Clinical and Postmortem Analysis of Retinal Tissue

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The authors of this abstract have indicated that they have no conflicts of interest that relate to the content of this abstract.

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Background

Introduction:

- Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by memory impairment and abnormal behavior[1].
- Aβ is a biomarker of (AD) in human 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 is to quantify oligomeric Aβ in human retinal tissue, a region described to contain aggregated Aβ in AD progression **(Figure 1).**
- While there are ongoing clinical trials of antibodies against oligomeric Aβ, our study is the first that assesses and quantifies AβO's in clinically and neuropathologically characterized postmortem retinal tissue.

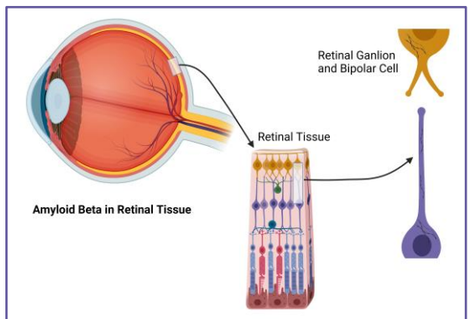

Figure 1: Oligomeric Aβ Hindering Synapse Propagation
- Possible oligomerization of Amyloid beta interfering with retinal ganglion and bipolar neuron connections

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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 **(Figure 2)**.
- We used humanized, affinity-matured, IgG2 mAb selective biotinylated antibody (ACU-193) to detect soluble Aβ oligomers. ACU-193 antibody was shared by Dr. Klein's laboratory. The antibody was optimized using 1:3000 for 15 minutes in formic acid and biotinylated.

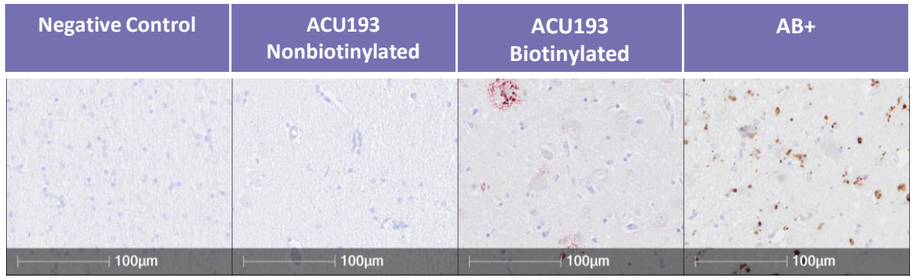

Figure 2: Optimization of ACU193 biotinylated antibody on case A17-111

- QuPath, a quantitative pathology, and bioimage program was used to detect AβO immunoreactive retinal tissue in digitized whole slide images. Once percentage of positive tissue area was measured, one-way ANOVA and t-tests were performed to compare AβO's in controls and different stages of AD.

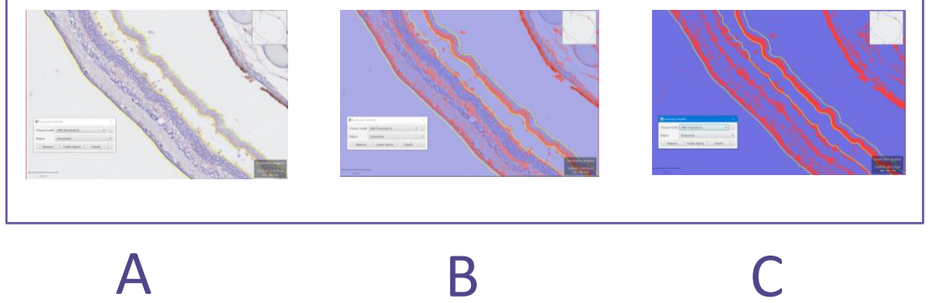

Figure #3: QuPath Threshold Module
Computer generated threshold of retinal tissue to depict how the percentage of positive tissue was calculated on the whole-slide image. These images are taken from QuPath and illustrate the same portion of the retina but having the thresholder at 100% transparency and 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.
- However, statistically significant differences were not observed when comparing different forms of AD
- CERAD Power Calculation: Sparse/Mod: 44/44; Sparse/Freq: 25/25; Mod/Freq: 80/80

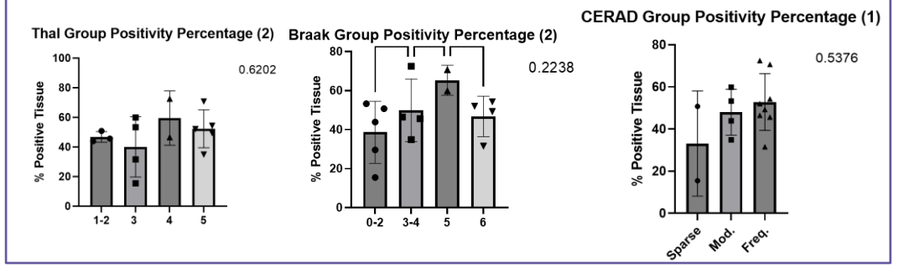

Figure 4: Percent Positivity by Clinical Classification

Case Nbs	Clinical (cntl, MCI, AD)	Thal	Braak	CERAD
4	Likely control	2/3	2/3	Mod/Freq
2	AD	5	6	Freq
1	"Slight AD", Vascular Dementia	3	0	Sparse
1	"Early Dementia", CVA	1	2	Sparse
7	Dementia	3/4/5	4/5/6	Mod/Freq

Figure 5: Clinical Classification of Cases;
Cntl: Control, MCI: Mild Cognitive Impairment, AD: Alzheimer's Disease

Conclusions

- The retinal ACU193 immunohistochemical percent positivity observations in different stages of AD cases highlight the presence of AβO's in the post-mortem human retinal in healthy controls and in AD progression.
- The progression of well-established neuropathologically classified AD stages (e.g Braak) may correlate with retinal oligomeric Aβ levels.
- Larger sample numbers are necessary to comprehensively assess and quantify retinal oligomeric Aβ. Such studies will further explore AβO's potential as a biomarker in AD.
- This project highlights the possibility and potential for advancing the analysis of other retinal proteins

Reference

(1) "Alzheimer's Disease." Johns Hopkins Medicine, https://www.hopkinsmedicine.org/health/conditions-and-diseases/alzheimers-disease.
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(4) Figure 1 created using BioRender.com

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