Differential Associations with Macular Inner Retinal Thickness Measures in a Large Cohort: The UK Biobank

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Conflict of interest:

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Abstract

Objective: To describe and compare associations with macular retinal nerve fiber layer (mRNFL), ganglion cell complex (GCC) and ganglion cell–inner plexiform layer (GCIPL) thickness in a large UK cohort.

Design: Cross-sectional study

Participants: We included data from 42,044 participants of the UK Biobank, a large-scale multisite cohort study. Mean age of participants was 56 years; 53% were women

Methods: Spectral-domain optical coherence tomography macular images were automatically segmented and analyzed. Corneal compensated intraocular pressure (IOPcc) was measured with the Ocular Response Analyzer. Univariable and multivariable linear regression was used to examine associations with mean mRNFL, GCC and GCIPL thickness. Factors examined were age, sex, ethnicity, height, BMI, smoking status, alcohol intake, Townsend deprivation index, education level, diabetes status, spherical equivalent, and IOPcc.

Main outcome measures: mRNFL, GCC and GCIPL.

Results: In addition to confirming previously reported associations with thinner inner retinal thickness (older age, male sex, higher BMI and diabetes), we identified several novel independent associations. Thinner inner retina was associated with frequent alcohol intake (most significant for GCIPL: -0.46 µm for daily or almost daily intake compared to special occasion only or never [95% CI -0.61, -0.30]; P=1.1x10^{-8}), greater social deprivation (most significant for GCIPL: -0.28 µm for most deprived quartile compared to least deprived quartile [95% CI -0.42, -0.14]; P=6.6x10^{-5}), lower educational attainment (most significant for mRNFL: -0.36 µm for less than O level compared to degree level [-0.45, 0.26]; P=2.3x10^{-14}), and non-White ethnicity (most significant for mRNFL comparing Blacks to Whites: -1.65 µm [95% CI -1.86, -1.43]; P=2.4x10^{-50}). IOPcc was most significantly associated with GCIPL (-0.04 µm/mmHg [95% CI -0.05, -0.03]; P=4.0x10^{-10}) and was not significantly associated with mRNFL (0.00 µm/mmHg [95% CI -0.01, 0.01]; P=0.77). The variables examined explained a greater proportion of the variance of GCIPL (11%) than GCC (6%) or mRNFL (7%).

Conclusion: The novel associations we identified may be important to take into account when using inner retinal parameters as a diagnostic tool. Associations were generally strongest with GCIPL, particularly for IOP. This suggests GCIPL may be the superior inner retinal biomarker for macular pathophysiological processes, and especially for glaucoma.

Damage to macular retinal ganglion cells (RGCs) occurs early in glaucoma[1] and spectral-domain optical coherence tomography (SD-OCT) measurements of the inner retina at the macula have been shown to be useful for detecting glaucoma.[2,3] Different commercially available SD-OCT devices report different segments of inner retinal macular thickness; commonly reported segments are the ganglion cell complex (GCC; macular retinal nerve fiber layer [mRNFL] + ganglion cell layer [GCL] + inner plexiform layer [IPL]) and the ganglion cell–inner plexiform layer (GCIPL; GCL + IPL). Both GCC and GCIPL thickness have been reported to be comparable to circumpapillary retinal nerve fiber layer (cRNFL) thickness at diagnosing glaucoma.[4,5] Macular GCC and GCIPL measurements have been shown to be helpful in the detection of glaucoma progression,[6,7] and may be superior to cRNFL measurements at detecting progression in severe disease.[8–10] A meta-analysis reports similar accuracy of GCC and GCIPL measurements for glaucoma diagnosis,[11] which is in agreement with studies that conducted head-to-head comparisons of GCC and GCIPL diagnostic accuracy within the same study participants.[12–14]

Understanding epidemiological associations with macular inner retinal measurements is important to help define normal ranges in population subgroups, and may shed light on pathophysiological mechanisms underlying glaucoma. Comparing strengths of associations between mRNFL, GCC and GCIPL may provide insight into their relative potential as biomarkers. Using data from a very large adult cohort, the UK Biobank, we aimed to describe and compare basic demographic, socioeconomic, anthropometric, lifestyle and ocular associations with mRNFL, GCC and GCIPL.

Methods

UK Biobank

The UK Biobank is a very large multisite cohort study established by the Medical Research Council, Department of Health, Wellcome Trust medical charity, Scottish Government and Northwest Regional Development Agency. Detailed study protocols are available online (http://www.ukbiobank.ac.uk/resources/ and http://biobank.ctsu.ox.ac.uk/crystal/docs.cgi). A baseline questionnaire, physical measurements, and biological samples were undertaken in 22 assessment centers across the UK between 2006 and 2010. All UK residents aged 40 to 69 years who were registered with the National Health Service (NHS) and living up to 25 miles from a study center were invited to participate. The study was conducted with the approval of the North-West Research Ethics Committee (ref 06/MRE08/65), in accordance with the principles of the Declaration of Helsinki, and all participants gave written informed consent. This research has been conducted using the UK Biobank Resource under Application Number 2112.

Participants completed a touch-screen self-administered questionnaire and underwent physical examination at a baseline assessment. Table 1 summarizes the ascertainment of the baseline assessment variables used in the current study. Body mass index (BMI) was calculated as weight/height^2 (Kg/m^2). We selected these variables *a priori* to examine the basic descriptive epidemiology of inner retinal morphology, including demographic, socioeconomic, anthropometric and basic lifestyle factors. We additionally examined diabetes status as a potentially important confounder, given that diabetes is relatively common and has known retinal sequalae.

Ophthalmic assessment

Ophthalmic assessment was not part of the original baseline assessment and was introduced as an enhancement in 2009 for 6 assessment centers which are spread across the UK (Liverpool and Sheffield in North England, Birmingham in the Midlands, Swansea in Wales, and Croydon and Hounslow in Greater London). SD-OCT imaging of both eyes was performed using the Topcon 3D OCT- 1000 Mark II in a dark room without pupil dilation using the 3-dimensional 6x6 mm^2 macular volume scan mode (512 A scans per B scan; 128 horizontal B scans in a raster pattern). The right eye was imaged first. Version 1.6.1.1 of the Topcon Advanced Boundary Segmentation (TABS) algorithm was used to delineate the inner and outer retinal surfaces.[15] Quality control to exclude images of poor quality has been described in detail previously.[16] We excluded scans with an image quality score (signal strength) less than 45. Additionally, several segmentation indicators were calculated which

also served to identify poor scan quality or segmentation failures; we excluded the poorest 20% of images for each of these indicators. The inner limiting membrane (ILM) indicator was a measure of the minimum localized edge strength around the ILM boundary across the entire scan; this is useful for identifying blinks, scans that contain regions of severe signal fading, and segmentation errors. The validity count indicator is used to identify scans with a significant degree of clipping in the OCT scan's z-axis dimension. The motion indicators use both the nerve fiber layer and the full retinal thicknesses, from which Pearson correlations and absolute differences between the thickness data from each set of consecutive B-scans are calculated. The lowest correlation and the highest absolute difference in a scan serve as the resulting indicator scores and serve to identify blinks, eye motion artifacts, and segmentation failures. It should be noted that the image quality score and the above-mentioned indicators are usually highly correlated. We used average thickness parameters derived from the macula-6 grid. Participant-level mRNFL, GCC and GCIPL thicknesses (µm) were calculated as the mean of right and left eye values for each participant with good quality images available for both eyes. If data were only available for one eye, we considered that value for the participant.

Participant intraocular pressure (IOP, mmHg) was measured once for each eye using the Ocular Response Analyzer (ORA; Reichert, Corp., Buffalo, NY). Participants who reported eye surgery within the previous 4 weeks or participants reporting an eye infection were precluded from having IOP measured. The ORA is a non-contact tonometer that measures the force required to flatten the cornea using a jet of air. Unlike conventional non-contact tonometry, the ORA measures two pressures; firstly, when the cornea flattens on inward motion, and secondly when the cornea is flattened on outward motion. The average of these two pressures has been calibrated to derive a Goldmann-correlated IOP (IOPg) and the difference between these two pressures has been shown to be related to the biomechanical properties of the cornea.[17] A linear combination of these two pressures has been developed to derive a corneal-compensated IOP (IOPcc).[18] We used IOPcc for our primary analyses as it is thought to provide the most accurate assessment of true IOP and least affected by corneal properties.[19] To handle extreme values of IOP, we excluded the top and bottom 0.5% of IOP measurements. We excluded participants with a history of laser or surgery for glaucoma, eye injury, corneal graft surgery, or refractive laser surgery as these participants are likely to have IOP altered from physiological levels. For patients using IOP-lowering medication (n = 1,151), we imputed pre-treatment IOP by dividing by 0.7 based on the mean IOP reduction achieved by medication.[20] This approach has been used successfully in genome-wide association studies of IOP.[21,22] Additionally, in sensitivity analyses, we used IOPg with imputed pre-treatment IOP, and also IOPg and IOPcc following exclusion of participants using IOP-lowering medication. Refractive status of both eyes was measured by autorefraction (Tomey RC5000; Erlangen-Tennenlohe). Spherical equivalent was calculated as the

sphere + 0.5 * cylinder. We excluded participant eyes with high refractive error (< -6 D or > +6 D). We calculated participant-level IOP and spherical equivalent as the mean of right and left eye values if data were available for both, or as either the right or left eye value if data were only available for one eye.

Statistical analyses

Demographic, systemic and ocular characteristics for included participants were described, stratified by sex. Comparisons between men and women for each of the variables were made using the independent sample t-tests for continuous variables and chi-squared tests for categorical variables. We first examined crude associations with mRNFL, GCC and GCIPL using univariable linear regression. Variables significantly associated with any of mRNFL, GCC and GCIPL at a $P < 0.01$ level were then considered together in a multivariable linear regression model for each of mRNFL, GCC and GCIPL. Given weight and BMI are highly correlated, we only considered one of the parameters in multivariable analyses; we selected BMI based on stronger univariable associations. Similarly, given IOPg and IOPcc are highly correlated , we considered only one of the parameters in multivariable analyses; IOPcc was selected on the basis that it better reflect true physiological IOP.[19] To determine whether the associations we identified were primarily driven by participants with established glaucoma, we carried out the same multivariable analyses following exclusion of participants with self-reported (touch-screen questionnaire) or hospital admission coded (ICD 10) glaucoma, and excluding any participants using IOP-lowering medication (n = 41,449 following exclusion of 595 participants). We also conducted sensitivity analyses for the associations of mRNFL, GCC and GCIPL with IOP, as primary analyses included IOP measurements that were imputed for pre-treatment levels in participants using glaucoma medication. Firstly, rather than imputing pre-treatment IOP, we conducted analyses using current IOP even if using IOP-lowering medication, and additional analyses excluding participants using IOP-lowering medication. We also conducted further analyses using IOPg rather than IOPcc. To examine how much of mRNFL, GCC and GCIPL variance are explained by the factors we examined, we calculated R^2 statistics for the multivariable regression models. Stata version 15.1 (StataCorp LP, College Station, TX, USA) was used for all statistical analyses.

Results

In total, SD-OCT images from 67,310 UK Biobank participants were available at the time of this analysis. Following image segmentation and quality control, there were 45,815 participants with mRNFL, GCC and GCIPL thickness measurements. There were complete data for all exposure (age, sex, ethnicity, weight, height, BMI, smoking status, alcohol intake, deprivation score, diabetes status, education level, spherical equivalent, IOP) and retinal thickness variables for 42,044 participants; all analyses were conducted using these participants. The mean age of included participants was 56 years and 53% were women. Table 2 summarizes mean mRNFL, GCC and GCIPL as well as demographic, systemic and ocular factors for included participants.

Univariable associations with mRNFL, GCC and GCIPL are shown in Table 3. There were significant associations with at least one of mRNFL, GCC and GCIPL for all examined variables except for height which was not significant associated with any thickness parameter (all $P > 0.30$; Table 3). Height was therefore not carried forward for the multivariable analyses. Both weight and BMI were significantly associated with all three thickness parameters; given their collinearity, only BMI was carried forward for multivariable analyses as described in the Methods. Both IOPg and IOPcc were significantly associated with GCC and GCIPL (both $P < 0.001$), but not with mRNFL (both $P \geq 0.12$). Given the collinearity between IOPg and IOPcc, only IOPcc was carried forward for multivariable analyses, as detailed in the Methods.

Multivariable associations with mRNFL, GCC and GCIPL are shown in Table 4. Age was strongly associated with a thinner mRNFL, GCC and GCIPL; the association appeared stronger for GCC and GCIPL than for mRNFL. Related to the strength of association and the very large sample size, the *P*-values for associations with age were extremely small; for GCC and GCIPL, the *P*-values were smaller than can be handled by most modern statistical software ($P < 10^{-300}$). Men had significantly thinner mRNFL and GCC than women (both $P \leq 7.1 \times 10^{-23}$), and thinner GCIPL of borderline significance ($P = 0.042$). Asian and Black participants had thinner mRNFL, GCC and GCIPL than White participants. Participants with higher BMI had thinner mRNFL, GCC and GCIPL (all $P \leq 1.5 \times 10^{-8}$). Daily or almost daily alcohol intake was associated with thinner mRNFL, GCC and GCIPL when compared to participants who drank least (never or special occasions only). There was no significant difference in thickness parameters for participants reporting less frequent alcohol intake (Table 4). Participants in the most deprived quartile of the Townsend deprivation index had significantly thinner GCC and GCIPL (both $P \leq 1.2 \times 10^{-4}$) than participants in the least deprived quartile; the difference was only borderline significant for mRNFL ($P = 0.012$). There was evidence of progressively thicker mRNFL, GCC and GCIPL with higher educational attainment (Table 4). Participants with self-reported diabetes had thinner

mRNFL, GCC and GCIPL (all $P < 0.003$). There were very strong and highly significant associations between thickness parameters and spherical equivalent and these were in different directions for mRNFL compared to GCC and GCIPL. A more myopic refraction was associated with a thicker mRNFL ($P = 1.1 \times 10^{-251}$) but a thinner GCC ($P = 1.2 \times 10^{-93}$) and GCIPL ($P < 10^{-300}$). IOPcc was not associated with mRNFL, but was negatively associated with both GCC ($P = 5.8 \times 10^{-5}$) and GCIPL ($P = 4.0 \times 10^{-10}$) thickness. Of the three multivariable models, the R^2 was greatest for the GCIPL model indicating that the explanatory variables we assessed explained more of the variance of GCIPL (11%) than mRNFL (7%) or GCC (6%) (Table 4). The same multivariable analyses were also conducted following exclusion of participants using glaucoma medication and/or self-reported glaucoma or hospital ICD 10 coded glaucoma (Table S1, available at www.aaojournal.org). Associations were very similar for all variables, apart from IOP for which the associations were less significant. There was no longer a significant association between IOP and GCC, and the association between IOP and GCIPL was less significant ($P = 3.9 \times 10^{-5}$).

We also conducted sensitivity analyses for the associations between IOP and inner retinal thickness measures, as described in the Methods. Results were similar when we examined IOPg instead of IOPcc (Table 5). Also, results were similar if we either excluded participants using IOP-lowering medication or used current treated IOP rather than imputing the pre-treatment IOP (Table 5). For all analyses, IOP was not associated with mRNFL and was more significantly associated with GCIPL than GCC. Again, the model R^2 was greatest for the GCIPL models (Table 5).

Discussion

Our study is the largest to date examining the epidemiology of macular inner retinal anatomy. We confirm previously reported associations with age, sex, BMI, diabetes and refractive error, and have identified multiple novel associations with thinner inner retina at the macula including non-White ethnicity, frequent alcohol intake, greater social deprivation, lower educational attainment and higher IOP. This is also the first study to examine how epidemiological associations vary between different inner retinal parameters, namely mRNFL, GCC and GCIPL.

Older age was strongly associated with thinner inner retinal thickness, in agreement with previous studies.[23–26] This association was apparent for all three inner retinal parameters, but was strongest and most significant for GCC and GCIPL. While it is not possible to infer a causal effect of inner retinal thinning due to aging from a cross-sectional study, it is unlikely an association this strong is due to a cohort effect. Comparing the age coefficients (Table 4) with the mean thickness values in our study (Table 2) derives a yearly percentage decline in thickness of 0.14% for mRNFL, 0.18% for GCC and 0.20% for GCIPL; this is also in keeping with previous studies.[23–26]

We found men to have thinner macular inner retinas, and this was most apparent for mRNFL. Thinner GCIPL in men was previously reported in a multiethnic volunteer study of 282 normal participants.[23] Other studies found no significant association between inner retinal thickness and sex,[24,26] and one study from a subset of the Singapore Chinese Eye Study (SCES) found women to have thinner inner retina.[25] While it is possible that the relationship between sex and macular inner retinal thickness varies between populations, it is more likely that the variation in results is stochastic due to the smaller sample sizes and resultant statistical power of previous studies. Our finding of a thinner inner retina in men may be aligned with the greater susceptibility to glaucoma reported in men.[27]

Higher BMI was associated with thinner inner retina, in agreement with a study of British twins which reported thinner GCC with higher BMI.[24] We observed the association with similar strength for mRNFL, GCC and GCIPL, suggesting the association is not related specifically to RGCs. Also in agreement with this is the previously reported association of higher BMI with thinner macular total retina thickness in the UK Biobank.[16]

We observed thinner inner retinas in participants with diabetes; this association was more significant for mRNFL than for GCC or GCIPL. This is in agreement with small case-control studies that have reported thinner inner retinas in participants with diabetes compared to controls,[28–30] and has led to the hypothesis that diabetic peripheral neuropathy and inner retinal thinning may share common

biological pathways.[31] Interestingly, laser treatment for proliferative diabetic retinopathy without macular oedema has been shown to cause an increase in GCIPL thickness.[32]

We observed very strong associations of spherical equivalent with inner retinal thickness and, strikingly, the associations were in a different direction for mRNFL than for GCC and GCIPL. Our finding of thinner GCC and GCIPL with increasing myopia is in agreement with previous reports.[23–26] Our finding of a thicker mRNFL with increasing myopia is novel, to the best of our knowledge. Analyzing the relationship between refractive error and retinal thickness is extremely difficult due to the issue of magnification effects which cannot be accurately accounted for. The grid within which the SD-OCT measurements are made will cover different absolute amounts of the macula depending on the refractive status of the eye. Due to this, the foveal pit will take up a different proportion of the grid simply due to refractive error induced magnification effects. In longer, myopic eyes, the grid will cover a larger proportion of the macula than in shorter, emmetropic eyes. This will result in the thickest parts of the inner retina proportionally covering less of the grid in myopic eyes, potentially explaining the thinner GCC and GCIPL. Additionally, the foveal pit will make up proportionally less of the imaged and analyzed grid in myopic eyes than emmetropic eyes. If the foveal pit affects RNFL more than GCC or GCIPL, then this may explain why RNFL is thicker in SD-OCT images of myopic eyes. With the current data in our study, we do not believe it is possible to determine true differences in inner retinal thickness by refractive error as we are unable to distinguish the contribution due to magnification.

We identified several novel associations with inner retinal thickness parameters. Asian and Black participants had thinner mRNFL, GCC and GCIPL than White participants. While this may be in part reflecting a greater susceptibility to glaucomatous processes in non-White people, as suggested from epidemiological data,[27] this more likely reflects ethnically-determined differences in baseline retinal anatomy. This highlights the importance of taking ethnicity into account when defining normal ranges for diagnostic tests for glaucoma.

Frequent alcohol intake was associated with thinner mRNFL, GCC and GCIPL compared to rare or no alcohol intake. This is in agreement with a study examining cRNFL (i.e. circumpapillary rather than macular measures);[33] Lamparter and colleagues reported thinner cRNFL in participants of the Gutenberg Health Study whose alcohol intake was high according to WHO guidelines (≥ 10g/d for women; ≥ 20g/d for men).[33] Our findings support the assertion that RNFL thinning may occur as a result of chronic alcohol intake and in a dose-dependent manner.[33] It is not possible to determine from our study what mechanisms may be underlying the association with alcohol. Potential mechanisms may include direct effects of alcohol on retinal ganglion cells, or indirect effects via dehydration.

We found more socially deprived participants to have thinner inner retinas, particularly for GCC and GCIPL. This is consistent with the previously reported association of social deprivation with self-reported glaucoma in UK Biobank.[34] We also found less educated participants to have thinner mRNFL, GCC and GCIPL. This is consistent with a scanning laser ophthalmoscopy study of cRNFL in participants of another, independent UK cohort of older adults.[35] Interestingly, the association of a thicker GCC and GCIPL in more educated participants is strong enough to outweigh the expected thinner GCC and GCIPL we may expect to see given the association between education and myopia,[36] even in unadjusted analyses (Table 3). From our cross-sectional study, it is not possible to know if less educated participants had thinner inner retinas at baseline, or whether this is something that developed over time as a result of lack of education. Another study of UK Biobank participants reported that baseline mRNFL predicted future cognitive decline.[37] If inner retinal thickness is causally associated with cognitive health, this may explain the relationship with education that we observed with more cognitively able people with thicker inner retinas being more likely to remain in education for longer.

Typically, in epidemiological studies, if a significant association is not found, it may be the case that a true association does not exist or that the study was underpowered to detect a true association. With the huge sample size in our study, it is unlikely that a biologically meaningful association will not be identified if it truly exists. Strong associations in our study (e.g. age and spherical equivalent) were so statistically significant that the *P*-value was so small the statistical software could not distinguish it from zero ($P < 10^{-300}$). We did not find associations between inner retinal thickness and height or smoking status. Given the statistical power, our study provides good evidence for no true association between inner retinal thickness and height or smoking. The lack of association with smoking suggests that inflammatory mechanisms do not have a prominent role in pathophysiological processes underlying variation in inner retinal thickness.

The effect sizes for the associations we report are modest in magnitude, but important when considered in the context of the standard deviation (SD) of the retinal thickness parameters and when compared to the association with age (a well-established important association of inner retinal thickness that is corrected for in diagnostic tests). For example, the thinner mRNFL observed in men had a magnitude of 17% of the SD of mRNFL and is equivalent to the magnitude of thinner mRNFL observed in participants that were 15 years older. Similarly, the thinner GCC seen in Black compared to White participants had a magnitude of 26% of the SD of GCC, equivalent to being 10 years older. Collectively, the predictor variables we examined explained a considerable proportion of the total variance of inner retinal thickness; 6.7% for mRNFL, 5.6% for GCC and 11.2% for GCIPL (Table 4).

We found higher IOP to be associated with a thinner GCC and GCIPL. If we consider glaucoma as a complex disease with multiple underlying etiological processes and with a phenotypic spectrum from normal to severe disease, it is likely that variation in inner retinal anatomy in a population may be reflecting the pre-clinical disease spectrum and may be secondary to these etiological processes. Therefore, determinants of inner retinal thickness variation may also be determinants of the glaucomatous process. On this basis, we would expect to see an association between IOP and inner retinal thickness, given the strength of IOP as a risk factor for glaucoma.[38] The association with IOP in our study was most significant for GCIPL, potentially suggesting GCIPL as a superior biomarker for glaucomatous processes. We found no significant association between IOP and mRNFL, potentially suggesting mRNFL to be a less effective biomarker for glaucomatous processes. This is in contrast to the well-established role of cRNFL as a biomarker in the management of glaucoma.[39] Our data suggest that, at the macula, variation in mRNFL within a population may be more influenced by factors other than glaucomatous processes.

Overall, the predictor variables we examined explained twice more of the variance of GCIPL than of either GCC or mRNFL. This suggests that GCIPL is better reflecting the biological processes that these variables contribute to and may therefore be a superior biomarker for pathophysiological processes influencing RGC health in general.

The major strength of our study is the very large sample size which afforded sufficient power to definitively determine which factors were or were not associated with inner retinal thickness. Limitations of our study include the reliance on automated segmentation of the retina. While we applied strict quality control criteria, and manually checked a proportion of scans,[16] it was not feasible to manually check all scans for accurate segmentation. Additionally, it was not possible to reliably segment the boundary between the GCL and IPL meaning we could not examine these layers individually. Another limitation of the UK Biobank is that it is a volunteer cohort, and participants are likely healthier than the general population. Furthermore, our quality control process excluded participants and this could also lead to selection bias. This may limit the generalizability of our results, though it seems unlikely that that directions of association with inner retinal thickness would be differential by selection.

In summary, we present the largest epidemiological study of inner retinal anatomy to date. We identified novel associations with thinner inner retina, including non-European ethnicity, frequent alcohol intake, greater social deprivation and lower educational attainment. These associations were statistically independent from each other and warrant further investigation to help determine if they are causal and what the underlying mechanisms may be. Stronger associations were seen with GCIPL

362 compared to mRNFL or GCC, particularly for IOP, suggesting GCIPL may be a superior biomarker for
363 macular pathophysiological processes and especially for glaucoma.

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References:

1. Hood DC, Raza AS, de Moraes CG V., et al. Glaucomatous damage of the macula. Prog Retin Eye Res 2013;32:1–21.

2. Kim KE, Park KH. Macular imaging by optical coherence tomography in the diagnosis and management of glaucoma. Br J Ophthalmol 2018;102:718–724.

3. Oddone F, Lucenteforte E, Michelessi M, et al. Macular versus Retinal Nerve Fiber Layer Parameters for Diagnosing Manifest Glaucoma: A Systematic Review of Diagnostic Accuracy Studies. Ophthalmology 2016;123:939–949.

4. Mwanza JC, Durbin MK, Budenz DL, et al. Glaucoma diagnostic accuracy of ganglion cell-inner plexiform layer thickness: Comparison with nerve fiber layer and optic nerve head. Ophthalmology 2012;119:1151–1158.

5. Tan O, Chopra V, Lu ATH, et al. Detection of Macular Ganglion Cell Loss in Glaucoma by Fourier-Domain Optical Coherence Tomography. Ophthalmology 2009;116.

6. Shin JW, Sung KR, Park SW. Patterns of Progressive Ganglion Cell–Inner Plexiform Layer Thinning in Glaucoma Detected by OCT. Ophthalmology 2018:1–11.

7. Hou HW, Lin C, Leung CKS. Integrating Macular Ganglion Cell Inner Plexiform Layer and Parapapillary Retinal Nerve Fiber Layer Measurements to Detect Glaucoma Progression. Ophthalmology 2018;125:822–831.

8. Hammel N, Belghith A, Weinreb RN, et al. Comparing the Rates of Retinal Nerve Fiber Layer and Ganglion Cell–Inner Plexiform Layer Loss in Healthy Eyes and in Glaucoma Eyes. Am J Ophthalmol 2017;178:38–50.

9. Lavinsky F, Wu M, Schuman JS, et al. Can Macula and Optic Nerve Head Parameters Detect Glaucoma Progression in Eyes with Advanced Circumpapillary Retinal Nerve Fiber Layer Damage? Ophthalmology 2018:1–6.

10. Zhang X, Dastiridou A, Francis BA, et al. Comparison of Glaucoma Progression Detection by Optical Coherence Tomography and Visual Field. Am J Ophthalmol 2017;184:63–74.

11. Kansal V, Armstrong JJ, Pintwala R, Hutnik C. Optical coherence tomography for glaucoma diagnosis: An evidence based meta-analysis. PLoS One 2018;13:e0190621.

12. Kim HJ, Park KH, Kim YK, Jeoung JW. Evaluation of Layer-by-Layer Segmented Ganglion Cell Complex Thickness for Detecting Early Glaucoma According to Different Macular Grids. J Glaucoma 2017;26:712–717.

13. Pazos M, Dyrda AA, Biarnés M, et al. Diagnostic Accuracy of Spectralis SD OCT Automated Macular Layers Segmentation to Discriminate Normal from Early Glaucomatous Eyes. Ophthalmology 2017;124:1218–1228.

14. Chien JL, Ghassibi MP, Patthanathamrongkasem T, et al. Glaucoma diagnostic capability of global and regional measurements of isolated ganglion cell layer and inner plexiform layer. J Glaucoma 2017;26:208–215.

15. Keane PA, Grossi CM, Foster PJ, et al. Optical coherence tomography in the UK Biobank study - Rapid automated analysis of retinal thickness for large population-based studies. PLoS One 2016;11:1–15.

16. Patel PJ, Foster PJ, Grossi CM, et al. Spectral-Domain Optical Coherence Tomography Imaging in 67 321 Adults: Associations with Macular Thickness in the UK Biobank Study. Ophthalmology

2016;123:829–40.

17. Luce DA. Determining in vivo biomechanical properties of the cornea with an ocular response analyzer. J Cataract Refract Surg 2005;31:156–62.

18. Luce D. Methodology for Corneal Compensated IOP and Corneal Resistance Factor for An Ocular Response Analyzer. Invest Ophthalmol Vis Sci 2006;47:2266.

19. Chan MPY, Grossi CM, Khawaja AP, et al. Associations with Intraocular Pressure in a Large Cohort: Results from the UK Biobank. Ophthalmology 2016;123:771–82.

20. van der Valk R, Webers CAB, Schouten JSAG, et al. Intraocular pressure-lowering effects of all commonly used glaucoma drugs: a meta-analysis of randomized clinical trials. Ophthalmology 2005;112:1177–85.

21. Hysi PG, Cheng C-Y, Springelkamp H, et al. Genome-wide analysis of multi-ancestry cohorts identifies new loci influencing intraocular pressure and susceptibility to glaucoma. Nat Genet 2014;46:1126–30.

22. Khawaja AP, Cooke Bailey JN, Wareham NJ, et al. Genome-wide analyses identify 68 new loci associated with intraocular pressure and improve risk prediction for primary open-angle glaucoma. Nat Genet 2018;50:778–782.

23. 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. Investig Ophthalmol Vis Sci 2011;52:7872–7879.

24. Bloch E, Yonova-Doing E, Jones-Odeh E, et al. Genetic and environmental factors associated with the ganglion cell complex in a healthy aging british cohort. JAMA Ophthalmol 2017;135:31–38.

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

26. Kim NR, Kim JH, Lee J, et al. Determinants of perimacular inner retinal layer thickness in normal eyes measured by fourier-domain optical coherence tomography. Investig Ophthalmol Vis Sci 2011;52:3413–3418.

27. Tham Y-C, Li X, Wong TY, et al. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology 2014;121:2081–90.

28. van Dijk HW, Kok PHB, Garvin M, et al. Selective loss of inner retinal layer thickness in type 1 diabetic patients with minimal diabetic retinopathy. Investig Ophthalmol Vis Sci 2009;50:3404–3409.

29. Pekel E, Tufaner G, Kaya H, et al. Assessment of optic disc and ganglion cell layer in diabetes mellitus type 2. Med (United States) 2017;96:1–4.

30. Ng DSK, Chiang PPC, Tan G, et al. Retinal ganglion cell neuronal damage in diabetes and diabetic retinopathy. Clin Exp Ophthalmol 2016;44:243–250.

31. Dehghani C, Srinivasan S, Edwards K, et al. Presence of Peripheral Neuropathy Is Associated With Progressive Thinning of Retinal Nerve Fiber Layer in Type 1 Diabetes. Invest Ophthalmol Vis Sci 2017;58:BIO234-BIO239.

32. Kim JJ, Im JC, Shin JP, et al. One-year follow-up of macular ganglion cell layer and peripapillary retinal nerve fibre layer thickness changes after panretinal photocoagulation. Br J Ophthalmol 2014;98:213–217.

33. Lamparter J, Schmidtmann I, Schuster AK, et al. Association of ocular, cardiovascular, morphometric and lifestyle parameters with retinal nerve fibre layer thickness. PLoS One 2018;13:1–11.

34. Shweikh Y, Ko F, Chan MPY, et al. Measures of socioeconomic status and self-reported glaucoma in the U.K. Biobank cohort. Eye (Lond) 2015;29:1360–7.

35. Khawaja AP, Chan MPY, Broadway DC, et al. Laser scanning tomography in the EPIC-Norfolk Eye Study: principal components and associations. Invest Ophthalmol Vis Sci 2013;54:6638–45.

36. Mountjoy E, Davies NM, Plotnikov D, et al. Education and myopia: Assessing the direction of causality by mendelian randomisation. BMJ 2018;361.

37. Ko F, Muthy ZA, Gallacher J, et al. Association of Retinal Nerve Fiber Layer Thinning With Current and Future Cognitive Decline: A Study Using Optical Coherence Tomography. JAMA Neurol 2018:1–8.

38. de Voogd S, Ikram MK, Wolfs RCW, et al. Incidence of open-angle glaucoma in a general elderly population: the Rotterdam Study. Ophthalmology 2005;112:1487–93.

39. Tatham AJ, Medeiros FA. Detecting Structural Progression in Glaucoma with Optical Coherence Tomography. Ophthalmology 2017;124:S57–S65.