Hyperspectral analysis of amyloid beta (Aβ) evolutional changes in preclinical to late-stage Alzheimer's disease using matched brain and retinal tissue

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Background

- Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by memory impairment and abnormal behavior[1].
- Aβ is a biomarker of AD in humans and accumulates in the brains of AD mouse models. Amyloid beta has also been shown to instigate several features of pathologic AD[2,3].
- The aim of this study was to elucidate the neuropathologic sequence and corresponding [RetiSpec] hyperspectral imaging (HSI) signals of Aβ evolutional changes in preclinical to late-stage AD using both retinal and brain samples.
- A pathological Aβ protein aggregation is hypothesized to progress as described in figure 1

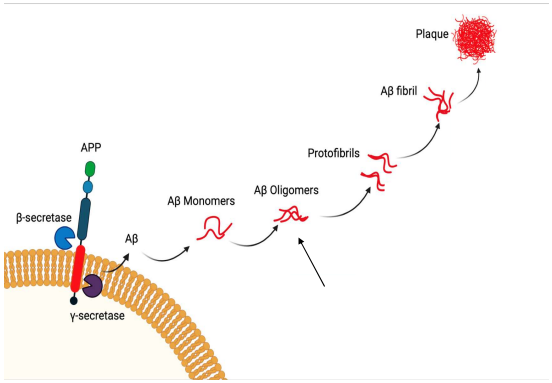

Figure 1: Amyloid-beta pathological aggregation progression[3]

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Methods

- Immunohistochemistry was performed on human retinal samples (n=15) obtained from the Baltimore Longitudinal Study of Aging (BLSA) and Johns Hopkins University Alzheimer's Disease Center. These cases encompass the entire spectrum of AD as indicated by Thal phase, in addition to CERAD and BRAAK stages **(Table 1)**.

- Each retinal 10-micron section is analyzed in 6 anatomic retinal territories including: temporal central, temporal peripheral, temporal extreme periphery, nasal central, nasal peripheral and nasal extreme periphery. Five regions of interest (ROIs), each containing ≥150 pixels, were scanned using Cytoviva Hyperspectral Imaging microscope as described previously (5). The spectral information was extracted using ENVI, version 5.5 (Exelis, McLean, VA). Correlation between the degree of AD pathology and HSI signal (ΔOD 460-480 nm) was determined using Graphpad Prism 9.0.

- We used humanized, affinity-matured, IgG2 mAb selective biotinylated antibody (ACU-193) to detect soluble Aβ oligomers.

n = 15	Clinical	Thal	Braak	CERAD-NP
4	Likely control	2/3	2/3	Mod/Freq
2	Dementia	5	6	Freq
1	Dementia	3	0	Sparse
1	Dementia	1	2	Sparse
7	Dementia	3/4/5	4/5/6	Mod/Freq

Table 1: Clinical Classification of Cases

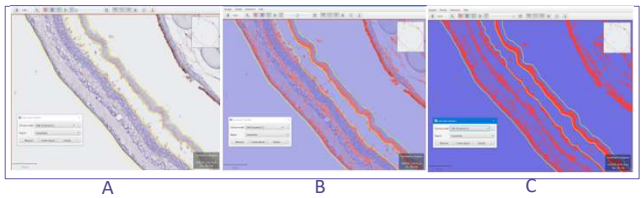

Figure 2: QuPath Threshold Module
Computer generated threshold of retinal tissue to depict how the percentage of positive tissue was calculated on the whole-slide image. These images are taken from QuPath and illustrate the same portion of the retina but having the thresholder at 100% transparency and decreasing to better show what cells were marked as positive.

Results

- When assessing the distribution of AβO's present from 15 retinal tissue samples, we observed 29-53% retinal tissue area positivity in controls with lower levels of pathologic AD and 15-72% retinal tissue area positivity in the AD group.
- Level of AD pathology demonstrated an effect on retinal HSI (rHSI) spectra (450nm-600nm) in regions near the optic disc and ventral periphery.
- Intermediate AD pathology showed the strongest rHSI signature followed by high, then low AD pathology samples.
- Correlation of the Braak neurofibrillary tangle stage and ΔOD offered a Pearson r score of 0.79 (p = 0.004).

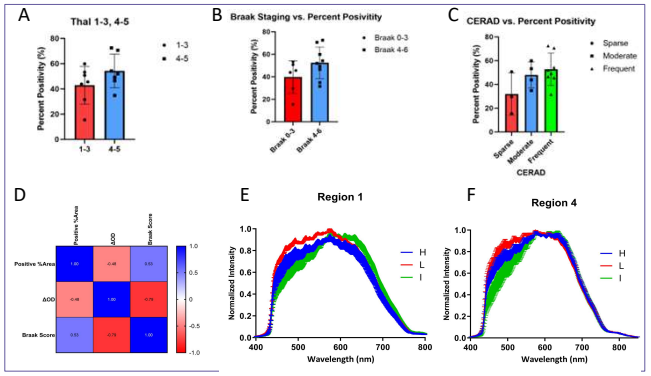

Figure 3: Amyloid-beta oligomer percent positivity and rHSI signal demonstrate statistically significant relationship with brain pathology

Conclusion

- First study to assess and quantify AβOs in clinically, neuropathologically, and hyperspectrally characterized postmortem matched brain and retinal tissues
- Progression of well-established neuropathologically classified AD stages (e.g Braak) may correlate with retinal oligomeric Aβ levels measured using rHSI.
- Certain retinal regions that share nervous tissue with brain are more likely sensitive to rHSI-mediated detection of AD pathology
- Larger samples needed to comprehensively assess and quantify AβOs
- This study provides evidence that may inform future clinical contexts of use for [RetiSpec] rHSI as a potential diagnostic, prognostic, and disease monitoring tool that may augment the current clinical gold-standard of Aβ-PET

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