Henry Marshall, Sean Mullany, Ayub Qassim, Owen Siggs, Mark Hassall, Bronwyn Ridge, Thi Nguyen, Mona Awadalla, Nicholas H. Andrew, Paul R. Healey, Ashish Agar, Anna Galanopoulos, Alex W. Hewitt, Stuart MacGregor, Stuart L. Graham, Richard Mills, Angela Shulz, John Landers, Robert J. Casson, Jamie E. Craig

Cardiovascular disease predicts structural and functional progression in early glaucoma

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TITLE: Cardiovascular Disease predicts structural and functional progression in early glaucoma

AUTHORS: Henry Marshall[1], Dr Sean Mullany MD[1], Dr Ayub Qassim, MBBS[1], Dr Owen Siggs DPhil[1], Dr Mark Hassall, DPhil[1], Bronwyn Ridge [1], Thi Nguyen BMSc (Optom)[1], Dr Mona Awadalla MBBS,[1] Dr Nicholas Andrew MBBS,[1] A/Prof Paul R Healey, PhD [5,6], Dr Ashish Agar, PhD[7,8], Dr Anna Galanopoulos, FRANZCO[2], A/Prof Alex W Hewitt, PhD [3], A/Prof Stuart MacGregor PhD, Prof Stuart L Graham PhD[4], A/Prof Richard Mills PhD, A/Prof John Landers, PhD [1], Prof Robert J Casson PhD[2], Prof Jamie E Craig DPhil [1] (corresponding author)

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ADDRESS FOR REPRINTS: Flinders University, Department of Ophthalmology, Bedford Park, South Australia, 5042

RUNNING HEAD: Association of cardiovascular disease with baseline and longitudinal progression in glaucoma

ABSTRACT

Purpose: To investigate the association between cardiovascular disease and baseline structural defects and longitudinal disease progression in glaucoma.

Design: Prospective longitudinal cohort study of pre-perimetric and perimetric glaucoma.

Participants: 2122 eyes from 1089 participants recruited to the Progression Risk of Glaucoma: RElevant SNPs with Significant Association (PROGRESSA) study were evaluated for baseline and longitudinal structural thinning on Spectral Domain Optical Coherence Tomography (SD-OCT), and visual field progression on Humphrey Visual Field (HVF) assessment.

Methods: Eyes were classified as showing either solely mGCIPL, solely pRNFL, or both mGCIPL and pRNFL structural defects at study enrolment. The cardiovascular disease and medication characteristics of these study groups were compared to a subgroup of stable control eyes. The cardiovascular characteristics of eyes showing SD-OCT or HVF progression were compared to stable control eyes.

Outcome Measures: Baseline and longitudinal SD-OCT thinning and HVF progression.

Results: Eyes with solely mGCIPL structural defects at baseline had a higher prevalence of hypertension (OR: 2.47; 95% CI: [1.52, 4.02]; P<0.001), myocardial infarction (OR: 5.49 95%CI: [1.67, 18.07]; P=0.005), aspirin use (OR: 2.60; 95% CI: [1.28, 5.27; P=0.007), statin use (OR: 2.85; 95% CI: [1.61, 5.06]; P<0.001), and antihypertensive use (OR: 2.74; 95% CI: [1.68, 4.46]; P<0.001) than control eyes. Eyes with solely pRNFL structural defects did not exhibit a higher prevalence of cardiovascular disease or medication use than control eyes.

Review of longitudinal SD-OCT and HVF data (mean follow up: 5.71±1.48 years) showed that systemic hypertension was associated with an increased risk of structural progression (OR: 1.72; 95% CI: [1.02, 2.89]; P=0.034) and visual field progression (OR: 1.74; 95% CI: [1.16, 1.26]; P=0.021). The use of antihypertensive treatment was also associated with an increased risk of structural progression (OR: 1.59; 95%CI: [1.08, 2.37]; P=0.019), and visual field conversion (OR: 1.68; 95%CI: [1.11, 2.54]; P=0.013). A 1 standard deviation increase in systolic blood pressure was associated with a greater risk of structural progression (OR: 1.34; [1.09; 1.65]; P=0.005) and greater risk of visual field progression (OR: 1.26; 95%CI: [1.01, 1.56]; P=0.039). The association between

systolic blood pressure and structural progression was comparable to that observed between IOP and structural progression (OR: 1.31; 95% CI: [1.05, 1.63]; P=0.018).

Conclusion: Cardiovascular disease is an important risk factor for baseline mGCIPL structural change and for disease progression in glaucoma. Treatment of hypertension may be important in preventing structural and functional progression in early glaucoma, although further investigation to understand the effect of disease and treatment is required.

PRÉCIS

Cardiovascular disease, especially hypertension, is associated with macula GCIPL structural defects at baseline in glaucoma, and is predictive of both structural and visual field progression in early glaucoma.

ABBREVIATIONS:

ANOVA: Analysis of Variance

DDLS: Disc Damage Likelihood Scale

GLM: Generalised linear model

HVF: Humphrey Visual Field

IOP: Intraocular Pressure

mGCIPL: macula Ganglion Cell Inner Plexiform Layer

ONH: Optic Nerve Head

pRNFL: Peripapillary Retinal Nerve Fibre Layer

PROGRESSA: Progression Risk of Glaucoma: RElevant SNPs with Significant Association

SD-OCT: Spectral Domain Optical Coherence Tomography

INTRODUCTION

Glaucoma is an optic neuropathy in which raised intraocular pressure (IOP) is a major risk factor for retinal ganglion cell degeneration.[1,2] Several large population studies have identified cardiovascular disease to also be a risk factor for the diagnosis of primary open-angle glaucoma.[3–6] Potential shared patho-etiological mechanisms including microvascular damage and ocular perfusion pressure abnormalities have been proposed.[3,7] In this model, retinal vascular hypoperfusion is hypothesised to predispose retinal ganglion cells to injury at an IOP comparable to the normal population.[3,8,9]

Retinal ganglion cell degeneration may be clinically monitored using Spectral-Domain Optical Coherence Tomography (SD-OCT) thickness measurements of the peripapillary Retinal Nerve Fiber (pRNFL) and the macula Ganglion Cell Inner Plexiform Layer (mGCIPL). Although pRNFL and mGCIPL SD-OCT thickness measurements have comparable sensitivity and specificity for glaucoma diagnosis,[2,10–14] emerging evidence suggests that the site of earliest structural defect may hold clinical importance.[15–17] For instance, our group have recently demonstrated that mGCIPL loss precedes pRNFL loss in glaucoma with lower average IOP.[18] We therefore hypothesised that alternate pathways, such as vascular dysfunction, may be implicated in cases exhibiting first structural damage in the mGCIPL.

To test this hypothesis, this study characterised the baseline structural phenotype of eyes enrolled into the Progression Risk of Glaucoma: RElevant SNPs with Significant Association (PROGRESSA) study. It first compared the prevalence of cardiovascular risk factors within each endophenotype group to a subgroup of stable control eyes, before evaluating the association between these risk factors with both structural and functional progression.

METHODS

Study Overview

This study investigated the relationship between systemic cardiovascular risk factors and baseline structural change and longitudinal progression in pre-perimetric and perimetric glaucoma. Eyes studied were selected from the PROGRESSA study, which is an ongoing, longitudinal, prospective, multi-centre observational cohort study of early glaucoma cases and glaucoma suspects in Australia; PROGRESSA has been described elsewhere.[19]

Study Groups Definition

In this study, we primarily compared eyes that showed structural defects at baseline to a subgroup of structurally and functionally stable, control eyes. Baseline structural defects were defined by the presence of detectable thinning on either pRNFL or mGCIPL SD-OCT imaging. Eyes in this cohort were then classified based according to the site of their defect as either: "solely mGCIPL structural defects", "solely pRNFL structural defects" or "both mGCIPL and pRNFL structural defects" at baseline.

The subgroup of stable, control eyes were obtained from structurally and functionally stable patients. Inclusion into this group required that the patient did not exhibit a structural defect at baseline, and showed no event-based longitudinal SD-OCT progression, nor visual field progression in either eye during study involvement. From each patient, we then randomly selected one eye to analyse per control participant using a random number generator. In essence, although these patients were recruited into PROGRESSA due to a clinically suspicious disc (*i.e.* DDLS $\geq$1 at enrolment), they did not exhibit any structural or functional change over the study period.

This 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.

Ocular and Systemic Characteristics of study population

Ocular phenotypic data was obtained through 6-monthly ophthalmologic examinations, as per PROGRESSA protocol. Clinical ophthalmic data included: best corrected central visual acuity, IOP measurement (using Goldmann applanation tonometry), and corneal pachymetry. Comprehensive

previous ocular and medical history was obtained by the recruiting clinician using a standardised general health questionnaire at the time of study enrolment. This questionnaire specifically addressed cardiovascular risk factors including: hypertension, diabetes, myocardial infarction, and clinically diagnosed cerebrovascular events (strokes and transient ischaemic attacks). Vascular disease features addressed in the questionnaire included: history of migraine and history of Raynaud's disease. Furthermore, we elected to evaluate the association with common cardiovascular medications. Additional clinical cardiovascular data included blood pressure measurement and serum lipid profiles determined from baseline blood tests.

Optical Coherence Tomography imaging

OCT imaging was performed using the CIRRUS SD-OCT (Software Version 9.5; Carl Zeiss, Meditec; Dublin, CA). Peripapillary RNFL thickness was measured using the optic disc 200x200 cube scan, and macula GCIPL thickness was measured using the macula 512x128 cube scan. SD-OCT scanning was performed by an experienced operator using CIRRUS FastTrac eye-tracking technology with fixation centred on the optic disc and fovea. OCT imaging was performed at the time of enrollment to PROGRESSA and at each subsequent six-monthly visit. All SD-OCT scans were evaluated for image quality prior to assessment of baseline structural defects by a single investigator (HM). Scans with a signal strength <6, a significant acquisition artefact, or a non-glaucomatous pathology were excluded from analyses.

Baseline structural assessment was undertaken by evaluation of the SD-OCT 6x6mm² pRNFL and the elliptical mGCIPL thickness deviation maps that were obtained at an eye's enrolment into PROGRESSA. Baseline structural defects were defined by the presence of a region of 4 x 4 pixels (1 superpixel) encoded red on the respective thickness deviation maps, indicating that the thickness in this corresponding region of the en face image is below the lower age-adjusted 99th percentile threshold.

Figure 1A below depicts a case classified as "solely pRNFL structural defect," whereas Figure 1B below depicts a case classified as "solely mGCIPL structural defect." We then sought to compare the prevalence of cardiovascular risk factors in each structural phenotype to control eyes.

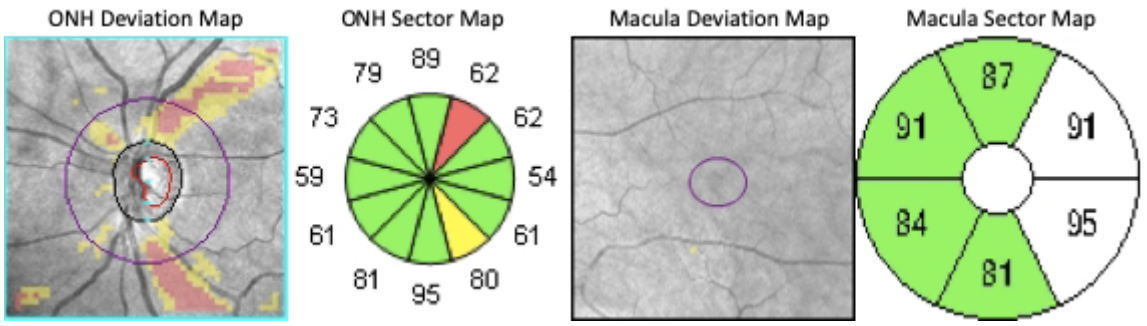

Figure 1A: A representative case of solely pRNFL structural defect

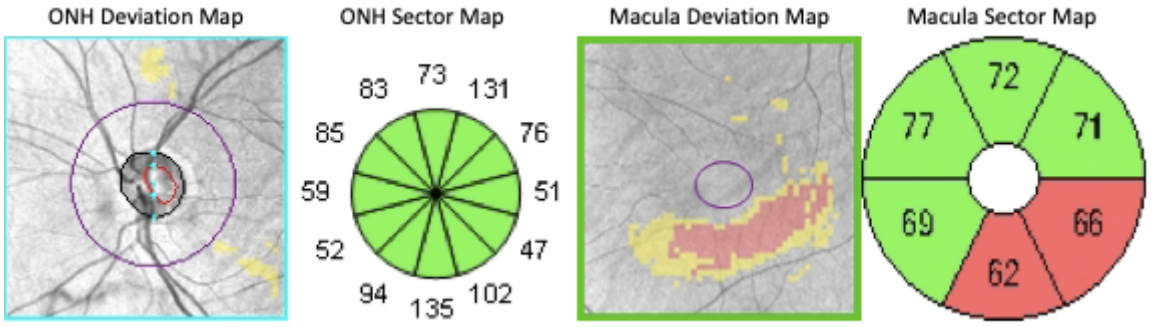

Figure 1B : A representative case of solely mGCIPL structural defect

Figure 1A and Figure 1B depict the ONH Deviation map, ONH Sector Thickness diagram, the Macula Cube Deviation map and the Macula Cube Sector Thickness diagram for two eyes in this study. The optic disc and macula OCT scans were performed at the same visit in both cases.

ONH: optic nerve head; pRNFL: peripapillary retinal nerve fibre layer; mGCIPL: macula ganglion cell inner plexiform layer; OCT: optical coherence tomography.

Assessment of longitudinal SD-OCT optic nerve head and macula scans was performed using commercially available CIRRUS HD-OCT event-based Guided Progression Analysis (GPA). Event-based progression was identified using a replicated "likely loss" criteria as previously described.[18,20] In addition, we used longitudinal trend analysis data to evaluate the effect of cardiovascular disease features on the rates of mGCIPL and pRNFL thinning.

Visual Field Assessment

We reviewed the baseline Humphrey Visual Field (HVF) 24-2 SITA Standard tests for all eyes included in the study to classify eyes as either early manifest glaucoma (EMG) or glaucoma suspect (GS) at study enrolment. EMG eyes were defined by the presence of a reproducible glaucomatous visual field defect, (as per a modified Hoddapp-Parrish-Anderson [HPA] criteria), on consecutive reliable HVF assessments at baseline.[21] A reliable HVF was defined by a fixation loss less than 33% and a false positive rate less than 33%. We defined a glaucomatous visual field defect as an abnormal

Glaucoma Hemifield Test (GHT) or Pattern Standard Deviation (PSD) <5% and three contiguous HVF locations with pattern deviation defect <5% significance level, reproducible in the same HVF zone on two successive HVF tests. If the GHT and the PSD were normal, then the three contiguous HVF locations were required to have a pattern deviation defect at <1% significance level on two successive HVF tests. GS cases did not demonstrate glaucomatous visual field defect at baseline as per criteria above albeit having optic discs features of possible or likely glaucoma (DDLS grade 1-2). Visual field progression for both GS and EMG eyes was defined by the presence of a new reproducible visual field defect, as per aforementioned criteria on two consecutive reliable visual fields.

Statistical Analyses

Univariate analyses of ocular and systemic characteristics between the outcome of baseline SD-OCT assessment and baseline ocular or systemic features were undertaken using a stepwise protocol. Initially, an analysis of variance (ANOVA) was implemented to compare study groups for a given ocular or cardiovascular parameter. Blood pressure and intraocular pressure measurements were adjusted for treatment using censored normal regression.[22] The p-value threshold was adjusted to 0.002 to account for multiple hypothesis testing (Bonferroni method). We then undertook pairwise comparisons between study groups and control eyes for variables with an ANOVA p-value <0.002 using univariate generalised linear modelling (GLMs) with mixed-effects.[23] GLMs with mixed effects were fitted with the *glmer* function from the *lme4* package (v1.1-18-1) in R (v3.4.1, RCore Team, Austria). The p-value threshold was adjusted for family-wise error rate (Bonferroni method; adjusted p-value threshold: 0.017). We then undertook a multivariate sub-analysis of EMG eyes with variable selection using criterion based procedures to verify the observed association between cardiovascular disease parameters and perimetric glaucoma. We subsequently investigated the influence of those variables predictive of baseline structural phenotype on longitudinal structural and functional progression using SD-OCT and HVF assessments. To do so, multivariable logistic and cox regression survival analyses compared the prevalence of cardiovascular characteristics between control eyes to eyes demonstrating the following outcomes: any structural progression (mGCIPL or pRNFL), mGCIPL structural progression, pRNFL structural progression and visual field progression. The p-value threshold for statistical significance in multivariable sub-analyses was 0.05.

Tabulated data presents mean ± standard deviation for continuous variables and prevalence (%) for discrete variables within each structural phenotype or control group. Post-hoc comparisons between structural phenotype groups (solely mGCIPL, both mGCIPL and pRNFL, or solely pRNFL) and control group were undertaken for variables with a p-value < 0.002 for analysis of variance, and are

described as mean difference and 95% confidence interval for continuous variables and odds ratio and 95% confidence interval for discrete variables.

RESULTS

Study group Characteristics

The baseline optic disc and macula deviation thickness maps of 2122 eyes from 1089 patients enrolled in the PROGRESSA study between May 2012 and March 2017 were reviewed for evidence of baseline structural thinning. 76 eyes were excluded due to poor quality scans, the presence of significant acquisition artefacts, or non-glaucomatous pathology. The SD-OCT baseline deviation thickness maps of 2046 eyes were assessed for the presence of structural defects. 315 eyes (16%) demonstrated structural defects solely on mGCIPL deviation thickness maps, 953 eyes (46%) demonstrated structural defects on both mGCIPL and pRNFL deviation thickness maps, and 316 eyes (16%) demonstrated structural defects solely on pRNFL deviation thickness maps. Of the 462 eyes that did not demonstrate any structural defects on baseline mGCIPL or pRNFL deviation thickness maps, 274 (59%) subsequently demonstrated glaucomatous SD-OCT or HVF deficits during monitoring. The remaining 187 eyes (from 94 patients) which demonstrated neither baseline structural defects nor structural progression over a minimum of 3 years (mean±SD; 5.13±1.38years) years were used as an internal, non-disease control group. One eye was randomly selected for analysis from each control participant. The mean age at baseline was 64.0 (SD 11.7 years), and 34% of all non control eyes were classified as early manifest glaucoma at baseline (Figure 2).

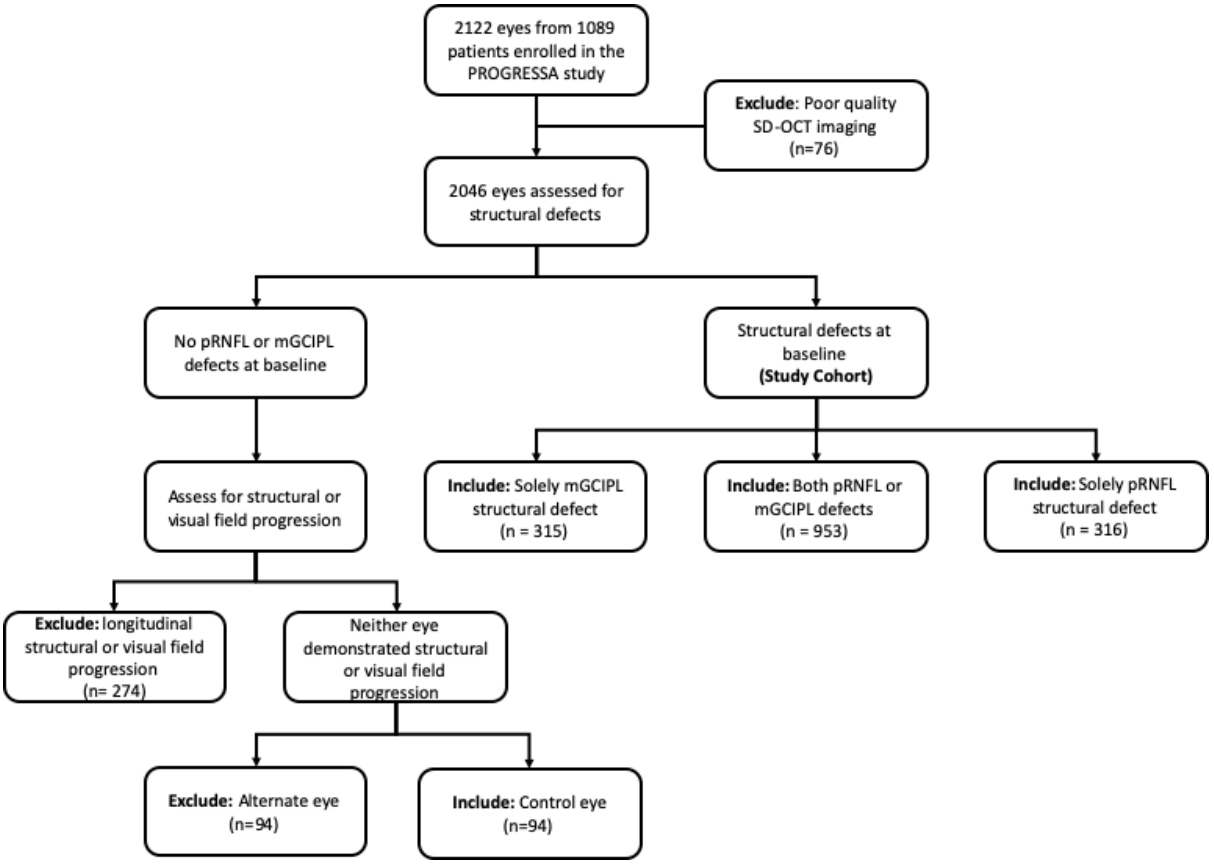

Figure 2: Schematic illustration of study characterisation

SD-OCT: Spectral-Domain Optical Coherence Tomography; pRNFL: peripapillary Retinal Nerve Fibre Layer; mGCIPL: macular Ganglion Cell Inner-Plexiform Layer

Baseline Ocular Characteristics

Univariate analysis of baseline ocular parameters demonstrated that eyes with solely pRNFL defects had a higher baseline IOP (mean difference: 4.45mmHg; 95%CI: [3.52; 5.37]; P<0.001), worse mean deviation (MD) (mean difference: -0.74; 95% CI: [-1.06, -0.42]; P<0.001) and more myopic spherical equivalent refraction (mean difference: -0.87; 95%CI: [-0.16, -1.57]; P=0.012) compared to control eyes. Eyes with solely mGCIPL defects also had a worse MD (mean difference: -1.06; 95%CI: [-0.65, -1.47]; P<0.001) were older (mean age difference: 5.45 years; 95%CI: [2.43; 8.47]; P=0.002), and more likely to be on topical glaucoma medication (OR: 6.30; 95%CI: [2.82,14.12]; P<0.001) than control eyes. Eyes with both mGCIPL and pRNFL defects had higher IOP (mean IOP difference: 1.56; 95% CI: [0.57, 2.56]; P<0.001) worse MD (mean IOP difference: -1.62; 95%CI: [-1.33 -1.91]; P<0.001), were older (mean age difference: 3.74; 95%CI: [0.94, 6.54]; P<0.001) and more likely to be on topical glaucoma medications (OR: 10.23; 95%CI: [5.63, 26.93]; P<0.001) when compared to control eyes at baseline.

Table 1: Comparison of Ocular characteristics between study groups

	Solely mGCIPL defects (n= 316)	**Both pRNFL and mGCIPL defects (n=949)**	**Solely pRNFL defects (n=315)**	**Control eyes (n=94)**	**Global P-value**
Baseline Ocular Parameters					
Baseline IOP (mmHg)	17.13±5.64	19.02±6.58	20.14±5.18	16.82±4.27	**<0.001**
Post-hoc comparison	0.33 [-1.68, 2.34] P=0.966	2.20 [0.19, 4.20] P=0.018	3.34 [1.39, 5.29] **P<0.001**		
VCDR	0.64±0.08	0.69±0.12	0.65 ±0.08	0.63±0.11	0.126
MD at Baseline (dB)	-1.24±2.26	-1.62±2.13	-0.48 ±1.77	0.33±1.08	**<0.001**
Post-hoc comparison	-1.57 [-2.08, -1.05] **(P<0.001)**	-1.95 [-2.43, -1.46] **(P<0.001**	-0.81 [-1.22, -0.39] **(P<0.001)**		
Central corneal thickness (µm)	551.52±31.0	540.2±35.9	544.96±37.5	543±41.7	0.037
Spherical equivalent (D)	-0.23±2.93	-1.08±2.77	-0.39±1.86	0.48±2.22	**<0.001**
Post-hoc comparison	-0.71 [-1.40, 0.02] (P=0.140)	-1.56 [-2.19, -0.93] **(P<0.001)**	-0.97 [-1.35, -0.38] **(P=0.012)**		
Age (years)	66.21±9.70	64.22±9.92	61.63±10.74	61.00±10.97	**<0.001**
Post-hoc comparison	5.20 [2.72, 7.67] **(P=0.002)**	3.22 [0.91, 5.53] **(P=0.003)**	0.64 [-2.05, 3.31] (P=0.520)		
Gender (% Female)	60.44	56.82	50.40	57.89	0.055
Ocular History					
Early Manifest Glaucoma (%)	34.60	51.62	38.60	NA	NA
Topical glaucoma medication (%)	34.04	51.02	31.73	6.67	**<0.001**

Post-hoc comparison	6.30 [2.82,14.12] **(P<0.001)**	10.23 [5.63, 26.93] **(P<0.001)**	5.210 [2.32,11.70] **(P<0.001)**		
SLT (%)	6.10	7.79	5.40	6.81	0.163
Trabeculectomy (%)	0	0	0	0	NA
Cataract Surgery at Baseline (%)	23.79	20.72	17.08	15.79	0.0784
Disc Haemorrhage (%)	0.3	2.55	0.3	0	NA
Glaucoma Family History (%)	45.58	50.82	56.77	64.44	0.062

Baseline Cardiovascular Disease Characteristics

Prevalence of cardiovascular risk factors was compared between eyes with structural defects and control eyes. Eyes with solely mGCIPL structural defects demonstrated a higher prevalence of hypertension (OR: 2.47; 95%CI: [1.52, 4.02]; P<0.001), and history of myocardial infarction (OR: 5.49 95%CI: [1.67,18.07]; P=0.005) than control eyes. Eyes with both mGCIPL and pRNFL defects were associated with a higher prevalence of hypertension (OR: 1.80; 95%CI: [1.14, 2.83]; P=0.016) than control eyes. No differences were observed between eyes with solely pRNFL defects and control eyes. (Table 2).

Table 2: Comparison of cardiovascular comorbidity between study groups and control groups

	Solely mGCIPL defects (n=316)	**Both pRNFL and mGCIPL defects (n=949)**	**Solely pRNFL defects (n=315)**	**Control eyes (n=94)**	**Global P-value**
Past Medical History					
Diabetes (%)	15.4	14.3	13.1	12.8	0.7
Hypertension (%)	53.7	45.9	39.0	31.9	**0.001**
Post-hoc comparison	2.47 [1.52, 4.02] **(P<0.001)**	1.80 [1.14, 2.83] (P=0.016)	1.36 [0.84, 2.22] (P=0.215)		
Myocardial Infarction (%)	15.3	7.9	5.8	3.2	**<0.001**
Post-hoc comparison	5.49 [1.67,18.07] **(P=0.005)**	2.59 [0.80, 8.37] (P=0.111)	1.85 [0.53, 6.43] (P=0.33)		
Stroke/TIA (%)	7.4	5.3	0.9	1.0	**<0.001**
Post-hoc comparison	7.37 [0.98,55.3] (P=0.059)	5.15 [0.70,37.70] (P=0.16)	0.90 [0.09, 8.76] (P=0.931)		
Raynaud's (%)	8.6	5.0	5.8	4.3	0.163
Migraine (%)	20.5	19.0	22.7	20.2	0.353
Blood Pressure and Cholesterol Measurements*					
Systolic Blood Pressure (mmHg)	138.91±21.7	138.6±23.0	133.9±21.2	126.9±27.3	**<0.001**
Post-hoc comparison	1.01 [0.99, 1.02] (P=0.051)	1.01 [0.99, 1.02] (P= 0.062)	1.001 [0.98,1.01] (P=0. 807)		
Diastolic Blood Pressure (mmHg)	78.8±13.6	77.8±14.7	75.7±13.4	73.5±15.3	0.011
Total Cholesterol (mg/dL)	199.5±49.9	202.2±51.9	203.1±52.0	205.8±49.5	0.103
HDL Cholesterol (mg/dL)	58.7±15.5	57.6±18.5	62.9±19.1	60.1±15.6	0.005

***Note:** values adjusted for the presence of antihypertensive/lipid lowering therapy for all eyes.

Baseline Cardiovascular Medication Characteristics

Participants with eyes demonstrating solely mGCIPL structural defects were more likely to have been prescribed regular aspirin (OR: 2.60; 95% CI: [1.28, 5.27; (P=0.007), statins (OR: 2.85; 95% CI: [1.61, 5.06]; P<0.001), and antihypertensives (OR: 2.74; 95%CI: [1.68, 4.46]; P<0.001) than controls. Participants with eyes exhibiting both mGCIPL and pRNFL structural defects had a higher prevalence of antihypertensive use (OR: 1.82; 95% CI: [1.16, 2.86]; P=0.007) and statin use (OR: 2.01; 95% CI: [1.17, 3.46]; P=0.009) than controls . The cardiovascular medication profiles of participants with solely pRNFL structural change did not differ from controls (Table 3). Figure 2 summarises the differences in cardiovascular disease features and medication profiles of each subgroup for each variable for which a statistically significant distribution p-value (P<0.002) was observed.

Table 3: Comparison of cardiovascular medication between study groups and control eyes

	Solely mGCIPL defects (n=316)	**Both defects (n=949)**	**Solely pRNFL defects (n=315)**	**Control eyes (n=94**	**Global P-value**
Medication					
Aspirin (%)	23.6	14.9	9.9	10.6	**<0.001**
Post-hoc comparison	2.60 [1.28, 5.27] **(P=0.011)**	1.47 [0.75, 2.90] (P=0.265)	0.92 [0.43, 1.96] (P=0.835)		
Clopidogrel (%)	6.0	31.6	29.7	0	0.008
Statin (%)	38.7	30.7	21.4	18.1	**<0.001**
Post-hoc comparison	2.85 [1.61, 5.06] **(P<0.001)**	2.01 [1.17, 3.46] **(P=0.009)**	1.23 [0.68, 2.23] (P=0.486)		
Antihypertensive (%)	56.2	46.1	37.7	31.9	**<0.001**
Post-hoc comparison	2.74 [1.68, 4.46] (**P<0.001**)	1.82 [1.16, 2.86] **(P=0.007)**	1.29 [0.79, 2.10] (P=0.307)		
Beta blocker (%)	17.3	9.4	6.4	8.5	**<0.001**
Post-hoc comparison	2.24 [1.03, 4.90] (P=0.042)	1.12 [0.53, 2.39] (P=0.767)	0.73 [0.31, 1.724] (P=0.477)		
ACE Inhibitor (% eyes)	11.8	13.3	9.23	8.5	0.166
Angiotensin Receptor Blocker (%)	21.7	16.0	15.4	8.0	0.045
Calcium Channel Blockers (%)	7.7	6.2	4.2	2.1	0.250
Metformin (%)	7.7	7.8	9.0	7.5	0.967
Insulin (%)	2.2	2.2	2.6	0	0.938

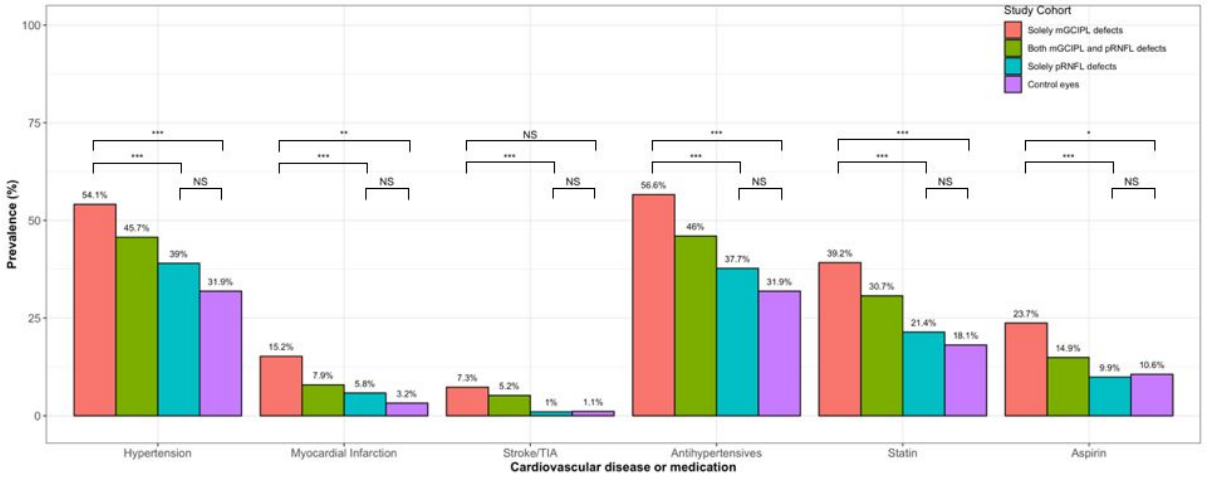

Figure 2: Bar graph summarising the prevalence of systemic cardiovascular characteristics and medications between study groups.

*** : P<0.001; NS: Non significant

P-values depicted for comparisons between solely mGCIPL and controls (top row), solely mGCIPL and pRNFL (middle row), and solely pRNFL and control (bottom row).

A subanalysis assessed to what extent the age difference between those eyes with solely mGCIPL defects and control eyes could have confounded our primary analysis. After accounting for age, eyes with solely mGCIPL defects had a higher prevalence of hypertension (OR: 1.99; 95% CI: [1.18, 3.37]; P=0.010), and myocardial infarction (OR: 4.13; 95% CI: [1.22,13.96]; P=0.023). Eyes with solely mGCIPL defects also had a higher prevalence of treatment with antihypertensive medications (OR: 2.17; 95% CI: [1.28,3.67]; P=0.004), and statin use (OR: 2.29; 95% CI: [1.26 ,4.19]; P=0.007). Treatment with Aspirin (OR: 1.97; 95% CI: [0.83, 4.70]; P=0.089) and beta-blockers (OR: 1.95; 95% CI: [0.71 5.36]; P=0.109) were no longer significantly associated with the mGCIPL structural phenotype although a trend remained.

Sub-analysis of Ocular and Systemic Risk factors in Early Manifest Glaucoma cases

A subanalysis of the 721 eyes classified as EMG on baseline visual field assessment was undertaken to further evaluate our findings in the setting of clearly defined perimetric glaucoma. 109 (15%) eyes were classified as demonstrating solely mGCIPL structural defects, 490 (68%) eyes were classified as demonstrating both pRNFL and mGCIPL defects, and 122 (17%) eyes were classified as demonstrating solely pRNFL structural defects. In this analysis, we compared the prevalence of cardiovascular disease and medications in each structural phenotype to control eyes. Analyses were undertaken using separate multivariate models for cardiovascular disease and for cardiovascular medication. This was done to reduce the collinearity that occured between disease and disease treatment, for instance hypertension and antihypertensive treatment. Analyses were also undertaken including age as a covariate.

Multivariate analysis of cardiovascular disease identified a higher prevalence of hypertension (OR: 2.59 95%CI [1.26, 5.31]; P<0.001) and myocardial infarction (OR: 5.04; 95%CI: [1.68, 21.11]; P=0.005) in those EMG eyes demonstrating solely mGCIPL structural change compared to control eyes. Eyes with solely mGCIPL structural change also had a higher prevalence of stroke/TIA but this did not reach statistical significance on multivariate assessment accounting for age (P=0.061). Eyes with both mGCIPL and pRNFL change had a higher prevalence of hypertension than control eyes but this also did not reach significance after accounting for age (P=0.082).

Perimetric glaucoma patients with predominantly mGCIPL thinning exhibited a higher prevalence of treatment with antihypertensives (OR: 3.55; 95%CI: [1.92, 6.59]; P<0.001), statins (OR: 2.06; 95%CI: [1.26,4.84]; P=0.008) and aspirin use (OR: 2.39 95%CI: [1.06, 5.35]; P=0.034) than control eyes. Perimetric glaucoma patients showing both mGCIPL and pRNFL structural change had a higher prevalence of antihypertensive use but this did not reach statistical significance (P=0.102).

Perimetric glaucoma with predominantly pRNFL structural change were not associated with a higher prevalence of any cardiovascular disease or medication compared to control eyes. These patients however did exhibit a higher baseline IOP than control eyes (Mean difference: 2.05mmHg 95% CI: [1.09, 3.92]; P<0.001).

Figure 3 illustrates the prevalence of cardiovascular disease and medications characteristics between structural endophenotypes in cases with EMG and control eyes.

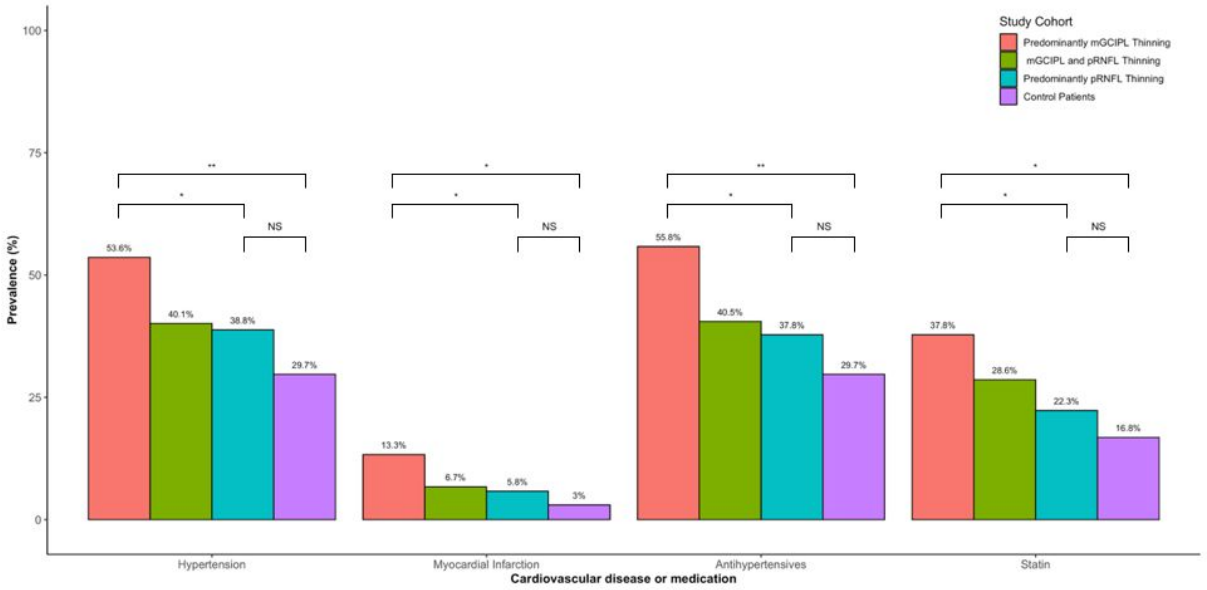

Figure 3: Bar graph comparing prevalence of cardiovascular treatment and disease in EMG eyes

*** : P<0.001; **: P<0.01; *: P<0.05; NS: Non significant

P-values depicted for comparisons between solely mGCIPL and controls (top row), solely mGCIPL and pRNFL (middle row), and solely pRNFL and control (bottom row) after adjustment for age.

Longitudinal Structural and Functional Progression

We reviewed longitudinal SD-OCT and HVF data for all PROGRESSA eyes to assess the association of cardiovascular disease/medications with structural and/or functional progression (mean duration of monitoring: 5.71±1.48 years). Analyses accounted for age and baseline IOP as covariates.

A past medical history of hypertension was associated with an increased likelihood of any structural progression (OR: 1.72; 95%CI: [1.02, 2.89]; P=0.034), mGCIPL structural progression (OR: 2.09; 95%CI: [1.24, 3.52]; P=0.023), pRNFL structural progression (OR: 1.78; 95%CI: [1.05, 2.98]; P=0.042) and was also associated with a faster rate of average mGCIPL thinning on trend analysis (estimate: -0.07um/yr 95%CI: [-0.01, -0.12]; P=0.011). Hypertension was additionally associated with a greater likelihood of visual field progression, as determined by HPA criteria (OR: 1.74; 95%CI: [1.16, 2.61]; P=0.021).

The presence of antihypertensive treatment was associated with an increased risk of any structural progression (OR: 1.59; 95%CI: [1.08, 2.37]; P=0.019), mGCIPL structural progression (OR: 1.57; 95%CI: [1.03, 2.49]; P=0.036) and a faster rate of average mGCIPL thinning (estimate: -0.057um/year; 95%CI: [0.03, 0.08]; P=0.017). Antihypertensive treatment was also associated with an increased risk of visual field progression during study involvement (OR: 1.68; 95%CI: [1.11, 2.54]; P=0.013).

Further evaluation of blood pressure explored the relationship between recorded blood pressure at enrolment and longitudinal SD-OCT and HVF progression. Systolic and diastolic blood pressure measurements were adjusted for antihypertensive therapy by fitting a censored regression model. A higher systolic blood pressure was associated with a greater risk of structural progression (OR: 1.013/mmHg; 95% CI: [1.004; 1.022], P=0.005), mGCIPL structural progression (OR: 1.022/mmHg; 95%CI: [1.012, 1.032]; P=0.003) and a greater risk of visual field progression (OR: 1.01/mmHg; 95% CI: [1.001, 1.021]; P=0.039). A higher systolic blood pressure was associated with pRNFL structural progression(P=0.083), but this did not reach significance after accounting for age and IOP . Diastolic blood pressure was also associated with a greater risk of any structural progression (OR: 1.02/mmHg; [1.01, 1.03]; P=0.024), and mGCIPL progression (OR: 1.03/mmHg; 95% CI: [1.02, 1.06]; P<0.001). Diastolic blood pressure was not associated with visual field progression (P=0.158).

Comparative analysis between IOP and systolic blood pressure showed that a 1 standard deviation (SD) increase in systolic blood pressure had a similar association with structural progression as a 1 SD increase in IOP (Systolic BP OR: 1.34; [1.09; 1.65]; P=0.005, IOP OR: 1.31; 95% CI: [1.05, 1.63];

P=0.018). IOP was however more strongly associated with visual field progression than systolic blood pressure (Systolic BP OR: 1.26; 95%CI: [1.01, 1.56]; P=0.039, IOP OR: 1.58; 95% CI: [1.24, 2.02]; P<0.001). Baseline IOP was not associated with mGCIPL structural progression (P=0.199).

Assessment of the relationship between blood pressure and IOP observed that a 10mmHg increase in baseline systolic blood pressure was associated with a 0.47mmHg increase in baseline IOP (coefficient: 0.047mmHg; 95% CI: [0.027, 0.061]; P<0.001; R-squared: 2.01%). The presence of systemic hypertension was additionally associated with a higher baseline IOP, after accounting for age and antihypertensive treatment (mean difference: 2.02mmHg; 95% CI: [1.32, 2.72]; P<0.001).

DISCUSSION

This study has shown systemic cardiovascular disease to be an important risk factor for structural and functional progression in early glaucoma. We initially identified that systemic cardiovascular disease characteristics, such as hypertension and myocardial infarction, as well as medications, such as antihypertensives, aspirin and statins, were predictive of mGCIPL structural defects at baseline. Following a review of longitudinal progression data, hypertension, antihypertensives use and higher systolic blood pressure were associated with an increased risk of structural and functional progression in these patients. The effect sizes of which were comparable to that observed for baseline IOP.

One must consider our findings in conjunction with the existing literature surrounding cardiovascular disease and glaucoma. Several large clinical studies have shown hypertension to be an important risk factor for glaucoma diagnosis and progression.[3–5,24,25] The present study has further characterised this risk by identifying associations between hypertension and baseline mGCIPL thinning, longitudinal mGCIPL and pRNFL thinning, and visual field progression. It also identified that a higher systolic blood pressure was associated with an increased risk of visual field progression and mGCIPL progression. The observed relationship between blood pressure and IOP implies that the effects of hypertension on glaucomatous progression may be partly mediated by IOP pathways.[26] The association between mGCIPL progression and blood pressure, but not IOP, postulates that vascular pathways may be particularly important in glaucomatous damage of the macula. These findings provide support for the treatment of poorly controlled hypertension in glaucoma, as proposed by the Blue Mountains Eye Study.[4,25,27–30]

Other major studies have also shown hypotension to be a risk factor for glaucoma.[4,25,27–30] This has led the Los Angeles Eye Study to propose a U-shaped relationship between blood pressure and glaucoma. In this model, both hypotension and hypertension are risk factors for glaucoma. Our study identified an increased risk of structural and functional progression in those subjects treated with antihypertensive medications. Previous groups have proposed that antihypertensive use may be a risk factor for glaucomatous progression by causing nocturnal hypotension.[31–33] In the absence of ambulatory blood pressure monitoring, it is plausible that these patients were experiencing this phenomenon. Alternatively, antihypertensive therapy may simply be a surrogate marker for hypertension in this study. Further investigation of these possibilities is clearly warranted.

Similarly, the interaction between hypercholesterolemia, statin use and primary open angle glaucoma is debated in the literature.[34] A meta-analysis by Wang and colleagues revealed a 1.37 relative risk of

glaucoma in individuals with hypercholesterolemia, but Kang et *al.* more recently pooled 3 large population based studies and found no association between hypercholesterolemia and glaucoma.[35,36] Further in this debate, statins have been proposed to be protective against glaucoma, with some studies postulating that the degree of neuroprotection is proportional to the duration of statin use.[37,38] In the present study, statin use was predictive of baseline mGCIPL defects. However, we were unable to demonstrate an association between measured cholesterol parameters and structural phenotype, which may be a consequence of the high prevalence of statin use in our study leading to lower cholesterol levels in those on treatment. In the absence of historical cholesterol data, we favour the hypothesis that statin use in our study may represent a surrogate marker of hypercholesterolemia in those exhibiting mGCIPL structural change.

Finally, Mondal et *al.* have demonstrated that myocardial infarction was associated with worsening visual field in a prospective study of 62 patients with stable open angle glaucoma.[39] Our current study has developed upon this work by illustrating that myocardial infarction was associated with a thinner mGCIPL at baseline in a substantially higher powered longitudinal prospective study. Further study is required to understand the nature of, and mechanism for the observed association between ischemic heart disease, baseline structural characteristics, and glaucoma progression.

Although we have identified that cardiovascular phenomena are clearly associated with macular structural defects, we recognise several study limitations. It is unknown if the structural changes observed on macular SD-OCT analysis are related to glaucoma, to cardiovascular disease, or to both diseases. We did however identify that cardiovascular disease was associated with baseline structural phenotypes in perimetric glaucoma cases, and with longitudinal visual field progression, which supports that notion that these macula changes are truly glaucomatous defects, and important in considering visual morbidity and progressive visual field loss in glaucoma. The age difference between patients with mGCIPL baseline change and the subgroup of stable patients introduces a potential for age associated bias in the cardiovascular phenotypes. We addressed this limitation by accounting for age in a sensitivity analysis of our preliminary findings and in all subsequent multivariate comparisons. We classified structural phenotypes by assessing mGCIPL and pRNFL deviation maps. A number of studies have independently shown that mGCIPL and pRNFL deviation maps have comparable glaucoma diagnostic capability.[14,40–42] Our use of the 24-2 Humphrey visual field test to assess visual field progression may miss up to 50% of macula defects, which are typically deeper and closer to fixation and better detected on 10-2 perimetry.[43,44] 10-2 field testing was not part of the study protocol and we may expect stronger associations between cardiovascular disease and visual field progression if this form of perimetry was available. Finally, the enrollment for this study

predated the commercial availability of OCT-Angiography. In the future we will evaluate the association between cardiovascular risk factors, blood vessel phenotypes, and macula thinning.

This study evaluated structural and functional phenotypes of glaucoma. Using a combination of baseline and longitudinal SD-OCT imaging of the mGCIPL and the pRNFL, Humphrey visual field assessment and optical coherence tomography angiography, we have highlighted the importance of cardiovascular disease in both structural and functional disease progression. The importance of these parameters far exceeded that of other clinical covariates such as Raynaud's disease, migraine and diabetes, which often receive more clinical attention in risk stratification. The strong associations observed in this study between cardiovascular parameters and glaucoma phenotype and progression, underscore the need for mechanistic research to determine the best way to slow disease progression in ways that do not depend solely on lowering intraocular pressure.

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