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Recent advancements toward non-invasive imaging of retinal amyloid-beta for early detection of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive impairment suggested to be induced by the accumulation of amyloid-β (Aβ) in the brain, especially in the hippocampus. Cerebral Aβ deposits may be detected through positron emission tomography (PET) as early as two decades before clinically diagnosed AD-associated dementia, which provides the opportunity for early therapeutic interventions (Wang and Mao, 2021). PET may not be suitable for AD screening since it is invasive, costly, and inaccessible for routine clinical use or population screening. Aβ deposits have also been identified throughout the retina, which is a developmental outgrowth of the diencephalon and shares physiological and pathological pathways with the central nervous system (London et al., 2013). Patients with mild cognitive impairment and early AD are reported to have visual disturbances involving visual field loss with reported thinning of the retinal layers including the retinal nerve fiber layer, ganglion cell layer, and inner plexiform layer (Koronyo-Hamaoui et al., 2011; Wang and Mao, 2021). Retinal Aβ deposits have been detected prior to the manifestation of cerebral Aβ deposits in transgenic mice models of AD (Koronyo-Hamaoui et al., 2011; Habiba et al., 2021). Since the retina provides an easily accessible location for non-invasive imaging, retinal Aβ may have the potential to be a surrogate for cerebral Aβ and a biomarker for the detection of AD prior to irreversible cognitive impairment. Several techniques have been explored for imaging retinal Aβ, including the use of curcumin and hyperspectral imaging, which have been shown to differentiate AD patients and normal subjects *in vivo* (Koronyo et al., 2017; Hadoux et al., 2019). These non-invasive, imaging studies have also characterized retinal Aβ in human subjects and found correlations between retinal Aβ and cerebral manifestations including increased cerebral Aβ load and low cognitive assessment scores (Hadoux et al., 2019; Dumitrascu et al., 2020). However, further investigations with larger sample sizes and longitudinal studies are needed to determine if retinal Aβ can be applied in routine clinical settings and potentially for population-based screening.

Aβ originates from proteolytic cleavage of amyloid precursor protein, which is expressed in various tissues including in the retina (Wang and Mao, 2021). The main alloforms of Aβ identified for AD in the brain and retina are $Aβ_{42}$ and $Aβ_{40}$. These monomers can spontaneously aggregate into oligomers, which can then self-assemble into β-pleated sheets and form plaques (Naaman et al., 2020). Retinal Aβ in postmortem human tissue has been identified throughout the retinal layers both intracellularly and extracellularly. These deposits ranged from 5 µm to 20 µm with larger deposits resembling classical cerebral Ab deposits (Wang and Mao, 2021). Investigations into non-invasive imaging of retinal Aβ have focused on extrinsic fluorophore labeling or autofluorescence.

In the past decade, curcumin, a natural and safe fluorophore, has been studied to determine its utility for characterizing retinal Aβ *in vivo*. When delivered systemically, curcumin has a high affinity for Aβ oligomers, β-pleated sheets, and plaques with a higher affinity for $Aβ_{42}$ than $Aβ_{40}$ (Dumitrascu et al., 2020). Lipidation of curcumin (Longvida) allows improved bioavailability and stability during oral ingestion (Koronyo et al., 2020). In APP/PS1, a double transgenic AD mice model, increased retinal Aβ plaques are detected with curcumin in comparison to wild type mice with retinal Aβ being identified as early as 2.5 months without manifestation of cerebral Aβ plaques, which were detected at 5 months (Koronyo-Hamaoui et al., 2011). In human postmortem tissue, curcumin staining of whole-mount retinas showed increased Aβ load mostly in the inner retinal layers and periphery of the superior quadrant in both mild cognitive impairment and AD patients in comparison to normal controls (Koronyo-Hamaoui et al., 2011; Koronyo et al., 2017). For *in vivo* imaging, oral Longvida curcumin showed a 2.1-fold increase in retinal Aβ for AD patients in comparison to normal controls with retinal Aβ load also concentrated in the inner retinal layers and periphery of the superior quadrant especially surrounding the retinal vasculature (**Figure 1**; Koronyo et al., 2017). Increased Aβ load in the same region is found to correlate with decreased hippocampal volume and is associated with lower cognitive assessment scores. Similarly, subjects with mild cognitive impairment had elevated peripheral superior retinal Aβ levels in comparison to normal controls (Dumitrascu et al., 2020).

Curcumin labeling with optical imaging appears to provide an *in vivo*, non-invasive, and accessible method for characterizing and quantifying retinal Aβ, however further investigations are necessary to determine if it can be applied in large-scale, population-wide screening for early detection of AD and monitoring disease stages. Currently, it is unclear why retinal Aβ appears to concentrate in the peripheral superior quadrant of the retina. Patients with early AD have loss of visual field in the inferior quadrant, which corresponds to the superior quadrant of the retina. Thinning of the retinal nerve fiber layer, ganglion cell layer, and inner plexiform layer was also observed in the superior quadrant of AD patients (Wang and Mao, 2021). Future investigations into the underlying mechanism of AD progression may elucidate why this region of the retina is more vulnerable to AD pathology. While the measurement of curcumin labeling has been shown to have intraocular and interocular repeatability, future studies with larger sample sizes are necessary to determine if retinal Aβ distributions and manifestation are applicable and generalizable in different populations (Dumitrascu et al., 2020). Larger sample sizes with additional characterization of curcumin labeled Aβ may also help differentiate AD from other amyloidogenic diseases like glaucoma and age-related macular degeneration, which share similar presentations of Aβ in the retina (Wang and Mao, 2021). Like AD, glaucoma can present with Aβ deposits in the inner retinal layers with a similar pattern of retinal ganglion cell loss (Koronyo et al., 2017). Likewise, AD can present with Aβ in drusenoid deposits that are similar in appearance to reticular pseudodrusen

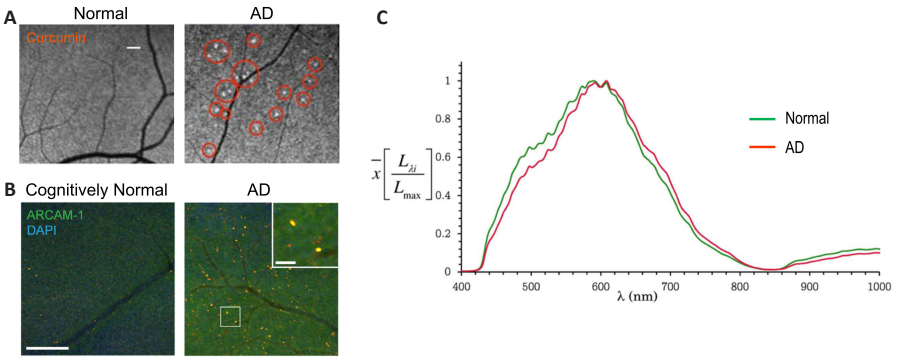

Figure 1 | **Detecting retinal amyloid-β (Aβ) in Alzheimer's disease (AD) using curcumin, amyloid-targeting fluorescent probe (ARCAM-1), and hyperspectral imaging (HSI) in humans.**
(A) *In vivo* imaging of oral Longvida curcumin fluorescence in the superior temporal retinal region of a normal subject and AD patient. Curcumin positive spots representing Aβ circled in red. Scale bar: 400 µm. Reproduced from Koronyo et al. (2017), under the open-access terms of the Creative Commons Attribution License (CC-BY). (B) ARCAM-1 staining for Aβ in whole-mount retina of cognitively normal human and AD patient. Counterstained with DAPI. Scale bar (full): 300 µm. Scale bar (magnified): 50 µm. Reproduced from Cao et al. (2021), under open-access terms of the Creative Commons Attribution-Non-Commercial-No Derivatives 4.0 International License. (C) HSI spectra differences are most prominent between 450 and 580 nm when comparing retinal tissue of normal control and AD patient. Differences proposed to be due to increased Aβ load in AD tissue. Reproduced from More and Vince (2015), under open access terms of the ACS AuthorChoice License.

of age-related macular degeneration, which may also contain Aβ (Wang and Mao, 2021). Additional studies with increased sample sizes and longitudinal follow-up may help determine a threshold for curcumin labeled retinal Aβ load that can help predict the development of AD-associated disease progression with associated cognitive decline and cerebral atrophy.

Other extrinsic probes for labeling retinal Aβ have also been explored for accessible and non-invasive screening for AD including Aβ specific camelid-derived antibody fragments (nanobodies), amyloid-targeting fluorescent probe (ARCAM-1), and CRANAD-X probes for near-infrared fluorescence imaging (Yang et al., 2019; Cao et al., 2021; Habiba et al., 2021). All of these alternative probes have been able to differentiate APP/PS1 mice from wild type mice. ARCAM-1 and near-infrared fluorescence imaging were conducted *in vivo*. Like curcumin fluorescence imaging, Aβ specific nanobodies have also shown that Aβ oligomers, concentrated in the inner retinal layers, were detected in the retina of 3-month-old APP/PS1 mice with cerebral Aβ not detected until 8 months (Habiba et al., 2021). Furthermore, in comparison to cognitively normal controls, ARCAM-1 has shown increased retinal Aβ load in postmortem retinal tissue of AD patients with increased accumulation of Aβ in the superior quadrant of the retina (**Figure 1**; Cao et al., 2021). Additional research into these probes is needed to determine if they can be safely and effectively used *in vivo* for human subjects. Currently, curcumin imaging requires a 4-day loading protocol through oral ingestion and has moderate affinity to proteins other than Aβ like hyperphosphorylated Tau, which has also been detected and observed to have a neurodegenerative effect in the retinas of AD patients (Mutsuga et al., 2012; Dumitrascu et al., 2020; Wang and Mao, 2021). With further development, these alternative fluorescent probes may have the possibility of providing a shorter loading time, potentially through intravascular injection rather than oral ingestion, and be more specific for Aβ.

Recent advances have also been made in detection of retinal Aβ without an extrinsic fluorescent probe by using hyperspectral imaging (HSI). For HSI, a series of image frames are collected across the retina and across many wavelengths of light within a given range, which combines into a data cube that contains both spectral and spatial information (Hadoux et al., 2019). This data cube provides a spectral signature representing the scattering of light in the overall imaged area, which can be utilized to non-invasively analyze the structural and morphological properties of the retina (More and Vince, 2015). As shown in APP/PS1 mice tissue, Aβ, especially $A\beta_{42}$ oligomers, have been shown to have a distinctive, measurable effect on the spectral signature that affects the HSI spectra in proportion to the Aβ load. With increased Aβ load, the total scattering of light in the tissue was decreased directly due to Aβ aggregation or due to its subsequent neurotoxic effects (More and Vince, 2015). In APP/PS1 mice, these Aβ associated changes in HSI signatures indicated increased retinal Aβ load, which occurred prior to measurable cognitive decline and distinguished these transgenic mice from wild type mice. In comparison to normal retinal tissue, Aβ had a similar effect on the spectral signature of postmortem retinal tissue from AD patients (**Figure 1**; More and Vince, 2015). For *in vivo* imaging in human subjects, the HSI data cube needs to be further processed due to high ocular variability between subjects (Hadoux et al., 2019). Components of the eye with a known effect on the spectral signature like the lens, macular pigment, and hemoglobin were corrected for the raw HSI data. The resulting retinal spectral signature indicated that the main difference between normal controls and patients with high cerebral Aβ load (as seen on PET imaging), was the presence of retinal Aβ in the high cerebral Aβ cases (Hadoux et al., 2019). The regions of the retina with the most discrimination between high cerebral Aβ load cases and controls were predictively in the superior quadrant of the retina, but also in the fovea. However, it is unclear if the difference in HSI spectra between the high cerebral load cases and controls were solely due to retinal Aβ rather than a combination of other AD-associated retinal changes like hyperphosphorylated tau and inflammation (Hadoux et al., 2019). Additional studies with larger sample sizes that further characterize the effects of different AD-associated mechanisms on the HSI spectra of the eye are necessary to better understand the utility of this method for detecting retinal Aβ and potentially AD *in vivo*.

Despite advances in the understanding of AD, a method that is suitable for population-wide screening of AD is still under development. Since retinal Aβ load elevations have been observed prior to irreversible cerebral neurodegeneration and cognitive decline, numerous studies have explored non-invasive imaging of these Aβ aggregations for detecting AD. Methods for detecting Aβ retinal include extrinsic fluorophore labeling, which has been shown to be successful in allowing visualization of these deposits. However, only curcumin, which requires a lengthy oral loading period, has proven to be applicable for living human subjects. While HSI imaging without an extrinsic fluorophore cannot allow visualization of retinal Aβ deposits, it has also been successful in detecting the presence of increased retinal Aβ load *in vivo* for human subjects. Nevertheless, further exploration is warranted to better characterize the manifestation of retinal Aβ and validate these methods in larger, more heterogeneous populations prior to application in clinical settings.

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