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Retinal Imaging

Retinal ganglion cell–inner plexiform layer thickness is nonlinearly associated with cognitive impairment in the community-dwelling elderly

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Abstract

Introduction: Thinning of optical coherence tomography–measured retinal nerve fiber layer thickness and ganglion cell–inner plexiform layer (GC-IPL) thickness has been found in patients with Alzheimer's disease. However, the association of these retinal markers and cognition in nondemented elders may not be linear.

Methods: This cross-sectional study included 227 community-dwelling elders (age 65+ years). Multivariable regression analyses were performed to investigate the association between retinal nerve fiber layer/GC-IPL and global/domain-specific cognition.

Results: The performance of global cognition decreased as mean GC-IPL of bilateral eyes deviated from the sample mean (77.5 μm) (quadratic GC-IPL: $\beta = -0.49 \times 10^{-2}$; 95% confidence interval: -0.74×10^{-2} to -0.23×10^{-2}). Similar associations were also found for logical memory. No significant association was observed between retinal nerve fiber layer and cognition.

Discussion: Either thinning or thickening of GC-IPL was associated with poor cognition in nondemented elderly (a U-shaped association). GC-IPL may serve as a noninvasive preclinical predictor of Alzheimer's disease.

Keywords: Retina; Alzheimer's disease; Biomarkers; Preclinical AD; Cognitive impairment; Optical coherence tomography; OCT; Ganglion cell–inner plexiform layer; GC-IPL; Retinal nerve fiber layer; RNFL; Retinal ganglion cell; Amyloid hypothesis; Synaptic dysfunction; Dendritic pathology

1. Background

An era of population aging is under way, and the estimated global prevalence of dementia in population aged over 60 years was 5.2% in 2015 [1]. Dementia is a major public health burden, and its prevalence is expected to rise exponentially in the next two decades. The leading type of dementia, Alzheimer's disease (AD), is a neurodegenerative disease that progresses slowly over time before reaching a

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full-blown clinical manifestation. The transitional phase from normal cognition to AD is recognized as mild cognitive impairment (MCI). Because of the irreversibility of AD and the lack of effective treatment other than symptomatic treatment for severe stage, current research efforts are shifting toward the preclinical stage to slow down disease progression and to improve treatment outcomes. Therefore, biomarkers for identifying high-risk elders at preclinical AD are essential for early detection. Brain magnetic resonance imaging, positron emission tomography imaging, and cerebrospinal fluid amyloid β42/tau protein are promising preclinical markers of AD [2]. However, the concerns of high cost and invasiveness preclude them from the routine screening for dementia.

Being an extension of the central nervous system (CNS), the retina shares many histological, physiological, and embryological features with the brain. Subtle neural changes in the retina may reflect the pathological degeneration of the CNS during the disease process of multiple sclerosis [3,4], Parkinson's disease [5,6], and AD [7]. Currently, noninvasive *in vivo* quantification of retinal neural tissue can be conducted quickly and automatically at low cost by using optical coherence tomography (OCT). Therefore, in the contrast of amyloid imaging or cerebrospinal fluid study, OCT has a good potential to serve as a noninvasive tool for early and massive screening of cognitive impairment in the community [8]. The axons of retinal ganglion cells (RGCs) extend from the inner retina and form direct synaptic connections with the CNS in the thalamic region. The standard OCT protocol generates two quantitative RGC measures. The retinal nerve fiber layer (RNFL) thickness represents the quantity of axons, and the ganglion cell–inner plexiform layer (GC-IPL) thickness reflects the quantity of cell bodies and dendrites of RGCs. In this study, the thickness of RNFL is referred to "RNFL," and the thickness of GC-IPL is referred to "GC-IPL."

A recent systematic review and meta-analysis [9] found that AD patients showed a significant thinning of GC-IPL compared with that of controls. In addition, the thinning of GC-IPL showed borderline significance while comparing MCI to the controls using paired-eye data; however, this association became significant when single-eye data were applied. Overall, prior studies have been focused on AD patients, but studies on MCI or nondemented (preclinical phase) elders are limited. Therefore, this study aimed to explore the association of OCT-measured retinal biomarkers (RNFL and GC-IPL, using both bilateral and unilateral eye data) with global and domain-specific cognition in community-dwelling, nondemented elderly people, adjusting for important covariates.

2. Methods

2.1. Study population

This cross-sectional study is from the 2nd follow-up (2015–2017) of an ongoing cohort study called the Taiwan Initiative for Geriatric Epidemiological Research (2011–present). Taiwan Initiative for Geriatric Epidemiological Research recruited 605 community-dwelling elders (aged 65 + years) who received annual elderly health checkups at National Taiwan University Hospital, Taipei, Taiwan, at baseline (2011–2013). Cognitive impairment was the primary outcome, and the cognition of each participant in this cohort was assessed every 2 years. In the 2nd follow-up, OCT and fundus photography were performed to collect retinal data from 342 participants.

Participants with any of the following conditions or diseases were excluded from this study (n = 115): (1) history of clinically diagnosed AD, use of medication(s) for AD treatment, or a cognitive score suggesting undiagnosed dementia [Montreal Cognitive Assessment–Taiwanese version (MoCA-T) score < 21] (n = 28); (2) history of stroke (n = 16); (3) history of CNS insults including head injury, brain tumor ≥3 cm in diameter, and brain surgery (n = 51); (4) history of epilepsy (n = 1); (5) history of Parkinson's disease (n = 5); (6) fundus photography and OCT showing glaucomatous characteristics (localized nerve fiber layer wedge defect, diffuse or localized disc rim thinning, and disc hemorrhage), significant optic atrophy, or retinal vascular diseases (e.g., diabetic retinopathy, retinal artery/vein occlusion, hypertensive retinopathy) in either eye as judged by an ophthalmologist who was blinded to participants' cognition (n = 45); or (7) undetermined apolipoprotein (*APOE*) ε4 status (n = 4). Furthermore, we excluded participants for whom bilateral RNFL and GC-IPL data were unavailable (n = 83). After the exclusion of all ineligible patients, 227 participants were included in this study to assess the associations of RNFL and GC-IPL with global and domain-specific cognitive function. The research plan, informed consent, questionnaires, and application forms were approved by the research ethics committee at National Taiwan University Hospital. Written informed consent was obtained from all study participants.

2.2. Cognitive assessment

Both global and domain-specific cognition were assessed by a battery of neuropsychological tests. All cognitive tests were performed by a trained research assistant. We used the MoCA-T to assess global cognition. Participants with MoCA-T scores less than 24 and 21 (cutoffs used for Taiwanese population) were defined as having global cognitive impairment and likely to have dementia, respectively [10]. Participants with MoCA-T scores less than 21 were excluded because of the chance of undiagnosed dementia. For domain-specific cognition, the Wechsler Memory Scale–Third Edition was used to assess the domains of logical memory (theme I and II and recall I and II) and attention (digit span–forward and backward). For verbal fluency tests, each participant named as many fish, vegetables, and fruits as they could within 1 minute per category. For executive function, the trail making tests

A and B were administered. Data on the eleven aforementioned domain-specific cognitive variables were obtained from these neuropsychological tests. To facilitate comparisons across different cognitive variables, we standardized the score of each domain-specific variable into a Z score based on the mean and standard deviation (SD) of the raw score. The larger the score, the better the individual's cognition.

2.3. Retinal biomarkers: OCT measurements for RGCs

After pupil dilation using 1% tropicamide and 2.5% phenylephrine hydrochloride, all participants were examined using fundus photography and OCT. We used Cirrus HD-OCT (Carl Zeiss Meditec, Inc., Dublin, CA, USA) to perform one macular cube 512 × 128 scan and one optic nerve head cube 200 × 200 scan of each eye to acquire the GC-IPL and RNFL data, respectively. The average and sectorial GC-IPL or RNFL from both eyes were automatically reported based on the standard scan protocols. For GC-IPL, data from six sectors (superior, superior-temporal, inferior-temporal, inferior, inferior-nasal, and superior-nasal) and the mean of all sectors of each eye (i.e., right-eye GC-IPL and left-eye GC-IPL) were recorded. For RNFL, data from four sectors (superior, temporal, inferior, and nasal) and the mean of all sectors of each eye (i.e., right-eye RNFL and left-eye RNFL) were recorded. In our main analyses, we used the average of right-eye and left-eye data for the two retinal biomarkers, respectively, which referred to mean GC-IPL and RNFL of bilateral eyes.

All fundus photography and OCT reports for each participant were scrutinized by an ophthalmologist who was blinded to the participants' cognitive function. If the RNFL and GC-IPL measurements were unreliable due to poor data quality (e.g., motional artifact, signal defect in the scanning region due to media opacity, very poor signal strength) or certain retinopathies that significantly interfered with measurement (e.g., macular edema, macular atrophy, macular hole, epiretinal membrane, or posterior staphyloma), the data were coded as "missing." In addition, participants with age-related macular degeneration were identified based on the inspection of their retinal imaging by the same ophthalmologist. Because age-related macular degeneration is known to be associated with both increasing risk of dementia [11] and GC-IPL thinning [12], this eye condition was included as a covariate in multiple regression analysis.

2.4. Covariates

Demographic data (age, sex, and years of education), medical history (hypertension and diabetes mellitus), and lifestyle information (smoking status and alcohol consumption) were obtained from self-reported questionnaires. Body mass index was obtained from the data of a routine health checkup. Physical function was assessed by the activities of daily living and instrumental activities of daily living scales. Physical activity was evaluated by a short version of the International Physical Activity Questionnaire. Depressive symptoms were quantified by the Center for Epidemiological Studies–Depression scale. A comorbidity of diabetes was defined as self-reported diagnosis (yes/no), use of antidiabetic drugs (yes/no), or fasting blood glucose $\geq$ 126 mg/dL. A history of hypertension was defined as a self-reported diagnosis (yes/no), use of antihypertensive medication (yes/no), systolic blood pressure $\geq$ 140 mm Hg, or diastolic blood pressure $\geq$ 90 mm Hg.

2.5. Laboratory assays

A blood sample was collected from each participant at the baseline (2011–2013). Genomic DNA was extracted from the buffy coats using a QuickGene-Mini 80 system (Fujifilm, Tokyo, Japan). *APOE* ε4 status was determined by TaqMan genomic assays using an ABI 7900HT fast real-time PCR system (Applied Biosystems Inc., Foster City, CA, USA).

2.6. Statistical analysis

For descriptive analysis of population characteristics, we used Student's t-test and the Mann-Whitney U test for continuous variables and the chi-squared test and Fisher's exact test for categorical variables to compare the distribution of each variable between elders with normal and impaired global cognition (MoCA-T score $\geq$ 24 vs. $<$ 24). The multivariable linear regression models were used to estimate the linear change in global or domain-specific cognitive function for each 1 μm increase in the mean RNFL or mean GC-IPL of bilateral eyes (i.e., β coefficients). Because no significant findings were observed for the linear associations between retinal markers (GC-IPL and RNFL) and cognition, we used general additive model (GAM) to further examine a possible nonlinear relationship between them. If a nonlinear relationship was found between retinal markers and cognition, the original and additional mean-centered quadratic retinal variables, that is $[(\text{retinal marker}) - \text{mean}(\text{retinal marker})]^2$, were used to assess their relations to global or domain-specific cognition in the multivariable linear regression models. For significant associations identified in the aforementioned analyses, further analyses of RNFL or GC-IPL were performed for the right and left eyes separately (additionally adjusted for OCT signal strength for unilateral eye data) and for each sector. Finally, a sensitivity analysis was performed to compare findings from elders with both bilateral RNFL and GC-IPL data (the main analyses mentioned previously) with findings from elders who had data of at least one eye. All statistical analysis was performed using SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA).

Table 1
Characteristics of the study population

Variables	Global cognition		*P* value
	Normal (n = 215)	Impaired (n = 12)	
	Mean ± SD, n (%)		
Age (years)	**71.1 ± 4.6**	**74.5 ± 5.5**	**.03**
Gender (women)	121 (56.3)	8 (66.7)	.56
Education (years)	**14.0 ± 3.3**	**12.2 ± 4.4**	**.02**
APOE ε4 carriers	35 (16.3)	3 (25.0)	.43
Diabetes	37 (17.2)	1 (8.3)	.70
Hypertension	123 (57.2)	7 (58.3)	.94
BMI (kg/m^2)	23.5 ± 3.0	24.7 ± 3.7	.09
ADL score	99.3 ± 2.3	100 ± 0	.28
IADL score	7.9 ± 0.3	7.8 ± 0.6	.19
IPAQ (MET-min/week)	2029.7 ± 1816.3	1914.9 ± 1873.3	.65
CES-D score	2.35 ± 4.5	2.92 ± 4.0	.75
AMD	26 (12.1)	3 (25.0)	.19
Mean RNFL of bilateral eyes (μm)	91.6 ± 9.3	88.6 ± 10.1	.29
Mean GC-IPL of bilateral eyes (μm)	77.7 ± 6.9	73.4 ± 12.3	.25

Abbreviations: SD, standard deviation; *APOE*, apolipoprotein E gene; BMI, body mass index; ADL, activities of daily living; IADL, instrumental activities of daily living; IPAQ, International Physical Activity Questionnaire; MET, metabolic equivalent; CES-D, Center for Epidemiological Studies–Depression; AMD, age-related macular degeneration; RNFL, retinal nerve fiber layer thickness; GC-IPL, ganglion cell–inner plexiform layer thickness.

NOTE. Global cognitive function was assessed by the Montreal Cognitive Assessment–Taiwanese version (MoCA-T), with scores less than 24 indicating global cognitive impairment.

NOTE. *P* values were obtained using Student's t-test and the Mann-Whiney U test for continuous variables and the chi-squared test and Fisher's exact test for categorical variables. Numbers in bold indicate significant findings (*P* value $<$ .05).

3. Results

3.1. Characteristics of the study population

A total of 227 elders with bilateral retinal data were included for analysis. The distribution of global cognition (MoCA-T score) was skewed to the left (median = 28; SD = 2.1). The distributions of the two retinal biomarkers approximated the normal distribution (RNFL: mean = 91.4 μm, SD = 9.3 μm; GC-IPL: mean = 77.5, SD = 7.3 μm). Table 1 shows the demographic characteristics of the study population. Elders with impaired global cognition (MoCA-T score $<$ 24) were older (74.5 vs. 71.1 years old, $P = .03$) and less educated (12.2 vs. 14.0 years of education, $P = .02$) than cognitively normal elders (MoCA-T score $\geq$ 24). For other covariates, no significant difference was observed between elders with impaired and normal cognition.

3.2. Linear association of mean RNFL and mean GC-IPL of bilateral eyes with global and domain-specific cognition

The results of multivariable linear regression adjusted for age, sex, years of education, depressive symptoms, *APOE* ε4 status, diabetes, hypertension, and age-related macular degeneration are shown in Table 2. Overall, there was no significant linear association between the original retinal biomarkers (mean RNFL and mean GC-IPL of bilateral eyes) and global or domain-specific cognition in this elderly population.

Table 2
Linear association of mean RNFL and mean GC-IPL of bilateral eyes with global and domain-specific cognition (n = 227)

Cognitive variables	Mean RNFL (μm)	Mean GC-IPL (μm)
	β (95% CI) × 10^{-2}	
Global cognition (MoCA-T score)	−2.05 (−4.85, 0.76)	−0.78 (−4.49, 2.93)
Logical memory—theme I	−0.68 (−1.94, 0.59)	−0.72 (−2.39, 0.95)
Logical memory—recall I	−0.90 (−2.17, 0.36)	−1.35 (−3.01, 0.31)
Logical memory—theme II	−0.02 (−1.20, 1.16)	−0.17 (−1.72, 1.38)
Logical memory—recall II	−0.33 (−1.60, 0.93)	−0.58 (−2.24, 1.08)
Digit span—forward	−0.41 (−1.73, 0.92)	0.09 (−1.42, 1.61)
Digit span—backward	−0.47 (−1.65, 0.71)	−0.80 (−2.54, 0.94)
Digit span—total	−0.42 (−1.57, 0.72)	−0.41 (−1.97, 1.14)
Trail making test A	−0.30 (−1.09, 0.49)	0.54 (−0.50, 1.58)
Trail making test B	−0.08 (−1.32, 1.15)	0.40 (−1.23, 2.02)
Trail making test (B-A)	0.05 (−1.28, 1.38)	0.21 (−1.55, 1.96)
Verbal fluency test—fruits	−0.52 (−1.70, 0.67)	0.46 (−1.10, 2.01)
Verbal fluency test—fishes	−0.51 (−1.86, 0.85)	0.63 (−1.16, 2.42)
Verbal fluency test—vegetables	−0.81 (−2.02, 0.39)	−0.36 (−1.95, 1.23)
Verbal fluency test—total	−0.73 (−1.91, 0.44)	0.21 (−1.34, 1.76)

Abbreviations: RNFL, retinal nerve fiber layer thickness; GC-IPL, ganglion cell–inner plexiform layer thickness; CI, confidence interval; MoCA-T, Montreal Cognitive Assessment–Taiwanese version.

NOTE. Global cognition is represented by the original MoCA-T score. To facilitate comparisons, we standardized domain-specific cognition scores as Z scores.

NOTE. All linear regression models were adjusted for age, sex, years of education, apolipoprotein E (*APOE*) gene ε4 status, Center for Epidemiological Studies–Depression (CES-D) score, history of diabetes, hypertension, and age-related macular degeneration (AMD).

3.3. Nonlinear association of mean RNFL and mean GC-IPL of bilateral eyes with global and domain-specific cognition

The spline curves from the GAM demonstrated the nonlinear relationship (an inverted U shape) between mean GC-IPL of bilateral eyes and global cognition (MoCA-T, $P < .001$) adjusted for various covariates (Fig. 1). A similar inverted U-shaped relationship was observed between GC-IPL and logical memory (Fig. A.1, Appendix A). However, for mean RNFL of bilateral eyes, the spline curves did not show the existence of a nonlinear association with global cognition ($P = .21$, Fig. 1) or domain-specific cognition (Fig. A.2, Appendix A).

Based on the association suggested by the GAM, a quadratic term of mean-centered GC-IPL was included in the models to assess its association with global and domain-specific

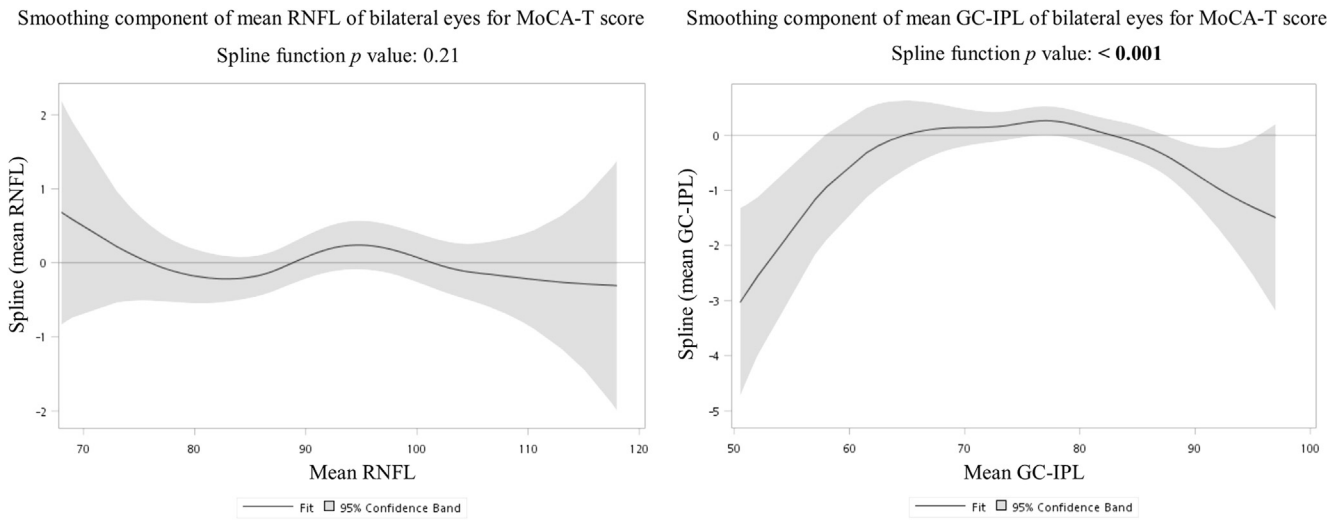

Fig. 1. Spline curves relating mean RNFL and mean GC-IPL of bilateral eyes with global cognition (MoCA-T score) from generalized additive models (GAMs, n = 227). All models were adjusted for age, sex, years of education, apolipoprotein E (*APOE*) gene ε4 status, Center for Epidemiological Studies—Depression (CES-D) score, history of diabetes and hypertension, and age-related macular degeneration (AMD). Numbers in bold indicate significant findings (*P* value < .05). Abbreviations: MoCA-T, Montreal Cognitive Assessment-Taiwanese version; RNFL, retinal nerve fiber layer thickness; GC-IPL, ganglion cell–inner plexiform layer thickness.

cognition (Table 3). We found that mean GC-IPL of bilateral eyes had a nonlinear association with global cognition (MoCA-T) [quadratic GC-IPL: $\beta = -0.49 \times 10^{-2}$, 95% confidence interval (CI): -0.74×10^{-2}, -0.23×10^{-2}]. The performance of global cognition (MoCA-T) decreased as mean GC-IPL of bilateral eyes deviated from the sample mean (77.5 μm). Mean GC-IPL of bilateral eyes values beyond 1.5 SD (±11.0 μm) from the mean (77.5 μm) was associated with a 0.59-point decrease in MoCA-T score after adjusting for other covariates.

Mean GC-IPL of bilateral eyes had a similar nonlinear association with logical memory–theme I (quadratic GC-IPL: $\beta = -0.18 \times 10^{-2}$, 95% CI: -0.29×10^{-2}, -0.05×10^{-2}), logical memory–recall II (quadratic GC-IPL: $\beta = -0.16 \times 10^{-2}$, 95% CI: -0.27×10^{-2}, -0.04×10^{-2}), and the trail making test A (quadratic GC-IPL: $\beta = -0.08 \times 10^{-2}$, 95% CI: -0.15×10^{-2}, -0.01×10^{-2}). Mean GC-IPL of bilateral eyes had linear and nonlinear associations with logical memory–recall I (linear GC-IPL: $\beta = -2.16 \times 10^{-2}$, 95% CI: -3.85×10^{-2}, -0.47×10^{-2}; quadratic GC-IPL: $\beta = -0.19 \times 10^{-2}$, 95% CI: -0.31×10^{-2}, -0.08×10^{-2}). No significant association was observed in the other cognitive domains (attention and verbal fluency).

3.4. Nonlinear association of unilateral GC-IPL with global and domain-specific cognition

We also investigated the separate associations of right- and left-eye GC-IPL with cognitive function (Table 3). Because a nonlinear association between GC-IPL and global cognition (MoCA-T) was suggested by the GAM (Fig. 1 and Fig. A.1, Appendix A), both linear and quadratic terms of GC-IPL were included in the models to assess the association with global and domain-specific cognition.

We found that right-eye GC-IPL was nonlinearly associated with global cognition (MoCA-T) (quadratic GC-IPL: $\beta = -0.18 \times 10^{-2}$, 95% CI: -0.37×10^{-2}, -0.004×10^{-2}, Table 3). The performance of global cognition (MoCA-T) decreased as right-eye GC-IPL deviated from the sample mean (77.8 μm). Right-eye GC-IPL values beyond 1.5 SD (±11.7 μm) from the sample mean (77.8 μm) were associated with a 0.25-point decrease in MoCA-T scores after adjusting for other covariates (data not shown).

Similar nonlinear associations were observed for logical memory–theme I (quadratic GC-IPL: $\beta = -0.16 \times 10^{-2}$, 95% CI: -0.24×10^{-2}, -0.08×10^{-2}) and logical memory–recall II (quadratic GC-IPL: $\beta = -0.09 \times 10^{-2}$, 95% CI: -0.17×10^{-2}, -0.01×10^{-2}). Linear and nonlinear associations were observed for logical memory–recall I (linear GC-IPL: $\beta = -2.13 \times 10^{-2}$, 95% CI: -3.88×10^{-2}, -0.39×10^{-2}; quadratic GC-IPL: $\beta = -0.11 \times 10^{-2}$, 95% CI: -0.19×10^{-2}, -0.03×10^{-2}).

Left-eye GC-IPL was nonlinearly associated with global cognition (MoCA-T) (quadratic GC-IPL: $\beta = -0.37 \times 10^{-2}$, 95% CI: -0.57×10^{-2}, -0.17×10^{-2}, Table 3). The performance of global cognition (MoCA-T) decreased as left-eye GC-IPL deviated from the sample mean (77.2 μm). Left-eye GC-IPL values beyond 1.5 SD (±11.6 μm) from the sample mean (77.2 μm) were associated with a 0.49-point decrease in MoCA-T scores after adjusting for other covariates (data not shown).

Table 3
Nonlinear association of bilateral or unilateral GC-IPL with global and domain-specific cognition (n = 227)

	GC-IPL (μm)					
	Bilateral eyes		Right eye		Left eye	
	Linear term	Quadratic term	Linear term	Quadratic term	Linear term	Quadratic term
Cognitive variables	β (95% CI) × 10^{-2}					
Global cognition (MoCA-T score)	−2.81 (−6.57, 0.94)	**−0.49 (−0.74, −0.23)**	−2.63 (−6.55, 1.28)	**−0.18 (−0.37, −0.004)**	−2.79 (−6.34, 0.76)	**−0.37 (−0.57, −0.17)**
Logical memory—theme I	−1.48 (−3.19, 0.23)	**−0.18 (−0.29, −0.05)**	−1.43 (−3.16, 0.30)	**−0.16 (−0.24, −0.08)**	**−1.71 (−3.33, −0.08)**	−0.09 (−0.19, 0.0005)
Logical memory—recall I	**−2.16 (−3.85, −0.47)**	**−0.19 (−0.31, −0.08)**	**−2.13 (−3.88, −0.39)**	**−0.11 (−0.19, −0.03)**	**−2.11 (−3.72, −0.50)**	**−0.13 (−0.22, −0.04)**
Logical memory—theme II	−0.47 (−2.09, 1.15)	−0.07 (−0.18, 0.04)	−0.19 (−1.83, 1.46)	−0.06 (−0.14, 0.01)	−0.77 (−2.30, 0.77)	−0.03 (−0.12, 0.05)
Logical memory—recall II	−1.23 (−2.94, 0.49)	**−0.16 (−0.27, −0.04)**	−1.29 (−3.04, 0.47)	**−0.09 (−0.17, −0.01)**	−1.27 (−2.90, 0.35)	**−0.10 (−0.19, −0.01)**
Digit span—forward	0.07 (−1.52, 1.65)	−0.004 (−0.11, 0.10)	0.06 (−1.67, 1.55)	0.02 (−0.05, 0.10)	0.06 (−1.43, 1.56)	−0.03 (−0.12, 0.05)
Digit span—backward	−0.74 (−2.56, 1.08)	0.01 (−0.11, 0.14)	−0.78 (−2.63, 1.06)	0.06 (−0.03, 0.15)	−0.39 (−2.11, 1.33)	−0.03 (−0.13, 0.07)
Digit span—total	−0.39 (−2.02, 1.23)	0.01 (−0.11, 0.12)	−0.49 (−2.14, 1.16)	0.05 (−0.03, 0.13)	−0.19 (−1.72, 1.35)	−0.04 (−0.12, 0.05)
Trail making test A	0.20 (−0.88, 1.27)	**−0.08 (−0.15, −0.01)**	0.34 (−0.77, 1.45)	−0.02 (−0.07, 0.03)	0.09 (−0.92, 1.10)	**−0.07 (−0.12, −0.01)**
Trail making test B	0.04 (−1.65, 1.73)	−0.09 (−0.20, 0.03)	−0.02 (−1.76, 1.71)	−0.02 (−0.11, 0.06)	0.16 (−1.41, 1.75)	−0.08 (−0.17, 0.01)
Trail making test (B-A)	−0.05 (−1.89, 1.78)	−0.06 (−0.19, 0.06)	−0.20 (−2.10, 1.68)	−0.02 (−0.11, 0.07)	0.15 (−1.56, 1.87)	−0.06 (−0.16, 0.03)
Verbal fluency test—fruits	0.15 (−1.47, 1.77)	−0.07 (−0.18, 0.04)	−0.04 (−1.69, 1.61)	−0.07 (−0.15, 0.01)	0.02 (−1.52, 1.55)	−0.02 (−0.10, 0.07)
Verbal fluency test—fishes	0.35 (−1.52, 2.21)	−0.07 (−0.20, 0.06)	0.34 (−1.56, 2.24)	−0.03 (−0.12, 0.06)	−0.13 (−1.87, 1.61)	−0.04 (−0.14, 0.06)
Verbal fluency test—vegetables	−0.72 (−2.37, 0.94)	−0.09 (−0.20, 0.03)	−0.64 (−2.33, 1.06)	−0.05 (−0.13, 0.03)	−0.94 (−2.49, 0.62)	−0.06 (−0.15, 0.03)
Verbal fluency test—total	−0.16 (−1.77, 1.45)	−0.09 (−0.20, 0.02)	−0.19 (−1.84, 1.45)	−0.06 (−0.13, 0.02)	−0.47 (−1.98, 1.03)	−0.05 (−0.13, 0.04)

Abbreviations: GC-IPL, ganglion cell–inner plexiform layer thickness; CI, confidence interval; MoCA-T, Montreal Cognitive Assessment–Taiwanese version.

NOTE. Global cognition is represented by the original MoCA-T score. To facilitate comparisons, we standardized the other domain-specific cognition scores as Z scores.

NOTE. Mean-centered GC-IPL, $[(\text{GC-IPL})—\text{mean}(\text{GC-IPL})]^2$, was used as the quadratic term.

NOTE. All linear regression models were adjusted for age, sex, years of education, apolipoprotein E (*APOE*) gene ε4 status, Center for Epidemiological Studies–Depression (CES-D) score, history of diabetes and hypertension, and age-related macular degeneration (AMD).

NOTE. Optical coherence tomography (OCT) signal strength of each eye was additionally adjusted in the models for the unilateral eye data.

NOTE. Numbers in bold indicate significant findings (P value $< .05$).

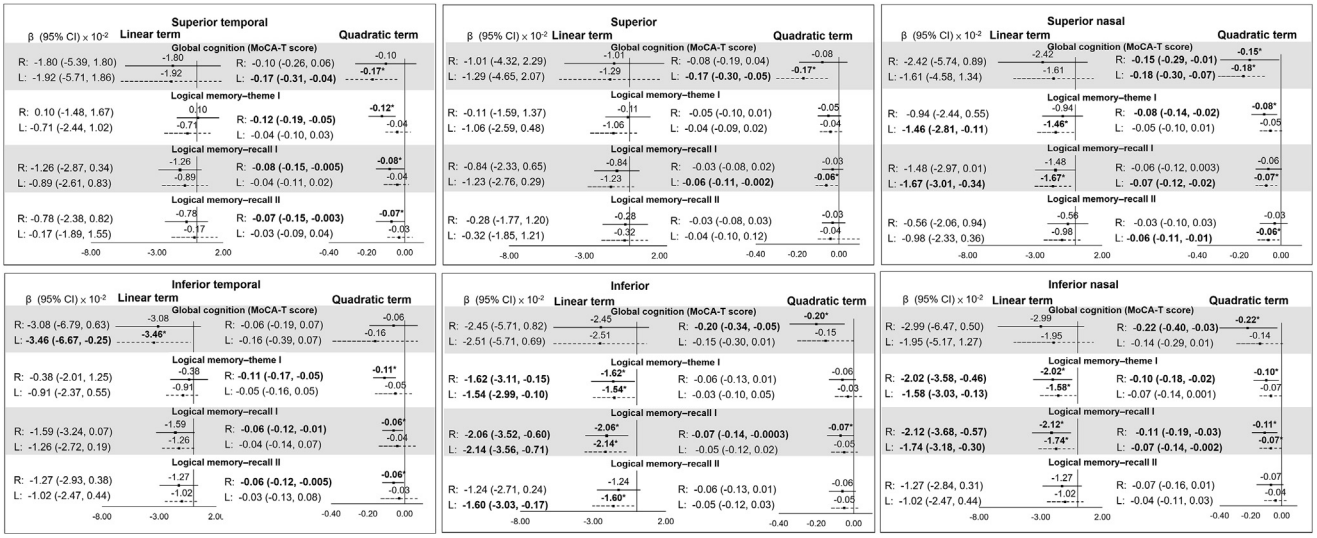

Fig. 2. Nonlinear association of sectorial GC-IPL with global cognition and logical memory (n = 227). Global cognition is represented as the original MoCA-T score. Logical memory scores were standardized as Z scores. R: Right eye; L: left eye. Mean-centered GC-IPL, $[(\text{GC-IPL}) - \text{mean}(\text{GC-IPL})]^2$, was used as the quadratic term. All linear regression models were adjusted for age, sex, years of education, apolipoprotein E (*APOE*) gene ε4 status, Center for Epidemiological Studies–Depression (CES-D) score, history of diabetes and hypertension, and age-related macular degeneration (AMD). Optical coherence tomography (OCT) signal strength of each eye was additionally adjusted in the models for unilateral eye data. Numbers in bold indicate significant findings (*P* value < .05). *Significant findings (*P* value < .05). Abbreviations: GC-IPL, ganglion cell–inner plexiform layer thickness; CI, confidence interval; MoCA-T, Montreal Cognitive Assessment—Taiwanese version.

Similar nonlinear associations were observed for logical memory–recall II (quadratic GC-IPL: $\beta = -0.10 \times 10^{-2}$, 95% CI: -0.19×10^{-2}, -0.01×10^{-2}) and the trail making test A (quadratic GC-IPL: $\beta = -0.07 \times 10^{-2}$, 95% CI: -0.12×10^{-2}, -0.01×10^{-2}). Linear association was observed for logical memory–theme I (linear GC-IPL: $\beta = -1.71 \times 10^{-2}$, 95% CI: -3.33×10^{-2}, -0.08×10^{-2}). Both linear and nonlinear associations were observed for logical memory–recall I (linear GC-IPL: $\beta = -2.11 \times 10^{-2}$, 95% CI: -3.72×10^{-2}, -0.50×10^{-2}; quadratic GC-IPL: $\beta = -0.13 \times 10^{-2}$, 95% CI: -0.22×10^{-2}, -0.04×10^{-2}). No significant association was observed for the other cognitive domains (attention and verbal fluency).

3.5. Nonlinear association of sectorial GC-IPL of bilateral eyes with global cognition and the domain of logical memory

To further clarify the association between specific sectors of GC-IPL and cognitive function, we analyzed all 6 sectorial GC-IPL values of each eye. Both global cognition and logical memory were significantly related to GC-IPL, and logical memory was the only domain to show such a relation before the data were subdivided into 6 sectors. These two cognitive variables were further assessed for sectorial data. We found that GC-IPL in the majority of sectors showed a significant nonlinear association with global cognition or logical memory, similar to the results for the GC-IPL as a whole (Fig. 2, sectors of the right and left eyes).

3.6. Sensitivity analysis with expanded sample size: nonlinear association between bilateral or unilateral GC-IPL and global cognition or the domain of logical memory

For sensitivity analysis, we performed the same regression analysis in expanded populations: (1) participants who had bilateral GC-IPL data but might not have bilateral RNFL data (n = 244); (2) participants who had right-eye GC-IPL data but might not have left-eye GC-IPL data or bilateral RNFL data (n = 263); and (3) participants who had left-eye GC-IPL data but might not have right-eye GC-IPL data or bilateral RNFL data (n = 268) (Table B.1, Appendix B). Overall, the results of the sensitivity analysis were consistent with the findings from participants with both bilateral RNFL and GC-IPL data.

4. Discussion

To the best of our knowledge, our study is the first to demonstrate a significant nonlinear association between mean GC-IPL of bilateral eyes and cognitive function in the elderly after adjusting for important covariates. We found inverted U-shaped relationships between mean GC-IPL of bilateral eyes and both global cognition and logical memory. This association remained significant in the analyses of unilateral GC-IPL data and most of the sectorial GC-IPL data. No association was observed between mean RNFL of bilateral eyes and global or domain-specific cognition.

Our finding suggested that thinning of GC-IPL was associated with poor cognition, which was consistent with previous studies. A previous study showed that

GC-IPL was significantly lower in elders with AD or MCI than in cognitively normal controls [13]. In addition, thinning of GC-IPL was also associated with increased disease severity, impaired performance in the memory domain [14,15], and a reduction of total brain volume in the occipital and temporal lobes (especially gray matter volume) in cognitively impaired (AD or MCI) elders [16].

By contrast, we also found that thickening of GC-IPL was related to poor cognition, which has not been reported in prior studies. Although thinning of GC-IPL was consistently related to AD in previous studies, a recent meta-analysis did not show a clear result in MCI patients [9]. It is possible that the true relationship between GC-IPL and cognition is nonlinear, based on the results from the GAM in this study. A recent cross-sectional study examined 63 cognitively normal adults who had a parent with AD and suffered from subjective memory complaints [17]. The study found that OCT-measured IPL volume was elevated in participants with high-level cerebral amyloid β (Aβ) burden, which partially supports our findings.

In AD pathogenesis, it is widely accepted that synaptic dysfunction and the subsequent neuronal death globally affect the CNS even before the clinical manifestations appear [2]. Similarly, RGC dendritic pathology and apoptosis have been demonstrated in a transgenic mouse model of AD [18–21]. Moreover, Aβ, amyloid precursor protein, and tau protein were detected in the GC layer and IPL of the retina, along with increased microglial and astrocytic activity [18–20,22–24].

GC-IPL as measured by OCT macular scanning reflects the *in vivo* condition of the dendrites and cell bodies of RGCs. Because most of our participants are cognitively normal (94.7% with a MoCA-T score $\geq$ 24), thinning and thickening of GC-IPL both imply that the pathology of RGCs may occur at the preclinical stage of AD. We postulated that Aβ deposition and neuroinflammation at the dendrites of RGCs are involved in the pathogenesis of thickening of GC-IPL. During the course of pathological cognitive decline, it is possible that synaptic dysfunction and the corresponding pathological dendritic changes were followed by the loss of RGCs in the early stage of AD; this neuronal loss gradually became significant and could be identified by thinning of GC-IPL. Our hypothesis is supported by a recent study using triple-transgenic AD mouse model, which found that Aβ plaques, tau tangles, astrogliosis, and neurodegeneration could be observed in the RGC layer at the presymptomatic stage [18]. However, further longitudinal studies are warranted to clarify our findings.

As a whole, our study showed consistent findings between bilateral and unilateral eye data. In addition, except statistical significance, the magnitude and direction were similar between right and left eyes for the association of GC-IPL with some cognitive domains (logical memory theme–I: −0.16 vs. −0.09; trail making test A: −0.02 vs. −0.07). Because the 95% CIs were partially overlapped between right and left eyes, this indicated small differences between them, which may be attributable to a random effect.

The present study has several strengths. First, to the best of our knowledge, this is the first study to explore the association between OCT-measured retinal markers and cognition in community-dwelling nondemented elders. Our findings provided evidence that GC-IPL might serve as an early and noninvasive biomarker of dementia. Second, in addition to global cognition, we assessed 11 domain-specific cognitive variables, which spanned the domains of logical memory, attention, executive function, and verbal fluency. These data enable a deeper understanding of the relation between retinal biomarkers and specific cognitive domains.

This study has some limitations. First, the cross-sectional design could not clarify the temporal relationship between changes in retinal biomarkers and cognitive decline. Second, the majority of our study population are Chinese elders living in Taipei metropolitan area with a high socioeconomic status, and they were volunteers from the health checkup program at a teaching hospital, which may limit the generalizability to a broader elderly population. Third, we did not acquire data on eyeball axial length, which is used to identify myopia and may influence OCT-measured RNFL and GC-IPL. However, the effect of axial length may be limited because elders with pathological high myopia of either eye tend to have posterior staphyloma and poor OCT quality and were therefore excluded when the ophthalmologist evaluated the imaging data.

In conclusion, our study suggested that either thinning or thickening of GC-IPL, as measured by OCT, was associated with poor cognition in community-dwelling elders. This indicates that GC-IPL might be an early biomarker of cognitive impairment. Because OCT is a fast and noninvasive examination, it could serve as an early screening tool to identify preclinical AD, which would help elders proactively care for their health by modifying lifestyle variables before the occurrence of irreversible AD. Mounting evidence supports that subtle changes in OCT-measured retinal biomarkers, especially RNFL and GC-IPL, may reflect neurodegeneration in the CNS. However, the underlying mechanism and its temporal relationship with the course of cognitive decline remain unclear. Further experimental and longitudinal studies are warranted to characterize RGC pathology in neurodegenerative disorders and to clarify the temporal relationship between retinal markers and cognitive decline.

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Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dadm.2018.10.006.

RESEARCH IN CONTEXT

1. Systematic review: Subtle neural layer changes in the retina may reflect the pathological neurodegeneration in the disease process of Alzheimer's disease (AD). Past studies found that thinning of optical coherence tomography–measured retinal nerve fiber layer and ganglion cell–inner plexiform layer thickness (GC-IPL) are associated with the risk of AD. However, the associations of these retinal markers with cognition in nondemented community-dwelling elders are inconclusive.
2. Interpretation: We found that either thinning or thickening of GC-IPL is associated with poor cognition in nondemented elders (a U-shaped association), which imply that the synaptic pathology and subsequent neuronal loss of retinal ganglion cells may occur at the preclinical stage of AD.
3. Future directions: GC-IPL may serve as a noninvasive preclinical predictor of AD. Future longitudinal studies are warranted to assess the nonlinear association of GC-IPL and cognitive decline to predict AD risk at the preclinical stage.

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