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

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Credits: Broad Proteomics Platform – Steve Carr, Eric Kuhn, Allie Cocco
Stuart Schreiber, Sonia Vallabh
CSF PrP as a pharmacodynamic biomarker

target PrP RNA in the brain with antisense

measure PrP in CSF

DNA → RNA → PrP<sub>C</sub> → PrP<sub>Sc</sub> → cell death
Why are pharmacodynamic biomarkers important?

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.

- 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


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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

- RPKPGGWTGGSR (all)
- YPGQGSPGGNR (all)
- PIIHFGSDYEDR (human)
- ESQAYYQR (human/cynomolgus)
- ESQAYYDGR (mouse/rat)
- VVEQMCITQYER (human)
- VVEQMCITQYEK (cynomolgus)
- VVEQMCVTQYQK (mouse/rat)
- GENFTETDVK (all)

N-terminus: RPKPGGWTGGSR
C-terminus: GENFTETDVK

Disulfide bond: PIIHFGSDYEDR
N-linked glycans: VVEQMCITQYER
Shedding: VVEQMCITQYEK
Design of multiple reaction monitoring (MRM) mass spec protocol

CSF with 0.03% CHAPS → spike $^{15}$N protein → denaturation reduction → alkylation → trypsin digest → spike heavy peptides → StageTip desalt → StageTip elute → LC-MS/MS

Legend:
- endogenous light PrP
- uniformly $^{15}$N-labeled PrP
- synthetic heavy peptides
Relative abundance of the different PrP peptides

A

B

C

light

$^{15}\text{N}$

light:$^{15}\text{N}$
Technical variability is smaller than biological variability for all peptides

<table>
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<tr>
<th>codons</th>
<th>peptide</th>
<th>mean intra-day CV</th>
<th>mean inter-day CV</th>
<th>inter-individual CV</th>
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<tr>
<td>25-37</td>
<td>RPKPGGWNTGGSR</td>
<td>10%</td>
<td>16%</td>
<td>80%</td>
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<tr>
<td>38-48</td>
<td>YPGQGSPGGNMR</td>
<td>12%</td>
<td>22%</td>
<td>52%</td>
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<tr>
<td>137-148</td>
<td>PIHFGSDYEDR</td>
<td>10%</td>
<td>12%</td>
<td>56%</td>
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<tr>
<td>195-204</td>
<td>GENFTETDVK</td>
<td>9%</td>
<td>12%</td>
<td>58%</td>
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<tr>
<td>209-220</td>
<td>VVEQMCITQYER</td>
<td>9%</td>
<td>12%</td>
<td>54%</td>
</tr>
<tr>
<td>221-228</td>
<td>ESQAYYQR</td>
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<td>18%</td>
<td>70%</td>
</tr>
</tbody>
</table>

- 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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