

Novel fluid biomarker studies in AD: Progress Update

Global Biomarkers Standardization Consortium (GBSC)



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Chief Technology Officer
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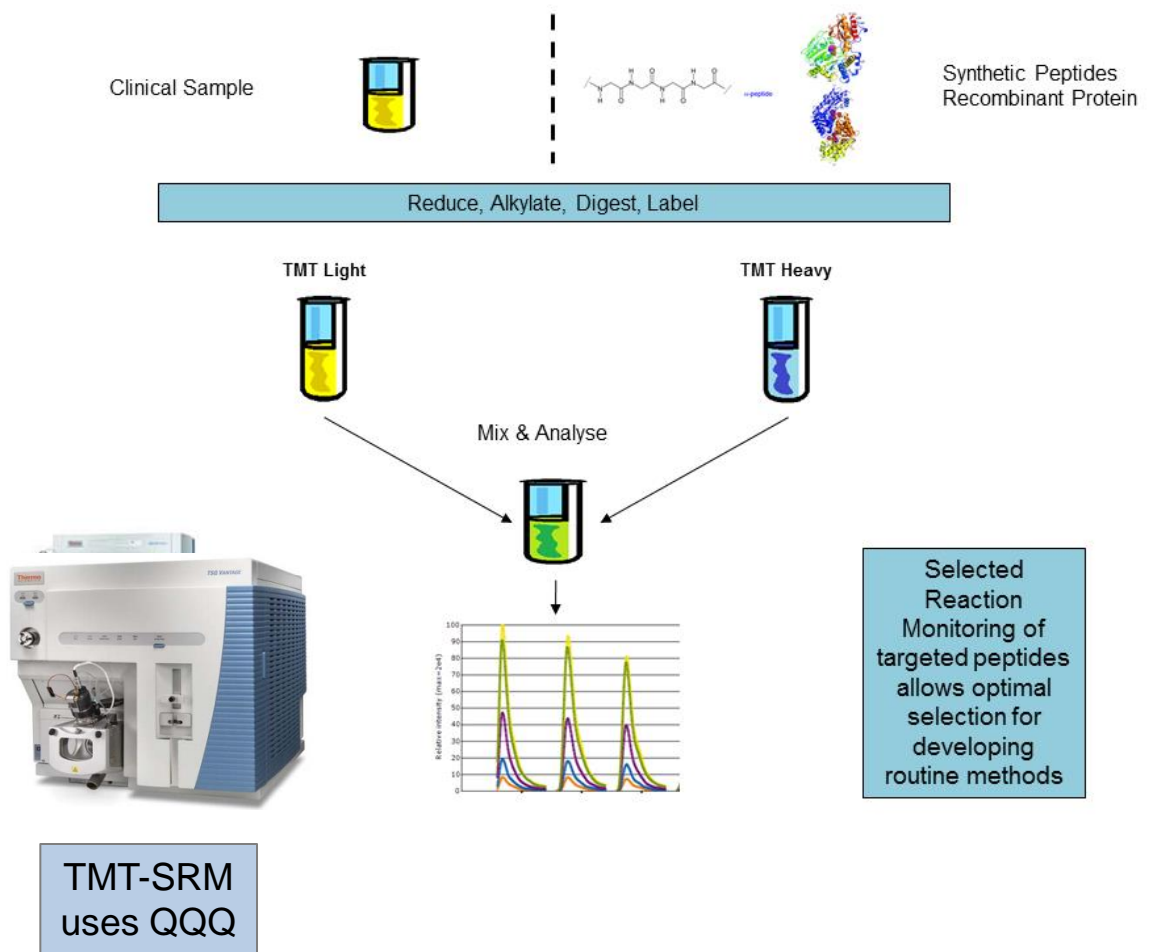
Agenda

- A novel 16 CSF protein panel by targeted mass spectrometry
- The role of post-translational modifications as sensitive biomarkers
- Proteome Sciences AAIC presentations

TMT-SRM 16-protein CSF Panel for Alzheimer's Disease

Protein	Disease Function
Amyloid-like protein 1	Post-synaptic function, neurite outgrowth, neuronal apoptosis
Amyloid beta A4 protein (APP)	Cleaved to form Amyloid beta, neurite outgrowth, regulator of lipid oxidation, promotes tau aggregation
Beta-2-microglobulin	Member of MHC complex, amyloid clearance, amyloid aggregation
Complement C3 alpha chain	Inflammatory response, vascular permeability, synaptic loss
Complement C3 beta chain	CR1-mediated phagocytosis
Chromogranin A	Major protein of dense-core synaptic vesicles, synaptic function
Complement factor H	Regulator of complement activation
Cystatin C	Amyloid binding and clearance
Serum amyloid P-component	Amyloid binding and clearance
Clusterin alpha chain	Amyloid binding and clearance
Clusterin beta chain	Amyloid binding and clearance
Apolipoprotein E	Amyloid binding and clearance
Alpha-2-macroglobulin	Amyloid binding and clearance
Secretogranin-2	Neuroendocrine secretion, synaptic function
Gelsolin	Inhibiting amyloid fibrils, prevents apoptosis
Fibrinogen gamma chain	Vascular function, control of fibrinolysis, amyloid binding

- Amyloid-like protein 1**
- Amyloid beta A4 protein
- Beta-2-microglobulin
- Complement C3 alpha
- Complement C3 beta
- Chromogranin A
- CFH
- Cystatin C
- Serum amyloid P-component
- Clusterin alpha
- Clusterin beta
- Apolipoprotein E
- Alpha-2-macroglobulin
- Secretogranin
- Gelsolin
- Fibrinogen gamma chain

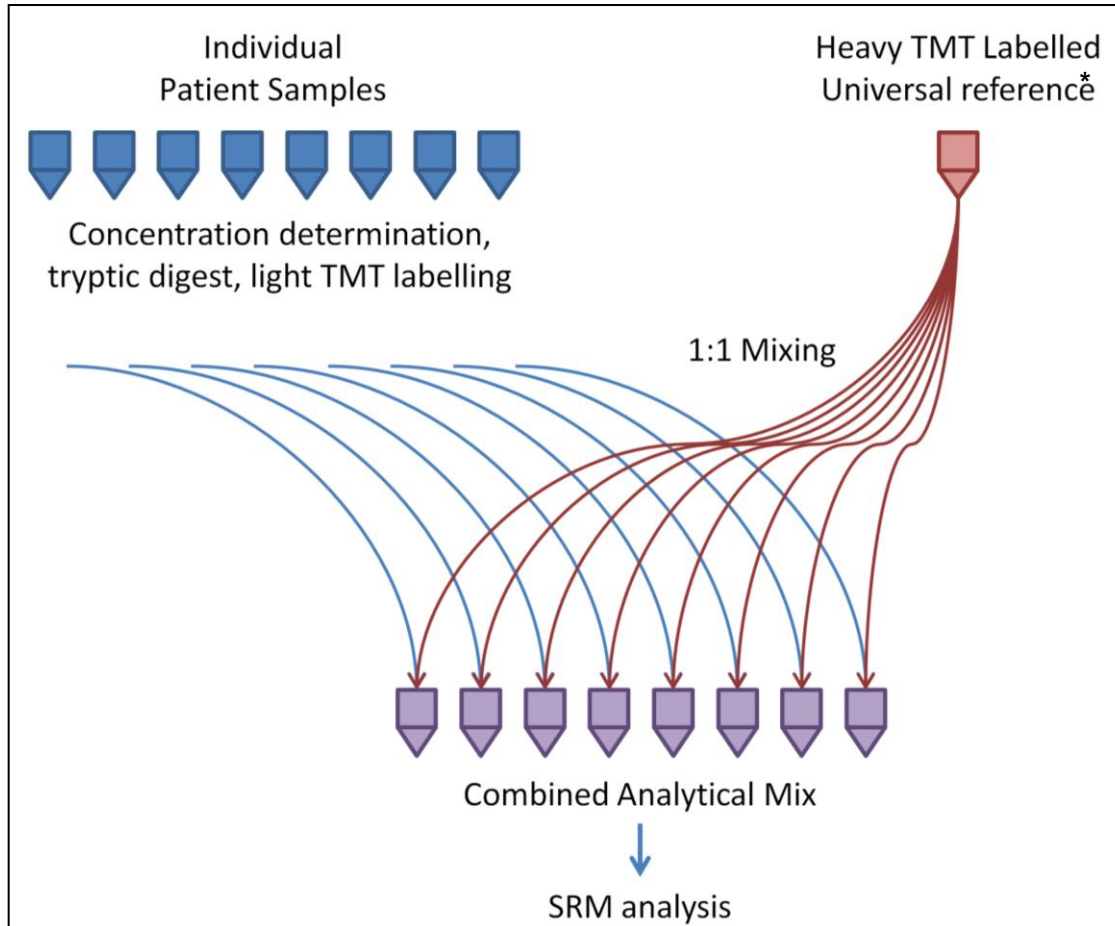


16 proteins - 31 distinct epitopes - 236 Tx measured

- CSF samples have been provided by Zetterberg/Blennow in Mölndal (Sweden)
- 31 control and 31 AD CSF samples
- Following metadata was also provided
 - Diagnosis, gender, age, and concentration of tau, beta-amyloid and phospho-tau
- Metadata of vial type and protein concentration after Bradford assay was added

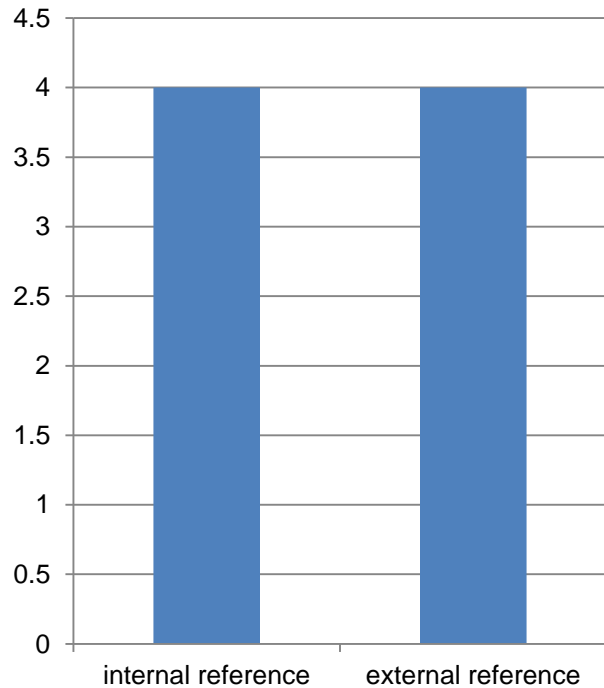
- Isotopic TMTduplex workflow
- Samples are individually labelled with TMTzero
- Universal reference (Seralab #CSF-123-S-26975) is stored pre-labelled with TMTsixplex tag in 50 μ L aliquot. Each vial is thawed and centrifuged prior to analysis
- Sample and Universal reference mixed 1:1 and measured in triplicate (repeat runs never run consecutively)
- 3 reference samples (1:1 mix of Universal Reference) performed during the 186 sample runs
- TSQ Vantage with 1h nLC gradient 2 μ L CSF o/c
- Each sample analysed in triplicate
- PinPoint 1.1 including manual editing

Validation study using 31 AD vs 31 Control CSF samples

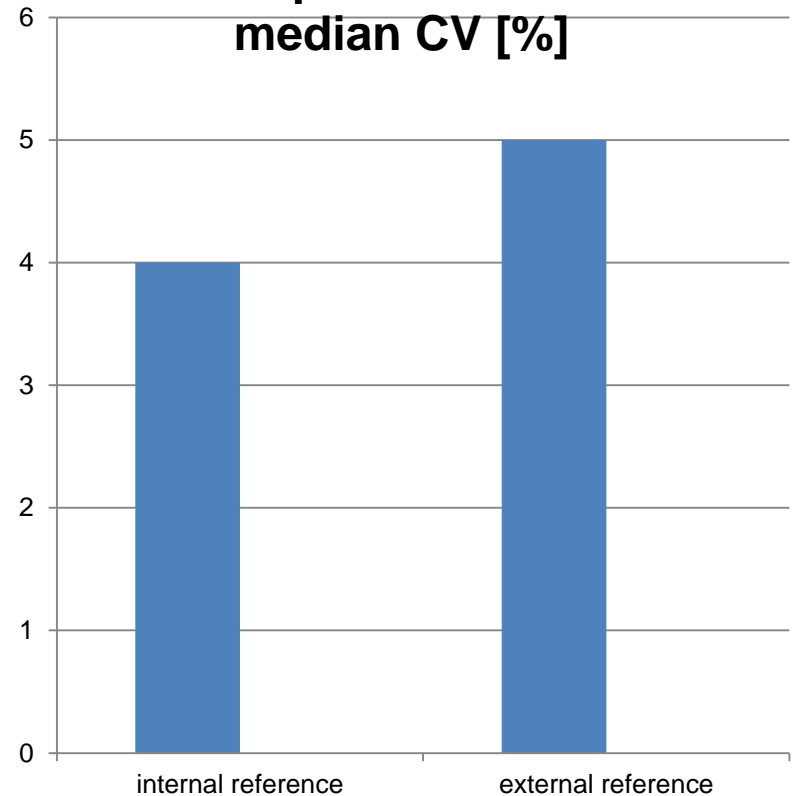


* - Universal Reference is bulk manufactured from hospital remnant CSF, aliquoted and stored @ -80°C

Protein level median CV [%]



Peptide Level median CV [%]



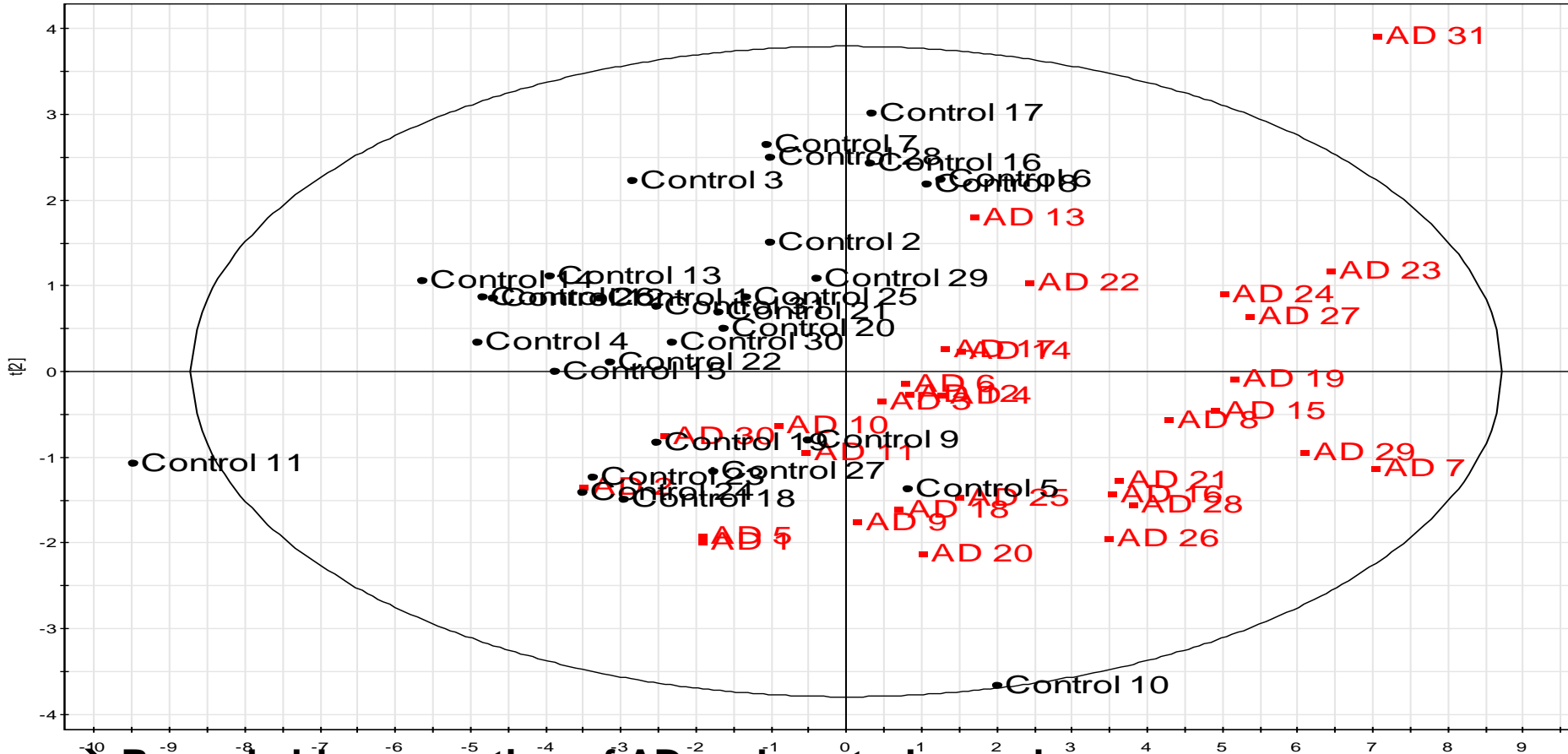
Overall assay precision is comparable at both protein and individual peptide level

Several epitopes within each protein can be measured independently of influence by PTM's

PCA – score plot disease PC1/PC2

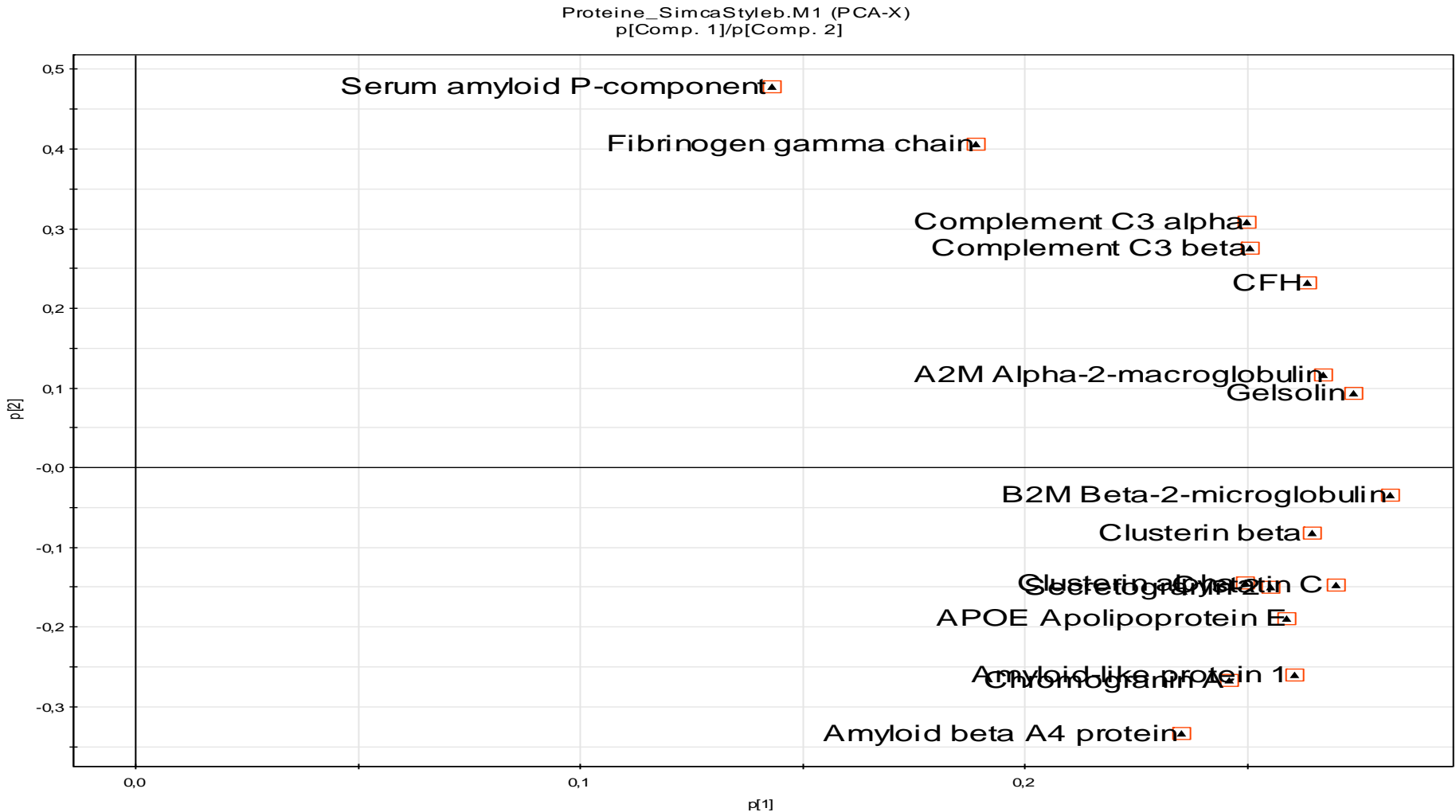
Proteine_SimcaStyleb.M1 (PCA-X)
 t[Comp. 1]/t[Comp. 2]
 Colored according to Obs ID (Sample_short)

■ AD
 ● Control



→ Remarkable separation of AD and control samples

R2X[1] = 0,741894 R2X[2] = 0,140655
 Ellipse: Hotelling T2 (0,95)



→ Most analytes have strong influence on outcome of separation

	LME pvalues					
	DiagnosisControl	VialType2	VialType3	GenderM	Age	Pconc
Amyloid_like_protein_1	0.0000	0.6432	0.9271	0.0535	0.0770	0.0004
Amyloid_beta_A4_protein	0.0000	0.2592	0.6043	0.0031	0.0440	0.9096
B2M_Beta_2_microglobulin	0.0000	0.5256	0.6413	0.2995	0.1700	0.0000
Complement_C3_alpha	0.0012	0.0284	0.4616	0.2152	0.0664	0.0000
Complement_C3_beta	0.2538	0.9547	0.2732	0.0752	0.0291	0.0000
Chromogranin_A	0.0000	0.3268	0.1429	0.4223	0.3022	0.0226
CFH	0.0001	0.2173	0.7809	0.7652	0.0109	0.0000
Cystatin_C	0.0000	0.9546	0.4786	0.9425	0.8475	0.0000
Serum_amyloid_P_component	0.1098	0.2290	0.0172	0.1405	0.0133	0.0000
Clusterin_alpha	0.0000	0.9062	0.0620	0.0067	0.2830	0.0006
Clusterin_beta	0.0000	0.7321	0.0203	0.0114	0.0116	0.0000
APOE_Apolipoprotein_E	0.0000	0.2538	0.0853	0.0219	0.4609	0.0000
A2M_Alpha_2_macrolobulin	0.0000	0.4538	0.5398	0.6799	0.1139	0.0000
Secretogranin_2	0.0000	0.0883	0.3439	0.4935	0.0004	0.0000
Gelsolin	0.0000	0.9234	0.4585	0.6393	0.0004	0.0000
Fibrinogen_gamma_chain	0.5471	0.6928	0.3594	0.8410	0.0000	0.0000

P values < 0.05 are marked in red

- 13 analytes have a significant p value (< 0.05) for diagnosis
- Protein concentration has a high influence (except for Amyloid beta A4)

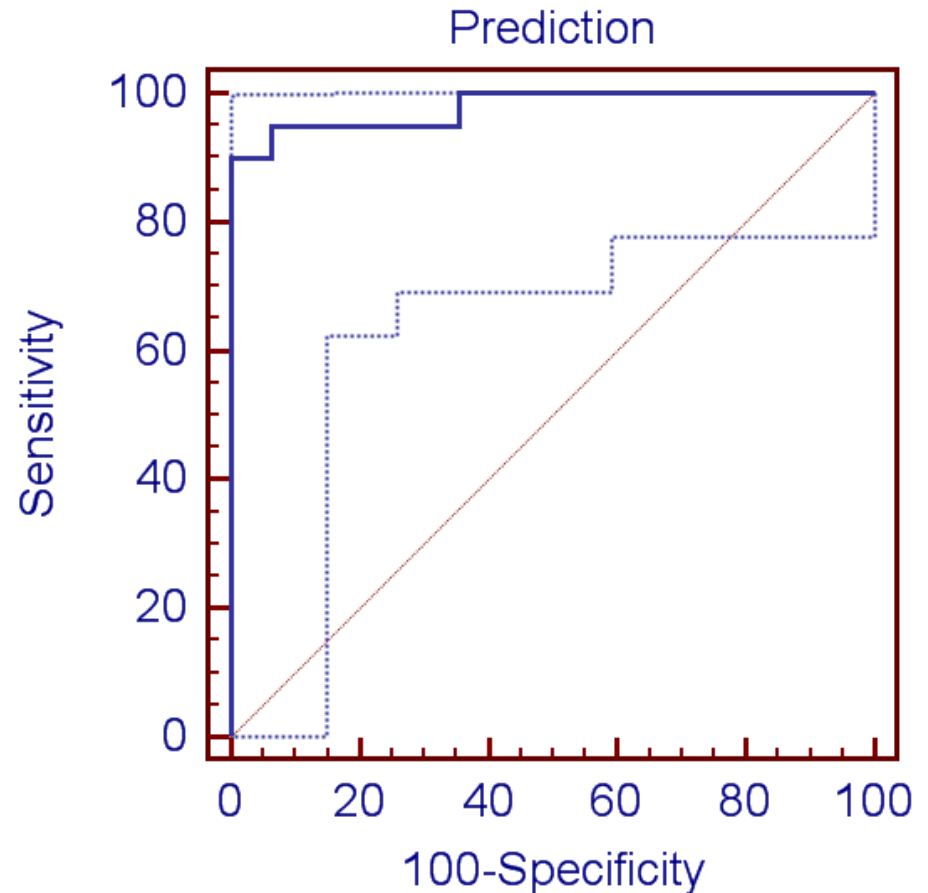
GMDH Model calculated based on individual protein expression relative to Universal CSF Reference

$$\begin{aligned}
 Y1 = & 1*(-1.18589) + \\
 & \text{Amyloid_like_protein_1}*0.591252 + \\
 & \text{Complement_C3_alpha}*0.526865 - \\
 & \text{Complement_C3_beta}*0.614223 + \\
 & \text{Age}*0.0193848 + \\
 & \text{Secretogranin_2}*0.138263 - \\
 & \text{Gelsolin}*0.470016
 \end{aligned}$$

$Y1 > 0.5 = \text{AD}$

$Y1 < 0.5 = \text{No AD}$

AUC = 0.979

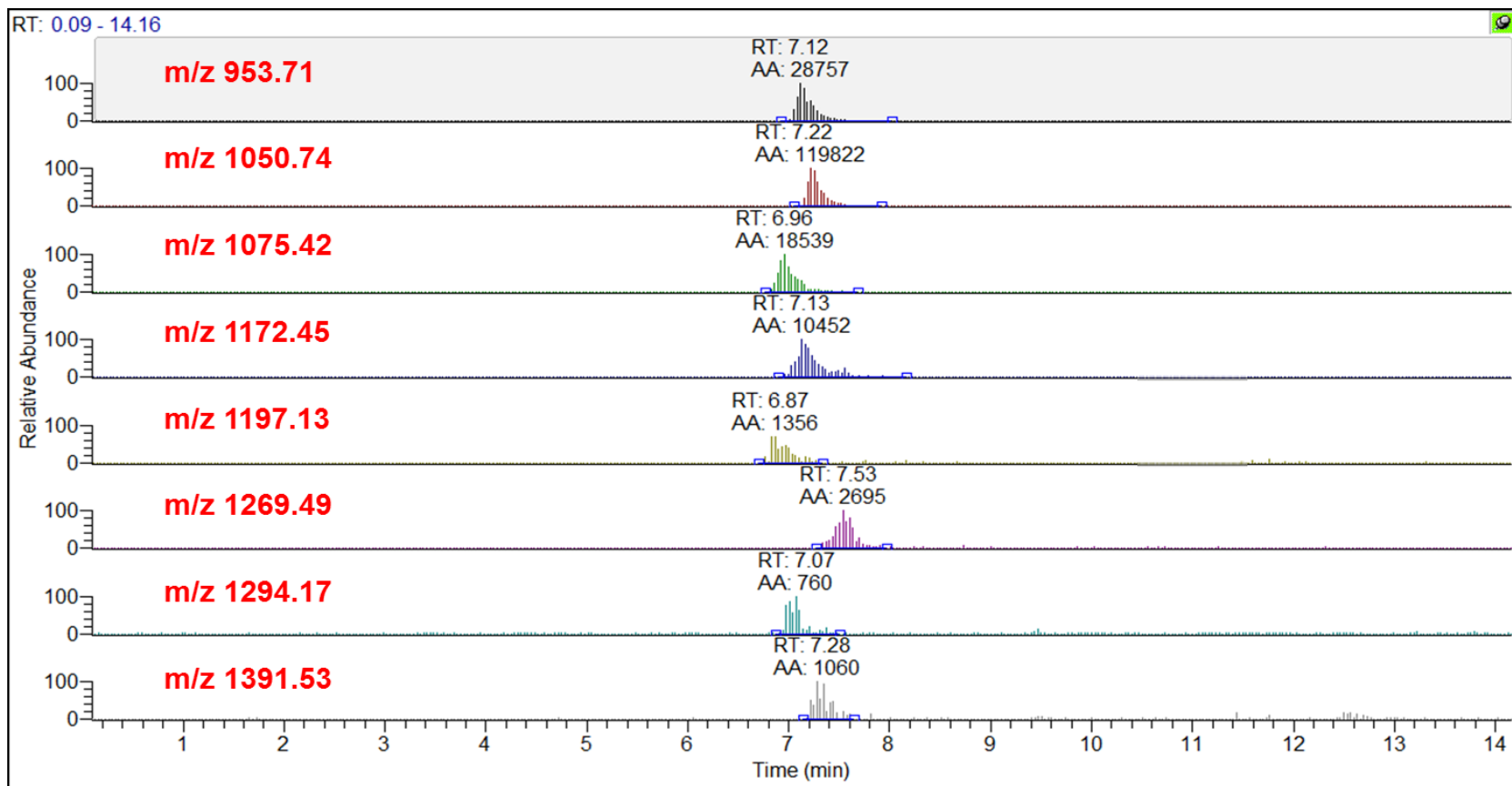


- QC (SDS-PAGE, Bradford assay), TMT processing for isotopic TMTduplex, nLC-SRM data acquisition, MS data analysis and statistical analysis has been completed successfully
- Results demonstrate excellent precision of the assay
- 16 protein analytes have been evaluated for feasibility as biomarkers in an initial cohort of 62 samples
- The CSF 16plex assay provides diagnostic performance comparable to existing CSF biomarkers eg Amyloid and pTau
- The CSF 16plex assay is available via PS Biomarker services for the analysis of further CSF cohorts
- Contact glenn.barney@proteomics.com for sales enquiries

Clusterin PTM Assay

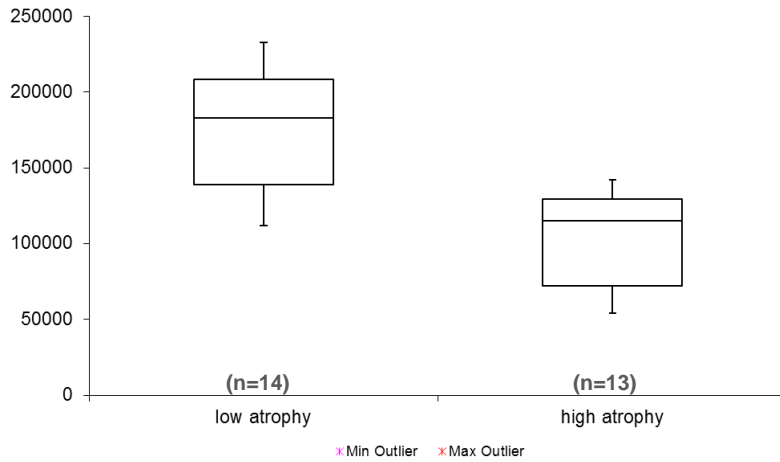
- Several 2DE spots containing Clusterin found to correlate with AD progression (Thambisetty *et al.*, 2010) but exact molecular forms of clusterin within each spot remained to be elucidated
- Do alterations in glycosylation patterns of clusterin have biomarker utility ?
- Performed a detailed glycoproteomics profiling of plasma clusterin to obtain a comprehensive description of glycoforms associated with the alpha and beta chains of the protein
- Site specific changes observed with significant correlation to the extent of atrophy

Selective Quantitation of eight glycoforms of Clusterin

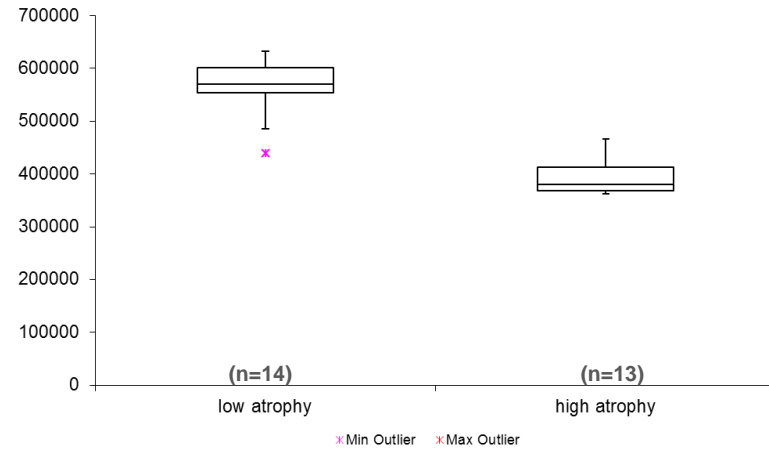


Measured in a cohort of high and low atrophy individuals (n=27 overall)

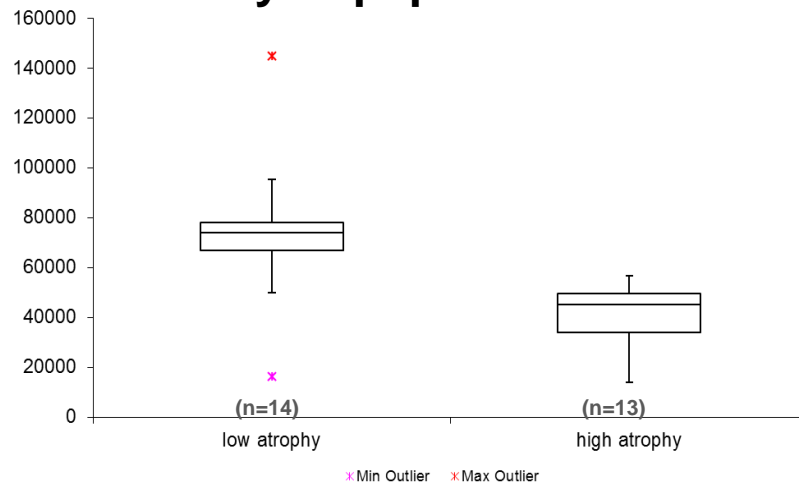
Glyco-peptide #1



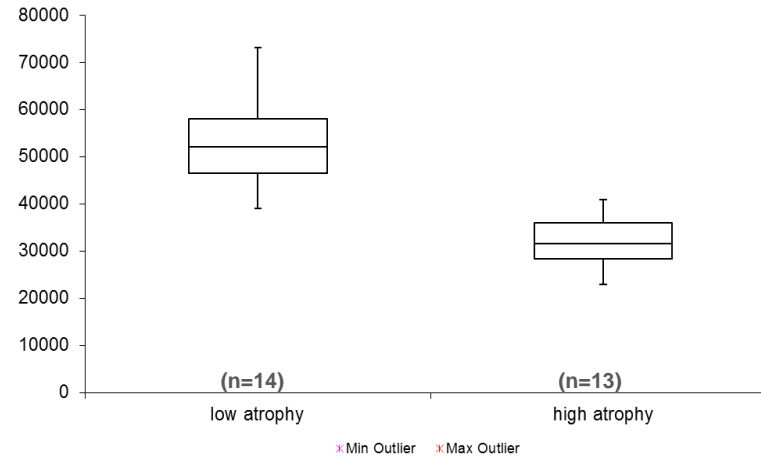
Glyco-peptide #3



Glyco-peptide #2



Glyco-peptide #4



- A novel CSF 16plex neurodegeneration assay
- Unravelling the role of CK1delta/epsilon inhibitors in modulating tau phosphorylation & cell signalling pathways using global phosphoproteomics in a transgenic tauopathy model of AD
- Structural Elucidation of Glycosylation profiles of human plasma Clusterin in AD patients
- Exploration of plasma biomarkers for AD using isotopic TMT & a combined directed / data-dependant acquisition nLC-MS-MS method
- Microglia Activation in CSF from patients with AD using a novel MS approach

Thanks for Listening

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