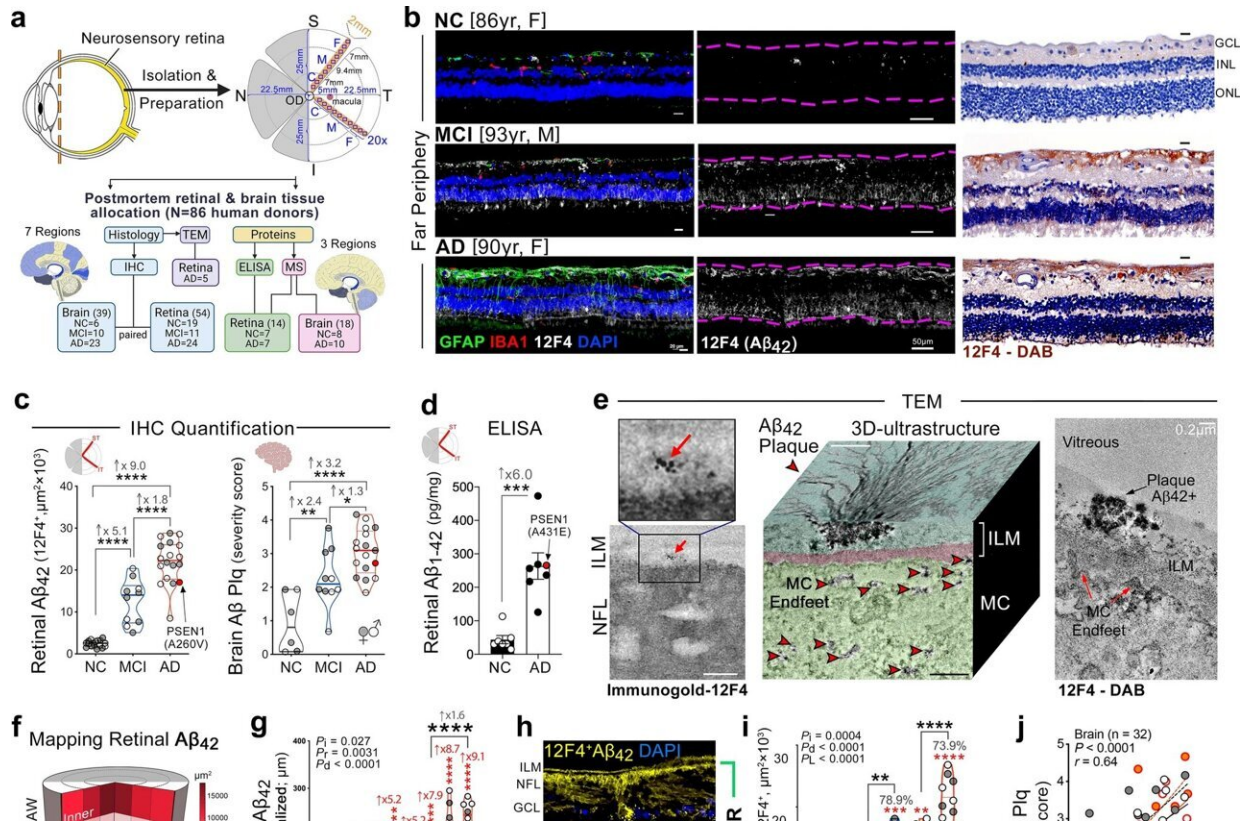# New insights into eye damage in Alzheimer's disease patients

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Spatiotemporal distribution of $A\beta_{42}$ burden in retinas of MCI and AD patients and relations to brain pathology and cognition. **a** Illustration depicts analyzed retinal cross-sections in predefined geometrical regions including superior- and inferior temporal (ST/IT) strips (orange) extending from the optic disc (OD) to the ora serrata and separated into subregions: central (C), mid-periphery (M) and far periphery (F). Schematic flow-diagram describes human donor eyes and brains allocated for histological and protein analyses (N = subjects). **b** Fluorescence micrographs of retinal cross-sections from MCI and AD patients

compared to normal cognition (NC) controls. Tissues were immunolabeled for GFAP$^+$-macroglia (green), IBA1$^+$-microglia (red), 12F4$^+$-Aβ_{42} (white), and DAPI$^+$-nuclei (blue; dashed lines indicate margins of analyzed layers between the inner and outer limiting membranes–ILM/OLM). Scale bar: 50 μm. Right micrographs are from the same individuals immunolabeled with 12F4$^+$-Aβ_{42} using peroxidase-based 3,3′diaminobenzidine (DAB) and hematoxylin counterstaining. Scale bar: 20 μm. **c** Violin plots display quantitative-IHC analysis of retinal (r)Aβ_{42}-immunoreactive area in age- and sex-matched patients with premortem clinical diagnoses of NC ($n = 17$), MCI ($n = 10$), or AD ($n = 18$), and paired-brain (b)Aβ-plaque severity scores in NC ($n = 6$), MCI ($n = 10$), and AD ($n = 17$) patients. Red circle represents an ADAD patient with an A260V mutation in presenilin-1 (*PSEN1*). **d** Retinal Aβ_{1-42} levels determined by ELISA are shown in an additional cohort of NC and AD patients ($n = 14$; ADAD patient with *PSEN1*-A431E mutation, red circle). **e** TEM-micrographs from AD patients' retina: Left, 12F4$^+$-immunogold Aβ_{42}-positive black puncta signals at high-magnification (red arrow) in the ILM/innermost layers. Scale bar: 200 nm. Middle: 3D-reconstruction of vertical/en face TEM images show rAβ_{42} plaque ultrastructure with fibril arms emanating from its dense core and Aβ-containing deposits (red arrowheads). Scale bar: 1 μm. Right, Aβ_{42} plaque (black arrow) and deposits within Müller cell (MC) endfeet (red arrows). Scale bar: 0.2 μm. **f** Pie charts display Aβ_{42} distribution across the inner retina (IR), outer retina (OR), and C, M, and F subregions: raw data and normalized per retinal thickness (density); higher burden in darker red. **g** Violin plot displays rAβ_{42} density for C, M, and F subregions. **h** Definition of inner retina (IR) and outer retina (OR) in a cross-section. Scale bar: 10 μm. **i** Aβ_{42} burden in IR vs. OR; percentages indicate rAβ_{42} area in IR of total area. Statistics: red or blue asterisks mark significance relative to NC or MCI, respectively. P_d–diagnostic groups; P_r–C, M, vs. F subregions; P_L–IR vs. OR layers; P_i–interactions. **j** Scatterplot presents correlations between rAβ_{42} area and Aβ plaques in total brain (gray) or EC (orange). **k–l** Mid-sagittal brain illustration and heatmap show color-grading magnitude of Pearson's correlation coefficient (r) values with multivariable Holm-Bonferroni adjusted *P*- values (asterisks) between rAβ_{42} burden and brain pathology: Aβ-(P)laques, neuropil threads (NT), and neurofibrillary tangles (NFT) in the hippocampus (Hipp), superior (S.) frontal (F. Ctx) and temporal (temp, T. Ctx) gyrus, S. parietal lobule (P. Ctx), entorhinal (EC), primary visual (PV), and visual association (VA) cortices. **m** Pearson's correlation between

rAβ^{42} burden and BRAAK stage. **n** Subjects were stratified based on high(H) or low(L) brain ATN-histopathology severity and plotted based on rAβ_{42} burden; extrapolated dotted-gray line marks rAβ_{42} level separating ATN^H from ATN^L individuals. **o** Pearson's correlations between rAβ_{42} area or bAβ burden and the Mini-Mental State Examination (MMSE)-cognitive scores. Data points are presented with group means ± SEMs. Filled and empty circles represent women and men, respectively. Median and lower and upper quartiles are indicated on each violin plot. *P