Progress in detecting prions and diagnosing prion diseases

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Transmissible spongiform encephalopathies (prion) diseases
Slow, fatal, transmissible neurodegenerative diseases

BSE
(mad cow disease)

chronic wasting disease
(CWD)

scrapie

Kuru, Creutzfeldt-Jakob disease (CJD)
Human TSE (prion) diseases

**SPORADIC:**
- Creutzfeldt-Jakob disease (sCJD)
- 1 case per 2 million people annually worldwide
- accounts for 95% of human TSE
- no known prion protein mutations
- probably spontaneous disease

**FAMILIAL:**
- familial CJD
- Gerstmann-Sträussler-Scheinker syndrome
- fatal familial insomnia
- prion protein mutations

**ACQUIRED:**
- kuru
- iatrogenic CJD (from medical mistakes)
  - neurosurgery, dura mater and corneal transplants, growth hormone
- variant CJD (from BSE-infected cattle)
TSE prions: a strange new class of infectious agent

- Little immune response by host
- No genes of its own
- Hard to decontaminate

An infectious protein

Purified prion amyloid
How infectious proteins (prion) reproduce: an abnormal form of a host protein

Shape change, clumping

normal protein

Infectious pathologic (prion) form

Host cell

Input: 1 infectious unit
Output: 1-100 billion infectious units!
Spreading routes for human prions

Kraus, Groveman & Caughey, Ann Rev Micro 2013
The need for practical, sensitive detection of prions

Definitive diagnostic tests
• preclinical
• early clinical

Rapid, sensitive assays for prion infectivity
• diagnosis
• detecting contamination:
  • blood
  • transplanted organs
  • foods, feeds, dietary supplements
  • other agricultural products
  • biotechnology products
  • pharmaceuticals
  • medical devices
  • cosmetics
  • environment

Current diagnosis of sCJD

Probable CJD
- Clinical features (remarkably heterogeneous)
- EEG
- MRI
- 14-3-3 protein or tau tests (cerebrospinal fluid)

Definite CJD
- PrP<sub>CJD</sub> deposition in brain tissue

Living patients
Biopsy or autopsy

In progress:
Definitive tests based on detecting PrP<sub>CJD</sub> in living patients
Need multiple tests:

- primary & confirmatory
- improve specificity, minimize false positives
- cope with wide range of sample types and applications
Plate-based fluorescence detection of prion-seeded PrP amyloid (Real-time Quaking-Induced Conversion: RT-QuIC)

- Sample + Normal PrP protein + Amyloid stain

96-well plate → Shaking fluorescence plate reader

ThT fluorescence (sum of 8 replicate wells)

- Scrapie brain
  - $10^{-3}$ - $10^{-8}$
- Normal brain
  - $10^{-9}$
  - $10^{-10}$
  - $10^{-4}$ - $10^{-7}$

- Infectious PrP seed → Normal PrP (sensor or substrate) → PrP amyloid with fluorescent stain

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

J. Wilham  C. Orrú
RT-QuIC tests for TSE prions

**Demonstrated applications:**
- human variant CJD, sporadic CJD, genetic TSEs
- rodent-adapted scrapie
- sheep scrapie (classical & Nor98)
- deer/elk CWD
- cattle BSE (classical & L-type)

**Accessible diagnostic specimens:**

**Cerebrospinal fluid** (humans, hamsters, cervids, sheep):
- Orrù et al., *mBio* 2015

**Nasal fluid, brushings** (humans, hamsters):

**Blood** (humans, sheep, hamsters, mice):
- Orrù et al, *mBio* 2011

**Saliva** (deer):

**Urine** (deer):
Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion

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Real Time Quaking-Induced Conversion Analysis of Cerebrospinal Fluid in Sporadic Creutzfeldt–Jakob Disease

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RT-QuIC of CSF as a diagnostic test for human sCJD

➤ 77-89% sensitivity
(% sCJD giving positive test)

➤ 99-100% specificity
(% non-sCJD giving negative test)

First disease-specific diagnostic test not requiring brain biopsy or post-mortem analysis.

Implementation by many CJD diagnostic centers around the world.
New conditions improve speed and sensitivity of RT-QuIC testing of human cerebrospinal fluid for Creutzfeldt-Jakob disease

CD Orrú, BR Groveman, AG Hughson, G Zanusso, MB Coulthart and B Caughey, *mBio* 2015
New conditions improve speed and sensitivity of RT-QuIC testing of human cerebrospinal fluid for Creutzfeldt-Jakob disease

- Faster, stronger RT-QuIC responses using new conditions
  - Reduced from days to hours.
- Positive RT-QuIC assays from 46 of 48 CJD cases but not from 39 non-CJD patients
  - 96% sensitivity
  - 100% specificity
- Similar results obtained by 2 other labs.
- New conditions improve performance and practicality of definitive diagnostic test for CJD.

CD Orrú, BR Groveman, AG Hughson, G Zanusso, MB Coulthart and B Caughey, *mBio* 2015
Olfactory neural cells are a surface exposed “window to the brain”. Escada et al., 2009
Nasal brushing procedure for collecting diagnostic specimens for RT-QuIC

• Positive RT-QuIC assays from 42 of 43 CJD cases but not from 43 non-CJD patients
  ➢ ≥97% sensitivity
  ➢ 100% specificity

• RT-QuIC of CSF samples (old conditions) from the same patients
  ➢ 79% sensitivity
  ➢ 100% specificity

• Nasal brushings provide potential basis for a definitive, less-invasive, definitive antemortem diagnostic test for CJD.

• Brushings contained \(~10^5\)-\(10^7\) prion seeds.
  ➢ infectivity lining the nasal cavity???

Bank vole PrP (produced in bacteria) as an apparently universal sensor molecule (substrate) for RT-QuIC

- BV rPrP$^\text{sen}$ has detected all (n=28) types of prions tested so far by RT-QuIC, including 5 (red) not detectable previously.
- Sensitivity is often comparable to best known sensor(s) for that prion.

Discriminating sporadic and variant CJD using bank vole (BV) and hamster (Ha) sensor molecules

**Humans:** + using BV rPrP$_{Sen}$

Stronger + with Ha rPrP$_{Sen}$ → sCJD

Much weaker or neg with Ha rPrP$_{Sen}$ → vCJD

Conclusions: RT-QuIC assays

- Increasingly practical, sensitive and specific
- Bank Vole PrP: a universal (so far) sensor molecule for RT-QuIC
- Prion strain discrimination:
  - Relative detection with different rPrP sen substrates
  - Biochemical comparison of RT-QuIC reaction products
Future prospects…

➢ Similar assays might be possible for many protein misfolding diseases involving amyloids.

➢ Patients with early neurological signs could be tested with a battery of such tests to establish diagnoses.

➢ Asymptomatic people who are at risk could be monitored for signs of incipient pathogenesis.

  Appropriate treatments (as available) could be started ASAP

➢ Monitoring therapeutic trails

  - without always requiring a clinical endpoint
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Overview:

- Early, definitive diagnosis of prion diseases:
  - Provide answers to patients, family and medical staff
  - Reduce risks of transmission
  - Facilitate treatments (as they become available)
    - Start early
    - Preserve better quality of life
    - Limit damage to be undone

- Accurate tests are becoming much more practical for living patients.

- RT-QuIC testing on cerebrospinal fluid and nasal brushings:
  - 90-98% sensitive (percent CJD cases giving positive tests)
  - Almost 100% specific (percent non-CJD cases giving negative tests)
  - Positive tests in hours (rather than days)
  - Strain discrimination is sometimes possible.
  - How early can infections be detected? …to be determined.
  - CSF testing now available in US from National Prion Disease Pathology and Surveillance Center (Dr. Safar) and others internationally.

- A new RT-QuIC substrate (sensor protein) from bank voles allows detection of all prions tested so far, including 4 human prion disease types that were previously undetectable.

- Such tests for pathological prion protein in living patients could be helpful in therapeutic trials.

- Similar tests for other disease-associated misfolded proteins should eventually be possible (in principle) to help differential diagnosis of neurodegenerative diseases such as prion diseases, Alzheimer’s, Parkinson’s, tauopathies, etc.