APOE glycosylation in ADNI Methods

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# Introduction

We reported that APOE4 is less glycosylated in CSF but not in plasma(Hu et al., 2020) and CSF APOE glycosylation is positively correlated with CSF Abeta 42 levels(Meuret et al., 2023). Whether APOE4 glycosylation is associated with longitudenal cognitive change is not known.

# Summary (or Abstract)

We obtained 188 plasma and 188 CSF samples from ADNI, to apply our unique apoE mass spectrometric immunoassay. The assay simultaneously detects the three apoE isoforms and their glycoforms in a manner that preserves isoform- specific glycosylation. Each sample was analyzed only once.

**Samples**

Total of 188 plasma and 188 CSF samples were received from ADNI cohots and analyzed. 8 groups of samples were selected based on whether performance of delayed recall test was stable or decline in cognitively normal (CN) or cognitively impaired (LMCI) individuals and were stratified by APOE into APOE4 carriers and non-carriers. Out of the 188 plasma and 188 CSF samples, three (3)  plasma and seven (7) CSF samples failed the analyses, and are labeled as NAs. Thus, there is data for 185 PLASMA and 181 CSF samples.

# Methodology

Polyclonal goat anti-human antibody to apoE (Cat. No. 50A-G1) was obtained from Academy Biomedical (Houston, TX). Mass Spectrometric Immunoassay (MSIA)-Tips (991CUS01) were acquired from Thermo Fisher Scientific (Waltham, MA). Activation and derivatization of the microcolumns inside the MSIA Tips were performed on a Multimek 96 automated 96-channels pipettor (Beckman Coulter, Brea, CA). The MSIA Tips were first rinsed with 200 mM HCl (10 aspiration/dispense cycles, 100 μL each), followed by water (10 cycles) and acetone (10 cycles). Then, the microcolumns inside the tips were activated through Carbonyldiimidazole (CDI) rinses (100 mg/mL CDI dissolved in N-methylpyrrolidinone (NMP), 1000 cycles, 50 μL each), followed by two rinses with NMP (10 cycles each, 100 μL). The tips were immediately immersed into the wells of a 96-microwell microplate containing 5 μg apoE antibody/well (in 100 μL of 10 mM MES buffer), and 1000 cycles (50 μL each) were performed, allowing for antibody attachment to the activated microcolumns. This was followed by one rinse with ethanolamine (ETA) and two rinses with PBS (50 cycles each, 100 μL). Total time taken for activation and derivatization of 96 MSIA Tips was 1.5 h. The antibody-derivatized tips were stored at 4°C.

Immediately prior to performing the apoE assay, 40 μL of plasma were mixed with 110 μL of PBS, 0.1% Tween (PBST); 200 μL of CSF were mixed with 200 μL PBST. Higher volumes of CSF were needed because apoE concentrations in CSF are approximately ten-times lower compared to plasma. The antibody-derivatized tips were mounted onto the head of the Multimek 96 pipettor and first rinsed with PBST (10 cycles, 100 μL). The tips were then immersed into the wells of a microplate containing the analytical samples (150 μL of plasma mixture and 400 μL of CSF mixture, per well), and 500 cycles (100 μL each) were performed, allowing for affinity capture of apoE from the samples. Then, one rinse with PBST (100 cycles, 100 μL) and two rinses with water (10 cycles each, 100 μL) followed, to wash off the non-specifically bound proteins from the microcolumns. To elute the captured apoE, 5 μL of MALDI matrix (20 g/L sinapic acid in 33% (v/v) ACN and 0.4% (v/v) TFA) were aspirated into each tip, pushed up and down ten times, and then dispensed directly onto a 96-well formatted MALDI target. Sample spots were dried on a hot plate at 50°C.

Bruker’s Autoflex III MALDI-TOF instrument (Bruker, Billerica, MA) was utilized to acquire linear mass spectra. The instrument was operated in positive ion mode, and mass spectra were acquired in the mass range from 7 to 70 kDa, with a 700 ns delay, 20.00 kV and 18.45 kV ion source voltages, with signal suppression of up to 7,000 Da. A total of 10,000 laser-shots were acquired and summed for each mass spectrum.

The mass spectra were externally calibrated using human hemoglobin peaks ([M+2H]2 +, [M+H]+, and [2M+H]+). The spectra were baseline subtracted (Convex Hull algorithm, 0.8 flatness) and smoothed (Savizky Golay algorithm, 5 m/z width, and 1 cycle) using Flex Analysis software (Bruker Daltonics). The peak intensities of all isoforms and glycoforms were measured using Zebra 1.0 software (Intrinsic Bioprobes Inc.) and tabulated in a spreadsheet. For samples from heterozygous individuals, peak intensities were measured separately for the two isoforms and their corresponding glycoforms in each mass spectrum. The intensities of all apoE peaks (unglycosylated protein and all glycoforms) were separately summed for each isoform. To obtain the percent abundance, the peak intensity of each apoE isoform-specific signal was divided by the summed intensity of all peaks for that isoform. To compare the expression levels of the three different isoforms in heterozygous individuals (*ɛ*2/*ɛ*3, *ɛ*2/*ɛ*4, *ɛ*3/*ɛ*4), the percent abundances of the unglycosylated and glycosylated apoE were summed for each isoform, and then compared by dividing one with another (e.g., apoE4 divided by apoE2 for the *ɛ*2/*ɛ*4 phenotype). To get the total glycosylation level of each isoform, the percent abundance of all glycoforms for a specific isoform was summed and then divided by the total percent abundance for that isoform. Similarly, to obtain the secondary glycosylation abundance of each isoform in CSF, percent abundances of the secondary glycosylation glycoforms were summed and divided by the total percent abundance for that isoform.

# References­

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