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Journal

Investigative ophthalmology & visual science, 52(11)

ISSN

0146-0404

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Publication Date

2011-10-01

DOI

10.1167/iovs.11-7896

Peer reviewed

Profile and Predictors of Normal Ganglion Cell–Inner Plexiform Layer Thickness Measured with Frequency-Domain Optical Coherence Tomography

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PURPOSE. To describe the profile and identify the predictors of the ganglion cell–inner plexiform layer (GCIPL) thickness measured with frequency-domain optical coherence tomography (FD-OCT) in normal eyes.

METHODS. Two hundred eighty-two normal subjects underwent macular and optic disc scanning in both eyes with Cirrus high-definition (HD)-OCT (Carl Zeiss Meditec, Dublin, CA). Linear regression analyses were performed to determine the association between GCIPL thickness and age, sex, ethnicity (Europeans, Africans, Hispanics, Asians, and Indians), eye laterality, refraction, intraocular pressure, axial length, central corneal thickness, mean retinal nerve fiber layer (RNFL) thickness, disc and rim areas, cup-to-disc area, vertical and horizontal cup-to-disc diameter ratios, vertical rim thickness, and OCT signal strength.

RESULTS. The mean ($\pm$ SD) age was 46.2 ± 16.9 years (range, 18–84 years). The mean and minimum GCIPL thicknesses ($\pm$ SD) were 82.1 ± 6.2 and 80.4 ± 6.4 μ m, respectively. There were significant differences in GCIPL thickness between macular sectors ($P < 0.05$), except between the superotemporal and inferonasal sectors ($P = 0.63$). The superonasal sector had the thickest and the inferior had the thinnest GCIPL. The

GCIPL of the superior hemisphere was thicker than that of the inferior, and the nasal sector GCIPL was significantly thicker than the temporal one ($P < 0.001$). The average GCIPL did not differ between male and female subjects ($P = 0.16$) after adjustment for axial length and between ethnic groups ($P = 0.41$) after adjustment for age, axial length, and RNFL thickness. Significant predictors of mean GCIPL thickness were average RNFL thickness ($\beta = 0.37$, $P < 0.001$), age ($\beta = -0.083$, $P < 0.001$), axial length ($\beta = -0.87$, $P = 0.001$), and male sex ($\beta = -1.62$, $P = 0.005$).

CONCLUSIONS. The independent factors associated with thinner GCIPL include thinner RNFL, older age, longer ocular axial length, and being male. Although the magnitude of the effect of age, axial length, and sex are small, these factors should be taken into account when interpreting Cirrus HD-OCT-based GCIPL thickness measurements. (*Invest Ophthalmol Vis Sci.* 2011;52:7872–7879) DOI:10.1167/iovs.11-7896

A better understanding of complex diseases, such as glaucoma, requires assessment of the various demographic, environmental, genetic, and ocular factors that are believed to be involved in their occurrence. Studies have shown that some classic risk factors, such as elevated intraocular pressure (IOP), are not sufficient to explain and/or predict the risk of developing glaucoma. On the other hand, retinal nerve fiber layer (RNFL) thickness measured noninvasively by optical coherence tomography (OCT) is a reliable early marker of glaucoma risk and can predict the development of subsequent glaucomatous visual field defects.^{1,2}

Several studies have characterized the retinal and RNFL thickness profile in healthy eyes,^{3,4} whereas others have, in addition, investigated the effect of demographic and ocular factors on these structures.^{3,5–11} Because the pathogenesis of glaucoma involves the degeneration not only of axons, but also of cell bodies and dendrites, it is fundamental to know the normal thickness profile of the retinal ganglion cell (RGC) and the dendritic layers. The average macular ganglion cell–inner plexiform layer (GCIPL) thicknesses measured using Stratus OCT (Carl Zeiss Meditec, Dublin, CA) and ranging between 68 and 74.8 μ m have been reported in normal eyes.^{12–16} However, the limitations of these studies are that they included only a small number of eyes and did not provide sectoral thicknesses of GCIPL or explore the relationship between macular GCIPL thickness and demographic or ocular parameters. Although ethnicity, age, axial length, and disc area have been shown to be determinant factors of RNFL thickness,^{4,7,9,11,17–19} it remains unknown whether these factors also have an effect on other retinal layers. The recent development of more powerful intraretinal segmentation algorithms for some commercially available spectral-domain OCT devices now allows quantitative measurement of

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⁹The participating investigators and their centers are listed in the Appendix.

Supported by an unrestricted grant from Carl Zeiss Meditec, Inc. (Dublin, CA).

Submitted for publication May 17, 2011; revised July 17, 2011 and August 11, 2011; accepted August 17, 2011.

Disclosure: J.-C. Mwanza, Carl Zeiss Meditec (F), Optovue (F), Heidelberg Engineering (F); M.K. Durbin, Carl Zeiss Meditec (E); D.L. Budenz, Carl Zeiss Meditec (F), Optovue (F), Heidelberg Engineering (F); C.A. Girkin, Carl Zeiss Meditec (F); C.K. Leung, Carl Zeiss Meditec (F, C, R); J.M. Liebmann, Carl Zeiss Meditec (F), Optovue (F, C), Heidelberg Engineering (F), Topcon (F, C); J.H. Peace, Carl Zeiss Meditec (F); J.S. Werner, Carl Zeiss Meditec (F); G. Wollstein, Carl Zeiss Meditec (F)

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FIGURE 1. Horizontal scan of the macula showing an unsegmented (A) and a segmented (B) GCIPL, using the ganglion cell analysis algorithm in a normal eye. *Top and bottom white lines:* the outer boundaries of the RNFL and IPL, respectively. GCIPL thickness is the distance between the two lines.

individual retinal layers and their changes in glaucoma.¹⁴ The purpose of the present study was to describe the distribution profile of the GCIPL thickness in normal eyes and to assess the effects of demographic and clinical parameters on GCIPL thickness using frequency-domain optical coherence tomography (FD-OCT).

MATERIALS AND METHODS

Participants

Normal volunteers aged 18 years and older were recruited and invited to participate in the Cirrus Normative Database Study at seven ophthalmology clinics. Written informed consent was obtained from each participant after study approval by the institutional review board of each participating institution. The study protocol complied with the Declaration of Helsinki. Eligibility was determined for each subject through a complete ophthalmic evaluation, including measurement of visual acuity, slit lamp examination, IOP measurement, dilated fundus examination, and visual field testing. Exclusion criteria included contraindication of dilation or intolerance of topical anesthetics or mydriatics; IOP ≥ 22 mm Hg or any type of glaucoma in either eye; intraocular surgery in the study eye (except cataract or refractive surgery performed more than 1 year before enrollment); best corrected visual acuity worse than 20/40; evidence of diabetic retinopathy, macular edema, or other vitreoretinal disease; or evidence of optic nerve abnormality in either eye. Subjects were also excluded if they had a CDR asymmetry ≥ 0.2 and/or abnormal visual fields defined as

glaucoma hemifield test results outside normal limits, a pattern SD with a $P < 5\%$, or a cluster of three or more points in the pattern deviation plots in a single hemifield (superior or inferior) with $P < 5\%$, one of which must be $P < 1\%$.

OCT Scanning

Imaging was obtained using the Cirrus HD-OCT (Cirrus; Carl Zeiss Meditec, Dublin, CA). Two scans, including one macular scan centered on the fovea (macular 200×200 cube protocol) and one peripapillary scan centered on the optic disc (optic disc 200×200 axial protocol), were acquired through dilated pupils with the same Cirrus HD-OCT instrument in both eyes of the same subject. The macular GCIPL and peripapillary RNFL thicknesses and optic nerve head (ONH) parameters were measured automatically using the GC, the RNFL, and the ONH analysis algorithms, respectively. The ganglion cell analysis (GCA) algorithm was a prerelease version of software intended for release with the 6.0 software version of Cirrus. Figure 1 shows the GCIPL segmented and reported by the GCA for a typical normal eye. The GCIPL is segmented using a 3-D algorithm using the minimum cost graph traversal in which the cost images combine an edge image and positional cost information. The GCA reports the average of the GCIPL thicknesses over six sectoral areas (superotemporal, superior, superonasal, inferonasal, inferior, and inferotemporal) that form an elliptical annulus around the fovea, as well as the overall average for the annulus (Fig. 2). The annulus has an inner diameter of 1 mm vertically, which was chosen to exclude the portions of the fovea where the layers are very thin and difficult to detect accurately, and an outer diameter of 4 mm vertically, which was chosen to be where the ganglion cell layer (GCL) again becomes thin and difficult to detect. The annulus is stretched by a factor of 20% horizontally in an effort to match the normal distribution of the thickest part of the GCL. The size of the annulus is derived from a preliminary analysis of 47 GCIPL maps from normal eyes (average GCIPL thickness map is shown in Fig. 3), as segmented by the GCA algorithm. The HD-OCT does not account for magnification of the eye in acquisition or analysis. For this reason, we did an additional analysis, looking at how an adjustment for magnification based on axial length would affect these measurements. The average GCIPL values over sectoral areas and over the annulus was determined after the dimensions were adjusted based on a formula that corrects for magnification. The GCA also reports the minimum (lowest GCIPL thickness over a single meridian crossing the annulus), which is expected to be sensitive to focal glaucomatous damage.

The ONH algorithm was an updated algorithm version also intended for release with the 6.0 version of Cirrus. The RNFL algorithm was the version already available with the 3.0 (and later) version of the software. For the peripapillary RNFL, only the mean thickness was analyzed. The following ONH parameters were analyzed: disc area, rim area, CDR, VCDR, HCDR, and VRT. A schematic representation of the ONH parameters has been presented.²⁰ Subjects with scans showing algorithm segmentation failure, signal strength <6 in either eye, or artifacts due to eye movements or blinking were excluded from the study.

Statistical Analysis

The normality of the distribution of the study sample was assessed by means of the Shapiro-Wilk (SW) test, which is commonly used to test

FIGURE 2. OCT image of the macula with the elliptic annulus (dimensions: 1 and 4 mm for the inner and outer vertical diameter, respectively, and 1.2 and 4.8 mm for the inner and outer horizontal diameter, respectively) around the fovea. (A) The six macular sectors: S, superior; SN, superonasal; IN, inferonasal; I, inferior; IT, inferotemporal; ST, superotemporal. (B) The superior (SH) and inferior hemisphere (IH); (C) the temporal (T) and nasal (N) sectors.

FIGURE 3. Average GCIPL thickness of 47 normal right eyes as segmented by the GCA algorithm. Black circles around the fovea are placed 10 μm apart. The annulus is divided into six 60° sectors.

whether the input data are normally distributed. Failing the normality test ($P < 0.05$) shows with 95% confidence that the data do not fit the normal distribution, whereas passing the normality test ($P > 0.05$) only shows that no significant departure from normality was found. Differences in means between the sexes, right and left eyes, and age or ethnic groups were calculated with the Student's *t*-test. A macular symmetry analysis was performed by comparing thicknesses of the temporal and nasal sectors, and those of the superior and the inferior hemispheres (Fig. 2). Simple linear regression was used to assess the correlation between GCIPL thickness and age, ocular axial length, refraction, IOP, CCT (central corneal thickness), disc area, RNFL thickness, disc area, rim area, VRT (vertical rim area), CDR (cup-to-disc ratio), VCDR (vertical cup-to-disc ratio), HCDR (horizontal cup-to-disc ratio), and signal strength. Stepwise multivariate linear regression analysis was performed to test and estimate the effect of these factors as well as sex and ethnicity on GCIPL thickness measurements. Only data from one study eye chosen randomly per participant were entered in the statistical analysis (SPSS ver. 18.0; SPSS, Chicago, IL).

RESULTS

Demographic and Ocular Characteristics of Study Participants

Table 1 shows the demographic and ocular characteristics of the 282 participants. The mean age of the study population was 46.2 ± 16.9 (18–84) years. There were 122 (43.3%) subjects of European, 33 (11.7%) of Hispanic, 51 (18.0%) of African, 62 (22.0%) of Asian (Chinese, Korean, and Japanese), and 14 (5%) of Indian descent. Ocular biometric, IOP, and optic disc measurements were comparable in both eyes (all $P > 0.05$). The average visual field mean deviation was 0.02 ± 1.0 dB, with no significant differences between the sexes ($P = 0.85$), right and left eyes ($P = 0.07$), and ethnic groups ($P = 0.26$). Asians had a significantly higher IOP (15.1 ± 2.4 mm Hg) than individuals of European (13.9 ± 2.5 mm Hg, $P = 0.011$), Hispanic (13.6 ± 2.2 mm Hg, $P = 0.028$), and African (13.7 ± 2.2 mm Hg, $P = 0.015$) descent. Asians also had longer eyes (24.27 ± 1.26 mm) than Africans (23.65 ± 0.82 mm, $P = 0.017$) and Hispanics (23.63 ± 0.82 mm, $P = 0.039$). CCT was

significantly thinner in Africans (526.90 ± 28.07 μm) than in all other ethnic groups ($P < 0.05$), whereas Asians (537.61 ± 32.30 μm) had thinner CCT than did Hispanics (562.42 ± 40.56 μm , $P = 0.007$) and those of European descent (560.93 ± 35.14 μm , $P < 0.001$). Refraction, expressed as the spherical equivalent, differed significantly among ethnic groups ($P < 0.001$), but not between left and right eyes ($P = 0.81$). Both the optic disc area and CDR were smaller in people of European descent (1.68 ± 0.30 mm^2 and 0.43 ± 0.18 , respectively) than in those of African descent (1.91 ± 0.34 mm^2 , $P < 0.001$ and 0.53 ± 0.14 , $P = 0.007$, respectively). HCDR and VCDR showed the same trend as disc size and CDR. After adjustment for age, axial length, CCT, IOP, and disc size, Europeans showed a thinner RNFL (90.7 μm) than Hispanics (94.5 μm , $P = 0.026$) and Asians (96.0 μm , $P < 0.001$), but did not differ significantly from Africans (92.8 μm , $P = 0.18$) and Indians (93.4 μm , $P = 0.26$). The men (47.2%) and women (52.8%) were similar in all characteristics except axial length (24.13 mm vs. 23.75 mm, respectively, $P = 0.003$).

GCIPL Thickness Measurements

Table 2 presents the means of GCIPL parameters and their distribution characteristics; the mean and minimum, superotemporal, superior, superonasal, inferonasal, inferior, and nasal thickness measurements were normally distributed. Figure 4 illustrates the frequency distribution of the mean GCIPL in the study population. The frequency distribution and mean GCIPL thickness in the study participants stratified by age groups are presented in Table 3. The GCIPL thickness was stable between 18 and 49 years and then decreased progressively (Fig. 5A), with mean GCIPL significantly thicker in the 18- to 29-, 30- to 39-, and 40- to 49-year age group than in the 60- to 69- ($P = 0.008$, 0.002 , and 0.002 , respectively) and 70- to 84-year age groups ($P = 0.006$, < 0.001 , and < 0.001 , respectively). The comparison between younger (age < 50 years, $n = 156$) and older (age ≥ 50 years, $n = 126$) subjects revealed significantly thinner measurements of all GCIPL parameters in younger than older subjects (all $P < 0.001$). The average GCIPL thickness was 82.1 ± 6.2 μm (range, 68–101), whereas the minimum GCIPL thickness was 80.4 ± 6.4 μm (range, 64–98). Of the six macular sectors, the superonasal had the thickest whereas the inferior had the thinnest GCIPL. Figure 5B shows the thickness profile of the GCIPL in the six macular sectors. The sectors differed significantly from each other in GCIPL thickness ($P < 0.05$), except in the comparison between the superotemporal and inferonasal sector ($P = 0.63$). The symmetry analysis revealed that the GCIPL of the superior hemisphere was thicker than that of the inferior and that the nasal GCIPL was signifi-

TABLE 1. Demographic and Ocular Characteristics of Study Participants

Parameter	Mean (SD)	Range
Age, y	46.2 ± 16.9	18–84
SER, D	-1.00 ± 2.1	–10.6 to 6.25
Axial length, mm	23.94 ± 1.06	20–28
CCT, μm	549.58 ± 36.37	449–662
IOP, mm Hg	14.04 ± 2.41	8–20
Disc area, mm^2	1.76 ± 0.34	1.01–2.93
Rim area, mm^2	1.30 ± 0.21	0.72–2.27
CDR	0.46 ± 0.17	0.1–0.8
VCDR	0.45 ± 0.16	0.1–0.75
HCDR	0.1 ± 0.19	0.1–0.85
VRT, μm	842.33 ± 223.70	447.50–1662.74
RNFL thickness, μm	92.83 ± 9.44	70.35–121.10
OCT signal strength	9.3 ± 0.6	7.0–10

SER, spherical equivalent refraction.

TABLE 2. Distribution of Macular GCIPL Thickness

	Mean (SD)	Median	Min	Max	Kurtosis	Skew	SW
Mean GCIPL	82.1 (6.2)	82.3	68	101	−0.06	0.03	0.370
Minimum	80.4 (6.4)	81.0	68	98	−0.02	−0.11	0.299
Superotemporal	81.7 (6.1)	82.0	67	100	−0.05	0.17	0.066
Superior	83.6 (6.8)	84.0	66	101	−0.25	−0.03	0.365
Superonasal	84.4 (7.1)	84.0	65	104	−0.02	−0.00	0.450
Inferonasal	81.5 (7.0)	82.0	62	105	0.13	−0.11	0.175
Inferior	79.9 (6.5)	80.0	64	99	−0.15	−0.03	0.310
Inferotemporal	82.0 (6.1)	82.0	66	103	0.25	0.10	0.028
Superior hemisphere	83.1 (6.7)	83.0	65	104	−0.11	0.08	0.03
Inferior hemisphere	81.2 (6.6)	81.5	62	105	0.10	−0.03	0.006
Nasal	82.8 (7.2)	83.0	62	105	0.08	−0.06	0.12
Temporal	81.8 (6.1)	82.0	66	103	0.09	0.14	0.003

Data are the thickness in micrometers.

cantly thicker than the temporal one ($P < 0.001$). Subjects of European descent had significantly thinner average GCIPL than Hispanics ($P = 0.007$) and Asians ($P = 0.003$); however, these differences disappeared after controlling for age, axial length, and RNFL thickness differences ($P = 0.41$ and 0.14 , respectively). No other interethnic differences in GCIPL thickness were observed. The average GCIPL did not differ between the sexes ($P = 0.36$), but the men had significantly thicker superotemporal ($P = 0.026$) and inferotemporal GCIPL ($P = 0.041$) compared with the women; these differences persisted after controlling for axial length ($P = 0.008$ and 0.012 , respectively). There were no other differences between the men and the women or between the right and left eyes.

Correlations and Predictors of GCIPL Thickness

Simple linear regression analysis indicated statistically significant relationships between average GCIPL thickness and age ($R^2 = 0.14$, $P < 0.001$), axial length ($R^2 = 0.024$, $P = 0.009$), CCT ($R^2 = 0.024$, $P = 0.01$), OCT signal strength ($R^2 = 0.036$, $P = 0.001$), disc area ($R^2 = 0.029$, $P = 0.004$), rim area ($R^2 = 0.11$, $P < 0.001$), RNFL thickness ($R^2 = 0.42$, $P < 0.001$), and VRT ($R^2 = 0.025$, $P = 0.008$; Fig. 6). Sectoral GCIPL thicknesses also significantly correlated with all these factors (all $P < 0.05$). Of all GCIPL parameters, only the minimum ($R^2 = 0.014$, $P = 0.047$) and inferotemporal sector ($R^2 = 0.018$, $P = 0.025$) showed significant but weak correlations with IOP. When these correlations were performed separately in the group of younger and older subjects, only RNFL thickness ($R^2 = 0.35$, $P < 0.001$ and $R^2 = 0.38$, $P < 0.001$, respectively) and rim area ($R^2 = 0.085$, $P < 0.001$ and $R^2 = 0.098$, $P < 0.001$, respectively) were significantly related to GCIPL thick-

ness in both groups. The relationships with axial length ($R^2 = 0.084$, $P < 0.001$) and disc area ($R^2 = 0.072$, $P = 0.001$) were significant only in the younger subjects, whereas the relationships with age ($R^2 = 0.17$, $P < 0.001$), CCT ($R^2 = 0.087$, $P = 0.001$), and signal strength ($R^2 = 0.059$, $P = 0.006$) were significant in the older subjects only.

Table 4 shows results of stepwise multivariate linear regression analyses. Significant predictors of mean GCIPL thickness were mean RNFL thickness ($\beta = 0.37$, $P < 0.001$), age ($\beta = -0.083$, $P < 0.001$), axial length ($\beta = -0.87$, $P = 0.001$), and sex ($\beta = -1.62$, $P = 0.005$). A separate analysis including only 280 subjects with refractive errors greater than -8.00 D produced similar results. These four factors accounted for 47.2% of GCIPL thickness variability. After average RNFL thickness was removed from the model, the effect of axial length was nullified, whereas age ($\beta = -0.191$, $P < 0.001$) and sex ($\beta = -0.105$, $P = 0.023$) remained significant predictors (not shown in Table 4). When both RNFL thickness and age were removed, sex ($\beta = -0.102$, $P = 0.022$), and axial length ($\beta = -0.13$, $P = 0.005$) still had statistically significant effects. Extrapolation from regression equations indicated that average GCIPL thickness decreases by 0.101% per year of increased age and by 1.06% per each 1-mm increase in axial length. There were sectoral differences in the age-related GCIPL thinning rate, with the inferonasal and superonasal sectors showing the fastest and most similar thinning (0.13% and 0.12% per year, respectively), followed by the superior and inferior sectors (0.09% each), the superotemporal sector (0.074%), and the inferotemporal sector (0.072%). The effect of axial length was more pronounced in the inferior sector (1.34% decrease in thickness with each millimeter increase in axial length) and less pronounced in the superotemporal sector (0.86%). To confirm that GCIPL is not dependent on disc size, a separate multivariate analysis was performed that included only subjects of European and African descent because of the observed significant difference in optic disc size between these two groups. The factors entered in the model were RNFL thickness ($\beta = 0.39$, $P < 0.001$), age ($\beta = -0.07$, $P = 0.002$), CCT ($\beta =$

FIGURE 4. Distribution of mean GCIPL thickness in 282 normal volunteers (histograms) compared with normal distribution (solid curve).

TABLE 3. Mean GCIPL Thickness Stratified by Age Group

Age Group (y)	Subjects n (%)	Mean GCIPL Thickness (μm)	95% CI
18–29	59 (21.0)	83.6 ± 5.5	82.2–85.1
30–39	52 (18.4)	84.2 ± 5.3	82.8–85.7
40–49	45 (16.0)	84.4 ± 6.3	82.5–86.3
50–59	54 (19.1)	82.0 ± 5.4	80.5–83.5
60–69	41 (14.5)	79.6 ± 6.3	77.7–81.6
70–85	31 (11.0)	75.9 ± 4.8	74.1–77.7

FIGURE 5. Trend of mean GCIPL thickness in study participants stratified in age-groups (A) and profile of GCIPL thickness in the six macular sectors (B).

-0.025 , $P = 0.012$), and sex ($\beta = -1.70$, $P = 0.017$). None of the optic disc parameters influenced GCIPL thickness measurements. Because axial length correlated with age ($R^2 = 0.017$, $P = 0.025$), sex (point-biserial $R^2 = 0.03$, $P = 0.003$), and CCT ($R^2 = 0.03$, $P = 0.003$), a separate regression model assessing the influence of factors obtained from the fundus on average GCIPL after adjusting for these correlations produced slight changes in the results. Age, axial length, sex, and CCT were forced into the regression model. Age ($\beta = -0.86$, $P < 0.001$) and sex ($\beta = -1.60$, $P = 0.004$) remained statistically significant. Axial length ($\beta = -0.76$, $P = 0.007$) and average RNFL thickness ($\beta = 0.362$, $P < 0.001$), entered in a forward stepwise fashion, were also statistically significant. However, the total R^2 relating age, sex, and CCT to average GCIPL was 0.19, the addition of axial length increased the R^2 by 0.035 only, and the addition of RNFL increased the R^2 further by a substantial 0.26. In total, the combined influence of age, sex, CCT, axial length, and RNFL thickness explains approximately 50% of the variability in GCIPL thickness.

DISCUSSION

This study was performed to determine the profile and predictors of GCIPL thickness of adult individuals, to allow a better understanding of the distribution of normal macular GCIPL thickness, and to provide additional parameters that can be used in glaucoma diagnosis.

Our study showed a mean decrease in average GCIPL thickness with increasing axial length, in the magnitude of 1.06% per each millimeter increase in axial length. Despite some reports indicating the lack of association between axial length

and RNFL thickness,^{4,21-23} the weight of evidence points toward an axial length-related decrease in RNFL thickness. Variable thinning rates ranging between 0.01% and 7% have been reported.^{7,11,17-19} Kim et al.²⁴ recently found a GCC thinning rate of 0.17% per millimeter increase in axial length. The important question that needs answering is whether axial length determines the RNFL or GCIPL thickness or vice versa. While age-related thinning of GCL and RNFL actually corresponds histologically to a decrease in the number of cells and axons with aging, there is no basis to show that RGCs and their axons degenerate under the influence of increasing axial length, whether it precedes or follows changes in RNFL or GCIPL thickness, unless there are other factors, such as elevated IOP. It is a limitation of this study that the Cirrus GCA does not account for magnification errors during acquisition or analysis. The decrease in measured GCIPL thickness with axial length may be an effect of magnification on the area measured. There are several methods available for correcting distances measured in the back of the eye for magnification due to the optical characteristics of the eye.²⁵⁻²⁷ Although Littmann²⁷ provides a formula to correct for magnification based on corneal curvature, refractive error, and axial length, an alternate formula that utilizes only axial length is provided in Bennett et al.²⁵ Using the Bennett formula, the shortest eye in this study should have the linear dimensions of the sectors adjusted by a factor of 0.82, whereas the longest eye should be adjusted by a factor of 1.2. Making a magnification correction to each sectoral average based on the axial length would result in errors in the average GCIPL thickness that range from -13% to 4% . Although, most of the errors in measurement would be $<5\%$, this range was wide enough to reverse the relationship

FIGURE 6. Scatterplots of simple linear regression between mean GCIPL thickness and age (A), axial length (B), CCT (C), signal strength (D), disc area (E), rim area (F), mean RNFL thickness (G), and VRT (H).

TABLE 4. Stepwise Multiple Regression Analysis for the Association between GCIPL Thickness and Demographic and Ocular Parameters

Parameters	Mean		Superotemporal		Superior		Superonasal		Inferonasal		Inferior		Inferotemporal	
	β	P	β	P	β	P	β	P	β	P	β	P	β	P
Age, y	-0.083	<0.001	-0.061	0.001	-0.074	<0.001	-0.10	<0.001	-0.11	<0.001	-0.074	<0.001	-0.059	0.001
Sex	-1.62	0.005	-2.39	<0.001	—	—	-1.42	0.027	—	—	—	—	-2.21	<0.001
Asian	—	—	—	—	—	—	2.43	0.002	1.72	0.027	—	—	—	—
Eye	—	—	—	—	1.27	0.04	2.27	<0.001	1.85	0.003	—	—	—	—
Axial length, mm	-0.87	0.001	-0.71	0.009	—	—	-0.94	0.003	-1.08	0.001	-1.10	<0.001	-0.77	0.006
Mean RNFL, μm	0.37	<0.001	0.36	<0.001	0.40	<0.001	0.38	<0.001	0.37	<0.001	0.36	<0.001	0.35	<0.001

between axial length and average GCIPL thickness from negative ($\beta = -0.87$, $P < 0.001$) to positive ($\beta = 1.02$, $P < 0.001$). A similar effect of magnification correction on the relationship between axial length and RNFL thickness was recently reported by Kang et al.,²⁸ whereas others observed either no change²⁹ or nullification of the relationship.^{4,30} Since the Cirrus does not correct for this error, it is likely that the effect of eye magnification accounts for the dependence of GCIPL thickness on axial length.

The fact that the RNFL thickness was the strongest determinant of GCIPL thickness is not surprising, as the two are closely related. Indeed, earlier studies have used optic nerve axonal count to determine the number of RGCs, as each axon in the optic nerve represents one ganglion cell in the retina.^{31,32} From regression analysis, it was estimated that each micrometer decrease in RNFL thickness is accompanied with an $\sim 0.35\text{-}\mu\text{m}$ (0.43%) decrease in GCIPL thickness, indicating that the decrease in GCL thickness alone and thus that of the number of RGCs may be modest, as also previously suggested by Budenz et al.¹¹ Although one may argue that anatomically it is the GCIPL thickness that influences the RNFL thickness and not the other way around, it is important to bear in mind that it is still unknown whether axonal pathology precedes or follows RGC loss in glaucoma. However, because there is evidence of axonal degeneration can precede cell body degeneration in several neurodegenerative diseases including glaucoma, knowing whether the thickness of the GCIPL layer may be influenced by the thickness of the RNFL layer seems indicated. Thus, this analysis was performed on the basis that axonal dysfunction and degeneration may precede neuronal loss.

Age was a significant determinant of GCIPL thickness in the present study indicating a 0.102% decrease per year increase of age. Yearly age-related decline in RGC population expressed as a percentage of the total number have been estimated histologically at 0.07% to 0.61% by axonal count,³²⁻³⁶ 0.55% to 0.59% by cell count,³⁷⁻³⁹ and 0.1% to 0.35% by RNFL thickness measured using OCT.^{4,8,11,17,22,40-42} A recent analysis of the GCC using RTVue-1000 (Optovue, Inc., Fremont, CA) showed a 0.16% decrease per year,²⁴ which is in line with most OCT-based estimates. Clearly, there is a discrepancy in the age-related rate of RGC losses between imaging and histologic estimates that age itself does not explain. A similar observation was made by Harwerth et al.³⁹ after comparing data obtained from VF with normal glaucoma hemifield tests on SAP 24-2 and RNFL thickness measured using OCT of 55 patients aged 18 to 80 years (mean, 44.5 ± 17.3). After separately estimating the number of axons from SAP and RNFL thickness, they found an age-related thinning of RNFL of 0.27% per year and an age-related loss of RGCs of 0.50% per year from SAP. It is important to note that estimates from imaging studies do not account for contribution of other types of cells that are in the GCL (i.e., displaced amacrine cells)⁴³ or supporting cells contained in the RNFL. In consequence, there remain issues to be resolved as to the real rate of RGC loss due to aging. We found topographic variations in age and axial length-related thinning rate of GCIPL. This phenomenon has been reported for GCC²⁴ and peripapillary RNFL thicknesses^{4,10,17,22,41} and signifies, clinically, that attention should be paid to sectoral GCIPL thinning, as it may be useful in detecting glaucoma and its progression. The correlation of average GCIPL thickness with age was curvilinear, with a steeper drop beyond the age of 60 years, which agrees with other histomorphometric studies on age-related loss of optic nerve fibers.⁴⁴

It was interesting to note that both GCIPL and RNFL thicknesses were not associated with disc area, even when the analysis was performed on a subgroup that only included subjects of European and African descent, which significantly

differed in optic disc size. Because histologically the number of RGCs equals that of axons, this finding suggests that the number of axons within the RNFL is independent of disc size. The association between disc size and RNFL thickness and thus the number of axons has long been controversial. Indeed, some histologic^{33,44,45} and imaging studies^{5,8,23,24,46} have failed to find such an association, whereas others have observed an increase in axonal count^{34,47,48} or RNFL thickness^{4,7,9,11} with increasing disc size. The source of these discrepancies in disc size-dependent effect across histologic and imaging studies is a likely result of the large individual anatomic variability in the number of RGCs and in optic disc size.

It is well established from human and animal studies that there are large differences in RGC number between individuals. Because there is a direct relationship between the number of RGCs and the RGC layer thickness and because RGC count is not possible in living human eyes, GC IPL thickness was used in the present study to indicate the size of the RGC population within the macular area shown in Figure 2. Therefore, the variation in GC IPL thickness found in the present study reflects the real variation in the number of ganglion cells, as previously demonstrated in humans.⁴³

The average GC IPL thickness of 82.1 μm found in this study is 8.9% to 17% higher than values reported by earlier studies performed using time-domain OCT.^{12,14-16} This difference may be attributable in part to the fact that the other studies included the 1-mm diameter area near the fovea, whereas our study did not. This is a region where the GC IPL becomes 0. The difference in resolution between time-domain and spectral-domain OCT may explain, at least in part, the discrepancy in the results. In addition, the thickness of 73.5 μm reported by DeBuc et al.¹² may have been affected by insufficient power of light entering the eye and reflected from the retina, since their scans were acquired without pupil dilation. The thickness profile of macular sectors indicated that the superotemporal and inferior sectors had the thickest and thinnest GC IPL, respectively. In addition, nasal sectors were thicker than temporal ones, and the superior hemisphere had a significantly thinner GC IPL compared to the inferior. This is the first study reporting sectoral GC IPL thickness measurements within the macula. Histologic studies in human^{43,49} and monkey eyes⁵⁰ have shown that in the central retina, the nasal and superior sectors have more ganglion cells than do the temporal and inferior sectors, respectively. Thus, our findings concur with the known normal anatomic distribution of RGCs. Although the right and left eyes included in the present study were not fellow eyes from the same individuals, the lack of difference in average GC IPL thicknesses between eyes may correspond to histologic findings that there is a similarity between the two fellow eyes in ganglion cell topography.⁴³

Caution should be exercised when interpreting the results and implications of our findings. Since the distribution of subjects in the different ethnic groups was uneven, the conclusions about ethnicity must be interpreted with caution. Further studies with more subjects of non-European descent may be warranted to clarify whether ethnic differences in GC IPL thickness should be taken into account when diagnosing glaucoma by using GC IPL thickness measurements. Also ethnicity-specific databases may be needed in the future for reliable use of the normative database software. It is also important to bear in mind that the age-related thinning rate of the GC IPL reported in the present study is derived from regression analysis of cross-sectional, not longitudinal, data. Since it is not known to what extent one can accurately determine the longitudinal thinning rate of retinal layers based on cross-sectional data, it is advisable not to assume that the results reported herein are indicative of estimates that would be obtained if the same subjects were observed longitudinally.

In conclusion, we have generated a normal profile and predictors of GC IPL thickness using a new macular ganglion cell analysis algorithm for the device used in this study. The GC IPL thickness is dependent on age, axial length, and sex, but not on eye, CCT, or optic disc topographic measurements. This information may be useful in making clinical decisions in glaucoma and other optic neuropathies characterized by the loss of RGCs.

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APPENDIX

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