200-kHz swept-source OCT system at the bedside in the Duke intensive care nursery or Duke Regional Hospital. During the OCT imaging, the certified imager placed the imaging probe over the infant's eye while gently holding the infant's eyelid open with fingertips (Figure 1, A). The right and left eyes were imaged in the same direction. The OCT system had 2 ultracompact (UC), noncontact, handheld probes; we used the UC2 probe (scanning protocol: 6.93- × 6.39-mm scan with 512 A-scans per 112 B-scans at 0°) until October 2, 2018, after which we used the UC3 probe with a wider field of view (scanning protocol: 10 × 10 mm and 13 × 13 mm scans with 1000 A-scans per 256 B-scans at 0°).[45] In 14 imaging sessions, when infants had been transferred to a second hospital, we imaged the infants with a commercial spectral-domain OCT system (Envisu C2300, Leica, Research Triangle Park, North Carolina, USA; 10- × 10-mm volume scans). Our previous study showed excellent repeatability and reproducibility of RNFL thickness measurements based on different handheld OCT systems.[46]

All OCT volumes, which included the PMB, were automatically segmented using proprietary infant-specific software, the Duke OCT Retinal Analysis Program Marking Code Baby version 2.0 (MATLAB R2017b; Mathworks, Natick, Massachusetts, USA). The trained grader (K.P.W.) at the Duke Advanced Research in SS/SD-OCT Imaging Laboratory used the proprietary software to mark the center of the optic nerve and center of the fovea to define the organizing axis from the optic nerve to the fovea. The trained grader reviewed the automated segmentation and corrected errors of RNFL segmentation across an arc above and below the organizing axis at 1.5 mm from the optic nerve head center (Figure 1, B and C).[38,39] The average RNFL thickness was calculated at a radial distance of 1.5 mm from the optic nerve center across the PMB (arc from −15 to +15° relative to the organizing axis) and across both temporal quadrants (arc from −45 to +45° relative to the organizing axis).[38] Average RNFL thickness measurement was required to have a minimum of 90% of the specified arc segmented for inclusion in the analysis.

To assess the repeatability and reproducibility of the RNFL segmentation, we randomly selected 10 OCT volumes (1 volume per infant) that were originally segmented by the primary grader (K.P.W.) for RNFL in the PMB. Then, a secondary grader (D.T.) selected an alternative OCT volume from the same session for each eye. The primary and secondary graders independently segmented RNFL in the PMB in the original and alternative OCT volumes while blinded to the results from each other and the original RNFL segmentation.

• **STATISTICAL ANALYSIS:** Statistical analysis was performed using MATLAB (MathWorks) and R 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

TABLE 1. Characteristics of the Study Cohort

	Total Cohort	Extremely Preterm	Very Preterm
No. of infants	83	36	43
Gestational age (wks)	28.0 ± 2.5	25.6 ± 1.3	29.6 ± 0.98
Age at OCT imaging (wks)	36.1 ± 0.6	35.9 ± 0.6	36.2 ± 0.6
Birth weight (g)	974.4 ± 272.2	775 ± 192.2	1,119.3 ± 224.1
Sex			
Male	41 (49.4)	21 (58.3)	20 (46.5)
Race			
African-American	37 (44.6)	15 (41.7)	21 (48.8)
Asian	5 (6.0)	0 (0)	4 (9.3)
White	38 (45.8)	19 (52.8)	17 (39.5)
Mixed	3 (3.6)	2 (5.6)	1 (2.3)
Ethnicity			
Non-Hispanic	76 (91.6)	34 (94.4)	38 (88.4)
ROP stage at OCT imaging[a]			
Stage 0	44 (53.0)	10 (27.8)	30 (69.8)
Stage 1	15 (18.1)	5 (13.9)	10 (23.3)
Stage 2	22 (26.5)	19 (52.8)	3 (7.0)
Stage 3	2 (2.4)	2 (5.6)	0 (0)
Plus disease at OCT imaging			
None	78 (94.0)	31 (86.1)	43 (100)
Pre-plus or plus disease[b]	5 (6.0)	5 (13.9)	0 (0)
Treatment[c]			
None	75 (90.4)	29 (80.6)	42 (97.7)
Bevacizumab and laser photocoagulation	5 (6.0)	5 (13.9)	0 (0)
Laser photocoagulation	3 (3.6)	2 (5.6)	1 (2.3)

OCT = optical coherence tomography; ROP = retinopathy of prematurity.

Values are mean ± standard deviation and n(%).

[a]Six infants had 1-stage difference in the ROP stage between the right and left eyes. We used the higher ROP stage between the 2 eyes.

[b]All infants in this category had pre-plus or plus disease in both eyes.

[c]For infants who received treatments for ROP, both eyes received the same treatment, and the treatment was administered after the session of OCT imaging.

We reported descriptive statistics in mean ± SD unless otherwise specified. We assessed the intra- and intergrader reproducibility of RNFL thickness via Bland-Altman plots and intraclass correlation coefficients (ICCs). We compared RNFL thickness in the PMB and across both temporal quadrants using a paired *t*-test, and then investigated the intra-eye correlation of RNFL thickness in the PMB and across both temporal quadrants using Pearson correlation coefficients (r). For this analysis, we only included eyes that had RNFL thickness measurements in both regions. To assess the intereye relationship of RNFL thickness in the right and left eyes, we generated Bland-Altman plots and calculated r. Because we found high intra- and intereye correlations of RNFL thickness (predefined as $r \geq 0.7$),[47] we only used and reported the average PMB RNFL thickness of the right and left eyes in our subsequent analyses when data from both eyes of an infant were available.

To determine clinical predictors of RNFL thickness in preterm infants, we first performed univariable linear regression of PMB RNFL thickness with 7 factors of interest (ie, 1 at a time) that were proposed in previous studies in school-age children or adults.[22–31,33–36] The factors of interest included sex (male vs female), race (white vs non-white), ethnicity (Hispanic vs non-Hispanic), gestational age, birth weight, ROP stage, and presence of plus disease (none vs pre-plus or plus disease). We used the ROP stage and presence of plus disease at the selected sessions of OCT imaging. If the ROP stage or presence of plus disease differed between the right and left eyes, we used the data from the worse eye. The associations between RNFL thickness and findings in cranial ultrasound and magnetic resonance imaging will be reported in a separate paper. Then, we entered all significant factors (predefined as $P < .05$ in univariable linear regression) into a multivariable regression model to identify independent predictor(s) of RNFL thickness. We assessed the existence of collinearity among the variables (ie, multicollinearity) in the multivariable model using the variance inflation factor. We predefined a variance inflation factor <5 as an acceptable degree of collinearity.[48]

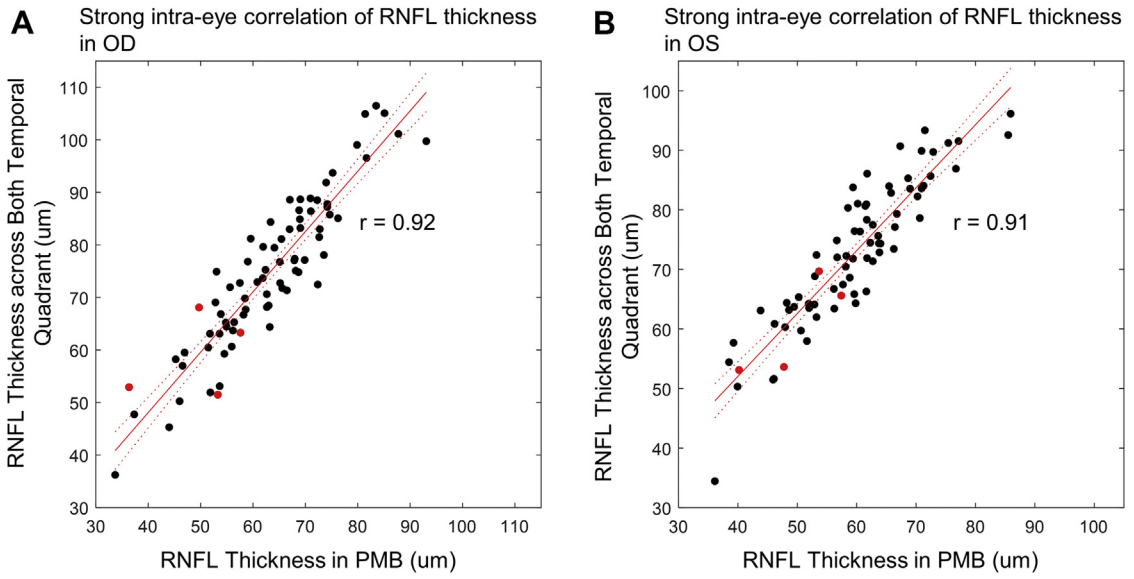

FIGURE 2. Strong intraeye correlation of retinal nerve fiber layer (RNFL) thickness in the papillomacular bundle (PMB) and across both temporal quadrants of (A) the right eyes (n = 78) and (B) the left eyes (n = 75). RNFL thickness across both temporal quadrants was significantly higher than RNFL thickness in the PMB for both right eyes (mean difference: 1.5 μm; 95% confidence interval: 10.1-12.9; $P < .001$) and left eyes (mean difference: 13.1 μm' 95% confidence interval: 14.3-12.0; $P < .001$). The data points of eyes with pre-plus or plus disease (solid red circles) are distributed evenly on both sides of the trendline.

We compared RNFL thickness between the following cohorts using unpaired *t*-tests: 1) EPT (gestational age <28 weeks) versus VPT (gestational age ≥28 weeks and <32 weeks) infants; 2) ELBW (birth <1,000 g) versus VLBW (birth weight ≥1,000 g and <1,500 g) infants; and 3) small for gestational age versus not small for gestational age infants. Small for gestational age was defined as <10th percentile of birth weight for gestational age by sex using a United States national reference for fetal growth.[49]

RESULTS

• **CHARACTERISTICS OF THE STUDY COHORT:** The demographics and clinical characteristics of the included 83 infants are in Table 1. The mean ± SD gestational age was 28.0 ± 2.5 weeks, and the birth weight was 974.4 ± 272.2 g. The PMA at which OCT images were captured was 36.1 ± 0.6 weeks. Among the included 83 infants, 36 (43.4%) infants were EPT, 43 (51.8%) infants were VPT, and 4 (4.8%) infants were born at or after 32 weeks. EPT and VPT infants appeared to have a comparable distribution of sex, race, and ethnicity. However, compared with VPT infants, EPT infants had lower birth weight and a higher percentage of ROP stage 2 (52.8% vs 7.0%). Also, all 5 infants diagnosed with pre-plus or plus disease were EPT.

• **INTRA- AND INTER-GRADER REPRODUCIBILITY OF RNFL THICKNESS:** The reproducibility of RNFL thickness in the PMB was evaluated based on 10 original and 10 alternative OCT volumes of 10 infants. The primary grader (K.P.W.) deemed 1 alternative OCT volume as ungradable and segmented RNFL in the remaining 19 OCT volumes. The secondary grader (D.T.) segmented RNFL in all 20 OCT volumes. Overall, we found excellent intra- and intergrader reproducibility of RNFL thickness (Supplemental Figures 2-4). RNFL thickness in the first and second attempt of segmentation in the original OCT volumes by the primary grader had a mean difference of 0.1 μm (95% limits of agreement: −4.4 to +4.6 μm), with an ICC of 0.98 (Supplemental Figure 2). Similarly, RNFL thickness measured based on the original and alternative OCT volumes were comparable for the primary grader (mean difference: −0.8 μm; 95% limits of agreement: −8.7 to +7.1 μm; ICC: 0.95) (Supplemental Figure 3, A) and the secondary grader (mean difference: 0.0 μm; 95% limits of agreement: −7.8 to +7.8 μm; ICC: 0.95) (Supplemental Figure 3, B). Moreover, RNFL thickness measured by the 2 graders had a mean difference of −0.8 μm based on the original OCT volumes (95% limits of agreement: −7.1 to +5.4 μm; ICC: 0.95) (Supplemental Figure 4, A) and a mean difference of 0.1 μm based on the alternative OCT volumes (95% limits of agreement: −5.4 to +5.5 μm; ICC: 0.98) (Supplemental Figure 4, B).

• **INTRA- AND INTEREYE RELATIONSHIP OF RNFL THICKNESS:** Among 159 eyes from 83 infants with RNFL thickness measurements within 36 ± 1 weeks PMA, all 159

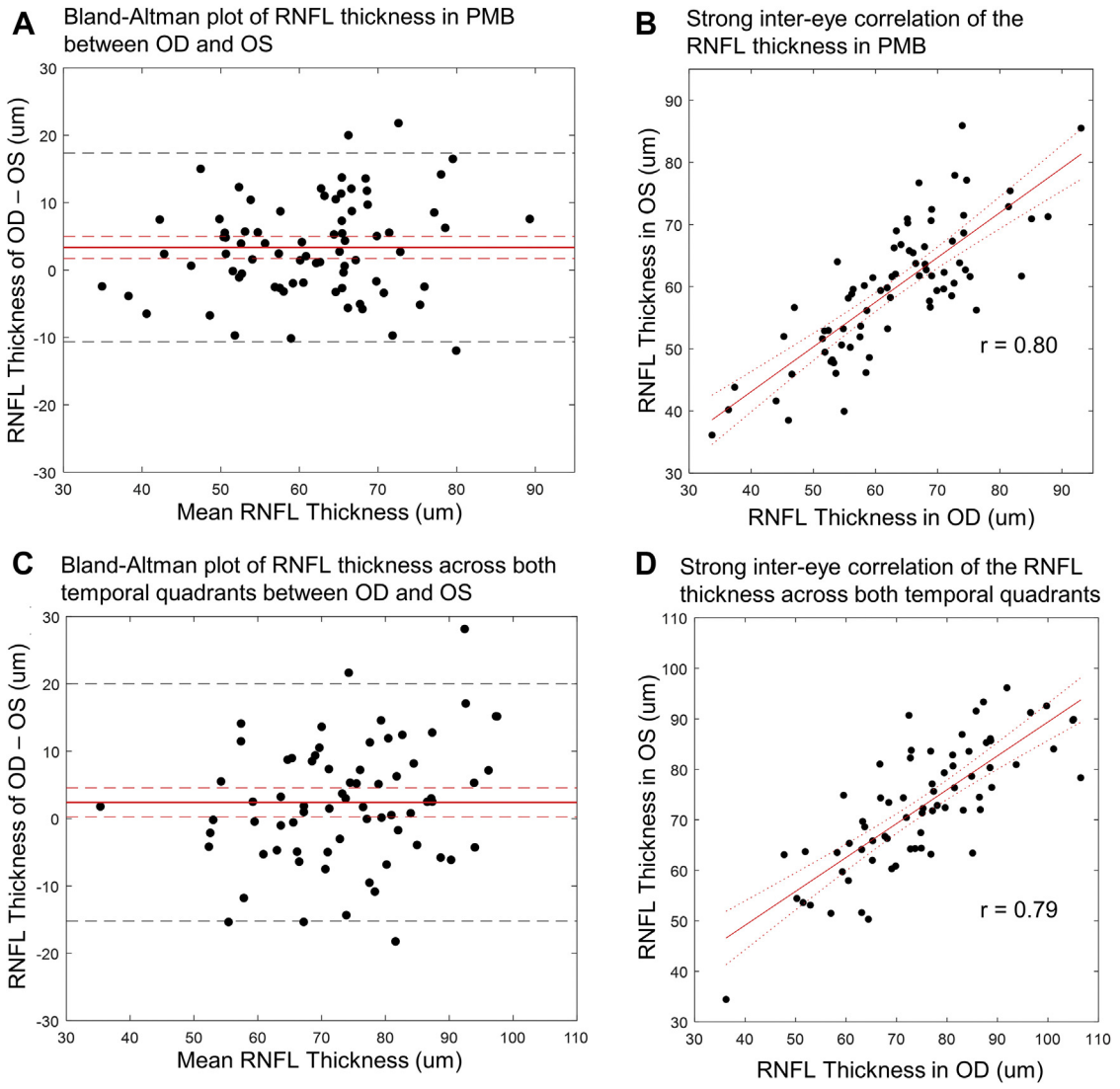

FIGURE 3. Intereye relationship of retinal nerve fiber layer (RNFL) thickness (A and B) in the papillomacular bundle (PMB) (n = 76 infants) and (C and D) across both temporal quadrants (n = 70 infants). (A and C) The solid red line represents the mean difference of RNFL thickness between the right and left eyes (the dashed red lines represent the 95% confidence interval [CI] for the mean). The dashed black lines represent 95% limits of agreement. The RNFL thickness was significantly higher in the right eyes than in the left eyes. The mean difference in the PMB RNFL thickness between the right and left eyes was 3.4 μm (95% CI: 1.7-5.0; $P <$ .001 (A), and the mean difference in RNFL thickness measured across both temporal quadrants was 2.4 μm (95% CI: 0.3-4.6; $P =$ .03 (C). (B and D) RNFL thickness was highly correlated between the right and left eyes (r = 0.80 in the PMB and 0.79 across both temporal quadrants). The dotted lines represent the 95% CI.

eyes had RNFL thickness measurements in the PMB, and 153 eyes had RNFL thickness measurements across both temporal quadrants. RNFL thickness across both temporal quadrants was significantly higher than RNFL thickness in the PMB for both right eyes (mean difference: 11.5 μm; 95% confidence interval [CI]: 10.1-12.9; $P <$ .001) and left eyes (mean difference: 13.1 μm; 95% CI: 14.3-12.0; $P <$ .001). Despite the differences, RNFL thickness in the PMB and across both temporal quadrants was highly correlated (r = 0.92 and 0.91 in the right and left eyes, respectively) (Figure 2, A and B).

RNFL thickness in the right eyes was slightly but significantly higher than RNFL thickness in the left eyes (Figure 3, A and C). The mean difference in the PMB RNFL thickness between right and left eyes was 3.4 μm (95% CI: 1.7-5.0; $P <$ .001), and the mean difference in RNFL thickness measured across both temporal quadrants was 2.4 μm (95% CI: 0.3-4.6; $P =$ .03). RNFL thickness was highly correlated between the right and left eyes (r = 0.80 in the PMB and 0.79 across both temporal quadrants) (Figure 3, B and D). Because of the high intra- and intereye correlations of RNFL thickness, we used and reported the

TABLE 2. Univariable and Multivariable Analysis of the Retinal Nerve Fiber Layer Thickness

	Univariable Analysis			Multivariable Analysis[a]		
Characteristic	Estimate (μm)	95% Confidence Interval (μm)	*P* Value	Estimate (μm)	95% Confidence Interval (μm)	*P* Value
Sex						
Male	2.5	(−2.2 to 7.2)	0.29			
Female	Reference					
Race						
White	−2.0	(−6.7 to 2.7)	0.40			
Non-White	Reference					
Ethnicity						
Non-Hispanic	4.4	(−4.0 to 12.8)	0.30			
Hispanic	Reference					
Gestational age (wks)	1.3	(0.4 to 2.2)	0.007	−1.0	(−2.3 to 0.3)	0.12
Birth weight (250 g)	5.2	(3.3 to 7.0)	< 0.001	6.2	(3.4 to 8.9)	< 0.001
ROP stage	−3.7	(−6.1 to −1.3)	0.003	−0.6	(−3.7 to 2.4)	0.68
Pre-plus or plus disease	−11.5	(−21.1 to −2.0)	0.02	−4.6	(−14.1 to 4.9)	0.34

ROP = retinopathy of prematurity.
[a]Only characteristics with $P < .05$ were included in the multivariable model.

average PMB RNFL thickness of the right and left eyes in our subsequent analyses. The distribution of PMB RNFL thickness of the entire cohort was a mean ± SD of 61.1 ± 10.7 μm and was well approximated by a normal distribution (Supplemental Figure 5). RNFL thickness across both temporal quadrants was 73.2 ± 12.9 μm.

• **ASSOCIATION BETWEEN CLINICAL FACTORS AND RNFL THICKNESS:** Table 2 shows the univariable regression coefficients for sex, race, ethnicity, gestational age, birth weight, ROP stage, and presence of pre-plus or plus disease. We found that RNFL thickness was not significantly associated with sex, race, or ethnicity (*P* value range: .29-.40). However, RNFL thickness was positively correlated with gestational age and birth weight (r = 0.30 and 0.53, respectively) (Figure 4, A and B). Moreover, RNFL thickness was negatively associated with the ROP stage (r = −0.32) (Figure 4, C) and presence of pre-plus or plus disease ($P = .02$) (Table 2). We determined RNFL thickness in ROP stages 0, 1, and 2 as 63.5 ± 9.9 μm, 62.9 ± 11.5 μm, and 56.4 ± 9.9 μm, respectively. RNFL thicknesses in the 2 infants with ROP stage 3 were 38.3 and 57.4 μm, respectively (Figure 4, C).

Multivariable analysis revealed that only birth weight was an independent predictor of RNFL thickness in preterm infants ($P < .001$) (Table 2). After adjustment for birth weight, gestational age, ROP stage, and presence of pre-plus or plus disease were not significantly associated with RNFL thickness in the multivariable model (*P* value range: .12-.68) (Table 2). The variance inflation factor of the multivariable model ranged from 1.28 to 2.48 (<5), indicating an acceptable degree of collinearity between variables.[48] Based on the univariable model (Table 2), we estimated that a 250 g increase in birth weight was associated with a 5.2 μm (95% CI: 3.3-7.0) increase in RNFL thickness.

• **RNFL THICKNESS IN INFANTS WITH DIFFERENT DEGREES OF PREMATURITY:** Compared with VPT infants (n = 43), EPT infants (n = 36) had significantly thinner RNFL (58.0 ± 10.7 μm vs 63.4 ± 10.7 μm; $P = .03$). However, after adjusting for birth weight, the difference was no longer statistically significant ($P = .25$). Using birth weight to classify the degree of prematurity, we divided the study cohort into ELBW (n = 42) and VLBW (n = 39) infants. RNFL thickness was 11.2 μm thinner in ELBW infants than in VLBW infants (55.5 ± 8.3 μm vs 66.7 ± 10.2 μm; $P < .001$). The difference remained statistically significant after adjusting for gestational age ($P < .001$). RNFL thickness in the small for gestational age cohort (n = 20) and in the not small for gestational age cohort (n = 63) was comparable (58.6 ± 9.2 μm vs 61.9 ± 11.1 μm; $P = .22$).

DISCUSSION

USING BEDSIDE SWEPT-SOURCE OCT SYSTEMS WITH CUSTOM-built, ultra-compact imaging probes, we successfully evaluated RNFL thickness in preterm infants at 36 ± 1 weeks PMA before any ROP treatment, with excellent intra- and intergrader reproducibility (ICC: 0.95-0.98). To our knowledge, this was the first study to report RNFL thickness in preterm infants within such a narrow age window (ie, 2 weeks); no previous studies have reported RNFL thicknesses in EPT and ELBW infants who often have the worst neurodevelopmental and visual outcomes. The direct

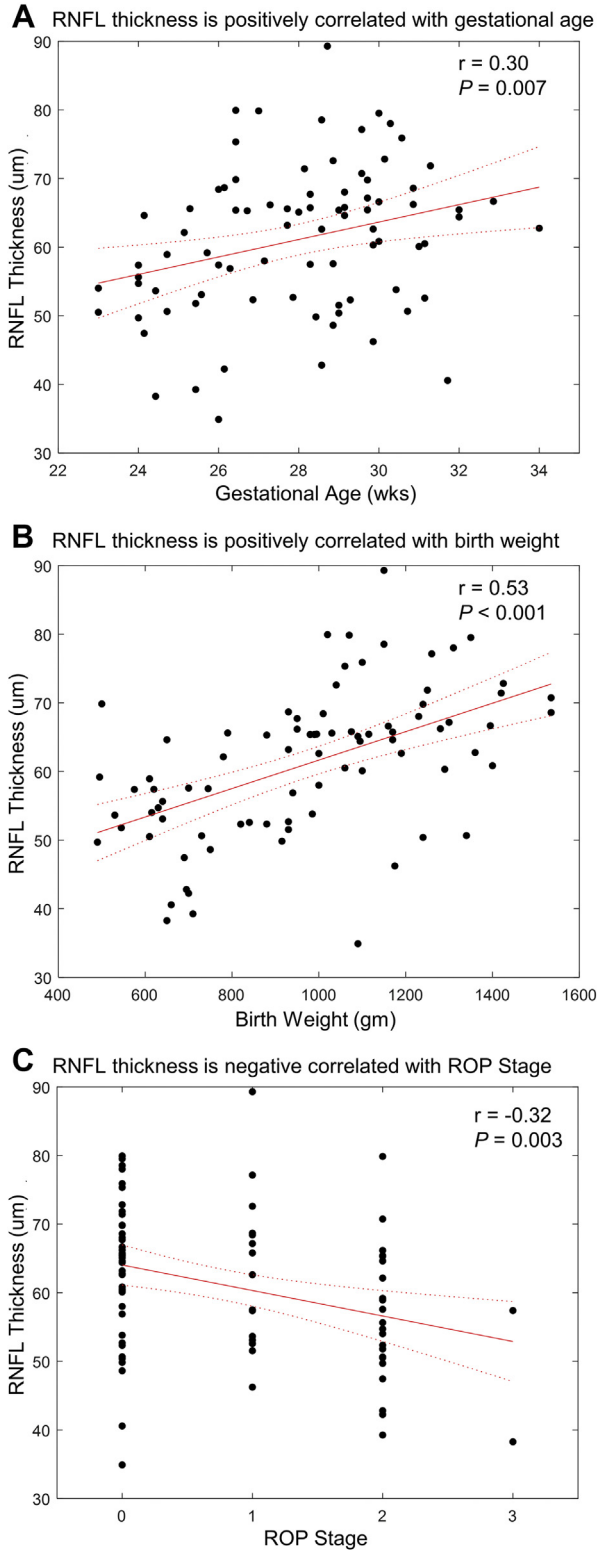

FIGURE 4. Correlation of retinal nerve fiber layer (RNFL) thickness with (A) gestational age, (B) birth weight, and (C) stage of retinopathy of prematurity (ROP) (n = 83 infants). In each scattered plot, the dotted lines represent the 95% confidence interval of the trendline (solid line). Based on the univariate analysis, RNFL thickness was positively correlated with gestational age and birth weight (*P* = .007 and < .001, respectively) (A and B). RNFL thickness was negatively associated with the ROP stage (*P* = .003 (C).

visualization of RNFL in infants allowed us to identify factors related to RNFL thickness near birth without confounding effects from later neurodevelopment. In the present analysis, we found high intra- and intereye correlations of RNFL thickness. Interestingly, RNFL was slightly but significantly thicker in the right eyes than the left eyes, consistent with previous reports in children and adults.[37,50–58] In our cohort, we found that the main factor that influenced RNFL thickness at 36 weeks PMA was birth weight, which implied that RNFL thickness near birth was mostly affected by intrauterine processes. We estimated that a 250 g increase in birth weight was associated with a 5.2 μm (95% CI: 3.3-7.0) increase in RNFL thickness. In contrast, sex, race, ethnicity, gestational age, ROP stage, and presence of plus disease were not significant predictors of RNFL thickness after adjustment for birth weight. We also reported and compared RNFL thicknesses in infants with varying degrees of prematurity. Compared with VPT infants, EPT infants had significantly thinner RNFL thicknesses (mean difference: 5.4 μm; $P = .03$), which was explained by differences in birth weight. Compared with VLBW infants, ELBW infants had largely reduced RNFL thicknesses (mean difference: 11.2 μm; $P < .001$). We did not find a significant difference in RNFL thicknesses between infants who were and were not small for gestational age ($P = .22$).

Several insights can be gained from our analysis. We discovered that RNFL across both temporal quadrants was >10 μm thicker than RNFL in the PMB among preterm infants. This finding was consistent with the RNFL thickness profile in healthy adults,[59] which suggested the physiological RNFL thickness profile might start at birth although the macula is not fully mature until years after birth.[60] The physiologically thinner RNFL in the PMB could be explained by a high concentration of parvocellular cells, which have small cell bodies and thin axons.[61] An alternative explanation for the apparent difference was that the retinal vessels in the vicinity of the peripapillary temporal quadrant might induce OCT artifacts, leading to artificially higher RNFL thickness measurements.[59,62] If this is the case, eyes with retinal vascular dilation (eg, pre-plus or plus disease) will likely have a higher difference in RNFL thickness between the temporal quadrant and PMB. However, our data in Figure 2 did not agree with this hypothesis and showed that the difference was comparable in eyes with and without pre-plus or plus disease. Future studies are needed to measure RNFL thickness in all peripapillary quadrants in infants to generate the entire RNFL thickness profile and correlate with pathological conditions in infancy or late life.

We were surprised to find RNFL thickness measured by OCT was slightly but significantly higher in the right eyes than in the left eyes among preterm infants (Figure 3, A

and C). The reason underlying the intereye difference in RNFL thickness remains unclear. Although we imaged the right and left eyes in the same direction when the infants were positioned horizontally, it was still possible that subtle differences in the way the imaging probe was held might have contributed to the intereye difference in RNFL thickness. Another possibility was that the physiological thickness of temporal RNFL might be slightly different between the right and left eyes in preterm infants. At least 10 previous studies in children and adults also found greater RNFL thicknesses (assessed by OCT) in the right eyes than in the left eyes.[37,50–58] For example, Park and associated reported that temporal peripapillary RNFL thickness in 121 adults (mean age: 43.2 years) was 6.2 μm greater in the right eyes than the left eyes ($P < .001$).[50] Similarly, Altemir and associates found a 3.6 μm thicker temporal RNFL in the right eyes than the left eyes in 357 healthy children (mean age: 9 years; $P < .0005$).[54] Moreover, a recent study performed single cell transcriptome profiling of retinal ganglion cells and found that the composition of retinal ganglion cell subtypes was different between the left and right eyes.[63] Certain retinal ganglion cell subtypes were significantly enriched (up to 3.8-fold) in the right eyes, whereas some other subtypes were enriched in the left eyes.[63] These findings in the intereye difference in RNFL could add to the existing literature on the left−right asymmetry in the development of brain, face, spine, limb, and visceral organs.[64–69] Future studies are needed to understand the mechanism responsible for the intereye difference in RNFL thicknesses and potential implications for neurodevelopment or ocular conditions. In addition, future studies that assess RNFL thickness with OCT should attempt to balance the number of the right and left eyes when comparing different clinical cohorts.

Our analysis showed that among 7 variables of interest, birth weight was the only significant independent predictor of RNFL thickness at 36 weeks PMA in preterm infants. These data suggested a higher birth weight was indicative of a greater reserve of retinal ganglion cells in infants, regardless of the duration of pregnancy and ROP stage. Thus, the reserve of retinal ganglion cells might be related to prenatal factors that affect the infant's birth weight through intrauterine processes. One potential factor is prenatal nutrition. Fetal malnutrition can disturb cellular growth and differentiation in the central nervous system in humans and animals.[70] A history of inadequate prenatal nutrition has been associated with a higher risk of optic nerve hypoplasia in adults.[71,72] For example, Garcia-Filion and associates found a much higher prevalence of low maternal pregnancy weight gain among offspring with optic nerve hypoplasia compared with population data (35% vs 3.7%).[72] Moreover, Lenzi and associates recently showed that a flaxseed-based diet (high in protein and lipids) in the pre- and postnatal period had a favorable influence on optic nerve development of rats, further supporting the potential role of nutrition in optic nerve development.[73] Another prenatal factor that may influence both birth weight and retinal ganglion cells is intrauterine exposure to toxins. Maternal use of recreational drugs, alcohol, or tobacco during pregnancy has been associated with an increased risk of optic nerve hypoplasia in offspring.[71] Also, Ashina and colleagues recently found that maternal smoking during pregnancy was independently associated with thinner RNFL in school-age children ($P < .001$).[23] In addition, maternal conditions, including preeclampsia and gestational diabetes, have also been associated with low birth weight and optic nerve hypoplasia in offspring.[71] The impact of these maternal diseases on RNFL thickness in infants remains unclear. Future studies are needed to investigate the relationship between these prenatal factors and RNFL thickness near birth, which may identify risk factors for suboptimal development of optic nerve and provide intervention opportunities during pregnancy to improve the ocular outcome of premature birth.

Perinatal events may also affect early optic nerve development in preterm infants. For example, PVL has been associated with cupping or small optic disc area in affected patients, possibly depending on the timing of insults,[74–77] which suggests that PVL may cause retrograde transsynaptic degeneration of retinal ganglion cells.[74–77] In our study, 3 infants had PVL, and they had much thinner RNFL thickness at 36 weeks PMA (34.9, 42.8, and 53.6 μm) compared with the rest of the cohort (61.8 ± 10.3 μm), consistent with this hypothesis.

The temporal RNFL was significantly thicker in prematurely born school-age children with a history of more severe versus milder ROP.[22,26–28] Although it is unclear why the thicker temporal RNFL exists, 2 hypotheses have been proposed. First, a pathologic intrauterine process related to the development of ROP may induce temporal RNFL thickening.[27] For example, the relatively vascular-depleted retina during the ROP development causes overproduction of hormones and growth factors, which may affect the development or distribution of RNFL.[27] If this hypothesis is correct, the thicker temporal RNFL should be observed in infants with higher ROP stages shortly after birth. However, we did not find a positive correlation between PMB RNFL thickness and ROP stage before or after adjustment for birth weight (Figure 4, C and Table 2). Similarly, our previous study, which included 57 VPT infants (24 infants with ROP stage 3 or 4) also did not reveal a significant relationship between the ROP stage and RNFL thickness at term-equivalent age.[38] Thus, a second and more plausible hypothesis is that the treatment for ROP or a later developmental process (eg, myopia development) may induce thicker temporal RNFL in later life.[15,22,27,28] For example, previous studies in school-age children demonstrated that, in addition to having a thicker temporal RNFL, children with a history of treated ROP also had a thinner RNFL in the superior and nasal peripapillary quadrants.[22,27,28] As Pueyo and associates proposed, these observations could indicate

ganglion cell axon reorganization to preserve macular area function after axonal injury induced by retinal laser photocoagulation.[28] An alternative explanation for these observations was that infants with severe ROP had an increased risk of developing myopia in later life,[78] and myopia might have induced the distinct RNFL distribution patterns (ie, thicker temporal RNFL and thinner superior and nasal RNFL).[15] To further understand the impact of ROP and ROP treatment on RNFL, future prospective longitudinal studies are needed to monitor RNFL thickness in infants with severe ROP over time.

Most previous studies in children and adults did not report RNFL thickness in the PMB but rather in temporal quadrants. Temporal RNFL thickness in our cohort at 36 weeks PMA (73.2 ± 12.9 μm) appeared comparable with multiple previous reports in children and adults with a history of prematurity (eg, 73.8 μm in children at approximately 13 years old,[31] and 67.7 and 70.6 μm in adults at 56.1 years[79]).[22,23,25,28,29,31,79] Few other studies reported higher temporal RNFL thickness in children born preterm (eg, 88.84 μm at 6.88 years).[26,27] The variation in RNFL thickness across different studies might be related to differences in imaging devices and cohorts (eg, degree of prematurity, comorbidities, and treatment). Another contributor is the change in RNFL thickness that occurs with ocular growth.[32] Future longitudinal studies are required to investigate the change of RNFL thickness in preterm infants.

• **STUDY LIMITATIONS:** The present study was not without limitations. First, the number of infants with ROP stage ≥3 was small in the present study, so we were unable to examine the relationship between severe ROP and RNFL thickness. Second, most our study cohort were EPT and VPT infants. We do not know if our findings are applicable to infants born after 32 weeks. Third, the infants who were small for gestational age did not exhibit a significant difference in RNFL thickness compared with infants who were not small for gestational age. This might be related to the comparable birth weight between the 2 cohorts (904.8 ± 265.4 g vs 996.5 ± 272.7 g; $P = .19$) or the small number of infants who were small for gestational age ($n = 20$). Fourth, although a few previous studies in the adult population found a significant relationship between demographic factors and RNFL thickness,[33–36] we did not find a significant difference in RNFL thickness by sex, race, and ethnicity in our infant cohort. It is possible that these differences might develop later in life or the sample size of the present study might not be large enough to detect a significant difference. Future studies with a large infant cohort might be needed further to investigate the relationship between demographic factors and RNFL thickness. Fifth, the present study was based on cross-sectional data without longitudinal follow-up. We are currently collecting RNFL thickness at longitudinal follow-up visits in this cohort, and we will investigate the longitudinal change of RNFL thickness in this cohort as well as infants with PVL in future analyses.

CONCLUSIONS

IN CONCLUSION, BIRTH WEIGHT, RATHER THAN GESTATIONAL age, was a significant independent predictor of RNFL thickness in preterm infants in our study, which suggested that an intrauterine process that negatively affects birth weight might reduce the reserve of retinal ganglion cells. We also demonstrated the excellent intra- and intergrader reproducibility of RNFL thickness assessed via bedside handheld OCT imaging in awake preterm infants. Despite the high intra- and intereye correlations of RNFL thickness, RNFL thickness measured by OCT was slightly but significantly higher in the right eyes than the left eyes, corresponding with previous findings in children and adults.

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