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Relationship of Macular Ganglion Cell Complex thickness to Choroidal Microvasculature Dropout in Primary Open Angle Glaucoma

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ABSTRACT

Background/Aims: To investigate the rate of ganglion cell complex (GCC) thinning in primary open-angle glaucoma (POAG) patients with and without deep-layer microvasculature dropout (MvD)

Methods: POAG patients who had at least 1.5 years of follow-up and a minimum of 3 visits were included from the Diagnostic Innovations in Glaucoma Study. MvD was detected at baseline by Optical Coherence Tomography angiography (OCT-A). Area and angular circumference of MvD were evaluated on en-face choroidal vessel density images and horizontal B-scans. Rates of global and hemisphere GCC thinning were compared in MvD and non-MvD eyes using linear mixed-effects models.

Results: Thirty-six eyes with MvD and 37 eyes without MvD of 63 patients were followed for a mean of 3.3 years. In 30 out of 36 eyes, MvD was localized in the inferotemporal region. While mean baseline visual field mean deviation was similar between the two groups (p=0.128), global GCC thinning was significantly faster in eyes with MvD than in those without MvD (mean differences: -0.50 (-0.83, -0.17) µm/year; P=0.003)). Presence of MvD, area and angular circumference of MvD were independently associated with a faster rate of thinning (P=0.002, P=0.031 and P=0.013, respectively).

Conclusion: In POAG eyes, GCC thinning is faster in eyes with MvD. Detection of MvD in OCTA images can assist clinicians to identify patients who are at higher risk for central macula thinning and glaucomatous progression and may require more intensive management.

Key words: Ganglion Cell Complex, Optical coherence tomography angiography, glaucoma, glaucoma progression

SYNOPSIS:

In this observational cohort study, glaucomatous eyes with deep-layer microvascular dropout (MvD) exhibited faster ganglion cell complex (GCC) thinning than those without MvD.

INTRODUCTION

Glaucoma is a chronic optic neuropathy characterized by progressive loss of retinal ganglion cells (RGCs) associated with deterioration of retinal nerve fiber layer (RNFL) and optic nerve head (ONH).[1] Among the multiple proposed mechanisms of optic nerve damage, microvascular changes of the ONH have been proposed as a potential factor in the development and progression of glaucoma.[2 3] The choroidal (or deep-layer) microvasculature within the parapapillary area (PPA) may be of particular interest because it mainly receives its blood supply by the short posterior ciliary arteries that also perfuse deep ONH tissues.[4]

Several studies using optical coherence tomography angiography (OCT-A) have demonstrated regional choroidal microvasculature dropout (MvD) in patients with glaucoma,[4 5] which corresponds to a perfusion defect identified with indocyanine green angiography.[6] The presence of MvD in glaucoma patients has been associated with parameters of disease severity, such as a thinner retinal nerve fiber layer (RNFL) and worse visual field (VF) mean deviation (MD).[4]

MvDs have been frequently found in the inferotemporal region within the beta zone of parapapillary atrophy (β-PPA), the region consistent with the macula vulnerability zone (MVZ); this corresponds to the superior paracentral VF area.[7] These findings suggested that the impaired choroidal perfusion in the form of MvD might be associated with initial parafoveal scotomas.[8] To our knowledge, there are no previous studies that focused on the macular GGC layer to estimate its future changes in eyes with MvD. Early detection of such macular structural damage is relevant to glaucoma management as the loss of central vision can markedly impact patients' quality of life (QoL).[9] Prompt identification of these changes may help the clinician to intensify their management to preserve a patient's QoL.[9 10]

The purpose of this study was to compare the rates of macular GCC thinning in glaucoma eyes with and without MvD at baseline. We also examined whether area, angular circumference (AC) and location of MvD were associated with more rapid changes of macular GCC in glaucoma patients.

MATERIALS AND METHODS

Participants

This longitudinal study included POAG patients enrolled in Diagnostic Innovations in Glaucoma Study (DIGS)[11 12] who underwent OCT-A (Angiovue; Optovue Inc., Fremont, CA). All participants from DIGS who met the inclusion criteria described below were included in the present study. Informed consent was obtained from all study participants. This study received the institutional review board approval of the University of California, San Diego (NCT00221897) and the methodology adhered to the tenets of the Declaration of Helsinki. This study included eyes with a minimum of 3 macular OCT scans and a minimum of 1.5 years of follow-up and a good quality baseline OCT-A ONH image.

Eyes were classified as glaucomatous if they had repeatable (at least 2 consecutive) abnormal VF test results and evidence of glaucomatous optic neuropathy – defined as excavation, the presence of focal thinning, notching of neuroretinal rim, or localized or diffuse atrophy of the RNFL on the basis of masked grading of optic disc photographs by 2 graders. An abnormal VF test was defined as a pattern standard deviation outside of the 95% normal confidence limits or a Glaucoma Hemifield Test result outside normal limits.

Inclusion criteria also included (1) older than 18 years of age, (2) open angles on gonioscopy, and (3) best-corrected visual acuity of 20/40 or better. Exclusion criteria included (1) history of trauma or intraocular surgery (except for uncomplicated cataract surgery or glaucoma

surgery), (2) coexisting retinal disease, (3) uveitis, (4) non-glaucomatous optic neuropathy, (5) axial length of 26 mm or more and (6) VF mean deviation (MD) <-8 dB at baseline.

Optical Coherence Tomography Angiography (OCT-A)

All participants underwent OCT-A and Spectral-Domain (SD)-OCT imaging using AngioVue imaging system (OptoVue, Inc., Fremont, CA). This existing commercially available SD-OCT platform provides both thickness and vascular measurements. With the simultaneously acquired OCT and OCT-A volume of the AngioVue scan and automated segmentation by the AngioVue software, thickness and vascular analyses can be derived from the same image.

Macula 3 x 3 mm^2 scans (304 B-scans x 304 A-scans per B-scan) centered on the fovea and ONH 4.5 x 4.5 mm^2 (304 B-scans x 304 A-scans per B-scan) centered on the ONH were acquired with the AngioVue OCT-A system (software version 2018.1.0.43). The retinal layers of each scan were segmented automatically by the AngioVue software.

For this study, whole en-face image vessel density (wiVD) and the en-face choroidal vessel density map was derived from the entire 4.5 x 4.5 mm^2 scan that was centered on the ONH. This en-face choroidal vessel density map contains layers below the retinal pigment epithelium, including the choroid and sclera. The macula cube scanning protocol was used to assess GCC thickness. GCC thickness regions of the whole image (wiGCC) were analyzed.

OCT-A and SD-OCT image quality review was completed according to the Imaging Data Evaluation and Analysis Regarding Center standard protocol on all scans processed with standard AngioVue software. Poor-quality images were excluded; these were defined as images with 1) low scan quality with quality index (QI) of less than 4; 2) poor clarity; 3) residual motion artifacts visible as irregular vessel pattern or disc boundary on the en-face

angiogram; 4) image cropping or local weak signal resulting from vitreous opacity; or 5) segmentation errors that could not be corrected.

Choroidal Microvasculature Dropout detection

Dropout was required to be present in at least 4 consecutive horizontal B-scans and also to be >200 µm in diameter in at least one scan and to be in contact with the OCT disc boundary. The optic disc boundary was automatically detected by the Optovue software as the Bruch's membrane/ RPE complex opening. In case of errors in disc demarcation, one trained observer masked to the clinical information of the subjects corrected the disc boundary manually by searching for the positioning of Bruch's membrane opening (BMO), as previously described.[13]

Two observers (EM and NEN), who were masked to the clinical characteristics of the participants, independently determined the presence or absence of MvD for each patient. Disagreements between the 2 observers about the presence MvD were resolved by a third adjudicator (SM).

Measurement of MvD area, circumferential angle and location

Optic disc and the PPA margins were detected by simultaneously viewing the stereoscopic optic disc photographs and the scanning laser ophthalmoscopic (SLO) images that were obtained along with the OCT-A images. MvD area was manually demarcated on en-face choroidal vessel density maps using the line tool provided by ImageJ software (Version 1.53; available at http://imagej.nih.gov/ij/download.html; *National Institute of Health, Bethesda, Maryland, USA)*. Littmann's formula was used to correct the ocular magnification in OCT-A.[14 15] Details of the formula are provided elsewhere.[15] The Avanti SD OCT has a default axial length of 23.95 mm and an anterior corneal curvature radius of 7.77 mm.

MvD angular circumference (AC) was measured as previously described.[16] In brief, the two points at which the extreme borders of MvD area met the ONH border were identified and defined as angular circumferential margins. The AC was then determined by drawing two lines connecting the ONH center to the angular circumference margins of the MvD. Both area and AC of the MvD were independently assessed by two trained graders who were masked to the clinical data for each patient, including the GCC and RNFL thickness. Both MvD area and angular circumference were defined as the mean of the measurements made by the two observers to minimize interobserver variation.

MvD area that included large retinal vessels was included as part of the MvD area if the MvD extended beyond the vessels. In cases where the retinal vessels were located at the border of the MvD, the area covered by the vessels was excluded from the MvD area. Reflectance or shadowing of the large vessels on the horizontal and en-face images were excluded from the quantitative analysis by the two independent observers masked to the patients' baseline characteristics. In an eye showing more than one MvD, the area and the angular extent of each MvD were calculated separately and also added together to determine the total area and the total angular extension of MvD for the eye.

The sectoral location of the dropout was determined based on the 8 separate sectors corresponding to those on the RNFL vessel density map of the OCTA. For each MvD, a line was drawn to equally bisect the angular circumferential margins of the MvD from the ONH center, as previously reported,[17] to define the location of the MvD. Disagreements between the 2 observers in determining the MvD location were adjudicated by the third experienced grader.

Statistical analysis

Patient and eye characteristics data were presented as mean (95% confidence interval (CI)) for continuous variables and count (%) for categorical variables. Interobserver reproducibility for the presence of MvD and for the measured MvD area and AC were assessed using k statistics (i.e. k value) and interclass correlation coefficient (ICC), respectively. Categorical variables were compared using the chi-square test. Mixed-effects modeling was used to compare ocular parameters among groups. Mixed-effects modeling was used to compare ocular parameters among groups. Evaluation of the effect of microvascular dropout (MvD) on the mean rates of change in wiGCC was performed using a linear mixed model with random intercepts and random slopes. In this model, the average values of the outcome variables were explored using a linear function of time, and random intercepts and random slopes were introduced with patient- and eye-specific deviations from this average value. This model can account for the fact that different eyes may have different rates of wiGCC thinning over time, while allowing for correlation between two eyes of the same individual.

Factors contributing to the rate of wiGCC were explored using linear mixed models. Potential predictors which were associated with the rates of wiGCC thinning during the follow-up in univariable analysis ($p<0.1$) were included in the multivariable model. Statistical analyses were performed using Stata version 16.0 (StataCorp, College Station, TX). P values of less than 0.05 were considered statistically significant for all analyses.

RESULTS

Eighty-two POAG eyes of 69 patients met the eligibility criteria. Of these, 9 eyes of 6 patients were excluded because of the poor quality of their OCT-A images, resulting in

inclusion of 73 eyes of 63 (33 male and 30 female) POAG patients. Mean follow-up (95%CI) was 3.3 (3.1, 3.5) years for both MvD and non-MvD eyes with an average of 4.2 (3.8, 4.7) and 4.4 (4.0, 4.7) OCT visits, respectively (p=0.664).

Among the 73 eyes, MvD was observed in 36 (49.3%) eyes. In 16 (45%) of these 36 eyes, MvD was located in the inferior region, whereas 15 eyes (42%) showed MvD in both inferior and superior sectors. Interobserver agreement in detecting the presence of MvD (95% CI) was excellent (kappa =0.92 (0.83, 1.00)). The intraclass correlation coefficient (ICC) for interobserver reproducibility in measuring the area and the angular circumference of MvD (95% CI) was 0.98 (0.97, 0.99) and 0.94 (0.90, 0.96), respectively.

Figure 1 shows a representative case of the relationship between MvD at baseline and GCC progressive thinning.

Table 1 compares the demographics and clinical characteristics of eyes with and without MvD. Mean age (95% CI) was 68.5 (65.1, 71.9) and 70.5 (66.9, 74,2) in the MvD group and non-MvD group, respectively (P=0.437). Mean baseline VF MD (95%) was -2.8 dB (-3.6, -2.0) and -2.1 dB (-2.7, -1.4) in the MvD eyes and non-MvD eyes, respectively (P=0.126). The groups were similar in age, gender, race, axial length, CCT, baseline IOP, glaucoma severity defined as VF MD and mean number of OCT follow-up visits. Disc hemorrhage was detected in 8 (22%) MvD eyes and 4 (10.5%) non-MvD eyes (P=0.156). Mean baseline GCC thickness (95% CI) was lower in the MvD group compared with the non-MvD group globally (89.0 µm (85.6, 92.4) vs. 94.1 µm (90.7, 97.5), P= 0.041) and in the inferior hemifield (P=0.003), but not in the superior hemifield (P=0.527).

Table 1. Characteristics of Eyes Categorized by Microvascular Dropout Group

Variables	MvD (+)	MvD (-)	P value
Characteristic	n=36 eyes of 30 patients	n=37 eyes of 33 patients	
Baseline age (years)	68.5 (65.1, 71.9)	70.5 (66.9, 74.2)	0.437
Gender (Female/ Male)	14/16	16/17	0.885
Race (African American/ Non-African American)	8/22	15/18	0.122
Self-reported hypertension, n (%)	21 (70.0)	20 (60.6)	0.435
Self-reported diabetes, n (%)	7 (23.3)	7 (21.2)	0.840
Axial length (mm)	24.2 (23.9, 24.5)	24 (23.7, 24.2)	0.181
CCT (μm)	534.0 (519.2, 548.8)	537.7 (525.2, 550.3)	0.694
Mean baseline IOP (mm Hg)	14.3 (12.9, 15.7)	15.0 (13.9, 16.1)	0.420
Mean IOP during follow-up (mm Hg)	14.5 (13.2, 15.8)	15.3 (14.1, 16.6)	0.360
Disease Severity by baseline 24-2 VF MD			0.155
Early glaucoma, Eye No. (%)	32 (88.9)	36 (97.3)	
Moderate and advanced glaucoma, Eye No. (%)	4 (11.1)	1 (2.7)	
MvD Location			
Inferior, Eye No. (%)	16 (45.7)		
Superior, Eye No. (%)	4 (11.4)		
Both hemispheres, Eye No. (%)	15 (42.9)		
Corrected MvD area (mm^2)	0.09 (0.04, 0.14)		
MvD angle (degree)	25.1 (15.3, 35.0)		
Disc hemorrhage, n (%)	8 (22.9)	4 (10.5)	0.156
Mean baseline VF MD (dB)	-2.8 (-3.6, -2.0)	-2.1 (-2.7, -1.4)	0.126

Mean baseline global GCC thickness (µm)	89.0 (85.6, 92.4)	94.1 (90.7, 97.5)	0.041
Mean baseline hemi-inferior GCC (µm)	86.5 (81.7, 91,3)	95.0 (91.9, 98,2)	0.003
Mean baseline hemi-superior GCC (µm)	91.1 (87.5, 94.8)	92.9 (88.9, 96.9)	0.527
Baseline cpRNFL thickness (µm)	75.6 (70.3, 81.0)	81.7 (77.2, 86.1)	0.056
Length of follow-up (years)	3.3 (3.1, 3.5)	3.3 (3.1, 3.5)	0.901
Number of OCTA visits	4.2 (3.8, 4.7)	4.4 (4.0, 4.7)	0.664

CCT = central corneal thickness; IOP = intraocular pressure; MD = mean deviation; MvD = microvascular dropout; VF = visual field. Values are shown in mean (95% confidence interval), unless otherwise indicated. Statistically significant P values are shown in bold.

Table 2 and **Figure 2** shows the rate of thinning in global GCC and hemi-sectoral GCC in eyes with and without MvD. A significantly faster mean rate of GCC thinning in MvD eyes compared to non-MvD eyes was found in mean global, hemi superior, and hemi inferior area (mean difference (95% CI): -0.50 (-0.83, -0.17) µm/year; P=0.003, -0.55 (-0.92, -0.19) µm/year; P=0.003, and -0.48 (-0.84, -0.11) µm/year; P=0.010, respectively). Similar results were found after adjusting for confounding factors age, baseline VF MD and mean IOP during follow-up. These findings were also similar when adjusted for GCC thickness instead of baseline 24-2 VF MD.

Table 2. Comparison of Rates of Whole Image Ganglion Cell Complex Thinning between dropout and Non-dropout Eyes

	MvD Group **Mean (95% CI)**	**Non-MvD Group** **Mean (95% CI)**	**Difference** **Mean (95% CI)**	**P value** **(adjusted)**
wiGCC Change Rate (µm/year)				
Mean Global	-1.35 (-1.59, -1.11)	-0.85 (-1.09, -0.62)	-0.50 (-0.83, -0.17)	**0.003 (0.002)**
Hemi Superior	-1.40 (-1.66, -1.14)	-0.85 (-1.10, -0.59)	-0.55 (-0.92, -0.19)	**0.003 (0.004)**
Hemi Inferior	-1.34 (-1.60, -1.08)	-0.87 (-1.12, -0.61)	-0.48 (-0.84, -0.11)	**0.010**

(0.014)

MvD = microvascular dropout; wiGCC = whole image ganglion cell complex. Values are shown in mean (95% confidence interval), unless otherwise indicated. Statistically significant P values are shown in bold.

*Adjusted to age, baseline 24-2 visual field mean deviation, and mean IOP.

Factors contributing to the rate of global GCC thinning during the follow-up are summarized in **Tables 3.** Multivariable analysis showed that presence of MvD, area and AC of MvD and mean IOP during follow-up were significantly associated with a faster rate of GCC thinning (P=0.002, P=0.038, P=0.013 and P=0.020, respectively) after adjusting for age, baseline VF MD and mean IOP during follow-up. These results were similar even after adjusting for baseline GCC thickness instead of baseline 24-2 VF MD. Similar results were found after including baseline IOP instead of mean IOP during follow-up (**Supplemental Table 1**).

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Table 3. Factors Contributing to the Rate of Whole Image Ganglion Cell Complex Thinning Over Time by Univariable and Multivariable Mixed Model Analysis

Variables	**Univariable Model**		**Multivariable Model 1**		**Multivariable Model 2**		**Multivariable Model 3**	
	β, 95 % CI	**P value**	**β, 95 % CI**	**P value**	**β, 95 % CI**	**P value**	**β, 95 % CI**	**P value**
Age, per 10 years older	0.05 (-0.11, 0.21)	0.551	0.01 (-0.14, 0.15)	0.945	0.00 (-0.15, 0.15)	0.992	0.00 (-0.16, 0.15)	0.968
Gender: Male/Female	-0.20 (-0.55, 0.14)	0.247						
Race:								
African American/ Non-African American	0.01 (-0.35, 0.37)	0.959						
Self-reported diabetes	0.22 (-0.20, 0.64)	0.309						
Self-reported hypertension	-0.04 (-0.40, 0.33)	0.848						
Axial length, per 1mm longer	0.11 (-0.09, 0.30)	0.294						
CCT, per 100 μm thinner	0.20 (-0.24,0.64)	0.379						
Baseline IOP, per 1 mm Hg higher	**-0.04 (-0.09, 0.00)**	**0.077**						

Mean IOP during follow-up, per 1 mm Hg higher	**-0.05 (-0.09, 0.00)**	**0.032**	**-0.05 (-0.10, -0.01)**	**0.012**	**-0.05 (-0.09, -0.01)**	**0.026**	**-0.05 (-0.10, -0.01)**	**0.020**
Baseline VF MD, per 1 dB worse	**-0.07 (-0.16, 0.01)**	**0.082**	-0.05 (-0.12, 0.03)	0.243	-0.05 (-0.13, 0.03)	0.201	-0.04 (-0.12, 0.04)	0.292
Baseline VF PSD, per 1 dB worse	0.01 (-0.07, 0.08)	0.858						
Follow-up period, per 1 year longer	0.13 (-0.16, 0.41)	0.387						
OCT number of scans	0.09 (-0.04, 0.22)	0.185						
Presence of MvD	**-0.50 (-0.83, -0.17)**	**0.003**	**-0.49 (-0.81, -0.18)**	**0.002**				
Corrected MvD area, per 1 mm^2 larger	**-0.99 (-1.85, -0.14)**	**0.022**			**-0.92 (-1.75, -0.08)**	**0.031**		
MvD angle, per 10 degrees wider	**-0.01 (-0.01, 0.00)**	**0.009**					**-0.05 (-0.09, -0.01)**	**0.013**

CCT = central corneal thickness; IOP = intraocular pressure; MD = mean deviation; MvD = microvascular dropout; OCT = optical coherence tomography; VF = visual field.

Values are shown in β coefficient (95% confidence interval). Statistically significant P values are shown in bold. Multivariable Model 1: presence of MvD; Multivariable Model 2: corrected MvD area; Multivariable Model 3: MvD angular circumference

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DISCUSSION

This study showed that, with more than 3 years of follow-up, the rates of progressive GCC thinning were significantly faster in eyes with MvD compared to non-MvD eyes, supporting the role of MvD as a predictor of glaucoma progression. Furthermore, larger areas and angular extensions of MvD showed faster rates of GCC loss when compared to eyes with smaller MvDs. These findings demonstrate a possible role for OCT-A choroidal vessel density assessment as a biomarker for predicting glaucomatous GCC thinning.

Previous studies revealed a significant association between MvD and other predictors of glaucoma progression, such as lamina cribrosa defects[18-21] and disc hemorrhage (DH).[22-25] Park et al. reported that MvD was topographically related to DH, and was also associated RNFL thinning.[26] Evidence of DH during the follow-up of glaucoma eyes has been reported as a relevant prognostic factor for accelerated central VF progression.[27] As MvD was measured in the choroidal layer, it largely represents a localized perfusion defect of the choriocapillaris and choroidal microvasculature in the β-PPA region, and may indicate impaired perfusion of deep-layer tissues of the prelaminar or laminar region of the ONH. Since IOP-independent factors, such as vascular insufficiency to the ONH, may play an important role in the prognosis of OAG[2], MvD may be associated with accelerated glaucoma progression. Indeed, higher rates of VF loss have been observed in MvD eyes compared to those without MvD, despite no significant differences in IOP between the two groups.[28] A recent study also showed that the presence of MvD was one of the strongest predictive factors for faster progressive RNFL thinning over 2.5 years of follow-up.[29]

In the present study, eyes with MvD showed faster GCC thinning compared to non-MvD eyes over more than 3 years of follow-up. In agreement with these findings, previous

investigators demonstrated a significant association between faster rate of central VF loss and MvD detected during follow-up, whereas the rates of VF progression in the peripheral VF region did not differ significantly between MvD and non-MvD groups.[28] In this earlier study, the progression rate of VF was faster in the superior than in the inferior 10° zone, suggesting that the location of MvD in the inferior areas might be topographically related to poor prognosis during the course of the disease.

Several hypotheses may explain the faster rate of central macula thinning in eyes with evidence of MvD. The large majority of the eyes showed MvD located in the inferotemporal (IT) area rather than in other regions, a location consistent with the macular vulnerability zone (MVZ) in the retina,[7] where most of the retinal ganglion cell axons from inferior macular region project. Moreover, the most common pattern of GCC thinning in glaucoma is the widening of an existing defect, followed by deepening.[30] Because GCC progression reflects the expansion or deepening of a pre-existing initial GCC defect in eyes with MvD, the rate of GCC thinning is likely to be faster in eyes with MvD than without it. Another explanation is that parafoveal scotoma and central macula thickness involvement in patients with early glaucoma are often associated with risk factors closely related to vascular dysregulation in the ONH tissues, such as hypotension, migraine, and disc hemorrhage.[27] As a consequence, eyes with signs of hypoperfusion to the ONH, such as those with MvD, may show a faster rate of GCC thinning compared to eyes without MvD.

Baseline macular GCC thickness of the inferior hemifield was shown to be predictive of central and peripheral VF progression in POAG.[31-33] Of note, the involvement of the central 10° of the VF has been strongly correlated with vision-related QoL.[9] Daily activities, such as walking, reading and driving, are more likely to be affected by initial parafoveal VF defects compared to initial arcuate defects in glaucoma patients. Given the

substantial impact of central VF on quality of life, meticulous assessment of the glaucomatous macular damage is recommended in imagining glaucoma patients with MvD.

In the present study, baseline inferior hemifield GCC was significantly thinner in the MvD eyes compared with non-MvD eyes, and MvD was mostly localized in the IT sector (7-8 o'clock). These relationships support the possibility of a common mechanism between the macular thinning and the choroidal vascular impairment. The rates of GCC thinning were significantly faster in the eyes with MvD in both superior and inferior hemifields compared to the non-MvD groups in this study. Similar results were shown by Lee et al., who reported faster rates of superior and inferior RNFL thinning in eyes with MvD in both superior and inferior hemispheres.[34]

It remains unclear whether the extent (the area and circumference) of MvD is associated with the rate of glaucomatous damage. Whereas some retrospective studies[17 35] found that the extent of MvD was positively associated with severity and rapid progression of VF loss, the area of MvD at baseline was not significantly associated with faster rate of RNFL thinning in the study by Kim et al.[16] In the present study, area and angular circumference of MvD at baseline were significantly associated with the rate of future GCC thinning, suggesting that the extent of MvD may represent an indicator of high-risk glaucoma patients.

Our study had several limitations. First, MvD size was measured on the en-face choroidal vessel density map, which itself is subject to many limitations. To minimize subjectivity in the measurement of MvD. We defined MvD as a complete loss of the choroidal microvasculature with a size of 200 micron or greater in diameter, a method that has been validated in previous studies.[19] In addition, large overlying retinal vessels or DHs may

project onto en-face choroidal vessel density images, and may induce projection artifacts or shadows or make it difficult to detect or define MvD boundaries. In order to reduce these potential false negatives, MvD was defined by 3 trained examiners using both en-face and B-scan images and we found an excellent interobserver agreement (k= 0.935) between graders. Second, subjects with advanced stages of glaucoma (i.e. eyes with baseline MD <-8 dB) were excluded. Thus, our results may not be generalizable to eyes with more advanced glaucoma. Third, MvD area and AC were measured on en-face images using the automatic demarcation of the BMO, and this was not accurately demarcated in some eyes. To overcome this limitation, disc margin errors were manually corrected before the quantitative analysis of MvD by a trained observer who was masked to the clinical characteristic of the subjects. Fourth, the en-face choroidal vessel density image was used to detect MvD. Although this slab includes both choroid and inner sclera, the choroid is not segmented specifically and one cannot assume that it represents only the choroidal layer. Finally, ocular magnification effects associated with axial length might have influenced MvD area as measured by OCT-A. However, eyes with axial length > 26 mm were excluded in the current study. Moreover, Littmann's formula was used to correct the magnification effect.

In conclusion, MvD is an independent predictor for accelerated GCC loss in eyes with glaucoma, especially in early stages of the disease. The rate of GCC thinning was faster in eyes with evidence of MvD and thinner GCC at baseline. The rate of GCC thinning was significantly higher in both superior and inferior regions and the extent of MvD was also associated with the rate of GCC thinning in the future. Especially with early POAG, these findings suggest that assessment of MvD is useful for detection of patients at a high risk of rapid progression who require more intensive observation and treatment.

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STATEMENTS

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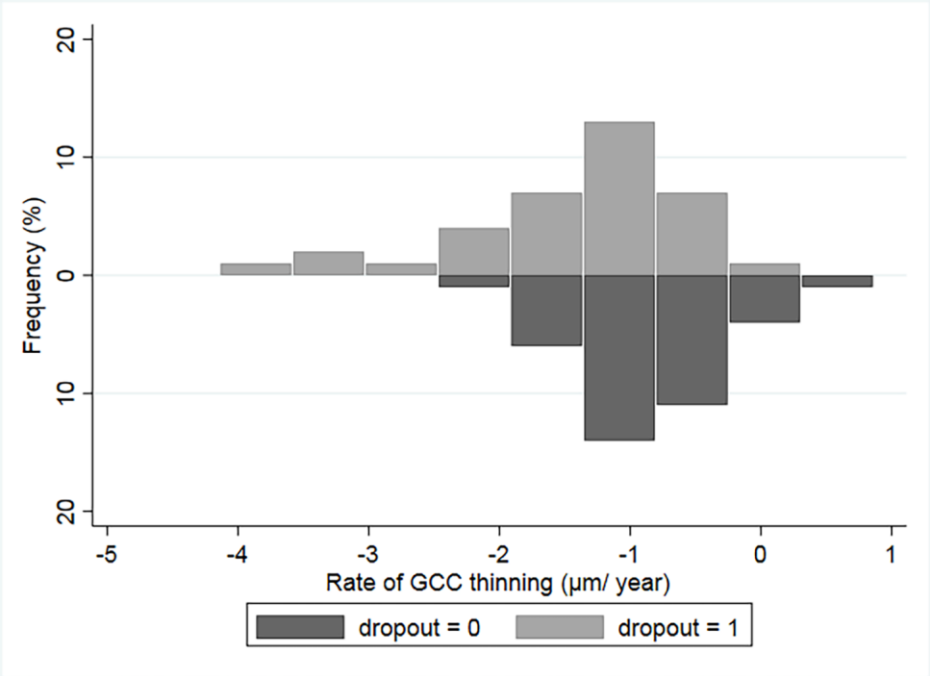
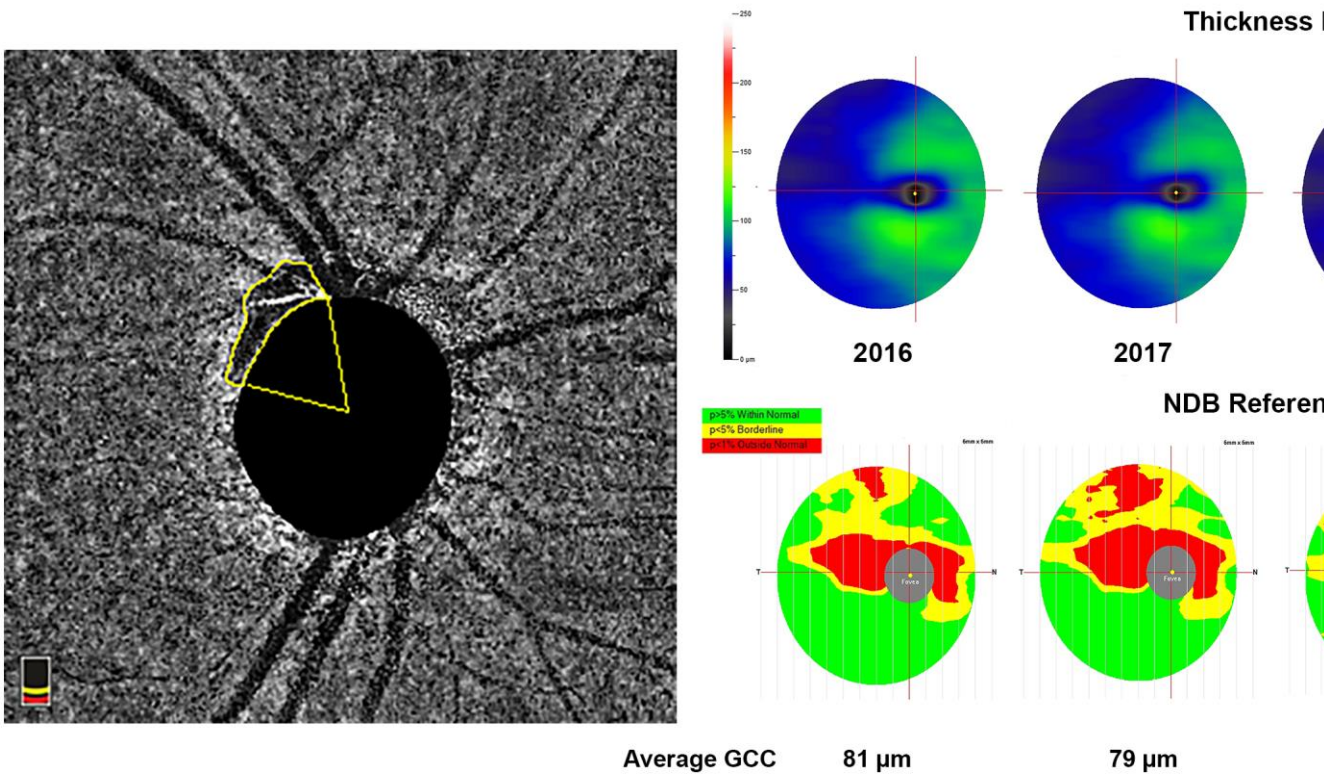
FIGURE LEGENDS

Figure 1. OCT-A En-face Choroidal vessel density Map showing supero-temporal MvD (Left) and subsequent hemi-superior GCC thinning (Right) in an open-angle glaucoma eye. OCT-A: Optic Coherence Tomography-Angiography; VD: Vessel Density; MvD: deep-layer Microvascular Dropout; GCC: Ganglion Cell Complex



473 Figure 2. Rates of GCC thinning (µm/year) in eyes with and without deep-layer

474 microvasculature dropout



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