Article

Determinants of Macular Layers and Optic Disc Characteristics on SD-OCT: The Rhineland Study

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Purpose: To investigate variation and determinants of macular layers, peripapillary retinal nerve fiber layer (pRNFL) and Bruch's membrane opening-minimum rim width (BMO-MRW) in the general population.

Methods: In 1306 participants, we performed spectral domain optical coherence tomography (SD-OCT) scans of the macula, pRNFL, and BMO-MRW, and assessed their determinants using multivariable regression. Intraindividual interocular differences were analyzed using Spearman's rank correlation analysis.

Results: Participant age ranged from 30 to 95 years (mean ± standard deviation, 56.1 ± 13.9) and 56% were women. Interocular correlation ranged from 0.63 to 0.93. Differences increased with age and were larger in persons with glaucoma or prior stroke. pRNFL and BMO-MRW decreased with increasing age. Except for RNFL, volumes of various inner macular layers and the outer nuclear layer (ONL) decreased with increasing age, more negative spherical equivalent (SE), and were lower in women compared to men. For some layers, age effects amplified over the life course. History of stroke was associated with smaller volumes of various layers, without reaching statistical significance. We found no association of further systemic parameters with any SD-OCT parameter.

Conclusions: We provide large-scale normative data from a Caucasian general population for various SD-OCT measures. Interocular variability increased with age and specific pathology. Factors, such as age, sex, refraction, and a history of stroke, were associated with various retinal assessments.

Translational Relevance: In clinical routine, our findings should be considered on a per eye basis when interpreting SD-OCT volumes, pRNFL, or BMO-MRW to avoid confounded results.

Introduction

Retinal layer thickness measurements with spectral domain optical coherence tomography (SD-OCT) are important clinical biomarkers for ophthalmic diseases, including glaucoma,[1,2] and are emerging as clinical biomarkers for neurodegenerative diseases, such as multiple sclerosis.[3,4] To date, however, we lack large-scale normative data for quantifiable SD-OCT outcomes, such as volume of the different macular layers from a general population.

The majority of published studies were performed using older SD-OCT devices with potentially lower scan resolution and/or did not consider systemic factors.[5–7] Today, more advanced SD-OCT devices and segmentation algorithms enable the automated and precise identification of retinal layers, including all macular layers, peripapillary retinal nerve fiber layer (pRNFL) as well as Bruch's membrane opening-minimum rim width (BMO-MRW).[8]

The clinical use of these quantifiable SD-OCT markers requires insight into the normal variation and determinants of those measures. These data can be provided by epidemiologic population studies. To

date, however, large-scale data on population variation and determinants of fully segmented macular layers and BMO-MRW in a general population are lacking. The few studies reporting normative values of macular layers and determinants were either based on small samples or did not investigate all macular layers separately.[9,10] The only available population study on determinants of BMO-MRW was conducted in an Asian population[11] and results may not be directly transferable to Caucasians.

Thus, we investigated ocular and systemic factors associated with measurements of all different macular layers, pRNFL, and BMO-MRW as well as their intraindividual interocular variation in a general population.

Methods

Study Population

This study was based on the Rhineland Study, a community-based prospective cohort study to which all inhabitants of two geographically defined areas in the city of Bonn, Germany, who were 30 years or older, were invited. Persons living in those areas were predominantly German with Caucasian ethnicity. Participation in the study was possible by invitation only. The only exclusion criterion was insufficient German language skills to provide informed consent. The study adhered to the tenets of the Declaration of Helsinki and had local ethical committee approval. All participants provided written informed consent. Our analyses were based on the first 1306 participants in the baseline examinations. SD-OCT data for the macular layers, pRNFL, and BMO-MRW were available for 1257, 1256, and 1267 persons, respectively, with complete SD-OCT data available for 1244 persons. The most frequent reason for missing SD-OCT data was technical issues, followed by low compliance during imaging resulting in low image quality.

Assessments

We assessed retinal layers using the Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany). The instrument combines OCT technology with a confocal scanning laser ophthalmoscope and provides an automatic real-time (ART) function that adjusts for eye movement and increases image quality.[12] Data on the individual corneal curvature (c-curve) of participants were entered before scan acquisition to adjust for corneal refraction.[13] The

SD-OCT imaging protocol includes a macular volume scan (97 horizontal B-scans on a $20° \times 20°$ field of imaging with 20 ART-frames) and two scan modalities around the optic nerve head (3.5 mm diameter circular scan with 100 ART-frames and 24 radial scans with 25 ART-frames each). We performed layer segmentation with the inbuilt segmentation algorithm of the Heidelberg Eye Explorer (HEYEX) on the macular volume scan. The HEYEX segmentation algorithm delineates the following macular layers: retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), and retinal pigment epithelium (RPE). Furthermore, the layers from RNFL to ONL are combined to inner retina (IRET), the photoreceptors and the RPE to outer retina (ORET), and all layers to total retina (TRET) measurements. For each layer the device reports the total volume (in mm^3) and the average thickness (in μm) in every sector of a 6 mm diameter Early Treatment Diabetes Retinopathy Study (ETDRS) grid centered on the fovea. We assessed pRNFL thickness and BMO-MRW based on the 3.5 mm circular scan and the 24 radial scans around the optic nerve head, which has been reported as highly precise.[14] Values for pRNFL thickness and BMO-MRW were calculated globally (G) and for six sectors (nasal [N], nasal superior [NS], nasal inferior [NI], temporal [T], temporal superior [TS], and temporal inferior [TI]), with T and N being twice the size of the other sectors.[15] Refraction and best corrected visual acuity (BCVA) were measured with an automated refractometer (Ark-1s; Nidek Co., Tokyo, Japan). IOP was measured using noncontact tonometry (TX-20; Canon, Tokyo, Japan). Spherical equivalent (SE) was calculated as the spherical value and half of the cylindrical value. Participants were dilated for imaging using standard mydriatic agents (tropicamide and phenylephrine). Axial length (AL) was assessed with the Pentacam AXL (Oculus, Wetzlar, Germany).

We investigated the influence of the following nonocular variables, which were selected a priori based on the literature and availability: hypertension (defined as systolic blood pressure [SBP] >139 mmHg and/or diastolic blood pressure [DBP] >89 mmHg and/or use of antihypertensive drugs), diabetes (defined as fasting glycated hemoglobin [HbA1c] > 6.4 % and/or the use of antidiabetic drugs), and self-reported history of stroke and glaucoma.

Data Analyses

Of the 1244 participants with complete SD-OCT data, the scans of six participants did not meet our predefined minimum quality standard of ≥20 dB (of a possible 40 dB) and, hence were excluded from the analyses. Lastly, we cleaned the data before further analyses according to Chauvenet's criterion.[16] This approach removes outliers above or below a certain number of standard deviations (SD) depending on sample size. Following Chauvenet's criterion, we excluded participants with total retinal volume above or below 3.48 SD from mean, leaving 1227 participants for the analyses.

We assessed intraindividual interocular mean and absolute differences and performed Spearman's rank correlation analysis for each layer. Since mean differences were virtually zero (Supplementary Fig. 1), we arbitrarily chose the right eye to be the study eye for all analyses requiring data from one eye only.

To investigate possible determinants of the volume or thickness of the layers, we performed multivariable linear regression including the measurement of each layer as the dependent variable. For ease of comparison between the different retinal layers, we standardized the layer volume and thickness measurements. Hence, the β-coefficients of the regression models indicate percentage of difference rather than absolute values.

We assessed the relations of age, sex, SE as a proxy for refraction, intraocular pressure (IOP), body mass index (BMI), smoking status, and medical history of hypertension, diabetes and stroke with macular layers' volume or thickness by entering them as independent variables in multivariable linear regression models. Additionally, we included age^2 in our models to investigate whether age had a nonlinear impact on our outcomes.

Due to technical issues at the start of the Rhineland Study, data were missing on smoking in the first 184 (15%) and on diabetes in the first 139 (11%) participants. Since participation order in the Rhineland Study was random with respect to measured variables, we imputed these missing variables with multiple imputations using chained equations.[17] Twenty complete imputed datasets were created and regression analyses were performed on each dataset individually. Note that we only imputed missing independent variables of the regression models, but no outcomes. Subsequently, the coefficients were pooled using Rubin's rules.[18] Restricting the analyses to only participants with complete data showed

Table 1. Characteristics of Participants Included in the Analyses

	n	Mean ± SD or *n* (%)
Age, years	1227	56.1 ± 13.9
Women	1227	687 (56)
BCVA	1221	1.1 ± 0.3
IOP, mmHg	1217	14.3 ± 3.2
SE, D	1220	−0.5 ± 2.5
AL, mm	1111	23.9 ± 1.3
BMI, kg/m²	1221	25.9 ± 4.7
Blood pressure, mmHg	1207	
SBP		128.6 ± 16.9
DBP		77.3 ± 9.8
Smoking status	1043	
Never		484 (46)
Former		424 (41)
Current		135 (13)
History of		
Glaucoma	1222	34 (3)
Diabetes mellitus	1088	56 (5)
Hypertension	1190	481 (40)
Stroke	1227	46 (4)

similar effect estimates as from the imputed datasets. As a further sensitivity analysis, we repeated all multivariable regression analyses excluding participants with known glaucoma.

To avoid α error accumulation due to multiple testing, we used a conservative Bonferroni correction and considered results statistically significant at the level α = 0.05/12 = 0.004. All analyses were performed with the statistical software RStudio (R version 3.4.1; RStudio, Inc., Boston, MA, available in the public domain at https://www.rstudio.com/).

Results

The age of the participants included in our analyses ranged from 30 to 95 years (mean [SD] 56.1 ± 13.9 years) and 56% were women (Table 1). Median BMI was 25.1 kg/m², indicating that more than half of our participants were overweight to a certain extent. Study participants with missing or omitted SD-OCT data were of similar age and sex compared to the study population (mean 59.6 ± 15.0 years old and 59% women), but slightly more myopic (mean −1.4 ± 5.2 diopters [D]).

Mean volumes of the macular layers of the right eye were 8.67 ± 0.40, 6.42 ± 0.39, and 2.25 ± 0.07

Table 2. Absolute Intraindividual Interocular Differences per Layer

Layer	All Participants Absolute Difference Mean (95% CI)	History of Stroke, $n = 46$ Absolute Difference Mean (95% CI)	History of Glaucoma, $n = 34$ Absolute Difference Mean (95% CI)
Total Retina	0.12 (0.11–0.13)	0.14 (0.06–0.21)	0.28 (0.12–0.44)
RNFL	0.06 (0.06–0.07)	0.06 (0.04–0.08)	0.07 (0.04–0.11)
GCL	0.03 (0.03–0.03)	0.03 (0.02–0.05)	0.06 (0.03–0.08)
IPL	0.03 (0.02–0.03)	0.02 (0.02–0.03)	0.06 (0.03–0.08)
INL	0.02 (0.02–0.03)	0.03 (0.02–0.04)	0.04 (0.02–0.07)
OPL	0.04 (0.04–0.05)	0.05 (0.04–0.07)	0.05 (0.03–0.06)
ONL	0.05 (0.05–0.05)	0.07 (0.04–0.09)	0.09 (0.06–0.12)
RPE	0.02 (0.01–0.02)	0.02 (0.01–0.02)	0.02 (0.01–0.02)
Inner retina	0.11 (0.10–0.12)	0.13 (0.07–0.18)	0.24 (0.12–0.37)
Outer retina	0.03 (0.03–0.04)	0.04 (0.02–0.07)	0.07 (0.03–0.10)
pRNFL	4.71 (4.34–5.08)	6.25 (1.27–11.23)	11.50 (4.98–18.02)
BMO-MRW	22.42 (21.33–23.52)	17.93 (13.55–22.31)	25.88 (17.89–33.86)

mm^3 for the total, inner, and outer retina, respectively. Mean global assessment was 99.6 ± 11.1 and 337.3 ± 60.1 μm for pRNFL thickness and BMO-MRW, respectively. Figures 1A and 1B depict the measurements of all different layers in the sectors of an ETDRS grid (mean $\pm$ SD).

Comparing the layers of the right and left eyes across individuals, Spearman's rank correlation of the total retinal volume was 0.91 and ranged from 0.63 in the OPL to 0.93 in the ONL. Except for macular RNFL ($r = 0.77$), the inner macular layers were highly correlated ($r = 0.87$ to $r = 0.91$). Correlation coefficients of left and right pRNFL and BMO-MRW were $r = 0.82$ and $r = 0.88$, respectively. Figure 2 shows a correlation matrix of all left and right eye measurements. No relevant changes were detected when excluding participants with an interocular difference of SE > 0.5, 1.0 or 1.5 D, respectively. Mean difference between the left and right eyes was close to zero for all macular layers, justifying the choice of the right eye as the study eye for further analyses. The average intraindividual interocular absolute difference was 0.12 mm^3 for the total retina, 4.7 μm for pRNFL, and 22.4 μm for the BMO-MRW (Table 2). In participants with self-reported stroke and glaucoma interocular absolute differences were larger for almost all layers except BMO-MRW, for which, however, confidence intervals were considerably wider (Table 2). Increasing age was associated with larger absolute differences in all layers except BMO-MRW, even after excluding participants with a history of stroke and glaucoma (data not shown).

The volumes of the macular GCL, IPL, INL, and ONL decreased with increasing age, as did pRNFL thickness and BMO-MRW (Tables 3, 4). Per decade, differences ranged from −1.1% to −3.0% for the macular layers and were −1.8% and −4.5% for pRNFL and BMO-MRW, respectively. Locally weighted scatterplot smoothing (LOWESS) regression plots of age with retinal layer measurements suggested an augmenting effect of age at higher ages for some layers (Figs. 3A, 3B) from the sixth decade onwards. We tested this by adding age^2 to the regression models and found, indeed, a significant nonlinear effect of age on GCL and IPL volume (both $P < 0.001$), and a borderline significant effect for total retina volume ($P = 0.001$), indicating a further acceleration of volume or thickness loss beyond the sixth decade.

More positive SE was associated with larger volumes of GCL, IPL, INL, and ONL (range, 0.6%–1.0% per D) and thicker pRNFL (0.7% per D). In contrast, the volume of macular RNFL was smaller with more positive SE (−1.0% per D) and tended to be higher with higher age. Volumes of the GCL, INL, and ONL were lower in women than men with differences ranging from −1.3% to −3.0%. For IPL the pattern was similar, but this did not reach statistical significance (−1.3%; 95% confidence interval [CI] = −2.2%; −0.4%). Additional adjustment for AL did not alter any of these associations (data not shown). Former smokers had lower volumes of macular GCL (−1.6%; 95% CI = −2.8%; −0.5%) and IPL (−1.3; 95% CI = −2.3%; −0.3%) and thinner pRNFL (−1.5%; 95% CI = −2.8%; −0.1%) as compared to never smokers, but these differences were not statistically significant after Bonferroni
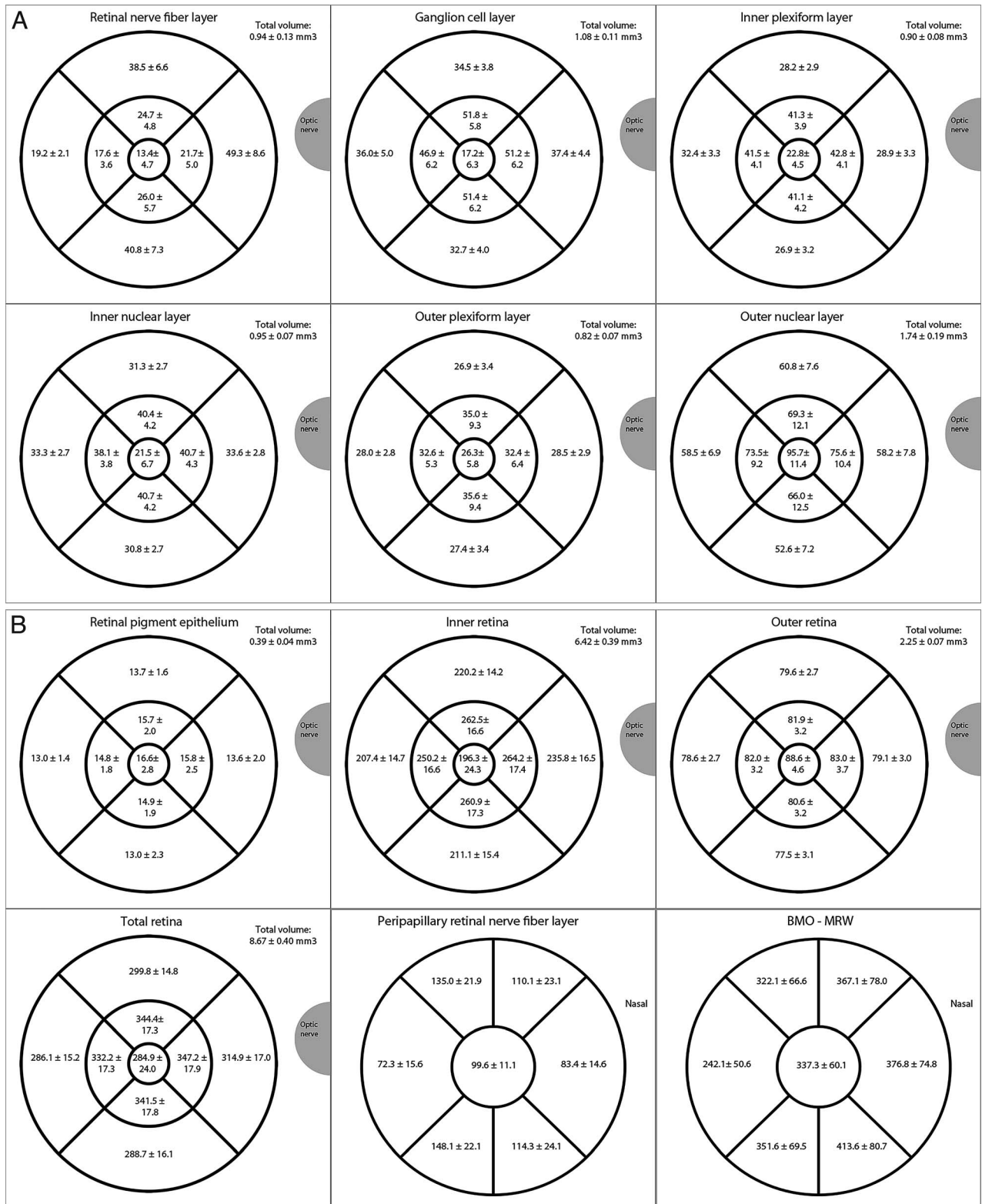
Figure 1. (A) Mean thickness and volume ($\pm$ SD) of macular layers, $n = 1227$. (B) Mean thickness and volume ($\pm$ SD) of macular layers, peripapillary RNFL, and BMO-MRW, $n = 1227$.

Table 3. Associations of Age, Sex, SE, IOP, and BMI with Macular Layers, pRNFL, and BMO-MRW (n = 1227)

	Age, per decade		Women vs. Men		SE, per D	
	Difference., % (95% CI)	P*	Difference, % (95% CI)	P*	Difference, % (95% CI)	P*
RNFL	0.84 (0.16; 1.51)	0.02	1.35 (−0.23; 2.93)	0.09	−1.02 (−1.34; −0.70)	<0.001*
GCL	−2.98 (−3.42; −2.55)	<0.001*	−1.59 (−2.61; −0.56)	<0.001*	0.77 (0.56; 0.98)	<0.001*
IPL	−2.22 (−2.61; −1.83)	<0.001*	−1.31 (−2.22; −0.39)	0.005	0.62 (0.43; 0.81)	<0.001*
INL	−1.09 (−1.43; −0.76)	<0.001*	−2.17 (−2.95; −1.39)	<0.001*	0.61 (0.45; 0.77)	<0.001*
OPL	0.46 (0.03; 0.90)	0.04	−0.06 (−1.08; 0.97)	0.91	0.01 (−0.20; 0.22)	0.93
ONL	−1.43 (−1.95; −0.91)	<0.001*	−3.00 (−4.22; −1.77)	<0.001*	1.01 (0.76; 1.26)	<0.001*
RPE	0.30 (−0.18; 0.78)	0.22	−1.46 (−2.60; −0.33)	0.01	0.19 (−0.05; 0.42)	0.12
Inner retina	−1.17 (−1.45; −0.90)	<0.001*	−1.36 (−2.01; −0.71)	<0.001*	0.43 (0.29; 0.56)	<0.001*
Outer retina	−0.14 (−0.29; 0.01)	0.07	−1.00 (−1.36; −0.64)	<0.001*	0.08 (0.00; 0.15)	0.04
Total retina	−0.91 (−1.12; −0.69)	<0.001*	−1.26 (−1.76; −0.76)	<0.001*	0.34 (0.24; 0.44)	<0.001*
pRNFL	−1.75 (−2.27; −1.23)	<0.001*	0.50 (−0.72; 1.73)	0.42	0.67 (0.42; 0.92)	<0.001*
BMO-MRW	−4.46 (−5.27; −3.64)	<0.001*	1.30 (−0.62; 3.22)	0.19	0.39 (0.00; 0.78)	0.05

Multivariable regression models adjusted for age, sex, SE, IOP, BMI, smoking status, hypertension, diabetes, and history of stroke.

* Bonferroni adjusted level of statistical significance (P < 0.004).

Table 3. Extended

	IOP, per mmHg		BMI, per kg/m²	
	Difference, % (95% CI)	P*	Difference, % (95% CI)	P*
RNFL	−0.08 (−0.32; 0.17)	0.54	−0.16 (−0.33; 0.02)	0.09
GCL	0.10 (−0.06; 0.26)	0.23	−0.01 (−0.12; 0.11)	0.88
IPL	0.11 (−0.03; 0.26)	0.12	0.03 (−0.07; 0.14)	0.52
INL	0.08 (−0.04; 0.20)	0.21	−0.01 (−0.10; 0.08)	0.85
OPL	−0.02 (−0.18; 0.14)	0.76	0.02 (−0.10; 0.13)	0.77
ONL	0.14 (−0.05; 0.33)	0.15	−0.11 (−0.25; 0.03)	0.13
RPE	0.07 (−0.11; 0.25)	0.45	−0.09 (−0.22; 0.03)	0.15
Inner retina	0.07 (−0.03; 0.17)	0.19	−0.04 (−0.12; 0.03)	0.25
Outer retina	0.04 (−0.01; 0.10)	0.14	−0.04 (−0.08; 0.00)	0.07
Total retina	0.06 (−0.02; 0.14)	0.12	−0.04 (−0.10; 0.02)	0.15
pRNFL	0.05 (−0.14; 0.24)	0.63	0.05 (−0.09; 0.19)	0.49
BMO-MRW	−0.06 (−0.36; 0.24)	0.69	0.19 (−0.03; 0.40)	0.09

correction. A similar pattern was seen for participants with a history of stroke, which was statistically significant for pRNFL and borderline statistically significant for GCL and IPL. Differences for pRNFL, GCL, and IPL were −4.9% (95% CI = −8.1%; −1.7%), −3.3% (95% CI = −6.0%; −0.7%) and −2.8% (95% CI = −5.2%; −0.4%), respectively (Table 4). We found no associations of IOP, BMI, current versus never smoking, hypertension, and diabetes with any SD-OCT outcome. After excluding participants with self-reported age-related macular degeneration (n = 36), epiretinal gliosis (n = 10), diabetic retinopathy (n = 4),

and glaucoma (n = 34) overall results did not change (data not shown).

Discussion

Our study provides normative data from a general population for quantifiable SD-OCT outcomes. We found that interocular variability can be substantial for some layers and increases with age and specific pathology (stroke, glaucoma). Beyond the sixth decade, the impact of age seems to accelerate. Our results implied that not only ocular but also systemic

Table 4. Associations of Smoking Status, Hypertension, Diabetes, and Stroke with Macular Layers, pRNFL and BMO-MRW (n = 1227)

	Former Smoking vs. Never Smoking		Current Smoking vs. Never Smoking	
	Difference, % (95% CI)	P*	Difference, % (95% CI)	P*
RNFL	0.23 (−1.59; 2.04)	0.81	2.66 (−0.18; 5.50)	0.07
GCL	−1.64 (−2.79; −0.49)	0.005	0.53 (−1.14; 2.20)	0.54
IPL	−1.30 (−2.33; −0.27)	0.013	0.72 (−0.77; 2.21)	0.34
INL	−0.29 (−1.20; 0.61)	0.52	0.29 (−1.01; 1.60)	0.66
OPL	−0.75 (−1.93; 0.42)	0.21	−0.25 (−1.97; 1.46)	0.77
ONL	0.65 (−0.73; 2.03)	0.35	0.03 (−2.03; 2.09)	0.98
RPE	−0.01 (−1.37; 1.35)	0.99	1.40 (−0.49; 3.30)	0.15
Inner retina	−0.36 (−1.09; 0.37)	0.34	0.59 (−0.52; 1.70)	0.29
Outer retina	0.20 (−0.23; 0.62)	0.37	0.17 (−0.44; 0.78)	0.59
Total retina	−0.22 (−0.78; 0.35)	0.45	0.48 (−0.38; 1.34)	0.27
pRNFL	−1.46 (−2.84; −0.08)	0.04	1.30 (−0.76; 3.35)	0.22
BMO-MRW	−0.12 (−2.26; 2.03)	0.91	0.32 (−2.96; 3.59)	0.85

Multivariable regression models adjusted for age, sex, SE, IOP, BMI, smoking status, hypertension, diabetes, and history of stroke.

* Bonferroni adjusted level of statistical significance (P < 0.004).

Table 4. Extended

	Hypertension		Diabetes		Stroke	
	Difference, % (95% CI)	P*	Difference, % (95% CI)	P*	Difference, % (95% CI)	P*
RNFL	0.19 (−1.80; 2.18)	0.85	0.26 (−3.70; 4.22)	0.90	0.55 (−3.57; 4.67)	0.79
GCL	−0.72 (−1.98; 0.55)	0.27	0.71 (−1.79; 3.21)	0.58	−3.33 (−6.01; −0.66)	0.015
IPL	−0.56 (−1.68; 0.56)	0.33	−0.08 (−2.32; 2.15)	0.94	−2.81 (−5.19; −0.42)	0.021
INL	0.24 (−0.71; 1.20)	0.61	0.40 (−1.47; 2.27)	0.67	−1.34 (−3.39; 0.70)	0.20
OPL	−0.36 (−1.61; 0.90)	0.57	−2.08 (−4.58; 0.43)	0.10	1.14 (−1.54; 3.82)	0.40
ONL	−0.97 (−2.46; 0.51)	0.20	−1.39 (−4.39; 1.61)	0.36	0.20 (−3.01; 3.40)	0.90
RPE	0.01 (−1.38; 1.39)	0.99	0.37 (−2.34; 3.08)	0.79	2.27 (−0.70; 5.24)	0.13
Inner retina	−0.50 (−1.30; 0.31)	0.23	−0.54 (−2.06; 0.98)	0.49	−0.84 (−2.55; 0.86)	0.33
Outer retina	−0.06 (−0.49; 0.38)	0.80	−0.40 (−1.25; 0.46)	0.36	0.29 (−0.64; 1.23)	0.54
Total retina	−0.38 (−1.00; 0.24)	0.23	−0.51 (−1.67; 0.66)	0.39	−0.55 (−1.86; 0.76)	0.41
pRNFL	−0.66 (−2.15; 0.83)	0.39	1.22 (−1.81; 4.25)	0.43	−4.89 (−8.09; −1.69)	0.003*
BMO-MRW	−0.25 (−2.59; 2.09)	0.83	−0.06 (−4.44; 4.32)	0.98	−4.17 (−9.19; 0.84)	0.10

factors must be considered when evaluating SD-OCT, in particular in longitudinal evaluations, as for example an incident stroke may confound the assessments.

We found that predominantly volumes of the inner macular layers (GCL, IPL, and INL) decreased with age and more negative SE and were lower in women compared to men. Our findings are in agreement with earlier studies that did not differentiate between single macular layers, but assessed the ganglion cell-inner plexiform layer (GC-IPL)[7] and the ganglion cell

complex (GCC), which is composed of RNFL, GCL, and IPL.[19,20] This age-related decline of GCL thickness has been reported as result of a loss of cells and axons with aging.[9] Our findings suggested that this cell loss accelerates with age from the sixth decade onwards. To a certain extent this may be due to higher prevalence of ocular conditions at older ages. However, prevalence of self-reported diseases was low and excluding these participants from the analyses did not change the results.

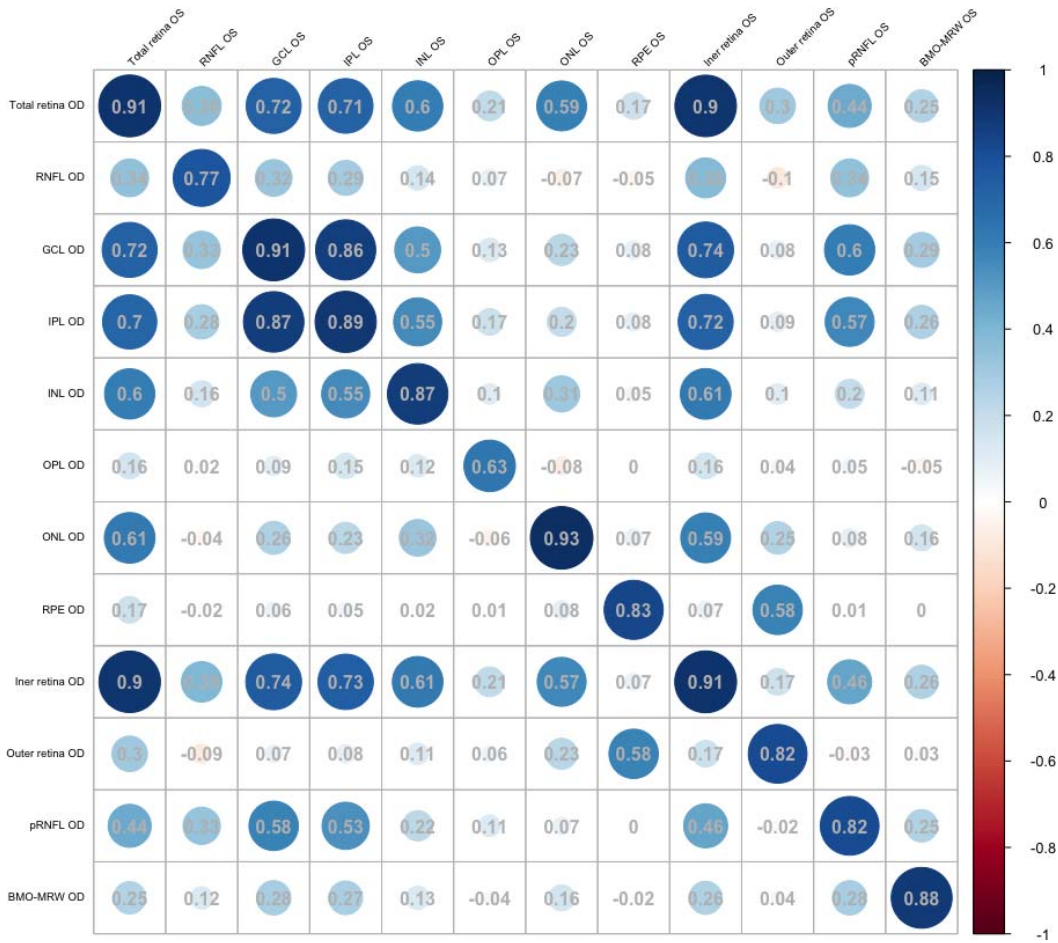Apart from the GCL and IPL, we found these

Figure 2. Interocular Spearman's rank correlation matrix of macular layers, peripapillary RNFL and BMO-MRW.

determinants to affect two further layers, the INL and the ONL. While the INL is composed of bipolar, horizontal, and amacrine cells in the inner macula, the ONL consists of the nuclear bodies of the photoreceptors. To our knowledge, our study is the first to describe these associations. A recent study on five macular layers reported no association of age with the OPL/ONL complex, but did not distinguish between OPL and ONL and was conducted in a much smaller sample.[9] In contrast, our findings suggested an age-related decrease in INL and ONL volume, which is likely to reflect cell decline in these layers.

Several studies have reported an association of SE and volume or thickness of retinal layer measurements, but the underlying mechanisms remain unclear.[7,21,22] Frequently suggested mechanisms are either axial stretching or artificially decreased measurements due to ocular magnification.[21,22] The

clinical relevance of adjusting for refraction in OCT imaging, however, seems obvious irrespective of the causal mechanism. We found that older age and more negative SE were associated with higher macular RNFL volumes, which has been described previously.[6,9] We hypothesize that foveal shape changes with aging, such as flattening of the foveal pit, may result in an altered measurement especially of the inner layers, which are extremely thin at the fovea. To our knowledge, only few and contradicting reports exist on determinants of foveal shape[23–25] and further research is warranted.

Women had lower volumes of different macular layers than men, which is in agreement with previous studies.[5,20] Though women tend to have shorter eyes in general,[26] the sex difference remained when controlling for AL or SE. While we cannot fully explain what underlies this sex difference, it is
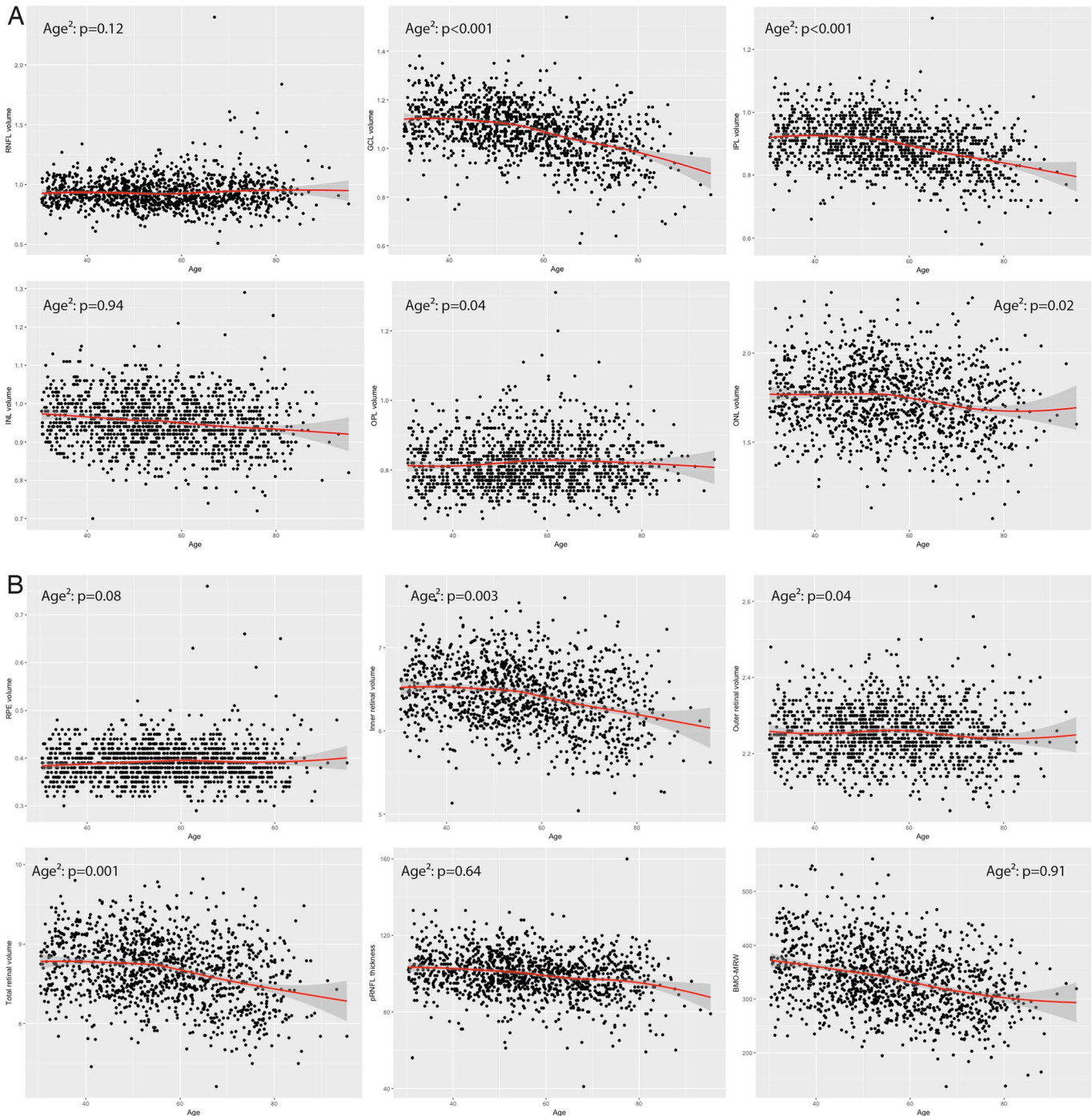
Figure 3. LOWESS regression plots of age and the respective layers.

important to take this into account when determining, for example, a normal range.

We observed no association of arterial hypertension and diabetes with any SD-OCT assessment, which partly contradicts previous reports on pRNFL.[27–29] However, we found a large effect of stroke on macular layer measurements, underlining the strong impact of (neuro)-vascular diseases on various retinal layers. Our results suggest that the retinal ganglion cells, their axons and their interconnections with other retinal cell types degenerate post stroke. However, we lack information on type

(ischemic/hemorrhagic) and localization of stroke. While we found that BMO-MRW declines by −4.5% per decade, the only other study on determinants of BMO-MRW reported a decrease of only −3.4% per decade in a Japanese population ($n = 258$).[11] The diversity in population characteristics may partly explain the difference in results. Furthermore, our analyses were adjusted for a larger number of potentially confounding variables, mostly systemic parameters, which were not considered in that previous study. Interestingly, we found the age-related decline of BMO-MRW (and less strongly of GCL and pRNFL assessments) already to be present between the ages of 30 to 50 (Figs. 3A, 3B). This may reflect that the ganglion cells and their axons are fully differentiated and that no further mitosis takes place as compensatory mechanism.

Our data depict potentially relevant intraindividual differences in various retinal layers between both eyes, which is in line with previous studies on pRNFL variability.[30,31] Mean differences between eyes were virtually zero and, hence, indicate no systematic differences between left and right eyes. Based on our data, arbitrarily choosing one eye to assess retinal layers in a research context is, therefore, appropriate. The absolute differences between eyes were smaller than interindividual SD, indicating less variation between eyes than between individuals. This does not apply to participants with previous stroke or known glaucoma, in which we found larger intraindividual differences. Moreover, we found that interocular differences increased with age, independent of stroke or glaucoma. To what extent this reflects clinically relevant accumulating pathology remains to be investigated.

Our results implied that, especially in longitudinal studies or clinical follow-up of SD-OCT imaging, an incident stroke should be considered as it may cause a decrease in the volume of various layers. In clinical monitoring of ophthalmic diseases, such as glaucoma, this may simulate an artificial aggravation.

The strengths of this study include the large community-based population with a wide age range from 30 to 95 years. The study protocol comprised comprehensive and standardized ocular and systemic phenotyping enabling the investigation of a variety of parameters. SD-OCT imaging was performed on both eyes using a state-of-the-art device with high resolution scans and automated layer segmentation, including the deeper outer retinal layers, on which we until now, lacked population data. To our knowledge, this study is one of the first to report on determinants of fully segmented macular layers, pRNFL, BMO-MRW, and the interocular variability in such a large Caucasian population. Yet, several limitations must be considered. Even though participation was possible by invitation only, a self-selection of more healthy participants may have occurred. This will probably not have affected our estimates of variability in a normal general population. However, it may have left our study underpowered to ascertain the effect of certain diseases, such as diabetes or stroke, for which prevalence was low. Furthermore, information on glaucoma and stroke was self-reported and less reliable than medical diagnoses. Any resulting misclassification most likely led us to underestimate related effect sizes in our study. We did not manually check segmentation. However, we excluded scans below a relatively strict quality threshold (20 dB), excluded outliers and performed sensitivity analyses excluding participants with self-reported eye diseases. Thus, given the large sample size remaining segmentation artefacts are unlikely to confound our results to a relevant extent.

In conclusion, we provide data on population variation and determinants of macular layers, pRNFL, and BMO-MRW from a large German, mostly Caucasian, population. Moreover, we established that interocular differences can be substantial for some layers and increase with ocular or systemic pathology and age. Our results help to evaluate the different retinal layers and should be considered in clinical settings and future research.

References

1. Mwanza JC, Oakley JD, Budenz DL, Anderson DR. Ability of cirrus HD-OCT optic nerve head parameters to discriminate normal from glaucomatous eyes. *Ophthalmology*. 2011;118:241–248.e1.

2. Gardiner SK, Fortune B, Demirel S. Localized changes in retinal nerve fiber layer thickness as a predictor of localized functional change in glaucoma. *Am J Ophthalmol*. 2016;170:75–82.

3. Jones-Odeh E, Hammond CJ. How strong is the relationship between glaucoma, the retinal nerve fibre layer, and neurodegenerative diseases such as Alzheimer's disease and multiple sclerosis? *Eye (London)*. 2015;29:1270–1284.

4. Balk LJ, Cruz-Herranz A, Albrecht P et al. Timing of retinal neuronal and axonal loss in MS: a longitudinal OCT study. *J Neurol*. 2016;263: 1323–1331.

5. Ooto S, Hangai M, Tomidokoro A et al. Effects of age, sex, and axial length on the three-dimensional profile of normal macular layer structures. *Invest Ophthalmol Vis Sci*. 2011;52: 8769–8779.

6. Demirkaya N, van Dijk HW, van Schuppen SM et al. Effect of age on individual retinal layer thickness in normal eyes as measured with spectral-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2013;54:4934–4940.

7. Mwanza JC, Durbin MK, Budenz DL et al. Profile and predictors of normal ganglion cell-inner plexiform layer thickness measured with frequency-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2011;52:7872–7879.

8. Chauhan BC, Burgoyne CF. From clinical examination of the optic disc to clinical assessment of the optic nerve head: a paradigm change. *Am J Ophthalmol*. 2013;156:218–227.e2.

9. Altay L, Jahn C, Arikan Yorgun M et al. Alteration of retinal layers in healthy subjects over 60 years of age until nonagenarians. *Clin Ophthalmol*. 2017;11:1499–1503.

10. Won JY, Kim SE, Park YH. Effect of age and sex on retinal layer thickness and volume in normal eyes. *Medicine*. 2016;95:e5441.

11. Araie M, Iwase A, Sugiyama K et al. Determinants and characteristics of Bruch's membrane opening and Bruch's membrane opening-minimum rim width in a normal Japanese population. *Invest Ophthalmol Vis Sci*. 2017;58:4106–4113.

12. Rougier M-B, Korobelnik JF, Malet F et al. Retinal nerve fibre layer thickness measured with SD-OCT in a population-based study of French elderly subjects: the Alienor study. *Acta Ophthalmol*. 2015;93:539–545.

13. Ctori I, Gruppetta S, Huntjens B. The effects of ocular magnification on Spectralis spectral domain optical coherence tomography scan length. *Graefes Arch Klin Exp Ophthalmol*. 2015;253:733–738.

14. Schrems-Hoesl LM, Schrems WA, Laemmer R, Kruse FE, Mardin CY. Precision of optic nerve head and retinal nerve fiber layer parameter measurements by spectral-domain optical coherence tomography. *J Glaucoma*. 2018;27:407–414.

15. Gmeiner JMD, Schrems WA, Mardin CY, Laemmer R, Kruse FE, Schrems-Hoesl LM. Comparison of Bruch's membrane opening minimum rim width and peripapillary retinal nerve fiber layer thickness in early glaucoma assessment. *Invest Ophthalmol Vis Sci*. 2016;57: OCT575–584.

16. Chauvenet W. A. *Manual of Spherical and Practical Astronomy,* 5th ed. Philadelphia: Lippincott Company; 1906:469–566.

17. White IR, Royston P, Wood AM. Multiple imputation using chained equations: issues and guidance for practice. *Stat Med*. 2011;30:377–399.

18. Rubin DB. *Multiple Imputation for Nonresponse in Surveys*. New York: John Wiley & Sons, Inc.; 1987.

19. Kim NR, Kim JH, Lee J, Lee ES, Seong GJ, Kim CY. Determinants of perimacular inner retinal layer thickness in normal eyes measured by Fourier-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2011;52:3413–3418.

20. Koh VT, Tham YC, Cheung CY, et al. Determinants of ganglion cell-inner plexiform layer thickness measured by high-definition optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2012;53:5853–5859.

21. Higashide T, Ohkubo S, Hangai M et al. Influence of clinical factors and magnification correction on normal thickness profiles of macular retinal layers using optical coherence tomography. *PloS One*. 2016;11:e0147782.

22. Hanno T von, Lade AC, Mathiesen EB, Peto T, Njlstad I, Bertelsen G. Macular thickness in healthy eyes of adults (N = 4508) and relation to sex, age and refraction: the Troms Eye Study (2007-2008). *Acta Ophthalmol*. 2017;95:262–269.

23. Gorrand JM, Delori FC. Reflectance and curvature of the inner limiting membrane at the foveola. *J Opt Soc Am A Opt Image Sci Vis*. 1999;16:1229–1237.

24. Dubis AM, McAllister JT, Carroll J. Reconstructing foveal pit morphology from optical coherence tomography imaging. *Br J Ophthalmol*. 2009;93:1223–1227.

25. Tick S, Rossant F, Ghorbel I et al. Foveal shape and structure in a normal population. *Invest Ophthalmol Vis Sci*. 2011;52:5105–5110.

26. Lee KE, Klein BEK, Klein R, Quandt Z, Wong TY. Association of age, stature, and education with ocular dimensions in an older white population. *Arch Ophthalmol*. 2009;127:88–93.

27. Mauschitz MM, Bonnemaijer PWM, Diers K et al. Systemic and ocular determinants of peripapillary retinal nerve fiber layer thickness measurements in the European eye epidemiology (E3) population. *Ophthalmology*. 2018;125:1526–1536.

28. van Dijk HW, Verbraak FD, Kok PHB et al. Decreased retinal ganglion cell layer thickness in patients with type 1 diabetes. *Invest Ophthalmol Vis Sci*. 2010;51:3660–3665.

29. van Dijk HW, Verbraak FD, Kok PHB et al. Early neurodegeneration in the retina of type 2 diabetic patients. *Invest Ophthalmol Vis Sci*. 2012;53:2715–2719.

30. Hwang YH, Song M, Kim YY, Yeom DJ, Lee JH. Interocular symmetry of retinal nerve fibre layer thickness in healthy eyes: a spectral-domain optical coherence tomographic study. *Clin Exp Opt*. 2014;97:550–554.

31. Al-Haddad C, Antonios R, Tamim H, Noureddin B. Interocular symmetry in retinal and optic nerve parameters in children as measured by spectral domain optical coherence tomography. *Br J Ophthalmol*. 2014;98:502–506.

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