Progress in detecting prions and diagnosing prion diseases

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Amyloid plaque Pam Caughey

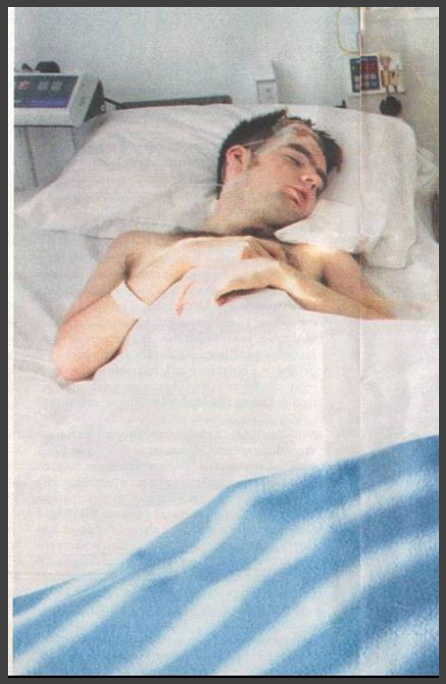
Transmissible spongiform encephalopathies (prion) diseases

Slow, fatal, transmissible neurodegenerative diseases

BSE
(mad cow disease)

chronic wasting disease
(CWD)

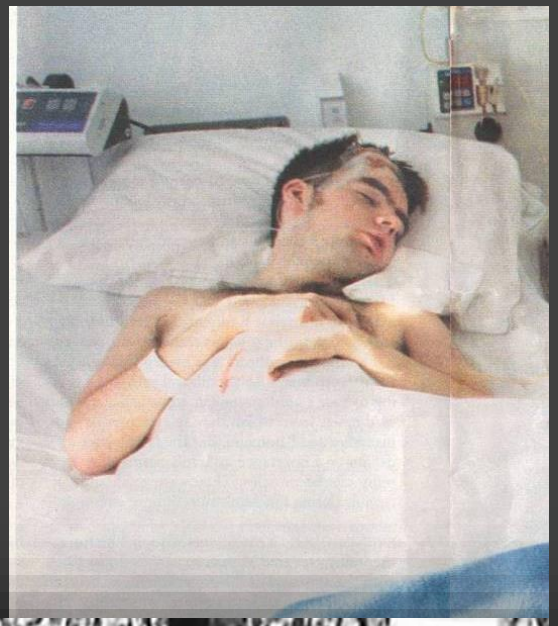
scrapie



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



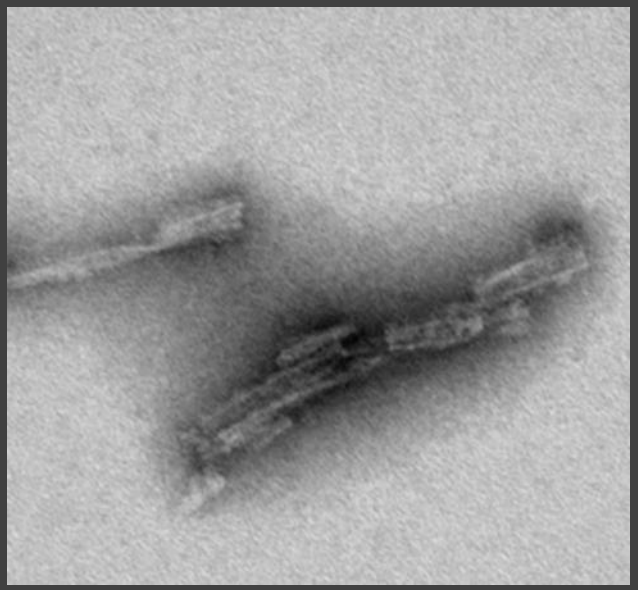
- FAMILIAL:
  - familial CJD
  - Gerstmann-Sträussler-Scheinker syndrome
  - fatal familial insomnia
  - prion protein mutations

- 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

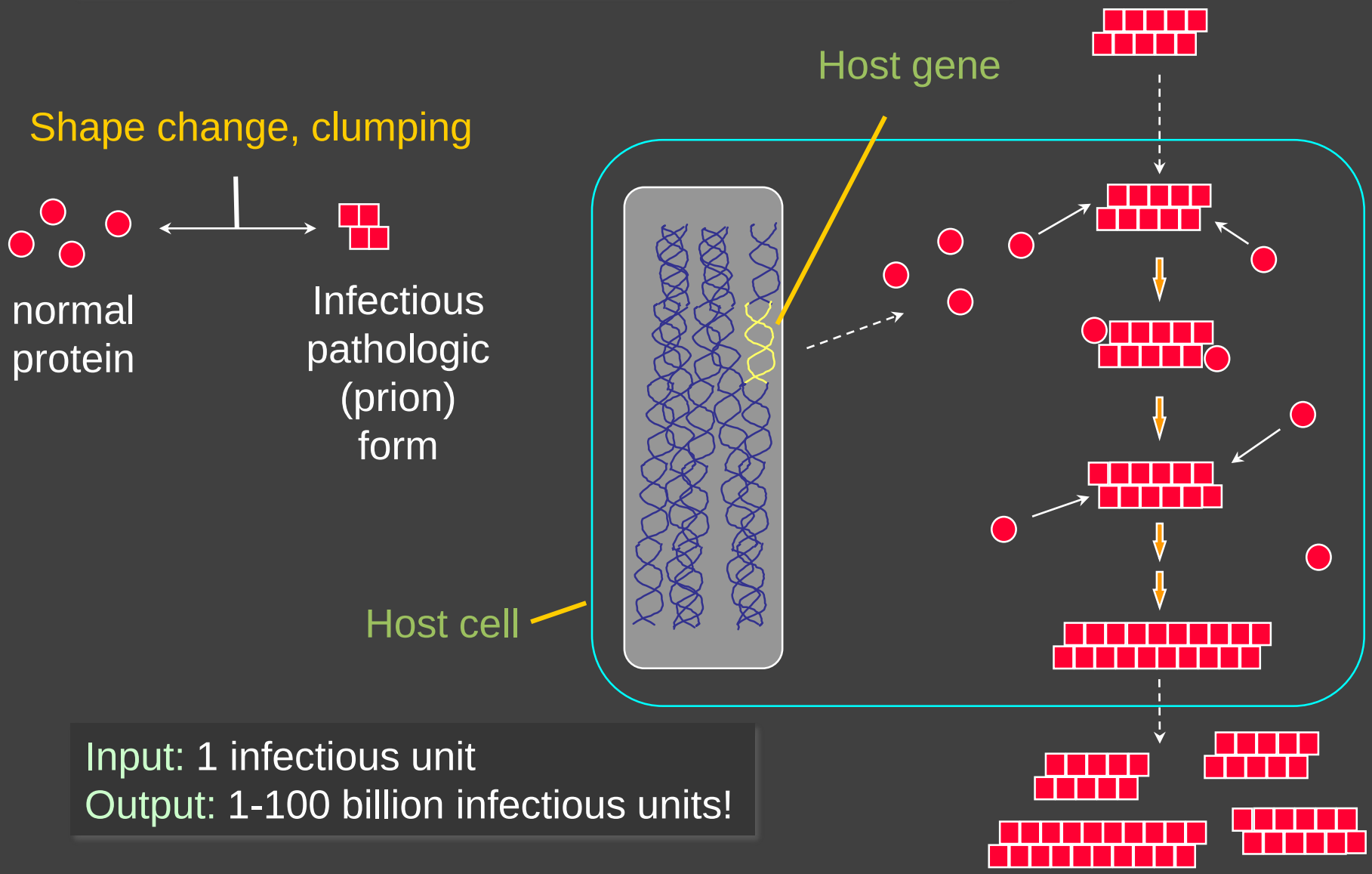
- Little immune response by host
- No genes of its own
- Hard to decontaminate

➡ An infectious protein



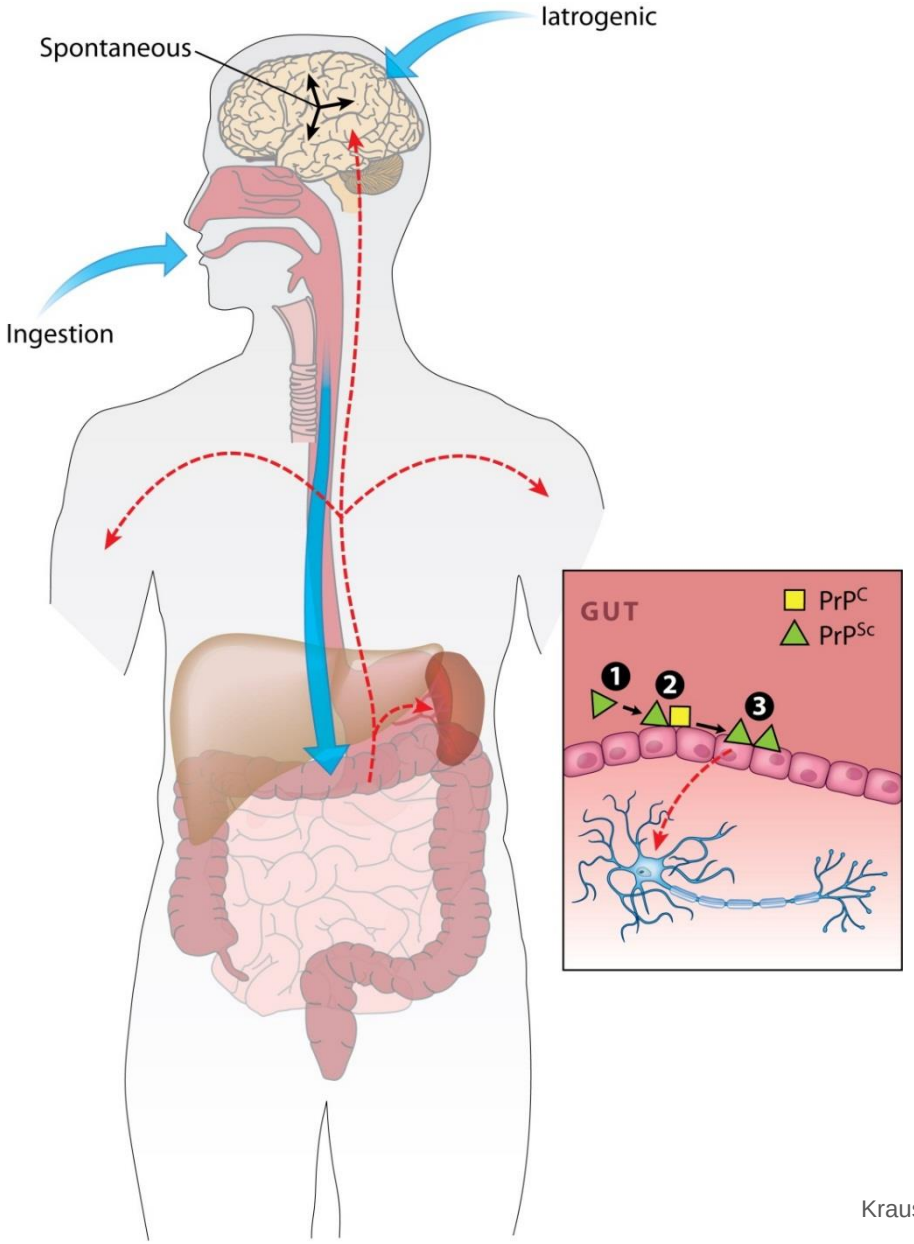
Purified prion amyloid

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



Input: 1 infectious unit
Output: 1-100 billion infectious units!

Spreading routes for human prions



Kraus, Groveman & Caughey, *Ann Rev Micro* 2013

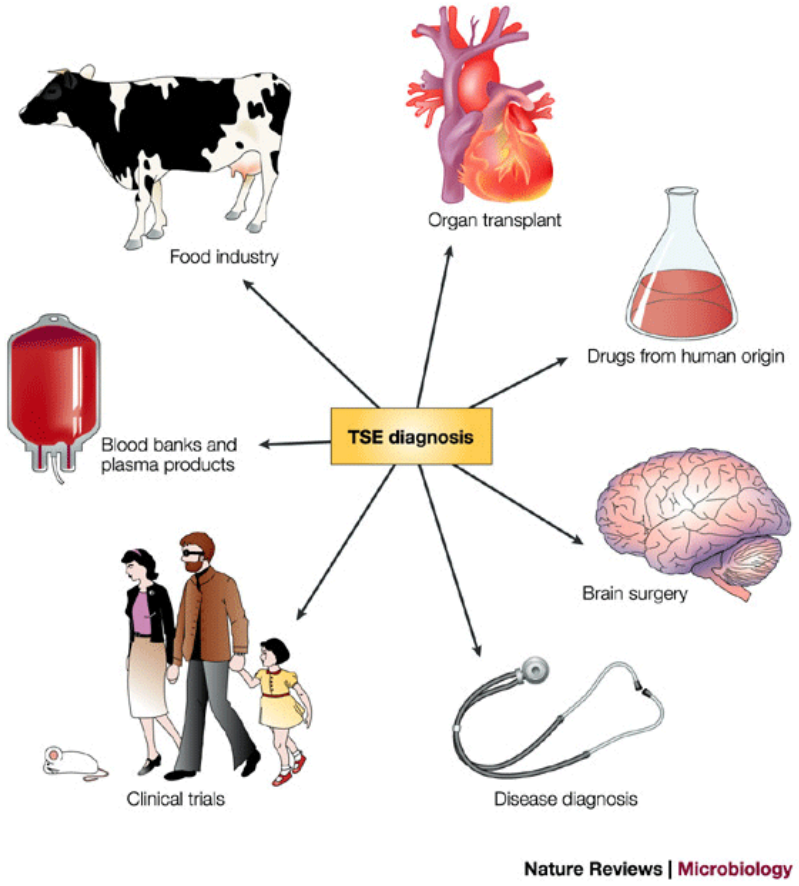
The need for practical, sensitive detection of prions

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



C. Soto, Nat. Rev. Microbiol., 2004

Current diagnosis of sCJD

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

] Living patients

Definite CJD
- PrP^{CJD} deposition in brain tissue

] Biopsy or autopsy

In progress:

Definitive tests based on detecting PrP^{CJD} in living patients

Table 1. Comparison of selected highly sensitive prion/PrP^{Sc} assays

Assay	Principle	Sensitivity: PrP^{res} (example)	Sensitivity: brain dil. (example)	Assay Time	Sample types	Comments	Refs.
PMCA	Seeded conversion of brain PrP^{C}; Sonicated; western blot	0.1 ag (hamster 263K et al.)	10^{-13} (hamster 263K et al.)	≤16 d	Brain, blood, feces, urine, spleen, milk, oral secretions, liver	Propagates infectivity; Sonication difficult to standardize	2, 3, 24–39
ASA	Seeded fibrillization of rec PrP^{C}; Shaken; Multiwell ThT detection	1 fg (hamster Sc237 et al.)	10^{-8} (hamster Sc237, sCJD et al.)	~1 d	Brain	Relies on decreased lag phase relative to spontaneous fibrillization	9, 43
S-QuIC	Seeded fibrillization of rec PrP^{C}; Shaken; western blot	0.1–100 fg (hamster 263K et al.)	10^{-10} (hamster 263K)	1–3 d	Brain, CSF	spontaneous fibrillization minimized	5,7
RT-QuIC	Seeded fibrillization of rec PrP^{C}; Shaken; Multiwell ThT detection	1 fg (hamster 263K et al.)	10^{-7-10} (hamster 263K, sCJD, vCJD)	~2 d	Brain, CSF, nasal fluids	No infectivity propagation; spontaneous fibrillization minimized; CJD diagnosis	6, 12, 15, 16, 18
eQuIC	Immunoprecip. + enhanced RT-QuIC; Multiwell ThT detection	1 ag (vCJD)	10^{-14} (sCJD, vCJD)	2–3 d	Blood plasma, brain	Captures seeding activity from inhibitory samples	8
Edgeworth	Steel bead capture + ELISA		10^{-10} (vCJD)	~2 d	Blood, brain	Captures PrP^{Sc} from inhibitory samples; e.g., vCJD blood samples	22

Need multiple tests:

- primary & confirmatory
- improve specificity, minimize false positives
- cope with wide range of sample types and applications

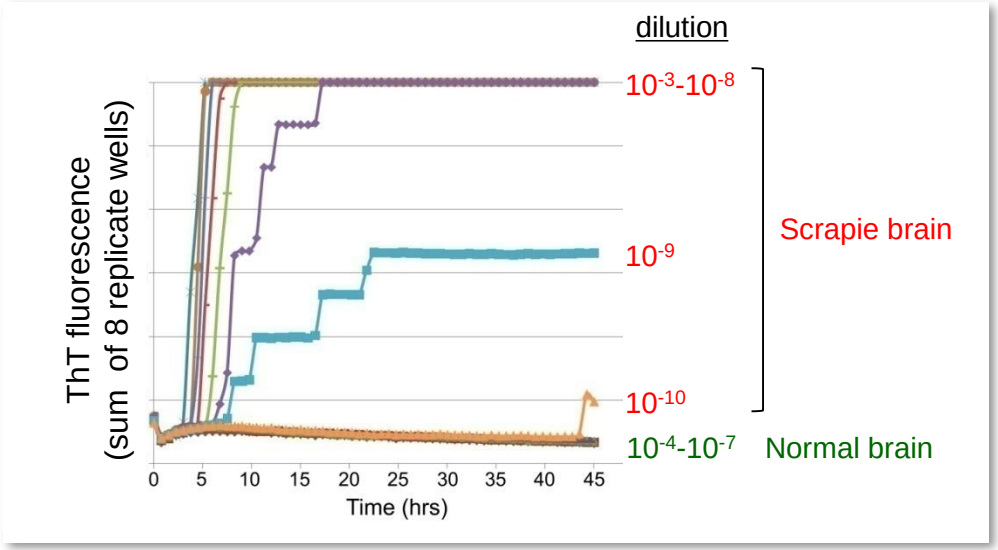
Orrù *et al*, **Prion** 2012

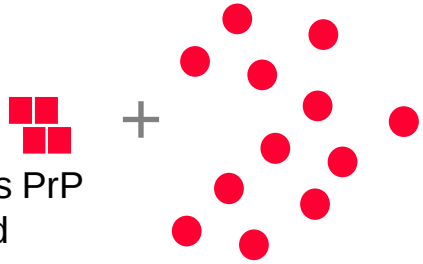
Plate-based fluorescence detection of prion-seeded PrP amyloid
(Real-time Quaking-Induced Conversion: RT-QuIC)

Sample + Normal PrP protein + Amyloid stain

96-well plate

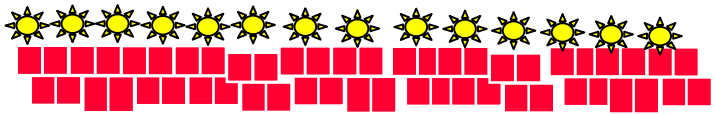
Shaking fluorescence plate reader





Infectious PrP seed

Normal PrP (sensor or substrate)

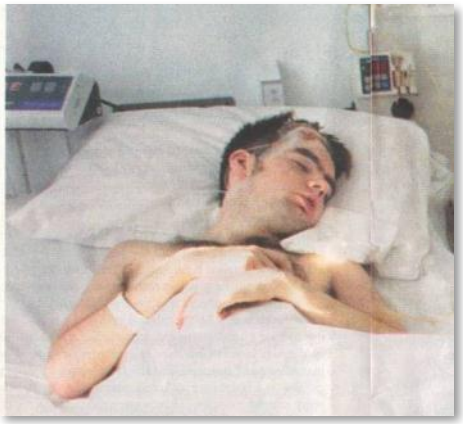


PrP amyloid with fluorescent stain

J. Wilham C. Orrú

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

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):

Wilham et al, ***PLoS Pathogens*** 2010
Atarashi et al, ***Nature Medicine*** 2011
Orrù et al, ***J Clin Micro*** 2012
McGuire et al, ***Ann Neurol*** 2012
Sano et al, ***PLoS One*** 2013
Haley et al, ***PLoS One*** 2013
Cramm et al, ***Mol Neurobiol*** 2014
Orrù et al., ***mBio*** 2015

Nasal fluid, brushings (humans, hamsters):

Wilham et al, ***PloS Pathogens*** 2010
Bessen et al, ***J Virol*** 2012
Orrù et al, ***New England J Med*** 2014
Zanusso et al., ***New England J Med*** 2014

Blood (humans, sheep, hamsters, mice):

Orrù et al, ***mBio*** 2011
Vascellari et al, ***PLoS One*** 2012
Elder et al, ***PLoS One*** 2013

Saliva (deer):

Henderson et al., ***PLoS One*** 2013

Urine (deer):

John et al, ***Prion*** 2013

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

nature medicine

2011

Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion

Ryuichiro Atarashi[1,2], Katsuya Satoh[1], Kazunori Sano[1,3], Takayuki Fuse[1], Naohiro Yamaguchi[1], Daisuke Ishibashi[1], Takehiro Matsubara[1], Takehiro Nakagaki[1], Hitoki Yamanaka[4], Susumu Shirabe[5], Masahito Yamada[6], Hidehiro Mizusawa[7], Tetsuyuki Kitamoto[8], Genevieve Klug[9], Amelia McGlade[9], Steven J Collins[9] & Noriyuki Nishida[1,3]

- 77-89% sensitivity

  (% sCJD giving positive test)

- 99-100% specificity

  (% non-sCJD giving negative test)

ANNALS of Neurology

2012

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

Lynne I. McGuire, PhD,[1] Alexander H. Peden, PhD,[1] Christina D. Orrú, PhD,[2] Jason M. Wilham, PhD,[2] Nigel E. Appleford, Cbiol,[3] Gary Mallinson, PhD,[3] Mary Andrews, BSc,[1] Mark W. Head, PhD,[1] Byron Caughey, PhD,[2] Robert G. Will, FRCP,[1] Richard S. G. Knight, FRCP,[1] and Alison J. E. Green, PhD[1]

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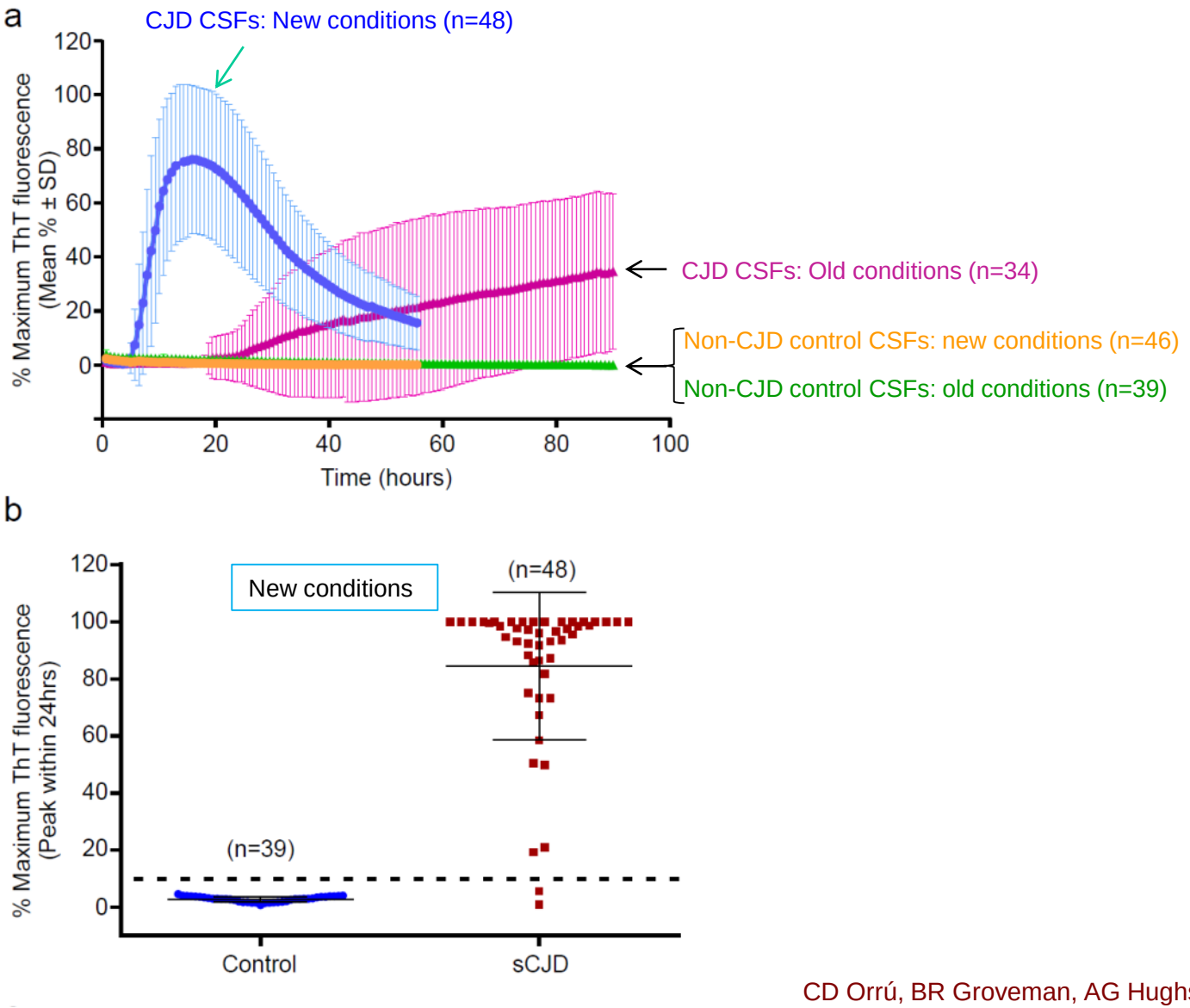
- First disease-specific diagnostic test not requiring brain biopsy or post-mortem analysis.
- Implementation by many CJD diagnostic centers around the world.

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

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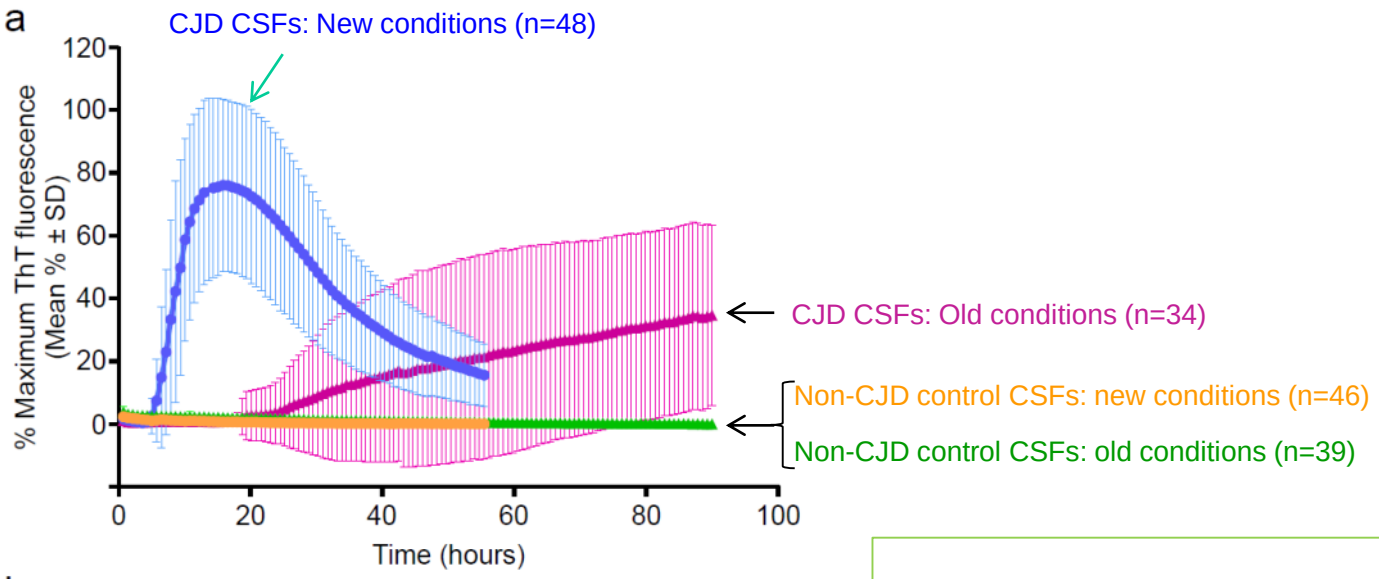
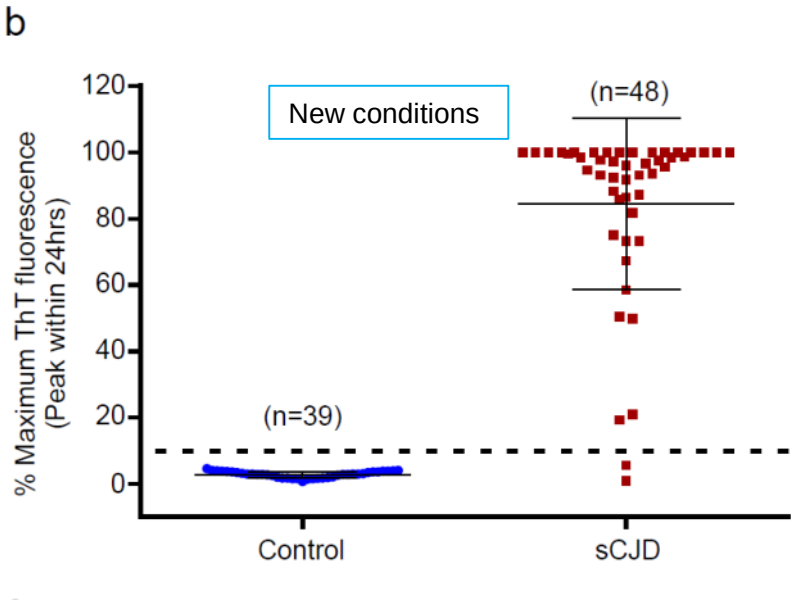
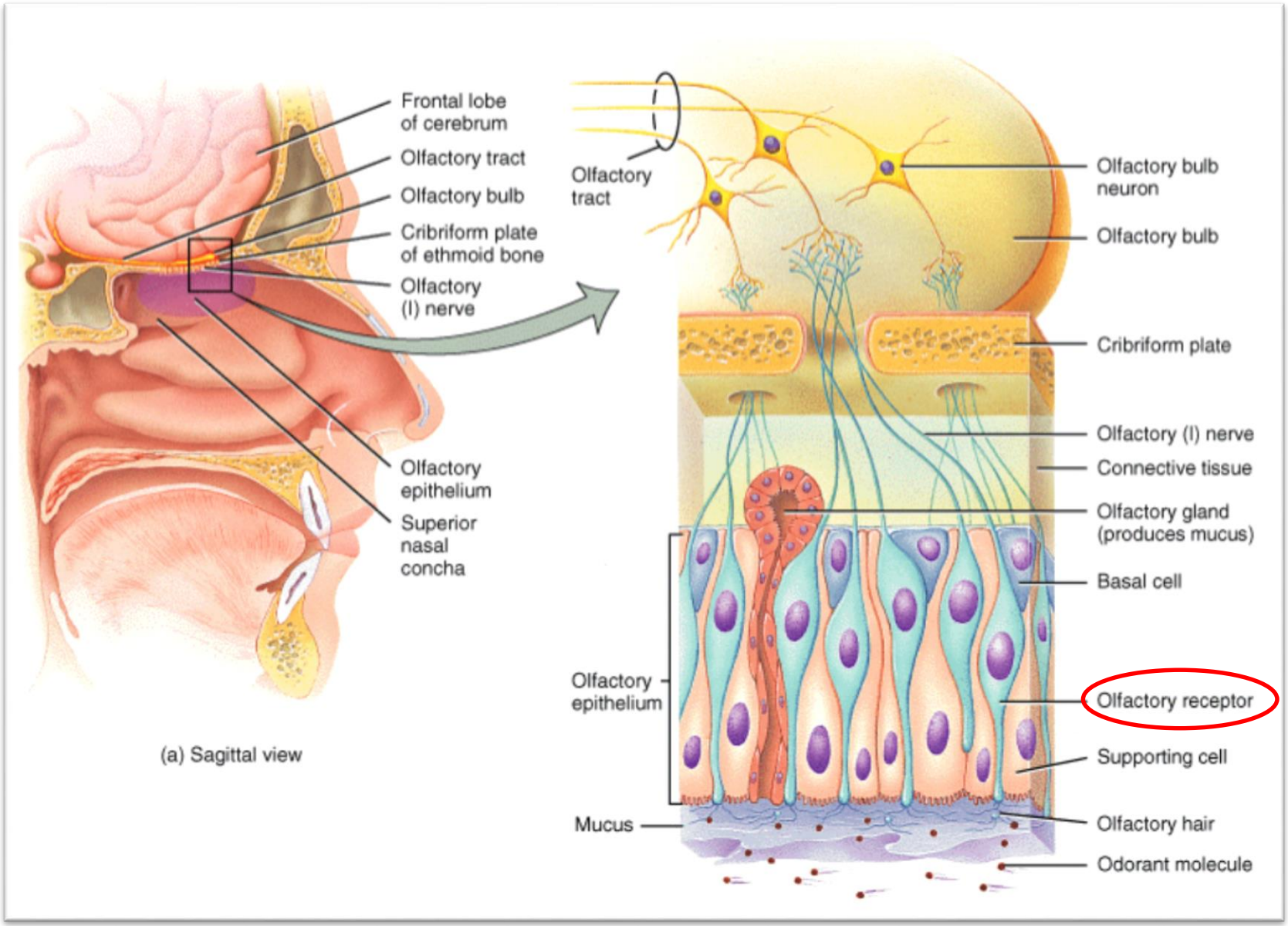




- Faster, stronger RT-QuIC responses using new conditions
  - Reduced from **days** to **hours**.
- Positive RT-QuIC assays from 46 of 48 CJD cases but not from 39 non-CJD patients
  - 96% sensitivity
  - 100% specificity
- Similar results obtained by 2 other labs.
- New conditions improve performance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

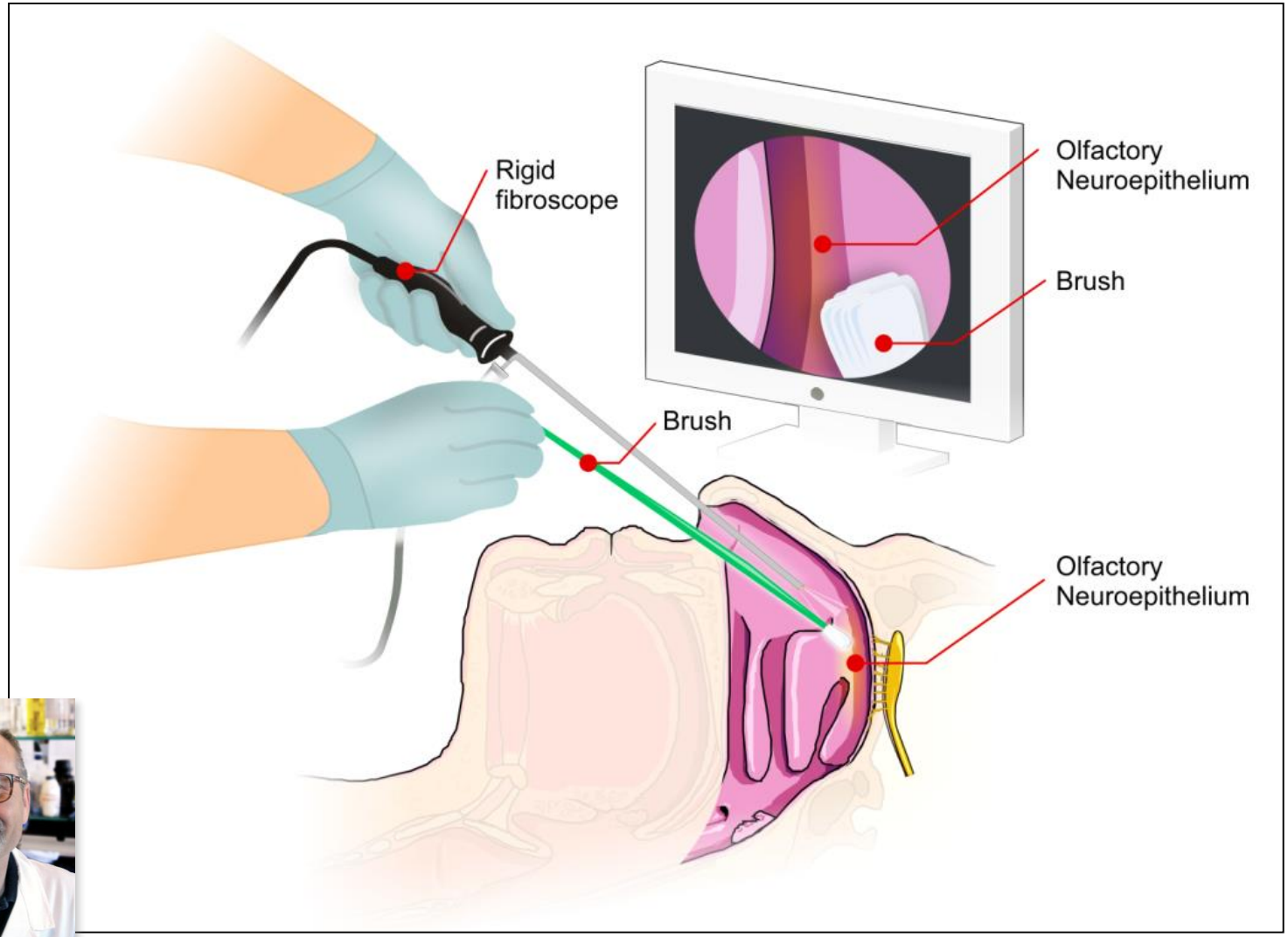
CD Orrú, BR Groveman, AG Hughson, G Zanusso, MB Coulthart and B Caughey, ***mBio*** 2015

Olfactory System



Olfactory neural cells are a surface exposed “window to the brain”. *Escada et al., 2009*

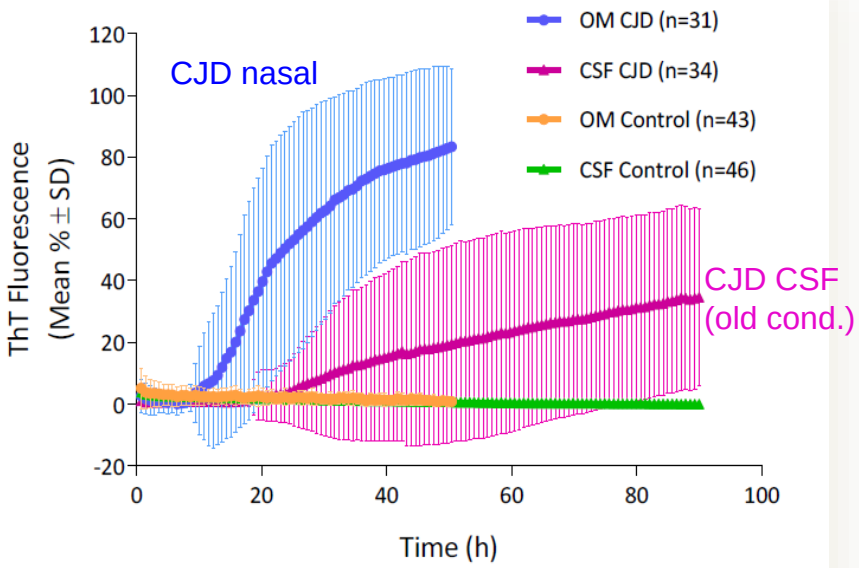
Nasal brushing procedure for collecting diagnostic specimens for RT-QuIC

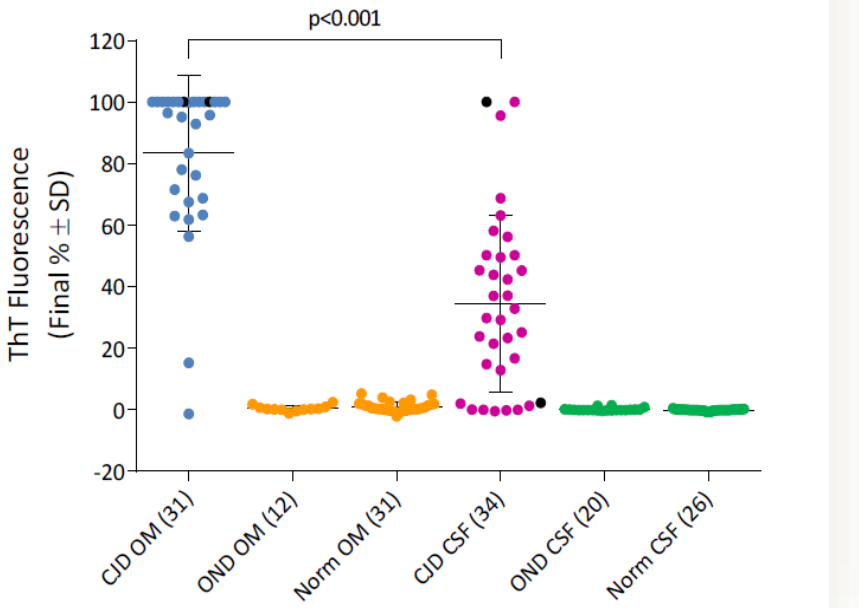


Gianluigi Zanusso

Orrù et al, *New England J Med* (in press)

RT-QuIC of nasal brushings (OM) in diagnosing sCJD in living patients

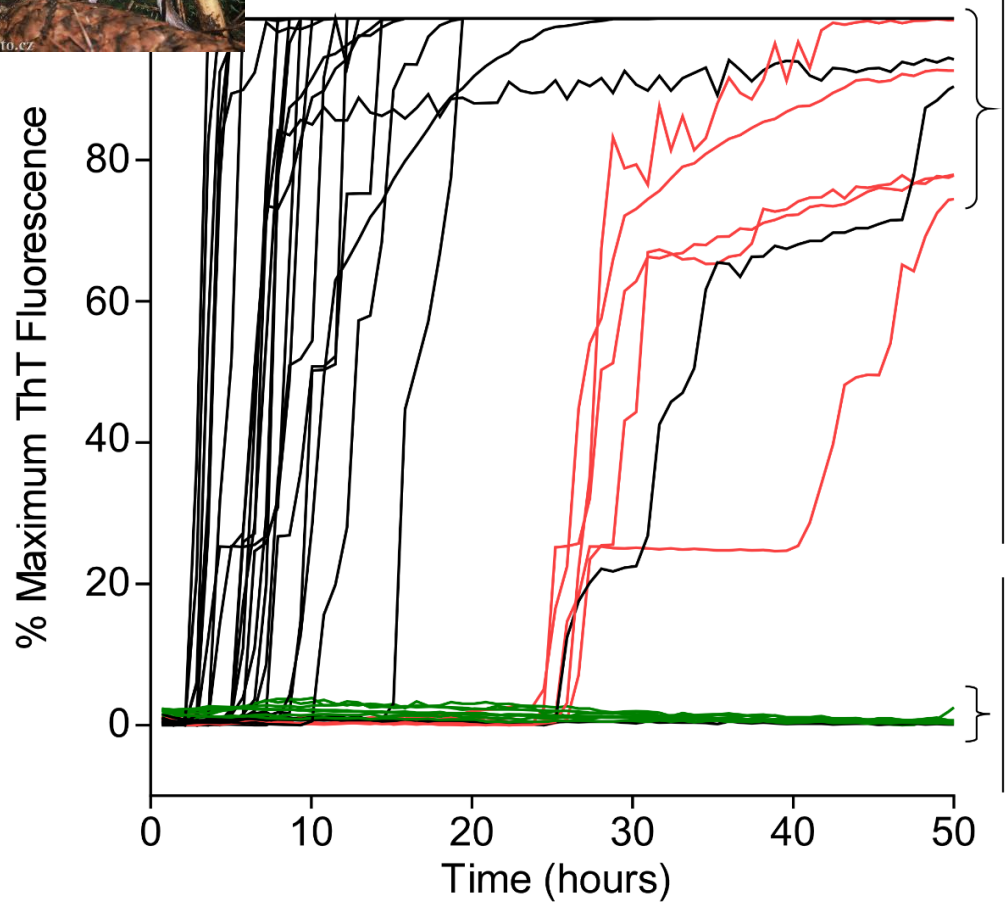




- Positive RT-QuIC assays from 42 of 43 CJD cases but not from 43 non-CJD patients
  - ≥97% sensitivity
  - 100% specificity
- RT-QuIC of CSF samples (old conditions) from the same patients
  - 79% sensitivity
  - 100% specificity
- Nasal brushings provide potential basis for a definitive, less-invasive, definitive antemortem diagnostic test for CJD.
- Brushings contained ~10^5-10^7 prion seeds.
  - infectivity lining the nasal cavity???

Orrú CD, Bongianni M, Tonoli G, Ferrari S, Hughson AG, Groveman BR, Fiorini M, Pocchiari M, Monaco S, Caughey B, Zanusso G. ***New Engl J Med*** (2014)
Zanusso G., Bongianni M, Caughey B, ***New Engl J Med*** (2014)

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

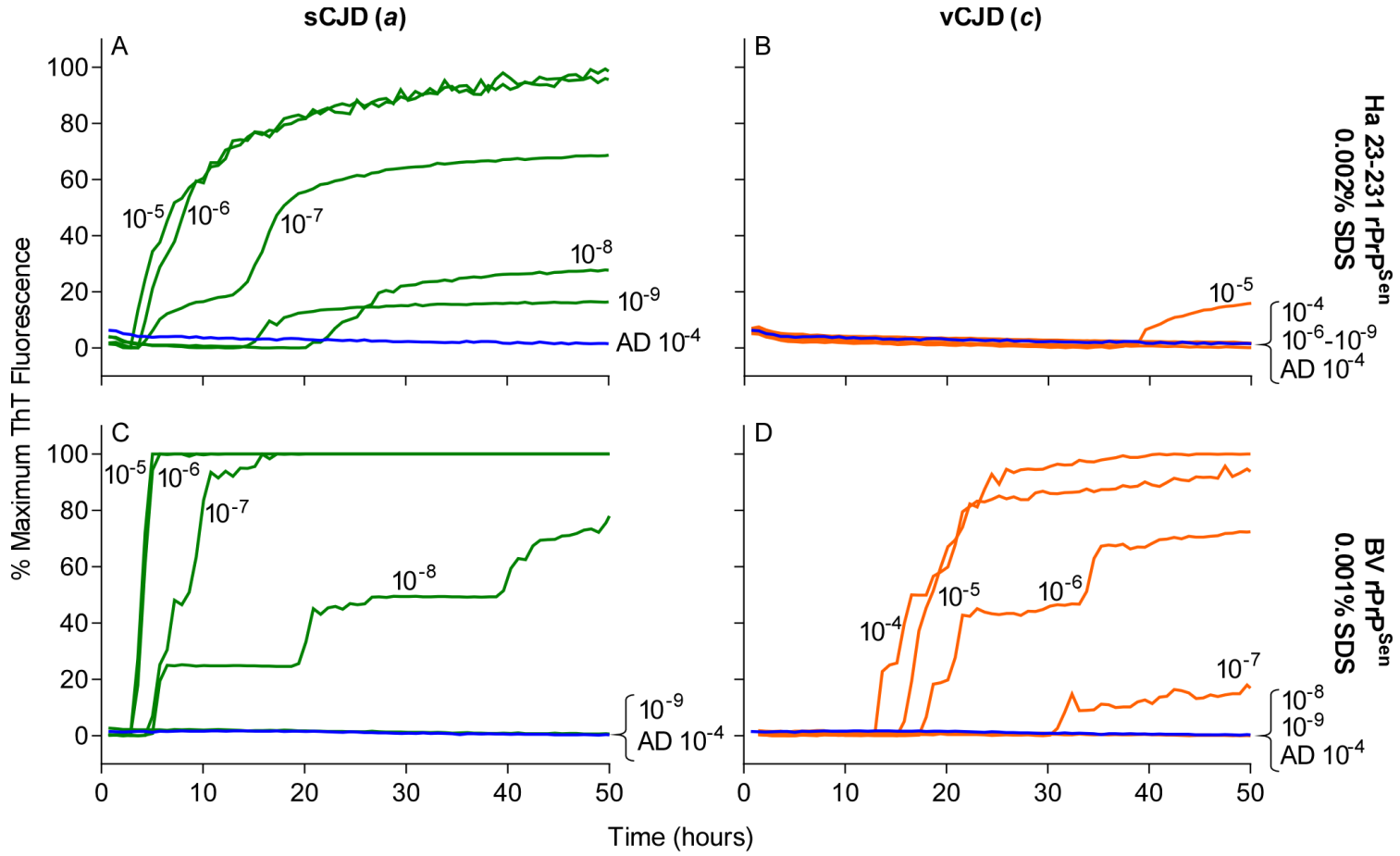


Human	Animal
sCJD Type 1	Classical Sheep Scrapie
sCJD Type 2	**Atypical Nor98 Sheep Scrapie**
vCJD	C-BSE Bovine
iCJD	L-BSE Bovine
F198S	CWD Elk
A117V	CWD Deer
H187R	263K Hamster
P102L*	Hyper Hamster
P102L	Drowsy Hamster
E200K	Chandler Mouse
V210I	ME7 Mouse
D178N (FFI)	87V Mouse
sFI	22L Mouse
6 Octarepeat insert	22L GPI^{-} Mouse
Alzheimer's Disease	Uninfected Sheep
Parkinson's Disease	Uninfected Bovine
Tumor	Uninfected Deer
Ischemia	Uninfected Elk
	Uninfected Mouse
	Uninfected Hamster

- BV rPrP^{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.

CD Orrù, BR Groveman, LD Raymond, AG Hughson, R Nonno, W Zou, B Ghetti, P Gambetti, B Caughey, ***PLoS Pathogens*** (in press)

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

CD Orrù, BR Groveman, LD Raymond, AG Hughson, R Nonno, W Zou, B Ghetti, P Gambetti, B Caughey, ***PLoS Pathogens*** (in press)

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.

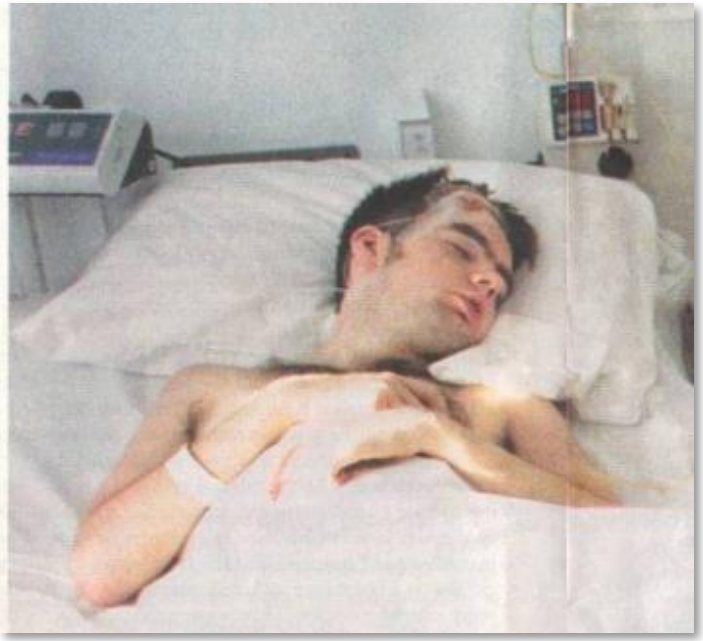
- Patients with early neurological signs could be tested with a battery of such tests to establish diagnoses.

- 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.