Liquid Chromatography-Mass Spectrometry (LC-MS): an innovative analytical approach to investigate the role of ApoE isoforms in Alzheimer's disease pathogenesis

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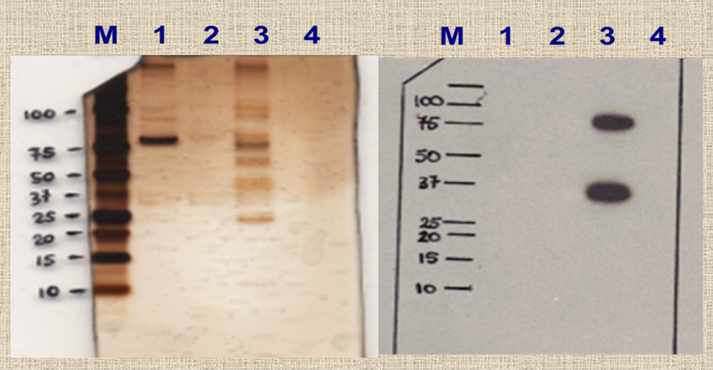
Introduction

Apolipoprotein E (ApoE) is an important triglyceride-cholesterol transporter and exists in three isoforms (ApoE2, ApoE3 and ApoE4). The ApoEε4 allele is the major genetic risk factor for Alzheimer's disease (AD) [1]. ApoE is also considered to be involved in clearance of amyloid-β (Aβ) peptides, that are known to aggregate and accumulate in senile plaques. We hypothesize Aβ accumulation in AD to result from reduced clearance capacity.

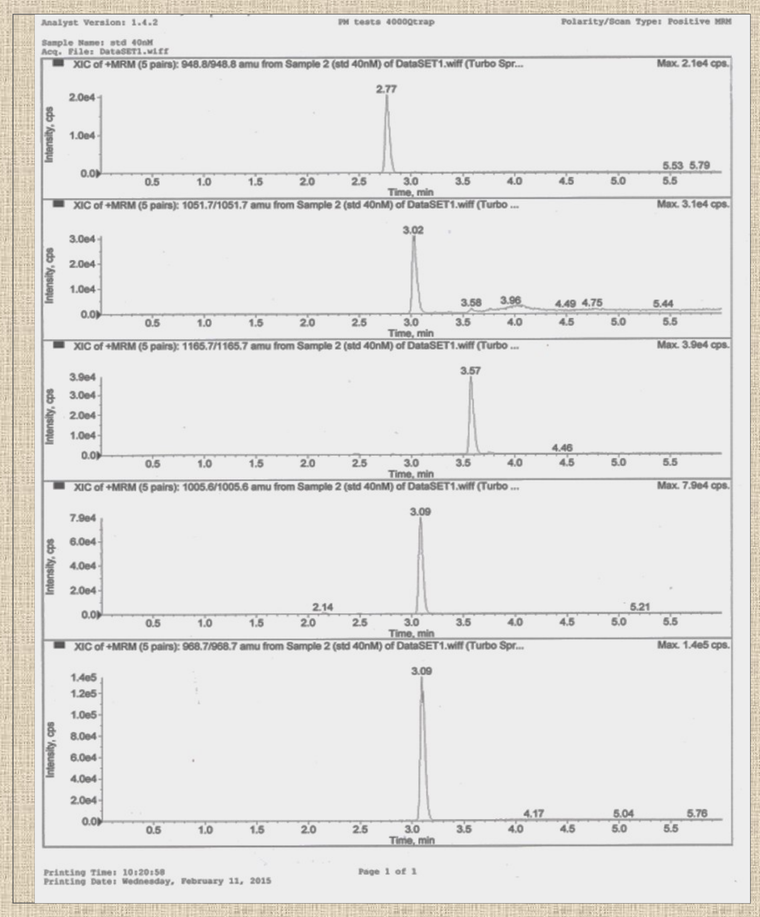
The aim of the project is to develop a reliable LC-MS/MS method to determine the amounts of the three ApoE isotypes in body fluids (plasma and CSF) [1]. Data on absolute levels of isotypes in homozygous and in heterozygous individuals may help us to better understand the contribution of ApoE genotype to the onset, progression and clinical features of AD.

Material and Methods

ApoE isoforms were purified by affinity chromatography from human body fluids (CSF and plasma) from genotyped ApoE homozygous individuals. Purity of the ApoE was checked by SDS-PAGE and Western blot analysis, while the concentration was measured by Enzyme-Linked ImmunoSorbent Assay (ELISA). The purified ApoE2, ApoE3 and ApoE4 were digested by trypsin [2,3], generating peptides that were analyzed by Liquid Chromatography-Mass Spectrometry (LC-MS/MS Applied Biosystems/MDS SCIEX 4000 Q TRAP). We used aliquots of ApoE peptides for initial experiments and to prepare calibration curves and "heavy peptides" with isotope valine (VAL-OH-13C5,15N) as internal standards (peptides prepared by Dr H. Hilkmann, NKI, Amsterdam) [1].



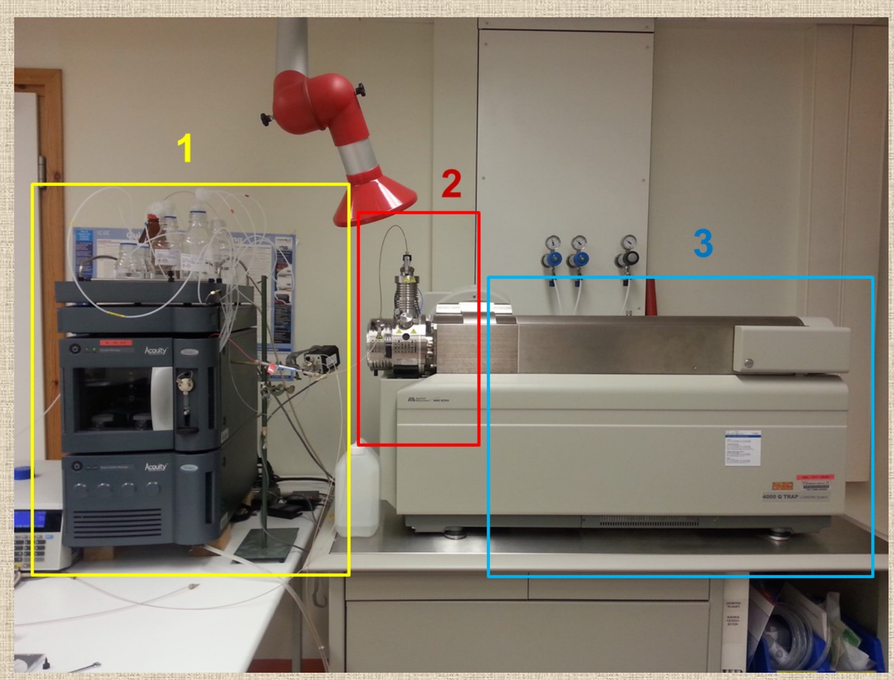
Quality of purified ApoE was checked by SDS-PAGE (left) and Western blot analysis (right). M: marker; 1: plasma (not digested); 2: plasma (digested); 3: ApoE3 enriched sample (not digested) in form of monomers and dimers; 4: ApoE3 enriched sample (digested).



Standard ApoE light peptides were analized as reference by LC-MS. The concentration used was 40 nM. Standard peptides are specific for the 3 ApoE isoforms. A: ApoE3 and 4; B: ApoE2; C: ApoE2 and 3; D: ApoE4; E: ApoE2, 3 and 4.

Results

We used light peptides specific for ApoE isoforms as standard to test the sensitivity of the machine and to see how peaks of interest looked like [1]. Then we tested ApoE enriched samples and plasma/CSF. The purified and digested ApoE2,3 and 4 samples gave peaks with expected retention times. Next, serial dilutions of these peptides, with a fixed amount of "heavy peptides" added as internal control, were shown to yield dose response curves.



LC-MS machine used. 1: LC part; 2: Ionization part; 3: Detector.

Conclusion

Using synthetic peptides for testing sensitivity indicated that the lowest limit of detection with our LC-MS/MS platform was 0.8 nM. Our results suggest that the method has sufficient specificity. Dilution ranges of synthetic peptides resulted in a dose response relationship that was linear over a wide range. Validation of the test is currently ongoing.

References

[1] Martìnez-Morillo et al. (2014). Assessment of Peptide Chemical Modifications on the Development of an Accurate and Precise Multiplex Selected Reaction Monitoring Assay for Apolipoprotein E Isoforms. J. Proteome Res. 2014, 13, 1077−1087.

[2] Van den Broek et al. (2013). Evaluation of Interspecimen Trypsin Digestion Efficiency Prior to Multiple Reaction Monitoring-Based Absolute Protein Quantification with Native Protein Calibrators. J. Proteome Res. 2013, 12, 5760−5774.

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