

**Erasmus MC**

Universitair Medisch Centrum Rotterdam



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

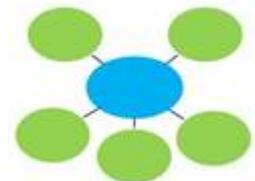
*Using a targeted proteomics approach*

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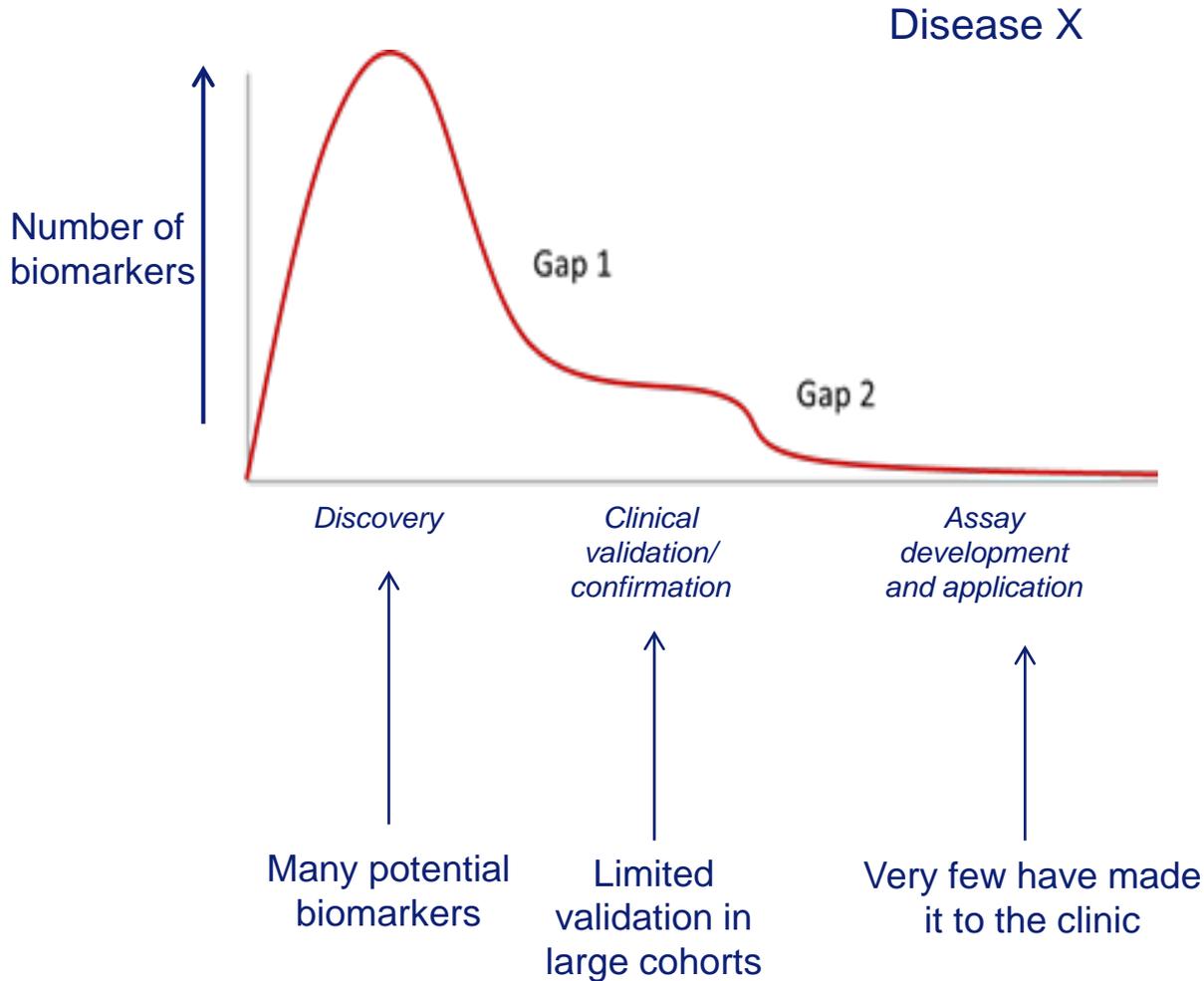
Skyline User Meeting, San Antonio, Texas

June 5th 2016



Biomarker Development Center

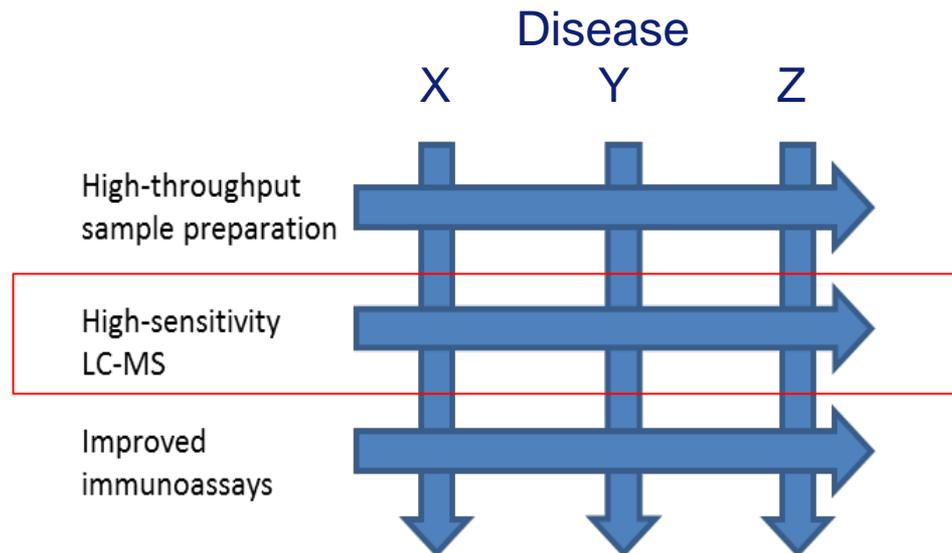
# Biomarker research faces several challenges



# Bridging the gaps

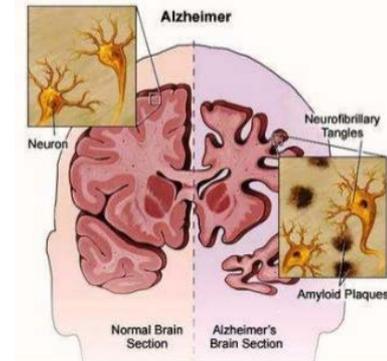
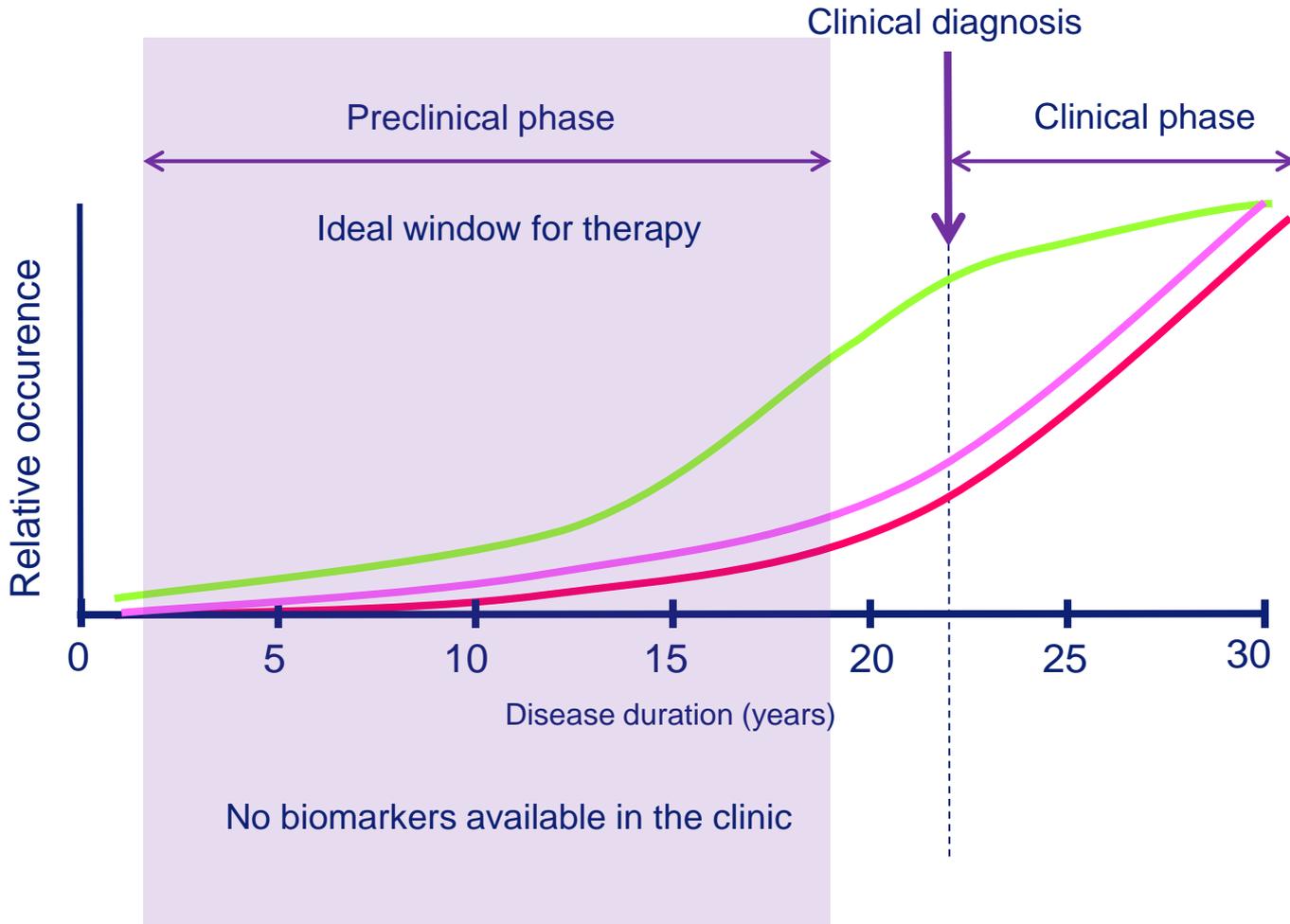
## Aims

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

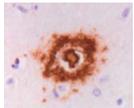


# Alzheimer's disease

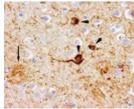
*A neurodegenerative disorder with a long preclinical phase*



— Aβ deposits

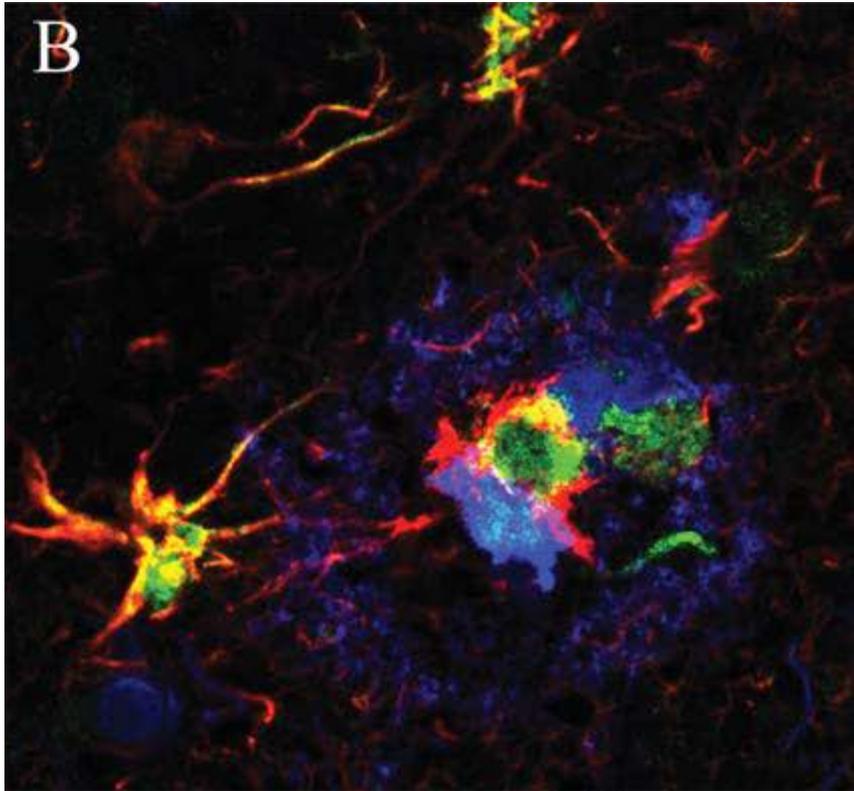


— Neurofibrillary tangles



— Cognitive impairment

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

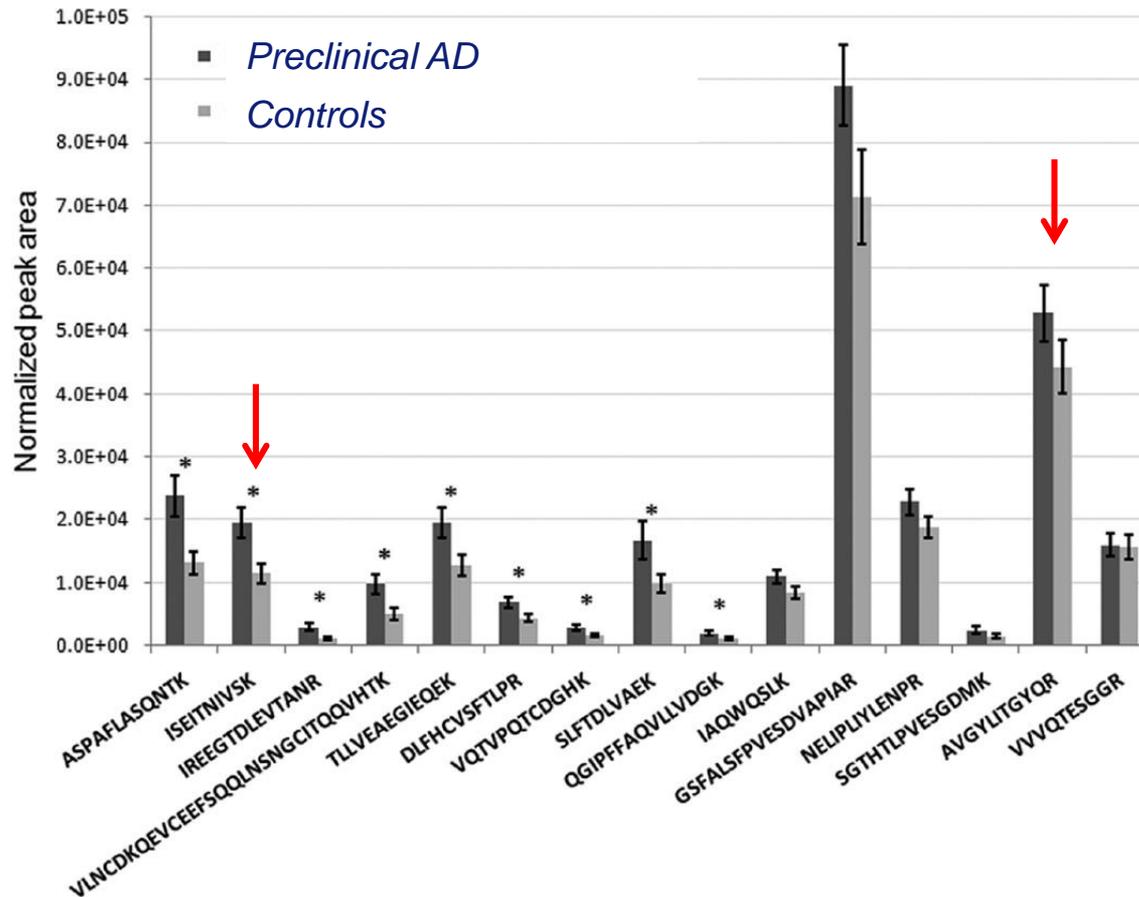


**PZP/ microglia/ Amyloid  $\beta$**

- Pregnancy Zone Protein (PZP/CPAMD6)
- Pan-protease inhibitor
- 73% sequence homology with  $\alpha$ 2-macroglobulin ( $\alpha$ 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 <b>K</b>
	AVGYLITGYQ <b>R</b>
α2MG	AIGYLITGYQ <b>R</b>

Method optimization

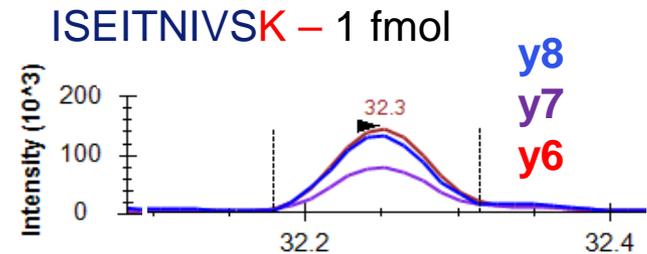
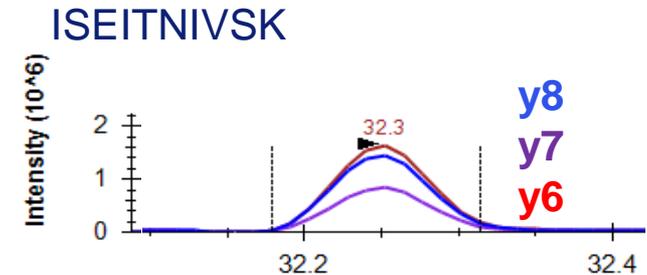
- Skyline
- Xevo TQ-S Triple Quadrupole
- nanoACQUITY UPLC
  - V/M Trap C18 column, 180µm x 20mm
  - 1.7µm BEH300 C18 analytical column, 75µm

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

## 3. Method optimization

- Transition selection

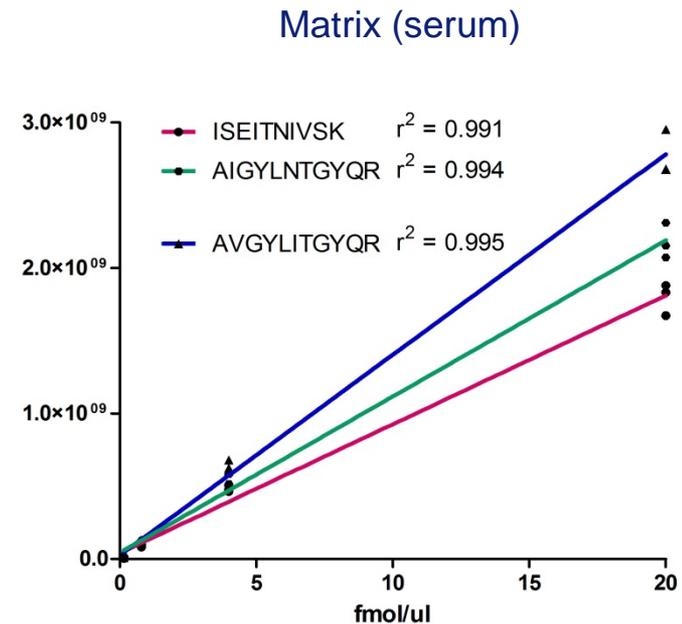
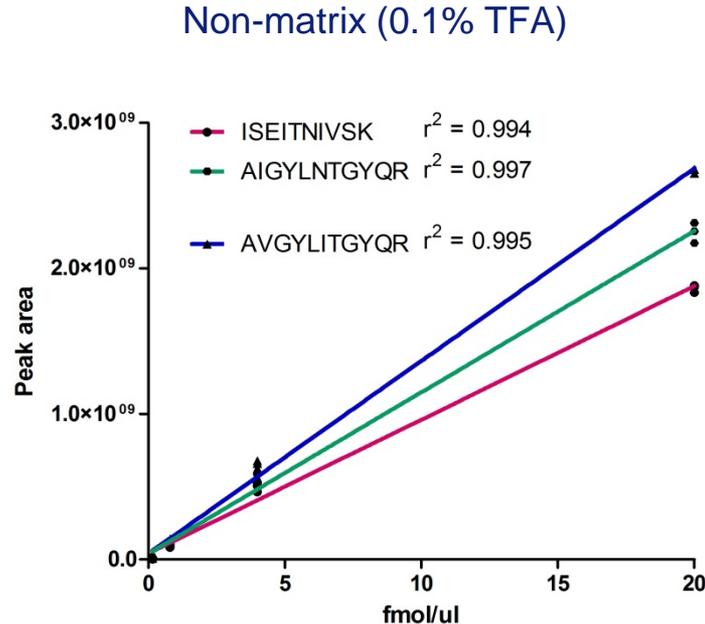
PZP peptides	y6	y7	y8	Collision energy
ISEITNIVSK (m/z 552.33 Da, +2)	661.39	774.47	903.52	15V
ISEITNIVSK (SIL) (m/z 556.33 Da, +2)	669.40	782.49	911.53	15V
	<b>y4</b>	<b>y6</b>	<b>y7</b>	
AVGYLITGYQR (m/z 620.84 Da, +2)	523.26	737.39	850.48	18V
AVGYLITGYQR (SIL) (m/z 625.84 Da, +2)	533.27	747.40	860.49	18V
<b>α2MG peptides</b>	<b>y6</b>	<b>y7</b>	<b>y9</b>	
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

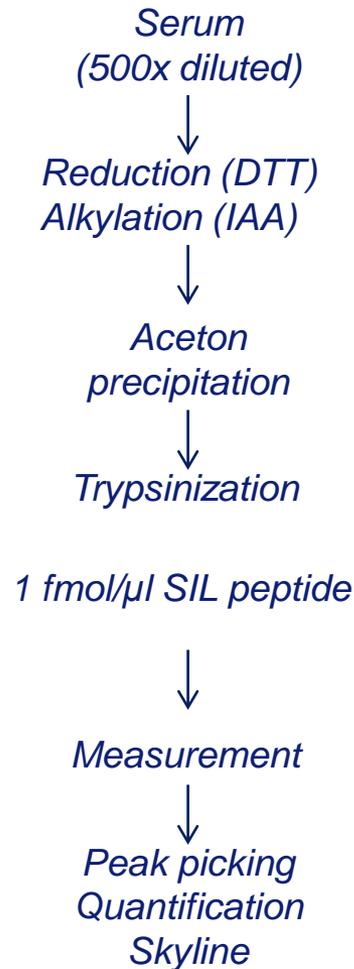
## 3. Method optimization

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

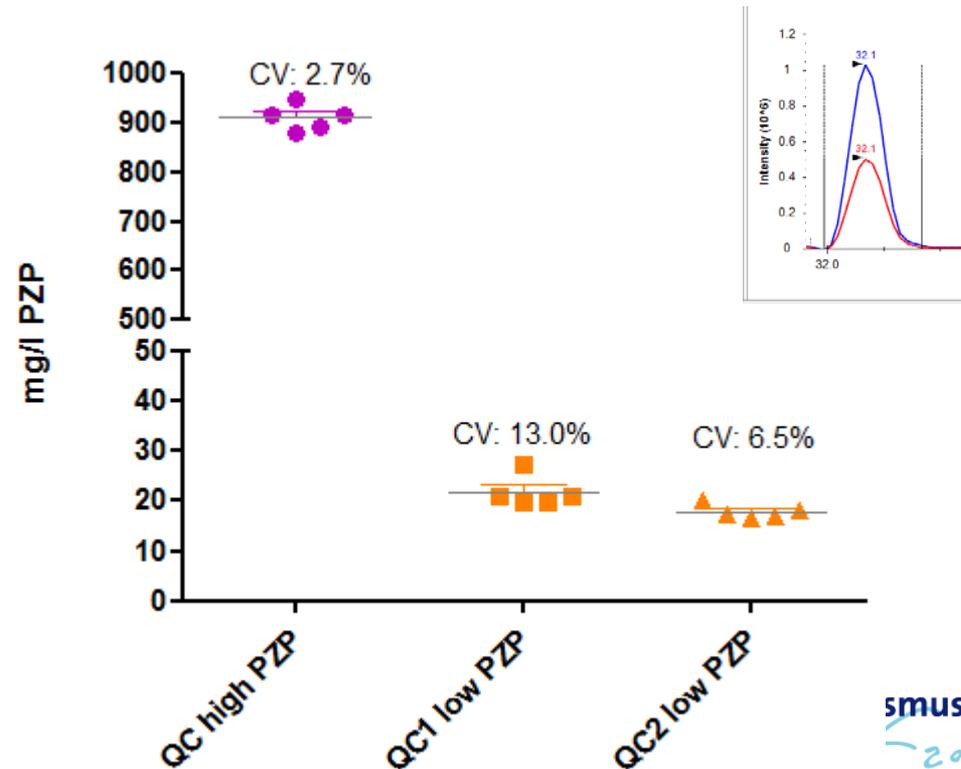


# Designing and optimizing a selected reaction monitoring assay for targeted PZP quantification in serum

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

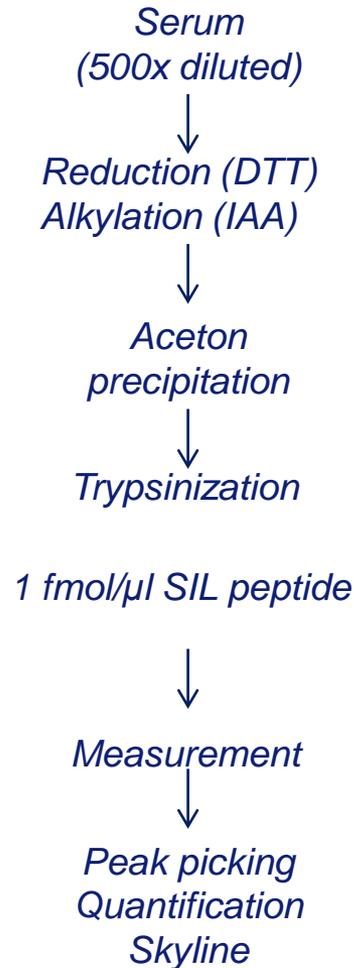


### Technical replicates - ISEITNIVSK

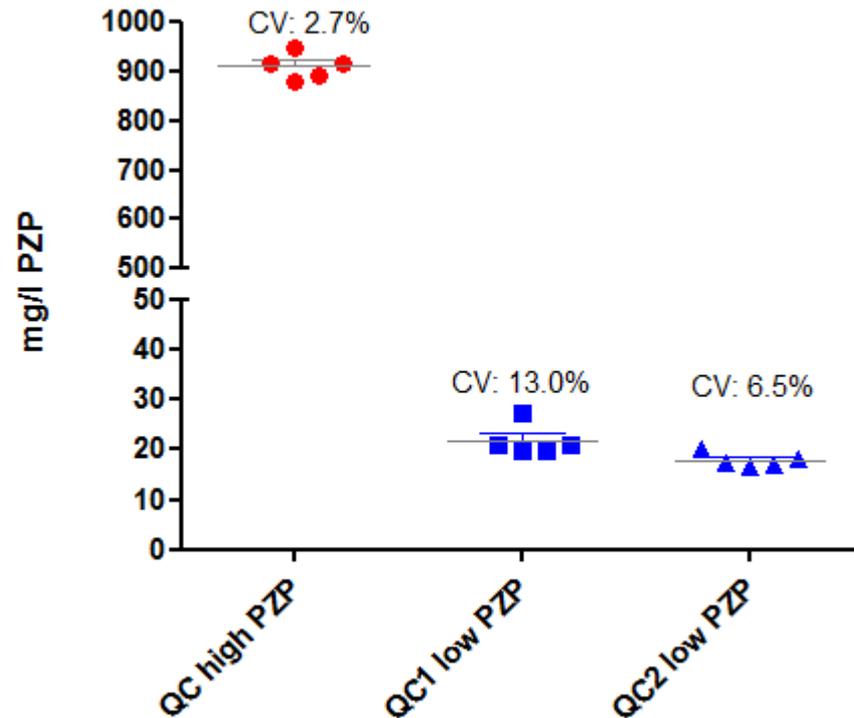


# Designing and optimizing a selected reaction monitoring assay for targeted PZP quantification in serum

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



Biological replicates - ISEITNIVSK

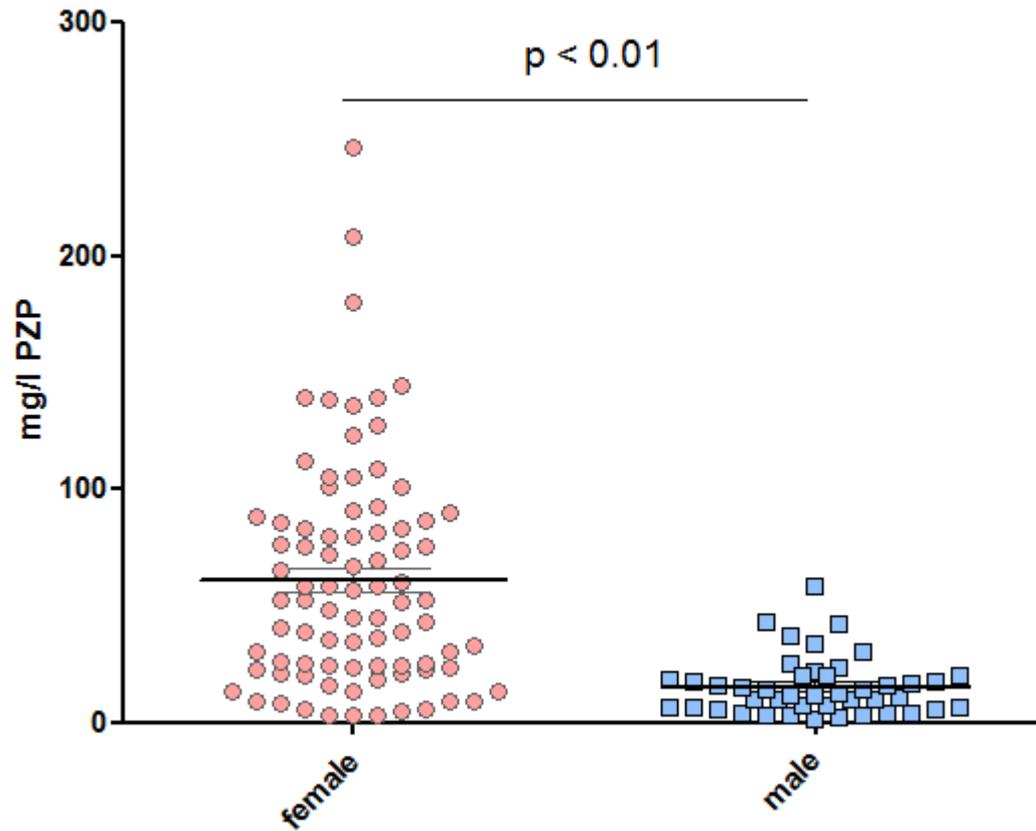


# Measuring PZP in a population cohort

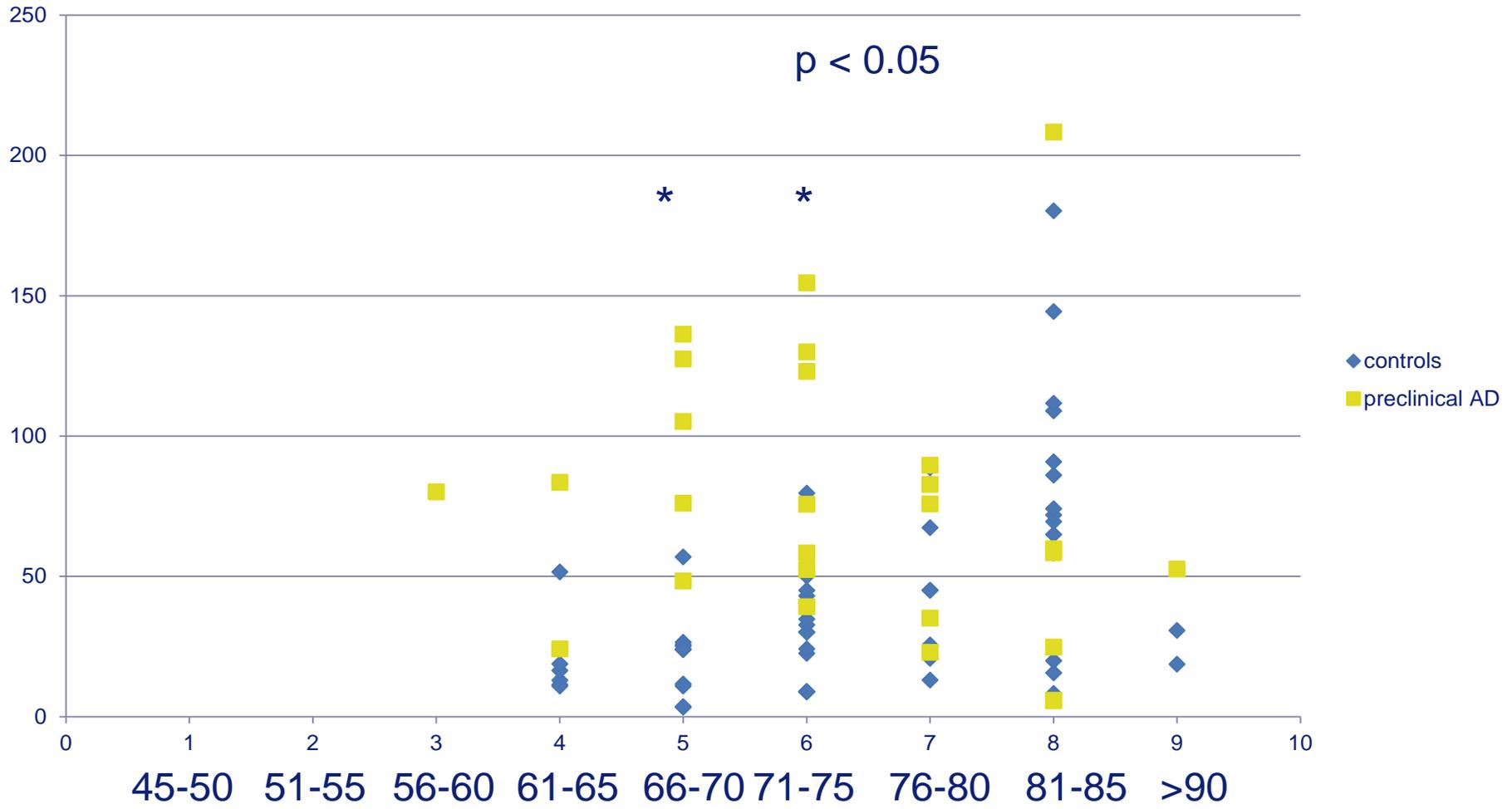
- Serum samples from the **Rotterdam Scan Study (RSS)**
  - Subset of the larger Rotterdam Study (15000 participants)
  - 1500 participants, no dementia at baseline
- 103 persons developed clinical AD (3 – 12 years later)
  - 42 male
  - 61 female
- 206 controls matched for age and gender



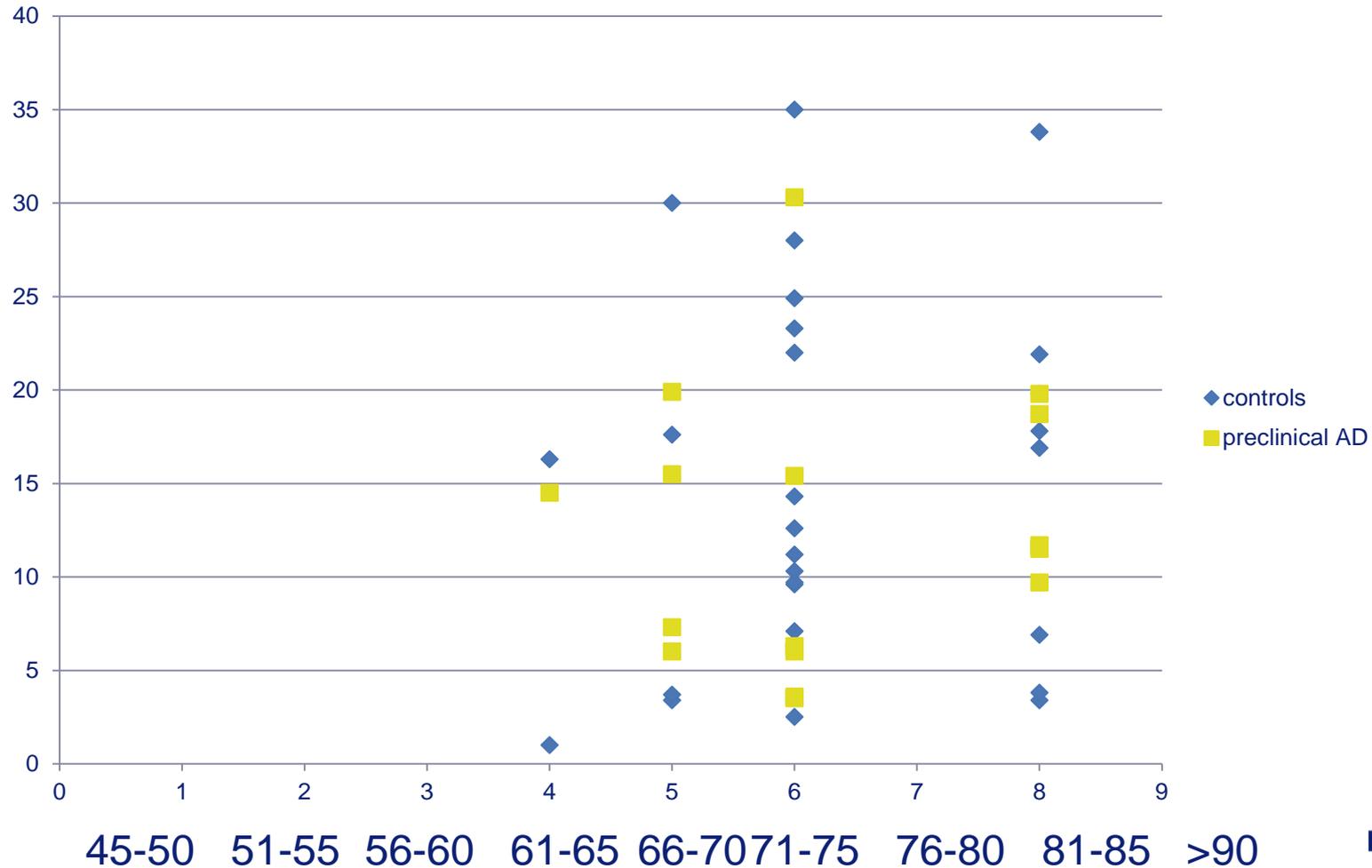
# PZP levels are significantly higher in female than in male participants



# 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



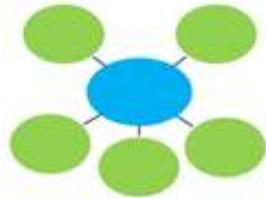
## Conclusion/discussion

- 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



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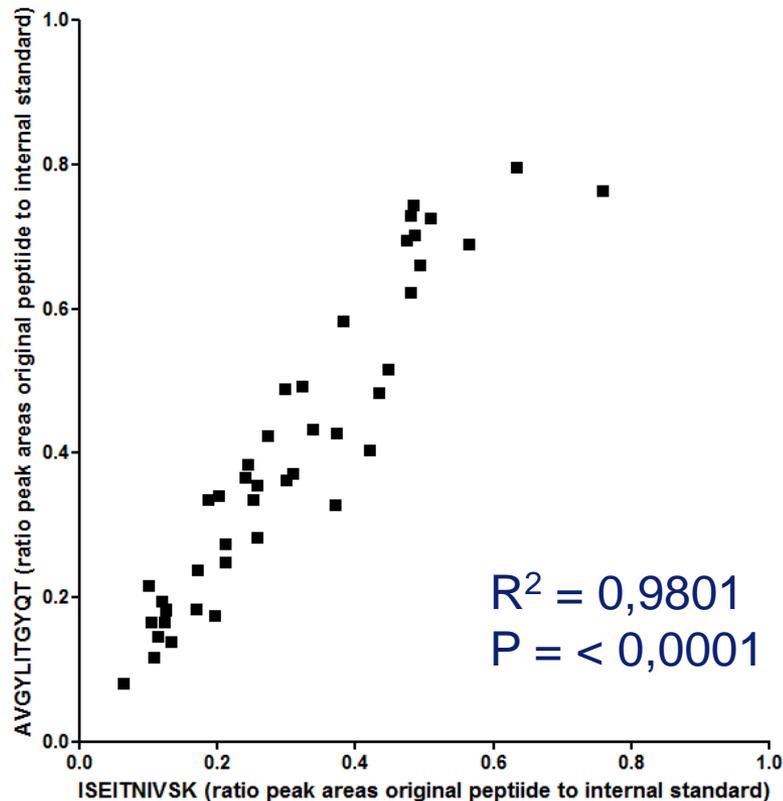


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# Designing and optimizing a selected reaction monitoring assay for targeted PZP quantification in serum

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



Strong correlation between PZP peptides ISEITNIVSK and AVGYLITGYQR

ISEITNIVSK used as quantifier