Domain-specific quantification of PrP in cerebrospinal fluid by targeted mass spectrometry

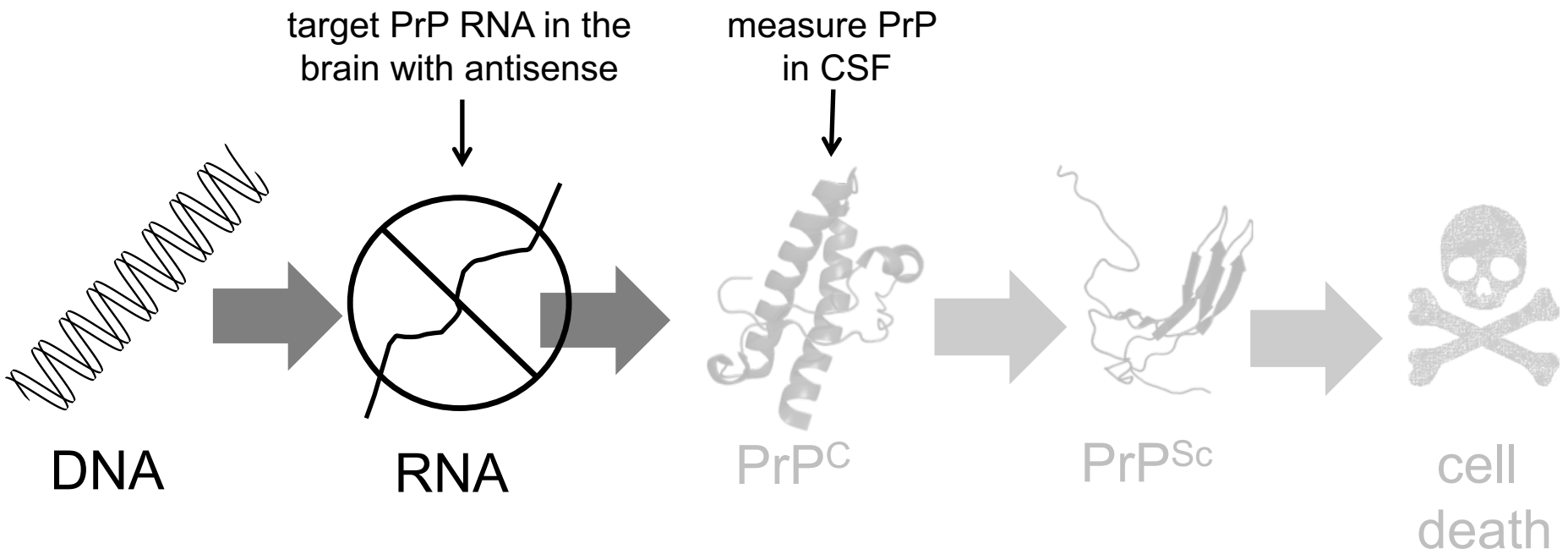
Pre-print: Minikel & Kuhn et al 2019, bioRxiv 591487
https://doi.org/10.1101/591487

Credits: Broad Proteomics Platform – Steve Carr, Eric Kuhn, Allie Cocco
Stuart Schreiber, Sonia Vallabh

Eric Vallabh Minikel
Presented at Prion2019, Edmonton, AB
May 21, 2019

BROAD INSTITUTE

CSF PrP as a pharmacodynamic biomarker



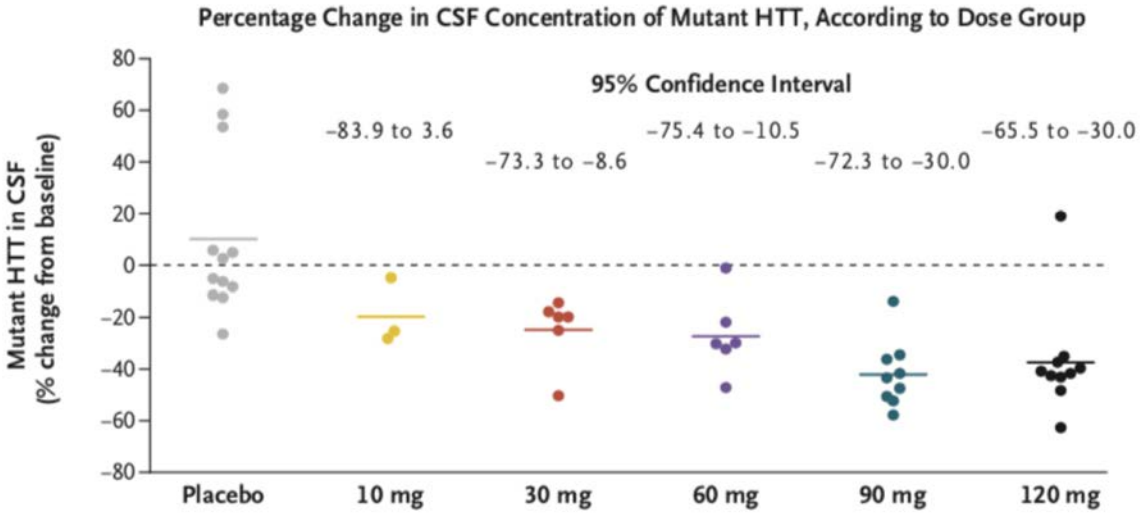
Why are pharmacodynamic biomarkers important?

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Targeting Huntingtin Expression in Patients with Huntington's Disease

Sarah J. Tabrizi, M.B., Ch.B., Ph.D., Blair R. Leavitt, M.D., C.M., G. Bernhard Landwehrmeyer, M.D., Edward J. Wild, M.B., B.Chir., Ph.D., Carsten Saft, M.D., Roger A. Barker, M.R.C.P., Ph.D., Nick F. Blair, M.B., B.S.,* David Craufurd, M.B., B.S., Josef Priller, M.D., Hugh Rickards, M.D., Anne Rosser, M.B., B.Chir., Ph.D., Holly B. Kordasiewicz, Ph.D., Christian Czech, Ph.D., Eric E. Swayze, Ph.D., Daniel A. Norris, Ph.D., Tiffany Baumann, B.S., Irene Gerlach, Ph.D., Scott A. Schobel, M.D., Erika Paz, B.S., Anne V. Smith, Ph.D., C. Frank Bennett, Ph.D., and Roger M. Lane, M.D.



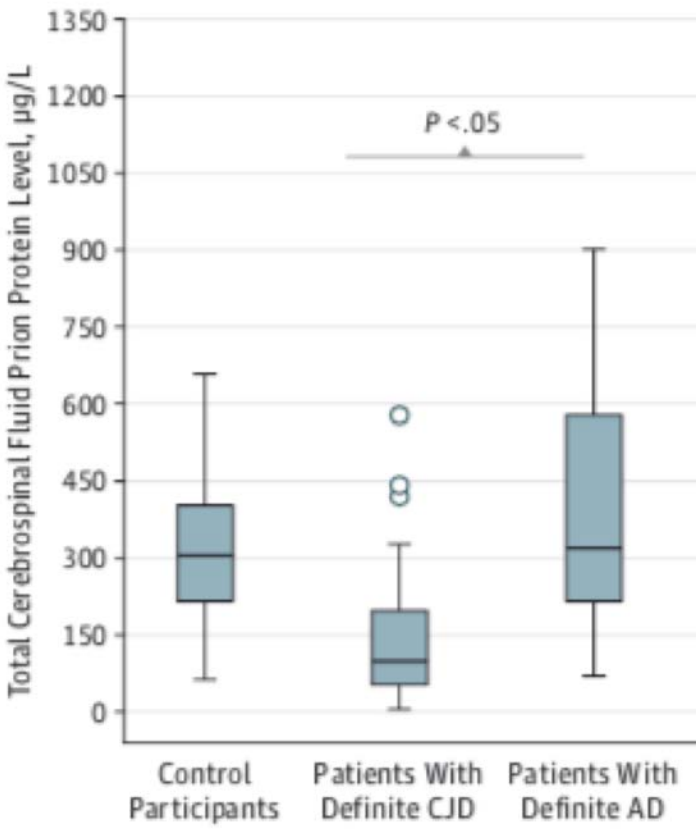
Pharmacodynamic biomarker can be measured in Phase I to...

- Confirm target engagement in living humans
- Guide dose selection for Phase II/III

Also has potential as surrogate endpoint

- See Sonia's talk! Thursday 2:55p

But, CSF PrP has also been studied as a disease biomarker



Journal of Alzheimer's Disease 17 (2009) 863–873
DOI 10.3233/JAD-2009-1110
IOS Press

Total Prion Protein Levels in the Cerebrospinal Fluid are Reduced in Patients with Various Neurological Disorders

Felix Meyne, Sara Friederike Gloeckner, Barbara Ciesielczyk, Uta Heinemann, Anna Krasnianski, Bettina Meissner and Inga Zerr*
National TSE Reference Center at Department of Neurology, Georg-August University, Göttingen, Germany

EJN EUROPEAN JOURNAL OF NEUROSCIENCE
European Journal of Neuroscience, Vol. 31, pp. 2024–2031, 2010 doi:10.1111/j.1460-9568.2010.07224.x
NEUROSYSTEMS

Codon 129 polymorphism and the E200K mutation do not affect the cellular prion protein isoform composition in the cerebrospinal fluid from patients with Creutzfeldt–Jakob disease

Matthias Schmitz,[1] Markus Schlomm,[2] Badiul Hasan,[1] Michael Beekes,[3] Eva Mitrova,[4] Carsten Korth,[5] Andreas Breit,[5] Julie Carimalo,[1] Joanna Gawinecka,[1] Daniela Varges[1] and Inga Zerr[1]
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[4]National Reference Center for Prion Diseases, Research Base of Slovak Medical University, Bratislava, Slovakia
[5]Institute of Neuropathology, Heinrich Heine University, Düsseldorf, Germany

Keywords: antibodies, CJD, CSF, isoform composition, PrP^{Sc}

Journal of Alzheimer's Disease 55 (2017) 1471–1480
DOI 10.3233/JAD-160740
IOS Press

Diagnostic Accuracy of a Combined Analysis of Cerebrospinal Fluid t-PrP, t-tau, p-tau, and $A\beta_{42}$ in the Differential Diagnosis of Creutzfeldt-Jakob Disease from Alzheimer's Disease with Emphasis on Atypical Disease Variants

Samir Abu Rumeileh[a], Francesca Lattanzio[a], Michelangelo Stanzani Maserati[b], Romana Rizzi[c], Sabina Capellari[a,b] and Piero Parchi[a,b,*]
[a]*Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy*
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[c]*Department of Neurology, IRCCS Arcispedale Santa Maria Nuova, Reggio Emilia, Italy*

Original Investigation

Association of Cerebrospinal Fluid Prion Protein Levels and the Distinction Between Alzheimer Disease and Creutzfeldt-Jakob Disease

Aline Dorey, PhD; Yannick Tholance, MSc, PharmD; Alain Vighetto, MD; Armand Perret-Liaudet, PharmD; Ingolf Lachman, PhD; Pierre Krolak-Salmon, MD, PhD; Uta Wagner, PhD; Hanne Struyfs, MSc; Peter P. De Deyn, MD, PhD; Benaissa El-Moualij, PhD; Willy Zorzi, PhD; David Meyronet, MD, PhD; Nathalie Streichenberger, MD; Sebastiaan Engelborghs, MD, PhD; Gabor G. Kovacs, MD, PhD; Isabelle Quadrio, PharmD, PhD

Cerebrospinal Fluid Total Prion Protein in the Spectrum of Prion Diseases

Anna Villar-Piqué[1] · Matthias Schmitz[1,2] · Ingolf Lachmann[3] · André Karch[4] · Olga Calero[5,6] · Christiane Stehmann[7] · Shannon Sarros[7] · Anna Ladogana[8] · Anna Poleggi[8] · Isabel Santana[9] · Isidre Ferrer[10,11] · Eva Mitrova[12] · Dana Žáková[12] · Maurizio Pocchiari[8] · Inês Baldeiras[9] · Miguel Calero[5,6] · Steven J. Collins[7,13] · Michael D. Geschwind[14] · Raquel Sánchez-Valle[15] · Inga Zerr[1,2] · Franc Llorens[1,11,16]

- CSF PrP drops by about half in symptomatic prion disease patients

Can CSF PrP work as a pharmacodynamic biomarker?

- Sonia has shown (Vallabh 2019 & MGH clinical study) that:
  - CSF PrP concentration can be precisely measured provided pre-analytical variability is minimized
  - CSF PrP is brain-derived
  - CSF PrP has good test-retest reliability in presymptomatic carriers
  - Pharmacodynamic effect of a PrP-lowering drug should be quantifiable in CSF

Prion protein quantification in human cerebrospinal fluid as a tool for prion disease drug development

Sonia M. Vallabh[a,b,c,1], Chloe K. Nobuhara[d], Franc Llorens[e,f], Inga Zerr[e], Piero Parchi[g,h], Sabina Capellari[g,i], Eric Kuhn[j], Jacob Klickstein[d], Jiri G. Safar[k,l], Flavia C. Nery[d,m], Kathryn J. Swoboda[d,m], Michael D. Geschwind[n], Henrik Zetterberg[o,p,q,r], Steven E. Arnold[d], Eric Vallabh Minikel[a,b,c,1], and Stuart L. Schreiber[a,1]

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Vallabh 2019, PMID: 30936307

Background

- Sonia has shown (Vallabh 2019 & MGH clinical study) that:
  - CSF PrP concentration can be precisely measured provided pre-analytical variability is minimized
  - CSF PrP is brain-derived
  - CSF PrP has good test-retest reliability in presymptomatic carriers
  - Pharmacodynamic effect of a PrP-lowering drug should be quantifiable in CSF
    - **Provided that there are no confounding disease-dependent changes in CSF PrP**
    - **CSF PrP concentration drops in symptomatic prion disease –** replicated by several investigators including Zerr, Llorens, Parchi, Schmitz, Dorey
    - **May limit use of this pharmacodynamic biomarker to presymptomatic individuals only**

Vallabh 2019, PMID: 30936307

Why does CSF PrP go down in symptomatic prion disease?

3 plausible biological reasons:

- PrP caught in plaques instead of shed into CSF?
- More PrP intracellular instead of on cell surface / shed into extracellular space?
- PrP downregulated as result of disease process? (Mays 2014)

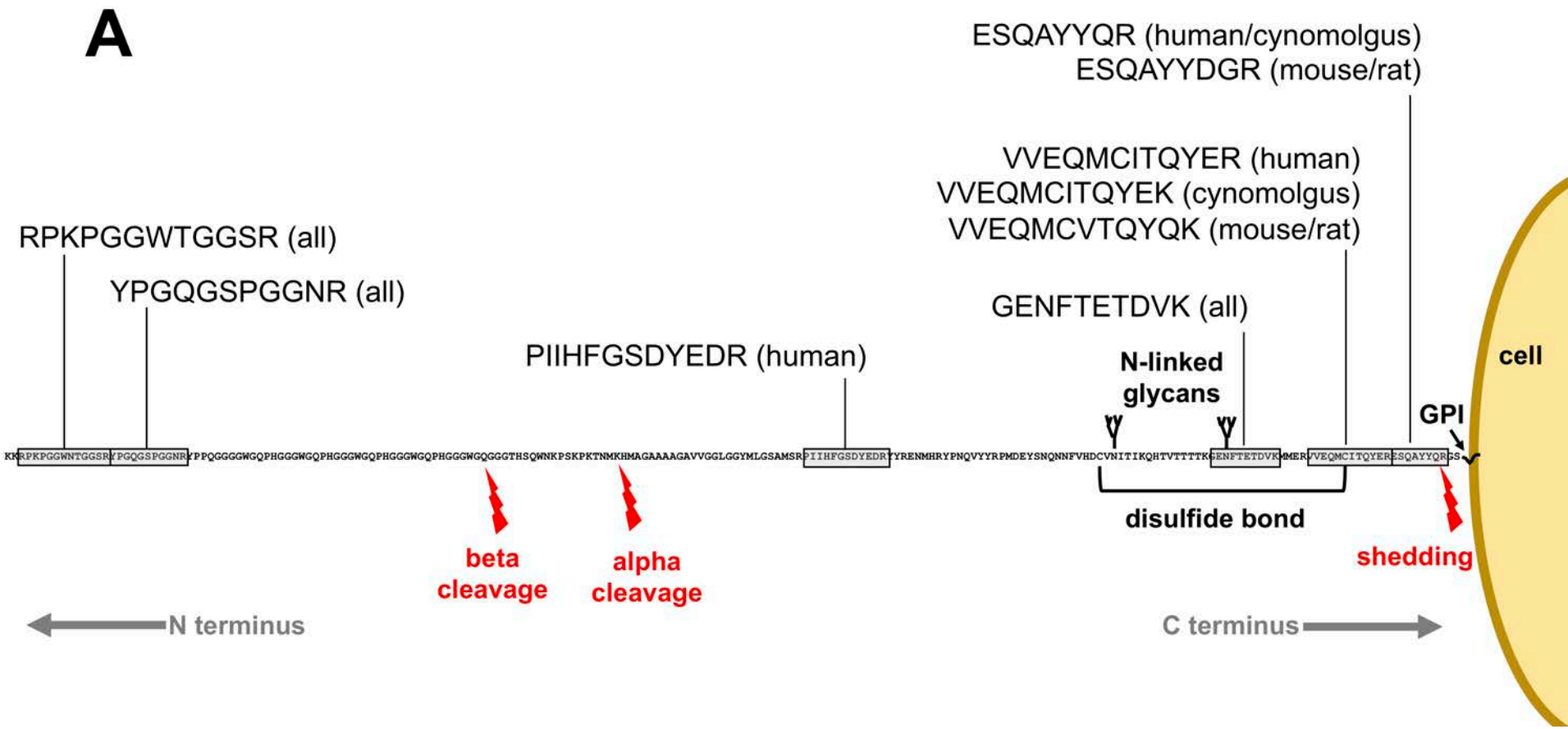
But also some possible artifactual reasons:

- ELISA epitopes misfolded / inaccessible to antibodies?
- PrP cleaved between the two ELISA epitopes?

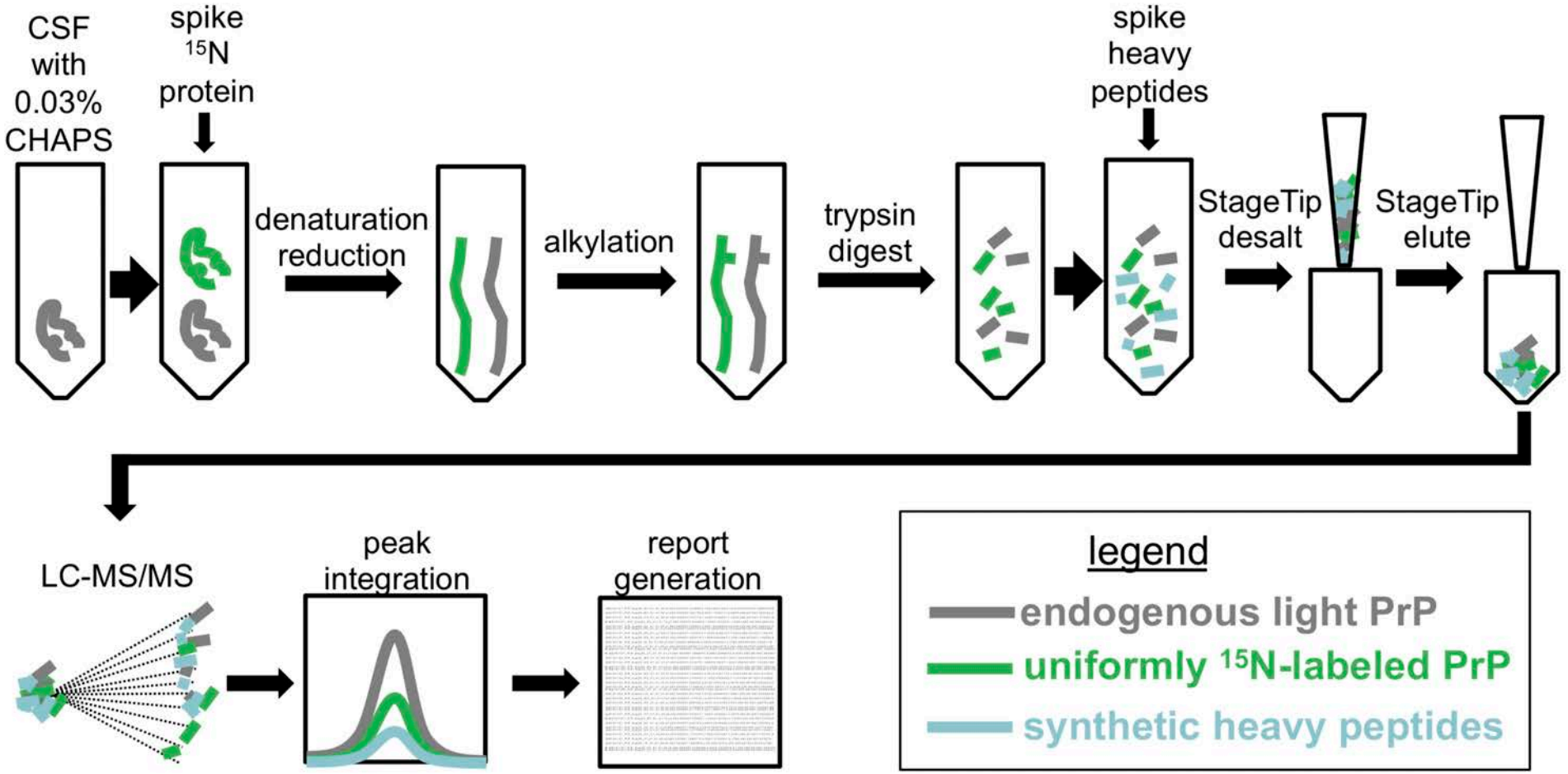
Need to develop an orthogonal method to quantify CSF PrP

- Determine if all domains of PrP are truly lowered in symptomatic CSF
- Cross-species sensitivity to support preclinical drug development
- Backup in case ELISA becomes unavailable, fails validation, etc.

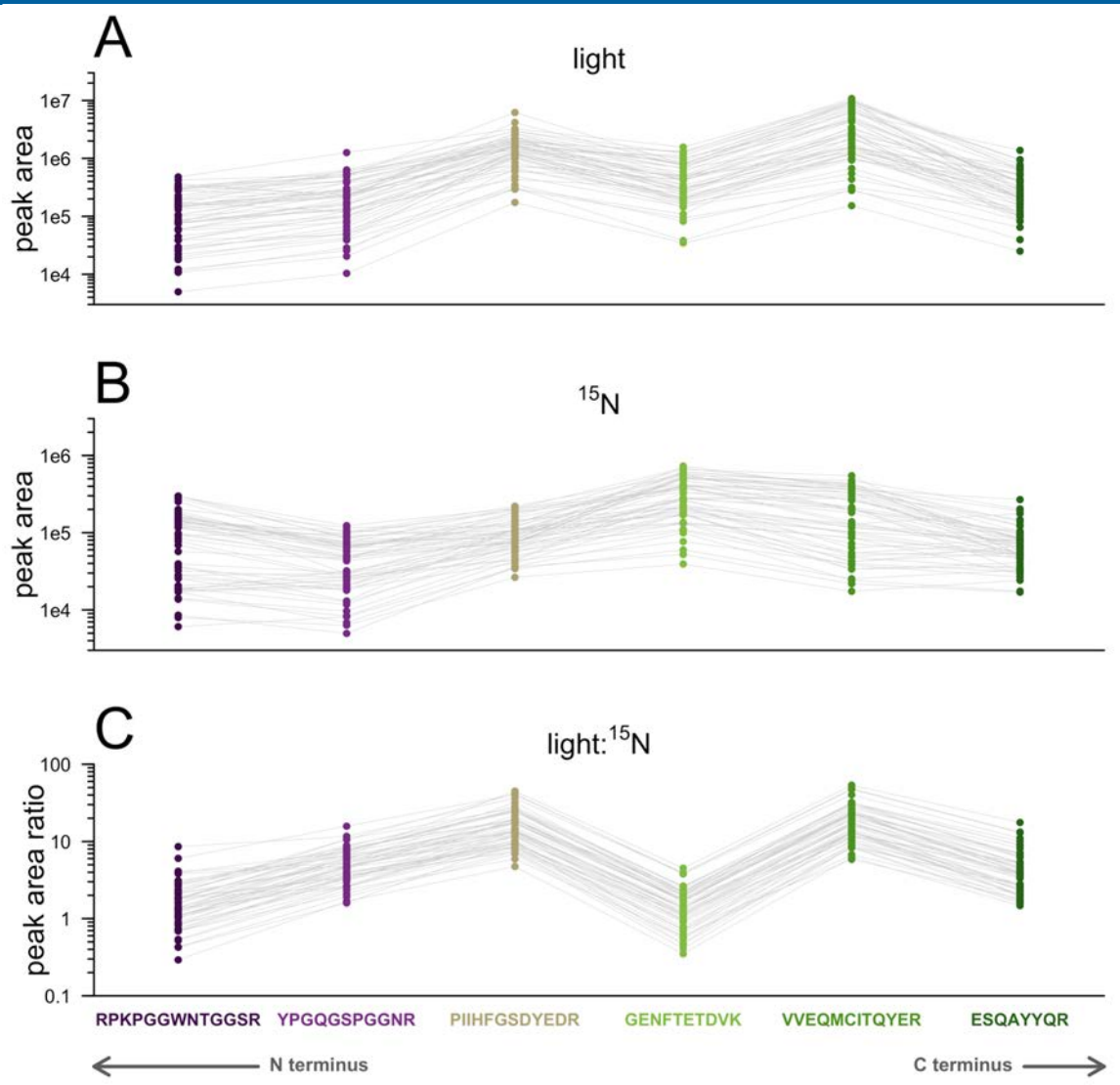
Choice of PrP tryptic peptides for monitoring



Design of multiple reaction monitoring (MRM) mass spec protocol



Relative abundance of the different PrP peptides



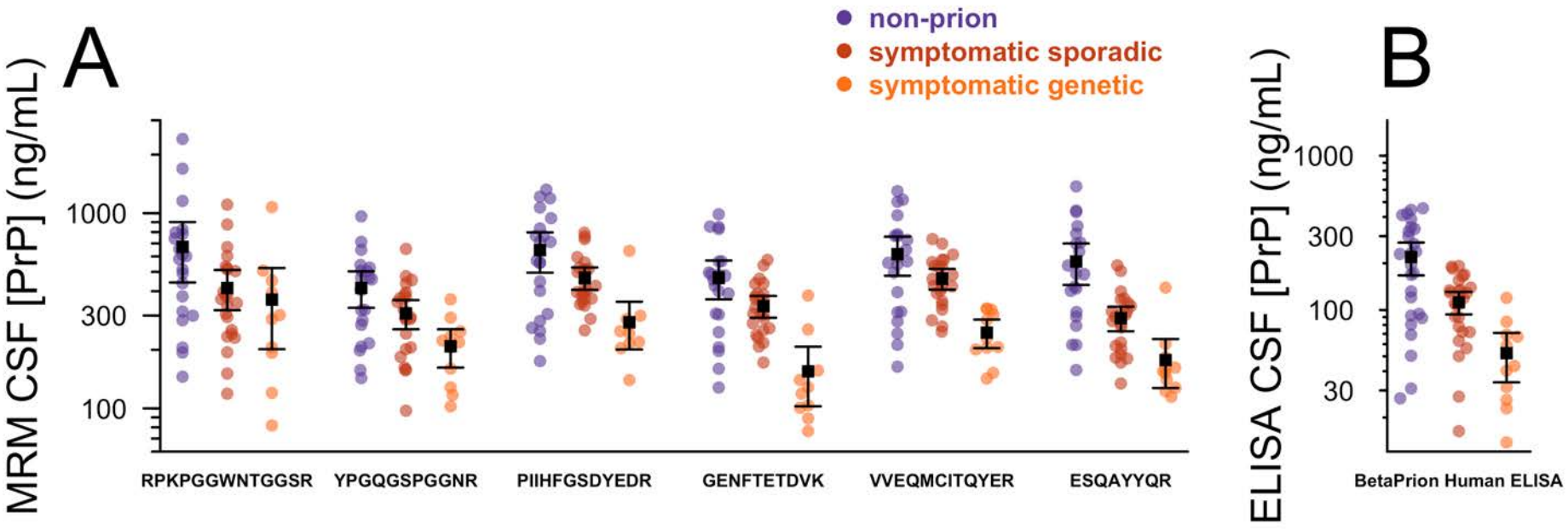
Technical variability is smaller than biological variability for all peptides

codons	peptide	mean intra-day CV	mean inter-day CV	inter-individual CV
25-37	RPKPGGWNTGGSR	10%	16%	80%
38-48	YPGQGSPGGNR	12%	22%	52%
137-148	PIIHFGSDYEDR	10%	12%	56%
195-204	GENFTETDVK	9%	12%	58%
209-220	VVEQMCITQYER	9%	12%	54%
221-228	ESQAYYQR	10%	18%	70%

- Each peptide is suited to independently report on the abundance of its domain of PrP in CSF

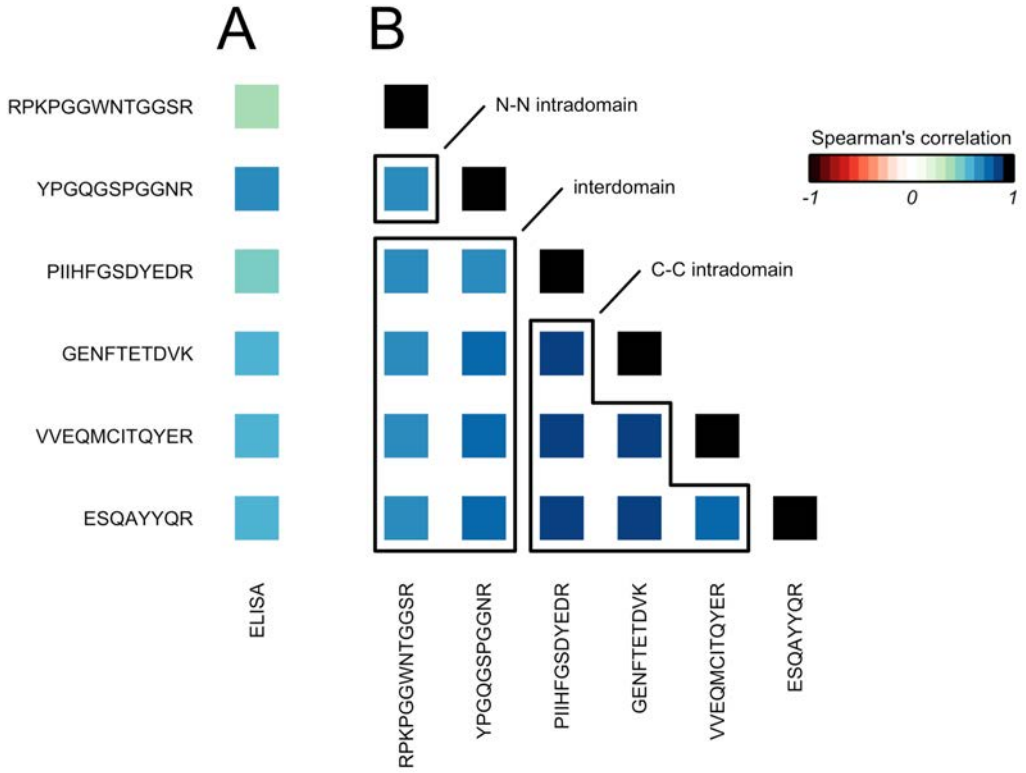
- Next: compare each peptide's abundance between diagnostic categories
  - *N*=55 blinded premortem CSF samples — rapidly progressive dementia cases referred to German & Italian prion surveillance later determined to be non-prion, sporadic, or genetic prion disease.

All PrP peptides are uniformly reduced in CSF in symptomatic prion disease



- Same pattern for each peptide as seen for ELISA in these same samples
- MRM (all peptides) and ELISA seem to be measuring the same thing

MRM and ELISA seem to be measuring the same thing



- Interpretation: both assays measure predominantly full-length PrP, **or** different PrP cleavage products may contribute but their relative abundance is not altered in the disease state

MRM cross-validates ELISA findings

- Detergent increases recovery of CSF PrP by MRM
- No correlation between MRM measurement of PrP concentration and CSF hemoglobin – confirms CSF PrP is from brain not blood
- Correlation between CSF PrP and CSF total protein is replicated by MRM
  - Might reflect true biology or pre-analytical factors, but is *not* just an ELISA "matrix interference" artifact — we confirm specificity of ELISA for PrP

Implications for developing PrP-lowering drugs

- Confirms that CSF PrP does go down in disease — pharmacodynamic readout of a PrP-lowering drug's effect may indeed be limited to pre-symptomatic individuals

- Confirms PrP is a simple, well-behaved analyte — supports its use as an endpoint

Thank you

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Funding: NIH F31 AI22592, Broad Next Gen Fund

Read the pre-print: Minikel & Kuhn et al 2019, bioRxiv 591487
https://doi.org/10.1101/591487