



Retinal glial changes in Alzheimer's disease: differential expression of glial markers in human post-mortem tissue

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Introduction

- The neuropathological hallmarks of Alzheimer's disease (AD), including amyloid beta and phosphorylated tau deposition, have been observed in the post-mortem human retina¹.
- This positions the retina as a potential source of non-invasive biomarkers for neurodegenerative disorders. In the brain, protein accumulation is associated with glial activation and neuronal loss².
- Therefore, we assessed features of glial activation in the retina of AD patients and non-demented controls as potential future biomarkers.

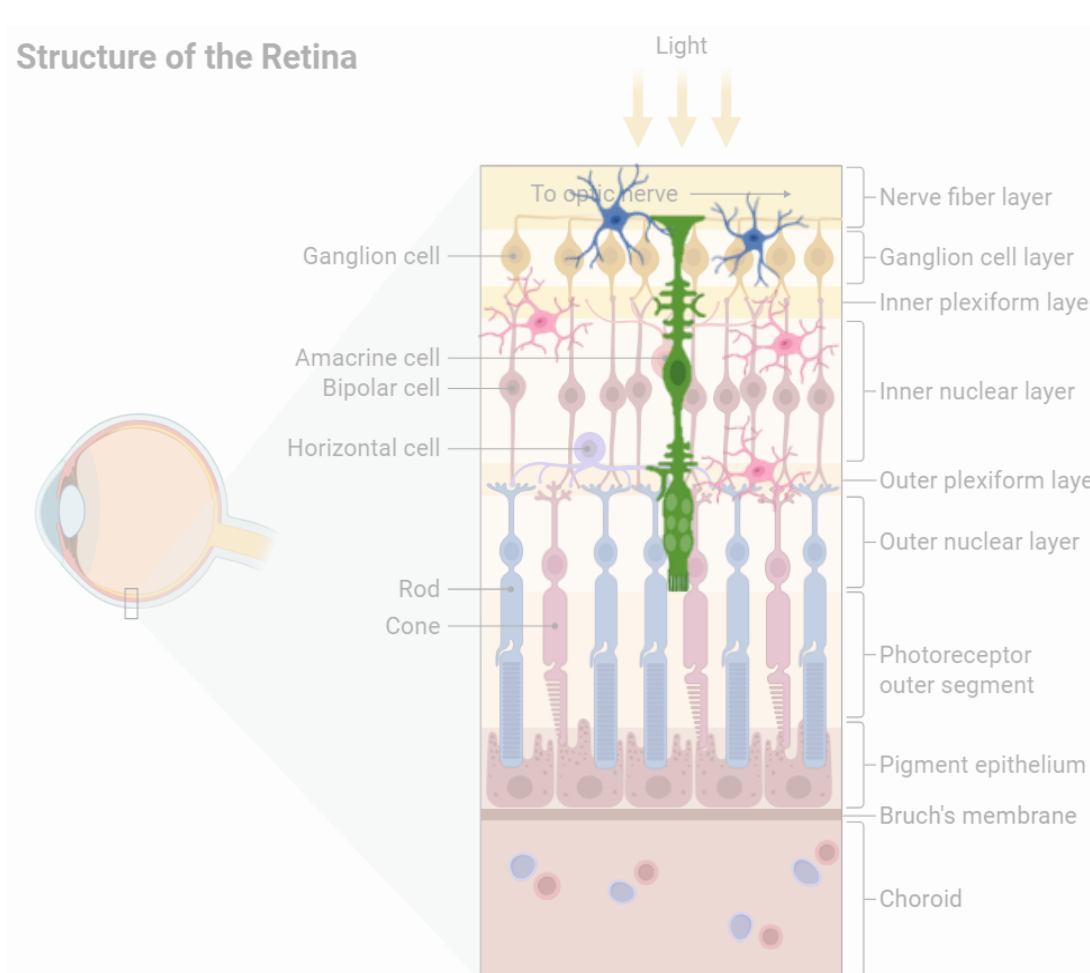
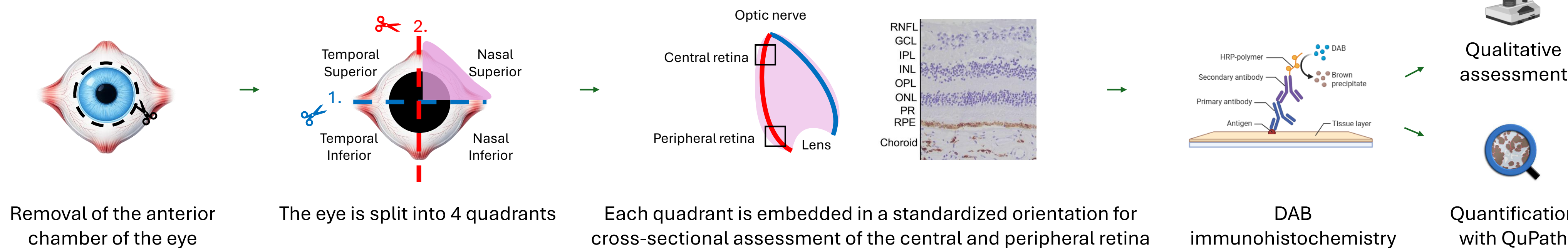


Figure 1. Illustration of the different cell types and cellular organization of the retina. A Müller glial cell spanning across all retinal layers is illustrated in green. *Adjusted image from BioRender.*

Methods

Eyes were obtained through the Netherlands Brain Bank. The neuropathological diagnosis was made following the NIA-AA criteria. The cohort included AD donors (n=11) and non-demented controls (n=13). Glial expression was assessed using DAB immunohistochemistry on 10 µm-thick formalin-fixed paraffin-embedded (FFPE) tissue sections of the superior quadrant of the retina, with primary antibodies targeting glial fibrillary acidic protein (GFAP), glutamine synthetase (GS), CD117, CD68. Stained sections were qualitatively analyzed, and protein expression was quantified in the peripheral and central retina using QuPath.

Immunohistochemistry workflow



Results

Table 1. Post-mortem cohort				Amyloid pathology	Tau pathology	Neuritic plaques
Group	N	Mean Age (SD)	Female/Total	ABC score (A0, A1, A2, A3)	ABC score (B0, B1, B2, B3)	ABC score (C0, C1, C2, C3)
AD	11	74.3 (10.0)	6/11	(0,0,0,11)	(0,0,3,8)	(0,1,3,7)
Control	13	82.5 (13.8)	9/13	(4,5,4,0)	(1,8,4,0)	(9,2,2,0)

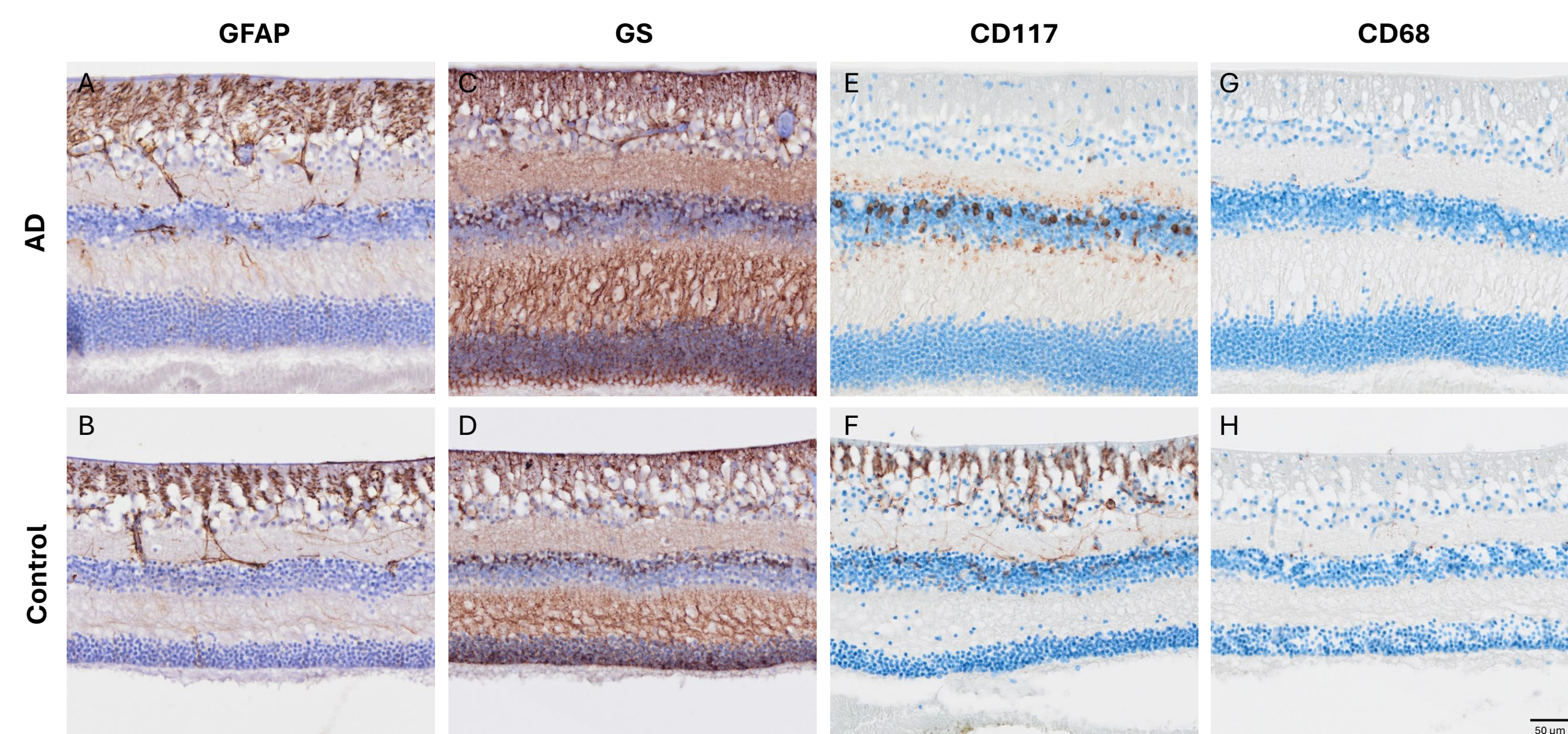
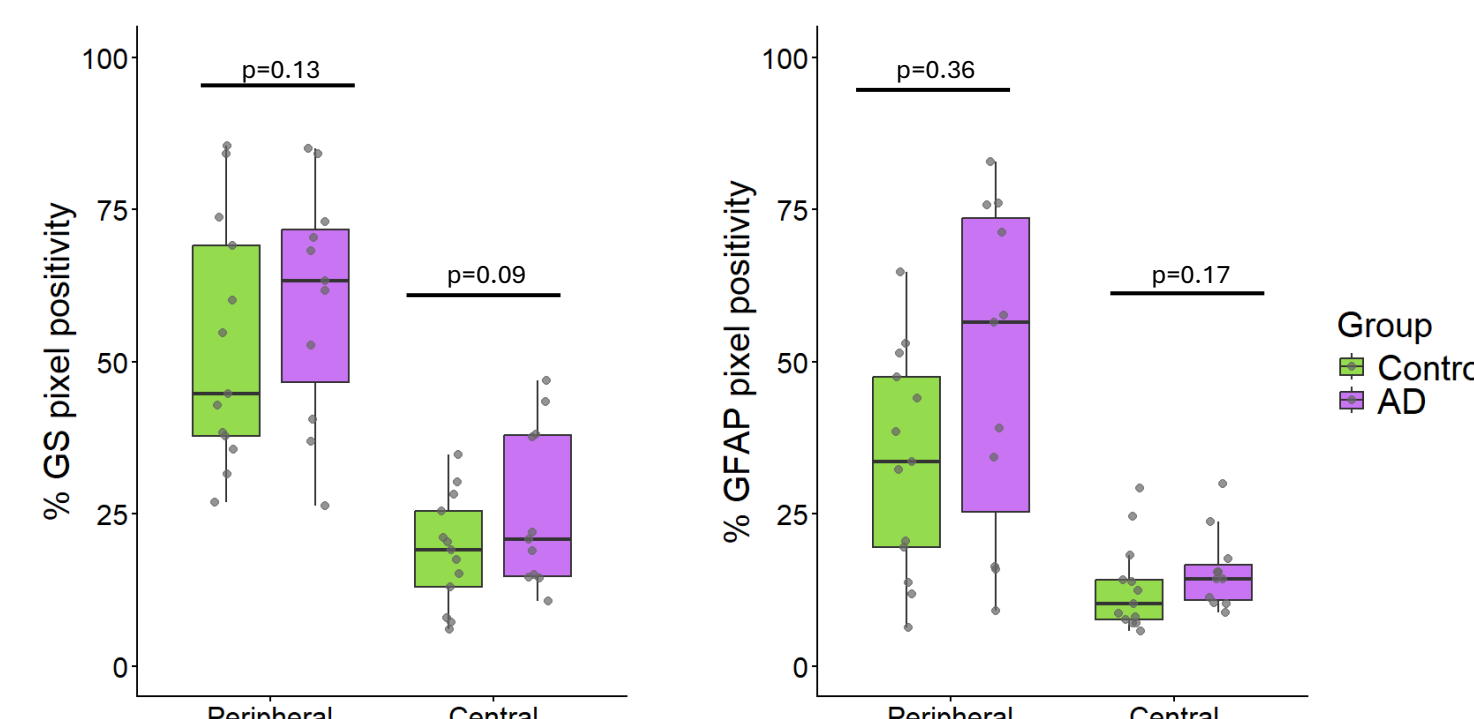
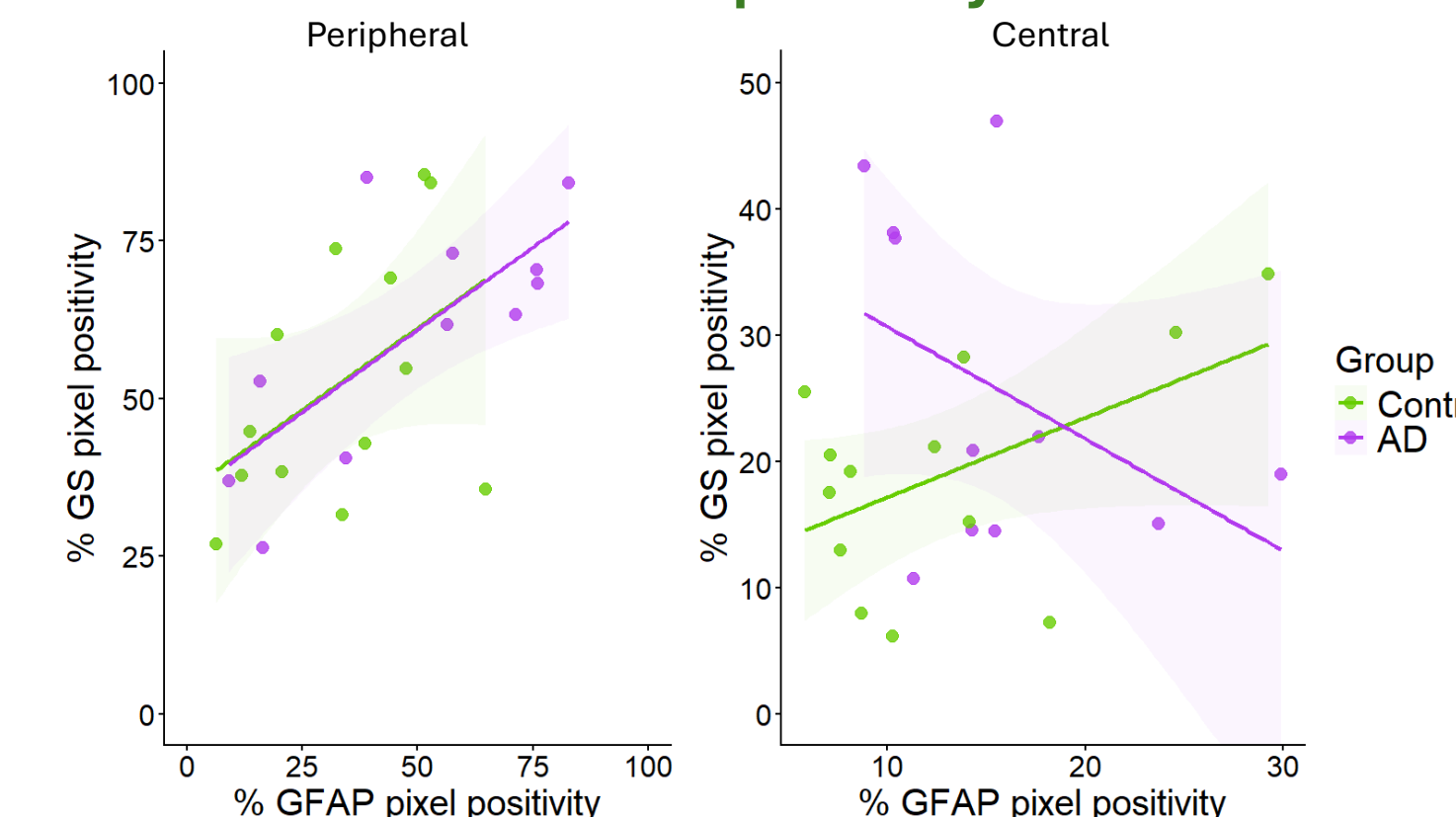


Figure 2. Immunohistochemistry in human post-mortem central retina. (A-B) GFAP immunostaining in AD and control retina shows mainly positivity in RNFL layer. (C-D) GS immunostaining in AD and control. (E-F) CD117 immunostaining in AD and control in which RNFL positivity is lost in the RNFL of AD retina (E). (G-H) CD68 immunostaining in AD and control.

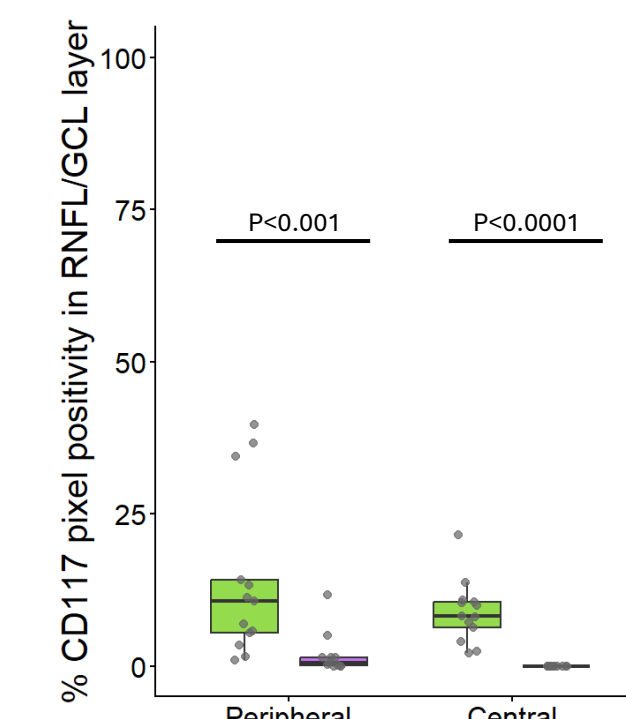
%GFAP and %GS immunopositivity in the retina are higher in AD, although not significantly



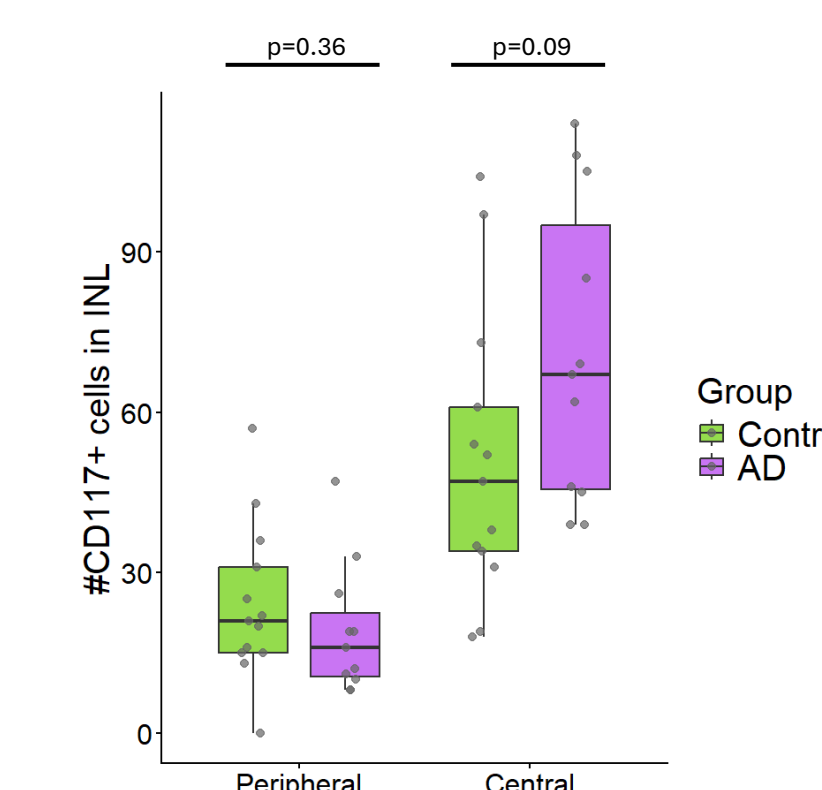
The central retina in AD shows an inverse association between GFAP and GS immunopositivity



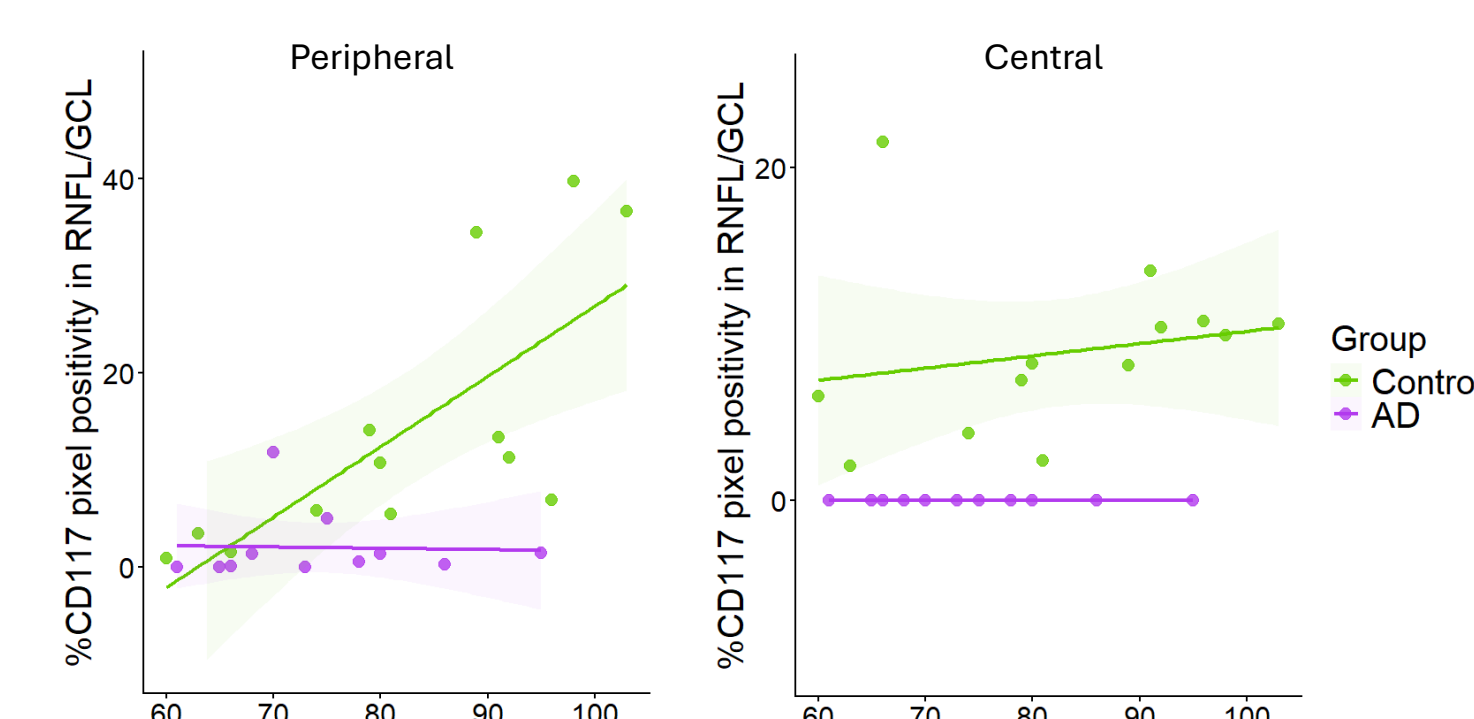
%CD117 immunopositivity is lower in the RNFL/GCL layer of the AD retina



Trend towards decreased peripheral and increased central INL CD117+ cell counts in AD retina



Loss of CD117 immunopositivity in AD is independent of age



CD117+ cells show limited overlap with calretinin+ and GS+ cells in control retina

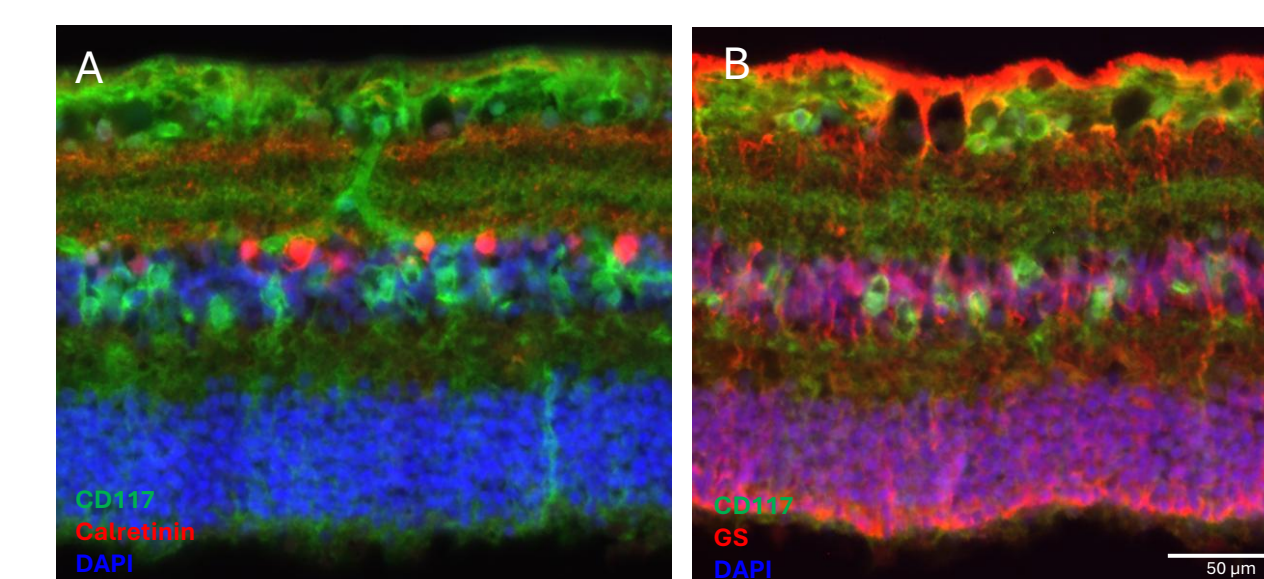


Figure 3. Immunofluorescence in human post-mortem retina. (A) CD117 (green), calretinin (red), DAPI (blue) immunofluorescence staining in control retina. (B) CD117 (green), GS (red), DAPI (blue) staining in control retina.

Conclusions

- In both AD and control donors, the glial reactive response is region-dependent.
- CD117-positive cells in the central INL layer are higher in AD retina compared to control.
- Loss of CD117-immunopositive cells in the RNFL/GCL layer of AD retina may suggest functional asthenia and represent a disease-associated retinal change.
- Astroglia in the central AD retina show an inverse association of GFAP and GS expression compared to controls, suggesting that astroglia are reactive, but cannot counteract excitotoxicity.
- No differences in CD68 expression were found between AD and control donors, implying a lack of phagocytosis in AD retinal microglia.

Future directions

- Assess additional glial markers, and their spatial distribution using retinal flatmount and punches.
- Expand the donor cohort to stratify groups based on clinical features including disease duration, AD subtypes, APOE status, ophthalmological comorbidities.