

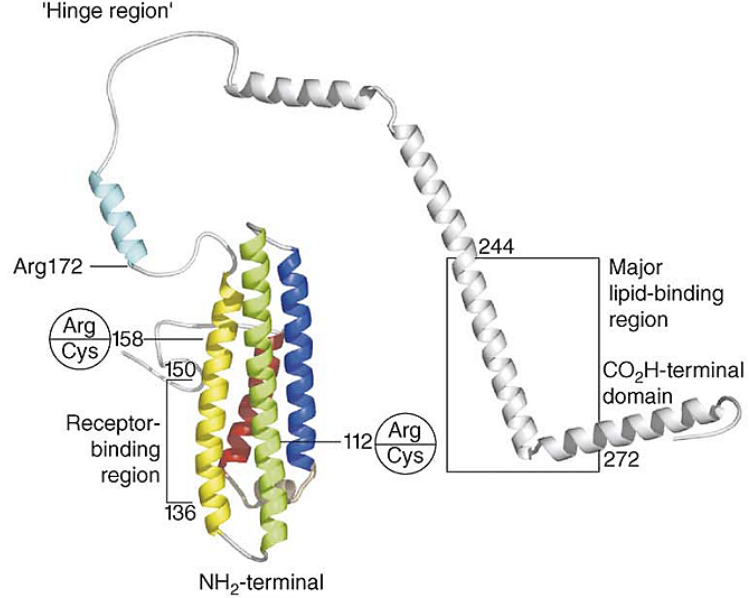
Differential Glycosylation of Apolipoprotein E in Alzheimer's Disease as a Molecular Signature

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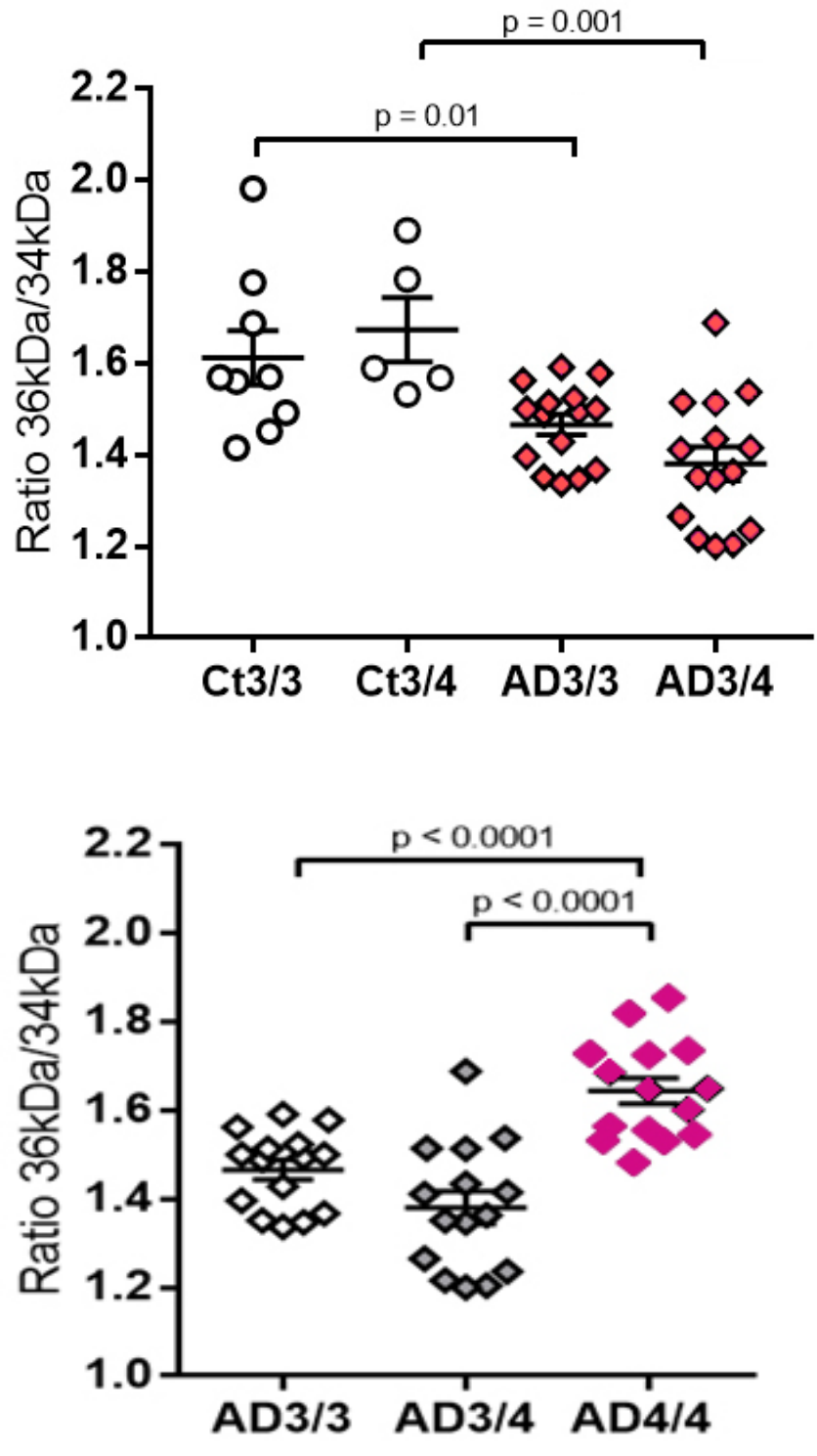
Introduction and Background

Apolipoprotein E (ApoE) is responsible for cholesterol transport and neuronal repair in the brain. There are 3 *APOE* alleles ($\epsilon 2$, $\epsilon 3$ y $\epsilon 4$) that drastically alter the risk of developing late-onset Alzheimer's disease: while *APOE* $\epsilon 2$ is protective and *APOE* $\epsilon 3$ is neutral, *APOE* $\epsilon 4$ represents a genetic risk factor. Under reducing conditions, human CNS ApoE resolves into two distinct **O-glycoforms** of approximately **36 kDa** and **34 kDa**, which can be capped with one or two sialic acid residues. Alterations in glycosylation have been documented in AD. **Aberrant glycan processing can lead to altered protein stability and dysfunctional cell signaling.**



• **ApoE2:** 112 = Cys; 158 = Cys.
• **ApoE3:** 112 = Cys; 158 = Arg.
• **ApoE4:** 112 = Arg; 158 = Arg

Building on this characterization, we previously demonstrated (Lennox MP et al. Alzheimer's Res Ther. 2022) that the CSF 36-kDa/34-kDa ratio decreases in AD relative to controls in *APOE* $\epsilon 3/\epsilon 3$ and $\epsilon 3/\epsilon 4$ carriers. Conversely, AD patients with *APOE* $\epsilon 4/\epsilon 4$ genotype exhibit a ratio closer to control levels rather than to other AD variants, highlighting a genotype-specific regulation of these glycoforms.



Aim

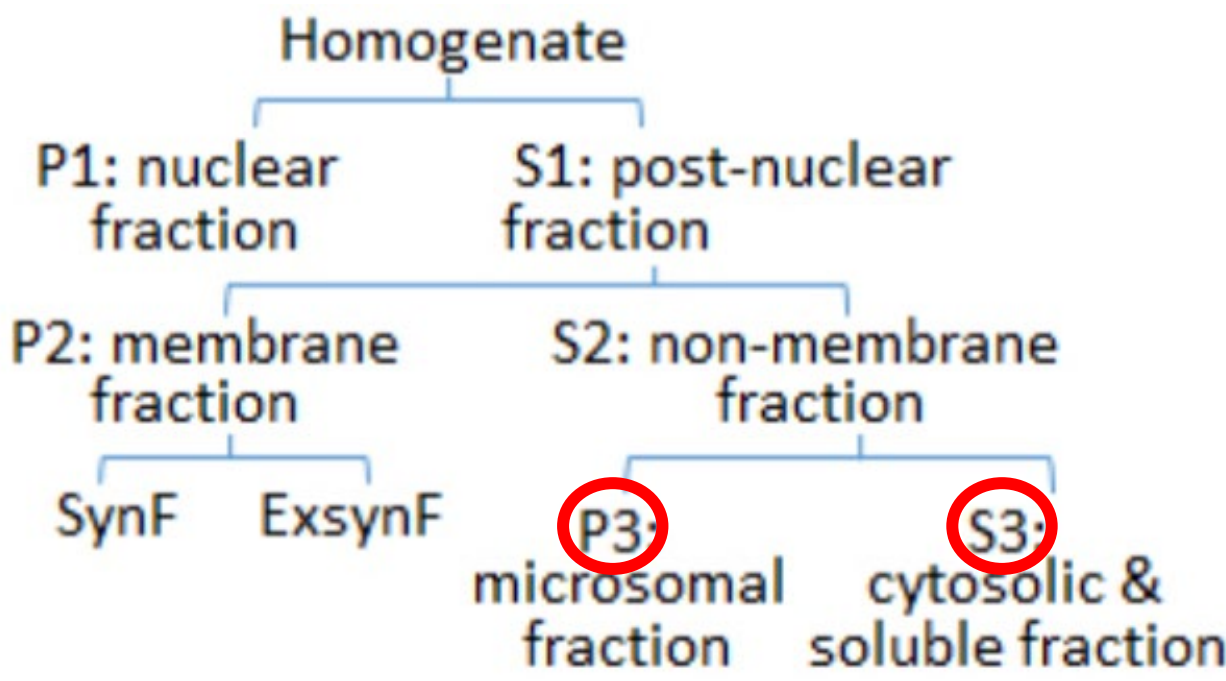
We aim to analyze the apoE glycosylation in the frontal cortex from AD and control individuals to identify any *APOE* genotype-related alteration in its glycosylation.

Materials and Methods

1. Cortex samples and homogenization

- **Human cortex** (~100 mg) .
→ Frontal Cortex: Control n= 23; AD (Braak I-II): n=6 (Braak III-IV): n= 8 (Braak V-VI): n = 18
- **Homogenization (10% w/v):** Extraction buffer (50 mM Tris-HCl pH, 150 mM NaCl, 5 mM EDTA, 0.5% Triton X-100, 1% NP-40, 0.5 mM PMS and protease inhibitors).
- **Lysate Processing:** Sonication followed by ultracentrifugation at 100,000xg 1h 4°C.

2. Fractionation Protocol

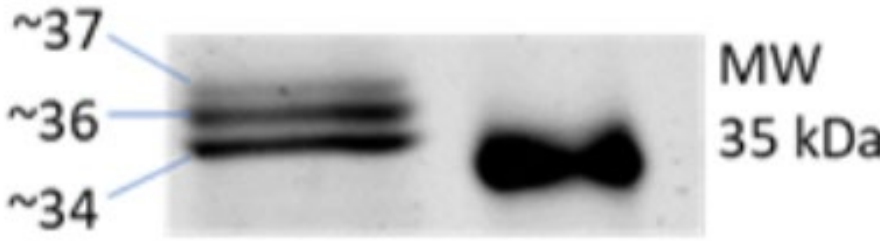


3. LECTIN BINDING ASSAY:

LECTINS	Specificity
	Monosaccharide/Oligosaccharide
<i>Maackia Amurensis</i> Lectin II (<i>MAL II</i>)	Sialic acid in an (α -2,3) linkage
<i>Jacalin</i>	O-glycosidically linked oligosaccharides, preferring the structure galactosyl (β -1,3) N-acetylgalactosamine

1. Cortex homogenates

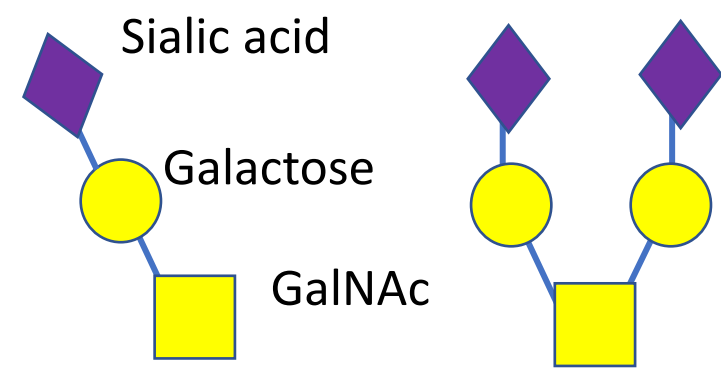
Human brain contains **two major ApoE glycoforms**



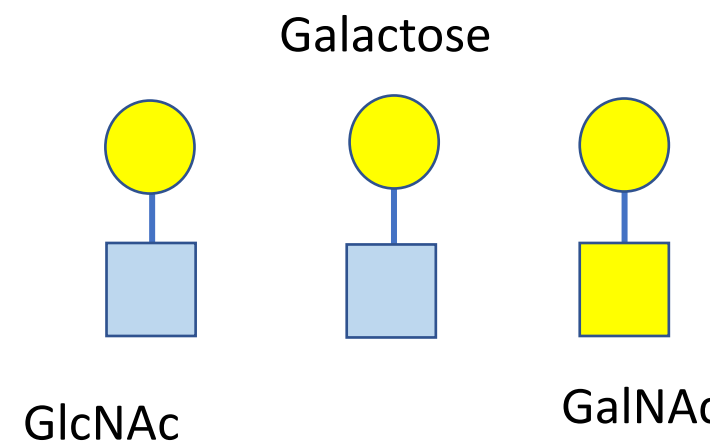
2. Fractionation protocol



MAAL-II (sialic acid)



Jacalin (O-glycans)

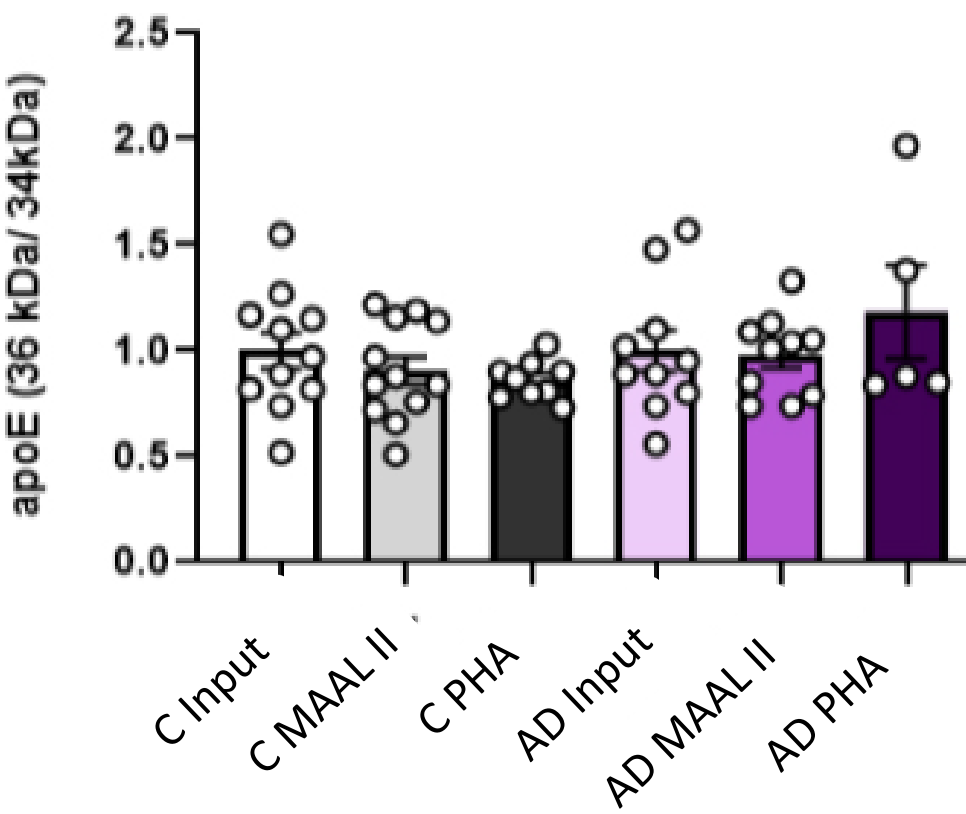
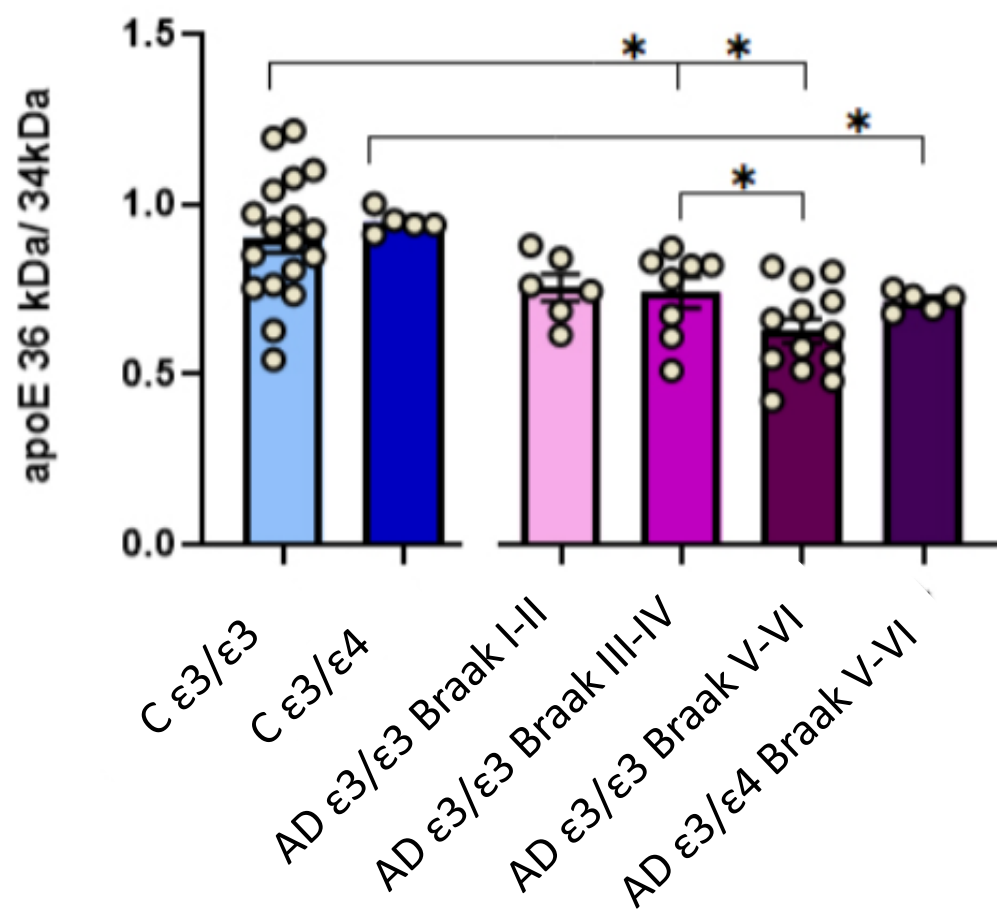


RESULTS

36/34kDa ApoE ratio in Cx Homogenates

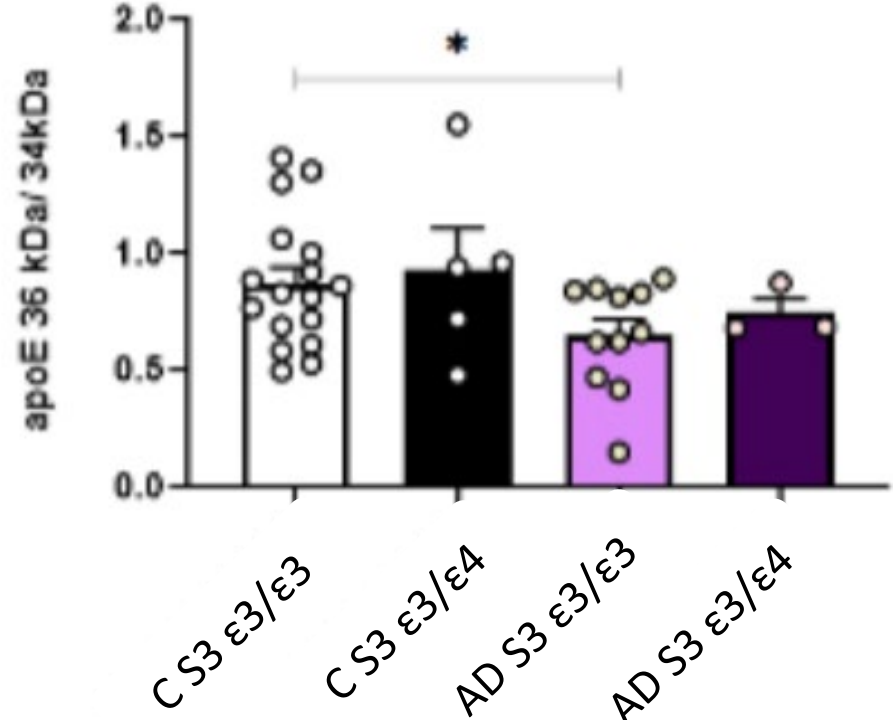
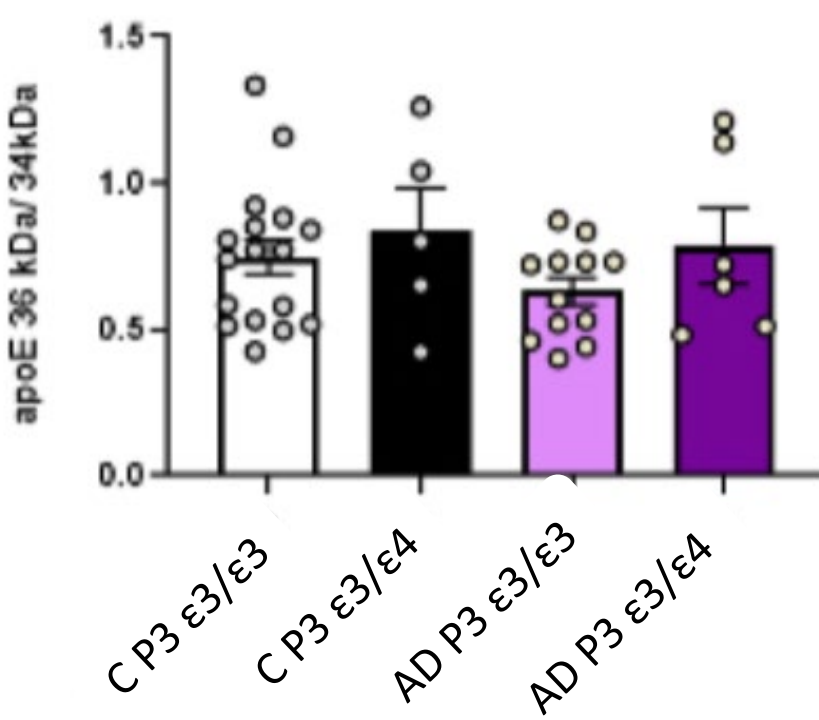
ApoE Ratio is lower in AD

No glycosylation differences between C and AD



36/34kDa ApoE ratio in Golgi Fraction

36/34kDa ApoE ratio in Secreted fraction



Similar ApoE ratio in Golgi

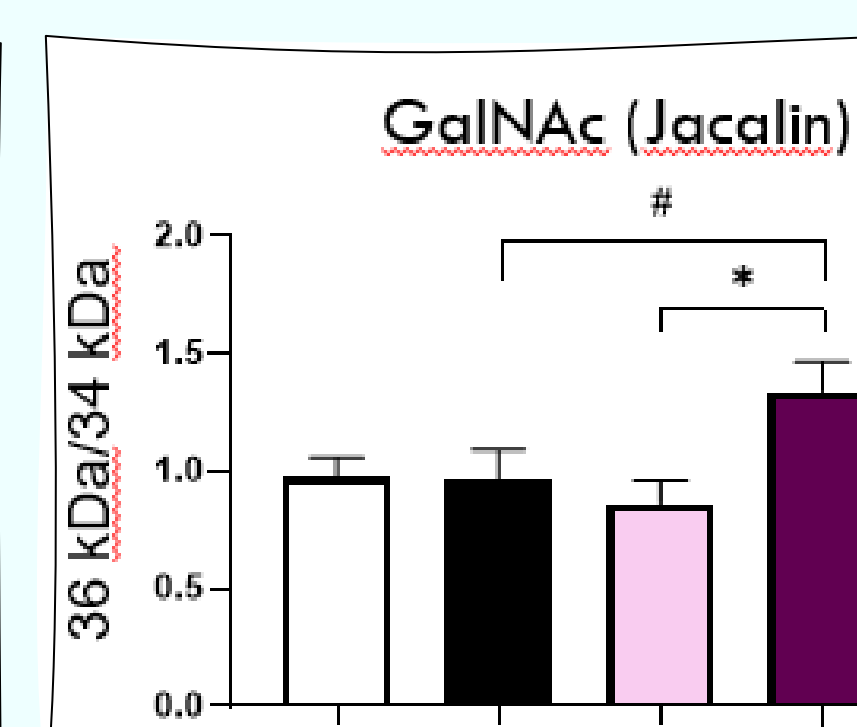
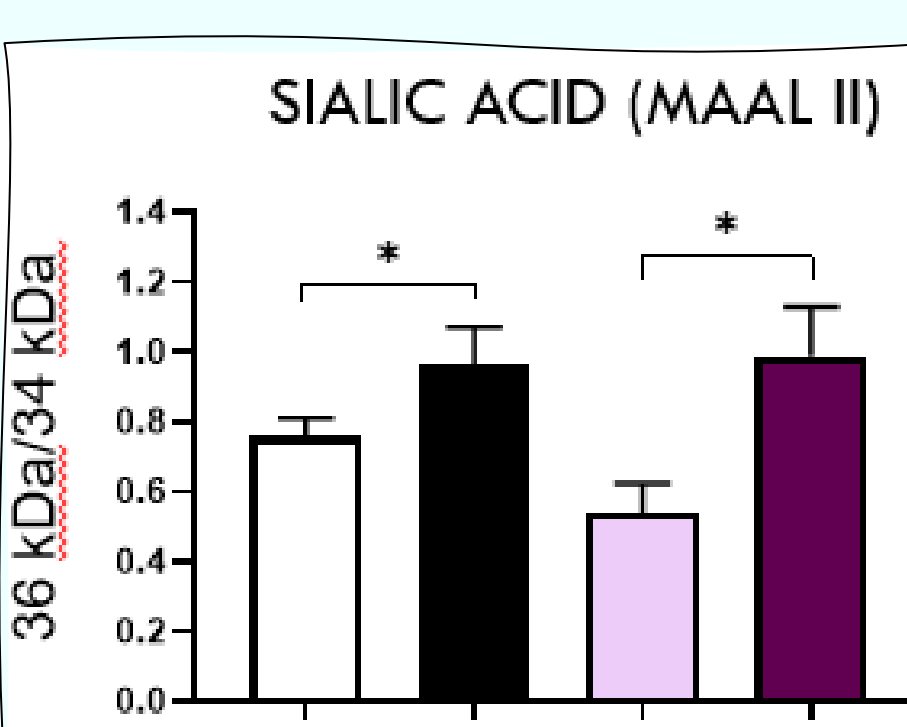
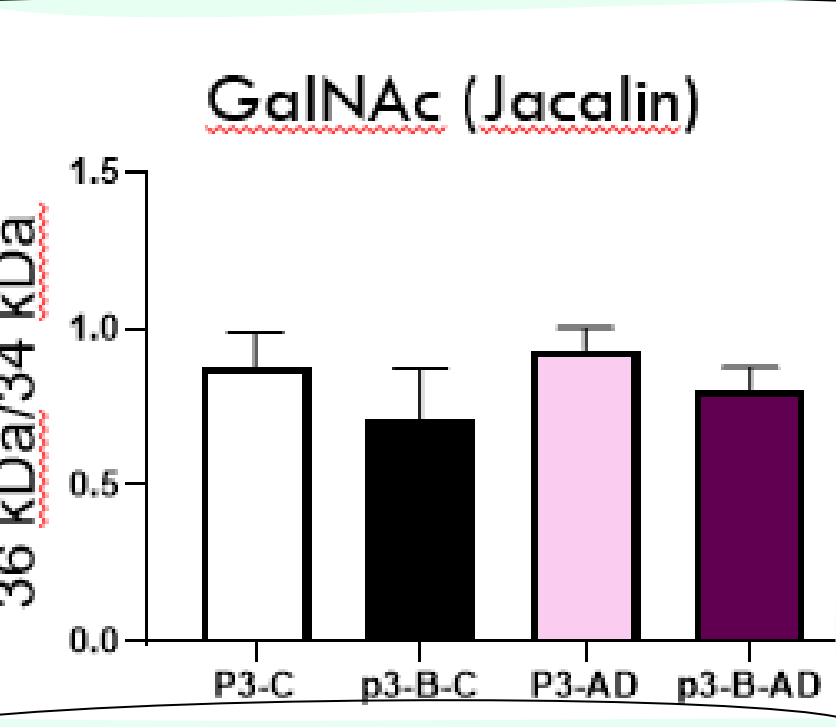
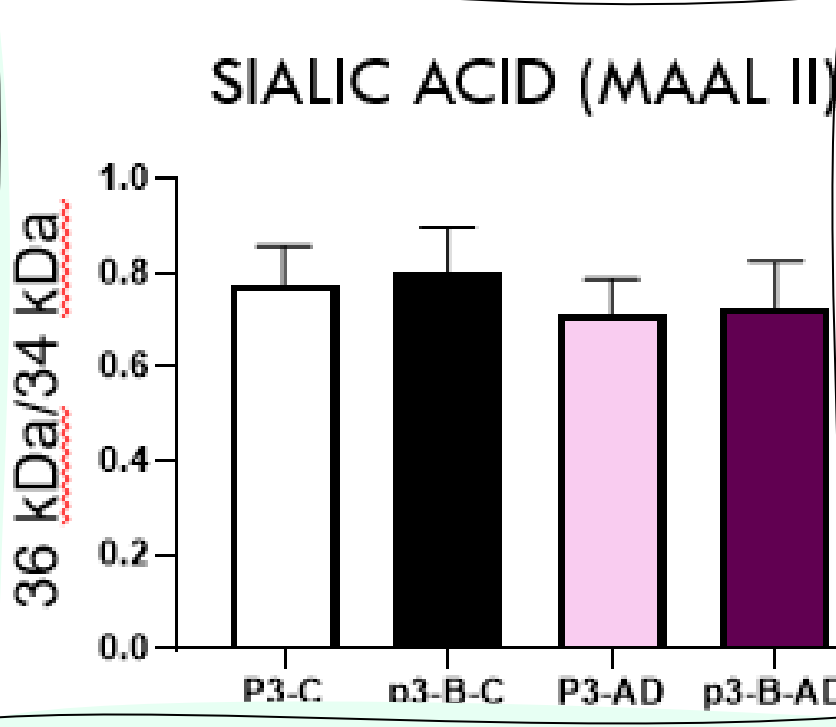
34kDa ApoE is more secreted in AD

Golgi Fraction

No differences in ApoE glycosylation in Golgi

Secreted ApoE

36kDa ApoE secreted is more glycosylated in AD



DISCUSSION

1. In the AD cortex, there is a different presence of ApoE glycoforms compared to controls, being 34kDa ApoE more abundant in AD.
2. The secreted 36 kDa glycoform in AD is more O-glycosylated and sialylated than the 34kDa compared to control.
3. In AD, the 34 kDa glycoform is more secreted than in controls, which have a similar secretion of 36kDa and 34kDa.
4. Pathological glycosylation of ApoE can contribute to disease progression.

TAKE-HOME MESSAGE

- **ApoE Shift:** The 34 kDa glycoform becomes the predominant ApoE species in the AD cortex.
- **Invariant Golgi Pool:** Intracellular ApoE glycosylation within the Golgi apparatus remains unaltered in AD.
- **Secreted Glycan Dysregulation:** Secreted ApoE in AD is mostly composed of the 34 kDa form, which is less glycosylated and sialylated. This loss likely impairs ApoE function, potentially contributing to the disease.