Development of an *Intravitam* Diagnostic Test for Human Prion Diseases using Real Time QuIC and Enhanced QuIC Assays

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Transmissible spongiform encephalopathies (TSEs)

BSE (mad cow disease)

Chronic wasting disease (CWD)

Scrapie

Kuru, Creutzfeldt-Jakob Disease (CJD)
Diagnosis of Sporadic Creutzfeldt-Jakob Disease

➢ Diagnostic tools:
  - EEG: periodic sharp waves complex
  - CSF: Positive 14-3-3 protein
  - MRI: Hyperintensity in the basal ganglia and cortical regions
  - PRNP: codon 129 polymorphism

➢ Lack of an Intravital Diagnostic test

➢ Definite Diagnosis is based on neuropathology & detection of pathological PrP in the brain
Plate-based fluorescence detection of PrP\textsubscript{res}-seeded rPrP amyloid ("Real-Time QuIC")

Sample + Recombinant PrP\textsubscript{sen} + Thioflavin T

96-well plate

Shaking fluorescence plate reader

ThT fluorescence
(sum of 8 replicate wells)

Scrapie brain

Normal brain

Dilution containing:
1 SD\textsubscript{50} (RT-QuIC): 50% positive Seeding Dose
1 LD\textsubscript{50} (bioassay): 50% Lethal Dose

End-point titrations of scrapie brain homogenate

RT-QuIC assay (2 days)

Hamster bioassay (6-12 months)

Quantitation by end-point dilution analysis
- similar to, and as sensitive as, hamster bioassay
- but much faster (1-2 days vs months), higher throughput, and cheaper

RT-QuIC analyses of human sCJD CSF samples

- 85-89% overall sensitivity
- 99(?) - 100% specificity
  • much better than other CSF markers

Olfactory neural cells are the only surface neural cells in our body. Olfactory mucosa could be considered a «window to the brain».

Escada P. A. et al., 2009
Detection of PrPres in sCJD Olfactory Epithelium


STUDY:
- 9 neuropathologically confirmed sCJD patients
- **autopsy:** brain, cribriforum plate with attached olfactory mucosa and the surrounding respiratory epithelium

RESULTS:
- In 9/9 patients PrPres was found in neuroepithelium & central olfactory pathway
- **No** PrPres was found in the same tissues from age matched controls

Could olfactory epithelium be used for *in vivo* diagnosis of sCJD?
Olfactory mucosa brushing procedure

Orru & Bongianni et al., submitted 2013
OM samples spiked with $4 \times 10^{-7}$ fold dilution of sCJD brain homogenate show strong increase in fluorescence.

Reactions seeded with $4 \times 10^{-8}$ fold dilutions (~2fg of protease-resistant PrP$_{\text{CJD}}$) give slower amplification kinetics.
sCJD Olfactory Mucosa samples have significant levels of RT-QuIC prion seeding activity

Orru` & Bongianni et al., submitted 2013
Neat CSF (magenta traces) and 1:250 dilutions of OM pellet (blue traces) samples from sCJD patients

Orru` & Bongianni et al., submitted 2013
Summary of RT-QuIC results: OM vs. CSF

<table>
<thead>
<tr>
<th>Subjects</th>
<th>RT-QuIC Positive OM</th>
<th>RT-QuIC Positive CSF</th>
<th>Definite diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sCJD (n=14)</td>
<td>14/14</td>
<td>11/14</td>
<td>10/14</td>
</tr>
<tr>
<td>MM (n=10)</td>
<td>10/10</td>
<td>7/10</td>
<td>MM1 (7/10)</td>
</tr>
<tr>
<td>MV (n=3)</td>
<td>3/3</td>
<td>3/3</td>
<td>MV1 (1/3) MV2 (1/3)</td>
</tr>
<tr>
<td>VV (n=1)</td>
<td>1/1</td>
<td>1/1</td>
<td>VV2</td>
</tr>
<tr>
<td>Other Neurodegenerative Disorders (OND)* (n=9)</td>
<td>0/9</td>
<td>0/6†</td>
<td>1/9†</td>
</tr>
<tr>
<td>Normal controls** (n=17)</td>
<td>0/17</td>
<td>0/7</td>
<td>-</td>
</tr>
</tbody>
</table>

All sCJD subtypes are readily detectable by RT-QuIC: only a single test with a single substrate is needed to screen all sCJD phenotypes.

Orru` & Bongianni et al., submitted 2013
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Gender</th>
<th>Codon</th>
<th>Clinical signs at onset</th>
<th>CSF 14-3-3/ Tau protein levels (pg/ml)</th>
<th>Typical MRI*</th>
<th>EEG (PSWCs)</th>
<th>Disease duration (months)</th>
<th>Diagnosis at the time of OM brushing</th>
<th>Definite diagnosis</th>
<th>RT-QuIC</th>
<th>OM log SD&lt;sub&gt;10/µl&lt;/sub&gt;</th>
<th>CSF log SD&lt;sub&gt;10/µl&lt;/sub&gt;</th>
<th>Brain log SD&lt;sub&gt;10/mg&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77/M</td>
<td>MM</td>
<td>Ataxia, visual hallucinations</td>
<td>1st positive/ 1297 2nd positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>+</td>
<td>3.85</td>
<td>7.20</td>
</tr>
<tr>
<td>2</td>
<td>50/F</td>
<td>MM</td>
<td>Ataxia, behavioural changes</td>
<td>Positive / &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>6</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>+</td>
<td>4.10</td>
<td>7.95</td>
</tr>
<tr>
<td>3</td>
<td>68/F</td>
<td>MV</td>
<td>Ataxia, dementia</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>No</td>
<td>16</td>
<td>Probable sCJD</td>
<td>sCJD MV1</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>7.45</td>
</tr>
<tr>
<td>4</td>
<td>73/M</td>
<td>MM</td>
<td>Depression</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>8</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>+</td>
<td>3.35</td>
<td>7.95</td>
</tr>
<tr>
<td>5</td>
<td>64/F</td>
<td>VV</td>
<td>Ataxia</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>Probable sCJD</td>
<td>sCJD VV2</td>
<td>+</td>
<td>+</td>
<td>3.35</td>
<td>7.45</td>
</tr>
<tr>
<td>6</td>
<td>64/F</td>
<td>MV</td>
<td>Depression, ataxia</td>
<td>1st positive/ 892 2nd positive/ &gt;2400</td>
<td>Yes</td>
<td>No</td>
<td>17</td>
<td>Probable sCJD</td>
<td>sCJD MV2</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>66/M</td>
<td>MM</td>
<td>Visual hallucinations</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>2</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>Neg.</td>
<td>ND</td>
<td>8.2</td>
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<tr>
<td>8</td>
<td>68/F</td>
<td>MM</td>
<td>Ataxia, visual hallucinations</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>7.45</td>
</tr>
<tr>
<td>9</td>
<td>77/M</td>
<td>MM</td>
<td>Ataxia</td>
<td>Positive/ ND</td>
<td>No</td>
<td>No</td>
<td>2</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>+</td>
<td>3.60</td>
<td>7.70</td>
</tr>
<tr>
<td>10</td>
<td>69/M</td>
<td>MM</td>
<td>Apraxia, epileptic seizures</td>
<td>Positive/ &gt;2400</td>
<td>No</td>
<td>No</td>
<td>2</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>Neg.</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>75/F</td>
<td>MM</td>
<td>Ataxia</td>
<td>1st negative/ ND 2nd positive/ ND</td>
<td>Yes</td>
<td>No</td>
<td>Still alive (2)</td>
<td>Probable sCJD</td>
<td>Pending</td>
<td>+</td>
<td>Neg.</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>12</td>
<td>29/F</td>
<td>MM</td>
<td>Depression, choreic movements</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>No</td>
<td>Still alive (8)</td>
<td>Probable sCJD</td>
<td>Pending</td>
<td>+</td>
<td>Neg.</td>
<td>3.60</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>65/M</td>
<td>MV</td>
<td>Depression, ataxia, extrapyramidal signs</td>
<td>1st positive/ 2297 2nd positive/ &gt;2400</td>
<td>Yes</td>
<td>No</td>
<td>Still alive (18)</td>
<td>Probable sCJD</td>
<td>Pending</td>
<td>+</td>
<td>Neg.</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>61/M</td>
<td>MM</td>
<td>Ataxia, cortical blindness</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>Still alive (5)</td>
<td>Probable sCJD</td>
<td>Pending</td>
<td>+</td>
<td>+</td>
<td>3.60</td>
<td>ND</td>
</tr>
</tbody>
</table>
Conclusions

- OM brush sampling is non-invasive, rapid, patient friendly
- RT-QuIC detection of prion seeding activity in OMs showed 100% sensitivity & specificity
- High sCJD prion seeding activity in OMs suggests that infectivity might be present: biosafety implications?
- Development of highly sensitive seeding assays of OM brushings for intravital diagnosis of other brain proteinopathies might be possible

Submitted manuscript:

“RT-QuIC of olfactory neuroepithelium brushings as a definitive intravital test for sCJD diagnosis”
Christina D. Orrú, Matilde Bongianni, Giovanni Tonoli, Sergio Ferrari, Andrew G. Hughson, Bradley R. Groveman, Michele Fiorini, Maurizio Pocchiarì, Salvatore Monaco, Byron Caughey & Gianluigi Zanusso
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