Development of an ante-mortem & pre-symptomatic diagnostic test for human prion diseases using RT-QuIC & eQuIC assays

Rocky Mountain Labs
National Institute for Allergy & Infectious Diseases

12th Annual CJD Foundation Family Conference

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Human prion diseases

- **INFECTIOUS:**
  - Kuru (cannibalism)
  - iatrogenic CJD (e.g. contaminated surgical instruments, prion-infected growth hormone injections)
  - variant CJD (from BSE-infected cattle)

- **FAMILIAL:**
  - prion protein mutations
  - familial CJD
  - Gerstmann-Sträussler-Scheinker syndrome (GSS)
  - fatal familial insomnia (FFI)

- **SPORADIC:**
  - no known prion protein mutations
  - probably spontaneous disease
  - 1 case per 2 million people annually worldwide
  - accounts for 95% of human TSE
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 prion-seeded PrP amyloid (Real-time Quaking-Induced Conversion: RT-QuIC)

- Extremely sensitive: up to 1 billion-fold amplification!
- Quantitative & Disease specific
- Much faster & cheaper than similarly sensitive tests

Wilham, Orrú, Bessen et al, PLoS Pathogens, 2010
RT-QuIC analysis of human cerebrospinal fluid (CSF)

- 85-89% overall sensitivity
- 99-100% specificity
  • much better than other CSF markers
Olfactory mucosa brushing procedure

Orru, Bongianni, Caughey & Zanusso et al., NEJM 2014
RT-QuIC analysis of olfactory mucosa samples from CJD & control patients
RT-QuIC of olfactory mucosa from CJD & control patients: Brush vs. Swab
### RT-QuIC of olfactory mucosa from CJD & control patients: Brush vs. Swab

<table>
<thead>
<tr>
<th></th>
<th>Brush 1</th>
<th>Swab 1</th>
<th>Swab 2</th>
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<tbody>
<tr>
<td>Patient 1</td>
<td>+</td>
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<td>Patient 2</td>
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<tr>
<td>Patient 18</td>
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</tr>
</tbody>
</table>

![Graph showing % Maximum ThT Fluorescence for different patients using Brush 1, Swab 1, and Swab 2](image-url)
RT-QuIC of olfactory mucosa from CJD & control patients: Brush vs. Swab
Higher temperatures allow faster identification of sCJD samples.
In vivo analysis of Olfactory Mucosa & Cerebro Spinal Fluid from sCJD patients and negative controls: preliminary update

Raymond G. & Orru C. & Caughey C., manuscript in preparation 2014
Faster & more sensitive RT-QuIC analysis of sCJD CSF

42°C

55°C

Average ThT Fluorescence

Time (h)
RT-QuIC detection of Genetic Human Prion Diseases

Orru C., Gambetti & Caughey, manuscript in preparation 2014
RT-QuIC detection of Genetic Human Prion Diseases

Orru C., Gambetti & Caughey, manuscript in preparation 2014
RT-QuIC analysis of olfactory mucosa samples from genetic CJD (E200K) & control patients
Conclusions

- Successfully tested additional pannel of sCJD OM & CSF samples
  - sensitivity ≥97% & specificity 100%
- Comparable sensitivity from samples collected by Cyto-brushes or Flocked swab
- Faster RT-QuIC detection of prion seeding activity in OM & CSF samples
- Efficient detection of prion seeding activity in gCJD$^{E200K}$ OM}
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[Image: A group photo of researchers outside Rocky Mountain Laboratories.]