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Innovation Applied

Development of multiplexed MSIA (Mass Spectrometric Immunoassay)-SRM assays for proteins associated with Alzheimer's Disease and application to clinical samples

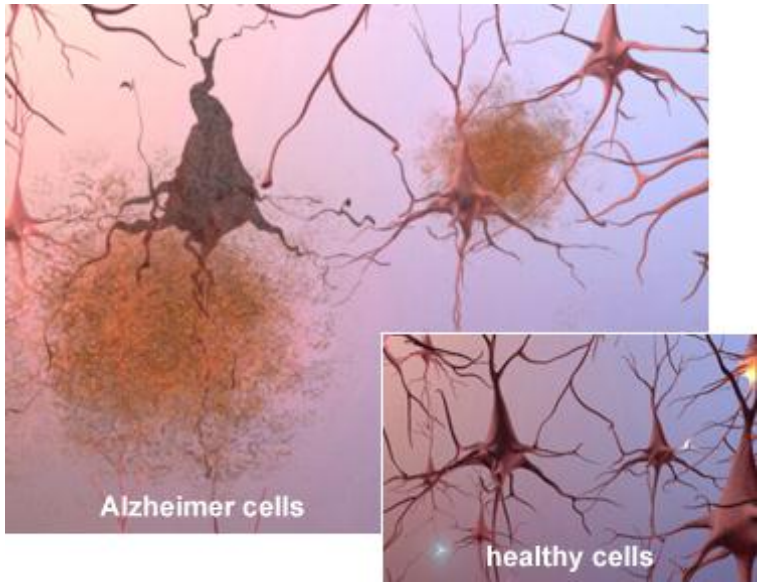
Mary F Lopez

BRIMS Biomarker Research Initiatives in Mass Spectrometry

Feb. 13, 2013

What is Alzheimer's and why is early detection important?

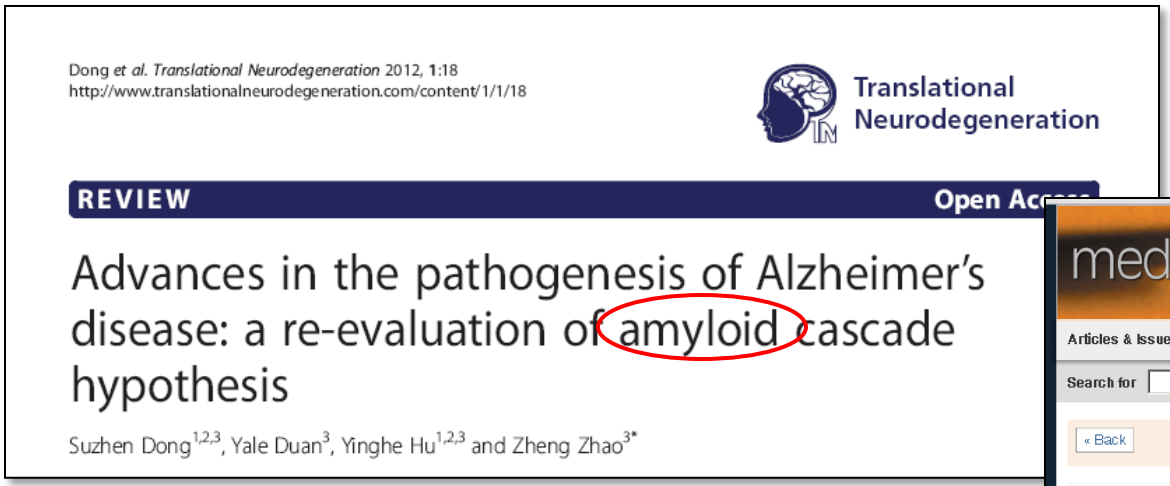
The hallmark pathology of AD is the extracellular deposition of beta amyloid plaques and intraneouronal tau deposition as neurofibrillary tangles

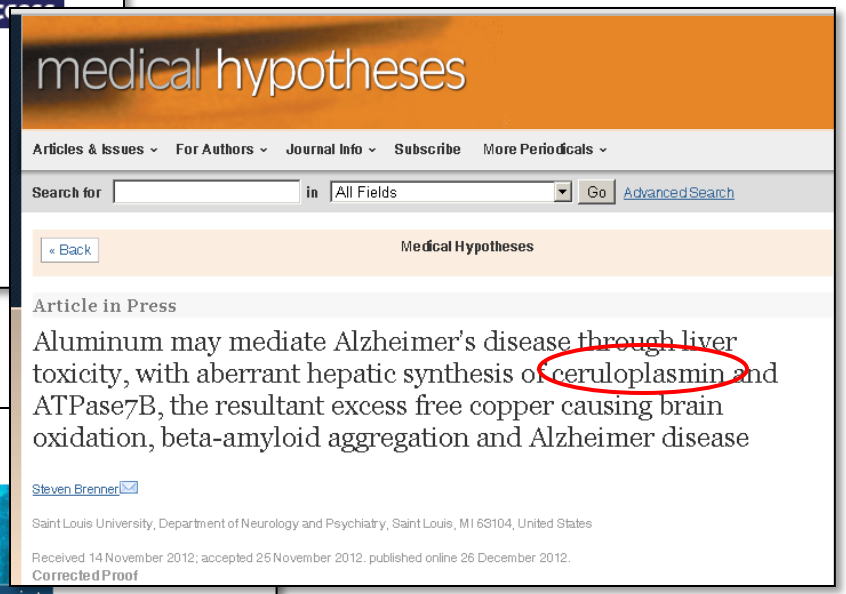


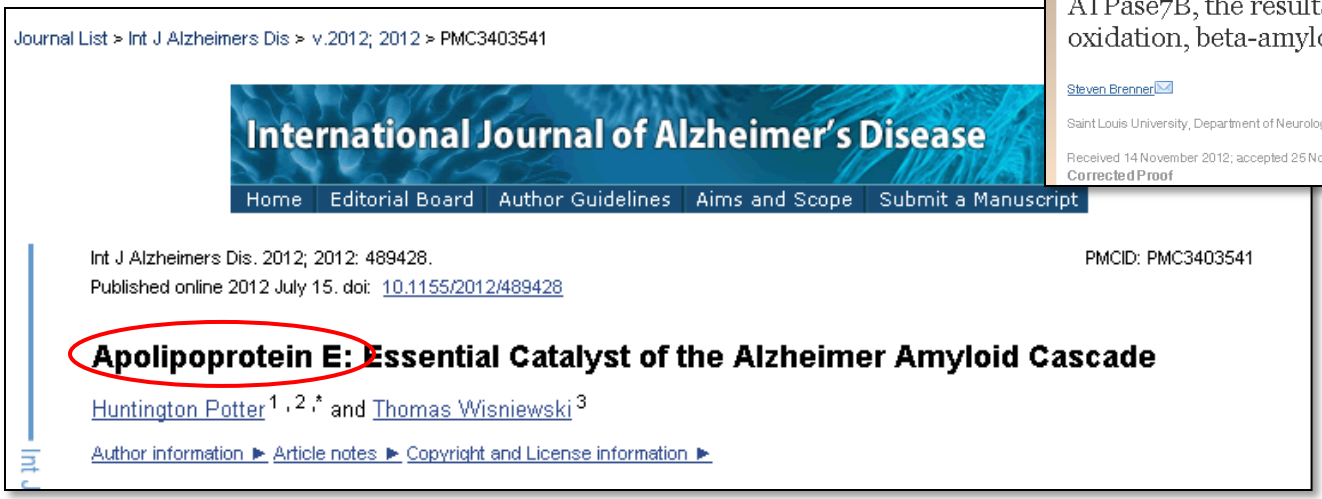
- AD is the most common cause of dementia among older people and affects an estimated 1 in 10 people over age 65. There is no known cure.
- At present, a definitive diagnosis can only occur after death.
- A blood test for early diagnosis could rule out other treatable causes of dementia.
- Early diagnosis could help plan for the future as well as provide opportunities to participate in clinical trials for new drugs.

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Several proteins, including amyloid beta, apolipoprotein E and ceruloplasmin have all been implicated in Alzheimer's disease (AD)







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Key observations and hypotheses of A beta, Apo E and CP involvement in AD

- The leading theory of AD pathophysiology is the Amyloid Cascade Hypothesis, which invokes the extracellular deposition of beta amyloid peptides (Aβ) in large fibrillar aggregates.
  - To date, several truncated variants of Aβ (1-38, 1-40, 1-42) have been found in brain and cerebrospinal fluid (CSF) while data from blood and plasma are more ambigous.
- Apolipoprotein E (Apo E) is thought to play a central role in the AD amyloid cascade leading to cognitive decline.
  - Apo E binds to Aβ and **both promotes** Aβ fibril **formation in the brain** and Aβ **clearance from the blood**.
  - The apparently contradictory fibril promotion and clearance roles of Apo E might be due to the different binding capacities of Apo E isoforms (Apo E2,3,4) for Aβ.
- Aluminum may cause hepatotoxicity with subsequent aberrant synthesis of proteins mediating copper homeostasis, such as ceruloplasmin and ATPase7B.
  - The resultant excess free copper, resulting in brain oxidation, protein degradation and beta-amyloid aggregation, are all features of Alzheimer disease.

How can we use mass spectrometry (MS) for the measurement of AD related proteins?

Selected Reaction Monitoring (SRM) targeted assays.



Precursor Ion M/Z
Pre set

fragmentation

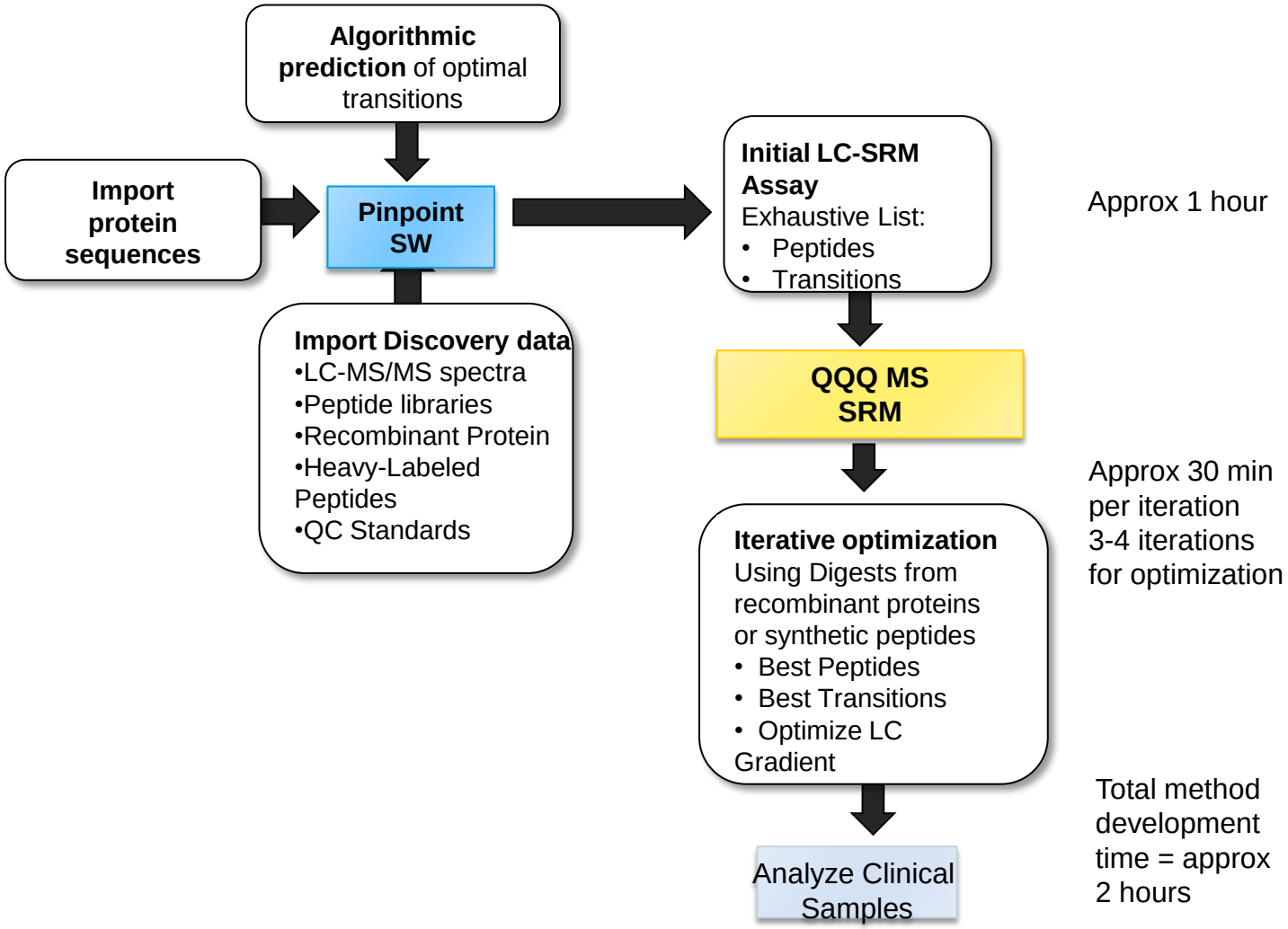
Product Ion M/Z
Pre set

Precursor +
Product Ion =
Transition

- In SRM, a triple quadrupole mass spectrometer functions as a set of very SPECIFIC and SENSITIVE filters to perform SRM targeted assays
- SRM assays are sensitive and robust for peptides and small molecules
- SRM assays may have enhanced selectivity *vs* ELISA or other methods
- SRM is high-throughput, hundreds of SRM transitions can be monitored per second
- Heavy isotope-labeled synthetic peptide standards can be used for very accurate quantification

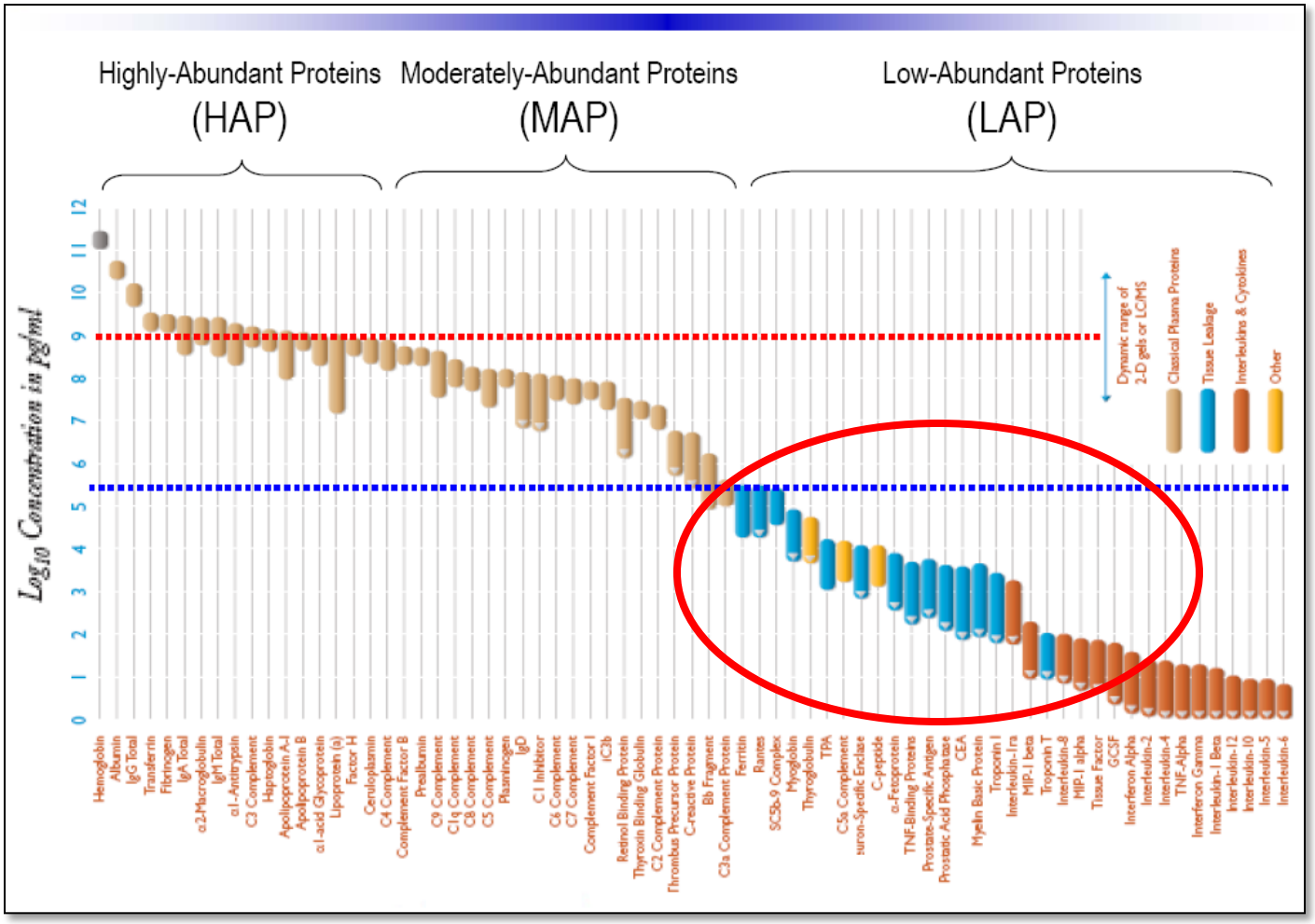
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SRM assay development workflow



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Caveat: The large dynamic range of proteins in blood presents a technical hurdle to the development of SRM assays biomarkers present in low abundance



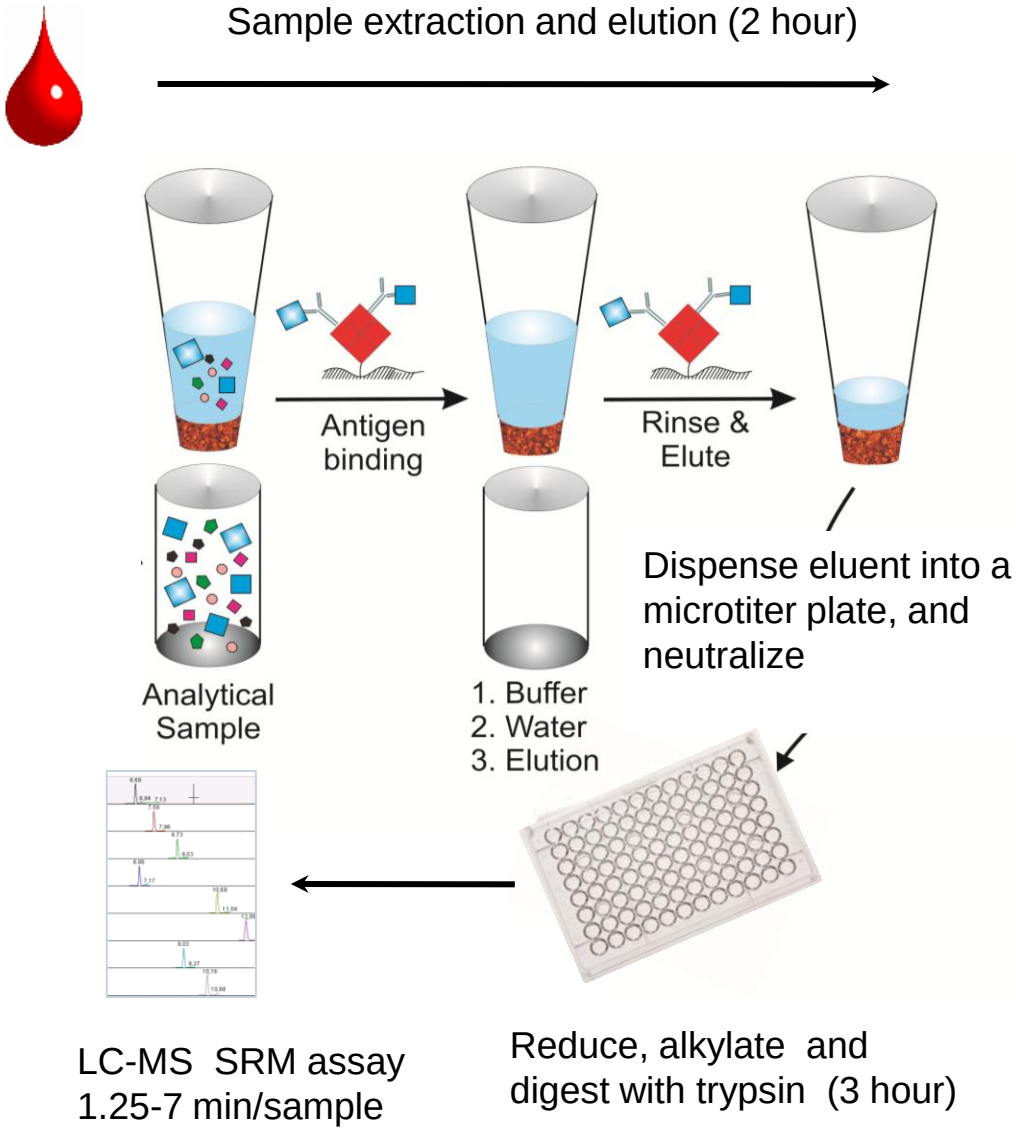
Low abundance analyte range *ca* <100pg/mL

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Sample preparation using Mass Spectrometric Immunoassay (MSIA) can provide 100X enrichment of lower abundance analytes

MSIA workflow

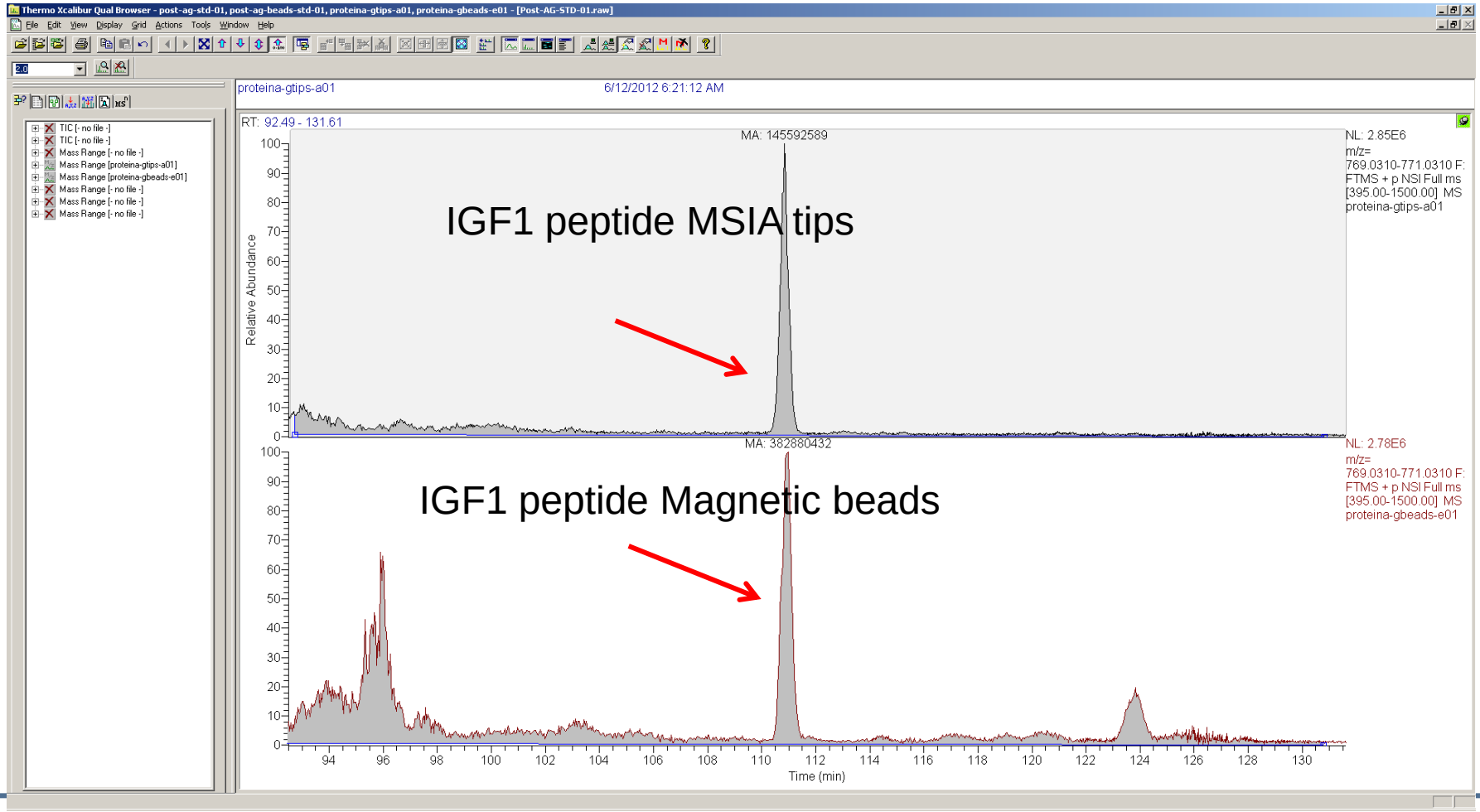
- Automated
- High-throughput
- Sensitive
- Selective
- Scalable
- Can be Multiplexed



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MSIA enrichment is more selective than other immunoenrichment techniques such as magnetic beads

Example: High resolution LC-MS of IGF1 peptide enriched from plasma using MSIA tips *vs* magnetic beads demonstrates improved signal-to-noise



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Collaboration with K Blennow Lab at the Sahlgrenska Institute to develop SRM A beta assays for serum and plasma

Biomark Med. 2012 Aug;6(4):409-17. doi: 10.2217/bmm.12.39.

Reference measurement procedures for Alzheimer's disease cerebrospinal fluid biomarkers: definitions and approaches with focus on amyloid β42.

Mattsson N, Zegers I, Andreasson U, Bjerke M, Blankenstein MA, Bowser R, Carrillo MC, Gobom J, Heath T, Jenkins R, Jeromin A, Kaplow J, Kidd D, Laterza OF, Lockhart A, Lunn MP, Martone RL, Mills K, Pannee J, Ratcliffe M, Shaw LM, Simon AJ, Soares H, Teunissen CE, Verbeek MM, Umek RM, Vanderstichele H, Zetterberg H, Blennow K, Portelius E.

Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy at University of Gothenburg, Sahlgrenska University Hospital, Mölndal, Sweden. niklas.mattsson@neuro.gu.se

Abstract

Cerebrospinal fluid (CSF) biomarkers for Alzheimer's disease (AD) are increasingly used in clinical settings, research and drug trials. However, their broad-scale use on different technology platforms is hampered by the lack of standardization at the level of sample handling, determination of concentrations of analytes and the absence of well-defined performance criteria for in vitro diagnostic or companion diagnostic assays, which influences the apparent concentration of the analytes measured and the subsequent interpretation of the data. There is a need for harmonization of CSF AD biomarker assays that can reliably, across centers, quantitate CSF biomarkers with high analytical precision, selectivity and stability over long time periods. In this position paper, we discuss reference procedures for the measurement of CSF AD biomarkers, especially amyloid β42 and tau. We describe possible technical approaches, focusing on a selected reaction monitoring mass spectrometry assay as a candidate reference method for quantification of CSF amyloid β42.

PMID: 22917143 [PubMed - indexed for MEDLINE]

All proteins and peptides targeted for the development of AD-related protein MSIA-SRM assays

Protein	Peptide sequence	
Abeta 1-38	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGG	
Abeta 1-40	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV	
Abeta 1-42	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA	
Apolipoprotein E Monitoring	ALMDETMK	All Apo E isoforms
	LGPLVEQGR	
	AATVGSLAGQPLQER	
	LEEQAQQIR	
	LQAEAFQAR	
	SWFEPLVEDMQR	
	VQAAVGTSAAPVPSDNH	
Apolipoprotein E Isoform specific	LAVYQAGAR	E4
	C[Carboxymethyl]LAVYQAGAR (aa 176 R→C)	E2, E3
	VEQAVETEPEPELR	E2,E3
	VEQAVETEPK (aa 31 E→K)	E4
	WELALGR	E2, E3
	WEPALGR (aa 46 L→P)	E4
	LGADMEDVC[Carboxymethyl]GR	E2
	LGADMEDVR (aa 130 C→R)	E3, E4
Ceruloplasmin	ALYLQYTDETFR	
	EYTDASFTNR	
	GAYPLSIEPIGVR	
	DIFTGLIGPMK	
	GEFYIGSK	

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Clinical samples, recombinant proteins and antibodies used for MSIA activation

Clinical samples were obtained with full IRB approval

Total Number of samples	Gender	Sample source	Sample Type	Diagnosis
20	10M, 10F	plasma	Normal	(20) Examination without complaint, suspected or reported diagnosis
20	10M, 10F	plasma	AD	
			AD	(9) Alzheimer's disease, unspecified
			AD	(2) F00.9 Dementia in Alzheimer's disease, unspecified
			AD	(2) F00.0 Dementia in Alzheimer's disease with early onset
			AD	(2) G30.0 Alzheimer's disease with early onset
			AD	(2) G30.8 Other Alzheimer's disease
			AD	(1) G30.1 Alzheimer's disease with late onset
			AD	(1) G30.9 Alzheimer's disease, unspecified
3	NA	CSF	AD	NA

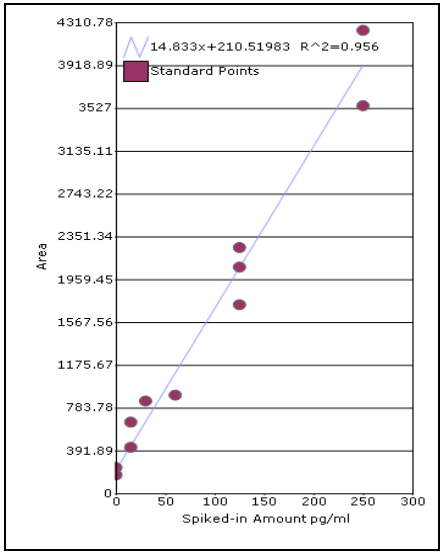
Recombinant proteins and antibodies were from commercially available sources

Protein or peptide	Recombinant	Antibody	Immunogen
Amyloid beta	Bachem H-2966 (1-38)	Covance SIG-39320-500	amino acids 3-8 of beta amyloid (EFRHDS)
	Bachem H-1194 (1-40)		
	Bachem H-1368 (1-42)		
Apolipoprotein E	OriGene PH300395	Academy Biomedical, 50-G1b, polyclonal	Apo E purified from human plasma
Ceruloplasmin	MP Biomedicals ICN19497401	Bethyl Laboratories A80-124	Human ceruloplasmin purified from plasma

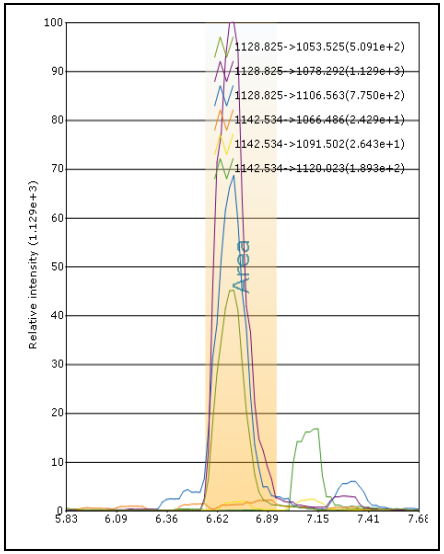
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Examples of SRM data, LLOQ and clinical ranges for targeted proteins/peptides

Abeta – aa 1-40
Calibration Curve
R2 = 0.95



Abeta – aa 1-42
Extracted ion chromatogram



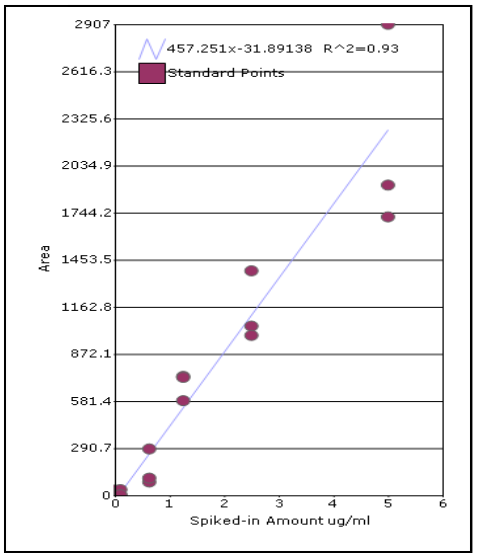
Protein	LLOQ*	Clinical range
Amyloid beta	50pg/mL	20-80pg/mL
Apolipoprotein E	10ng/mL	10-100ug/mL
Ceruloplasmin	62.5ug/mL	14-450ug/mL

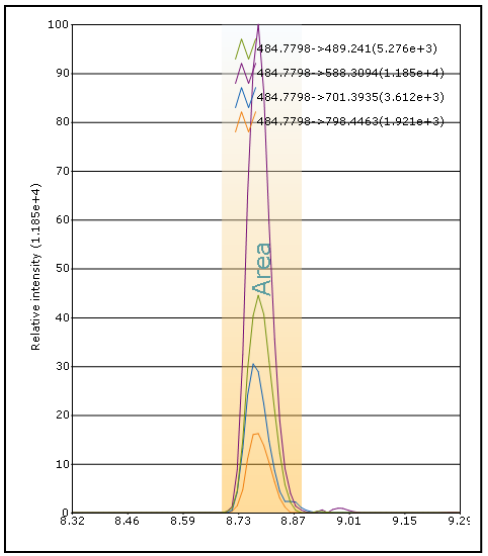
*Average of all monitoring peptides

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Examples of SRM data

Apo E
LGPLVEQGR
Calibration curve
R2 = 0.93

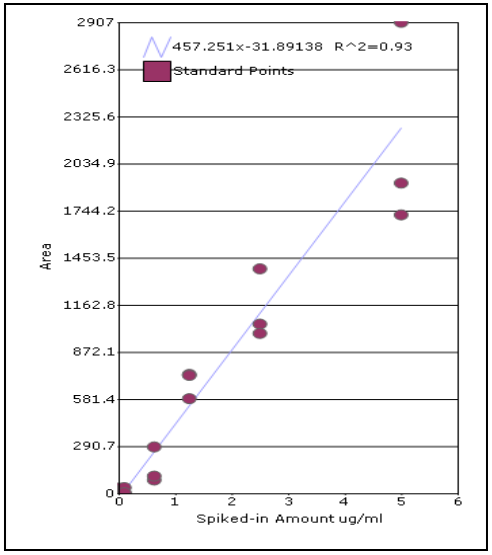


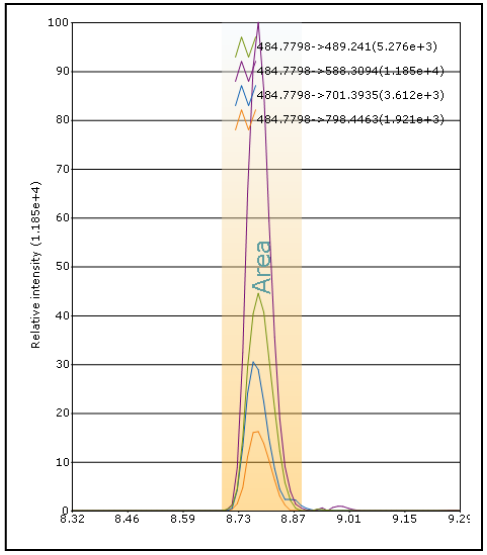


Apo E
LGPLVEQGR
Extracted ion
chromatogram

Ceruloplasmin
EYTDASFTNR
Calibration curve

R2 = 0.93



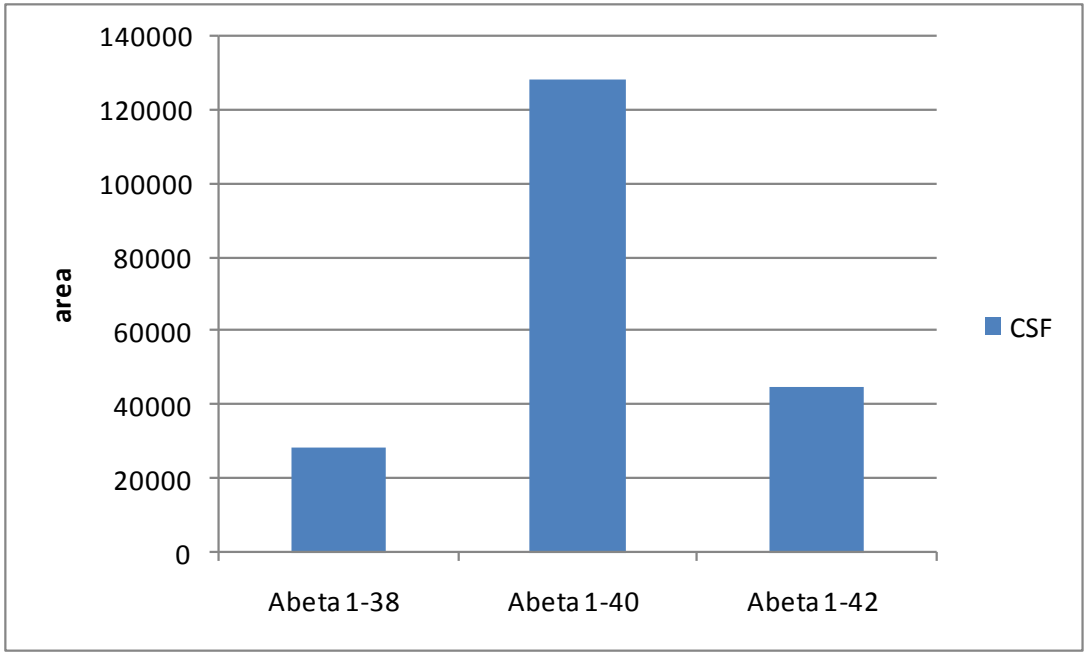


Ceruloplasmin
EYSDASFTNR
Extracted ion
chromatogram

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MSIA-SRM detection and quantification of Aβ peptides in human CSF

Consistent with our previously published data*, the MSIA-SRM assay detected all three Aβ fragments in CSF from patients with AD.

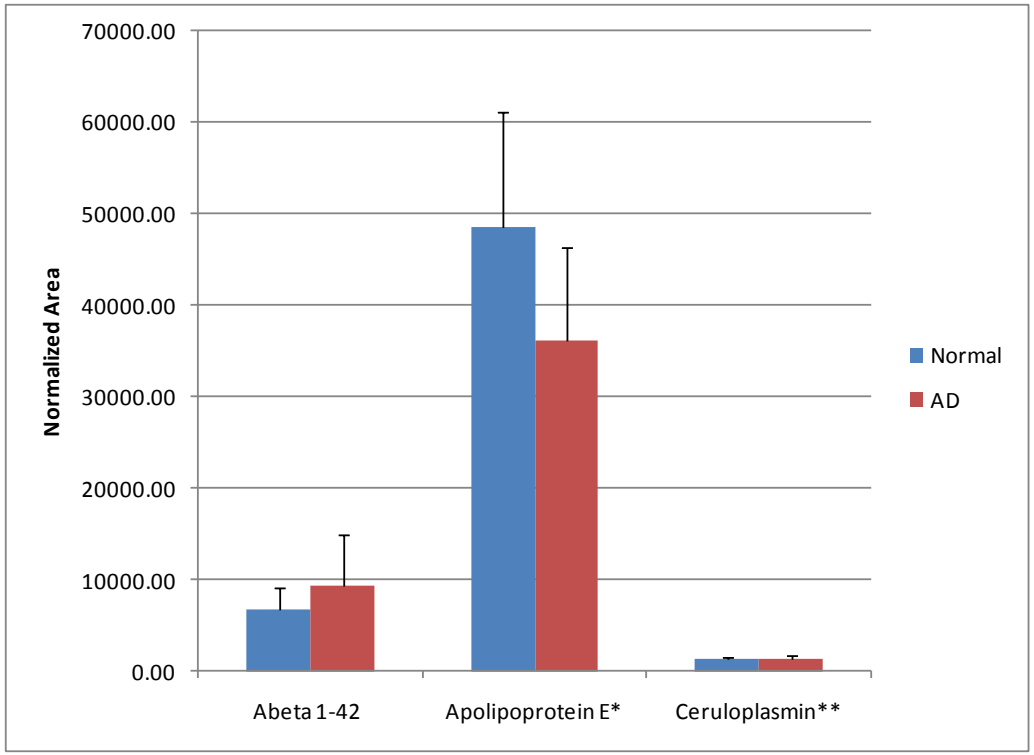


*Portelius E, Mattsson N, Andreasson U, Blennow K, Zetterberg H. Novel a β isoforms in Alzheimer's disease - their role in diagnosis and treatment. Curr Pharm Des. 2011;17(25):2594-602.

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Detection and quantification of Aβ, Apo E and CP in clinical samples

- When the MSIA-SRM assays were applied to the clinical plasma sample set, a differential abundance pattern was evident.
- The levels of Aβ1-42 were quantitatively increased in AD.
- Although we did detect Aβ1-40, the signal was not quantifiable and Aβ1-38 was either absent or below the level of detection.
- The overall Apo E abundance (as determined by the average of all monitoring peptides) was significantly higher in the control samples.
- CP abundance was not significantly different between AD and Normal.



* Average of 7 monitoring peptides

**Average of 5 monitoring peptides

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MSIA-SRM detection of isoform-specific Apo E peptides in AD and Control clinical samples

- Three isoform-specific Apo E peptides were detected in the clinical sample cohort.
- Two were at a very low frequency (5-15% in controls only) in our sample set.
- However, peptide LGADMEDVR, present in Apo E3 and 4, was found at approximately 1.4 X higher frequency in the AD samples.

Isoform	Mutation	Peptide sequence	Frequency of Detection (%) in Normal samples	Frequency of Detection (%) in AD samples
E4		LAVYQAGAR	100	100
E2, E3	176 R→C	C[Carboxymethyl]LAVYQAGAR	15	0
E2,E3		VEQAVETEPEPELR	100	100
E4	31 E→K	VEQAVETEPK	0	0
E2,E3		WELALGR	100	100
E4	46 L→P	WEPALGR	5	0
E2		LGADMEDVC[Carboxymethyl]GR	100	100
E3,E4	130 C→R	LGADMEDVR	28.5	40

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A growing number of MSIA-SRM assays have been developed to date…

- PTH
- IgF1
- A Beta, 1-38, 1-40, 1-42
- PCSK9
- EPO
- Beta 2 microglobulin
- Apo E2, 3, 4, 5 isoforms
- Apo CI
- Apo CIII
- Apo J clusterin
- Apo AI
- Ceruloplasmin
- PSA
- Procalcitonin
- C reactive protein
- VDBP

In Press

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Clinical Biochemistry

Available online 8 January 2013

In Press, Accepted Manuscript — Note to users



Rapid development of sensitive, high-throughput, quantitative and highly selective mass spectrometric targeted immunoassays for clinically important proteins in human plasma and serum

Bryan Krastins[a], Amol Prakash[a], David A. Sarracino[a], Dobrin Nedelkov[b], Eric E. Niederkofler[b], Urban Kiernan[b], Randall Nelson[c], Maryann S. Vogelsang[a], Gouri Vadali[a], Alejandra Garces[a], Jennifer N. Sutton[a], Scott Peterman[a], Gregory Byram[a], Bruno Darbouret[d], Johan Gobom[e], Erik Portelius[e], Josef Pannee[e], Kaj Blennow[e], Vathany Kulasingam[f], Lewis Couchman[g], Caje Moniz[g], Mary F. Lopez[a]

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[g] King's College Hospital, London, UK

Conclusion

- We have developed novel, multiplexed, high-throughput MSIA-SRM assays for 3 AD associated proteins.
- The assays demonstrate excellent linearity, sensitivity and precision for Aβ1-38, 1-40 and 1-42, CP and Apo E in plasma.
- We tested the assays with a small clinical cohort and demonstrated differential abundance of Aβ1-42 and ApoE in AD *vs* control, consistent with previously published data and the Amyloid Cascade Hypothesis.
- We demonstrated detection of Apo E-specific isoforms in blood plasma using MS.
- One of the Apo E isoforms, specific for E3,4 but not E2 displayed higher frequency in AD vs control samples. This observation is consistent with previously published genomic findings.
- Despite previously published results, we did not observe differential expression/abundance of ceruloplasmin in our cohort

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