Update Nella Diagnostica Molecolare Nelle Malattie da Prioni e Prion-like

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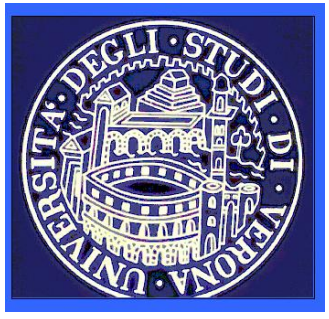


Riunione della Sezione Triveneto
Società Italiana di Neurologia
Cittadella, 17 Maggio 2019

Human Prion Diseases

Human Prion Diseases

Forms (Incidence)

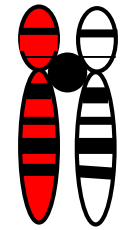
Etiology

Disease phenotype

Genetic (10%)

Prion Protein Gene Mutations

Autosomal dominant inheritance with a variability of penetrance mutation-dependent



Genetic Creutzfeldt-Jakob Disease (gCJD)
Fatal Familial Insomnia (FFI)
Gerstmann Straussler Scheinker syndrome (GSS)

Sporadic (90%)

Unknown

Creutzfeldt-Jakob Disease (sCJD)
Fatal Insomnia
VPSPr

Acquired (<1%)

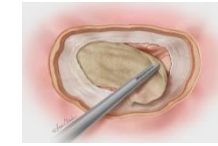
Exposure to prion sources of infectious material

→ Humans
Surgical/Medical procedures

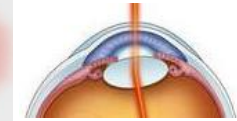
→ Animals
BSE related

Iatrogenic CJD (iCJD)

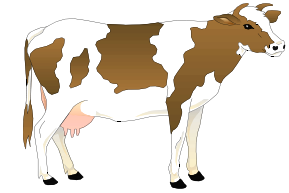
Dura mater transplants



Cornea



Growth hormone from cadaver



Variant CJD (vCJD)

Sporadic Creutzfeldt-Jakob Disease

Sporadic Creutzfeldt-Jakob Disease (sCJD)

➢ **Fatal Neurodegenerative disorder with a disease duration of less than 24 months**

➢ **Clinically characterized by a rapidly progressive dementia, with visual, cerebellar, pyramidal or extrapyramidal signs, myoclonus.**

Invariably evolving to akinetic mutism

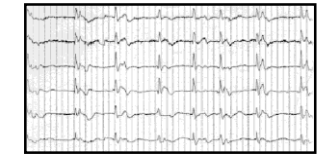
➢ **Diagnostic tools:**

- **EEG: Typical PSWCs**
- **CSF: Positive 14-3-3 protein or Prion detection by RT-QuIC assay**
- **MRI: High signal abnormailities in basal ganglia or at least two cortical regions either in DWI or FLAIR**
- ***PRNP*: codon 129 polymorphism**

➢ **Definite Diagnosis is based on demonstration of pathological PrP (PrP^{Sc}) in the nervous tissue**

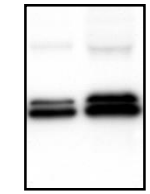
Probable Diagnosis

EEG



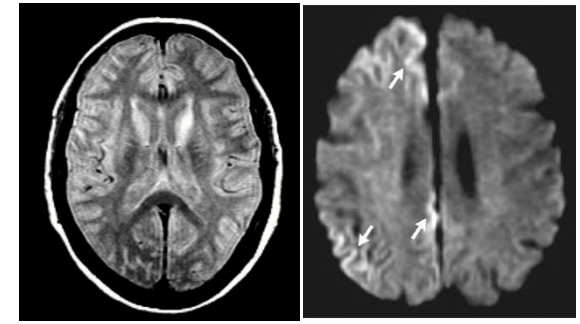
PSWCs

CSF



14-3-3 protein

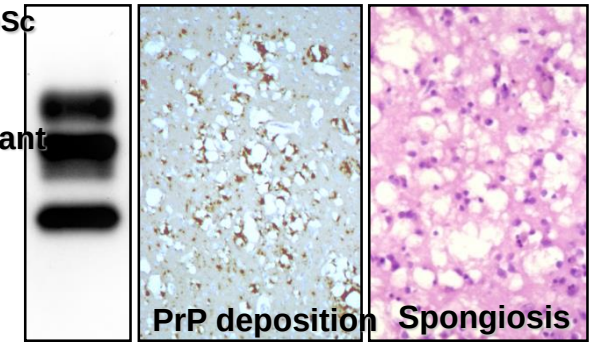
MRI



Definite Diagnosis

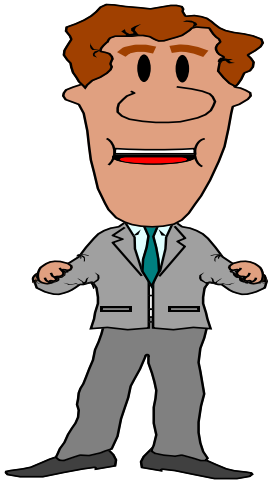
PrP^{Sc}

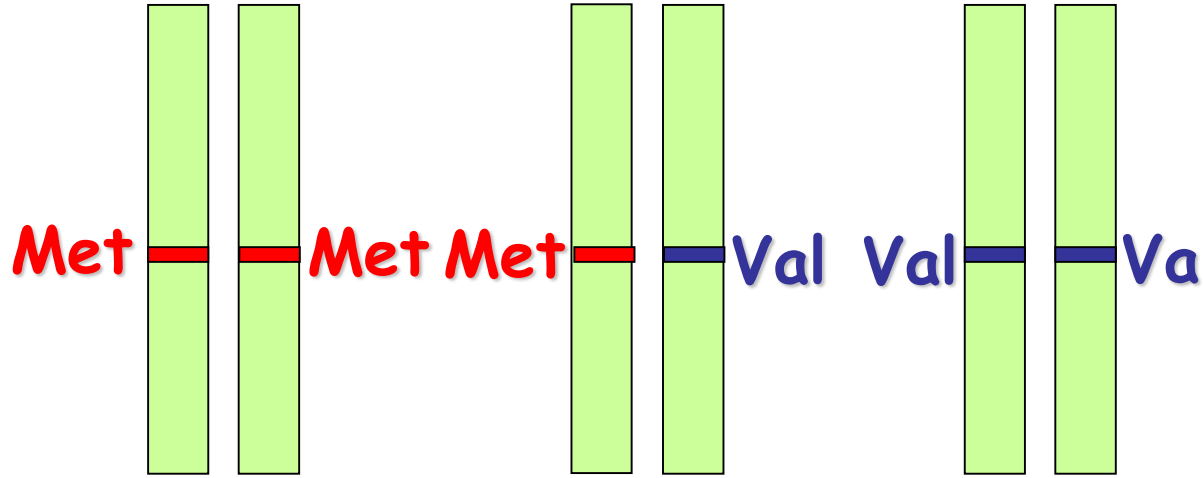
PK resistant PrP



PrP^{Sc} detection by immunoblot and immunocytochemistry

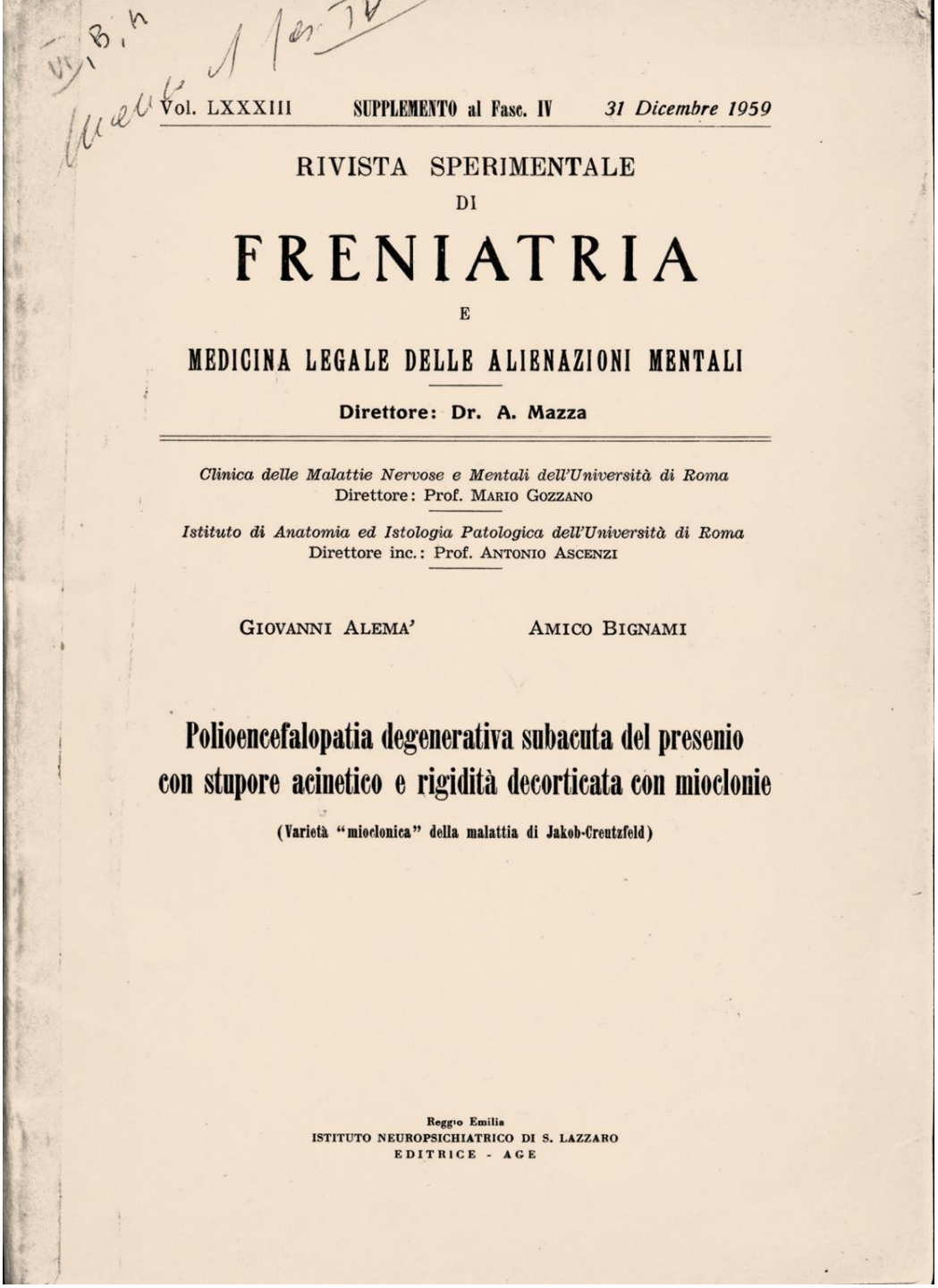
Influence of polymorphism Met/Val at codon 129 on Sporadic CJD susceptibility





Codon 129	Met/Met	Met/Val	Val/Val
Normal population	40 %	50 %	10 %
sCJD	75 %	10 %	15 %

- Determine susceptibility to prions
- Clinical disease modifier



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RIVISTA SPERIMENTALE
DI
FRENIATRIA
E
MEDICINA LEGALE DELLE ALIENAZIONI MENTALI

Direttore: Dr. A. Mazza

Clinica delle Malattie Nervose e Mentali dell'Università di Roma
Direttore: Prof. Mario Gozzano

Istituto di Anatomia ed Istologia Patologica dell'Università di Roma
Direttore inc.: Prof. Antonio Ascenzi

Giovanni Alema' Amico Bignami

Polioencefalopatia degenerativa subacuta del presenio con stupore acinetico e rigidità decorticata con mioclonie

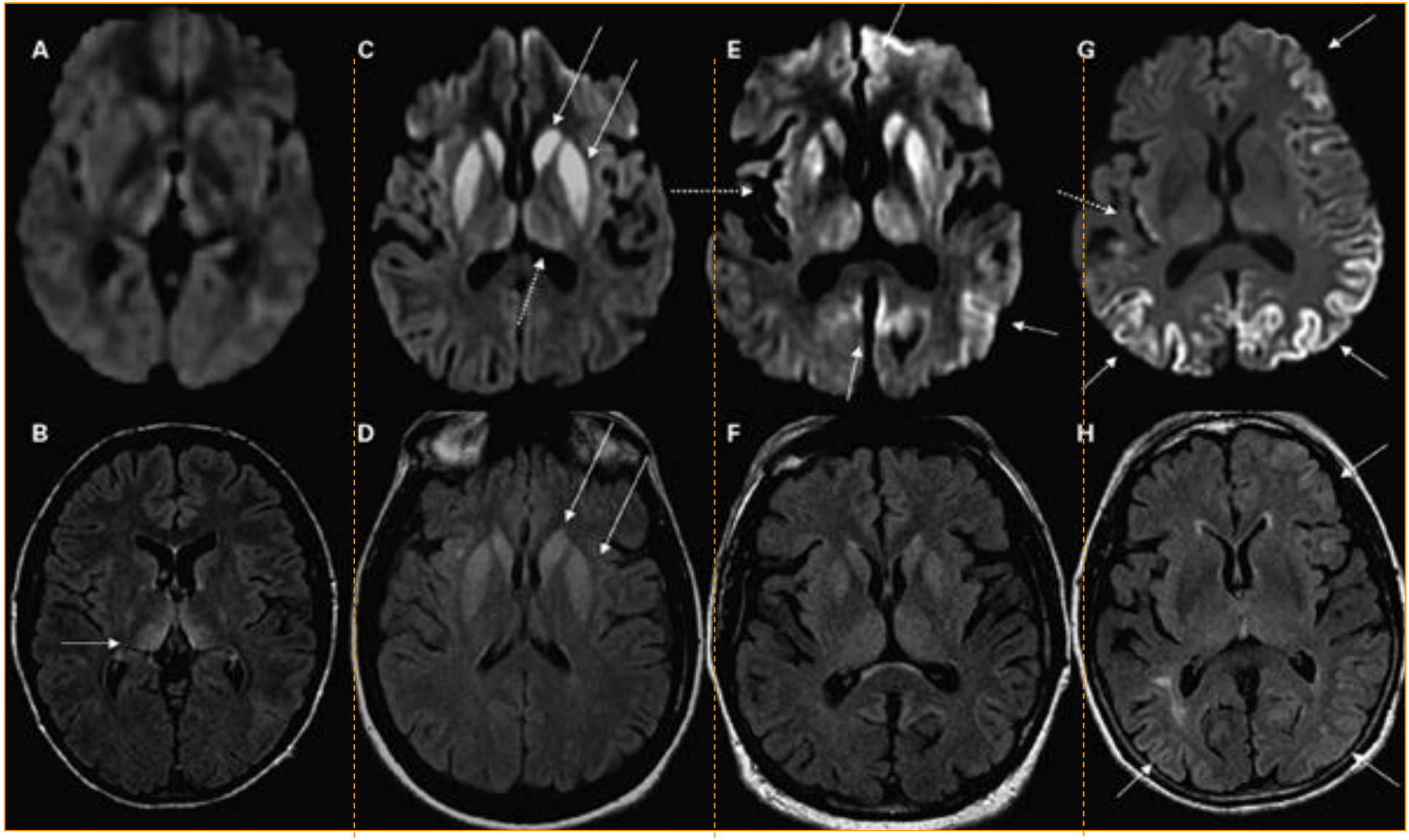
(Varietà "mioclonica" della malattia di Jakob-Creutzfeld)

Reggio Emilia
ISTITUTO NEUROPSICHIATRICO DI S. LAZZARO
EDITRICE - AGE

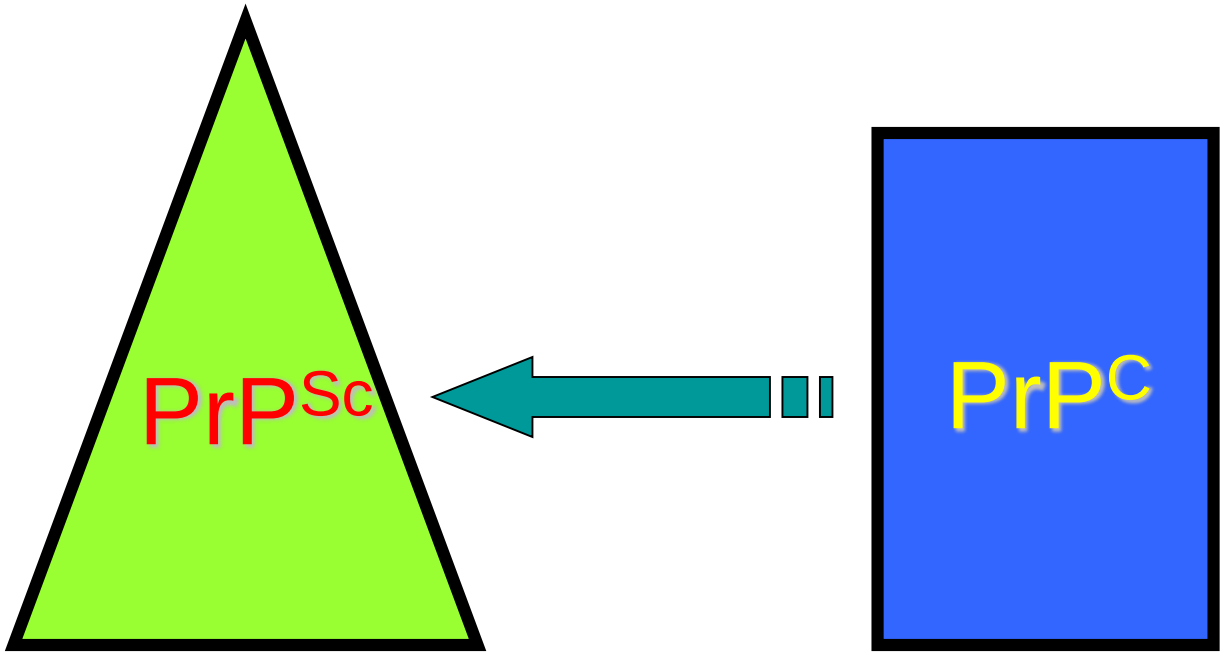
Clinical Variants of Sporadic Creutzfeldt-Jakob Disease based on Clinical Onset

- **Classic**: Cognitive Impairment, Ataxia, Myoclonus
- **Heidenhain**: Visual Disturbances and Hallucinations
- **Oppenheimer-Brownell or Ataxic**: Ataxia
- **Cognitive**: Cogntive Impairment, language and executive functions
- **Affective**: Depression
- **Amyotrophic**

MRI Lesion patterns in sCJD



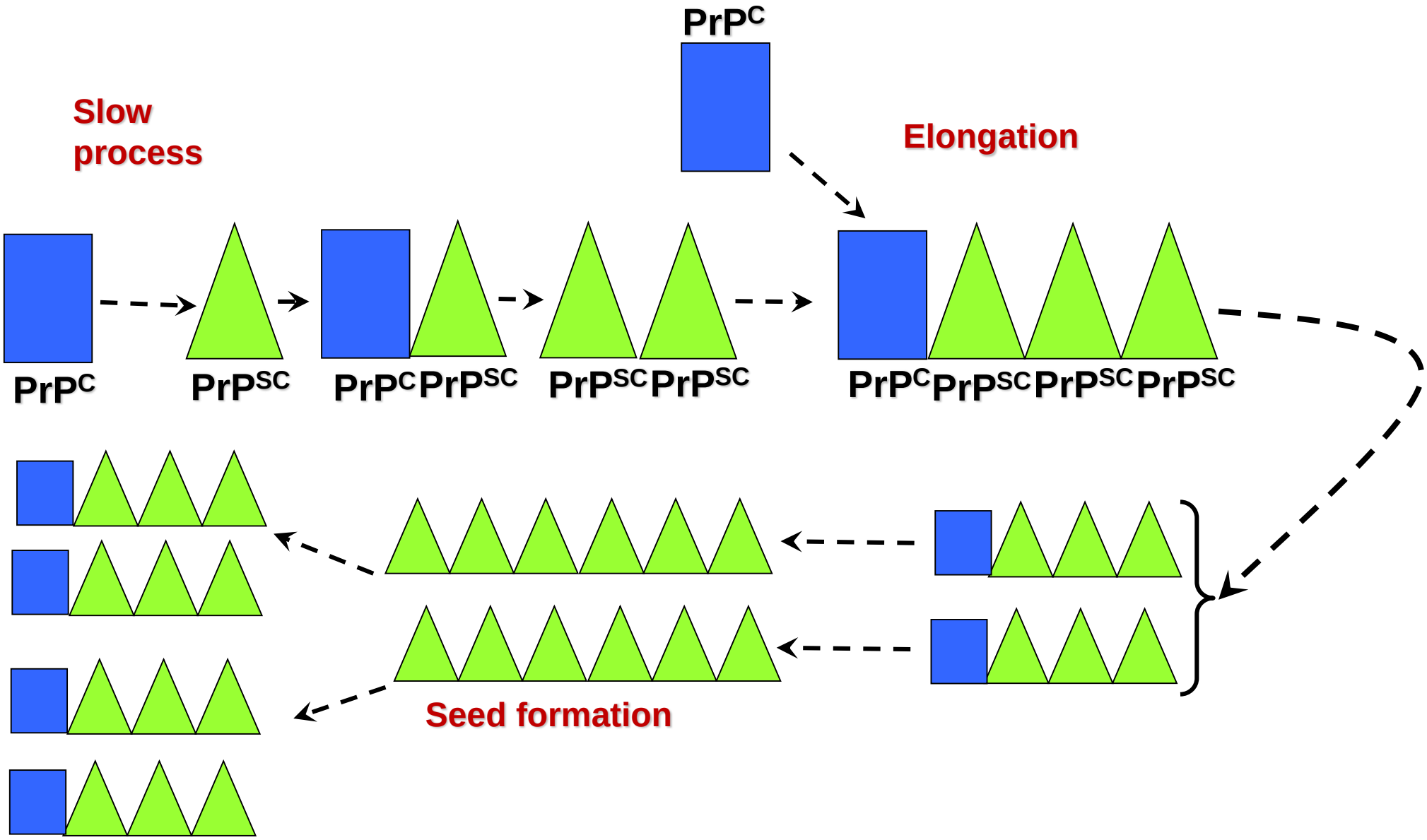
A tale of conversion of a “good” prion into a “bad” prion



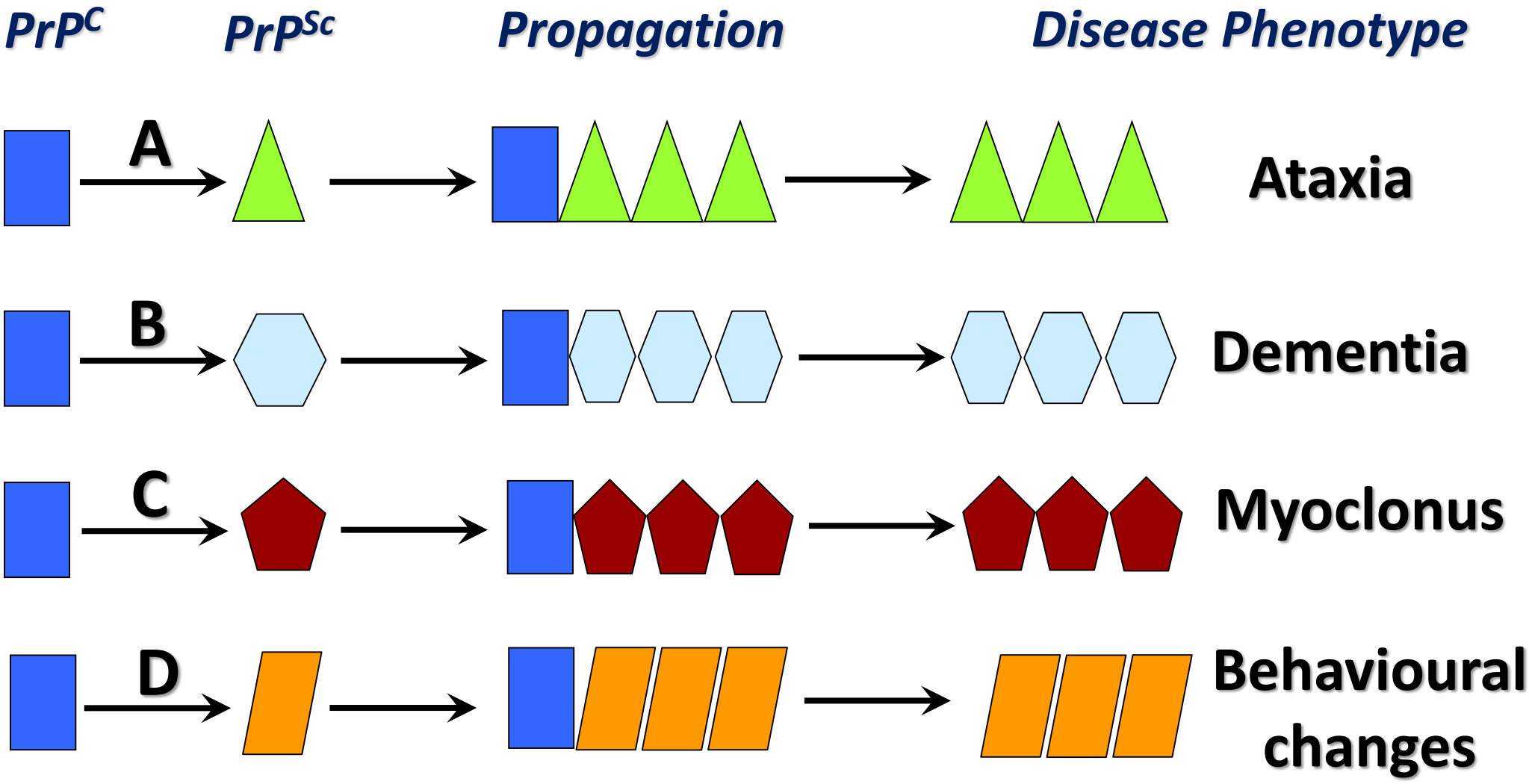
Pathological Prion Protein

The *Conditio sine qua non* is the presence of Cellular Prion Protein

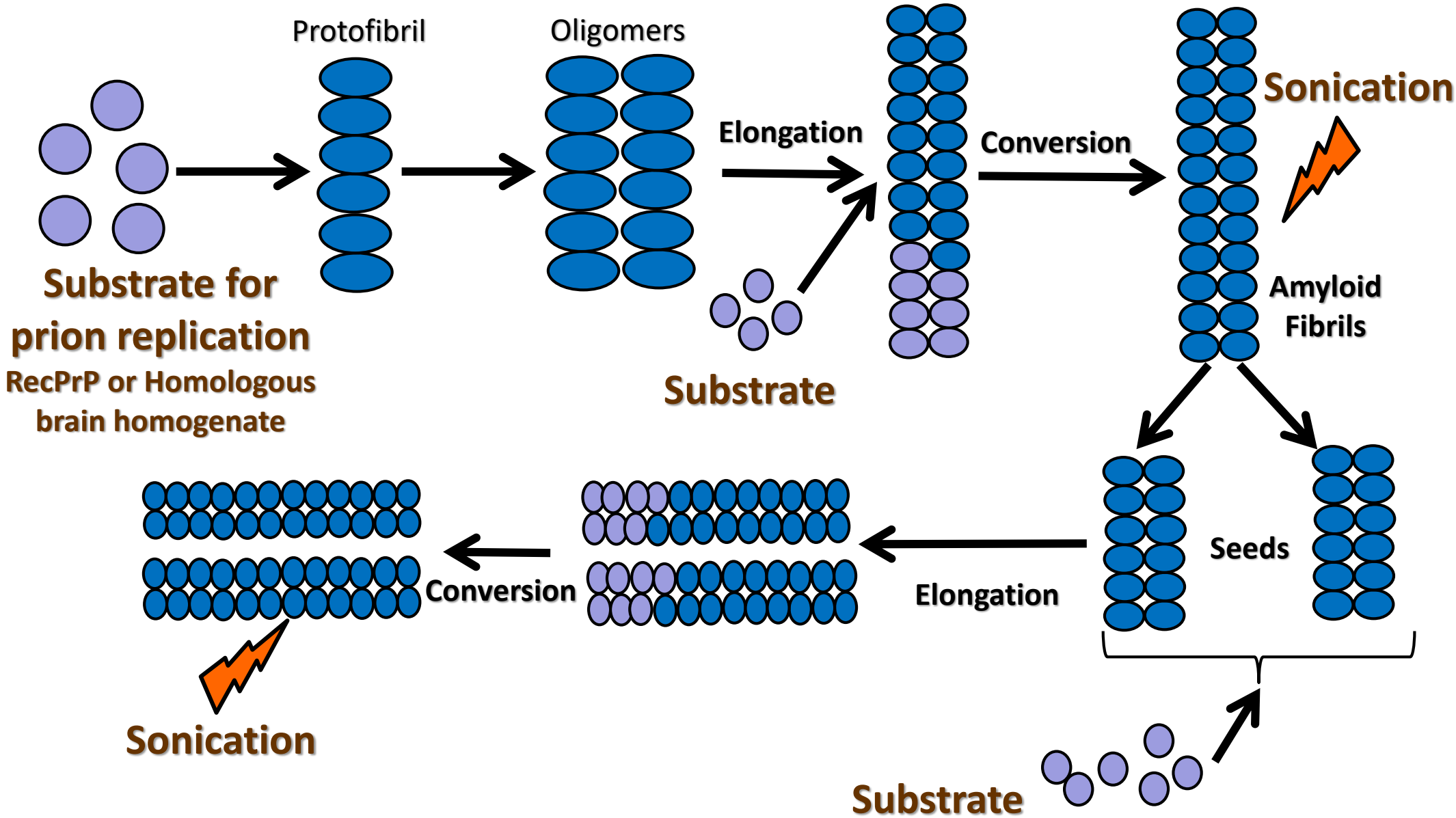
“Domino-like” Prion replication



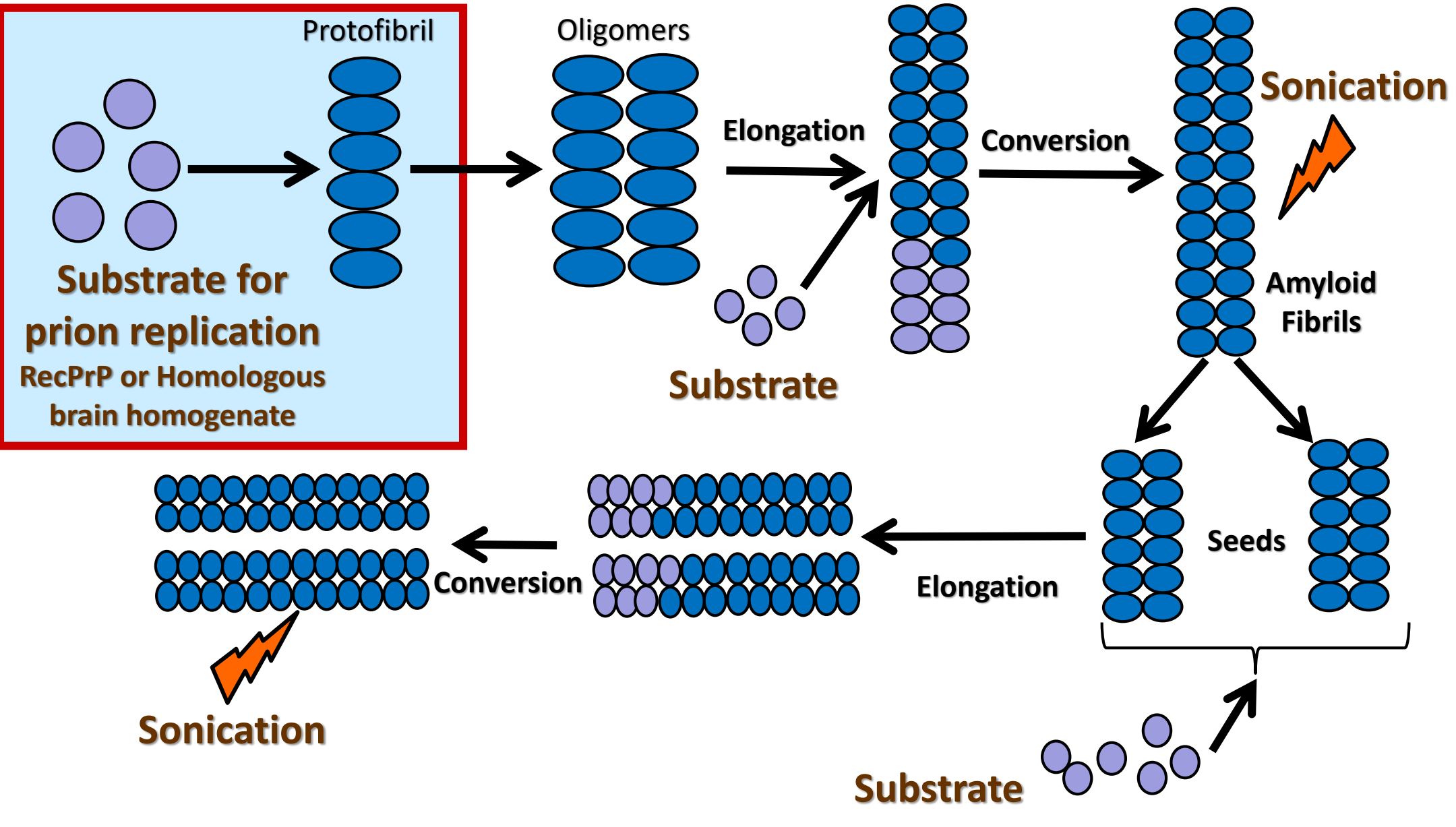
Different Prion Conformers Act as «Strains»



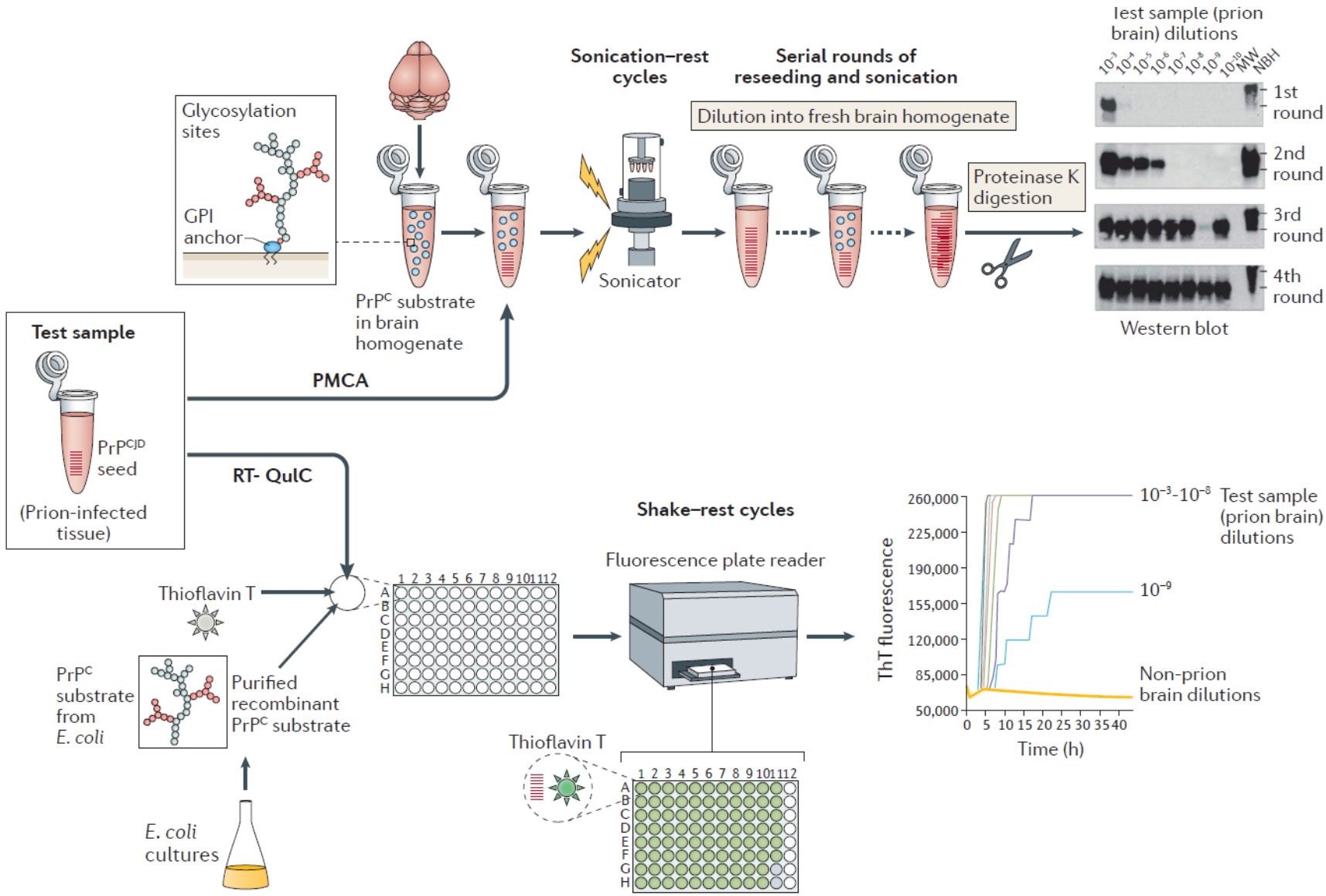
Principles of Prion Seeding Assays



Principles of Prion Seeding Assays



Diagnostic testing for CJD: PMCA vs RT-QuIC



Zanusso et al. 2016 NATURE REVIEWS | NEUROLOGY

Pros *vs* Cons

PMCA

- ✓ **Pros** Highly specific and sensitive
- ✓ **Cons** Provision of fresh brain tissue with PrP homologous to the testing sample
- ✓ **Cons** Low efficiency for sCJD prions
- ✓ **Cons** Time consuming (each round lasts 24-48 hours)
- ✓ **Cons** Technically demanding performed in few laboratories
- ✓ **Cons** Generation of Infectious prion, even spontaneosuly

RT-QuIC

- ✓ **Pros** Highly specific and sensitive
- ✓ **Pros** recPrP as substrate (non necessarily homologous) strains amplification is allowed by different recPrP
- ✓ **Cons** Low efficiency for vCJD prions
- ✓ **Pros** Rapid (hours), low cost
- ✓ **Pros** Set up in different laboratories without special training
- ✓ **Pros** Do not generate infectious prions

Real Time-Quaking Induced Conversion (RT-QuIC) In Creutzfeldt-Jakob Disease Diagnosis

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

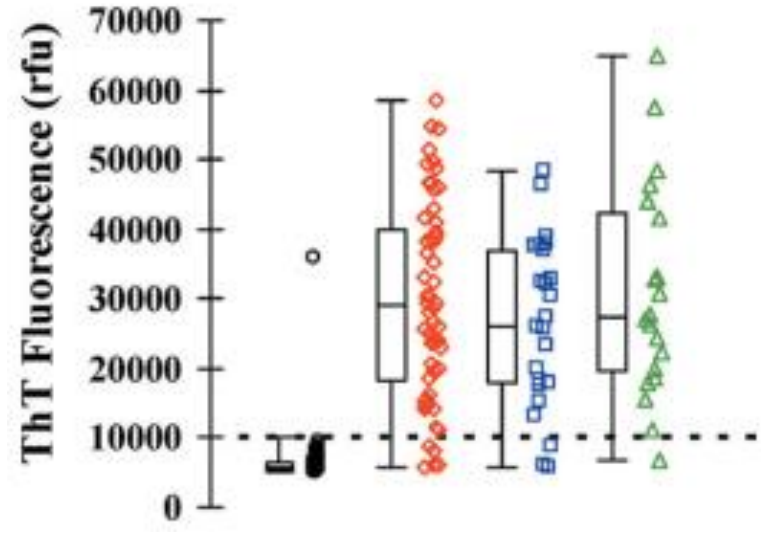
Lynne I. McGuire, PhD,[1] Alexander H. Peden, PhD,[1] Christina D. Orrú, PhD,[2] Jason M. Wilham, PhD,[2] Nigel E. Appleford, Cbiol,[3] Gary Mallinson, PhD,[3] Mary Andrews, BSc,[1] Mark W. Head, PhD,[1] Byron Caughey, PhD,[2] Robert G. Will, FRCP,[1] Richard S. G. Knight, FRCP,[1] and Alison J. E. Green,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

ANN NEUROL 2012;72:278–285

TABLE: The Sensitivity, Specificity, PPV, NPV, and Efficiency for Cerebrospinal Fluid RT-QuIC and 14-3-3 in Neuropathologically Confirmed sCJD for the Exploratory and Confirmatory Groups

	Exploratory Group		Confirmatory Group		Both Groups	
	RT-QuIC	14-3-3	RT-QuIC	14-3-3	RT-QuIC	14-3-3
sCJD	51/56	52/56	58/67	64/67	109/123	116/123
sCJD controls	1/52	23/52	0/51	13/51	1/103	36/103
Sensitivity, all	91%	93%	87%	96%	89%	94%
Sensitivity, MM	90%	97%	90%	93%	90%	95%
Sensitivity, MV	88%	82%	88%	100%	88%	95%
Sensitivity, VV	100%	100%	92%	100%	95%	100%
Specificity	98%	56%	100%	75%	99%	65%
PPV	98%	69%	100%	83%	99%	76%
NPV	91%	88%	85%	93%	88%	91%
Efficiency	94%	75%	92%	86%	93%	81%

Figures are given for each of the *PRNP* codon 129 genotypes of sCJD (MM, homozygous for methionine; MV heterozygous for methionine and valine; VV, homozygous for valine). The figures given are the number of positive or negative results over the total number of samples investigated. Efficiency was defined as: (true positives + true negatives)/total number tested.
NPV = negative predictive value; PPV = positive predictive value.



RESEARCH ARTICLE

January/February 2015 Volume 6 Issue 1 e02451-14 mBio mbio.asm.org 1

Rapid and Sensitive RT-QuIC Detection of Human Creutzfeldt-Jakob Disease Using Cerebrospinal Fluid

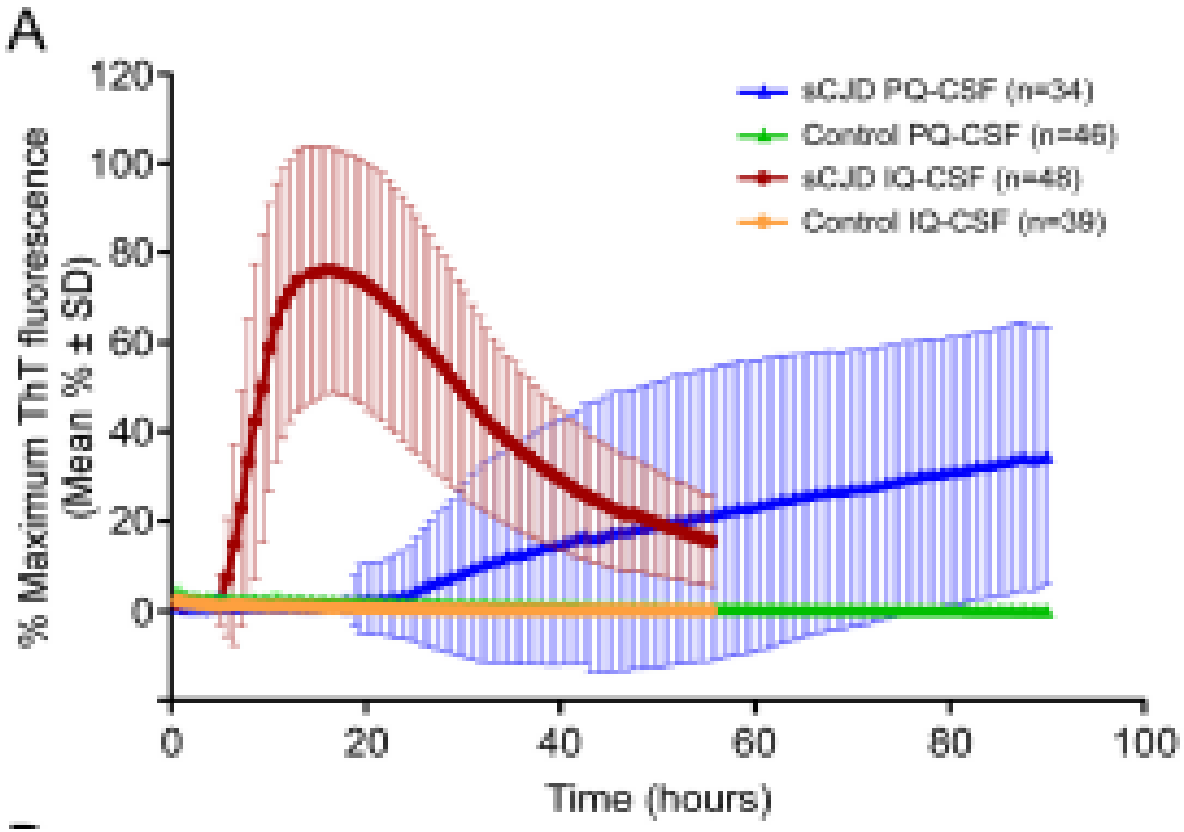
Christina D. Orrú,[a] Bradley R. Groveman,[a] Andrew G. Hughson,[a] Gianluigi Zanusso,[b] Michael B. Coulthart,[c] Byron Caughey[a]

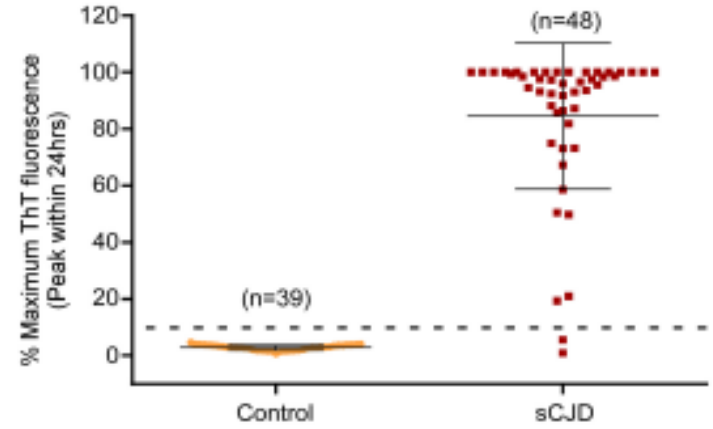
Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA[a]; Department of Neurological and Movement Sciences, University of Verona, Verona, Italy[b]; Canadian CJD Surveillance System, Public Health Agency of Canada, Ottawa, Ontario, Canada[c]

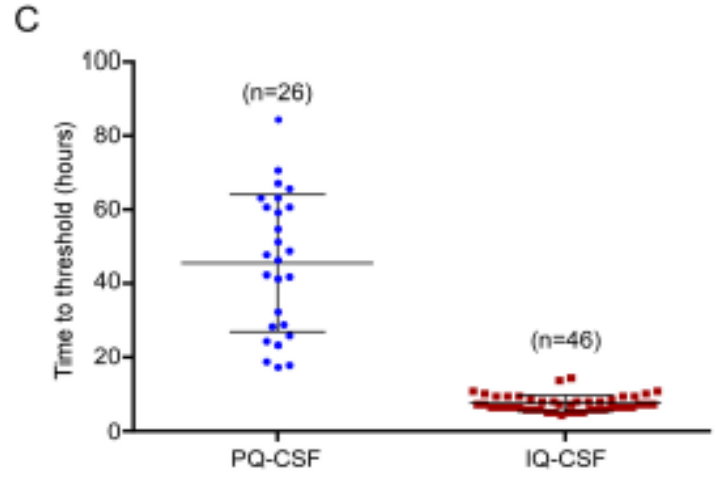
C.D.O., B.R.G., and A.G.H. contributed equally to this study.

PQ-CSF vs IQ-CSF assay

- 100% specificity
- Sensitivity increases from 70% to ~ 94%
- 24 Hours test





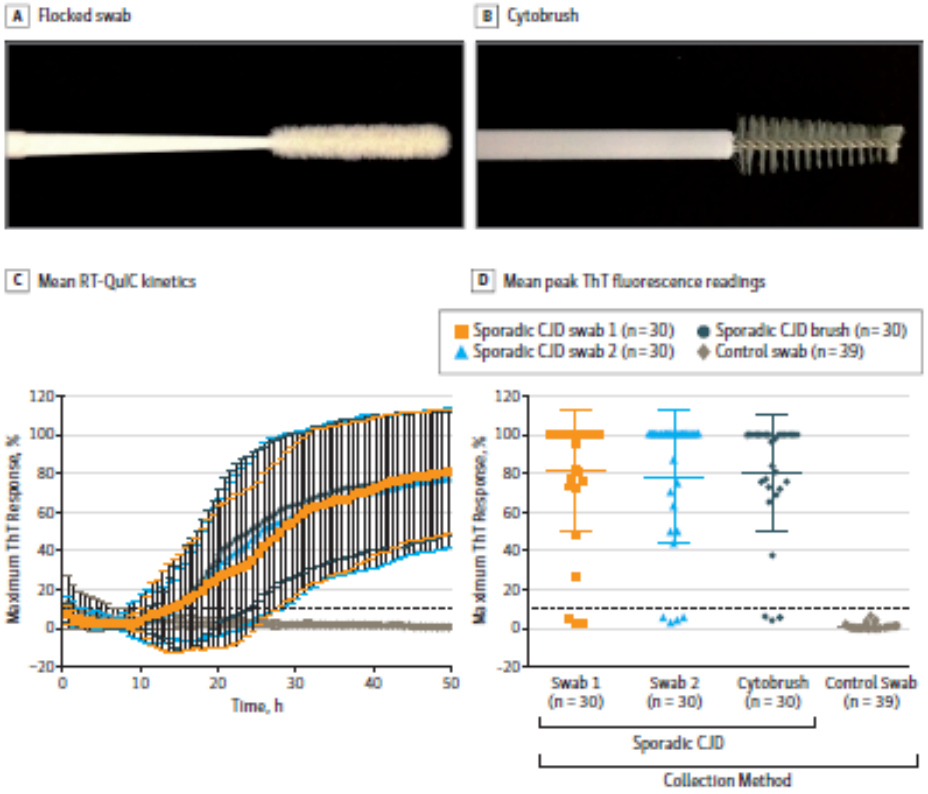


JAMA Neurology | Original Investigation

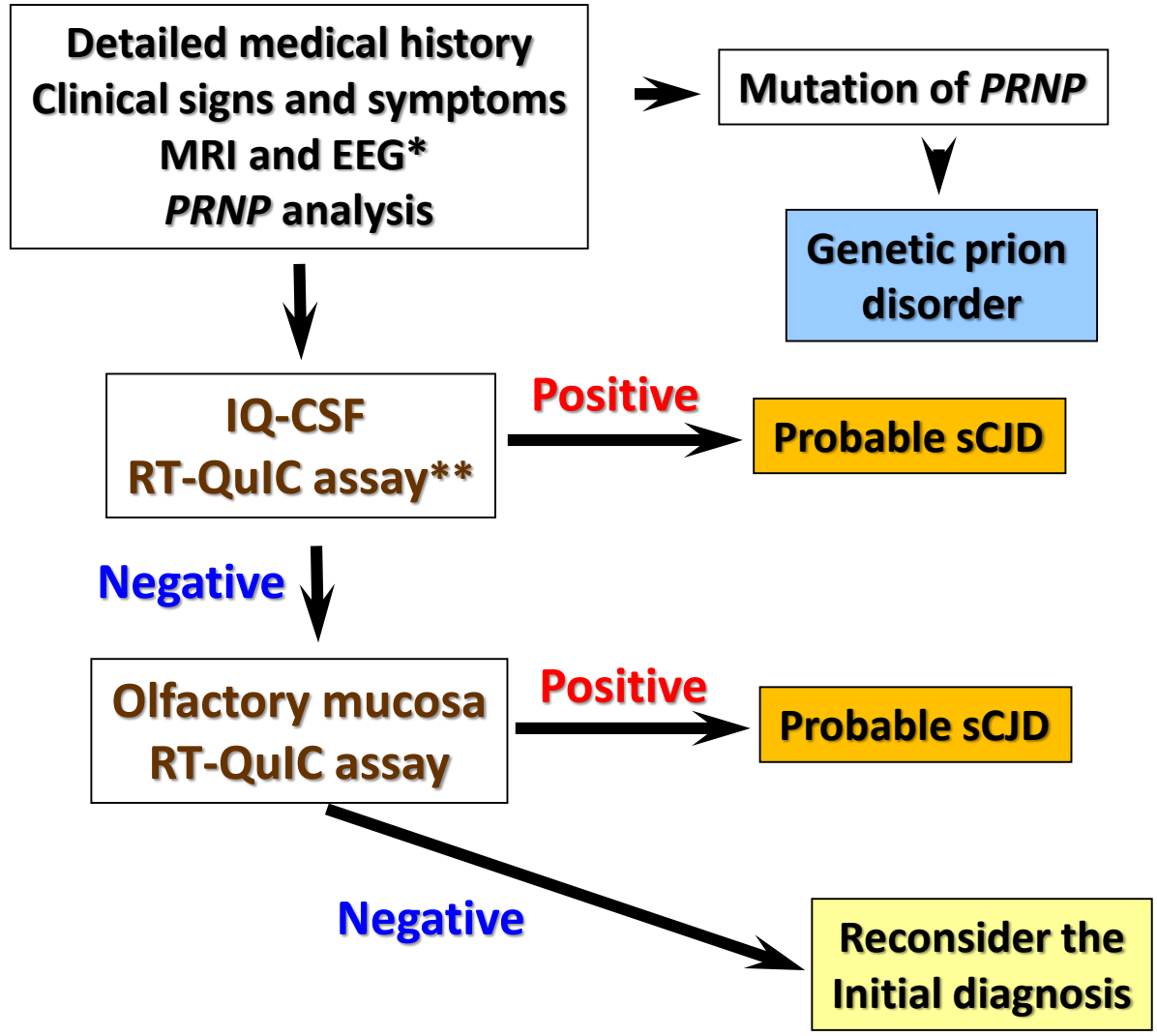
Diagnosis of Human Prion Disease Using Real-Time Quaking-Induced Conversion Testing of Olfactory Mucosa and Cerebrospinal Fluid Samples

Matilde Bongianni, PhD; Christina Orrù, PhD; Bradley R. Groveman, PhD; Luca Sacchetto, MD; Michele Fiorini, PhD; Giovanni Tonoli, MD; Giorgio Triva, BS; Stefano Capaldi, PhD; Silvia Testi, PhD; Sergio Ferrari, MD; Annachiara Cagnin, MD, PhD; Anna Ladogana, MD; Anna Poleggi, PhD; Elisa Colaizzo, MD; Dorina Tiple, MD; Luana Valanella, MD; Santina Castriciano, BS; Daniele Marchioni, MD; Andrew G. Hughson, MS; Daniele Imperiale, MD; Tatiana Cattaruzza, MD, PhD; Gian Maria Fabrizi, MD; Maurizio Pocchiari, MD; Salvatore Monaco, MD; Byron Caughey, PhD; Gianluigi Zanusso, MD, PhD

Figure 1. Olfactory Mucosa (OM) Sample Collection and Results of Real-Time Quaking-Induced Conversion (RT-QuIC) Assays



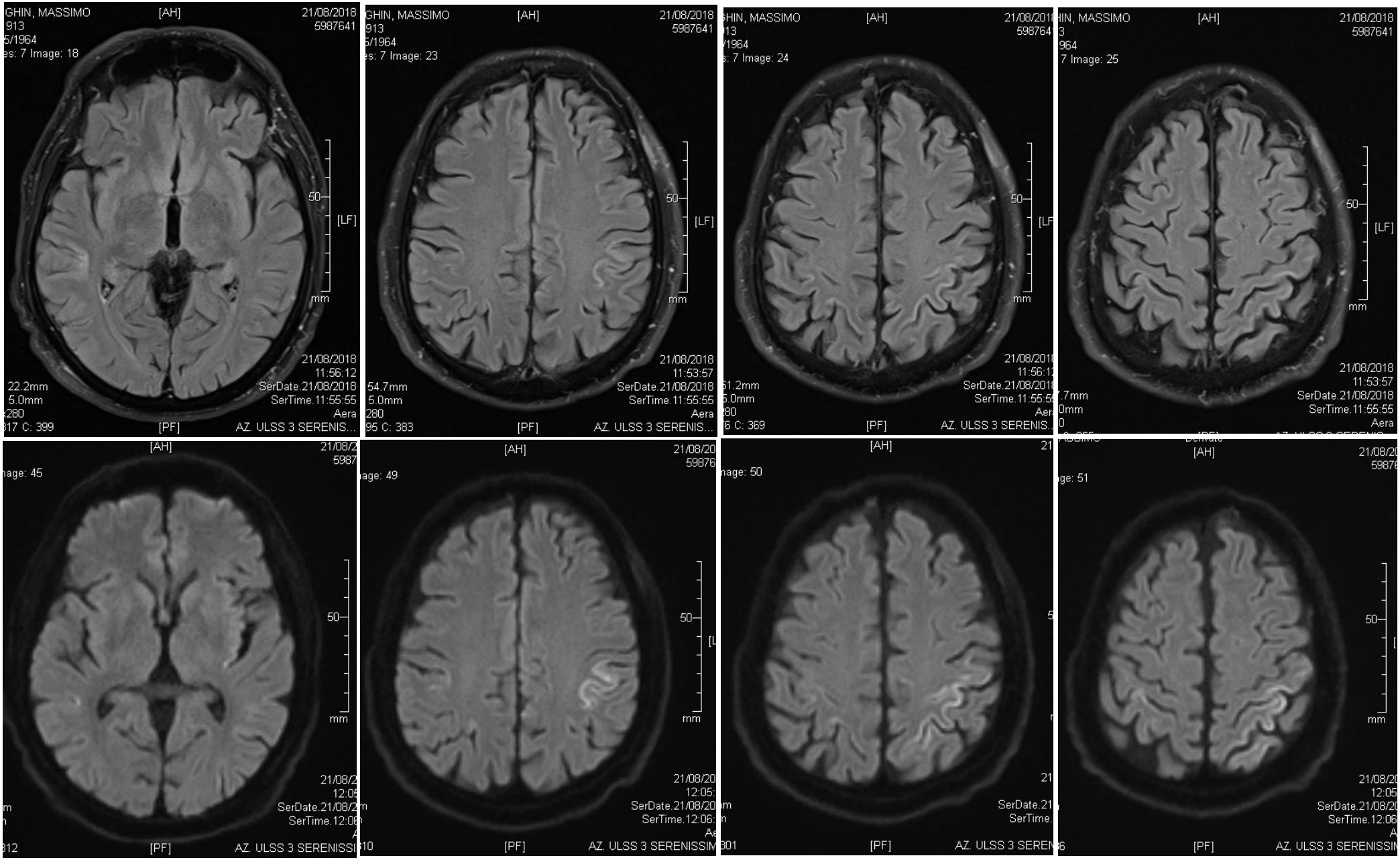
Diagnostic algorithm in Suspected Human Prion Disorder



Atypical sCJD cases 129MV

	Case 1	Case 2
Age	56 years Male	65 years Male
Disease onset	Transient diplopia	Paroxysmal episodes of light-headedness
Clinical evolution	6 months later transient dizziness Mild ataxia	16 months later behavioural visual and spatial disturbances, executive functions and memory impairment
EEG	Not specific	Not specific
MRI	Hyperintense signal in fronto-parietal cortexes	Hyperintense signal in Frontal temporal and parietal cortexes
CSF	14-3-3 Negative Tau 294 pg/mL	14-3-3 Negative Tau 266 pg/mL
RT-QuIC	Positive in CSF and OM	Positive in CSF and OM
PRNP	No mutation 129 MV	No mutation 129 MV

Case # 1



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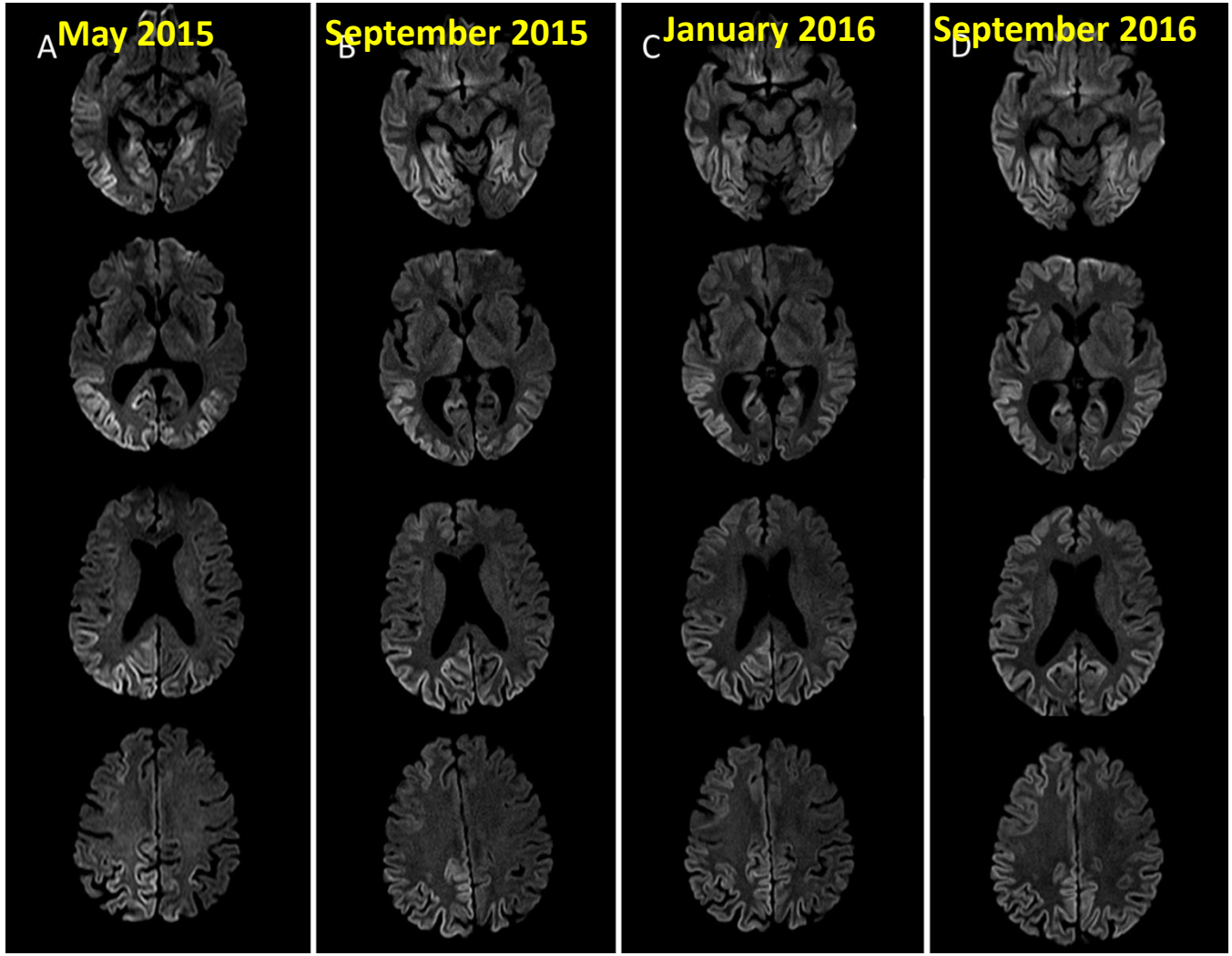
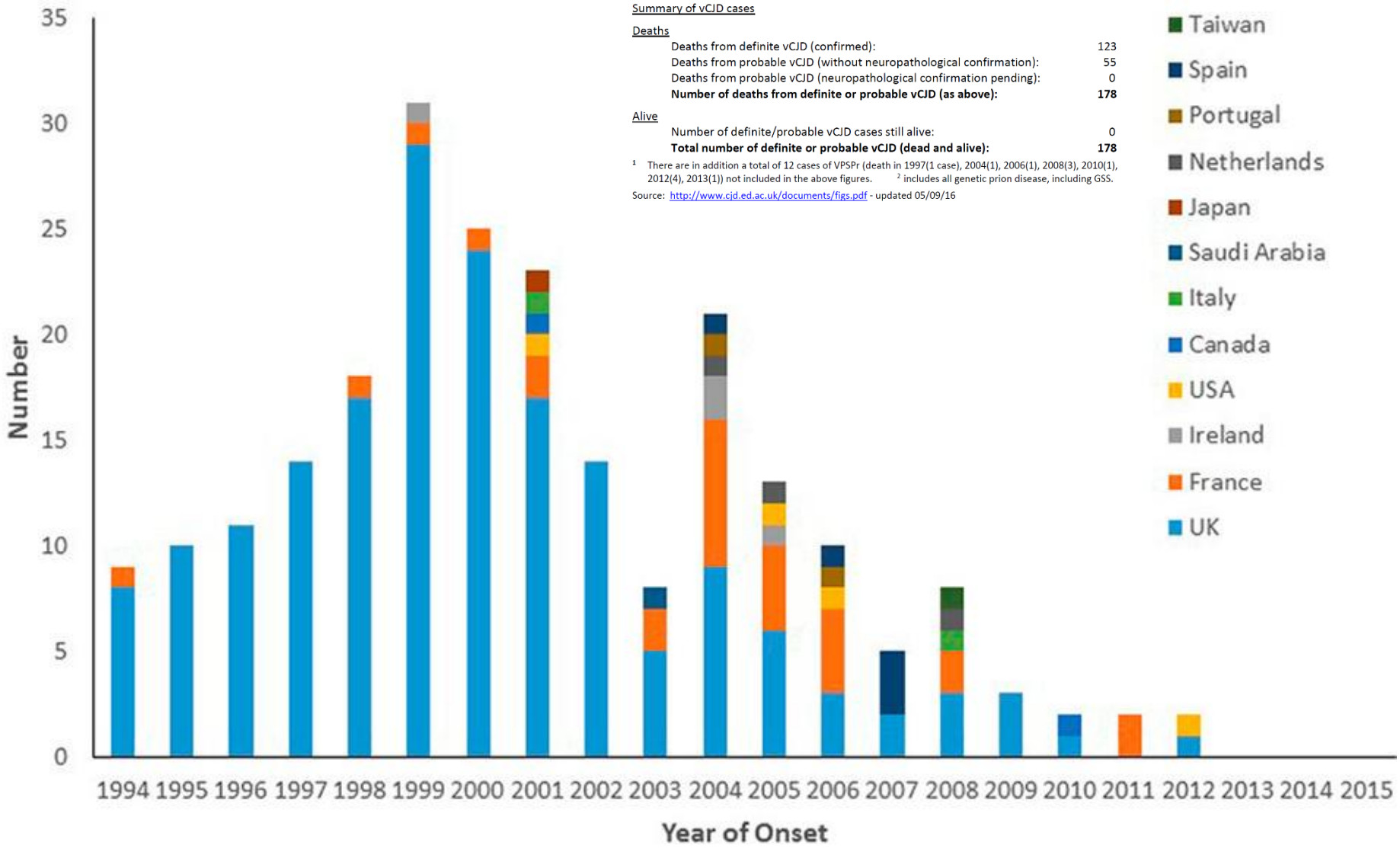
Case # 2



Clinical signs	**None**	**None**	**None**	**Dementia**
Neuropsychological assessment	**ND**	**Impaired face recognition**	**Mild visuo-spatial deficits**	**Pathological**
CSF	**ND**	**PQ-QuIC Negative**	**14.3.3 Negative** **PQ-QuIC: Negative** **OM Negative**	**14.3.3 Negative, Tau: 266 pg/mL** **IQ-QuIC: Positive** **OM Positive**

Protein Misfolded Cyclic Amplification (PMCA) In Variant Creutzfeldt-Jakob Disease Diagnosis

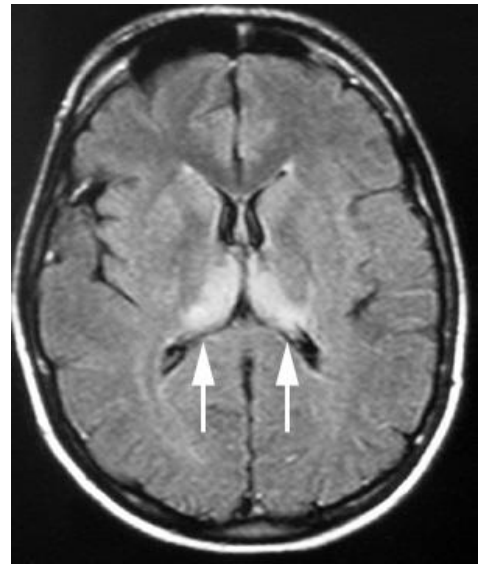
Variant CJD cases Worldwide



Disease phenotype of vCJD 129MM

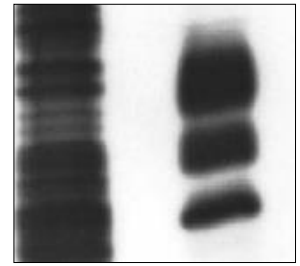
Mean age of onset	29 years
Disease Duration	14 months
Clinical disease	Early psychiatric symptoms Painful sensory symptoms Cerebellar ataxia Dementia in the late course
EEG	Not specific
MRI	Typical hyperintense signal in pulvinar
CSF	14-3-3 positive in 50%
Other tests	Tonsil biopsy
PrPTSE Glycotype **Neuropathology**	Type 2B Florid plaques

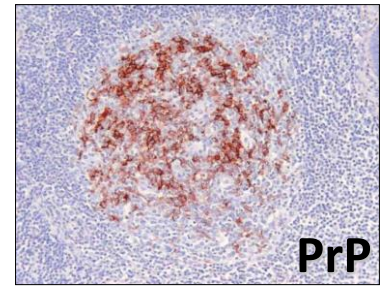
MRI



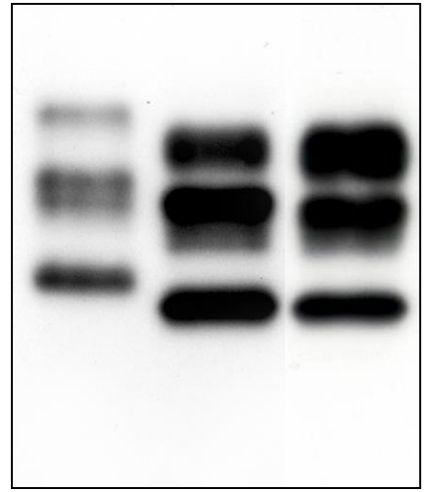
Tonsil biopsy

PK: - +



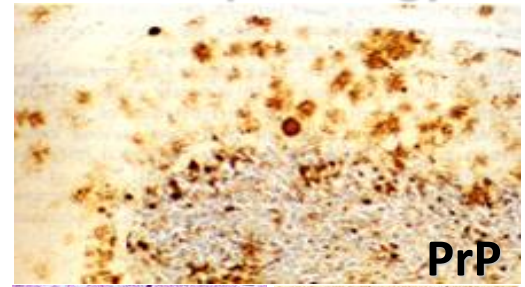


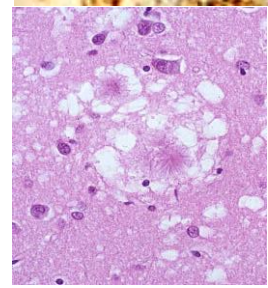
PrP^{TSE} Glycotype

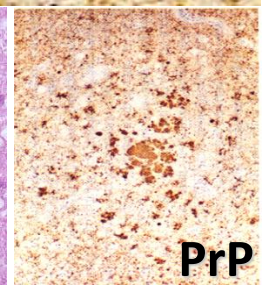


Type 1 Type 2A Type 2B

Neuropathology







Which Success in Human Prion Diseases

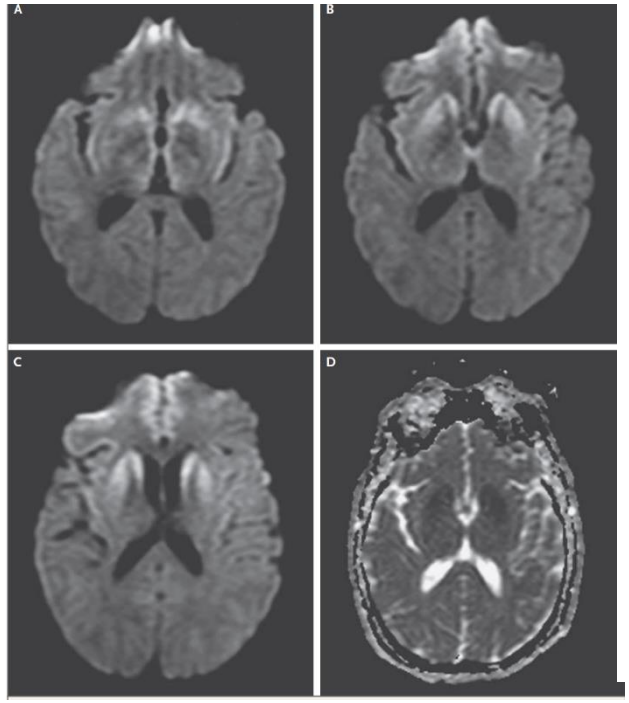
Forms (occurrence)	Etiology	Disease phenotype
Genetic (10%)	Prion Protein Gene Mutations Autosomal dominant inheritance with a variability of penetrance mutation-dependent	Genetic Creutzfeldt-Jakob Disease (gCJD) Fatal Familial Insomnia (FFI) Gerstmann Straussler Scheinker syndrome (GSS)
Sporadic (90%)	Unknown	Creutzfeldt-Jakob Disease (sCJD) Fatal Insomnia VPSPr
Acquired (<1%)	Exposure to prion sources of infectious material → Humans (surgical/medical procedures) → Animals BSE related	Iatrogenic CJD (iCJD) Dura mater transplants Cornea Growth hormone from cadaver Variant CJD (vCJD)

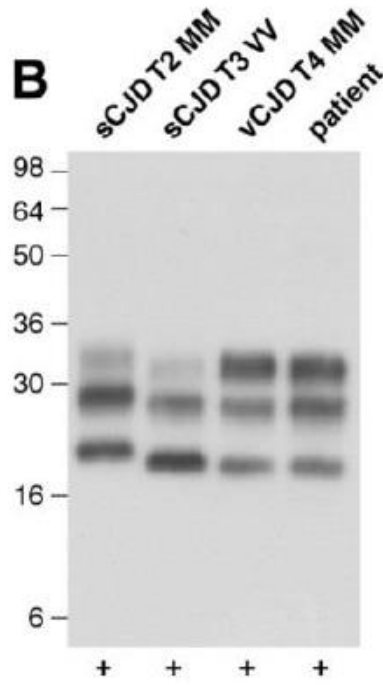
Prevention

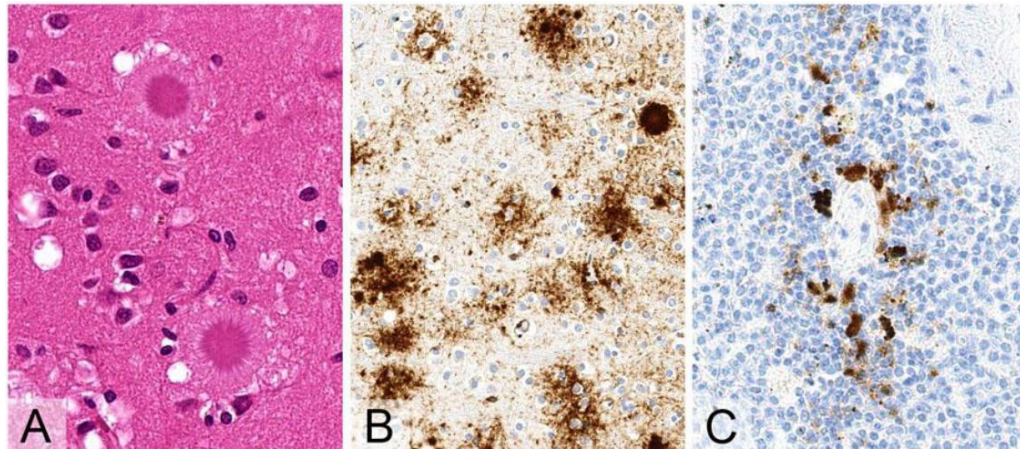
Variant Creutzfeldt–Jakob Disease in a Patient with Heterozygosity at *PRNP* Codon 129

Mok et al. N ENGL J MED 376;3 NEJM.ORG JANUARY 19, 2017

Age of onset	36 years
Disease Duration	15 months
Clinical disease	Behavioural changes Memory decline Ataxia, cerebellar signs Myoclonus
EEG	Not specific
MRI	Basal ganglia, insula and medial thalami
CSF	14-3-3 negative RT-QuIC negative
PrPTSE Glycotype	Type 2B Spleen positive
Neuropathology	Florid plaques







Diagnosis of Methionine/Valine Variant Creutzfeldt-Jakob Disease by Protein Misfolding Cyclic Amplification

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 24, No. 7, July 2018

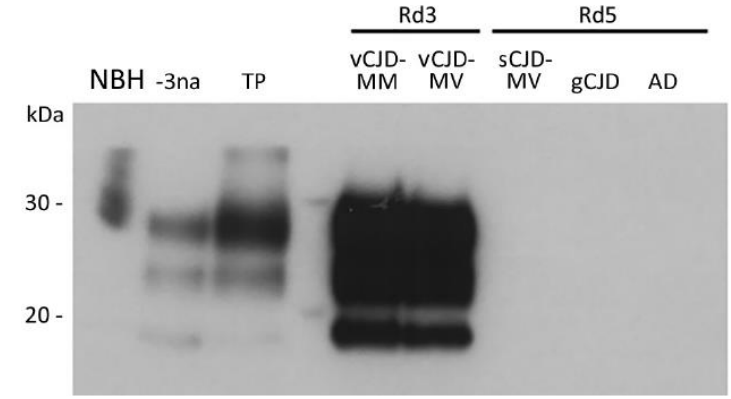
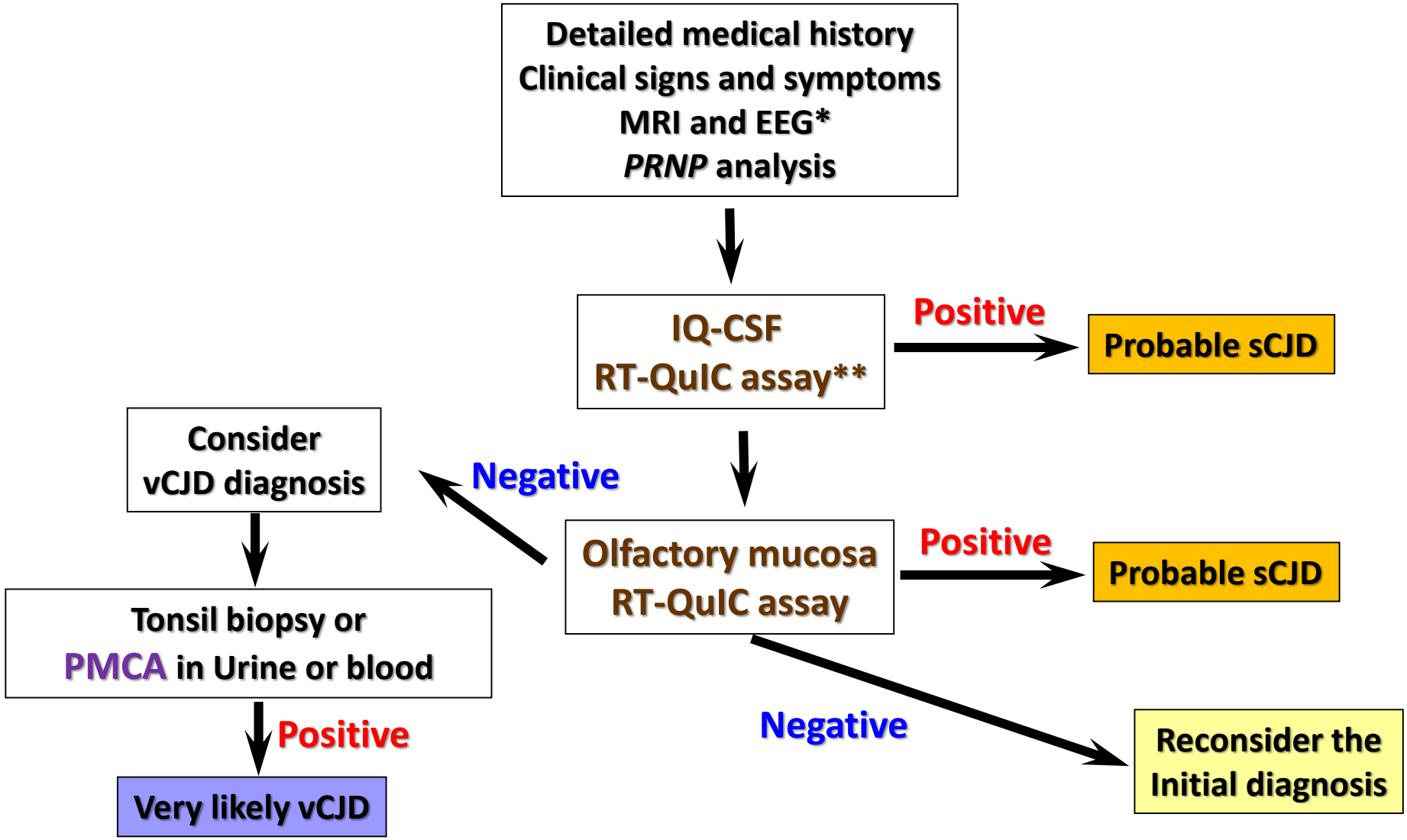


Table. Analysis of CSF samples from patients with CJD and controls by PMCA*

Diagnosis	No. patients with positive detection of PrP^{TSE} in CSF and codon 129 genotype/no. tested				Analytical performance, % (95% CI)
	Total	MM	MV	VV	
Clinical CJD					
Variant CJD	40/41†	37/38	1/1	NA	Diagnostic sensitivity 97.6 (87.1–99.9)
Definite	29/29	28/28	1/1	NA	
Probable	10/11	8/9	NA	NA	
Possible	1/1	1/1	NA	NA	
Sporadic CJD	0/23†	0/7	0/12	0/3	Analytic specificity 100 (93.7–100)
Definite	0/14	0/2‡	0/10	0/1‡	
Probable	0/9	0/5	0/2	0/2	
Genetic CJD	0/1	0/1	NA	NA	Analytic specificity 100 (93.7–100)
Non-CJD					Analytic specificity 100 (93.7–100)
Alzheimer's disease	0/12	ND	ND	ND	
Other nonneurodegenerative diseases	0/21	ND	ND	ND	

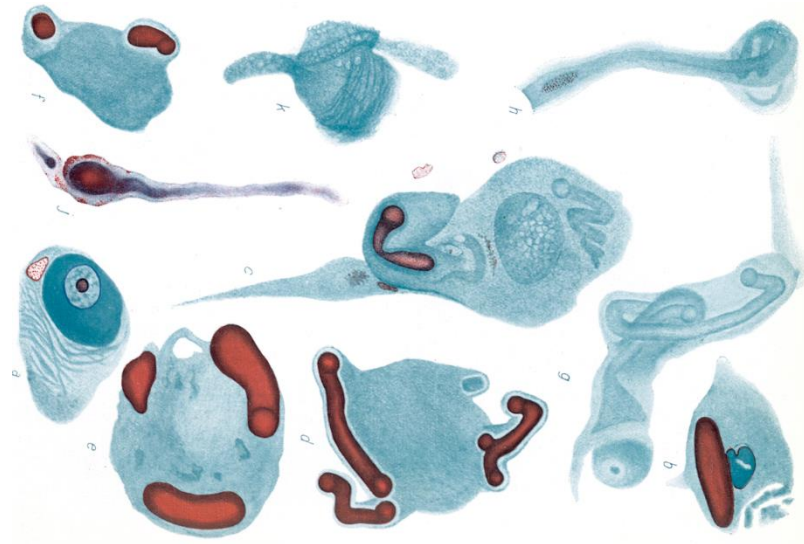
*CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; MM, methionine homozygous; MV, methionine/valine heterozygous; NA, not available; ND, not determined; PMCA, protein misfolding cyclic amplification; PrP^{TSE}, abnormal prion protein; VV, valine homozygous.
†Genotyping of the prion protein gene at codon 129 was not conducted for 2 patients with variant CJD and 1 patient with sporadic CJD.
‡The protease-resistant protein subtype was available for 3 patients with definite sporadic CJD and showed an equal distribution of MM1, MM2a, and VV2a.

Suspected Human Prion Disorder

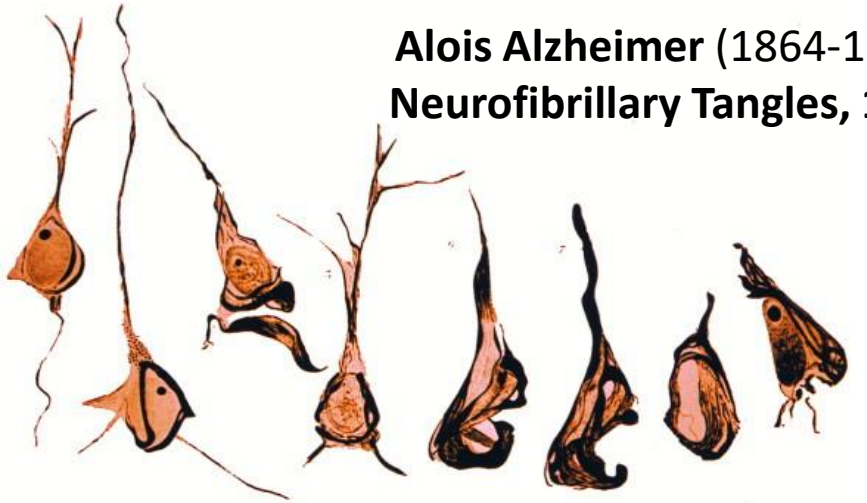


The origin of Misfolded Disorders

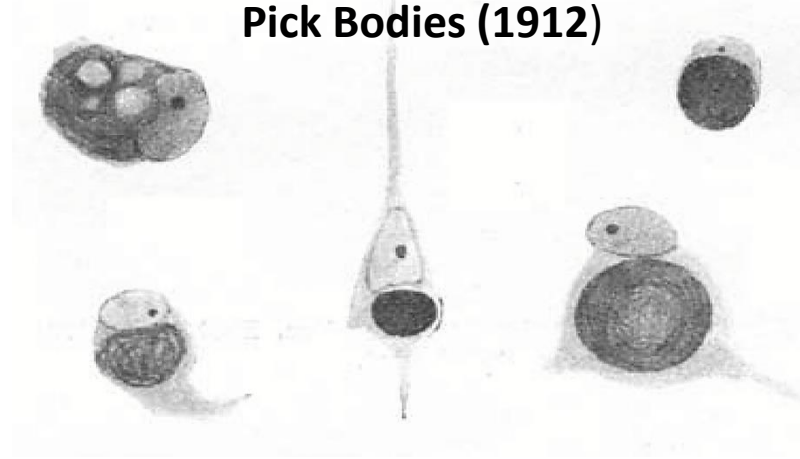
Friedrich Henrich Lewy (1885-1950)
Images of Lewy Bodies (1923)



Alois Alzheimer (1864-1915)
Neurofibrillary Tangles, 1911



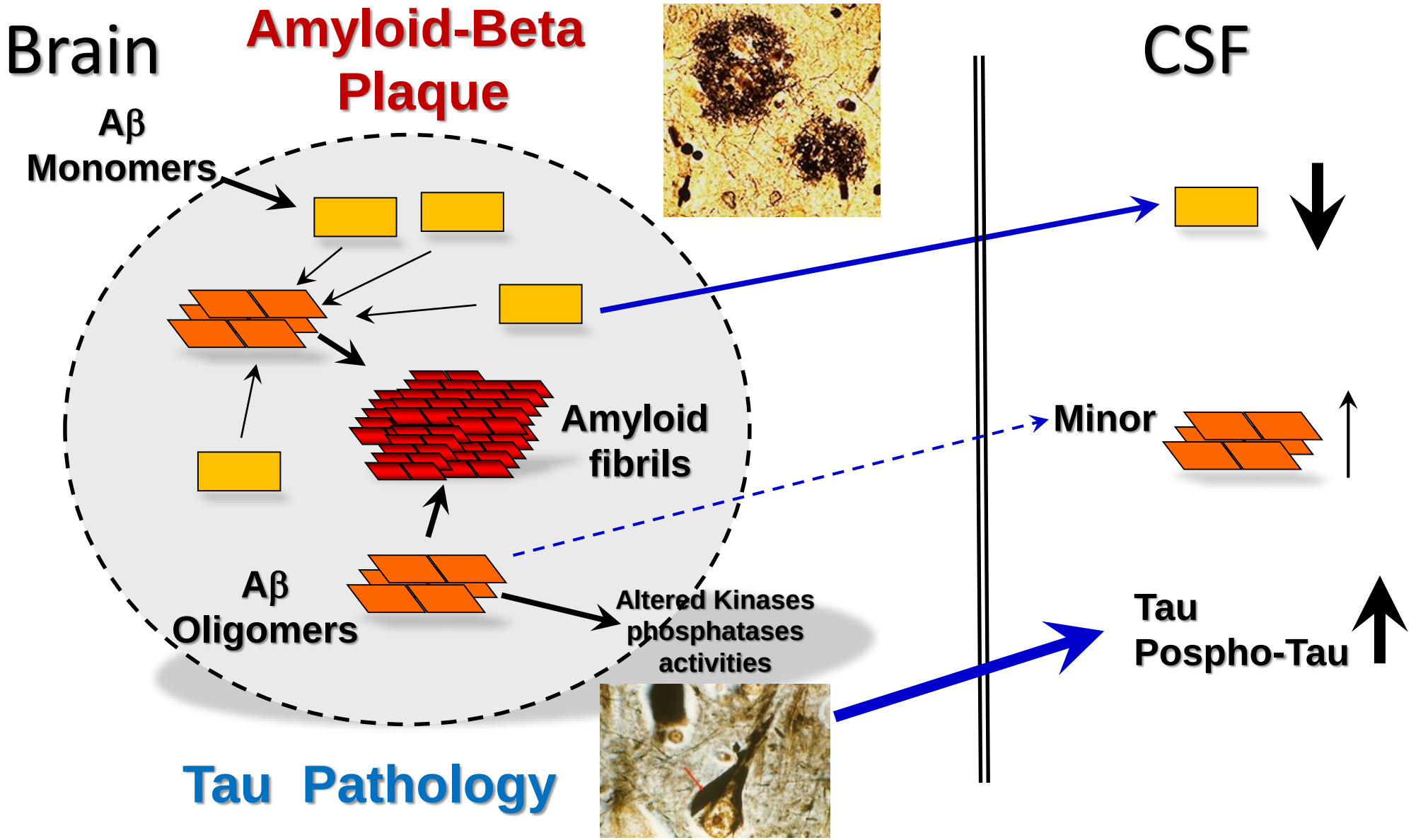
Friedrich Pick (1854-1924)
Pick Bodies (1912)



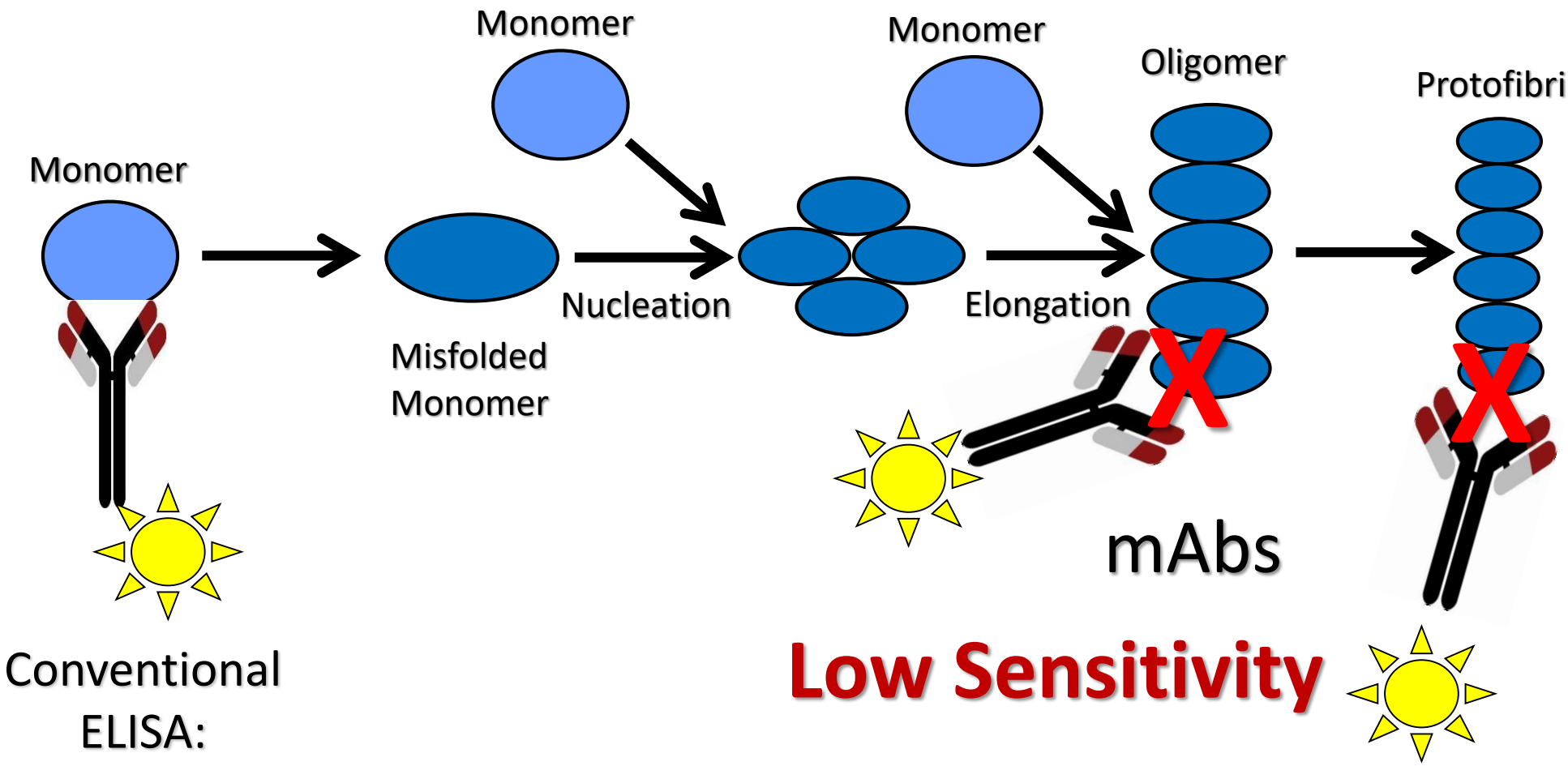
Cerebrospinal Fluid Biomarkers

Neurodegenerative Dementias	Disease Associated Proteins	CSF Measurement Specificity and Sensitivity
Alzheimer's Disease	Amylod beta / Tau	High
Dementia with Lewy Bodies	Alpha-Synuclein	Low
Frontotemporal Dementia	Tau	Low
Fronto-temporal Dementia and ALS	TDP43	Low

Aβ processing in the Brain and CSF changes

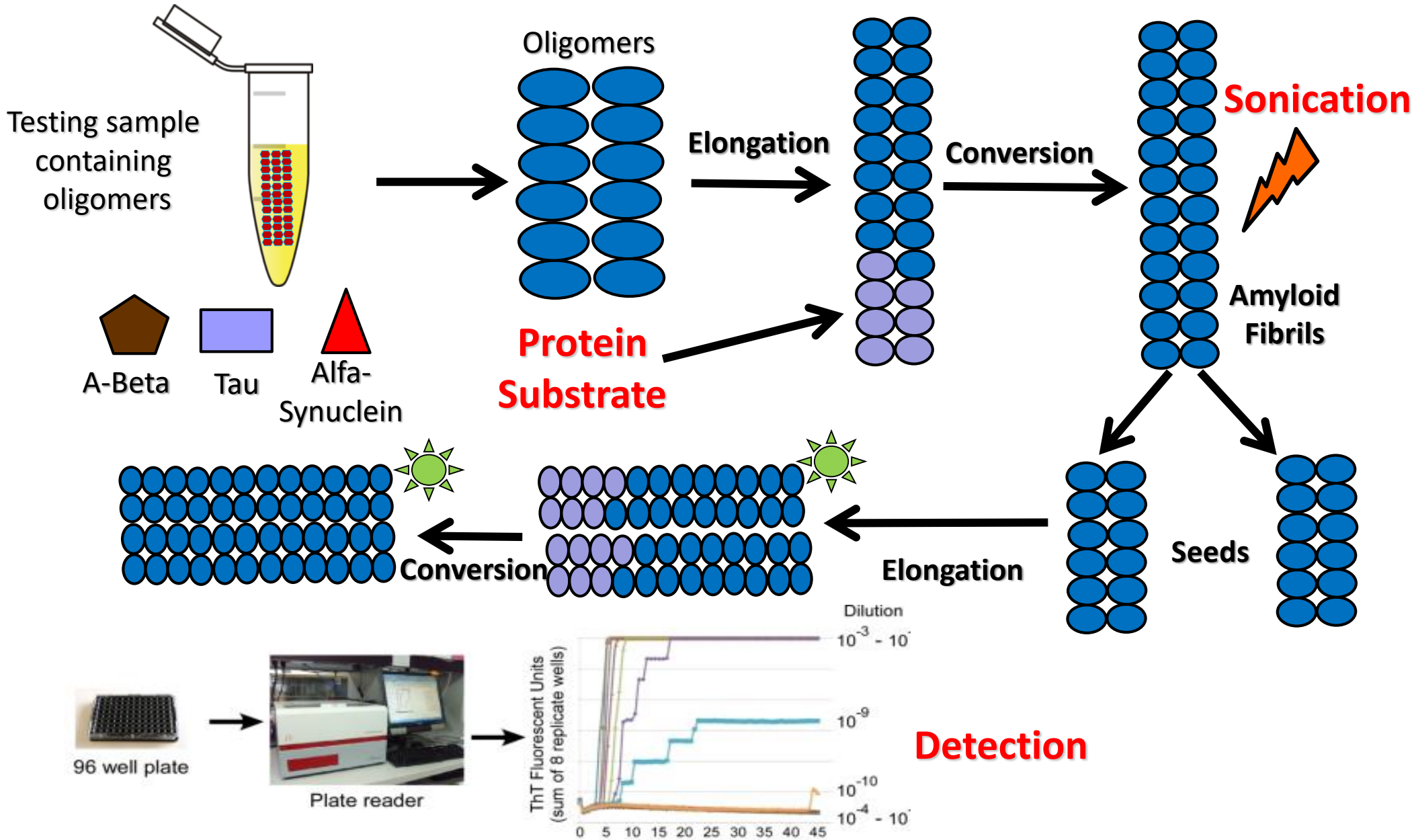


Oligomer Detection by ELISA



Conventional
ELISA:
A-β
Tau

Principle of Oligomer Seeding Assays



Why Use Oligomer Seeding Assays in Neurodegenerative Disorders

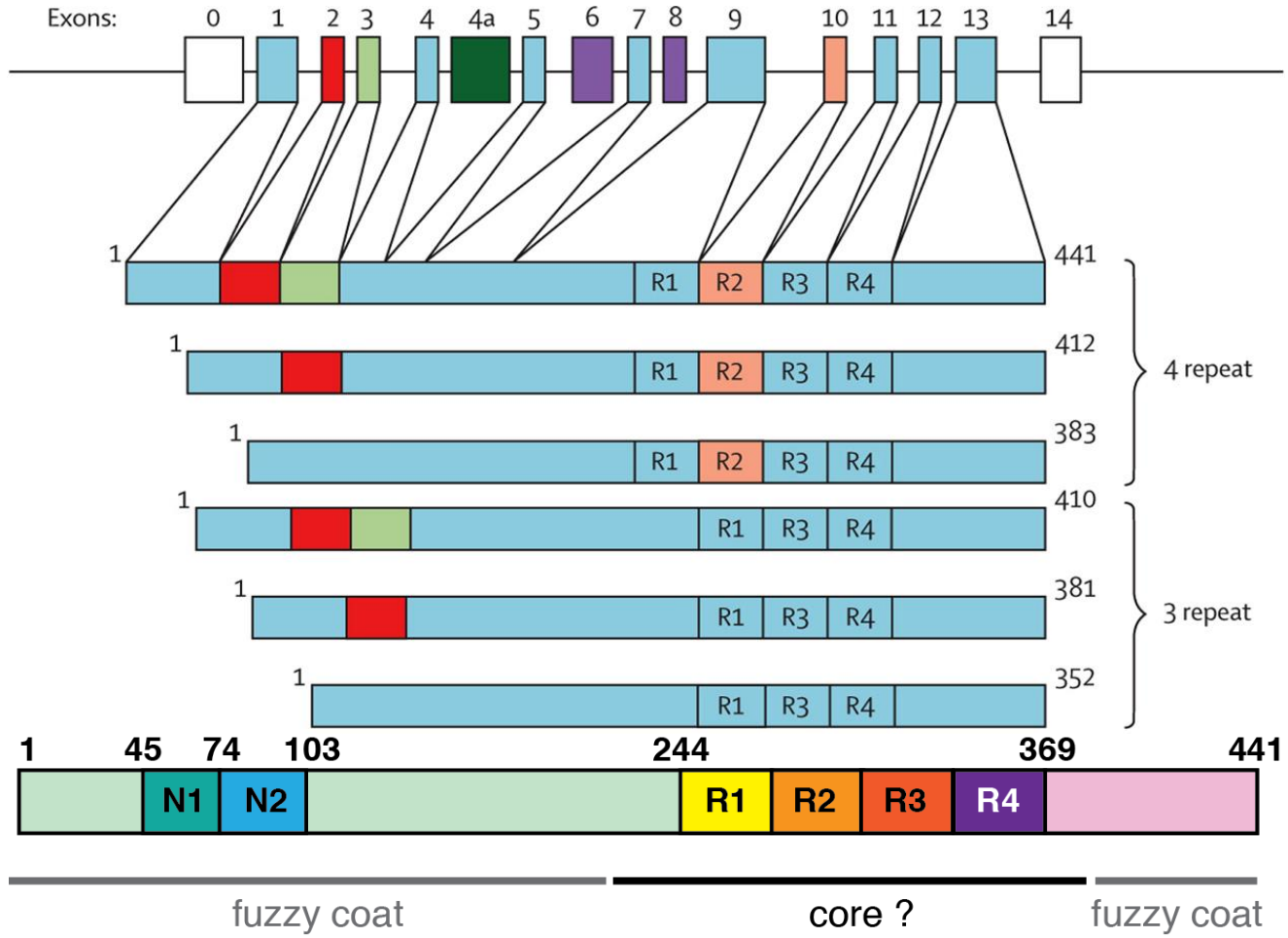
- ✓ **Identify oligomers**
- ✓ **Diagnostic purposes**

  Define the Molecular basis of a Neurodegenerative Disorder
- ✓ **Prognostic purposes**

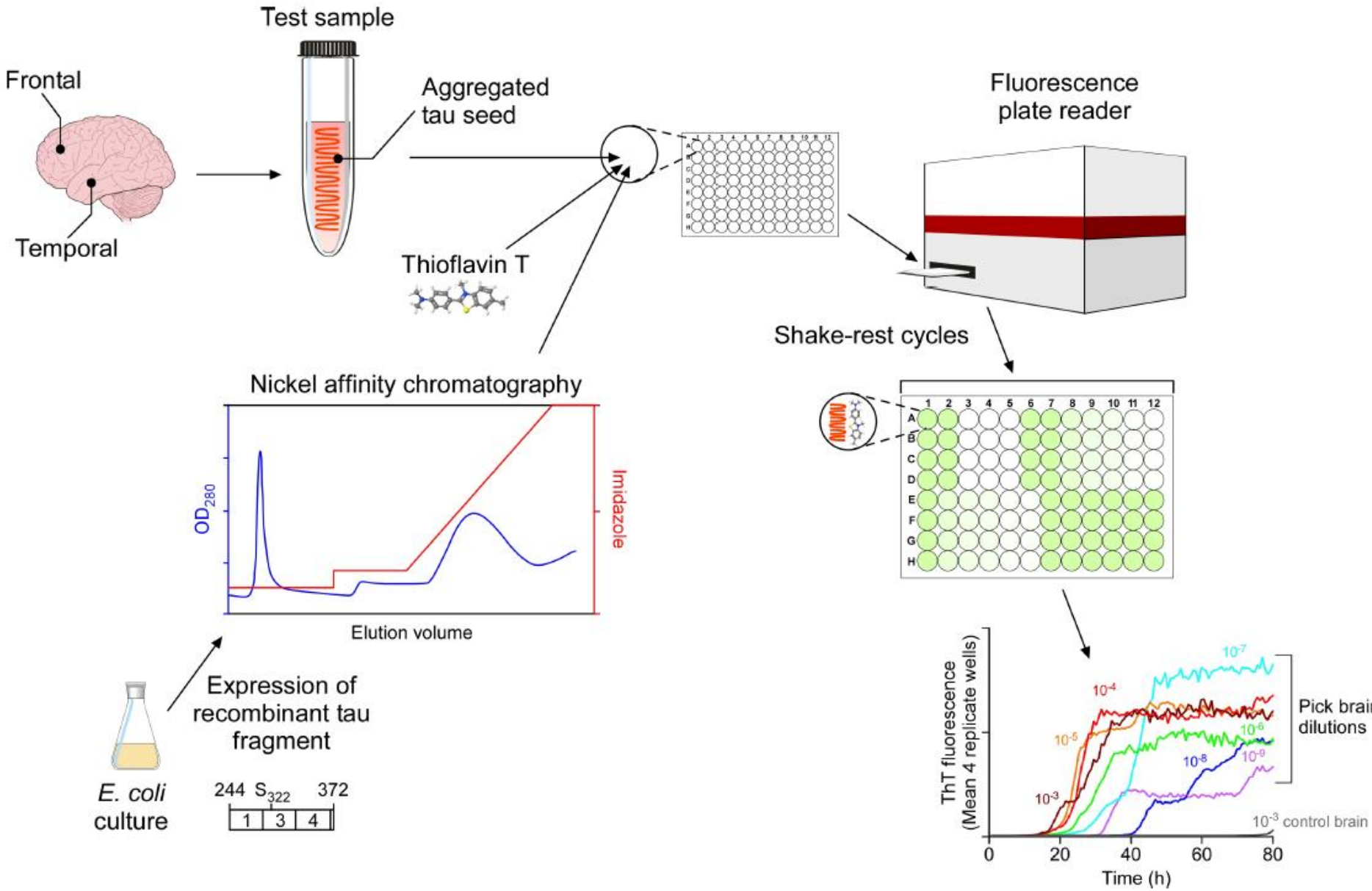
  Amyloid-Beta oligomers extent influences the progression of cognitive decline

  Define a Parkinsonism since the disease disability varies among different phenotypes
- ✓ **Potential therapeutic approaches**

Tau


Exons:
0 1 2 3 4 4a 5 6 7 8 9 10 11 12 13 14
1
441
R1 R2 R3 R4
412
383
4 repeat
410
381
352
3 repeat
1 45 74 103 244 369 441
N1 N2 R1 R2 R3 R4
fuzzy coat
core ?
fuzzy coat

Experimental procedure of Tau RT-QuIC



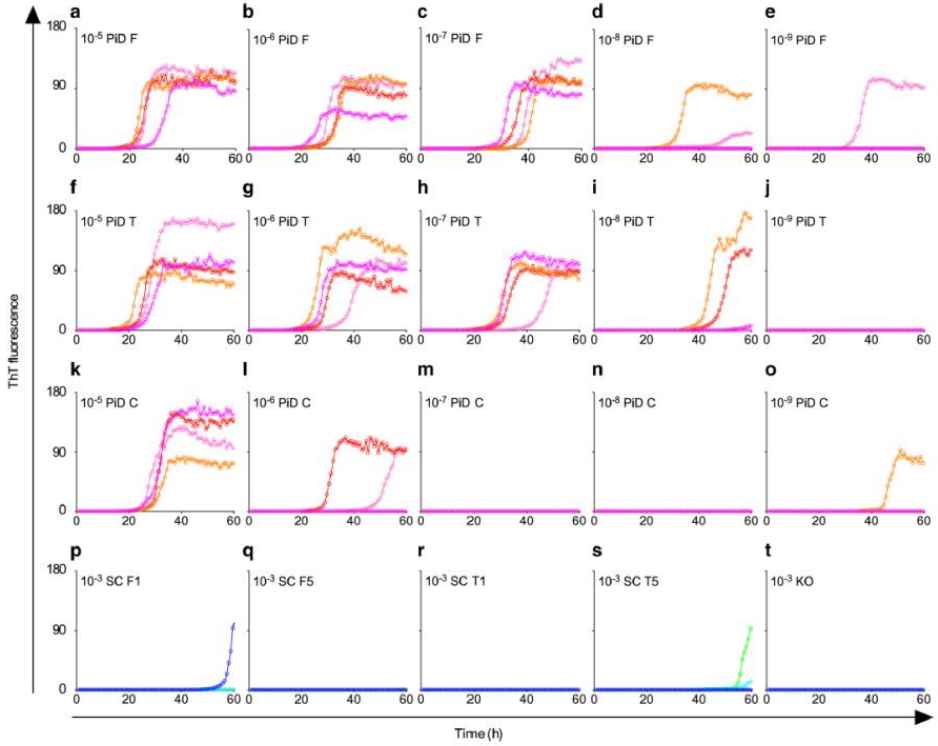
Acta Neuropathol (2017) 133:751–765
DOI 10.1007/s00401-017-1692-z

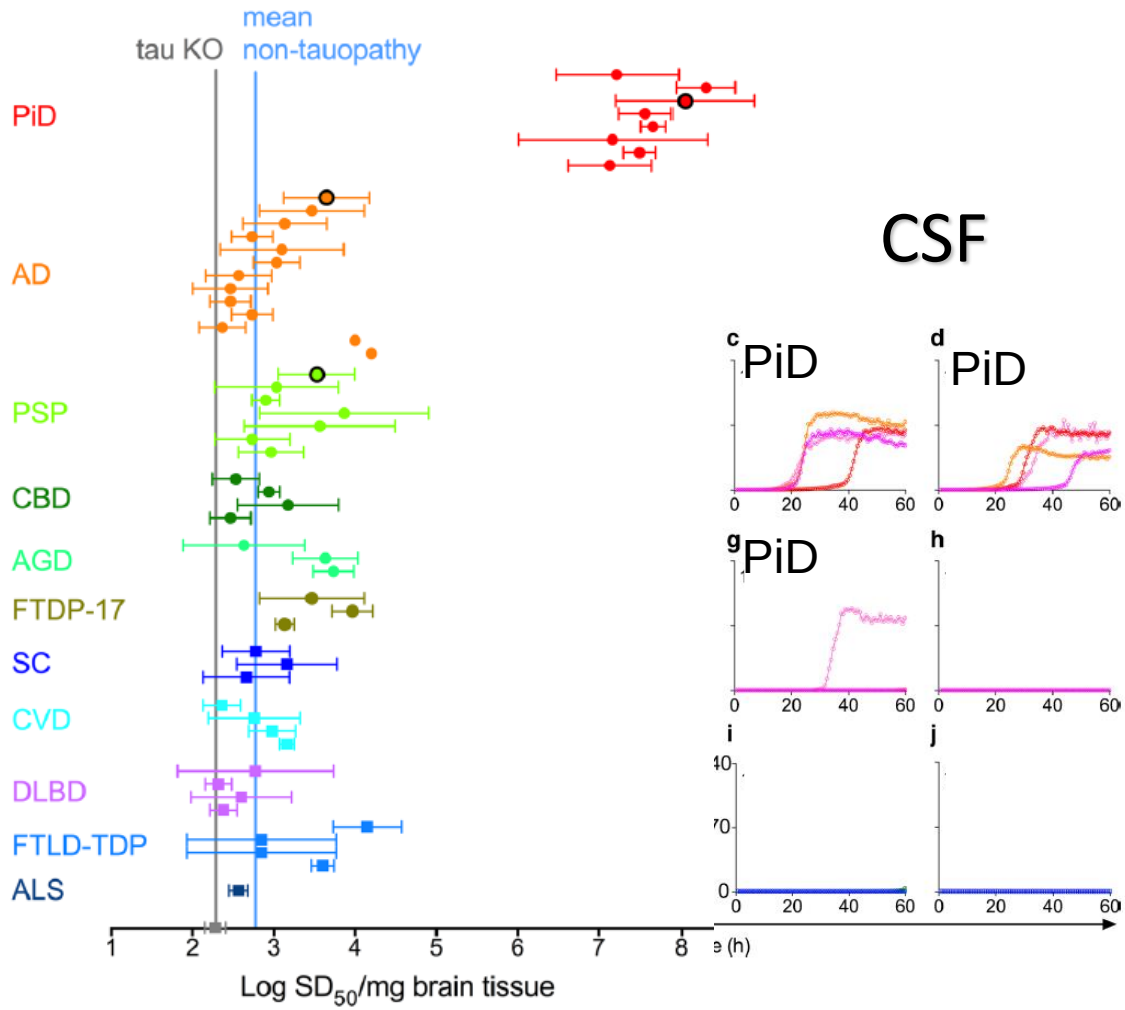
ORIGINAL PAPER

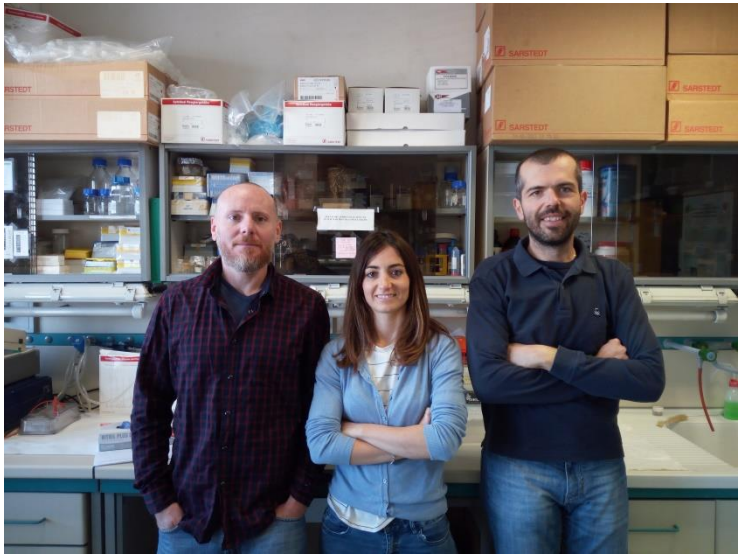
Ultrasensitive and selective detection of 3-repeat tau seeding activity in Pick disease brain and cerebrospinal fluid

Eri Saijo[1] · Bernardino Ghetti[2] · Gianluigi Zanusso[3] · Adrian Oblak[2] · Jennifer L. Furman[4] · Marc I. Diamond[4] · Allison Kraus[1] · Byron Caughey[1]

Detection limit in PiD brain tissue







Stefano Capaldi

Matilde Bongianni

Michele Fiorini

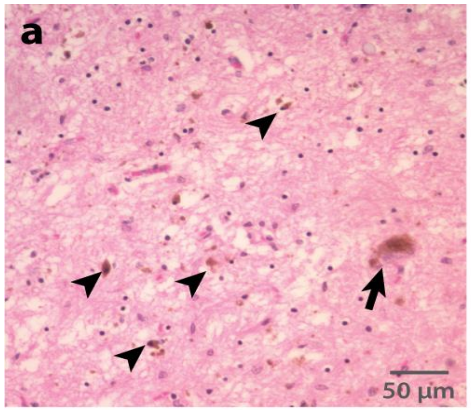
Alfa-Synuclein

Alpha-Synucleinopathies and Disease Phenotypes

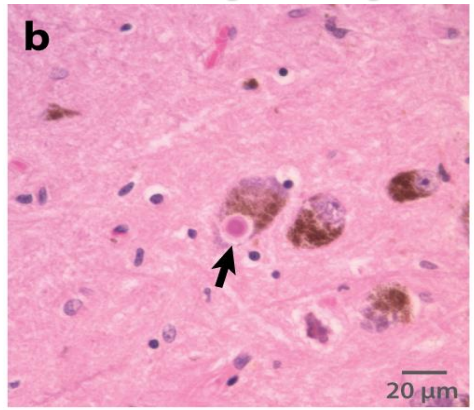
- ✓ Parkinson Disease
- ✓ Mutiple System Atrophy
- ✓ Lewy Body Dementia
- ✓ Pure Autonomic Failure

Lewy Body Pathology

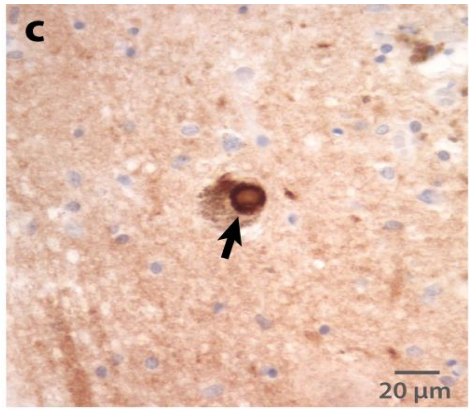
Neuronal loss gliosis



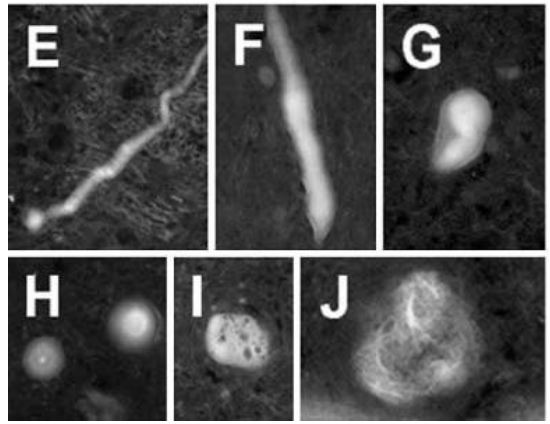
Lewy body

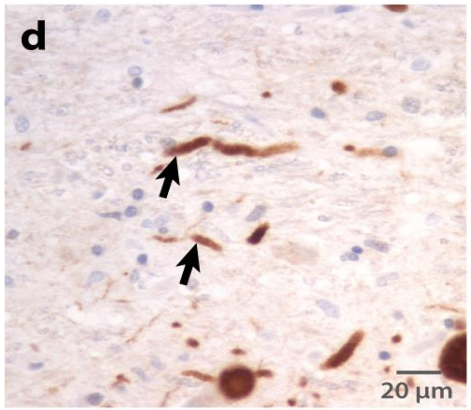


Lewy body
(α-synuclein)

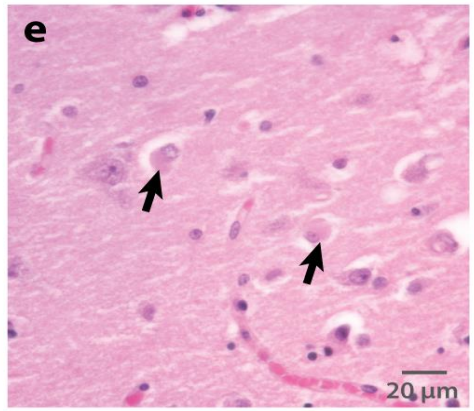


Thioflavin S

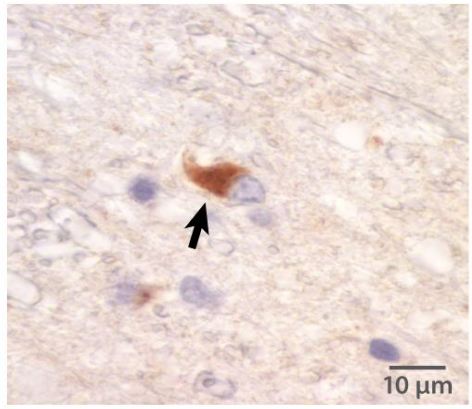




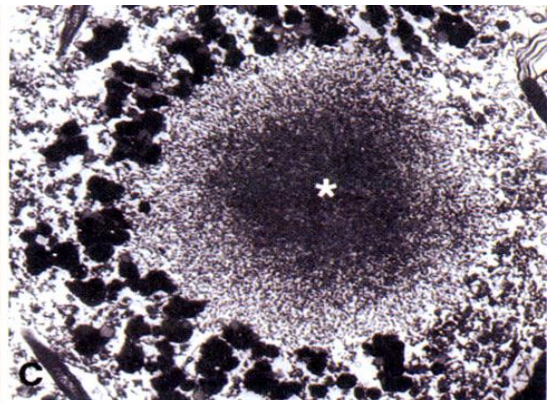
Lewy neurites
(α-synuclein)



Lewy bodies

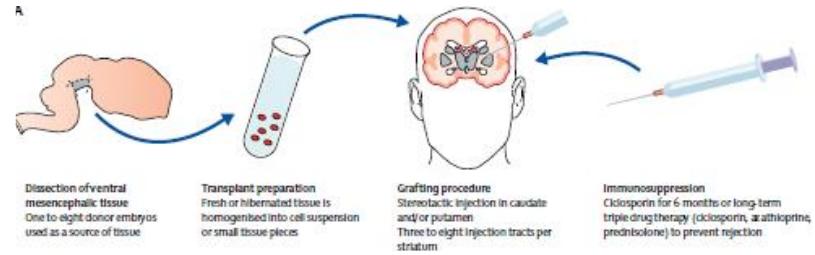


Oligodendroglial inclusion in MSA
(α-synuclein)



Shulman JM, et al. 2011.
Annu. Rev. Pathol. Mech. Dis. 6:193–222

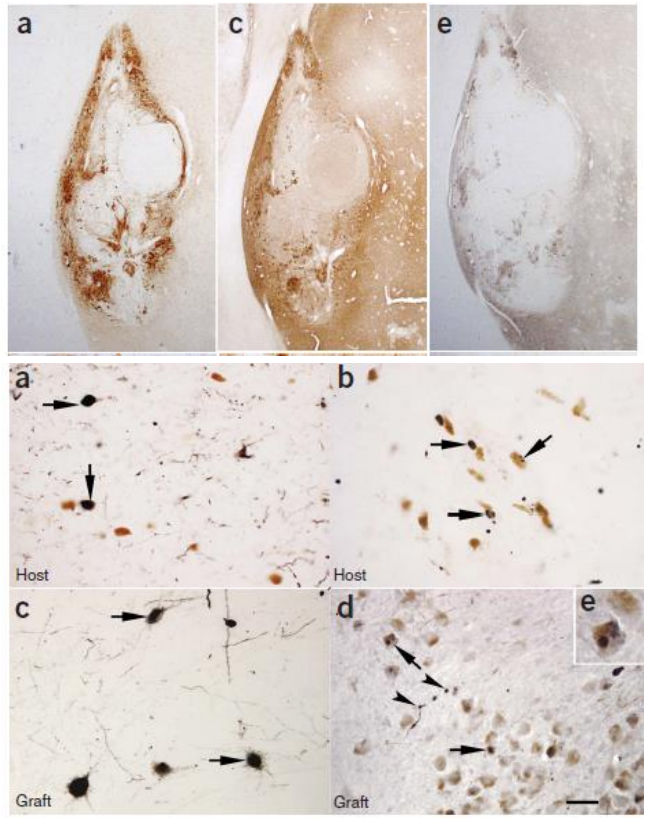
Prion-Like Propagation of Alpha-Synuclein aggregates



- ✓ Patients with PD 10-14 years after trapiantation of fetal midbrain showed alpha-synuclein inclusions in 2-5% grafted neurons at neuropathological examination
- ✓ Alpha-synuclein is detected in a non aggregated form after 4 yrs and in aggregated form after 14 yrs
- ✓ After 24 yrs 11-12% of grafted neurons exhibited alpha-synuclein inclusions

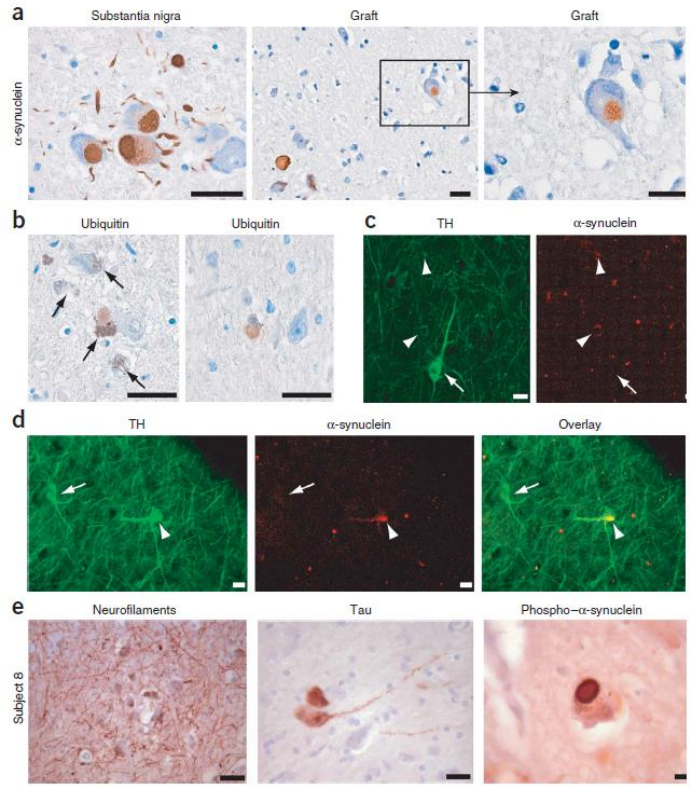
Lewy body–like pathology in long-term embryonic nigral transplants in Parkinson's disease

Jeffrey H Kordower[1], Yaping Chu[1], Robert A Hauser[2], Thomas B Freeman[3] & C Warren Olanow[4]



Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation

Jia-Yi Li[1], Elisabet Englund[2], Janice L Holton[3], Denis Soulet[1], Peter Hagell[4], Andrew J Lees[3], Tammaryn Lashley[3], Niall P Quinn[5], Stig Rehncrona[6], Anders Björklund[7], Håkan Widner[4], Tamas Revesz[3,9], Olle Lindvall[4,8,9] & Patrik Brundin[1,9]



NATURE MEDICINE VOLUME 14 | NUMBER 5 | MAY 2008

Seeding assays for α-synuclein

ANNALS of Clinical and Translational Neurology
Open Access
ANA American Neurological Association

BRIEF COMMUNICATION

Alpha-synuclein RT-QuIC in the CSF of patients with alpha-synucleinopathies

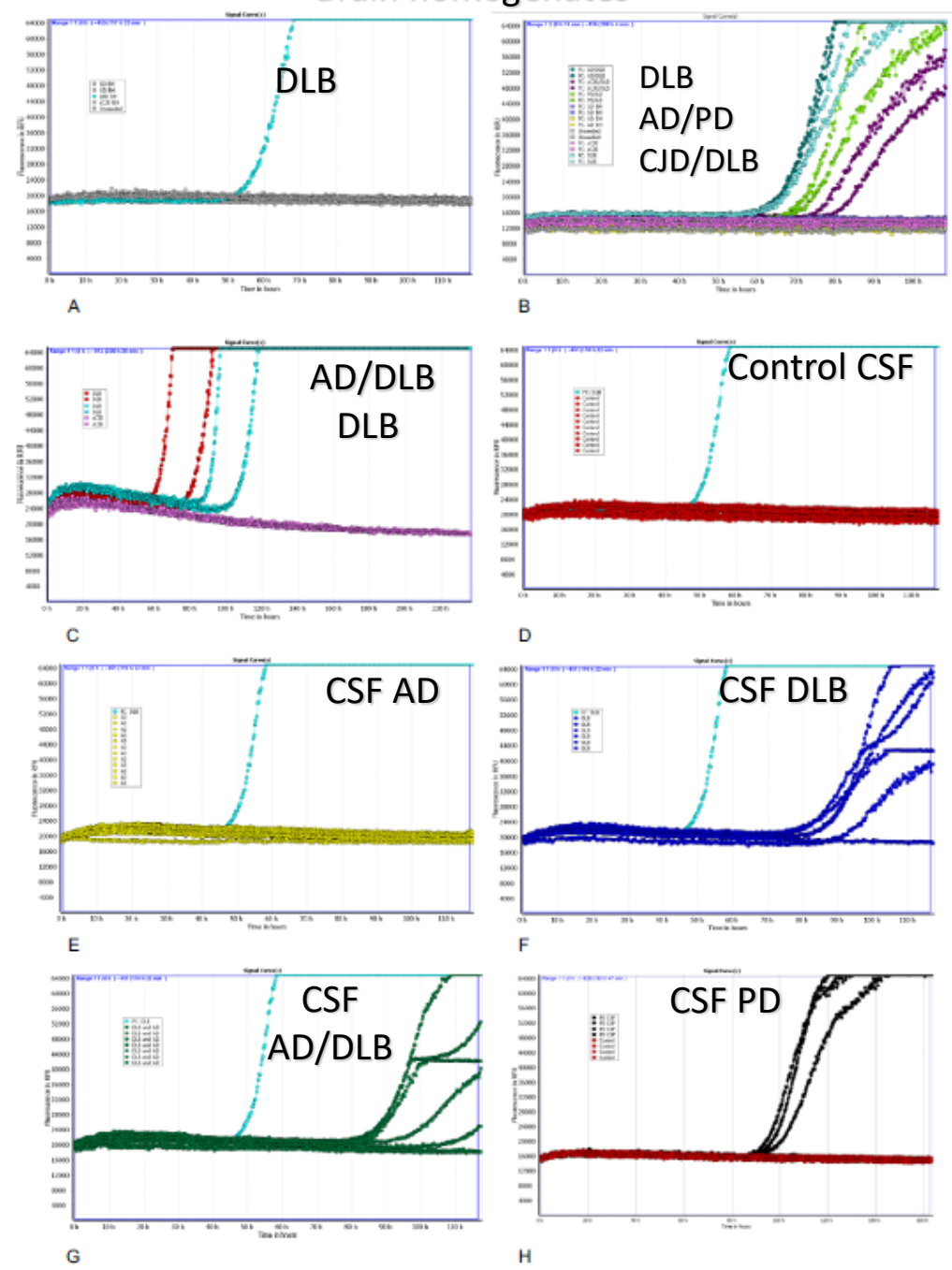
Graham Fairfoul[1], Lynne I. McGuire[1], Suvankar Pal[1,2], James W. Ironside[1], Juliane Neumann[3], Sharon Christie[4], Catherine Joachim[4], Margaret Esiri[4], Samuel G. Evetts[3], Michal Rolinski[3], Fahd Baig[3], Claudio Ruffmann[3], Richard Wade-Martins[5], Michele T. M. Hu[3], Laura Parkkinen[3] & Alison J. E. Green[1]

Table 2. Positive RT-QuIC reactions seeded with CSF samples from patients with neuropathologically confirmed DLB, mixed DLB/AD, AD with incidental LB, AD, PD, and healthy controls (Exploratory group) and patients with clinically diagnosed PD, at-risk PD, neuropathologically confirmed corticobasal degeneration and supranuclear palsy and PD controls (Confirmatory group).

	Number of positive RT-QuIC (%) using 5 μL	Number of positive RT-QuIC (%) using 10 μL	Number of positive RT-QuIC (%) using 15 μL
Exploratory patient group (*n*)			
AD with incidental LB (13)	2 (15%)	4 (31%)	2 (15%)
Healthy Controls (20)	0 (0%)	0 (0%)	0 (0%)
Mixed DLB/AD (17)	9 (53%)	11 (65%)	11 (65%)
Parkinson's disease (2)	2 (100%)	2 (100%)	2 (100%)
Progressive supranuclear palsy (2)	0 (0%)	0 (0%)	0 (0%)
Corticobasal degeneration (3)	0 (0%)	0 (0%)	0 (0%)
Pure AD (30)	2 (7%)	1 (3%)	0 (0%)
Pure DLB (12)	10 (83%)	11 (92%)	11 (92%)
Sensitivity (DLB)	83%	92%	92%
Specificity (vs. controls)	100%	100%	100%
Specificity (vs. AD)	93%	97%	100%
Specificity (vs. controls + AD)	96%	98%	100%
Confirmatory patient group (*n*)			
Parkinson disease (20)	–	–	19 (95%)
At-risk PD patients (3)	–	–	3 (100%)
Parkinson's disease controls (15)	–	–	0 (0%)
Sensitivity (PD)	–	–	95%
Specificity	–	–	100%

A positive RT-ASA response was classified as a relative fluorescence unit (rfu) value of >2SD above the mean of the negative controls at 120 h of at least one of the CSF duplicates.

Brain homogenates



Groveman et al. Acta Neuropathologica Communications (2018) 6:7
DOI 10.1186/s40478-018-0508-2

Acta Neuropathologica Communications

METHODOLOGY ARTICLE Open Access

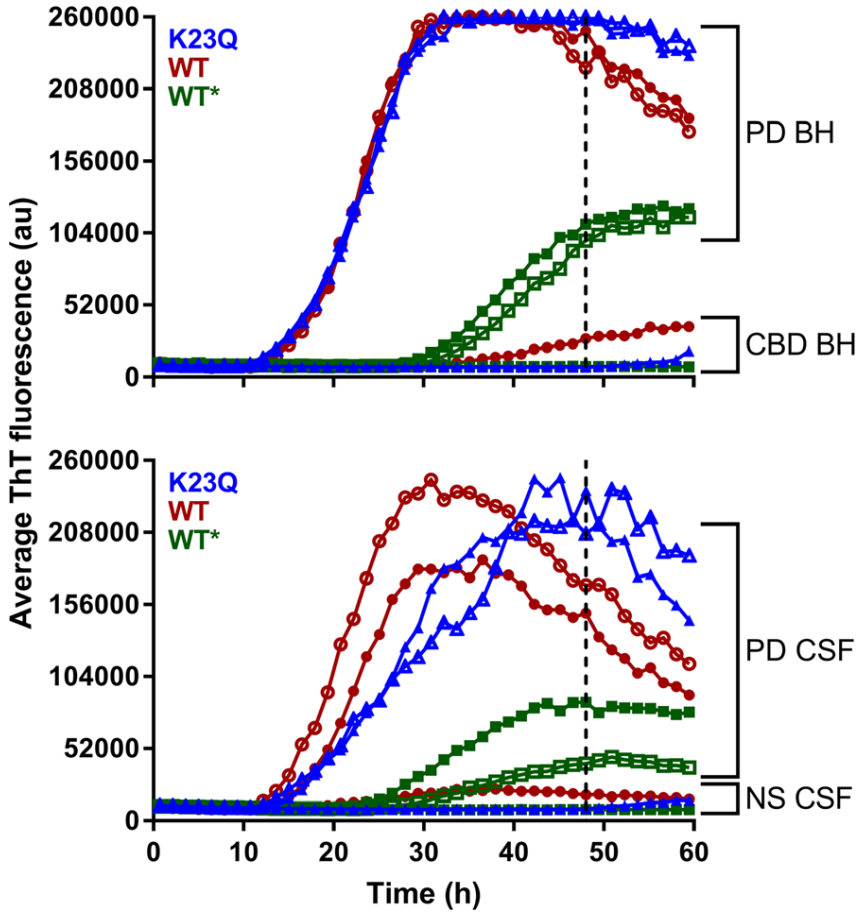
Rapid and ultra-sensitive quantitation of disease-associated α-synuclein seeds in brain and cerebrospinal fluid by αSyn RT-QuIC

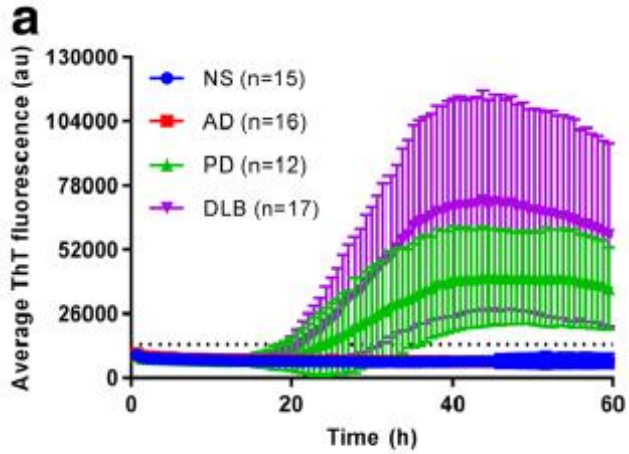
Bradley R. Groveman[1†], Christina D. Orrù[1†], Andrew G. Hughson[1], Lynne D. Raymond[1], Gianluigi Zanusso[2], Bernardino Ghetti[3], Katrina J. Campbell[1], Jiri Safar[4], Douglas Galasko[5*] and Byron Caughey[1*]

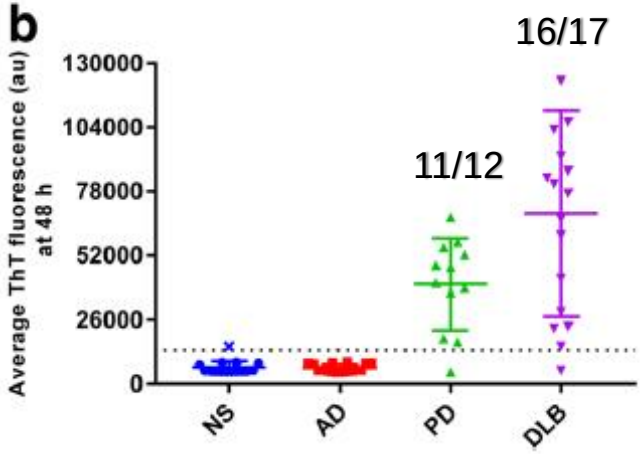
Table 1 Demographic data and cognitive impairment at the time of lumbar puncture (LP) in studied subjects

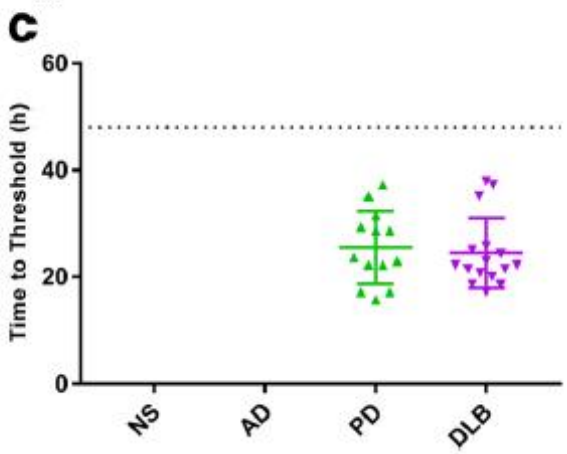
Final diagnosis	n	Age at onset (years)	Age at LP (years)	Mean interval between onset and LP (years)
Dementia with Lewy Bodies	17	69.6 ± 7.8	73.8 ± 7.8	4.2
Parkinson's Disease	12	63.1 ± 12.0	66.0 ± 12.9	2.9
Alzheimer's Disease	16	69.9 ± 9.1	73.9 ± 9.1	4
Control[b]	12	n/a	71.3 ± 7.0	n/a
Other[b]	3	65.7 ± 11.4	67.7 ± 10.7	2

CSF samples RT-QuIC testing by K23Q α-syn substrate



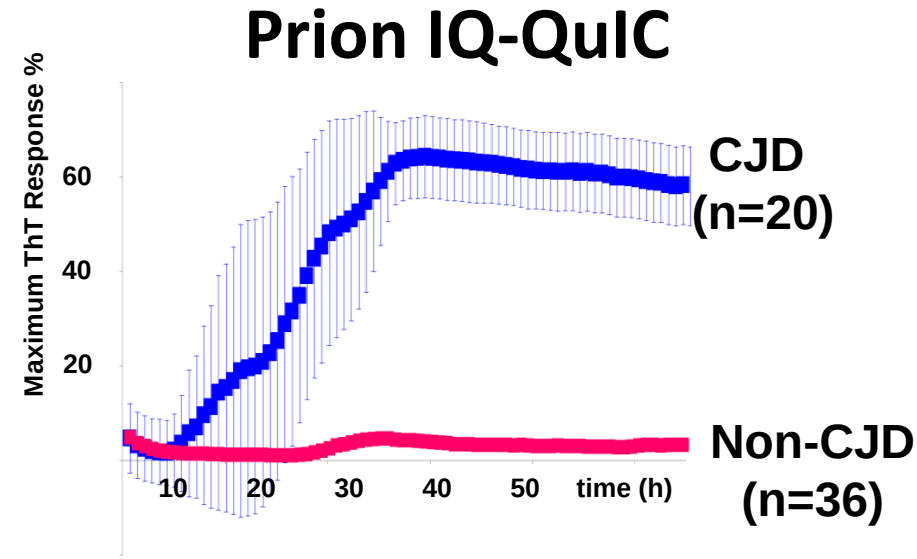


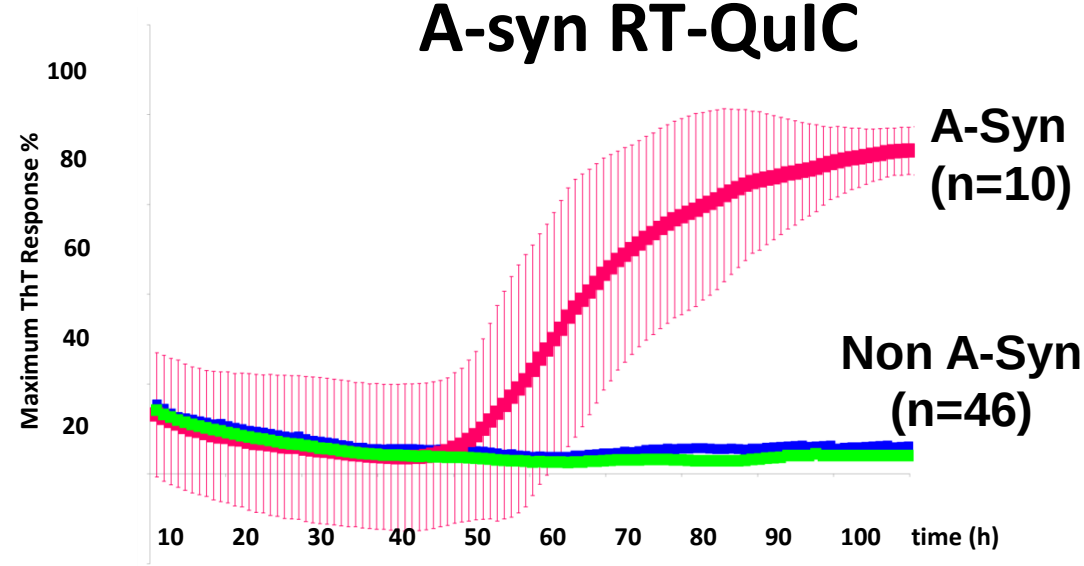




Alpha-Synuclein and Prion RT-QuIC in CSF of Definite Cases

Definite diagnosis	Final clinical diagnosis* (before RT-QuIC)	CSF RT-QuIC	
		PrP	α-syn
Alpha Synucleinopathies (n=10)		**0/10**	**10/10**
DLB (n=7)	Probable DLB (n=4)	0/7	7/7
	Possible DLB (n=1)		
	Possible CJD (n=2)		
AD/DLB (n=2)	Probable DLB (n=1)	0/2	2/2
	Possible CJD (n=1)		
MSA-C (n=1)	Possible DLB (n=1)	0/1	1/1
Prion diseases (n=20)		**20/20°**	**0/20**
Sporadic CJD (n=19)	Probable CJD (n=16)	19/19°	0/19
	Rapidly progressive dementia (n=3)		
Genetic CJD, E200K (n=1)	Probable CJD (n=1)	1/1	0/1
Other neurodegenerative diseases (n=8)		**0/8**	**0/8**
AD (n=4)	Probable DLB (n=1)	0/4	0/4
	Possible CJD (n=2)		
	Possible DLB (n=1)		
FTLD-TDP 43 (n=1)	Vascular PSP	0/1	0/1
PSP (n=2)	Probable CJD (n=1)	0/2	0/2
	PSP (n=1)		
CBD (n=1)	Probable CJD (n=1)	0/1	0/1
Other neurological diseases (n=18)		**0/7**	**0/18**
VD (n=4)	Possible CJD (n=4)	ND	0/18
Encephalitis (n=6)	Probable CJD (n=1)	0/1	
	Possible CJD (n=3)	0/1	
	Encephalitis (n=2)	ND	
Anti-IgLON5 disease (n=1)	Possible sporadic fatal insomnia	0/1	
PART (n=1)	Probable CJD	ND	
Brain tumor (n=2)	Possible CJD	0/1	
	Encephalitis	0/1	
Pontine myelinolysis (n=1)	Probable CJD (n=1)	ND	
Wernicke encephalopathy (n=1)	Possible CJD	ND	
Anoxic encephalopathy (n=2)	Probable CJD (n=1)	0/1	
	Autoimmune encephalitis (n=1)	0/1	



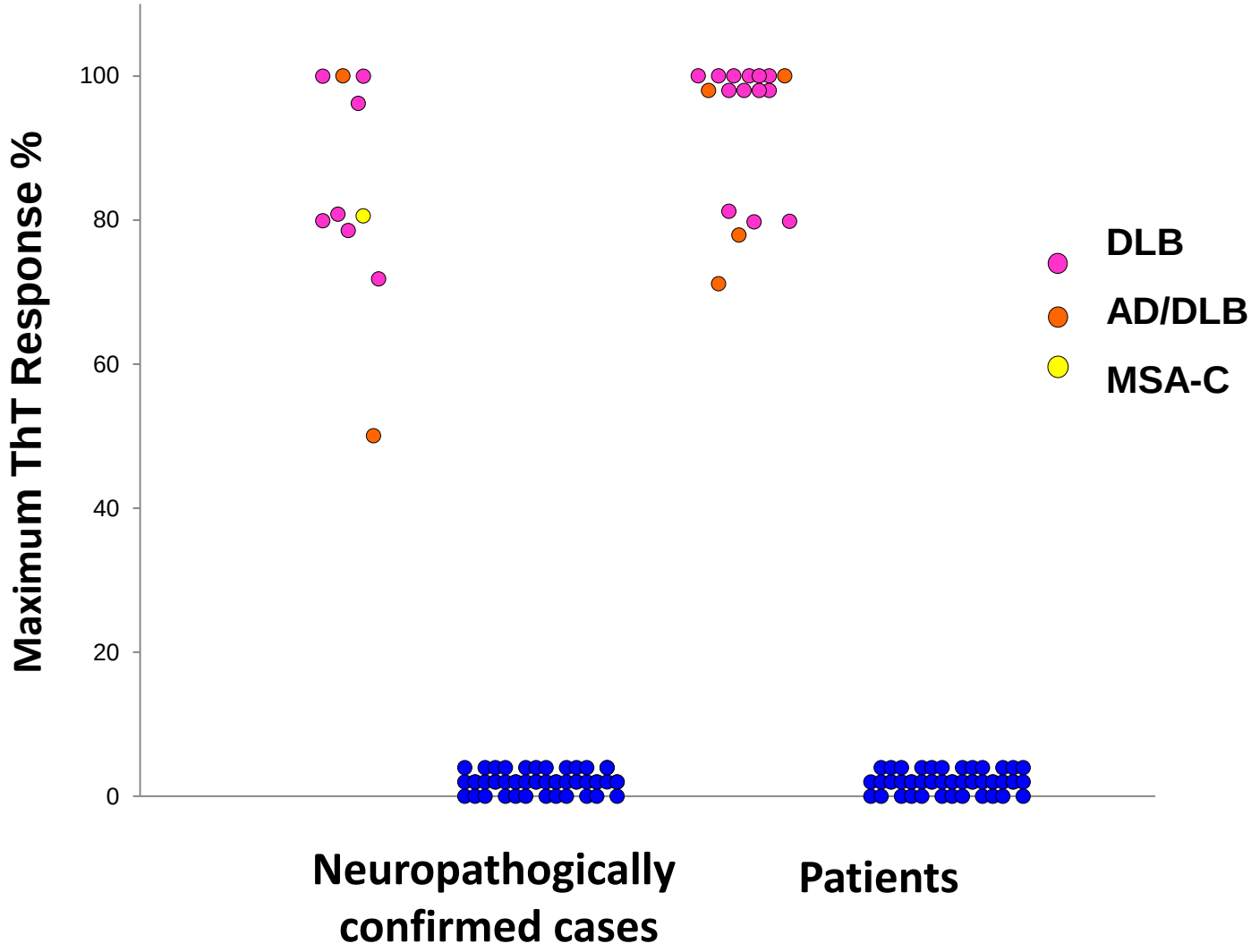


Bongianni et al. submitted

α-syn RT-QuIC for in CSF samples from patients with negative prion IQ-QuIC

Number of Patients	Clinical Diagnosis	IQ-QuIC	α-Syn RT-QuIC
16	Probable DLB	Negative	Positive (13) Negative (3)
6	Possible DLB	Negative	Negative
4	AD LB variant	Negative	Positive
9	Probable AD	Negative	Negative
4	NPH, WE	Negative	Negative

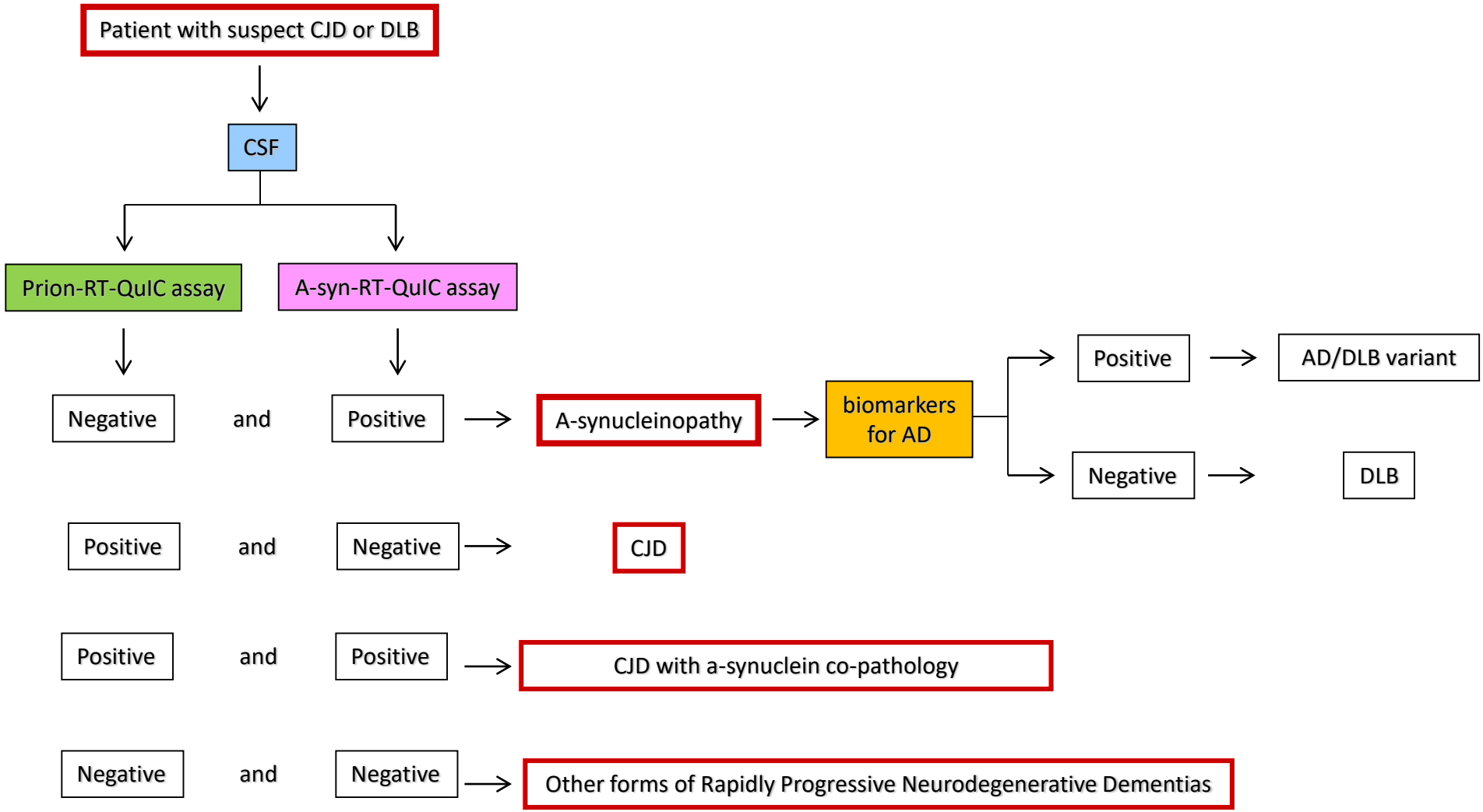
α-syn RT-QuIC assay in CSF samples of individual neuropathologically confirmed Diagnosis



RT-QuIC assay for alpha-Synuclein in CSF and OM samples from patients with clinical diagnosis of probable DLB

Patient	CSF	OM
1	POSITIVE	POSITIVE
2	POSITIVE	POSITIVE
3	POSITIVE	POSITIVE
4	POSITIVE	POSITIVE
5	POSITIVE	POSITIVE
6	POSITIVE	POSITIVE
7	POSITIVE	NEGATIVE
8	POSITIVE	NEGATIVE
9	POSITIVE	NEGATIVE
10	POSITIVE	POSITIVE
11	NEGATIVE	POSITIVE

Algorithm for *in vivo* differential diagnosis between CJD and DLB by prion and α-syn RT-QuIC assays on CSF samples



Summary

- ✓ RT-QuIC for a-syn on OM samples from patients with DLB is very promising
- ✓ We observed DLB cases RT-QuIC postive in the CSF and negative in OM but also *viceversa*.
- ✓ Although these data are preliminary, based on alimited numer of cases, it might be speculated that RT-QuIC for a-syn testings in CSF and/or OM might result with a high sensitivity

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