Associations With Retinal Nerve Fiber Layer Measures in the EPIC-Norfolk Eye Study

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PURPOSE. To describe GDxVCC retinal nerve fiber layer (RNFL) measures and associations in a predominantly white British population.

METHODS. The EPIC-Norfolk Eye Study is nested within a large multicenter cohort study, the European Prospective Investigation of Cancer. RNFL measurements were taken using the GDxVCC. Generalized estimating equation models were used to assess associations of RNFL measures with age, sex, body mass index (BMI), height, blood pressure, social class, education level, alcohol intake, smoking status, axial length, intraocular pressure, and lens status. Models were linearly adjusted for typical scan score to handle scans with atypical retardation.

RESULTS. There were complete data from 11,030 eyes of 6309 participants with mean age 68 years (48–90 years). Older age (−1.53 $\mu\text{m}/\text{decade}$ [95% confidence interval {CI} −1.73, −1.33], $P < 0.001$), male sex (−0.44 μm [95% CI −0.04, −0.84], $P = 0.031$), shorter axial length (−0.15 $\mu\text{m}/\text{mm}$ [95% CI −0.02, −0.28], $P = 0.024$), and pseudophakia (−0.49 μm [95% CI −0.94, −0.04], $P = 0.033$) were associated with thinner RNFL after adjustment for possible confounders. Higher BMI was associated with a thinner RNFL in men only (−0.30 $\mu\text{m}/5 \text{ kg}/\text{m}^2$ [95% CI −0.58, −0.02], $P = 0.039$).

CONCLUSIONS. This analysis of associations with RNFL thickness in a largely healthy population may provide insight into the determinants of glaucoma, suggesting higher risk in those who are older, in men, and in men with a higher BMI.

Keywords: scanning laser polarimetry, epidemiology, axial length, eye, glaucoma, body mass index

Assessment of the peripapillary retinal nerve fiber layer (RNFL) is an important part of the management of patients with suspected or established glaucoma. RNFL defects are seen prior to identifiable visual field loss in a significant proportion of patients with glaucoma.^{1,2} One technique for assessing RNFL is scanning laser polarimetry (SLP), which makes use of the birefringent properties of the RNFL.³ Area under the receiver operating characteristic curve (AUROC) statistics for SLP parameters in the diagnosis of early or more advanced glaucoma have been found to be in the region of 0.9.^{4,5} SLP has been found to be equal to other established methods of assessing RNFL for detecting glaucoma, namely optical coherence tomography (OCT) and scanning laser ophthalmoscopy.⁶

Examination of quantitative traits related to glaucoma (such as RNFL parameters) in healthy participants may provide insight into the etiology of glaucoma. Compared with examining glaucoma as a dichotomous outcome, quantitative trait analysis is less susceptible to misclassification bias and may provide more power for examining small associations. This approach has been successful in genetic association studies for glaucoma.⁷ The objectives of this report were to describe RNFL measures made with the GDxVCC in the EPIC-Norfolk Eye

Study and to describe associations with demographic, systemic, and ocular factors.

METHODS

Participants

EPIC-Norfolk, one of the United Kingdom arms of the European Prospective Investigation of Cancer (EPIC),⁸ is a prospective cohort study that recruited and examined 25,639 participants aged 40 to 79 years between 1993 and 1997.⁹ Recruitment was via general practices in the city of Norwich and the surrounding small towns and rural areas, and methods have been described in detail previously.⁹ Since virtually all residents in the United Kingdom are registered with a general practitioner through the National Health Service, general practice lists serve as population registers. This may therefore be considered a population-based study of persons receiving medical care in the Norwich region of the United Kingdom. Assessment of visual health formed part of the third health examination, and this is known as the EPIC-Norfolk Eye Study.¹⁰ In total, 8623 participants were seen for this examination between 2004 and

2011. The EPIC-Norfolk Eye Study was carried out following the principles of the Declaration of Helsinki and the Research Governance Framework for Health and Social Care. The study was approved by the Norfolk Local Research Ethics Committee (05/Q0101/191) and East Norfolk & Waveney NHS Research Governance Committee (2005EC07L). All participants gave written, informed consent.

Measurements

RNFL measurements were taken using the GDxVCC (Carl Zeiss Meditec, Inc., Dublin, CA), without pupil dilation, according to a standardized operating procedure. Spherical equivalent values derived from an autorefractor (Auto-Refractor 500, Humphrey Instruments, San Leandro, CA) were inputted. Initially a corneal scan was taken, followed by the RNFL scan. Scans were repeated to aim for a quality score of at least 7. The software automatically delineated an annulus, with an inner and outer diameter of 2.4 and 3.2 mm, respectively, centered on the optic disc. Only scans with a quality score of at least 7 were included in the analyses, based on manufacturer recommendation. Parameters considered were the average RNFL thickness and RNFL modulation (SD) within the annulus, and the nerve fiber indicator (NFI), a neural network-derived parameter designed to maximally discriminate between glaucomatous eyes and healthy controls.¹¹

Axial length was measured using a Zeiss IOLMaster Optical Biometer (Carl Zeiss Meditec Ltd., Welwyn Garden City, UK). Five measurements were taken per eye and a mean was calculated. Intraocular pressure (IOP) was measured using a noncontact appliance, the Ocular Response Analyzer (ORA; Reichert, Corp., Buffalo, NY). Three readings were taken per eye and the single best value of the Goldmann-correlated value was used (based on the best quality pressure waveform as assessed by the ORA software).

Height and weight were measured at the third health examination, with participants wearing light clothing and no shoes. Height was measured to 0.1 cm by using a stadiometer, and weight was measured to the nearest 0.1 kg by using digital scales (Tanita UK Ltd., Middlesex, UK). Body mass index (BMI) was calculated as weight/height². Self-reported alcohol intake and smoking status were also ascertained at the third health examination. Alcohol intake was calculated as units per week based on a questionnaire asking how much beer/cider/lager (half pints), wine (glasses), sherry/fortified wine (glasses), or spirits (single measures) were drunk for each day in the last week. Blood pressure was measured using an objective measurement device (Accutorr Plus; Datascope Patient Monitoring, Mindray UK, Ltd., Huntington, UK), also at the third health examination. Social class and educational level were ascertained at the first health examination. Social class was recorded according to the Registrar-General's occupation-based classification system and was based on the participant's last occupation if they were retired. Educational level was recorded and classified into four groups according to the highest qualification achieved.

Statistical Analysis

Comparisons were made between demographic characteristics of included and excluded participants. The independent samples *t*-test was used for continuous variables (which were normally distributed), and the χ^2 test for categorical variables.

Linear regression models were used to assess the association between RNFL measures and demographic, systemic, and ocular parameters. To handle data affected by atypical retardation pattern, regression models were linearly adjusted for typical scan score (TSS) based on a comparison of methods

detailed in the Supplementary Text and Supplementary Tables S1 and S2. Data from both eyes of each participant were considered, and generalized estimating equation models were used to account for the correlation between eyes. Initially, explanatory variables were included one at a time, adjusted for TSS only. Variables found to be significant at the $P < 0.1$ level were then included together in multivariable linear regression models, again adjusted for TSS. IOP, despite not being significant in the crude analyses, was included in the multivariable regression models given its known importance in the pathogenesis of glaucoma. Analyses were repeated stratified by sex, and any differences seen were assessed further by a Wald test of the relevant interaction term included in a model containing all participants.

For the main analyses presented, we did not exclude participants with glaucoma because we wanted to give an overview of RNFL epidemiology in the population as a whole. We subsequently repeated analyses following exclusion of participants who reported use of ocular hypotensive medication or a history of glaucoma surgery or laser.

Stata version 12.1 (StataCorp LP, College Station, TX) was used for all analyses and R version 2.15.1 (The R Foundation for Statistical Computing, Vienna, Austria) was used for plotting of figures.

RESULTS

There were complete data for RNFL measures and covariates from 13,886 eyes of 7101 participants. After exclusion of 2856 eyes (20.6%) with a poor quality RNFL scan, data from 11,030 eyes of 6309 participants were included in subsequent analyses. Compared to the rest of the baseline cohort originally

Figure. Plot of predicted RNFL average thickness by BMI for men and women (solid lines) with 95% CIs (dotted lines). Predicted values were derived from linear generalized estimating equation models, adjusted for age, height, systolic blood pressure, education, alcohol intake, axial length, lens status, IOP, and TSS.

TABLE 1. Regression Results With RNFL Measures as Dependent Variables

	Average Thickness, μm			Modulation, μm			Nerve Fiber Indicator		
	Coef	95% CI	P	Coef	95% CI	P	Coef	95% CI	P
Age, per decade	−1.65	(−1.82, −1.47)	<0.001	−1.41	(−1.54, −1.28)	<0.001	3.02	(2.71, 3.33)	<0.001
Sex									
Men	Ref			Ref			Ref		
Women	0.47	(0.20, 0.74)	0.001	0.01	(−0.20, 0.21)	0.94	−1.34	(−1.81, −0.87)	<0.001
BMI, per 5 kg/m ²	−0.09	(−0.25, 0.07)	0.26	−0.07	(−0.19, 0.05)	0.25	−0.02	(−0.29, 0.25)	0.91
Height, per 10 cm	0.06	(−0.09, 0.21)	0.44	0.20	(0.08, 0.31)	0.001	0.08	(−0.17, 0.34)	0.52
SBP, per 10 mm Hg	−0.08	(−0.16, −0.00)	0.05	−0.07	(−0.13, −0.01)	0.03	0.25	(0.11, 0.39)	0.001
DBP, per 10 mm Hg	0.13	(−0.02, 0.27)	0.08	0.12	(0.01, 0.22)	0.038	−0.17	(−0.42, 0.08)	0.18
Social class									
Professional	Ref			Ref			Ref		
Managerial/technical	−0.21	(−0.71, 0.28)	0.40	−0.06	(−0.43, 0.31)	0.75	0.34	(−0.51, 1.20)	0.43
Skilled nonmanual	−0.92	(−1.49, −0.36)	0.001	−0.42	(−0.85, −0.00)	0.049	1.26	(0.29, 2.24)	0.011
Skilled manual	−0.50	(−1.04, 0.04)	0.07	−0.21	(−0.61, 0.20)	0.31	0.73	(−0.20, 1.66)	0.12
Partly skilled	−0.10	(−0.70, 0.51)	0.76	−0.03	(−0.49, 0.43)	0.90	0.01	(−1.04, 1.06)	0.99
Unskilled	−1.23	(−2.26, −0.20)	0.019	−0.82	(−1.60, −0.05)	0.037	1.62	(−0.16, 3.40)	0.07
Education level									
Degree	Ref			Ref			Ref		
A level	−0.24	(−0.62, 0.13)	0.21	−0.06	(−0.34, 0.22)	0.67	0.13	(−0.52, 0.78)	0.70
O level	−0.20	(−0.70, 0.31)	0.44	−0.18	(−0.56, 0.19)	0.34	0.01	(−0.86, 0.87)	0.98
Less than O level	−0.90	(−1.31, −0.48)	<0.001	−0.45	(−0.76, −0.14)	0.005	0.77	(0.05, 1.49)	0.036
Alcohol intake									
No intake	Ref			Ref			Ref		
<7 units/wk	0.10	(−0.28, 0.48)	0.61	−0.11	(−0.39, 0.18)	0.46	−0.18	(−0.84, 0.47)	0.59
≥7 to <14 units/wk	0.11	(−0.28, 0.51)	0.58	0.13	(−0.17, 0.42)	0.39	−0.04	(−0.72, 0.64)	0.92
≥14 to <21 units/wk	0.17	(−0.31, 0.65)	0.48	0.31	(−0.05, 0.67)	0.09	−0.66	(−1.49, 0.17)	0.12
≥21 units/wk	0.41	(0.00, 0.82)	0.049	0.46	(0.15, 0.77)	0.003	−0.68	(−1.39, 0.02)	0.06
Smoking status									
Never	Ref			Ref			Ref		
Ever	−0.05	(−0.32, 0.22)	0.70	−0.06	(−0.26, 0.14)	0.56	0.05	(−0.41, 0.52)	0.82
Axial length, mm	0.29	(0.16, 0.41)	<0.001	0.04	(−0.06, 0.13)	0.42	0.74	(0.53, 0.95)	<0.001
IOP, mm Hg	0.00	(−0.03, 0.03)	0.75	0.01	(−0.02, 0.03)	0.45	0.03	(−0.02, 0.09)	0.23
Lens status									
Phakic	Ref			Ref			Ref		
Pseudophakic	−1.50	(−1.94, −1.06)	<0.001	−0.99	(−1.34, −0.64)	<0.001	4.20	(3.44, 4.97)	<0.001

Each explanatory variable was included one at a time, with adjustment for TSS only. DBP, diastolic blood pressure; SBP, systolic blood pressure. $P < 0.05$ are in bold.

recruited for EPIC-Norfolk ($n = 19,330$), these participants were significantly younger, of lower BMI, higher social class, and higher educational achievement level, and fewer were smokers (all $P < 0.001$). The mean age of included participants was 68 years (range, 48–90 years), and 56% were women. The cohort was 99.7% white.

Tables 1 and 2 summarize crude (adjusted for TSS only) and multivariable regression results examining associations with RNFL. Older age was significantly associated with thinner RNFL, less RNFL modulation, and a higher NFI in both crude and adjusted analyses. Women had significantly thicker RNFL and lower NFI in crude and adjusted analyses. Longer axial length was significantly associated with thicker RNFL, less RNFL modulation, and a higher NFI in adjusted analyses. Pseudophakic lens status was associated with thinner RNFL and a higher NFI after adjustment. Statistically significant crude associations observed between RNFL measures and height, blood pressure, social class, education, and alcohol intake were not evident following adjustment for covariates. IOP was not significantly associated with any RNFL measure in either crude or adjusted analyses.

Adjustment for TSS had large effects on the coefficients for age, height, and axial length compared with no consideration of TSS (Supplementary Text). However, similar results were

obtained using three different approaches for considering TSS (linear adjustment, quintile category adjustment, and exclusion of scans with TSS < 70; Supplementary Table S2).

After regression analyses were repeated separately for men and women, no significant differences in associations were found, except for BMI. Higher BMI was significantly associated with thinner RNFL and less RNFL modulation in men only (Table 3). In contrast, there was a trend for a lower NFI in women only. The Figure illustrates the predicted RNFL thickness by BMI for men and women.

Following exclusion of 196 participants who reported ocular hypotensive therapy ($n = 168$) or history of a glaucoma procedure ($n = 45$), crude and multivariable regression results were similar to results from the whole cohort, except for lens status. A comparison of the regression coefficients for the maximally adjusted models with RNFL average thickness as the dependent variable, before and after exclusion, is shown in Supplementary Table S3.

DISCUSSION

Glaucoma is an optic neuropathy characterized by accelerated loss of retinal ganglion cells (RGCs).¹² One of the manifestations of RGC loss is thinning of the RNFL.¹² If factors that

TABLE 2. Results From Three Multiple Regression Models, With RNFL Measures as the Dependent Variable

	Average Thickness, μm			Modulation, μm			Nerve Fiber Indicator		
	Coef	95% CI	P	Coef	95% CI	P	Coef	95% CI	P
Age, per decade	−1.53	(−1.73, −1.33)	<0.001	−1.50	(−1.65, −1.35)	<0.001	2.90	(2.56, 3.24)	<0.001
Sex									
Men	Ref			Ref			Ref		
Women	0.44	(0.04, 0.84)	0.031	−0.12	(−0.42, 0.17)	0.42	−1.15	(−1.83, −0.47)	0.001
BMI, per 5 kg/m ²	−0.07	(−0.22, 0.09)	0.38	−0.06	(−0.18, 0.06)	0.31	−0.13	(−0.39, 0.14)	0.34
Height, per 10 cm	0.00	(−0.22, 0.22)	0.99	0.08	(−0.08, 0.25)	0.32	−0.41	(−0.78, −0.03)	0.034
SBP, per 10 mm Hg	0.07	(−0.01, 0.16)	0.08	0.06	(0.00, 0.12)	0.05	−0.03	(−0.17, 0.11)	0.70
Education level									
Degree	Ref			Ref			Ref		
A level	−0.03	(−0.40, 0.34)	0.88	0.10	(−0.18, 0.37)	0.49	−0.03	(−0.66, 0.60)	0.92
O level	−0.22	(−0.71, 0.27)	0.38	−0.17	(−0.53, 0.20)	0.36	0.18	(−0.66, 1.02)	0.68
Less than O level	−0.38	(−0.80, 0.04)	0.08	−0.01	(−0.32, 0.31)	0.96	0.26	(−0.46, 0.98)	0.48
Test for trend			0.032			0.51			0.33
Alcohol intake									
No intake	Ref			Ref			Ref		
<7 units/wk	−0.04	(−0.42, 0.33)	0.82	−0.24	(−0.52, 0.03)	0.08	0.08	(−0.55, 0.72)	0.80
≥7 to <14 units/wk	−0.08	(−0.47, 0.30)	0.67	−0.05	(−0.33, 0.24)	0.76	0.17	(−0.49, 0.84)	0.61
≥14 to <21 units/wk	−0.07	(−0.55, 0.41)	0.78	0.04	(−0.31, 0.40)	0.81	−0.40	(−1.22, 0.41)	0.33
≥21 units/wk	0.09	(−0.32, 0.51)	0.66	0.07	(−0.24, 0.38)	0.67	−0.30	(−1.01, 0.42)	0.41
Axial length, mm	0.15	(0.02, 0.28)	0.024	−0.18	(−0.28, −0.09)	<0.001	1.08	(0.86, 1.30)	<0.001
IOP, mm Hg	−0.01	(−0.04, 0.02)	0.56	0.01	(−0.02, 0.03)	0.69	0.03	(−0.02, 0.08)	0.25
Lens status									
Phakic	Ref			Ref			Ref		
Pseudophakic	−0.49	(−0.94, −0.04)	0.033	0.15	(−0.21, 0.51)	0.42	2.25	(1.46, 3.03)	<0.001

All explanatory variables presented were included together in each of the models, and further adjusted for TSS. $P < 0.05$ are in bold.

determine RGC loss in glaucoma also affect RGC loss in normal individuals, examination of the determinants of RNFL measures in healthy individuals may provide insight into the etiology of glaucoma. The approach of examining a continuous quantitative trait related to glaucoma, rather than considering glaucoma as a dichotomous outcome, may provide increased power for finding small effect associations. For example, examining genetic associations with cup-to-disc ratio has identified polymorphisms subsequently found to be associated with glaucoma.¹³ To the best of our knowledge, this is the largest study describing the epidemiology of the RNFL at a population level.

We found RNFL average thickness to be significantly lower in older participants, independent of other covariables. This is in keeping with the wealth of published evidence using SLP^{14,15} and OCT.^{16–18} Given the cross-sectional nature of the EPIC-Norfolk Eye Study, it cannot be determined with certainty that the observed RNFL thickness decline is entirely due to ageing, or whether there may be an element of a cohort effect. Assuming the former, we observed a 1.53- μm decline in RNFL average thickness per decade of increasing age in the maximally adjusted model. This equates to a 2.6% decline per decade (based on a baseline RNFL thickness of 58.4 μm in those <60 years of age), which is similar to that of other

TABLE 3. Regression Coefficients for BMI in Models With RNFL Measures as Dependent Variables

	Average Thickness, μm			Modulation, μm			Nerve Fiber Indicator		
	Coef	95% CI	P	Coef	95% CI	P	Coef	95% CI	P
All participants: 11,030 eyes of 6309 participants									
Sex									
Men	Ref			Ref			Ref		
Women	0.43	(0.03, 0.83)	0.035	−0.13	(−0.42, 0.17)	0.41	−1.14	(−1.82, −0.46)	0.001
BMI, per 5 kg/m ²	−0.07	(−0.23, 0.08)	0.37	−0.06	(−0.18, 0.06)	0.31	−0.13	(−0.39, 0.14)	0.35
Men: 4812 eyes of 2784 participants									
BMI, per 5 kg/m ²	−0.30	(−0.58, −0.02)	0.039	−0.27	(−0.48, −0.06)	0.011	0.28	(−0.22, 0.78)	0.28
Women: 6218 eyes of 3525 participants									
BMI, per 5 kg/m ²	0.04	(−0.15, 0.22)	0.69	0.03	(−0.11, 0.17)	0.70	−0.32	(−0.62, −0.01)	0.045
All participants, with interaction term: 11,030 eyes of 6309 participants									
Test for interaction		$P = 0.054$			$P = 0.022$			$P = 0.062$	

Four types of model are presented: all participants, men only, women only, and all participants with an interaction term (sex $\times$ BMI). The test for interaction represents a Wald test of the interaction term. All models were adjusted for age, height, SBP, education, alcohol intake, axial length, lens status, IOP, and TSS. $P < 0.05$ are in bold.

published studies.^{17,18} A more novel finding was that RNFL modulation also declined with age and by a greater degree, with a 1.50- μ m (6.4%) decrease per decade.

We found women to have significantly thicker RNFL and lower NFI than men, both in a direction consistent with a lower prevalence of glaucoma. This did not appear to be due to a different rate of decline in thickness with age (β -coefficient [95% confidence interval {CI}] per decade older: $-1.53 \mu\text{m}$ [$-1.83, -1.23$] in men and $-1.53 \mu\text{m}$ [$-1.81, -1.26$] in women, both $P < 0.001$) and may therefore reflect a different baseline RNFL anatomy in men and women. This may in turn suggest a greater RGC reserve in women. However, no significant sex differences in RNFL measures were found in a recently published multicenter OCT study of 301 children.¹⁹ The evidence for a sex predilection in primary open-angle glaucoma is inconsistent,²⁰ though our results may provide indirect support for the finding of a Bayesian meta-analysis that suggested an increased risk in men (odds ratio 1.37 [95% credible interval 1.22 to 1.53]).²¹

There is consistent population-based evidence for the positive association between BMI and IOP.^{22–24} It is surprising, therefore, that there is growing evidence for a protective effect of increasing BMI on glaucoma risk.^{25–28} In two of these studies, the decreased risk was seen in women only.^{26,28} A postulated mechanism²⁸ for this sex-specific effect of BMI is that plasma estrogen levels are correlated with adiposity in postmenopausal women²⁹ and that neuroprotection may be mediated via estrogen receptors on RGCs.³⁰ We found increasing BMI to be associated with thinner RNFL and less RNFL modulation in men and lower NFI in women. Our results support a sex-specific association between BMI and RNFL health, and in the same direction as the two previously mentioned studies^{26,28} (women at less risk than men). It is possible that the sex difference observed for RNFL measures in our cohort, and for glaucoma in the literature,²¹ is explained in part by sex modifying the effect of BMI on RGC health and glaucoma risk.

There is conflicting evidence in the literature regarding the association of axial length and RNFL thickness. Most studies have reported lower RNFL thickness in eyes with longer axial lengths.^{17,18,31–33} The main reason studies using OCT have found a negative association with axial length is most likely due to uncorrected magnification error.³⁴ When ocular magnification was mathematically corrected for in one study, a significant negative association of RNFL thickness with axial length reversed into a significant positive association.³⁴ Similarly, Savini et al.³⁵ found a negative association between axial length and RNFL thickness, which became a positive association in the temporal sector following correction for ocular magnification. In contrast to OCT, the GDxVCC uses refractive error to partially correct for magnification due to axial length differences.³⁶ We found increasing axial length to be significantly associated with a thicker RNFL, consistent with the OCT studies that corrected for magnification. A recent systematic review and meta-analysis supported myopia as a risk factor for glaucoma.³⁷ This might suggest an expected thinner RNFL in myopes on average, though a thicker RNFL in longer eyes might be reflecting a different baseline anatomy, which could obscure any relative decrease due to glaucoma. It is also possible that the observed thicker RNFL in longer eyes we observed may be related to residual atypical retardation not fully adjusted for. In contrast to thicker RNFL, we found significantly less RNFL modulation in longer eyes. This may be reflecting a true anatomical difference in longer eyes or may be related to atypical retardation since it is unlikely that atypical retardation would have the same distribution as RNFL thickness.

We found pseudophakic participants to have significantly thinner RNFL and higher NFI after linear adjustment for confounders. This is in contrast to the described increase in RNFL thickness measurements and decrease in NFI shown to occur postoperatively in patients undergoing cataract surgery.^{38,39} A possible explanation is that patients with glaucoma may be exposed to treatments that hasten the formation of cataract, and hence this may result in bias toward thinner RNFL in pseudophakics. This is supported by the fact that we no longer found a significant association between lens status and RNFL thickness after exclusion of participants reporting use of ocular hypotensive medication or history of a glaucoma procedure (Supplementary Table S3).

Given the strength of IOP as a risk factor for glaucoma, it is surprising that IOP was not associated with RNFL measures in the EPIC-Norfolk cohort. Hospital-based studies have reported thinner RNFL in ocular hypertensive (OHT) patients compared with controls,⁴⁰ and a faster rate of RNFL thinning among glaucoma patients with higher IOP.⁴¹ However, to the best of our knowledge, an association between IOP and RNFL measures has never been reported at a population level. OHT patients derived from hospital clinics may be systematically different from healthy individuals measured with high IOP on one occasion. This may have implications for any population-based public health strategies for prevention of glaucoma. An alternative explanation for the lack of a significant association between RNFL parameters and IOP is that we could not include the effect of IOP on RNFL for those individuals who have had IOP-lowering therapy. Including these participants in the analysis is problematic because their measured IOP is therapeutically lowered. Excluding them may be losing the major driver for an association. We did not have data on pretreatment IOP for these participants.

Strengths of the present study include the population-based design and large sample size. Furthermore, detailed ophthalmic examination was undertaken allowing adjustment for important covariables in analyses. There are several limitations of the study design. The cohort is healthy and selected. It is likely that potential participants with significant visual impairment were not examined due to difficulty with required questionnaires or travel to the research clinic. The most likely effect of having a healthier cohort with truncated distributions is to reduce statistical power for detecting associations. Alternatively, if the direction of association in those with disease is opposite to that found in the healthy cohort, this may give biased results. However, this is unlikely. A significant proportion of GDxVCC scans have interpretation complicated by atypical retardation, and there may be residual bias in results despite adjustment for TSS. This issue mainly affects associations with age, height, and axial length and is discussed in more detail in the Supplementary Text. The GDx software has now been superseded by enhanced corneal compensation,⁴² which has been shown to be less affected by atypical retardation^{43,44} but was not available for this large population study, which commenced in 2004. It is also important to be cautious extrapolating findings regarding the physiological associations with RNFL measures in a largely healthy population to the pathogenesis of glaucoma. It may well be that what determines RGC loss in glaucoma does not affect RGC loss and therefore RNFL parameters in normal individuals. Furthermore, RNFL parameters may reflect other biological factors, such as the RNFL structure an individual is born with, and may not simply reflect RGC number.

In summary, in this predominantly white British population, significant associations were found between SLP-derived RNFL measures and age, sex, axial length, previous cataract surgery, and BMI (in men only). IOP was not associated with any RNFL measures.

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