

Erasmus MC

Universitair Medisch Centrum Rotterdam



Validating 'PZP' as a biomarker for preclinical Alzheimer's disease

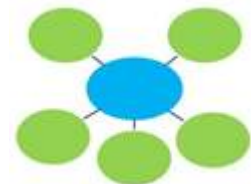
Using a targeted proteomics approach

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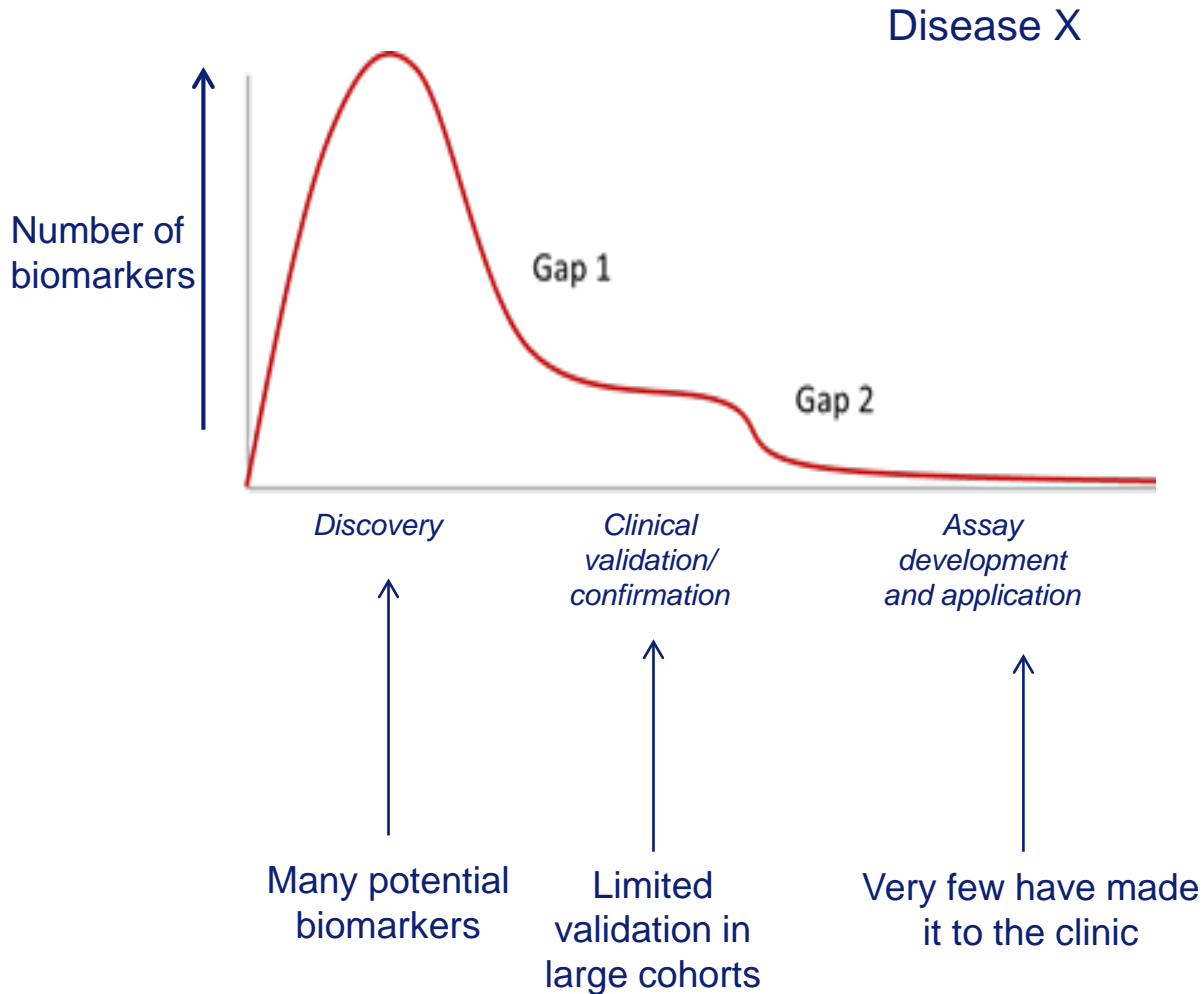
Skyline User Meeting, San Antonio, Texas

June 5th 2016



Biomarker Development Center

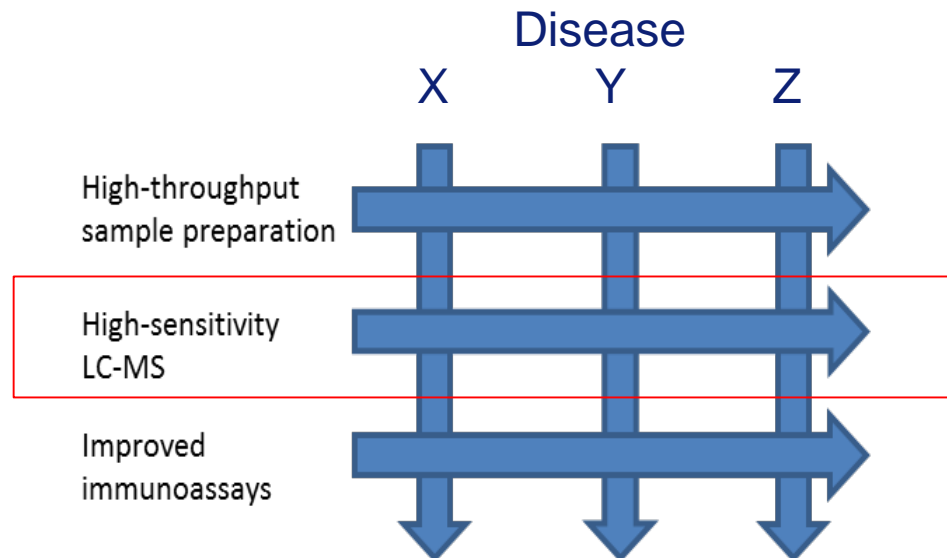
Biomarker research faces several challenges



Bridging the gaps

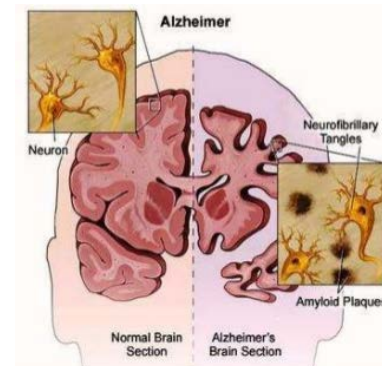
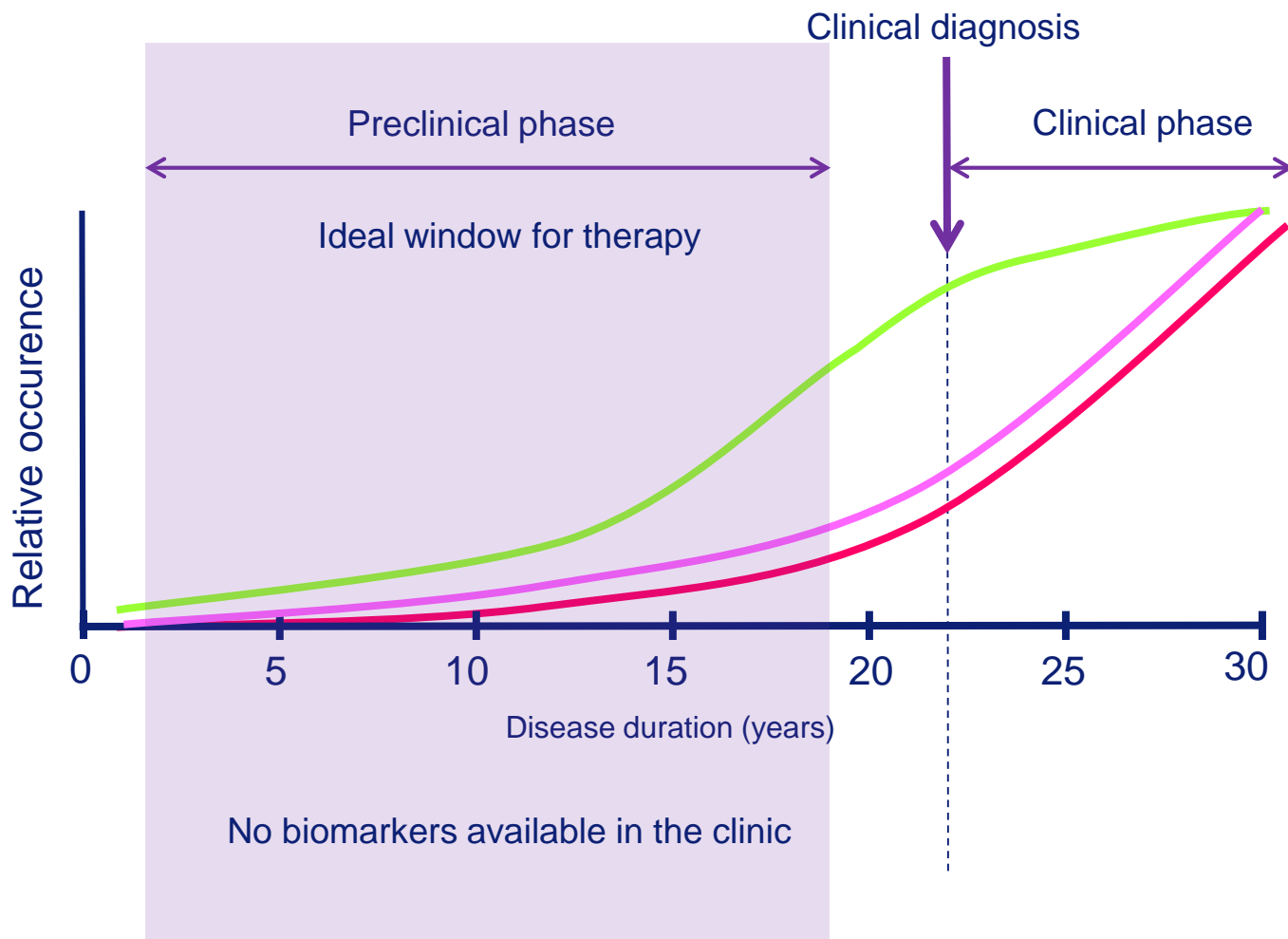
Aims

- Validate previously discovered biomarker candidates in large(r) population cohorts
- Develop assays that can be used in a clinical setting

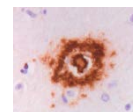


Alzheimer's disease

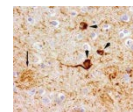
A neurodegenerative disorder with a long preclinical phase



— Aβ deposits

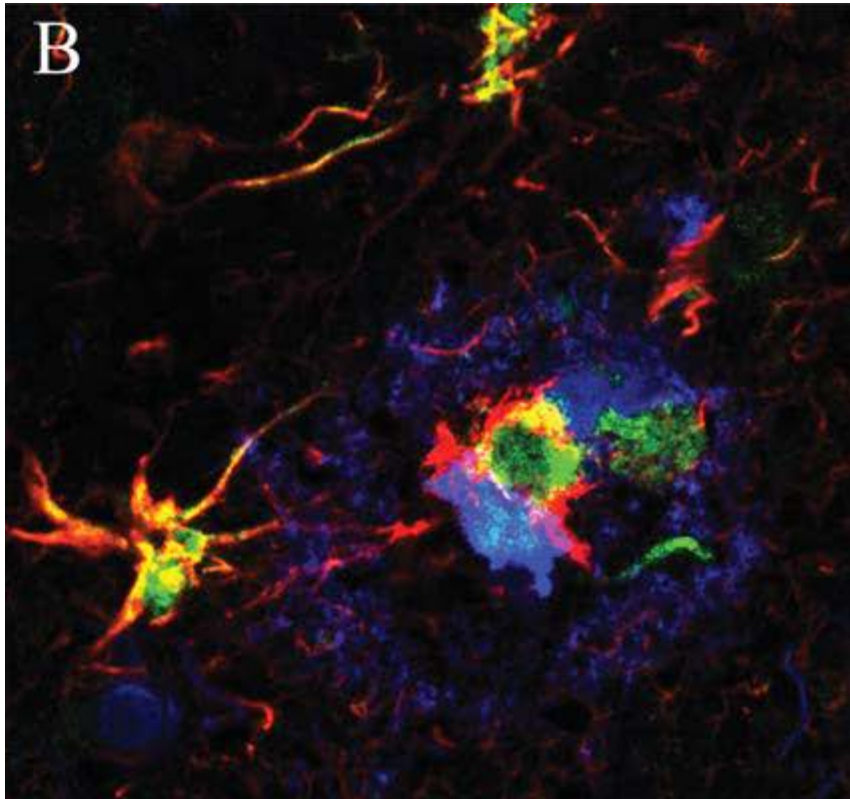


— Neurofibrillary tangles



— Cognitive impairment

PZP was previously identified as a potential serum biomarker for preclinical Alzheimer's disease

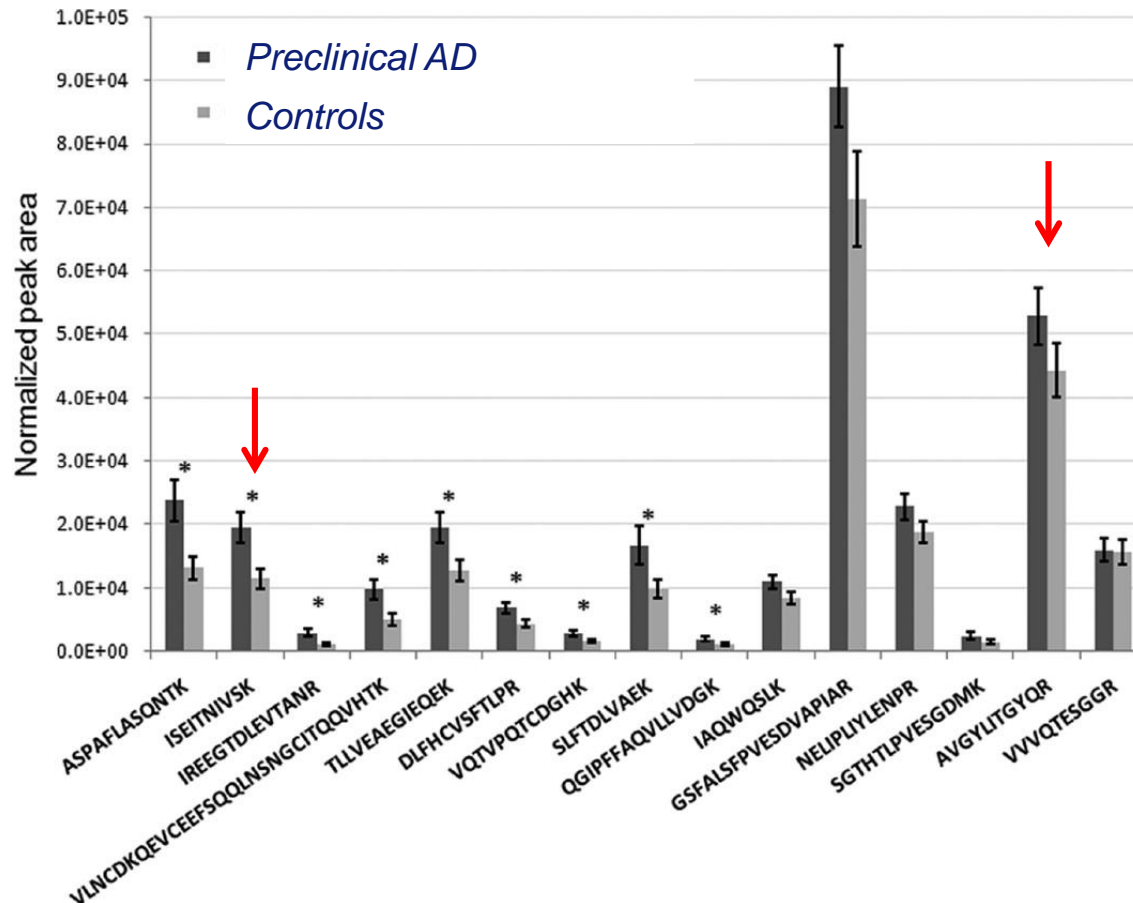


PZP/ microglia/ Amyloid β

- Pregnancy Zone Protein (PZP/CPAMD6)
- Pan-protease inhibitor
- 73% sequence homology with α 2-macroglobulin (α 2MG)
- Increased abundance in the AD brain
- Located to AD amyloid lesions

Targeted quantification of PZP in serum using SRM

Selection of PZP peptides to act as internal reference controls



15 unique PZP peptides observed in a shotgun proteomics approach

ISEITNIVSK
AVGYLITGYQR

Designing and optimizing an SRM assay for targeted PZP quantification in serum

Synthesis of selected peptides with stable isotope label (SIL) at **R** or **K**

- ≥98% pure
- Internal standard

Protein	Peptide sequence
PZP	ISEITNIVS K
	AVGYLITGYQ R
α2MG	AIGYLITGYQ R

Method optimization

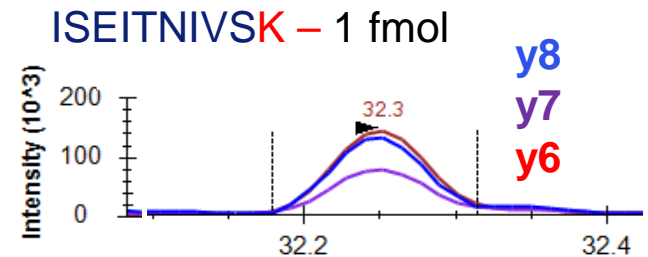
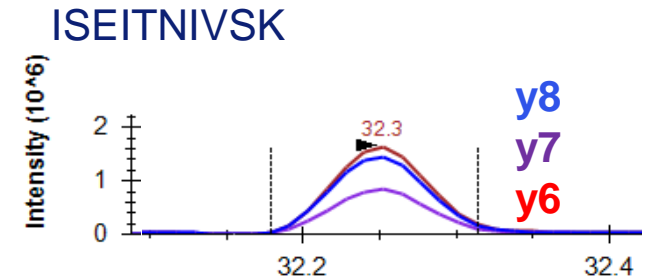
- Skyline
- Xevo TQ-S Triple Quadrupole
- nanoACQUITY UPLC
 - V/M Trap C18 column, 180µm x 20mm
 - 1.7µm BEH300 C18 analytical column, 75µm

Designing and optimizing an SRM assay for targeted PZP quantification in serum

3. Method optimization

- Transition selection

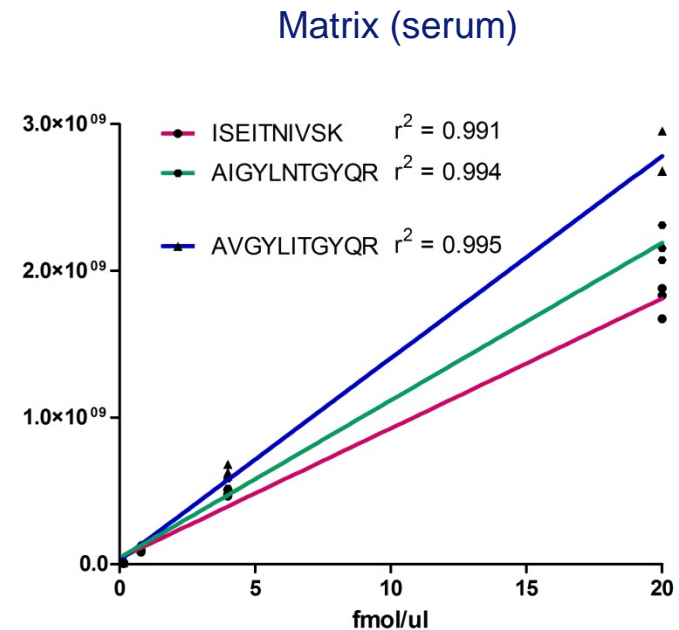
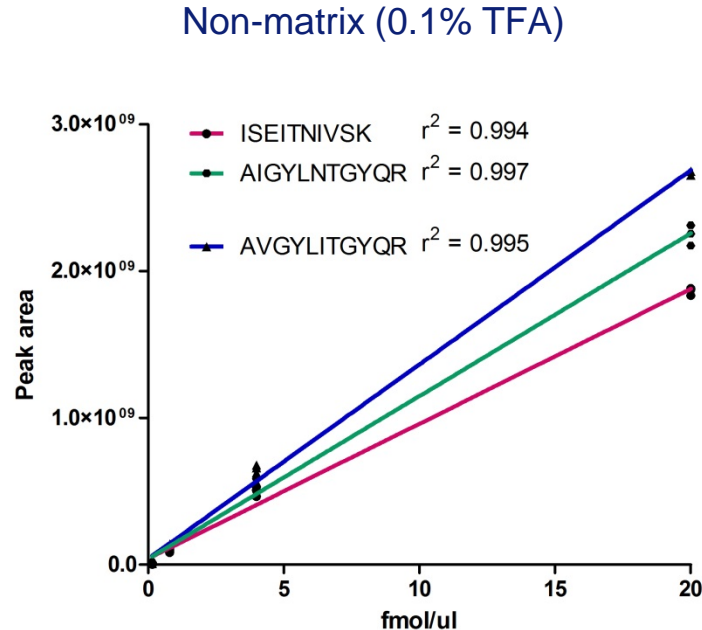
PZP peptides	y6	y7	y8	Collision energy
ISEITNIVSK (m/z 552.33 Da, +2)	661.39	774.47	903.52	15V
ISEITNIVSK (SIL) (m/z 556.33 Da, +2)	669.40	782.49	911.53	15V
	y4	y6	y7	
AVGYLITGYQR (m/z 620.84 Da, +2)	523.26	737.39	850.48	18V
AVGYLITGYQR (SIL) (m/z 625.84 Da, +2)	533.27	747.40	860.49	18V
α2MG peptides	y6	y7	y9	
AIGYLITGYQR (m/z 628.33 Da, +2)	738.35	851.44	1071.52	20V
AIGYLITGYQR (SIL) (m/z 633.33 Da, +2)	748.36	861.45	1081.53	20V



Designing and optimizing a selected reaction monitoring assay for targeted PZP quantification in serum

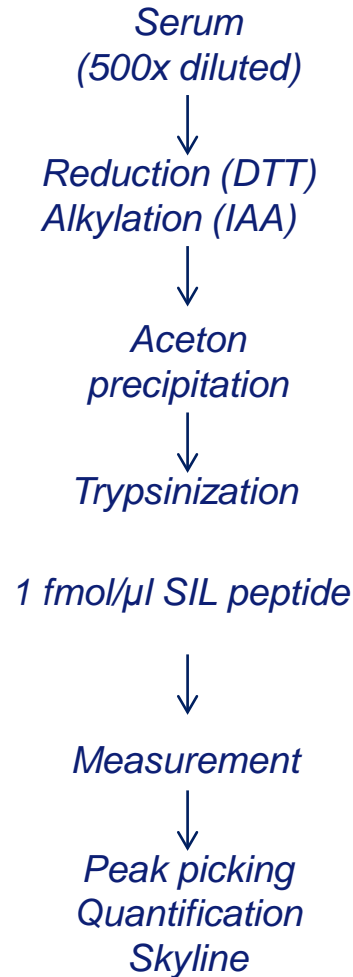
3. Method optimization

- Peptide calibration curves in 0.1% TFA and in biological matrix

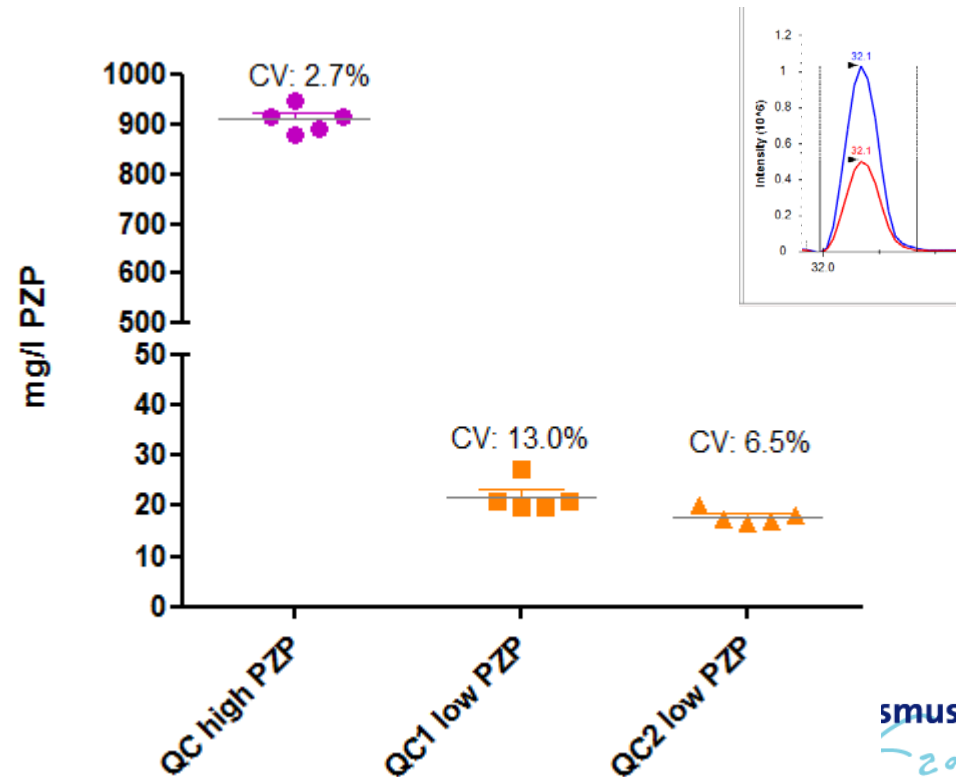


Designing and optimizing a selected reaction monitoring assay for targeted PZP quantification in serum

3. Method optimization - Measuring PZP in quality control serum samples

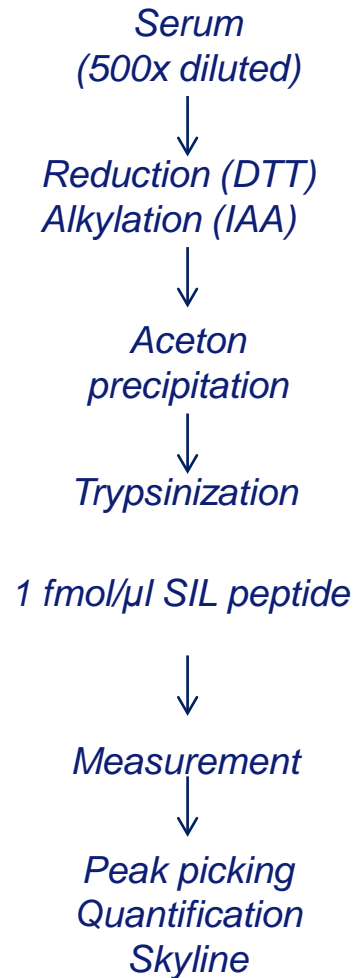


Technical replicates - ISEITNIVSK

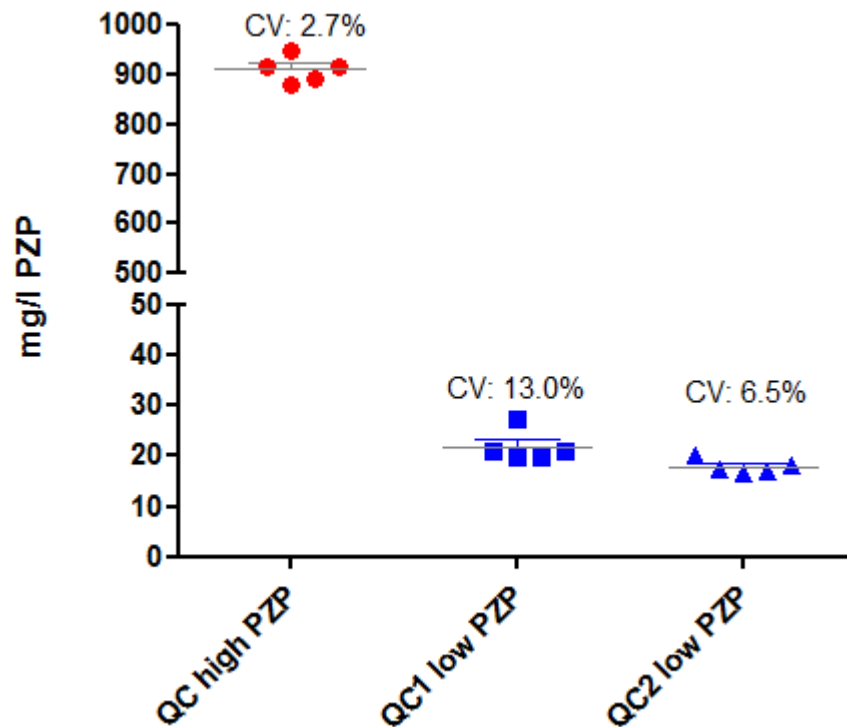


Designing and optimizing a selected reaction monitoring assay for targeted PZP quantification in serum

3. Method optimization - Measuring PZP in quality control serum samples



Biological replicates - ISEITNIVSK

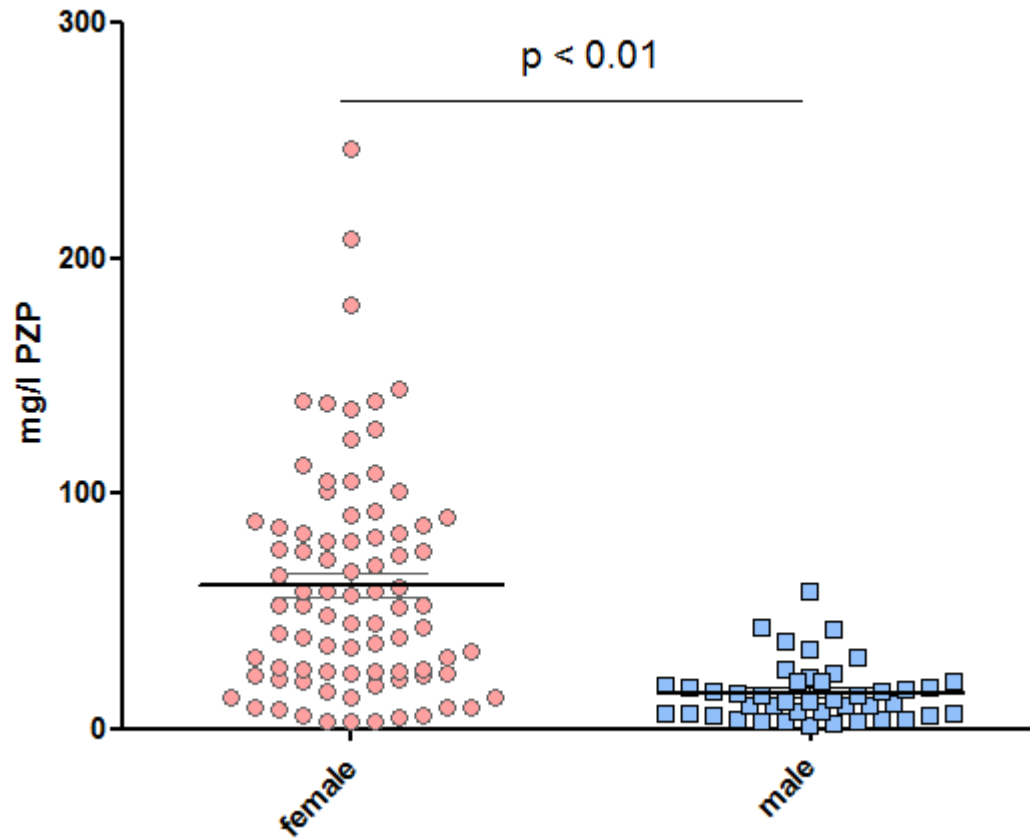


Measuring PZP in a population cohort

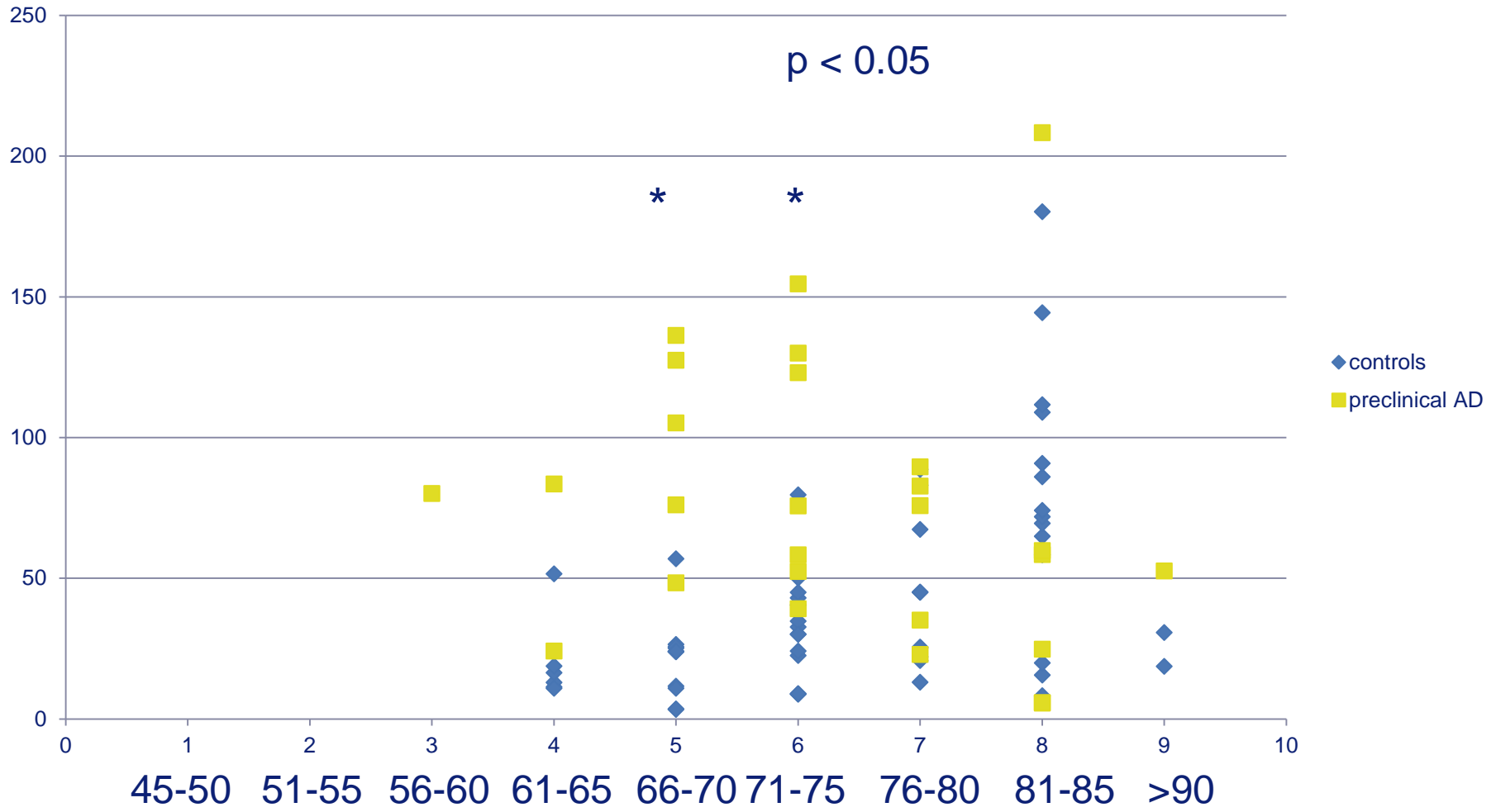
- Serum samples from the **Rotterdam Scan Study (RSS)**
 - Subset of the larger Rotterdam Study (15000 participants)
 - 1500 participants, no dementia at baseline
- 103 persons developed clinical AD (3 – 12 years later)
 - 42 male
 - 61 female
- 206 controls matched for age and gender



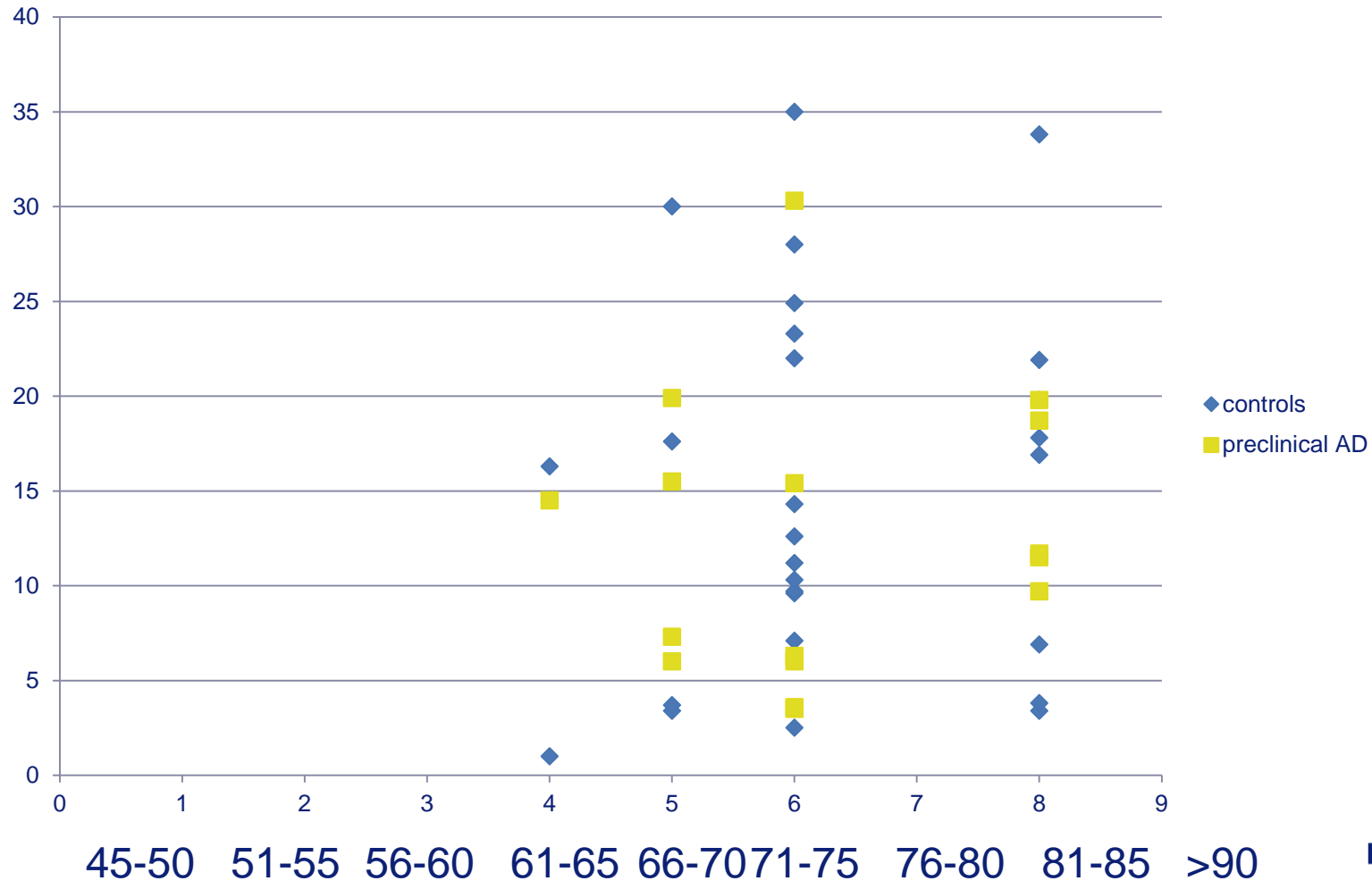
PZP levels are significantly higher in female than in male participants



Increased PZP levels in preclinical AD are only apparent in younger female cases



Age dependent increase in PZP was not observed in male preclinical AD cases



Conclusion/discussion

- Functional assay for quantification of PZP in serum
- Confirm that levels of PZP are increased in female preclinical AD cases in an age dependent manner
- Increase no longer apparent in older female participants
- Impacts the usefulness of PZP as a clinical biomarker

Acknowledgements



Arfan Ikram
Nakita Jainanduning
Christoph Stingl
Theo Luider

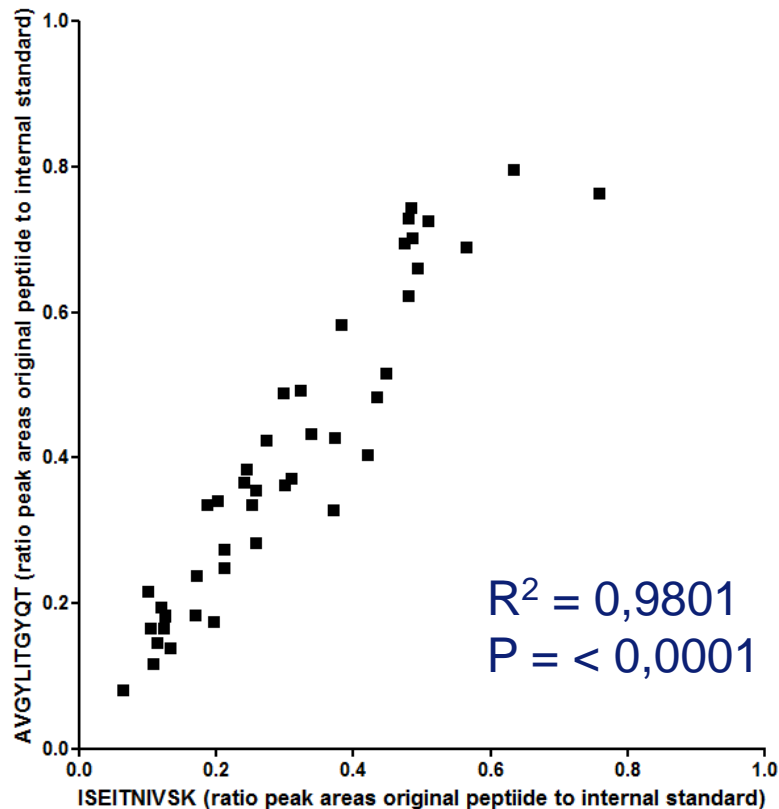


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Designing and optimizing a selected reaction monitoring assay for targeted PZP quantification in serum

3. Method optimization - Measuring PZP in quality control serum samples



Strong correlation between PZP peptides ISEITNIVSK and AVGYLITGYQR

ISEITNIVSK used as quantifier