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

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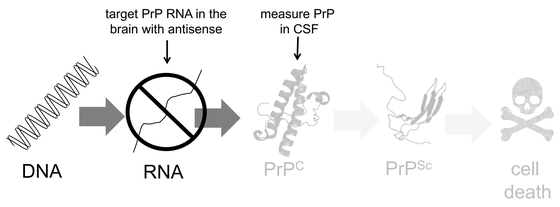
MOTIVATION

CSF PrP concentration will be important as a pharmacodynamic biomarker for PrP-lowering drugs.



PrP concentration as measured by ELISA drops in CSF during prion disease, potentially confounding the use of this biomarker in symptomatic patients.

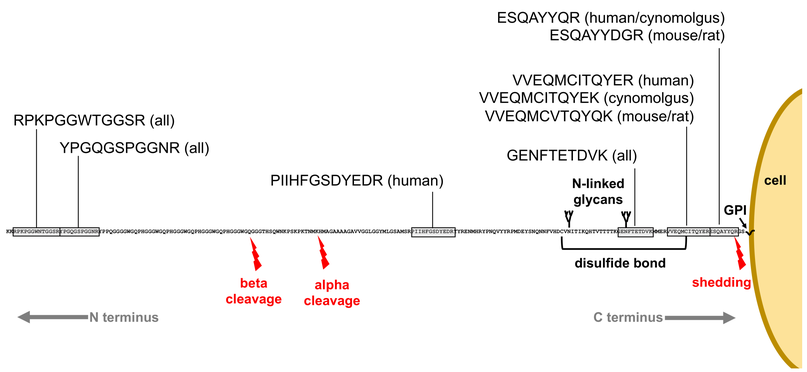
PrP misfolding and/or proteolysis could render PrP invisible to ELISA. What will we see if we measure different domains of PrP by observing individual peptides?

APPROACH

We developed prion protein multiple reaction monitoring (PrP MRM)— a targeted mass spectrometry assay to measure 9 tryptic peptides of PrP in CSF and brain.

We applied this assay to N=55 human CSF samples from individuals with or without prion disease to see how different PrP peptides behave.

CHOICE OF PEPTIDES

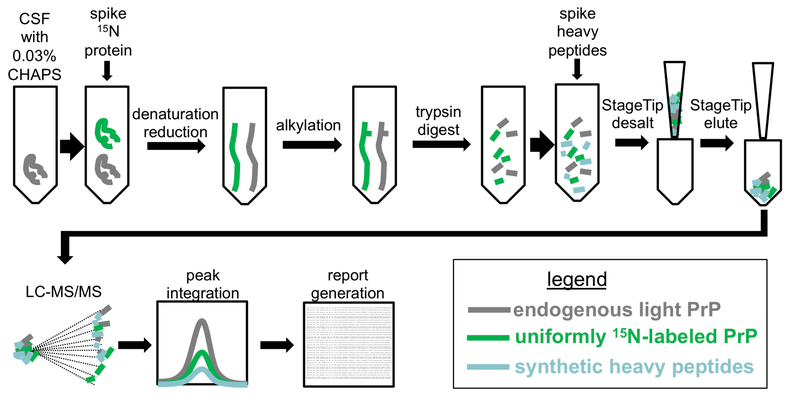


We selected nine peptides spanning N- and C-terminal domains of PrP, up and downstream of major proteolytic cleavage events.

We included orthologous peptides from preclinical species of interest.

Funding:
- NIH F31 AI22592
- Broad Next Gen Fund

METHOD DEVELOPMENT

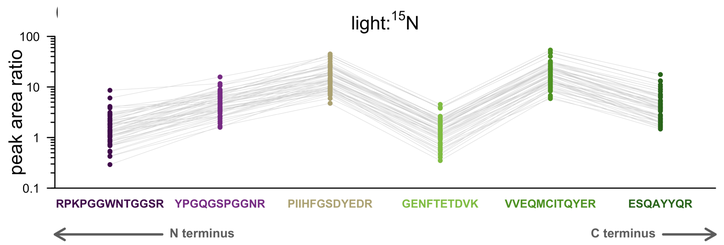


A denaturation step should help PrP to be quantified regardless of fold.

Trypsin digestion allows us to look at individual peptides regardless of whether they came from full-length or cleaved PrP.

Spiked known concentrations of isotopically labeled recombinant PrP and synthetic peptides permit quantification of endogenous PrP peptides.

PEPTIDE QUANTIFICATION



Peptides were quantified in human CSF in terms of light:^{15}N ratio. In general, high samples were high for every peptide, low samples were low for every peptide.

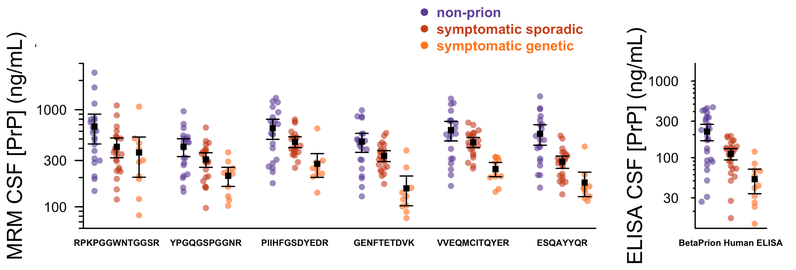
Some peptides were consistently higher than others. It is not clear how much this reflects abundance of different PrP isoforms in CSF, versus differences in mammalian vs. bacterially expressed PrP.

ANALYTICAL VALIDATION

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%

Signal (inter-individual variation) is larger than noise (technical replicate variation), so each peptide should be suited to independently report on the presence of its protein domain in human CSF.

COMPARISON ACROSS DIAGNOSTIC CATEGORIES

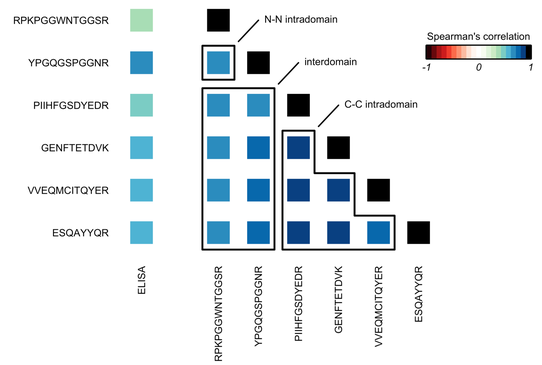


We analyzed CSF from *N*=55 rapidly progressive dementia cases referred for diagnostic testing and later found to have sporadic or genetic prion disease or a non-prion diagnosis.

Six of six peptides were uniformly reduced in prion disease CSF, mirroring the results from ELISA studies.

PrP MRM and ELISA may both measure predominantly full-length PrP, or, perhaps multiple isoforms of PrP contribute to CSF PrP, but their ratio does not change in the disease state.

MRM VS. ELISA



Across the N=55 samples, all peptides are strongly correlated with each other and with ELISA.

CONCLUSIONS

PrP is genuinely reduced in the CSF of symptomatic prion disease patients, and all domains of PrP are equally affected.

CSF PrP is likely to be most useful as a pharmcodynamic biomarker in pre-symptomatic individuals.

All peptides of PrP move in concert in the disease state. CSF PrP appears to be a simple, well-behaved analyte, supporting its use in trials.

LEARN MORE

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