INTRODUCTION

Premature birth can affect the prematurely born individual in many ways. Ocular sequelae include retinopathy of prematurity (ROP), which is one of the main neonatal morbidities following preterm birth (Express Group, 2010). It is a neurovascular disorder, characterized clinically by abnormal retinal vascular development (Hellström et al., 2013). However, even without ROP, premature birth has been shown to influence the development of the retina, including the fovea (Akerblom et al., 2011; Molnar et al., 2017). The fovea starts to develop at 12 weeks gestational age (GA); after mid-gestation, there is centrifugal displacement of the inner retinal cells to begin formation of the optic pit; and after term, a centripetal displacement of photoreceptors takes place to increase the foveal cone density. Cone density increases from birth until 15 months.
of age, when cone elongation begins and continues until around 13 years (Hendrickson et al., 2012; Provis et al., 1998). Central macular thickness and persistence of inner retinal layers are examples of foveal changes that have been associated with premature birth (Yanni et al., 2012).

Premature birth has been shown to influence the development of the central nervous system as well (de Kieviet et al., 2012; Saigal & Doyle, 2008). The retinal nerve fibre layer (RNFL) develops from the optic nerve and is part of the central nervous system. Studies on prematurely born children and adults have shown a thinner RNFL in the eyes of prematurely born individuals (Akerblom et al., 2012; Fieß, Schäffler, et al., 2022). This seems to be related to the brain structure and neurodevelopment (Rothman et al., 2015).

We have previously reported on the influence of premature birth on the visual function of a well-defined population of prematurely born young adults (Pétursdóttir et al., 2020). The aim of this study was to investigate the central retinal morphology in the same cohort and compare to term born controls.

2 | MATERIALS AND METHODS

2.1 | Participants

This study is part of a larger longitudinal, population-based follow-up study on the ophthalmological outcome of prematurely born individuals who were screened for ROP in the neonatal period and treated with cryotherapy when indicated. The prematurely born individuals were all part of a population-based study on the incidence of ROP, and thereafter took part in ophthalmological follow-up from 6 months until 10 years of age. Fifty-nine prematurely born individuals participated. They were born in Stockholm County, Sweden, between 1 November 1988 and 31 October 1990, with a birthweight (BW) of ≤1500 g. Forty-four controls were born at term in the same area, at the same time period, and with normal BWs. The criterion for ROP treatment in Sweden at that time was ROP stage 3 in at least four clock hours in zone II, with or without plus disease (Holmstrom et al., 1993). The study group was divided according to the degree of ROP in the neonatal period, that is, no ROP, untreated ROP, and treated ROP. At 2.5 years of age, neurological complication was defined as intraventricular haemorrhage grade 3 or 4 in the neonatal period and/or obvious neurologic sequelae (epilepsy, cerebral palsy or mental retardation) (Holmstrom et al., 1993, 1999; Holmstrom & Larsson, 2008).

Previous reports from the follow-up describe the study subjects as well as the control group in more detail (Pétursdóttir et al., 2020, 2021, 2022a, 2022b).

The study was conducted according to the Declaration of Helsinki and approved by the regional Ethical Review Board of Uppsala, Sweden (Dnr 2014/584). All participants signed a written consent before participation.

2.2 | Methods

Best-corrected distance visual acuity (VA) was assessed monocularly with the logarithmic Early Treatment Diabetic Retinopathy Study Research Group (ETDRS) (1985) chart at 4 m and contrast sensitivity (CS) with the Vistech Contrast Sensitivity Test System (VCTS 6500) (Vistech Consultants, USA). Contrast sensitivity was calculated as area under the curve (AUC) and has been described elsewhere (Pétursdóttir et al., 2020). Refraction in cycloplegia was assessed and spherical equivalent calculated.

Optical Coherence Tomography (OCT) scans were acquired using the spectral domain Cirrus HD-OCT 4000 (Carl Zeiss Meditec, Dublin, CA). The scans were performed through dilated pupils, and a single scan with signal strength ≥6 was selected for further analysis.

The Macular Cube scan 512 × 128, covering 6 × 6 mm of the retina with the fovea centred, was used for imaging the macular structure. The average retinal thickness was presented as numeric values for nine macular areas defined by the ETDRS Research Group (1). The central region is 1 mm in diameter (A1), the inner and outer circles are divided into four quadrants each, A2–A5 and A6–A9. The cube average thickness in micrometres and the total macular volume (cup volume, CV) in square millimetres were also noted. Furthermore, the ganglion cell-inner plexiform layer (GC-IPL) analysis segmentation algorithm was used to present the thickness of individual sectors of the macula superotemporal (ST), superior (S), superonasal (SN), inferonasal (IN), inferior (I), inferotemporal (IT), together with the average and minimum GC-IPL.

The RNFL thickness was measured with the Optic Disc Cube 200 × 200 protocol, and the average RNFL thickness and the RNFL thickness in different quadrants (S (superior), N (nasal), I (inferior) and T (temporal)) recorded.

2.3 | Statistical methods

Data are described descriptively using absolute frequencies for categorical variables and mean, standard deviation and range for numerical variables. Comparison between prematurely born and controls was performed using linear mixed models where all models included fixed effects of group, sex and refraction and subject number as random factors, taking into account repeated measures within subject (left and right eye). Results from mixed models are presented as estimated mean difference between groups with 95% confidence interval and p-value.

To explore risk factors regarding central macular thickness and RNFL within the prematurely born group, GA, BW, sex, neurological complications, ROP and treated ROP, were evaluated with univariable and multivariable linear mixed models.

No adjustments for multiplicity have been performed; thus, all p-values should be interpreted with that in mind.
3 | RESULTS

Fifty-nine prematurely born individuals and 44 full-term controls, all 25–29 years of age, participated in the study, see Table 1. One of the prematurely born individuals could not participate in the OCT assessments due to severe autism. This individual had no ROP in the neonatal period. Further, it was only possible to achieve good quality readings from the left eye of one prematurely born, because of very poor vision in the right eye. All 44 controls could complete the OCT assessments in both eyes. The outcome of logMAR VA, CS and refraction in the prematurely born and control groups have been described previously (Pétursdóttir et al., 2020, 2022a, 2022b).

The macular thickness in the nine EDTRS areas (A1-A9) are reported in Figure 1. The central area (A1) was significantly thicker (26.7 μm) in the prematurely born individuals compared to controls (p < 0.001), see also Figure 2. The ones without ROP had a mean thickness in A1 of 281 (SD 20) μm in the right eyes (REs) and 283 (SD 20) μm in the left eyes (LEs), and the ones with untreated ROP had a mean value of 281 (SD 22) μm in the REs and 280 (SD 24) μm in the LEs. The formerly treated children had the thickest central area, 296 (SD 26) μm in controls in the areas ranged from 3.1–4.8 μm in the LEs. The formerly treated children had the thickest central area, 296 (SD 26) μm in the LEs, and the ones with untreated ROP had a mean value of 281 (SD 22) μm in the REs and 280 (SD 24) μm in the LEs. The formerly treated children had the thickest central area, 296 (SD 26) μm in the REs and 303 (SD 29) in the LEs. The difference remained when excluding individuals with previous ROP, including treated ROP, and individuals with a neurological complication according to our definition (p < 0.001). There was no difference in the other macular areas nor the inner and outer circle of macula, see Figure 2. No difference was found in the total macular volume between prematurely born individuals and controls (REs 10.2 (SD 0.50), LEs 10.27 (SD 0.52) versus REs 10.34 (SD 0.47), LEs 10.35 (SD 0.48)).

The ganglion cell layer thickness in the six macular areas as well as average are given in Figure 3. The mean difference between prematurely born individuals and controls in the areas ranged from 3.1–4.8 μm, and the GC-IPL was statistically thinner in preterms in all areas (p < 0.05), see Figure 3. The average GC-IPL was in mean, 3.84 μm thinner in prematurely born individuals (p < 0.05) compared to controls and the minimum GC-IPL 8.02 μm thinner (p = 0.001). The statistical difference between preterms and controls remained after excluding those with previous ROP, including treated ROP, and those with previous neurology (p < 0.05 and p < 0.01).

Regarding optic nerve head OCT, the RNFL in the superior, inferior, temporal and nasal sectors together with the average RNFL thickness are given in Table 2 and Figure 4. The average RNFL thickness was significantly thinner in prematurely born individuals compared to controls, and in the inferior and nasal sectors of the optic nerve head, see Table 2. However, when excluding those with previous neurology according to our definition, and those with previous ROP (including treated ROP), the significant difference remained only regarding the average RNFL (difference = 5.38, confidence interval (CI) −9.47 to −1.29, p < 0.05) and the nasal sector (difference −9.57, CI −14.75 to −4.39, p < 0.01).

Within the prematurely born group, risk factors such as gestational age (GA), BW, sex, neurology, ROP and treated ROP, were evaluated with univariable and multivariable analyses. The statistically significant results from the multivariable analyses of the risk factors are presented in Table 3. The central macular thickness (A1) decreased by 3.65 μm with every additional week of GA, also illustrated in Figure 5, that is, low GA was a risk factor for thicker macula. Previous treatment of ROP was a risk factor for thicker central macula (p = 0.05), Table 3. Regarding the ganglion cell layer in the macula, no significant risk factors were found for reduced thickness. Treated ROP was found to be a risk factor for increased average RNFL, Table 3, which was most obvious in the temporal sectors, see Figure 4.

Within the prematurely born group, we also analysed the correlations between VA and CS (AUC) with the OCT measurements. The central macular thickness (A1) was not associated with VA nor CS. In multivariable analyses, thicker average GC-IPL was associated with

| TABLE 1 | Demographics of prematurely born individuals and controls. |
|---------|------------------|-----------|
|         | GA at birth (w) | BW (g)   |
|         | Mean (SD)       | Mean (SD) |
| Controls | 39–41           | 3000–4000 |
|          | N/A             | N/A      |
| Prematures | 29 (2)         | 1167 (237) |
|          | 24 to 34           | 700 to 1490 |
| No ROP | 30 (2)         | 1264 (195) |
|          | 26 to 34           | 711 to 1490 |
| Untreated ROP | 29 (2)       | 1082 (258) |
|          | 27 to 32           | 750 to 1466 |
| Treated ROP | 28 (2)       | 993 (201)  |
|          | 24 to 32           | 700 to 1380 |

Abbreviations: BW, birth weight; f, female; g, grams; GA, gestational age; L, left eye; m, male; N, number; n/a, not applicable; RE, right eye; ROP, retinopathy of prematurity; SD, standard deviation; w, weeks.

*In the eye with the most severe stage of ROP.
better logMAR VA (−0.0049, CI −0.0074 to −0.0025, \( p < 0.001 \)), see Figure 6, and better CS (0.0061, CI 0.0021 to 0.0101, \( p = 0.005 \)). Thicker RNFL was correlated with worse logMAR VA (0.0042, CI 0.0019 to 0.0065, \( p = 0.001 \)) and worse CS (−0.0058, CI −0.0098 to −0.0017, \( p = 0.007 \)).

4 | DISCUSSION

In the present study, retinal morphology was assessed with OCT in prematurely born individuals in adulthood, who had been screened for ROP, and compared with individuals born at term. We found that the central macular thickness was increased in individuals born preterm and that the macular ganglion cell layer was thinner, also when excluding those with previous ROP and those with neurological complications. Gestational age at birth and previous treatment of ROP were risk factors for a thicker macula, however, not for reduced GC-IPL. The average peripapillary RNFL was found to be thinner in the prematurely born group, also when excluding those with previous ROP and those with neurological complications. Within the prematurely born group, treated ROP was a risk factor for increased average RNFL. Further, both better VA and CS were associated with thinner optic nerve RNFL and thicker average GC-ILP.
Many OCT studies have reported on the macular retinal thickness and RNFL thickness in prematurely born children (Akerblom et al., 2011, 2012; Molnar et al., 2017), however, there are few studies performed in adults (Balasubramanian et al., 2019; Fieß, Pfisterer, et al., 2022; Nilsson et al., 2016). Nilsson et al. examined 14 ex-preterms, all treated with cryotherapy because of severe ROP, at an age of 24.5 years and found reduced foveal depth and altered distribution of RNFL in treated individuals compared to controls. Fieß et al. conducted a prospective study with 140 former preterm adults at the age of 18–51, born at a GA ≤32 weeks, of which 36 had previous ROP and seven had been treated, in a study on macular retinal and RNFL thickness. Balasubramanian et al. (2019) examined 133 extremely prematurely born individuals at 19 years of age and reported the central macular thickness.

The development of fovea and central macula has been evaluated in histological studies as well as in OCT studies. Maldonado and Toth (2013) used spectral domain OCT for analysing fovea in infants from postmenstrual age of 31 weeks up to adulthood. The authors found peripheral migration of the inner retinal layer and centripetal growth of the outer retinal layers in the first months of life. A progressive thinning of fovea and thickening of the parafoveal region with increasing age have been found (Maldonado & Toth, 2013; Vajzovic et al., 2012). In prematurely born children, a thicker macula has been found compared to full-term children, hypothesizing that the normal maturation of macula is disturbed by preterm birth (Venkataraman et al., 2023). Åkerblom et al. found that low GA was a risk factor for a thicker macula in preterm children; however, ROP was not. In contrast, Molnar et al. found that both GA and ROP were associated with increased macular thickness (Akerblom et al., 2011; Molnar et al., 2017). In the present cohort, we found that GA at birth remained correlated to a thicker macula also in adulthood, and that previous treatment of ROP was a risk factor for thicker macula in the multivariable analysis, in accordance with the study by Fieß, Pfisterer, et al. (2022).

The ganglion cell layer may be of interest in evaluating the optic nerve or visual pathway. It has been found to be reduced in optic nerve diseases and intracerebral lesions (Lee et al., 2018, 2019; Shinohara et al., 2022). As opposed to Balasubramanian et al., we found thinner GC-IPL in preterms than in full-term individuals and no impact regarding treated or untreated ROP (Balasubramanian et al., 2019). Similarly, GA or BW did not affect the GC-IPL in the preterms. Balasubramanian et al. found a weak correlation between decreased VA and thicker inner retinal layers (Balasubramanian et al., 2019), while we found no correlations between GC-IPL thickness and the best corrected VA or CS. The difference could be explained by different imaging techniques and software solutions.

Preterm birth affects the peripapillary RNFL thickness at school-age according to multiple previous studies (Akerblom et al., 2012; Fieß et al., 2017; Park & Oh, 2015; Ruberto et al., 2014). In accordance with Fieß et al., the average RNFL remained thinner in the prematurely born children than in controls. However, in the present study, we found that GA at birth remained correlated to a thicker macula also in adulthood, and that previous treatment of ROP was a risk factor for thicker macula in the multivariable analysis, in accordance with the study by Fieß, Pfisterer, et al. (2022). Nilsson et al. hypothesize that cryotherapy could be a factor leading to absence of foveal depression, thus a thicker macula (Nilsson et al., 2016). In the present study, we could not find any correlation between the best corrected VA and the thickness of central macula, in accordance with results from school-aged children (Akerblom et al., 2011; Molnar et al., 2017), albeit in contrast to Fieß, Pfisterer, et al. (2022). Likewise, there was no correlation with CS. The different results may be due to different cohorts in the studies.

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born individuals compared to controls even into adulthood (Fieß, Schäffler, et al., 2022). However, in the study by Nilsson et al., no difference was found regarding average RNFL (Nilsson et al., 2016).

In the present study, the former prematurely born individuals who had been treated for severe ROP in the neonatal period had a thicker average RNFL compared to untreated prematurely born (Table 3). We believe that this difference was explained by the thicker temporal sector, similar to the findings presented by Nilsson et al. and Fieß et al. who found thicker RNFL in the temporal sector in adults formerly treated for ROP (Fieß, Schäffler, et al., 2022; Nilsson et al., 2016). Our results contradict previous studies in children treated for ROP, in which the average RNFL was thinner or similar to that of untreated children (Akerblom et al., 2012; Fieß et al., 2017; Park & Oh, 2015). It is possible to hypothesize that there could be a change over time in the RNFL thickness from school age to adulthood in the treated individuals. A study of peripapillary RNFL in patients who underwent panretinal photocoagulation for proliferative diabetic retinopathy showed a reduction in average RNFL after 2 years (Kim

**FIGURE 3** (a, b) Ganglion cell-inner plexiform layer (GC-IPL) thickness (μm), mean (standard deviation) and range. (a) Prematurely born individuals. (b) Controls.
et al., 2012). However, the RNFL increased in the temporal sector; moreover, compared to control subjects, there was a significantly increased thickness, in line with the results from our study, in subjects formerly treated for ROP. One can only speculate whether the change comprises a compensatory regeneration in the nerve fibre layer around the optic nerve. Larger longitudinal studies over time in treated children might clarify this issue. Thicker peripapillary RNFL was correlated with worse logMAR VA and CS. A possible explanation is the effect of the temporal thickness seen in individuals formerly treated for ROP.

### Table 2

Retinal nerve fibre layer (RNFL) thickness in prematurely born individuals and controls as well as mean difference between the groups.

<table>
<thead>
<tr>
<th></th>
<th>Prematures</th>
<th>Controls</th>
<th>Mean difference prematures versus controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right eye</td>
<td>Left eye</td>
<td>Right eye</td>
</tr>
<tr>
<td>Average thickness (μm)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>90.96 (10.62)</td>
<td>92.27 (11.42)</td>
<td>96.55 (10.11)</td>
</tr>
<tr>
<td>Range</td>
<td>71 to 129</td>
<td>70 to 126</td>
<td>74 to 121</td>
</tr>
<tr>
<td>Superior quadrant (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>117.77 (15.02)</td>
<td>116.52 (15.84)</td>
<td>120.55 (15.26)</td>
</tr>
<tr>
<td>Range</td>
<td>82 to 185</td>
<td>83 to 165</td>
<td>89 to 161</td>
</tr>
<tr>
<td>Inferior quadrant (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>117.77 (15.02)</td>
<td>116.52 (15.84)</td>
<td>120.55 (15.26)</td>
</tr>
<tr>
<td>Range</td>
<td>82 to 185</td>
<td>83 to 165</td>
<td>89 to 161</td>
</tr>
<tr>
<td>Nasal quadrant (μm)</td>
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<tr>
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<td>117.77 (15.02)</td>
<td>116.52 (15.84)</td>
<td>120.55 (15.26)</td>
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<tr>
<td>Range</td>
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<td>89 to 161</td>
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<tr>
<td>Temporal quadrant (μm)</td>
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<td></td>
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<td>65.14 (27.75)</td>
<td>64.02 (6.96)</td>
</tr>
<tr>
<td>Range</td>
<td>39 to 190</td>
<td>36 to 179</td>
<td>49 to 82</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation.

### Figure 4

Retinal nerve fibre layer (RNFL) thickness (μm), in the preterm group according to 1: No ROP, 2: Untreated ROP and 3: Treated ROP. ROP, retinopathy of prematurity.

### 4.1 Strengths and limitations

This study is one of few conducted with formerly prematurely born adult individuals, where ROP and its treatment in the neonatal period were known. Thus, it provides an insight into the long-term effects of preterm birth on retinal structure. The prematurely born individuals have been followed since birth, and a majority of the control group has been followed from 10 years of age. One limitation was that no previous OCT had been performed in childhood, since the technique was not in
clinical use at that time. Longitudinal individual comparison could therefore not be performed. Further, the attendance rate of the original cohort was low.

5 | CONCLUSION

Central macular thickness was increased, and the macular ganglion cell layer was thinner in prematurely born young adults as compared to term born controls. Within the preterm group, we found that lower GA and treated ROP were risk factors for a thicker macula;

| TABLE 3 | Statistically significant risk factors regarding central macular thickness (A1) and average retinal nerve fibre layer (RNFL). |
|-----------------|---------------------------------|---------------------------------|
| Estimate        | Confidence interval              | p-Value                         |
| A1               |                                 |                                 |
| Gestational age (weeks) | −3.65 | −6.37 to −0.94 | 0.011 |
| Treated ROP     | 14.75                           | 0.33 to 29.18                   | 0.050 |
| RNFL            |                                 |                                 |
| Treated ROP     | 9.09                            | 2.54 to 15.64                   | 0.009 |

Abbreviation: ROP, retinopathy of prematurity.
however, we did not see the same risk factors for reduced GC-IPL. The prematurely born individuals had thinner average peripapillary RNFL than the controls. Within the group of prematurely born individuals, previous treatment for ROP was associated with thicker RNFL. Further, within the group of prematurely born individuals, thicker average GC-IPL and thinner optic nerve RNFL were associated with better VA and CS.

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