Genetic Risk of Cardiovascular Disease Is Associated with Macular Ganglion Cell–Inner Plexiform Layer Thinning in an Early Glaucoma Cohort

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Purpose: To evaluate the association between genetic risk for cardiovascular disease and retinal thinning in early glaucoma.

Design: Prospective, observational genetic association study

Participants: Multicohort study combining a cohort of patients with suspect and early manifest primary open-angle glaucoma (POAG), a cohort of patients with perimetric POAG, and an external normative control cohort.

Methods: A cardiovascular disease genetic risk score was calculated for 828 participants from the Progression Risk of Glaucoma: Relevant SNPs [single nucleotide polymorphisms] with Significant Association (PROGRESSA) study. Participants were characterized as showing either predominantly macular ganglion cell–inner plexiform layer (GCIPL), predominantly peripapillary retinal nerve fiber layer (pRNFL) or equivalent macular GCIPL and pRNFL spectral-domain OCT thinning. The cardiovascular disease genetic risk scores for these groups were compared to an internal reference group of stable suspected glaucoma and of an external normative population. Replication was undertaken by comparing the phenotypes of participants from the Australia New Zealand Registry of Advanced Glaucoma (ANZRAG) with the normative control group.

Main Outcome Measures: Spectral-domain OCT and Humphrey Visual Field (HVF) change.

Results: After accounting for age, sex, and intraocular pressure (IOP), participants with predominantly macular GCIPL thinning showed a higher cardiovascular disease genetic risk score than reference participants (odds ratio [OR], 1.76/standard deviation [SD]; 95% confidence interval [CI], 1.18–2.62; $P = 0.005$) and than normative participants (OR, 1.32/SD; 95% CI, 1.12–1.54; $P = 0.002$). This finding was replicated by comparing ANZRAG participants with predominantly macular GCIPL change with the normative population (OR, 1.39/SD; 95% CI, 1.05–1.83; $P = 0.022$). Review of HVF data identified that participants with paracentral visual field defects also demonstrated a higher cardiovascular disease genetic risk score than reference participants (OR, 1.85/SD; 95% CI, 1.16–2.97; $P = 0.010$). Participants with predominantly macular GCIPL thinning exhibited a higher vertical cup-to-disc ratio genetic risk score (OR, 1.48/SD; 95% CI, 1.24–1.76; $P < 0.001$), but an IOP genetic risk score (OR, 1.12/SD; 95% CI, 0.95–1.33; $P = 0.179$) comparable with that of the normative population.

Conclusions: This study highlighted the relationship between cardiovascular disease and retinal thinning in suspect and manifest glaucoma cases. *Ophthalmology Science 2022;2:100108 © 2021 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Glaucoma is an optic neuropathy in which elevated intraocular pressure (IOP) is the most important clinically modifiable risk factor.[1] Several large population studies have also identified that cardiovascular disease may be associated with a greater risk of primary open-angle glaucoma (POAG) developing.[2–4] Furthermore, cardiovascular risk factors have been specifically associated with early macular ganglion cell–inner plexiform layer

(GCIPL) thinning, as measured using spectral-domain OCT.[5]

A limitation of these observational studies is the inability to discern whether the relationship between cardiovascular disease and glaucoma is one of causation or correlation.[6] The incorporation of genetic data has been proposed as a solution to this predicament because it may mitigate the influence of environmental or behavioral confounders.[7]

The advent of large publicly available population-based data sets, such as the UK Biobank, has led to the development of numerous genetic risk scores for a range of different disease states and quantitative outcomes. In the context of cardio-vascular disease, Inouye et al[8] developed a genetic risk score that explained 26.8% of the estimated heritability for this trait and exhibited a stronger association with the development of future coronary artery disease end points than any conventional risk factor.

This genetic association study investigated the association between inherited risk of cardiovascular disease and glaucomatous retinal thinning. We hypothesized that a genetic risk score for cardiovascular disease end points would be associated with worse macular GCIPL thinning in participants recruited to the Progression Risk of Glaucoma Relevant SNPs [single nucleotide polymorphisms] with Significant Association (PROGRESSA) study.

Methods

Study Overview

This study used a cardiovascular disease genetic risk score to investigate the relationship between cardiovascular disease and glaucomatous structural phenotypes. It comprised 3 cohorts that included a discovery cohort of patients with suspected and early manifest POAG from the PROGRESSA study (n = 828),[9] a replication cohort of patients with perimetric POAG from the Australia New Zealand Registry of Advanced Glaucoma (ANZRAG; n = 664),[10] and a normative population cohort from the QSkin Sun and Health study (n = 17 642).[11]

In the discovery phase, patients from the PROGRESSA study were phenotyped based on baseline spectral-domain OCT findings as showing either predominantly macular GCIPL thinning, equivalent macular GCIPL and peripapillary retinal nerve fiber layer (pRNFL) thinning, or predominantly pRNFL thinning, as we described previously.[5] Initial analyses compared these phenotypic study groups with a reference group of patients with stable suspected glaucoma from the PROGRESSA study who did not exhibit structural change or visual field loss at baseline or during monitoring. Subsequent analyses then compared these study groups with a cohort of randomly sampled normative control participants from the QSkin Sun and Health study cohort. These findings were replicated by phenotyping participants enrolled in the ANZRAG study using the same methodology. In the absence of an internal reference group of patients with stable suspected glaucoma in this replication cohort, the cardiovascular genetic risk score of study groups was compared with participants from the QSkin Sun and Health study.

Cohort Descriptions

Baseline demographic features of the 3 cohorts are presented in Table 1. The PROGRESSA study is a longitudinal, prospective, multicenter observational cohort study of patients with early glaucoma and patients with suspected glaucoma in Australia.[12] Inclusion criteria requires the presence of either ocular hypertension (defined as IOP, >24 mmHg; central corneal thickness, <555 μm; and Disc Damage Likelihood Scale [DDLS] grade, 0 or 1),[13] an optic disc suspicious of glaucoma (DDLS grade, ≥1), or the presence of a glaucomatous visual field defect on 2 reliable visual fields, with a mean deviation of better than −6.0 dB. Participants with secondary forms of glaucoma, steroid-induced ocular hypertension, or retinal or neurologic causes of visual field loss were excluded from enrollment. In addition, for the purposes of this study, participants with high myopia (spherical equivalent, worse than −6.0 diopters in either eye) were excluded from analysis because of the confounding influence of high myopia on the spectral-domain OCT thickness deviation maps.[14–16] The QSkin Sun and Health study is a study of 43 794 randomly sampled participants 40 to 69 years of age from Queensland, Australia.[11] The ANZRAG study is an observational cross-sectional study of glaucoma in Australia and New Zealand in which longitudinal data are collected where possible.[10] The present study adhered to the tenets of the Declaration of Helsinki and followed the National Health and Medical Research Council statement of ethical conduct in research involving humans. Informed written consent was obtained from all participants, and the study was approved by the Southern Adelaide Clinical Human Research Ethics Committee.

Baseline Characteristics of Study Population

Baseline demographic, ocular, and cardiovascular data for PROGRESSA participants were obtained at study enrollment. Demographic and ophthalmic clinical data were obtained by the treating clinician through clinical ophthalmic examination and medical history. Ancestral history was self-reported by the study participant. Self-reported cardiovascular medical history was obtained using a standardized health questionnaire. This questionnaire specifically asked whether a participant had a history of hypertension or myocardial infarction. It also asked participants to list all current medications. Self-reported medical history and medication lists were then cross-referenced with notes from general practitioner referrals by the recruiting clinician at the time of enrollment. For the purpose of confirming correlation between the cardiovascular disease genetic risk score and cardiovascular disease in this population, a history of cardiovascular disease was arbitrarily defined by the presence of either hypertension, lipid-lowering therapy (statins, fibrates, or ezetimibe), or myocardial infarction.

Baseline demographic and ophthalmic data for ANZRAG participants were similarly obtained as part of a clinical ophthalmic examination and medical history at the time of enrollment by the treating clinician. Baseline demographic data were available for the QSkin Sun and Health Study participants. This was obtained through a postal questionnaire at the time of enrollment.

Structural Phenotype Definitions

Patients from the PROGRESSA and ANZRAG studies were phenotyped based on the presence of reproducible structural thinning on macular GCIPL or pRNFL spectral-domain OCT imaging. Patients with glaucoma in each cohort were characterized as showing one of the following: (1) predominantly pRNFL thinning, (2) equivalent macular GCIPL and pRNFL thinning, (3) predominantly macular GCIPL thinning, or (4) no detectable thinning at baseline.[5] Predominant pRNFL thinning was defined by at least 1 eye showing solely pRNFL structural defects (with the contralateral eye showing either no structural change, solely pRNFL structural change, or both pRNFL and macular GCIPL structural change; Fig 1A). Equivalent macular GCIPL and pRNFL thinning was defined by either both eyes showing pRNFL and macular GCIPL defects or 1 eye showing pRNFL and macular GCIPL defects in the absence of structural change in the contralateral eye (Fig 1B). Predominant macular GCIPL thinning was defined by at least 1 eye showing solely macular GCIPL structural change (with the contralateral eye showing either no structural change, solely macular GCIPL structural change, or both pRNFL and macular GCIPL structural change; Fig 1C). The reference group of PROGRESSA participants was

Table 1. Baseline Demographics of the 3 Cohorts

Variable	Progression Risk of Glaucoma: Relevant SNPs with Significant Association Study (n = 768)	Australia New Zealand Registry of Advanced Glaucoma Study (n = 664)	QSkin Sun and Health Study (n = 17 642)	P Value*
Age (yrs)	64.2 ± 10.3	72.6 ± 10.6	57.0 ± 7.89	<0.001
Sex (% female)	57.6	53.86	54.9	0.308
Ancestry (% European)	89.4	95.2	95.3	<0.001

Continuous variables are summarized as mean ± standard deviation, with discrete variables summarized as percentages.
*Analysis of variance.

defined as showing no detectable pRNFL or macular GCIPL thinning at baseline (Fig 1D) and no longitudinal structural or functional progression. In essence, although these patients were recruited into PROGRESSA because of a clinically suspicious disc (i.e., DDLS ≥1 at enrollment), they did not exhibit any clinically detectable disease progression during the monitoring period.

OCT Imaging

Structural phenotyping for patients in both the PROGRESSA and the ANZRAG cohorts was undertaken by reviewing the spectral-domain OCT thickness measurements of the pRNFL and the macular GCIPL. Patients in these studies undergo regular Cirrus HD-OCT optic disc 200 × 200 cube scans and macula 512 × 128 cube scans during study involvement (software version 9.5; Carl Zeiss Meditec, Dublin, CA), with fixation on the optic disc and the fovea using Cirrus Fastrac technology. Scans exhibiting signal strength of < 6, a significant acquisition artefact, or non-glaucomatous pathologic features were excluded from analysis. For patients in the PROGRESSA and ANZRAG studies, we reviewed the baseline and first follow-up spectral-domain OCT scans. Patients without CIRRUS spectral-domain OCT imaging were excluded from analysis.

Phenotyping was then undertaken by reviewing the 6 × 6 mm² pRNFL and the elliptical macular GCIPL thickness deviation maps for the respective optic disc or macula cube scans. For patients enrolled in the PROGRESSA and ANZRAG studies, a structural defect was defined by the presence of a reproducible region with measured thickness less than the lower normative population centile, as indicated by red discoloration of 4 × 4 pixels (>16 contiguous pixels) on the baseline and first follow-up pRNFL or macular GCIPL thickness deviation map.

Assessment of the association between cardiovascular disease genetic risk score and longitudinal structural progression was undertaken using Cirrus HD-OCT trend analysis software (software version 9.5). For each eye, the rate of average pRNFL thinning and the rate of average macular GCIPL thinning was obtained and used for analysis.

Baseline spectral-domain OCT macular GCIPL and pRNFL thickness deviation maps were reviewed for all participants in this study. Participants were then characterized as showing either predominantly pRNFL thinning (Fig 1A), equivalent macular GCIPL and pRNFL thinning (Fig 1B), predominantly macular GCIPL thinning (Fig 1C), or no structural change (Fig 1D). A structural defect was defined by the presence of a reproducible region with a measured thickness of less than the normative centile.

Visual Field Assessment

We reviewed the baseline Humphrey Visual Field (HVF) 24-2 Swedish Interactive Threshold Algorithm Standard tests for all eyes included in the study to classify participants as having either perimetric glaucoma or suspected glaucoma at study enrollment. Participants with perimetric glaucoma were defined by the presence of a reproducible glaucomatous visual field defect (as per a modified Hoddapp-Parrish-Anderson criteria) on consecutive reliable HVF assessments at baseline in at least 1 eye.[17] A reliable HVF was defined by a fixation of loss < 33% and a false-positive rate of < 33%. We defined a glaucomatous visual field defect as abnormal glaucoma hemifield test results or pattern standard deviation (PSD) of $P < 0.05$ and 3 contiguous HVF locations with pattern deviation defect at $P < 0.05$, reproducible in the same HVF zone on 2 successive HVF tests. If the glaucoma hemifield test results and the PSD were normal, then at least 1 of the 3 contiguous HVF locations was required to have a pattern deviation defect at $P < 0.01$ on 2 successive HVF tests. Patients with suspected glaucoma did not demonstrate glaucomatous visual field defect at baseline as per the criteria above, albeit having optic disc features of possible or likely glaucoma (DDLS grade, 1−2).[18] Baseline visual fields for perimetric participants were then assessed for the presence of paracentral visual field change, which was defined by the involvement of the central 16 points of the 24-2 HVF pattern without involvement of the corresponding temporal wedge.[9,19]

Genetic Risk Score Calculations

A per-allele weighted cardiovascular disease genetic risk score was calculated for each participant in the PROGRESSA study, the ANZRAG study, and the QSkin Sun and Health study. To do so, we used the per−single nucleotide polymorphism (SNP) summary statistics from a previously described genetic risk score for cardiovascular disease.[8] Briefly, Inouye et al[8] developed a genetic risk score for coronary artery disease from the per-allele effect sizes of 1.7 million SNPs with genome-wide association for this trait, after a meta-analysis of 3 genetic risk scores and validation in 482 629 participants from the UK Biobank. In addition, a per-allele weighted genetic risk score for IOP and vertical cup-to-disc ratio (VCDR) were also calculated using previously published summary statistics for these clinical variables. The derivation of these scores can be found elsewhere and did not include PROGRESSA participants.[12,20] In each instance, the calculated genetic risk score for each participant was normalized to the QSkin Sun and Health study cohort as Z scores. Participants in the PROGRESSA study were genotyped on HumanCoreExome arrays (Illumina); participants in the ANZRAG study were genotyped on Illumina Omni1M, OmniExpress, or HumanCoreExome arrays (Illumina); and participants from the QSkin Sun and Health study cohort were genotyped using the Illumina GSA array. After genotype imputation for all cohorts, genetic risk score calculations were undertaken using PLINK version 1.90 beta.[21]
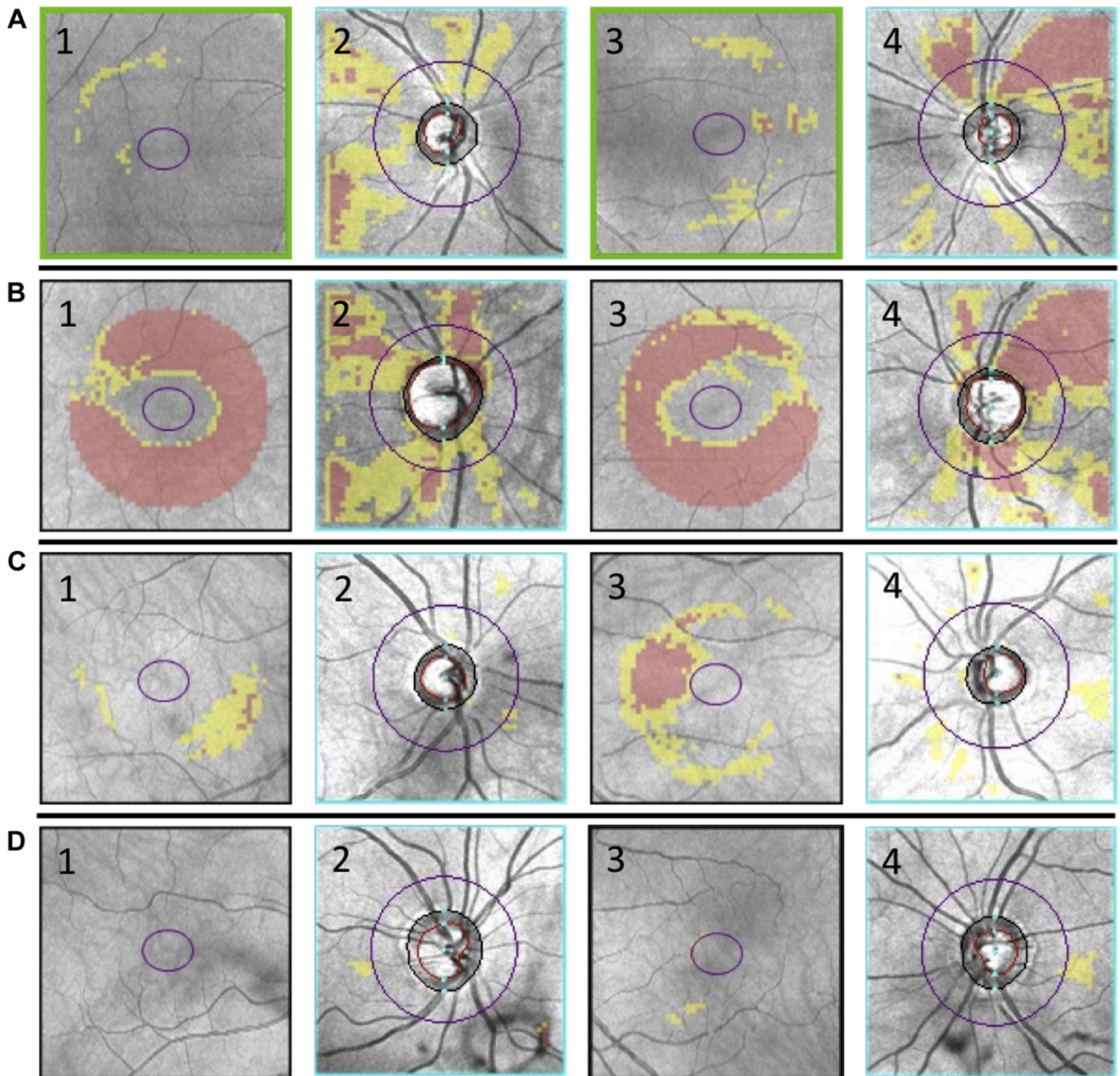
Figure 1. Illustration of baseline structural phenotypes. **A**, Participant demonstrating predominantly peripapillary retinal nerve fiber layer (pRNFL) thinning. **B**, Participant demonstrating equivalent macular ganglion cell–inner plexiform layer (GCIPL) thinning and pRNFL thinning. **C**, Participant demonstrating predominantly macular GCIPL thinning. **D**, Participant demonstrating no structural defects. In all panels, images are as follow: (1) right eye spectral-domain OCT macular GCIPL thickness deviation map, (2) right eye spectral-domain OCT pRNFL thickness deviation map, (3) left eye spectral-domain OCT macular GCIPL thickness deviation map, and (4) left eye spectral-domain OCT pRNFL thickness deviation map. A structural defect was defined by the presence of a visually reproducible region with thickness less than the lowest centile.

Statistical Analyses

The analysis of the association between a genetic risk score for cardiovascular disease and baseline spectral-domain OCT phenotyping was conducted through a stepwise protocol. Initially, preliminary analyses of variance (ANOVAs) were used first to assess for demographic differences between cohorts and second to compare the ocular and cardiovascular histories between structural phenotypes in the PROGRESSA cohort. An ANOVA was then implemented to compare the mean cardiovascular disease genetic risk score between structural phenotypes. Pairwise comparisons of cardiovascular disease genetic risk score between glaucoma phenotype groups and reference participants were then conducted as multivariate generalized linear modeling, which included age squared, sex, and IOP at enrollment as covariates. The *P* value threshold was adjusted for familywise error rate (Bonferroni method; adjusted *P* value threshold, 0.017). Secondary analyses were then undertaken in a subanalysis of patients with perimetric

glaucoma and by comparing the cardiovascular disease genetic risk score with the normative QSkin Sun and Health cohort.

Further analyses were undertaken comparing the genetic risk scores for the known glaucoma risk factors: IOP and VCDR. For each of these genetic risk scores, glaucoma phenotype groups were compared with the normative population from the QSkin Sun and Health Study. Comparisons between phenotype groups and internal reference participants from the PROGRESSA study were not conducted for these genetic risk scores because the reference group exhibited enrichment of these scores because of PROGRESSA being a cohort of patients with glaucoma. All comparisons between structural phenotypes and QSkin Sun and Health study participants included age, age squared, and sex as covariates. The *P* value threshold for statistical significance in all secondary analyses was set at 0.05. The findings from these analyses were then replicated by structurally phenotyping a cohort of participants from the ANZRAG study with perimetric glaucoma.

A secondary analysis was undertaken to investigate the association between paracentral visual field change and cardiovascular disease genetic risk. For this analysis, multivariate generalized linear modeling compared the cardiovascular genetic risk of PROGRESSA participants with perimetric glaucoma demonstrating paracentral visual field change with PROGRESSA reference participants and with QSkin Sun and Health control participants. Once again, age squared, sex, and IOP at enrollment were included in modeling as covariates, and the *P* value threshold was set at 0.05.

Multivariate linear regression analyses with mixed effects then assessed the association between cardiovascular disease genetic risk score and per-eye rate of average macular GCIPL thinning and rate of average pRNFL thinning. For this analysis, participants were grouped into high risk (>80th percentile of normative QSkin Sun and Health population) and low risk (<20th percentile of normative QSkin Sun and Health population) of cardiovascular disease, as per the stratifications previously used by Inouye et al.[8] Models were fitted using the lmerTest package version 3.1.2, with a random intercept per patient to account for intereye correlation. We additionally evaluated for correlation between the cardiovascular disease genetic risk score and the IOP and the VCDR genetic risk scores. This was undertaken by fitting a linear regression between the given genetic risk scores in the QSkin Sun and Health study cohort of normative participants. Tabulated summary data are presented as mean ± standard deviation for continuous variables and percentages for discrete variables within each structural phenotype or reference group.

Results

Patient Characteristics

The baseline macular GCIPL and pRNFL spectral-domain OCT thickness deviation maps of 1656 eyes from 828 genotyped participants (mean age, 64.4 ± 10.6 years; female sex, 55.2%) enrolled in the PROGRESSA study between May 2012 and January 2021 were reviewed for evidence of baseline structural defects. Fifty-one patients (6.2%) were excluded because of the presence of an acquisition artefact, nonglaucomatous pathologic features, or poor signal strength in at least 1 eye. A further 9 participants were excluded because of the presence of high myopia. The spectral-domain OCT baseline thickness maps of 768 remaining individuals were then assessed for characterization of baseline thinning. One hundred eighty-one patients showed predominantly macular GCIPL thinning, 284 patients showed equivalent macular GCIPL and pRNFL thinning, and 192 patients showed predominantly pRNFL thinning. One hundred eleven patients showed no detectable macular GCIPL or pRNFL thinning in either eye. Twenty of these participants were then excluded because of the presence of spectral-domain OCT or HVF progression during monitoring. The remaining 91 participants demonstrated neither structural or visual field change over a minimum of 3 years (mean ± SD, 5.34 ± 1.29 years) and were used as an internal reference group. At baseline, 30.4% (n = 203) of nonreference participants were classified as having perimetric glaucoma, and 16.5% (n = 106) of all nonreference participants did not have reliable visual fields.

The replication cohort was derived from 664 genotyped patients with nonadvanced POAG from ANZRAG with adequate Cirrus spectral-domain OCT macula and optic nerve head scans. Four hundred thirty participants showed equivalent macular GCIPL and pRNFL thinning, 114 participants showed predominantly macular GCIPL thinning, 92 participants showed predominantly pRNFL thinning, and 28 participants did not show any detectable macular GCIPL or pRNFL thinning in either eye. The mean age of this cohort was 72.6 ± 10.6 years and 53.85% were women.

The external normative control cohort comprised 17 642 genotyped participants from the QSkin Sun and Health Study. The mean age of this cohort was 64.1 ± 7.89 years, and 55.9% were women (Fig 2).

Preliminary Assessments

Preliminary evaluation of cardiovascular disease genetic risk score confirmed that a higher cardiovascular genetic risk score was associated with a greater likelihood of cardiovascular disease (OR, 1.32; 95% CI, 1.13−1.54; *P* = 0.001). No association was observed between cardiovascular disease genetic risk score and age (*P* = 0.667).

Association between Cardiovascular Disease Genetic Risk Scores and Structural Phenotype

Preliminary comparisons between structural phenotypes demonstrated a higher prevalence of cardiovascular disease in those participants with predominantly macular GCIPL thinning (*P* < 0.001, ANOVA; Table 2). Furthermore, participants with predominantly macular GCIPL thinning exhibited a higher prevalence of cardiovascular disease than the reference group after adjusting for age, sex, and baseline IOP (OR, 1.99; 95% CI, 1.06−3.73; *P* = 0.029).

Comparison of cardiovascular disease genetic risk score among structural phenotypes demonstrated significant differences between study groups (*P* < 0.001, ANOVA). Participants with predominantly macular GCIPL thinning demonstrated a higher cardiovascular genetic risk score than reference PROGRESSA participants (OR, 1.76/SD; 95% CI, 1.18−2.62; *P* = 0.005; Fig 3). This association was also present in a sensitivity analysis including a history of cardiovascular disease as a covariate (OR, 1.69/SD; 95% CI, 1.13−2.54; *P* = 0.010) and in a subanalysis comparing perimetric glaucoma participants with predominantly macular GCIPL thinning (n = 52) with
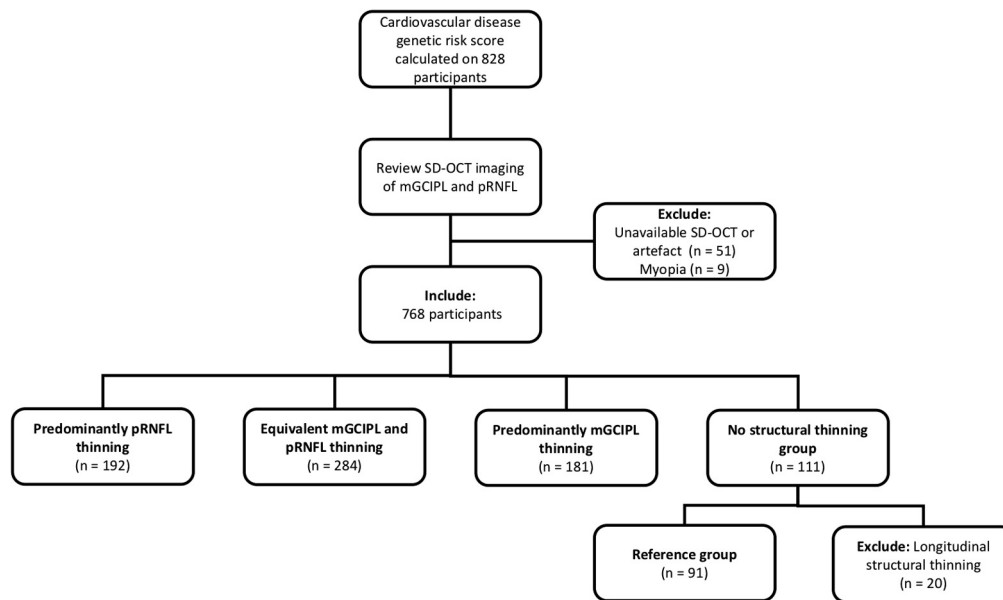
Figure 2. Schematic illustration showing Progression Risk of Glaucoma: Relevant SNPs [single nucleotide polymorphisms] with Significant Association study cohort (PROGRESSA). mGCIPL = macular ganglion cell−inner plexiform layer; pRNFL = peripapillary retinal nerve fiber layer; SD = spectral-domain.

reference participants (OR, 2.04/SD; 95% CI, 1.29−3.23; $P = 0.002$). To address the potential confounding influences of myopia on spectral-domain OCT imaging, a subgroup analysis was undertaken excluding those individuals with myopia (spherical equivalence worse than −0.5 diopters; OR, 1.83/SD; 95% CI, 1.11−3.05; $P = 0.004$).[14] Finally, a subanalysis to account for age differences between study groups compared the cardiovascular genetic risk score of predominantly macular GCIPL thinning group with 40 age-matched reference participants (mean age, 67.3 ± 6.8 years; $P = 0.890$ for age comparison). Participants with predominantly macular GCIPL thinning exhibited a higher cardiovascular genetic risk score than an age-matched reference group (OR, 2.02/SD; 95% CI, 1.31−3.14; $P = 0.001$).

In addition, participants with predominantly macular GCIPL thinning showed a higher cardiovascular disease genetic risk score than participants with predominantly pRNFL thinning (OR, 1.47/SD; 95% CI, 1.12−1.93; $P = 0.004$). Participants with predominantly pRNFL thinning exhibited a cardiovascular disease genetic risk score comparable with that of reference participants ($P = 0.354$).

Secondary analysis compared participants with predominantly macular GCIPL thinning with normative participants from the QSkin Sun and Health cohort. The PROGRESSA participants with predominantly macular GCIPL thinning showed a higher cardiovascular disease genetic risk score than QSkin Sun and Health study participants (OR, 1.32/SD; 95% CI, 1.12−1.54; $P = 0.002$). This observation was replicated in a subgroup analysis of 4057 randomly sampled age-matched QSkin Sun and Health study participants (mean age, 67.0 ± 1.8 years [$P = 0.681$]; OR, 1.30; 95% CI, 1.19−1.53 [$P = 0.001$]). Finally, it was also replicated in a subanalysis of White individuals to account for ethnic

differences between study groups (OR, 1.30; 95% CI, 1.08−1.57; $P = 0.005$).

Association between Cardiovascular Disease Genetic Risk and Structural Thinning in Australia New Zealand Registry of Advanced Glaucoma Cohort

The ANZRAG participants exhibiting predominantly macular GCIPL structural change showed a higher cardiovascular disease genetic risk score (OR, 1.39/SD; 95% CI, 1.05−1.83; $P = 0.022$) than participants from the QSkin Sun and Health Study. Participants with predominantly pRNFL thinning did not exhibit a higher cardiovascular disease risk than the normative population ($P = 0.758$), nor did participants with equivalent macular GCIPL and pRNFL thinning ($P = 0.338$).

Evaluation of Association with Longitudinal Disease Progression

Evaluation of the association between cardiovascular disease genetic risk score and longitudinal disease progression was undertaken by comparing the average rate of macular GCIPL thinning for participants in the high cardiovascular disease genetic risk (upper quintile) with participants in the low cardiovascular disease genetic risk (lower quintile) in the PROGRESSA study. Quintile thresholds were determined based on percentiles derived from normative control participants. Participants in the highest cardiovascular disease genetic risk score quintile demonstrated a faster rate of macular GCIPL thinning than participants in the lowest quintile (β coefficient, 0.093 μm/year; 95% CI, 0.002−0.18; $P = 0.044$). This association was observed in sensitivity

Table 2. Comparison of Clinical Risk Factors among Structural Phenotype Groups

Variable	Predominantly Peripapillary Retinal Nerve Fiber Layer Thinning (n = 192)	Equivalent Macular Ganglion Cell−Inner Plexiform Layer and Peripapillary Retinal Nerve Fiber Layer Thinning (n = 284)	Predominantly Macular Ganglion Cell−Inner Plexiform Layer Thinning (n = 181)	Reference Group (n = 91)	P Value*
Age (yrs)	61.7 ± 11.1	65.3 ± 10.1	67.7 ± 9.9	61.1 ± 10.5	<0.001
Sex (% female)	52.3	53.5	58.3	61.5	0.465
IOP (mmHg)	22.0 ± 6.2	21.0 ± 5.6	19.4 ± 4.9	19.2 ± 3.8	<0.001
Ancestry (% European)	89.0	88.4	89.1	96.1	0.436
Cardiovascular disease (%)	46.4	56.0	66.4	41.8	<0.001

IOP = intraocular pressure.
Continuous variables are summarized as mean ± standard deviation, with discrete variables summarized as percentages.
*Analysis of variance.

analyses that incorporated antiplatelet and antilipid therapy as covariates (β coefficient, 0.11 μm/year; 95% CI, 0.01−0.21; $P = 0.018$) and after accounting for the presence of IOP-lowering therapy (β coefficient, 0.09 μm/year; 95% CI, 0.001−0.18; $P = 0.041$). No difference was observed between the top and bottom quintiles when comparing the rate of pRNFL thinning ($P = 0.663$).

Secondary Analysis of Association between Intraocular Pressure and Vertical Cup-to-Disc Ratio Genetic Risk Scores and Structural Phenotype

Next, we explored the association of genetic risk scores for IOP and VCDR with these structural phenotypes. To
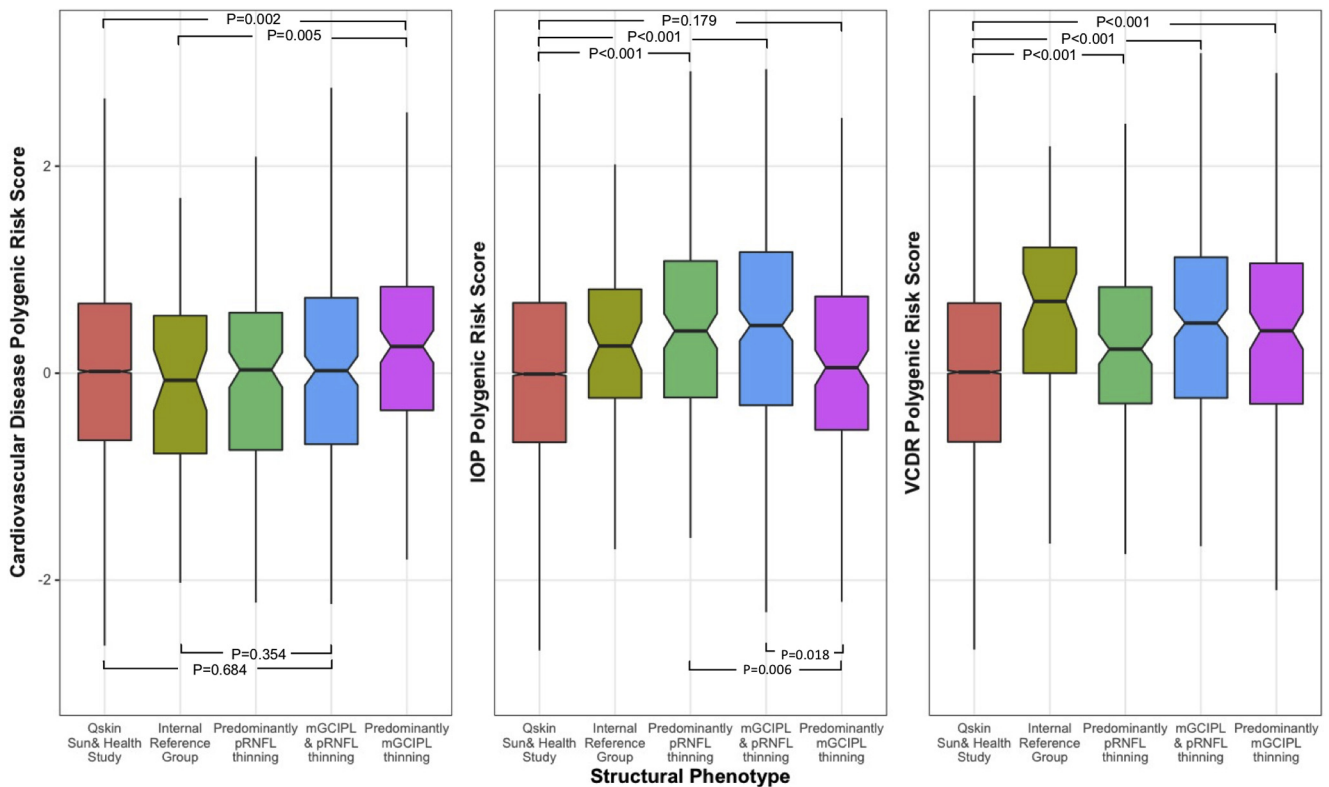
Figure 3. Box-and-whisker plot showing comparison of polygenic risk scores between phenotypes. **Left panel,** Comparison of cardiovascular disease polygenic risk score between structural phenotypes. **Middle panel,** Comparison of intraocular pressure (IOP) genetic risk score between structural phenotypes. **Right panel,** Comparison of vertical cup-to-disc ratio (VCDR) genetic risk score between structural phenotypes. The red box indicates QSkin Sun and Health Study participants, the yellow box indicates internal reference group, the green box indicates participants demonstrating predominantly peripapillary retinal nerve fiber layer (pRNFL) thinning, the blue box indicates participants demonstrating both macular ganglion cell−inner plexiform layer (mGCIPL) and pRNFL thinning; and the pink box indicates participants demonstrating predominantly macular GCIPL thinning.

circumvent the selection bias associated with using a cohort with glaucoma, comparisons of genetic risk scores were undertaken by comparing structural phenotypes with the normative population from the QSkin cohort, rather than the internal PROGRESSA reference group. Participants with predominantly macular GCIPL thinning showed a higher VCDR genetic risk score (OR, 1.48/SD; 95% CI, 1.24−1.76; $P < 0.001$), but a comparable IOP genetic risk score (OR, 1.12/SD; 95% CI, 0.95−1.33; $P = 0.179$) with the normal population. Participants with predominantly pRNFL thinning showed a higher IOP genetic risk score (OR, 1.46/SD; 95% CI, 1.25−1.72; $P < 0.001$) and a higher VCDR genetic risk score (OR, 1.28/SD; 95% CI, 1.09−1.50; $P < 0.001$) than the normal population. Finally, participants with both macular GCIPL and pRNFL thinning showed a higher IOP genetic risk score (OR, 1.46/SD; 95% CI, 1.28−1.66; $P < 0.001$) and a higher VCDR genetic risk score (OR, 1.56/SD; 95% CI, 1.36−1.78; $P < 0.001$) than normative participants (Fig 3).

Participants with predominantly macular GCIPL thinning showed a higher cardiovascular disease polygenic risk score than the external normative population ($P = 0.002$) and than the internal reference group ($P = 0.005$), but a comparable IOP genetic risk score to QSkin Sun and Health Study participants ($P = 0.179$). Participants with predominantly pRNFL thinning showed a higher IOP polygenic risk score than normative QSkin Sun and Health Study participants ($P < 0.001$), but a comparable cardiovascular disease polygenic risk score to internal reference group ($P = 0.354$) and to QSkin Sun and Health Study participants ($P = 0.684$). All study phenotypes demonstrated higher VCDR polygenic risk scores compared to the normative QSkin Sun and Health Study participants (all $P < 0.001$)

Association of Cardiovascular Disease Genetic Risk Scores with Paracentral Field Change

Macular GCIPL thinning was previously correlated functionally with paracentral 24-2 HVF change.[22] The PROGRESSA participants with paracentral visual field change at enrollment (n = 59 of 203) demonstrated a higher cardiovascular disease genetic risk score than PROGRESSA reference participants (OR, 1.85/SD; 95% CI, 1.16−2.97; $P = 0.010$). PROGRESSA participants with paracentral visual field also exhibited a higher cardiovascular disease genetic risk score than normative control participants (OR, 1.37/SD; 95% CI, 1.06−1.76; $P = 0.015$; $P = 0.026$). Replication of this finding was undertaken by reviewing the visual field data participants from the ANZRAG cohort. The ANZRAG participants with paracentral field change (n = 167) exhibited a higher cardiovascular disease genetic risk score than normative control participants (OR, 1.31; 95% CI, 1.04−1.65; $P = 0.021$) and than ANZRAG participants without paracentral field change (OR, 1.28/SD; 95% CI, 1.03−1.59; $P = 0.029$). Furthermore, ANZRAG participants with paracentral field change exhibited higher IOP polygenic risk scores (OR, 1.42; 95% CI, 1.14−1.76; $P = 0.002$) and a higher VCDR polygenic risk score (OR, 1.47; 95% CI, 1.16−1.86; $P = 0.001$) than normative control participants (Fig 4; Table 3).

The ANZRAG participants with paracentral visual field change demonstrated a higher cardiovascular disease polygenic risk score than normative control participants ($P = 0.021$) and than ANZRAG participants without paracentral visual field change ($P = 0.029$). The ANZRAG participants with paracentral visual field change also demonstrated a higher IOP and a higher VCDR polygenic risk score than normative control participants ($P = 0.002$ and $P = 0.001$, respectively).

Assessment of Pleiotropy

Linear regression using participants from the QSkin Health and Sun Study showed that higher cardiovascular disease genetic risk score was associated with a higher VCDR polygenic risk score (β coefficient, 0.05; 95% CI, 0.03−0.07; $P < 0.001$; $R^2 = 0.002$). This association was also present in the PROGRESSA study cohort (β coefficient, 0.09; 95% CI, 0.03−0.14; $P = 0.016$; $R^2 = 0.007$). No observed association was found between cardiovascular disease genetic risk score and IOP genetic risk score ($P = 0.194$).

Discussion

This genetic association study evaluated the relationship between cardiovascular disease and glaucoma. It identified that a higher cardiovascular disease genetic risk score was associated with a greater likelihood of baseline macular GCIPL structural thinning, longitudinal macular GCIPL progression, and parafoveal visual field change. Further analysis then evaluated the contribution of polygenic risk scores for IOP and VCDR on the site of earliest structural change.

This study builds on pre-existing literature investigating the association between cardiovascular disease and glaucoma. Multiple systematic reviews have illustrated that a history of cardiovascular disease may be an important risk factor for glaucoma diagnosis and progression.[23−25] Several smaller studies in turn have proposed that the macular ganglion cell complex may be particularly susceptible to cardiovascular dysfunction.[26,27] This study developed this work by illustrating that a higher cardiovascular disease genetic risk score was strongly correlated with both baseline and longitudinal ganglion cell layer thinning. Interestingly, participants demonstrating macular GCIPL thinning also exhibited an IOP genetic risk score comparable with that of the normative population. These findings corroborate previous work by our group and others that has shown that factors other than IOP may be particularly influential in glaucoma with early macular thinning.[9]

This study also demonstrated associations between cardiovascular disease genetic risk score and paracentral visual field progression. This finding corroborates other studies that proposed that systemic vascular risk factors may be implicated in parafoveal visual field change.[28−30] This analysis also showed that participants with paracentral visual field change also demonstrated higher IOP and higher
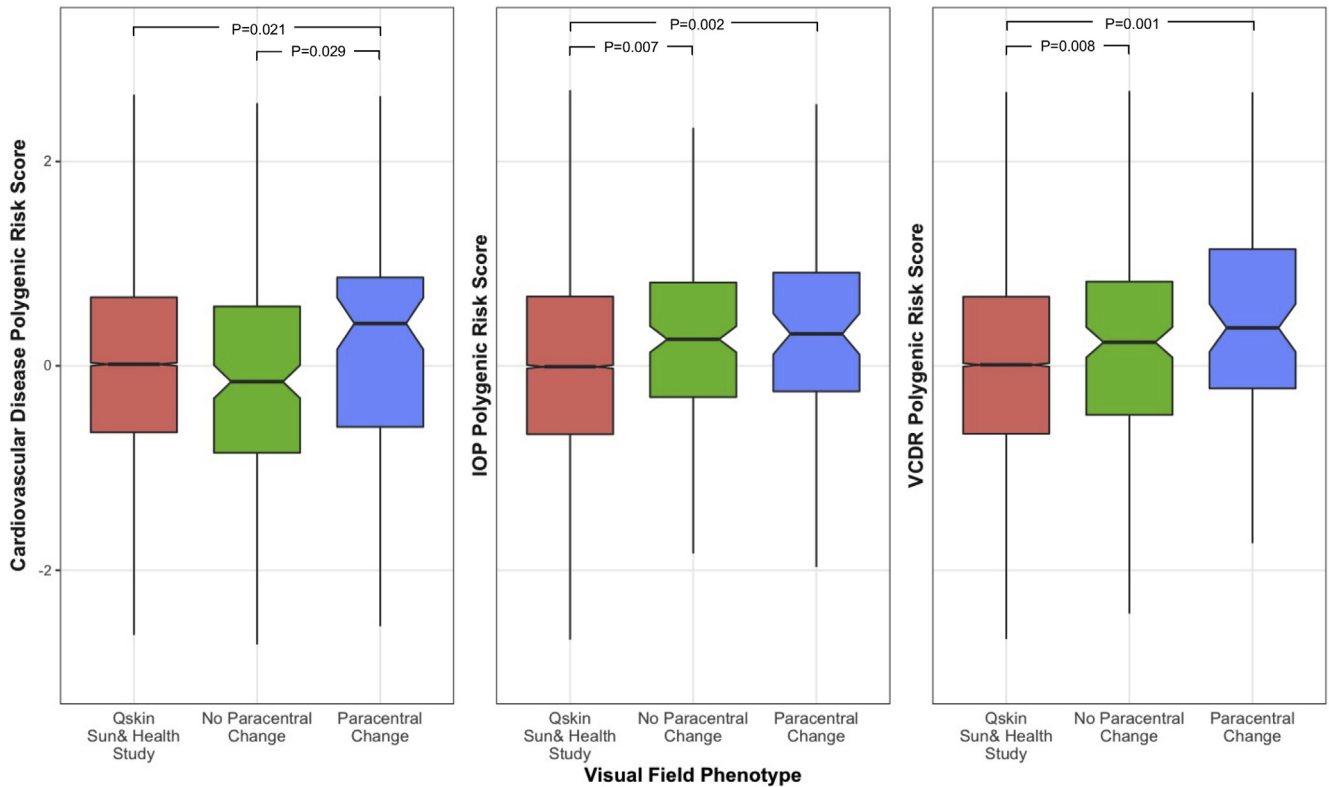
Figure 4. Box-and-whisker plot showing comparison of polygenic risk scores between visual field phenotypes in the Australia New Zealand Registry of Advanced Glaucoma (ANZRAG) study. **Left panel**, Comparison of cardiovascular disease polygenic risk score between visual field phenotypes. **Middle panel**, Comparison of intraocular pressure (IOP) genetic risk score between visual field phenotypes. **Right panel**, Comparison of vertical cup-to-disc ratio (VCDR) genetic risk score between visual field phenotypes. The red box indicates QSkin Sun and Health Study participants, the green box indicates ANZRAG participants demonstrating paracentral visual field change; and the blue box indicates ANZRAG participants who did not demonstrate paracentral visual field change.

VCDR polygenic risk scores, which highlights the multifactorial nature of disease progression in glaucoma. Nevertheless, we believe that these findings propose a possible structure–function relationship among cardiovascular disease risk, baseline macular GCIPL thinning, and subsequent paracentral field change.

It is also of interest that the cardiovascular disease genetic risk score was positively correlated with VCDR polygenic risk score. A plausible explanation is that variants for some genetic loci may be associated with both traits. For instance, common sequence variations at the CDKN2BAS1 locus, which are known risk factors for POAG diagnosis, have also been correlated with both a greater risk of coronary artery disease and a larger VCDR.[31–34] Although the power of this study is likely too small for a formal Mendelian randomization study, one may speculate that these

Table 3. Summary Characteristics of Participants with and without Paracentral Visual Field Change

Variable	No Paracentral Visual Field Defect (n = 144)	Paracentral Visual Field Defect (n = 59)	Univariate P Value*
Age (yrs)	67.8 ± 8.6	67.0 ± 10.1	0.747
Sex (% female)	53.5	61.2	0.247
IOP (mmHg)†	19.4 ± 6.8	20.5 ± 6.7	0.229
Mean deviation (dB)‡	−2.52 ± 1.67	−2.66 ± 2.13	0.567
VCDR§	0.71 ± 0.09	0.75 ± 0.08	<0.001

IOP = intraocular pressure; VCDR = vertical cup-to-disc ratio.
Continuous variables are summarized as mean ± standard deviation, with discrete variables summarized as percentages.
*Generalized linear modelling comparing early manifest glaucoma participants without paracentral field involvement and those with paracentral visual field involvement.
†Highest IOP.
‡Worst mean deviation between the 2 eyes.
§Highest VCDR between the 2 eyes.

pleiotropic pathways might explain the observed interrelationships among cardiovascular disease, VCDR, and macular GCIPL thinning that were observed in this study.

The use of polygenic risk scores in this study helps to circumvent the confounding issues that are prevalent among observational studies. Findings from epidemiologic studies may be influenced by reporting bias, environmental exposures, or reverse causality between risk factors and outcomes.[35–37] Because the genome is randomized and fixed from conception, polygenic risk score techniques are well suited to overcoming these issues by reducing the influence of environmental and behavioral exposures.[6] Accordingly, genetic association studies may be used to evaluate causation or to identify shared pathways to disease. The findings from this study propose that cardiovascular disease and macula GCIPL thinning in glaucoma may at least share some similar pathways.

We do recognize several limitations of our study design. Our results may have been confounded by the inclusion of cohorts with ancestry variation. However, the genetic risk score developed by Inouye et al[8] was validated in a population with diverse ancestry from the UK Biobank. In addition, the primary findings of this study were replicated in a subanalysis of participants of European descent. As such, we believe that the inclusion of all participants regardless of ancestry improves the generalizability of our results and helps to mitigate any potential selection biases. The use of arbitrary qualitative definitions of structural and functional change to phenotype participants in this study may also draw conjecture. Although these methods may fail to evaluate the association between these genetic risk scores and numerical outcomes, such as raw macular GCIPL thickness, they have the advantage of enabling the evaluation of risk factors that may cause either earlier or relatively greater macular or peripapillary thinning.

It is unclear if the retinal changes observed in this study reflect glaucomatous thinning because the centile thresholds are derived from the normative population.[38,39] In addition, macular GCIPL thinning was previously implicated in cardiovascular disease processes, and more broadly with other diseases of aging, such as Alzheimer's disease.[5,40] Regardless of cause, the observed genetic association between cardiovascular disease and visual field change indicates that these findings may hold functional implications in glaucoma-sensitive eyes. This methodology also included retinal areas that are less commonly associated with glaucoma diagnosis, such as the nasal pRNFL. Although including these regions limits the ability to assess the use of this genetic risk score to specifically predict glaucomatous change, it enables a more complete evaluation of the genetic association between cardiovascular disease and retinal thinning in glaucomatous eyes.

This study also arbitrarily defined cardiovascular disease by the presence of hypertension, lipid-lowering therapy, or history of myocardial infarction. The inclusion of lipid-lowering therapy in this definition may be criticized for being a surrogate marker for cardiovascular disease. However, Australian guidelines state that lipid-lowering therapy should be commenced in anyone with dyslipidemia, major risk factors for cardiovascular disease, or previous coronary artery disease.[41] In the absence of serum lipid profiling, we believe that this makes it suitable for the preliminary evaluation to verify the association between the genetic risk score developed by Inouye et al[8] and cardiovascular disease in this study population.

We also recognize limitations of using the QSkin Sun and Health study participants as a normative control group. It was beyond the scope of the QSkin Sun and Health study to collect ophthalmic medical history or to conduct spectral-domain OCT imaging of its participants. Therefore, this cohort is unlikely to reflect a truly healthy, non-glaucomatous population. However, the use of this cohort of more than 17 000 randomly selected normative participants did provide the opportunity to undertake robust analyses between a well-characterized glaucoma cohort and a population-based cohort. Although this population was not age matched to PROGRESSA study participants, we included age and gender as covariates in all analyses to account for demographic differences. We subsequently showed that glaucoma participants with predominantly macula GCIPL change demonstrated a higher cardiovascular genetic risk score than a normative population.

Similarly, one may question the use of patients with stable suspected glaucoma as an internal reference group because this group demonstrated enrichment of genetic risk scores for VCDR and IOP. This selection bias was accounted for by using the QSkin Sun and Health Study participants as a reference group in comparisons involving these genetic risk scores. However, this internal reference group did demonstrate a comparable cardiovascular disease genetic risk score to the normative population, which uniquely enabled the evaluation of the association between genetic risk for cardiovascular disease and macular GCIPL progression in glaucoma. We also recognize that this group was recruited as patients with suspected glaucoma, and hence does not reflect the normative, healthy population. Using a cohort of well-characterized patients with stable suspected glaucoma with longitudinal monitoring enables a robust analysis of risk factors contributing to structural and functional change in this disease.

Some limitations exist in the use of polygenic risk scores as instruments to assess causation. The large number of SNPs included in these genetic risk scores increases the likelihood of horizontal pleiotropy.[37,42] With an increasing number of included SNPs of small effect size, the usefulness of genetic risk scores to evaluate causation dimisnishes.[37,43] The genetic risk score developed by Inouye et al[8] contains more than 1.7 million SNPs. Furthermore, it is difficult to ascertain the specific mechanism explaining the observed correlations. As such, although the findings of this study suggest that glaucoma and cardiovascular disease may share common pathways, we recognize that one may not conclude direct causation explicitly between cardiovascular disease and glaucomatous thinning of the macula. Similarly, the clinical implication of these findings in individuals with a high genetic risk score remains unknown. By proposing that a shared pathway may exist, these observations suggest that a need to investigate cardiovascular disease and its sequelae in patients with glaucoma may exist.

Whether treating cardiovascular disease in high-risk individuals improves glaucoma outcomes remains to be discerned and is beyond the scope of this study.

This study evaluated the role of cardiovascular disease in glaucoma using a genetic risk score for coronary artery disease. It identified that a higher cardiovascular disease genetic risk score was associated with both structural and functional macular disease progression. This further highlights that cardiovascular disease may be an important risk factor for glaucomatous progression.

Footnotes and Disclosures

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