Novel retinal imaging biomarkers for cognitive decline: abridged secondary publication

CY Cheung *, VTT Chan, LWC Au, CC Tham, TCY Kwok, CTV Mok

KEY MESSAGES

1. There has been a search for Alzheimer disease (AD) biomarkers that facilitate early disease diagnosis while being non-invasive, widely available, and reliable. The retina, an extension of the central nervous system, offers a "window" for in vivo studies of the cerebral microvascular and neurodegenerative damage in AD.
2. In this prospective observational study, healthy controls and patients with AD or amnestic mild cognitive impairment underwent neuropsychological testing, retinal imaging, and neuroimaging to explore the associations of retinal changes with upstream pathological changes in AD and cognitive decline.
3. Compared with amyloid-β-negative individuals, amyloid-β-positive individuals had significantly thinner macular ganglion-cell inner plexiform layer thickness. Among amyloid-β-positive individuals, cognitively impaired individuals had significantly larger foveal avascular zone area, smaller fractal dimension, smaller skeleton density, larger vessel diameter index, and smaller inter-capillary area, compared with cognitively normal individuals. Global amyloid burden was associated with macular ganglion-cell inner plexiform layer thickness and foveal avascular zone area.
4. Central subfield thickness and mean cube thickness were associated with the progression of cognitive decline over 12 months.
5. Specific retinal microvascular abnormalities and retinal neuronal/axonal loss, measured using non-invasive retinal imaging technologies, may reflect cerebrovascular dysfunction and classic features of neuronal injury in the AD brain; moreover, they are associated with AD and can independently predict cognitive decline.

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[1] CY Cheung, [1] VTT Chan, [2] LWC Au, [1] CC Tham, [2] TCY Kwok, [2] CTV Mok

[1] *Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China*
[2] *Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong SAR, China*

* Principal applicant and corresponding author: carolcheung@cuhk.edu.hk

Introduction

Alzheimer disease (AD) is an incurable and progressive neurodegenerative disorder. Its pathological features are extracellular amyloid-β plaques and neurofibrillary tangles composed of intracellular hyperphosphorylated tau protein (p-tau). Advances in detecting cerebral spinal fluid and neuroimaging markers (eg, the use of Pittsburgh Compound-B to detect cerebral amyloid-β) improve the accuracy of AD diagnosis. However, clinical applications of these novel biomarkers are restricted by standardisation issues and invasiveness and by high costs and limited availability. Additionally, although various indirect manifestations of cerebral small vessel disease (eg, white matter lesions, lacunar infarcts, and cerebral microbleeds) have been associated with cognitive decline, direct in vivo visualisation of changes in cerebral small vessels (ie, cerebral arteriolar narrowing or capillary microaneurysms) remains difficult to achieve by current neuroimaging technology. Thus, there is an ongoing search for high sensitivity and specificity biomarkers that are reliable, non-invasive, accessible, efficient, and inexpensive.

The retina is a central nervous system tissue and displays physiological properties and regulatory mechanisms similar to those within the brain. The retina can be visualised and offers an excellent "window" for direct, non-invasive evaluation of AD-related changes in the central nervous system and microvasculature.[1,2] The advantages of retinal imaging include lower cost, lower invasiveness, and greater accessibility, compared with neuroimaging and cerebral spinal fluid markers. Moreover, common age-related ophthalmic diseases share risk factors with AD; thus, patients with such ophthalmic diseases may benefit from additional screening.

We investigated associations between retinal vascular and neuronal changes in AD. Our hypothesis was that specific retinal microvascular and neuronal abnormalities, measured using new non-invasive retinal imaging technologies, would reflect features of cerebrovascular dysfunction in AD, and that these abnormalities would be independently predictive of cognitive decline.

Methods

In this prospective observational study, patients with AD or amnestic mild cognitive impairment (aMCI) were recruited from the dementia/memory clinic at Prince of Wales Hospital, Hong Kong, whereas age-matched cognitively normal controls without objective cognitive impairment on formal neuropsychological testing were recruited from a community-based study. Standardised inclusion and exclusion criteria were used to control for other conditions with potential effects on retinal neuronal thickness and microvasculature.

All recruited individuals underwent neuropsychiatric assessments, including the Hong Kong List Learning Test, Mini-Mental State Examination (MMSE), and the Hong Kong version of the Montreal Cognitive Assessment. Cognitive diagnoses were made by an experienced dementia specialist in accordance with the 2018 NIA-AA research framework.[3]

All participants were invited to undergo retinal imaging at 1, 6, and 12 months, then annually thereafter at the CUHK Ophthalmic Research Centre. Imaging modalities included optical coherence tomography (OCT), OCT-angiography, and ultra-wide field scanning laser ophthalmoscopy.

Retinal capillary network imaging was performed by OCT-angiography using a swept-source OCT device. Slabs of superficial capillary plexus were automated and segmented by the built-in software. En-face images of the included OCT-angiograms were exported in greyscale from the built-in software, then imported into a customised MATLAB program for image analysis (Fig 1). Retinal capillary network measurements included foveal avascular zone (FAZ) area, FAZ circularity index, vessel density, fractal dimension, non-perfusion area, vessel diameter index, and inter-capillary area.

Ultra-wide field fundus images were obtained using an ultra-wide field scanning laser ophthalmoscope and then graded semi-automatically using the Singapore I Vessel Assessment software. Various retinal vascular parameters including retinal vessel calibre, tortuosity, and fractal dimension were calculated.

Retinal neuronal imaging was performed using macular and optic disk cube scan protocols. The built-in CIRRUS analysis software provided automated measurements of macular ganglion cell-inner plexiform layer (GC-IPL) thickness and retinal nerve fibre layer (RNFL) thickness for assessment of retinal neuronal and axonal loss (Fig 2).

A subset of participants underwent ^{11}C-PIB and ^{18}F-T807 positron emission tomography (PET)/computed tomography to quantify deposition of beta-amyloid and tau, respectively.[4] They also underwent magnetic resonance imaging (MRI) in accordance with standard protocols.

Results

We initially recruited 163 individuals, including 57 healthy controls, 53 patients with aMCI, and 53

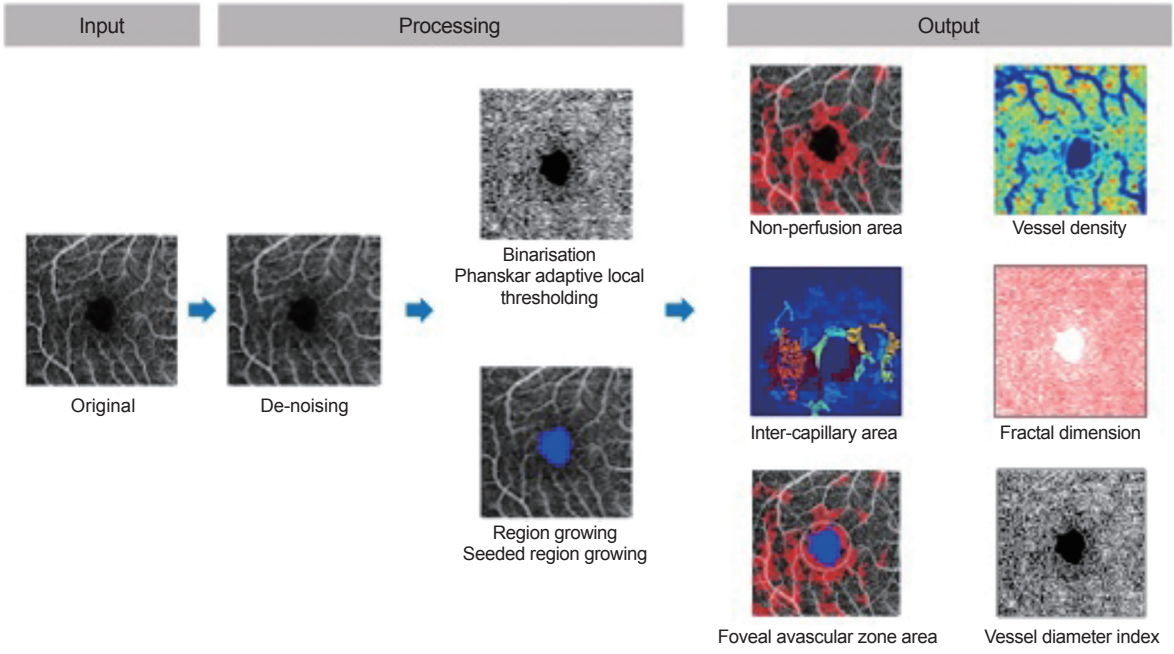

FIG 1. Retinal capillary network imaging via swept-source optical coherence tomography angiography: en-face images were exported in greyscale and then imported into a customised MATLAB program for image analysis.

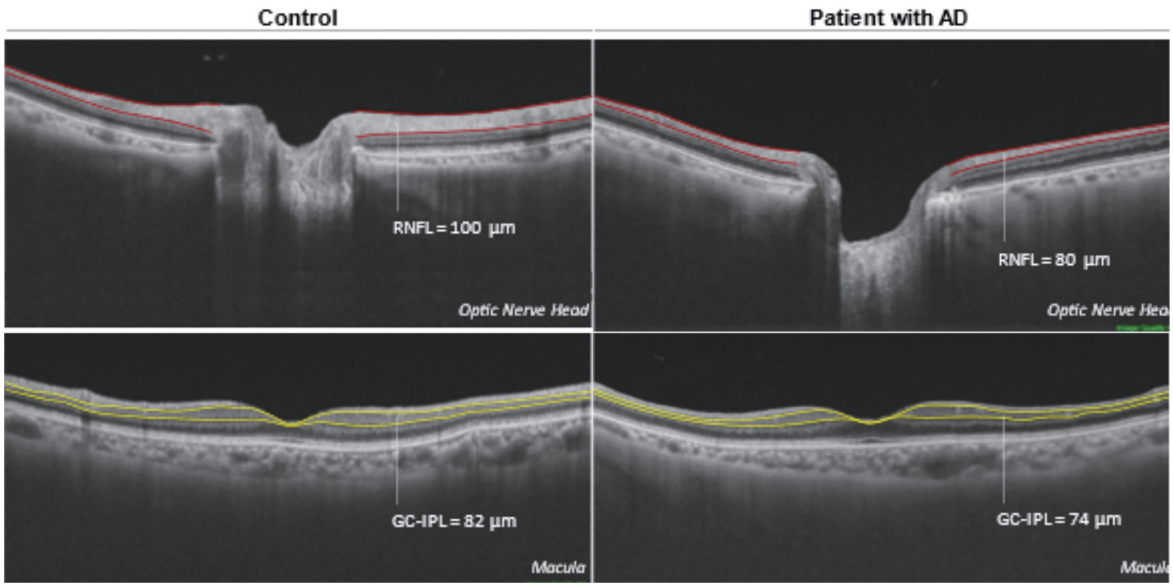

FIG 2. Spectral-domain optical coherence tomography: built-in CIRRUS analysis software provides automated measurements of macular ganglion-cell inner plexiform layer (GC-IPL) thickness and retinal nerve fibre layer (RNFL) thickness for assessment of retinal neuronal and axonal loss among healthy controls and patients with Alzheimer disease (AD).

patients with AD. During subsequent follow-up, diagnoses of some participants were revised and the distribution became 71 healthy controls, 40 patients with MCI, and 52 patients with AD. After recruitment, we excluded four healthy controls, six patients with MCI, and five patients with AD. Eventually, 148 participants were included in the analyses, including 67 healthy controls, 34 patients with aMCI, and 47 patients with AD. There were no significant differences among the groups, although there was a trend of retinal neuronal layer thinning, FAZ enlargement, and skeletal density reduction among patients with aMCI and patients with AD.

In total, 61 participants underwent PET: 31 healthy controls, 15 patients with aMCI, and 15 patients with AD. The diagnosis in one patient was modified from AD to aMCI by a neurologist; six patients with aMCI were amyloid-β-negative and reclassified as healthy controls. Eventually, 51 participants were included in analysis, including 22 amyloid-β-positive participants (11 with AD, six with aMCI, and five with normal cognition) and 29 amyloid-β-negative participants. Compared with amyloid-β-negative participants, amyloid-β-positive participants had significantly thinner macular GC-IPL. Among the amyloid-β-positive participants, cognitively impaired patients had significantly larger FAZ area, smaller fractal dimension, smaller skeleton density, larger vessel diameter index, and smaller inter-capillary area, compared with cognitively normal individuals. These differences remained significant after adjusting for age and sex.

Global amyloid burden was negatively associated with macular GC-IPL thickness (r= -0.26, 95% confidence interval [CI]= -0.49 to -0.02, P=0.03) and peripapillary RNFL thickness (r= -0.26, 95% CI= -0.52 to 0.01, P=0.06) and positively associated with FAZ area (r=0.38, 95% CI=0.11-0.66, P=0.01). Macular GC-IPL thickness, peripapillary RNFL thickness, central subfield thickness, FAZ area, fractal dimension, inter-capillary area, and non-perfusion area were also associated with amyloid and/or tau burden in specific brain regions.

Regarding correlation between retinal imaging parameters and MRI brain volume, macular RNFL thickness was positively associated with occipital lobe volume (r=0.46, 95% CI=0.15-0.76, P=0.001) and negatively associated with thalamus volume (r= -0.40, 95% CI=0.72 to -0.08, P=0.02). Non-perfusion area was positively associated with cingulate volume (r=0.76, 95% CI=0.16-1.36, P=0.01) and insula volume (r=0.70, 95% CI=0.09-1.31, P=0.03). Vessel density was negatively associated with occipital lobe volume (r= -0.40, 95% CI= -0.78 to -0.01, P=0.04) and insula volume (r= -0.55, 95% CI= -0.91 to -0.19, P=0.004). Inter-capillary area was positively associated with grey matter volume (r=0.35, 95% CI=0.01-0.70, P=0.04) and insula volume (r=0.44, 95% CI=0.11-0.77, P=0.01).

In total, 115 participants were followed up for ≥12 months and were included in longitudinal analyses; 36 of those participants had undergone PET scans. Twenty-five participants displayed a reduction of ≥3 points between the baseline and

most recent MMSE scores. Univariable analysis revealed that central subfield thickness (hazard ratio=1.612, 95% CI=1.054-2.468, P=0.0278) and mean cube thickness (hazard ratio=1.454, 95% CI=1.129-1.874), P=0.0038) were associated with a reduction of ≥3 points in MMSE score over 12 months. In multivariable analysis, these associations remained after adjusting for age and sex. However, macular GC-IPL thickness, peripapillary RNFL thickness, and OCT-angiography parameters were not associated with cognitive decline.

Discussion

Our findings revealed associations of retinal microvascular and neuronal changes with amyloid/tau burden and MRI brain volume. In particular, macular GC-IPL thickness, peripapillary RNFL thickness, and FAZ area were associated with quantitative measurements of amyloid burden in the brain. Retinal ganglion cells are neurons located in the ganglion-cell layers of the retina that receive visual information from photoreceptors and then project to the brain through the optic nerve. Therefore, amyloid and tau deposition along the axonal tracts of retinal ganglion cells may cause neuronal damage and subsequent thinning of the macular GC-IPL. Additionally, morphological and functional disruptions of cerebral capillary networks have been identified as precursors to AD-related neurodegenerative changes in animal models and post-mortem studies.[5] Changes in capillary morphology have also been identified as risk factors for small vessel diseases and neurodegeneration, which are associated with cognitive decline.[5] These findings may explain why an enlarged FAZ area, which indicates changes in capillary morphology, is associated with amyloid burden in the brain.

Our work demonstrates that retinal imaging has significant potential to identify AD-related retinal features, thereby facilitating the stratification of AD risk. Retinal imaging is non-invasive, easy to perform, and widely accessible and enables broader initial screening; patients with retinal changes can subsequently undergo more expensive brain imaging to diagnose specific subtypes of dementia. Importantly, retinal imaging enables the investigation of cerebral microvascular processes that currently cannot be discerned via MRI; these processes can be used to assess the pattern and extent of upstream brain pathology leading to cognitive decline and dementia.

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Disclosure

The results of this research have been previously published in:

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