Olfactory swabbing in prion and other neurodegenerative disorders

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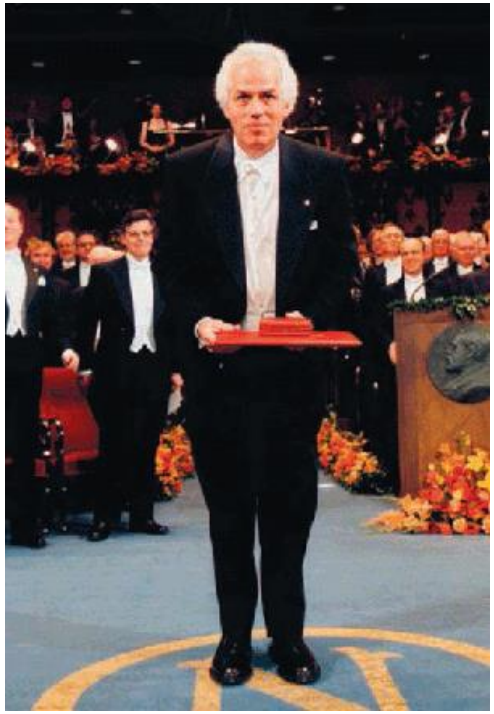
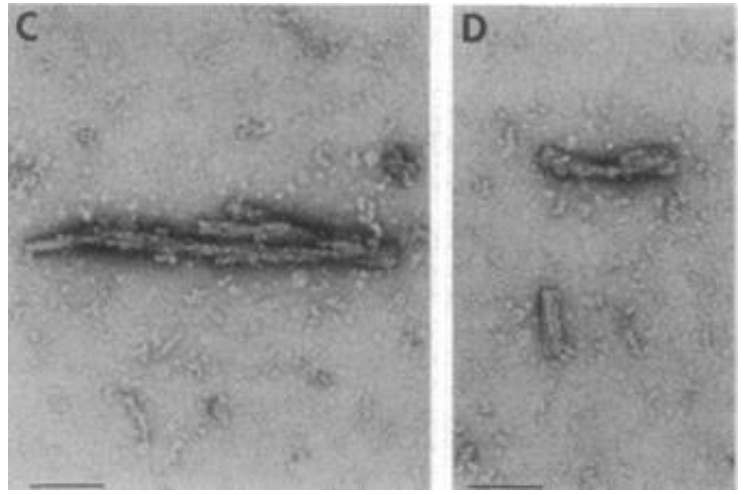
Nature of the Scrapie Agent: Self-replication and Scrapie

Nature 215, 1043–1044 (1967)

…Scrapie agent might be aberrant form of protein that spontaneously got made, and could serve as a template to induce production of more aberrant forms.

Proteinaceous Infectious Only



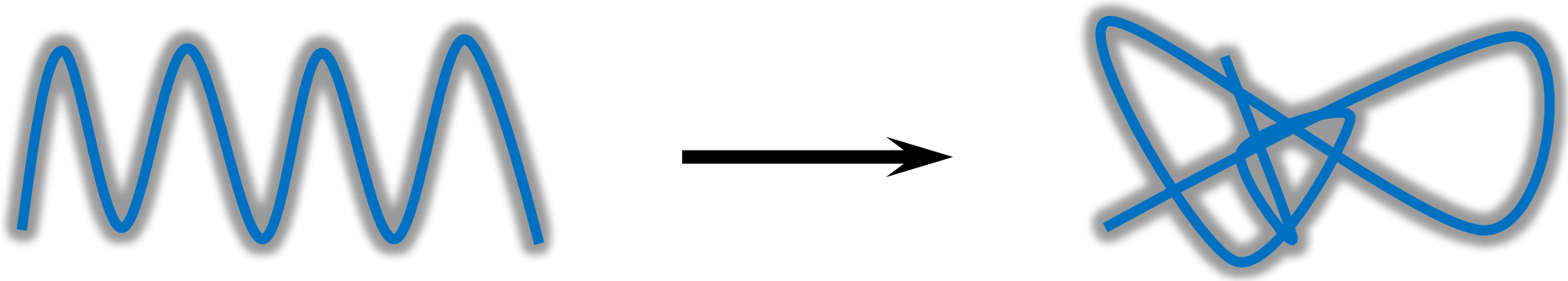


Science, 1982

Scrapie Associate Fibrils

The Meaning of *Prions*

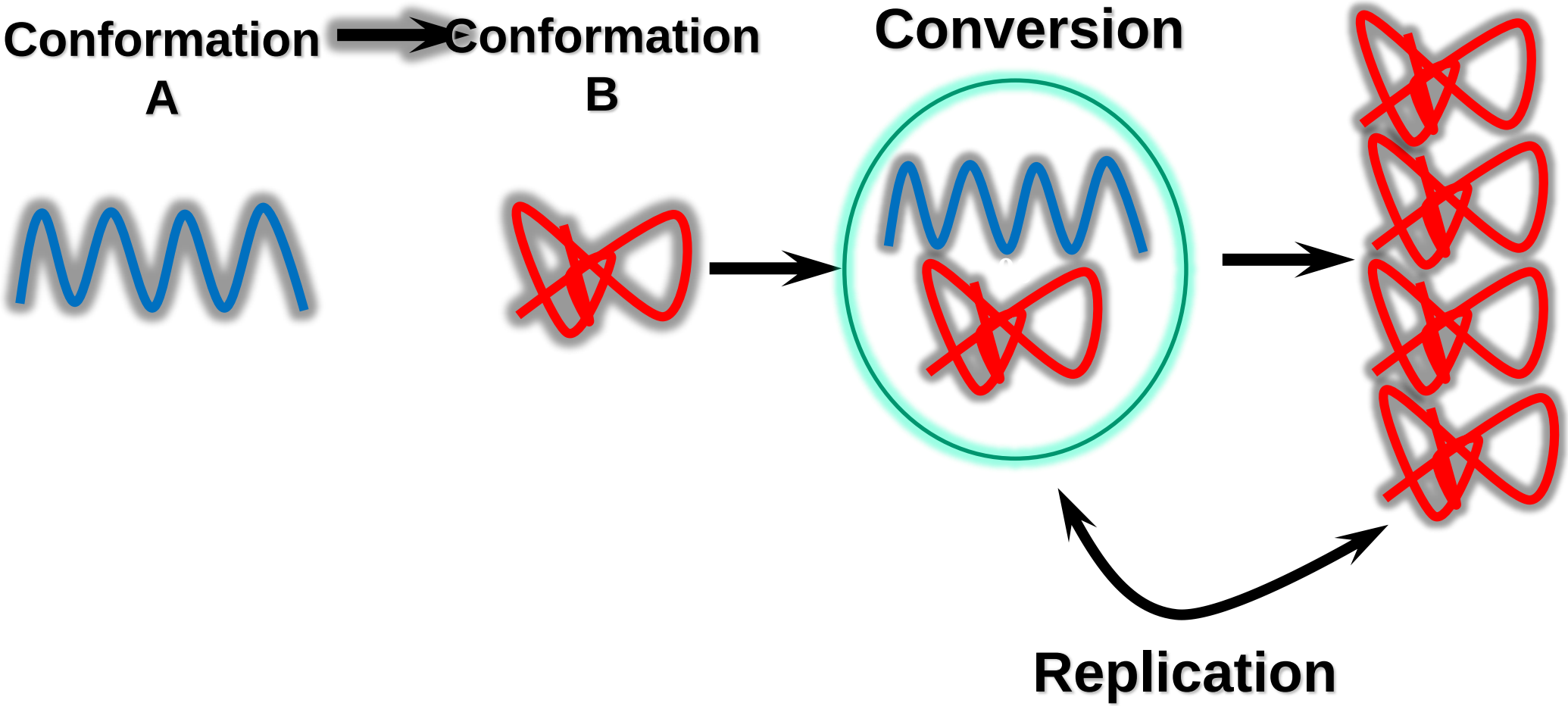
Conformational Changes of Prions



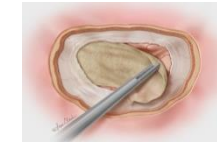
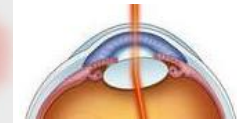
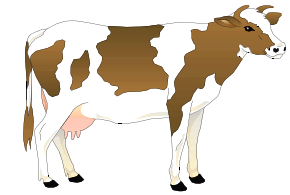
Normal Conformation A

Pathological Conformation B

Prion Propagation



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)

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

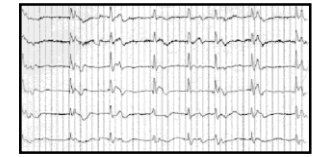
➢ **Diagnostic tools:**

- **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**

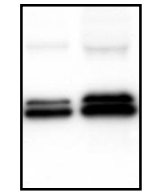
Probable Diagnosis

EEG



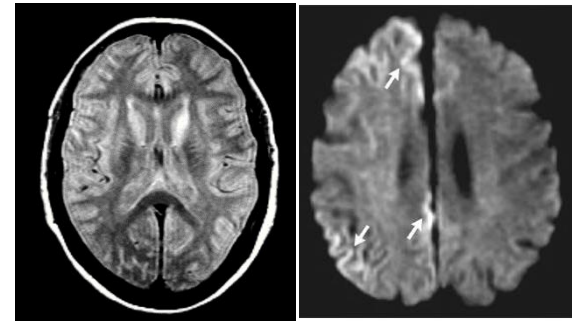
PSWCs

CSF



14-3-3 protein

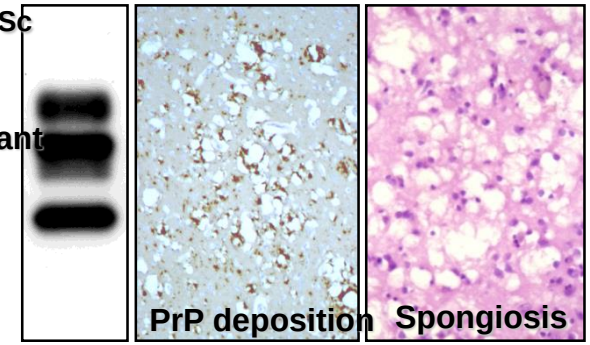
MRI



Definite Diagnosis

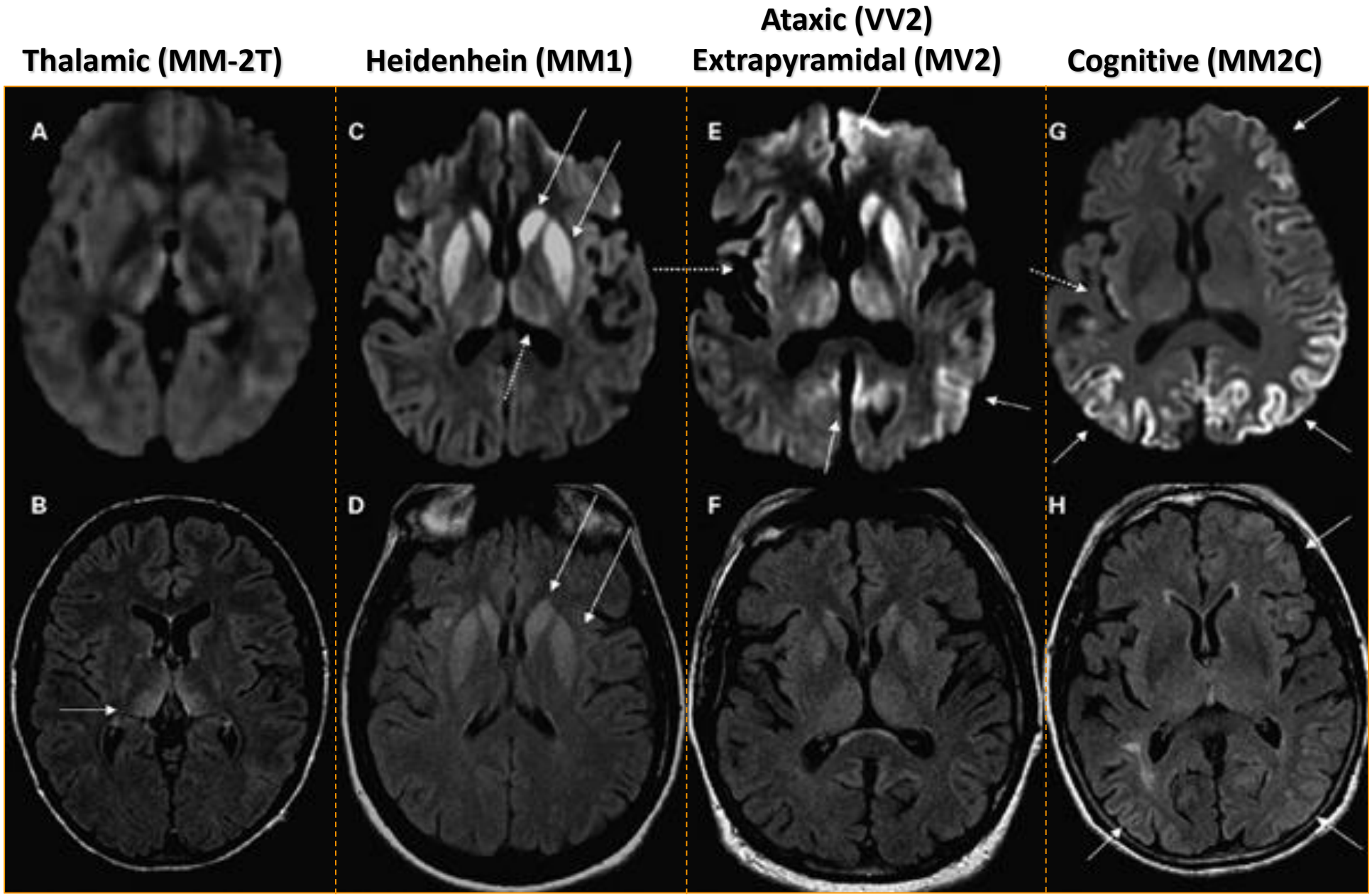
PrP^{Sc}

PK resistant PrP

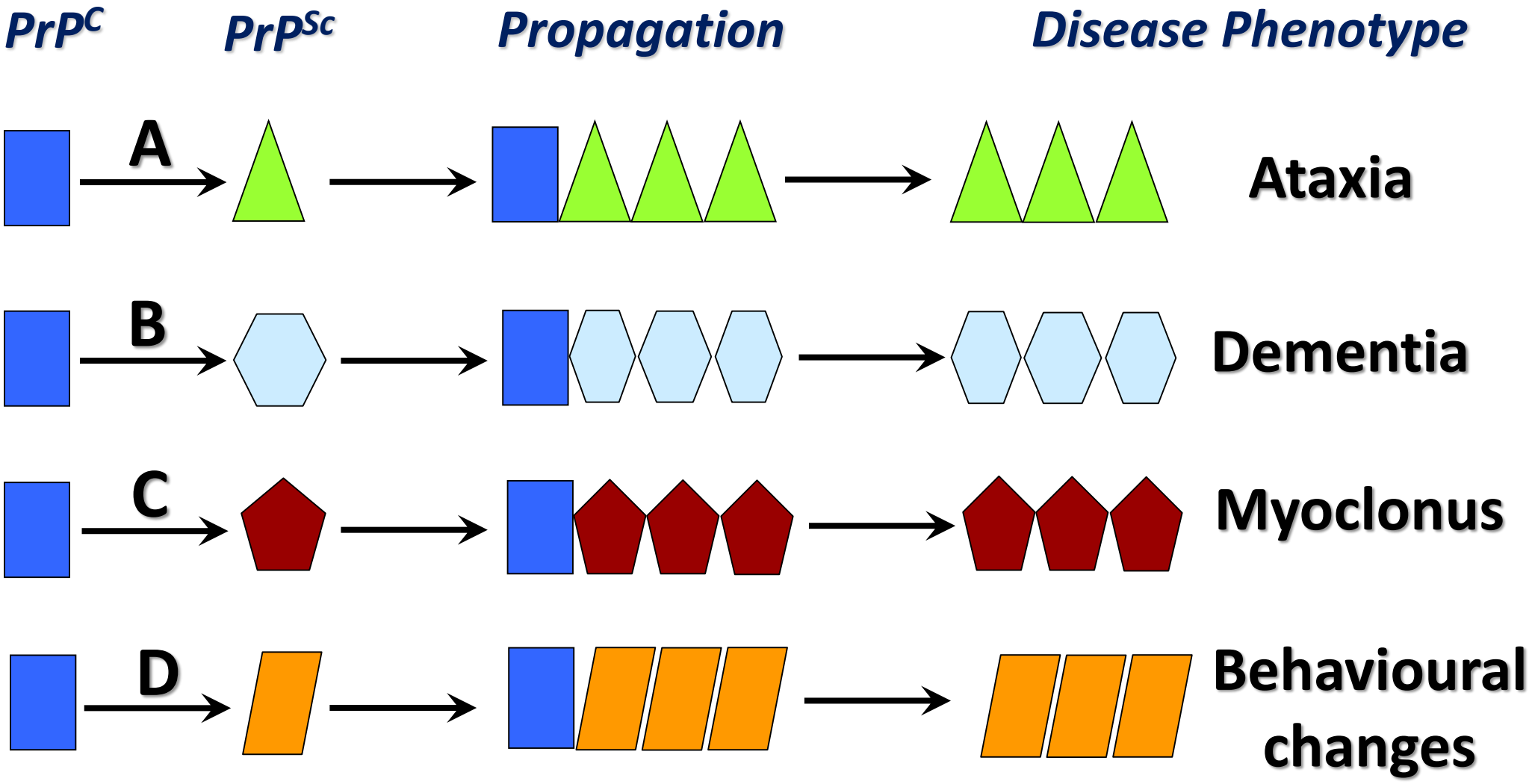


PrP^{Sc} detection by immunoblot and immunocytochemistry

MRI patterns in different molecular subtypes of sCJD

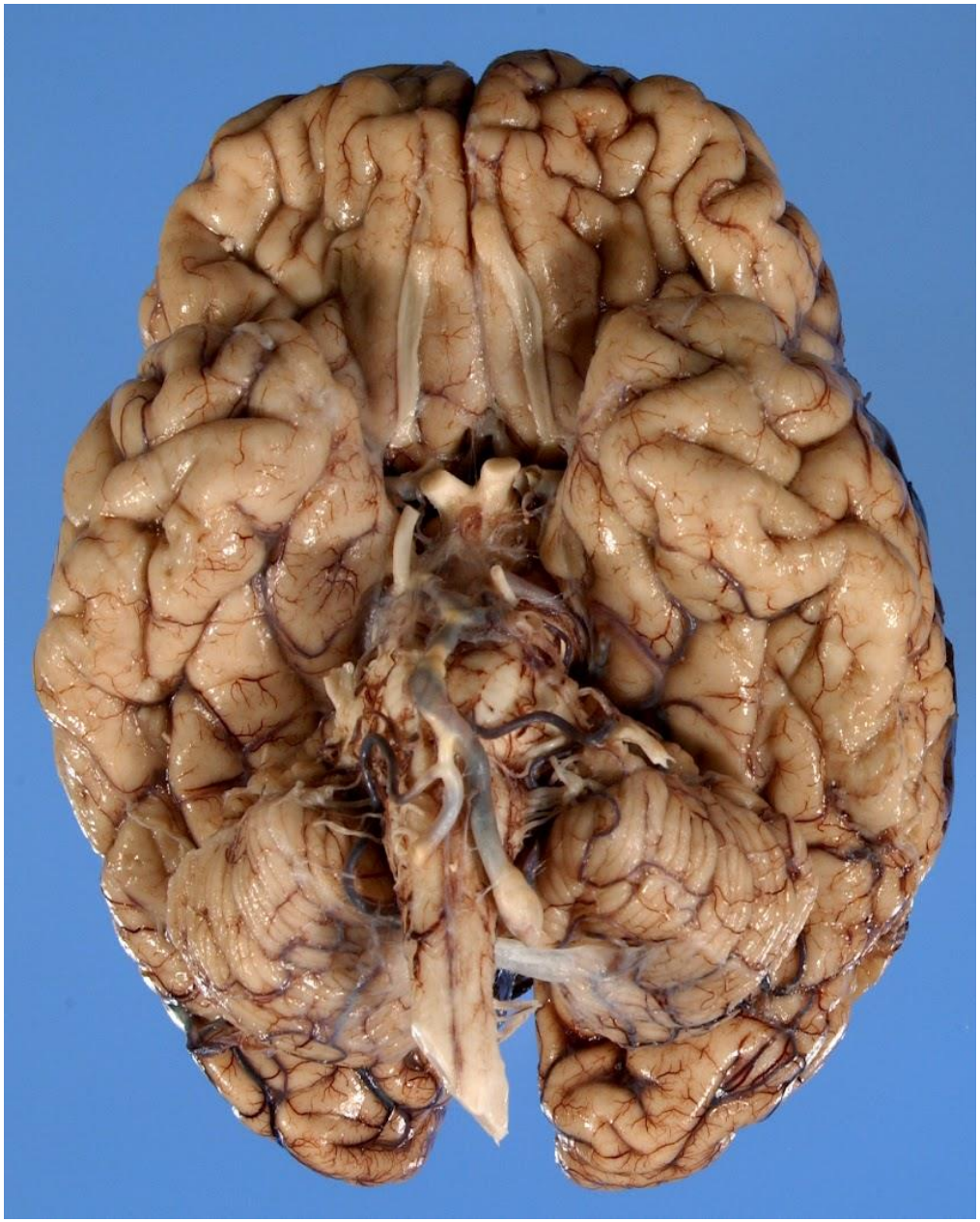


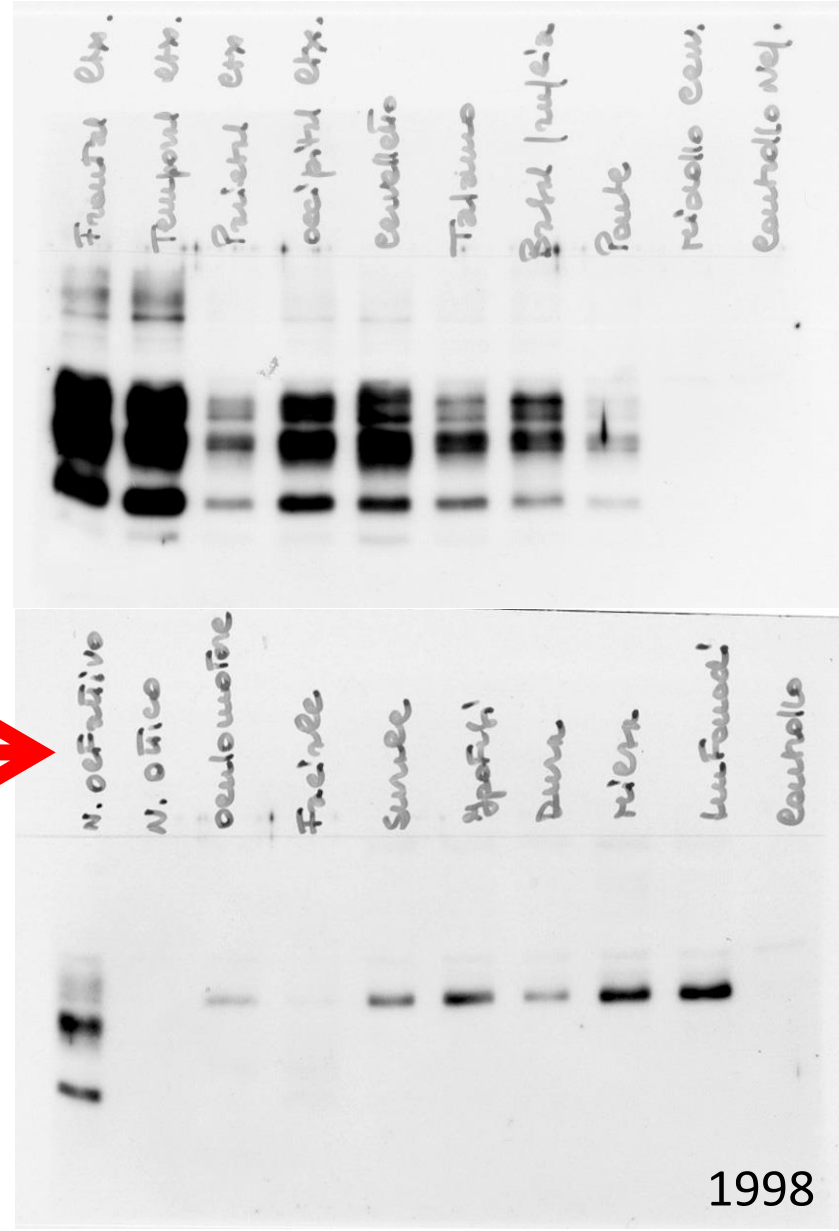
Different Prion Conformers Act as «Strains»



The Olfactory Story

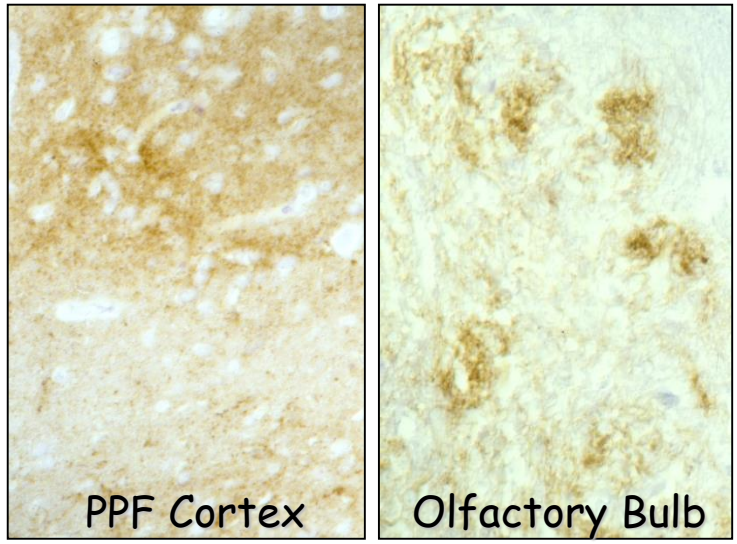
Is the Olfactory System involved in Human Prion Diseases ?



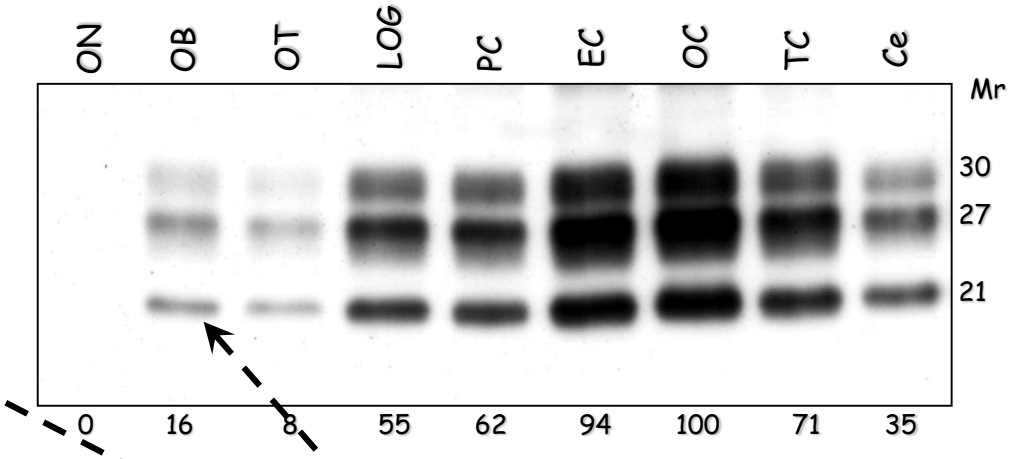


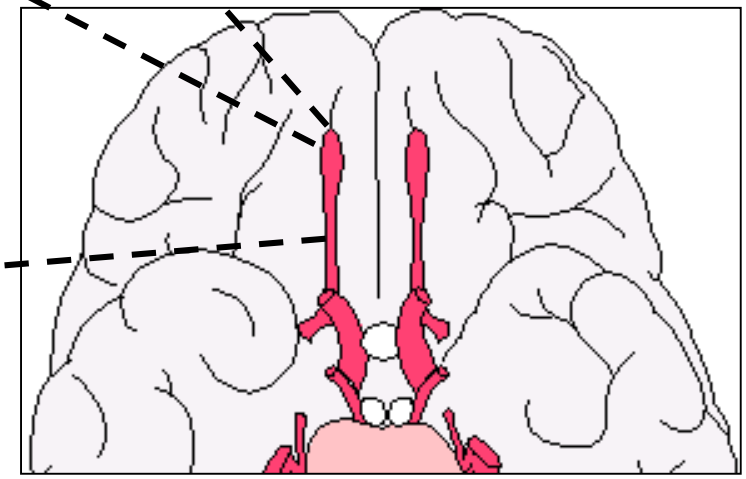
Pattern of PrP^{Sc} Deposition and Distribution in Olfactory Pathway of sCJD Met/Met-1

Synaptic Pattern of PrP Deposition

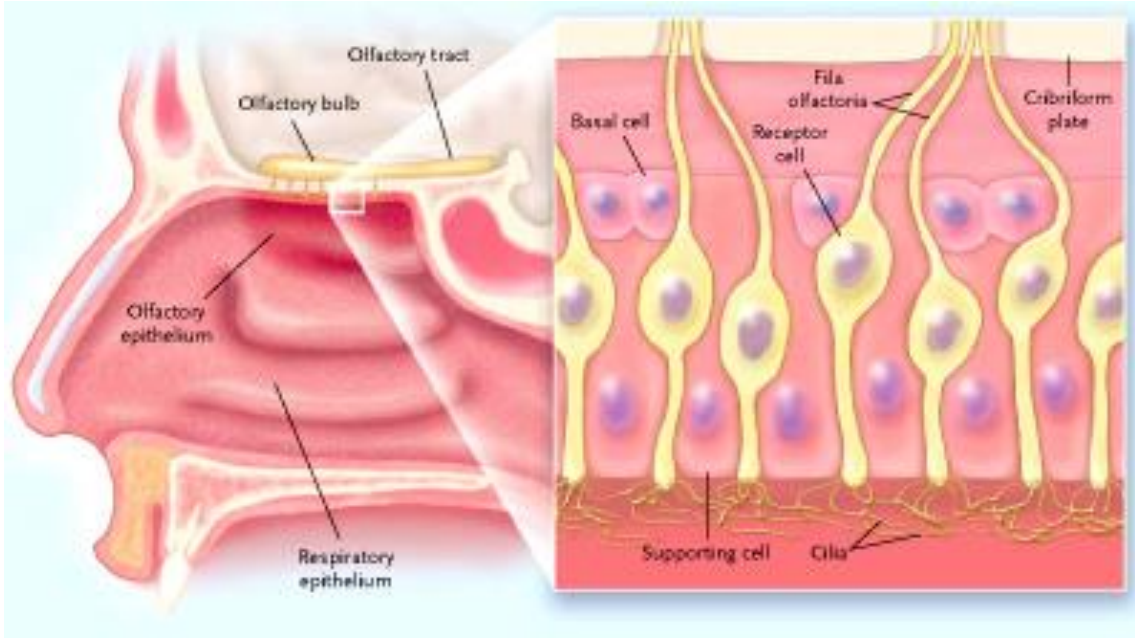


Immunoblot analysis of PrP Distribution

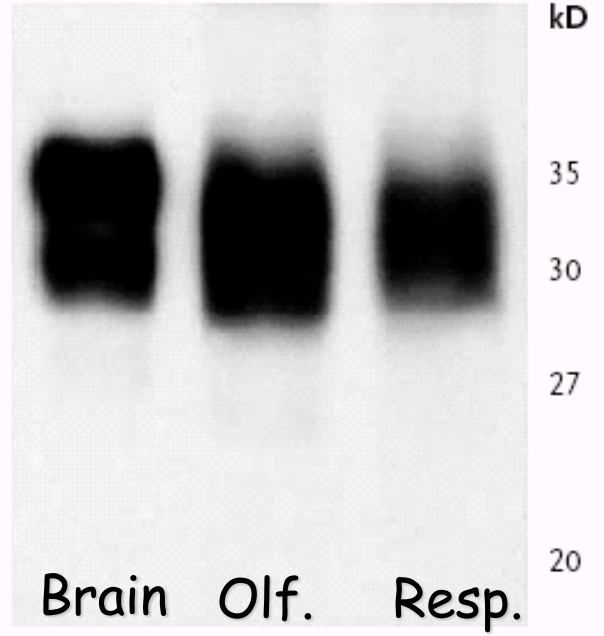




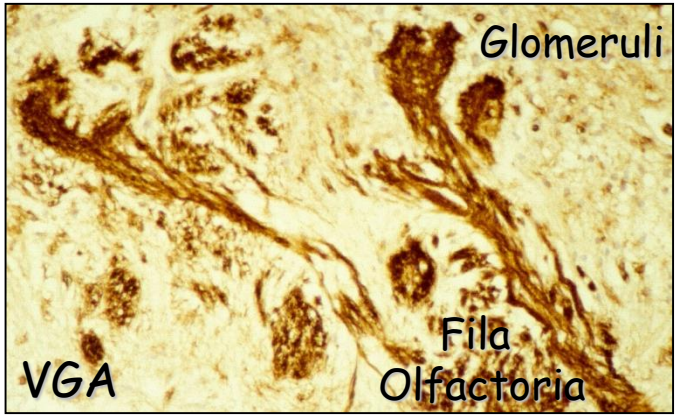
The Peripheral Olfactory system

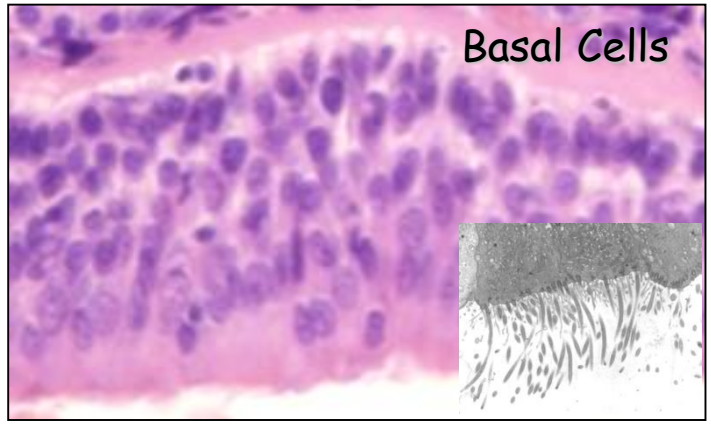


Expression of Cellular Prion Protein PrP^C in Olfactory and Respiratory Mucosa



Olfactory Mucosa





The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Detection of Pathologic Prion Protein in the Olfactory Epithelium in Sporadic Creutzfeldt–Jakob Disease

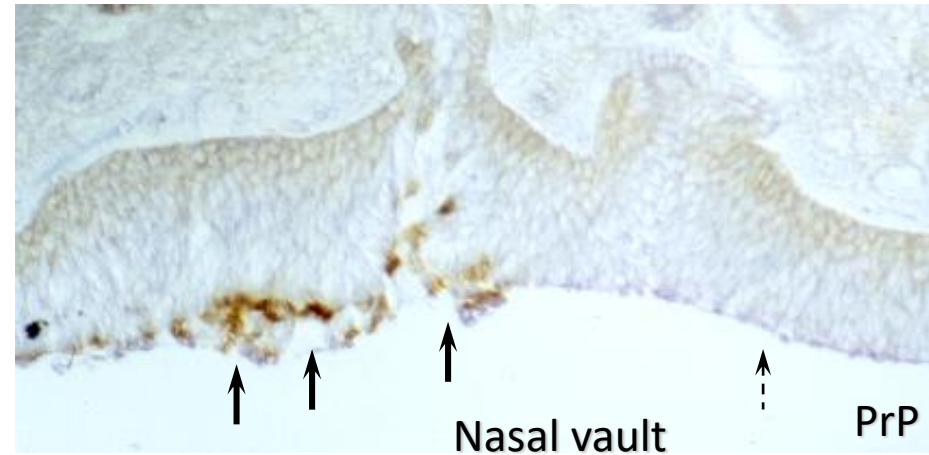
Gianluigi Zanusso, M.D., Ph.D., Sergio Ferrari, M.D., Franco Cardone, Ph.D., Paolo Zampieri, M.D., Matteo Gelati, Ph.D., Michele Fiorini, Ph.D., Alessia Farinazzo, Ph.D., Marina Gardiman, M.D., Tiziana Cavallaro, M.D., Marina Bentivoglio, M.D., Pier Giorgio Righetti, Ph.D., Maurizio Pocchiari, M.D., Nicola Rizzuto, M.D., and Salvatore Monaco, M.D.

Detection of PrP^{Sc} in the Olfactory Neuroepithelium in sCJD

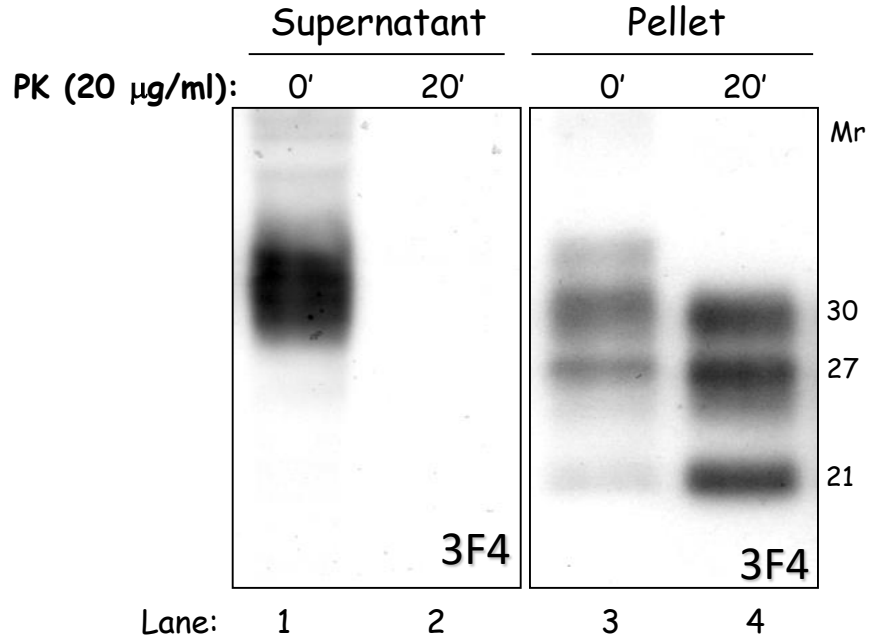
Table 1. Molecular and Clinical Characteristics of Nine Patients with Sporadic Creutzfeldt–Jakob Disease.*

Patient No.	Age at Onset (yr)/Sex	Codon 129/ PrP^{Sc}†	Signs at Onset	Duration	Clinical Evolution	EEG Findings‡
				mo		
1	72/M	Met/Met 21	Halluci-nations	5	Ataxia, myoclonus	PSWs
2	71/F	Met/Met 21	Halluci-nations	8	Dementia, ataxia, myoclonus	PSWs
3	73/F	Met/Met 21	Ataxia, dysarthria	16	Dementia	PSWs
4	74/F	Met/Met 21	Dementia	2	Ataxia	PSWs
5	69/F	Met/Met 21	Cortical blindness	5	Dementia, myoclonus	Diffuse slowing
6	59/M	Met/Met 21	Dementia	3	Myoclonus	PSWs
7	55/F	Met/Met 21	Anosmia, halluci-nations	5	Dementia	PSWs
8	52/M	Met/Met 21	Dementia	3	Ataxia, myoclonus	PSWs
9	64/F	Val/Val 19	Ataxia	5	Dementia	Diffuse slowing

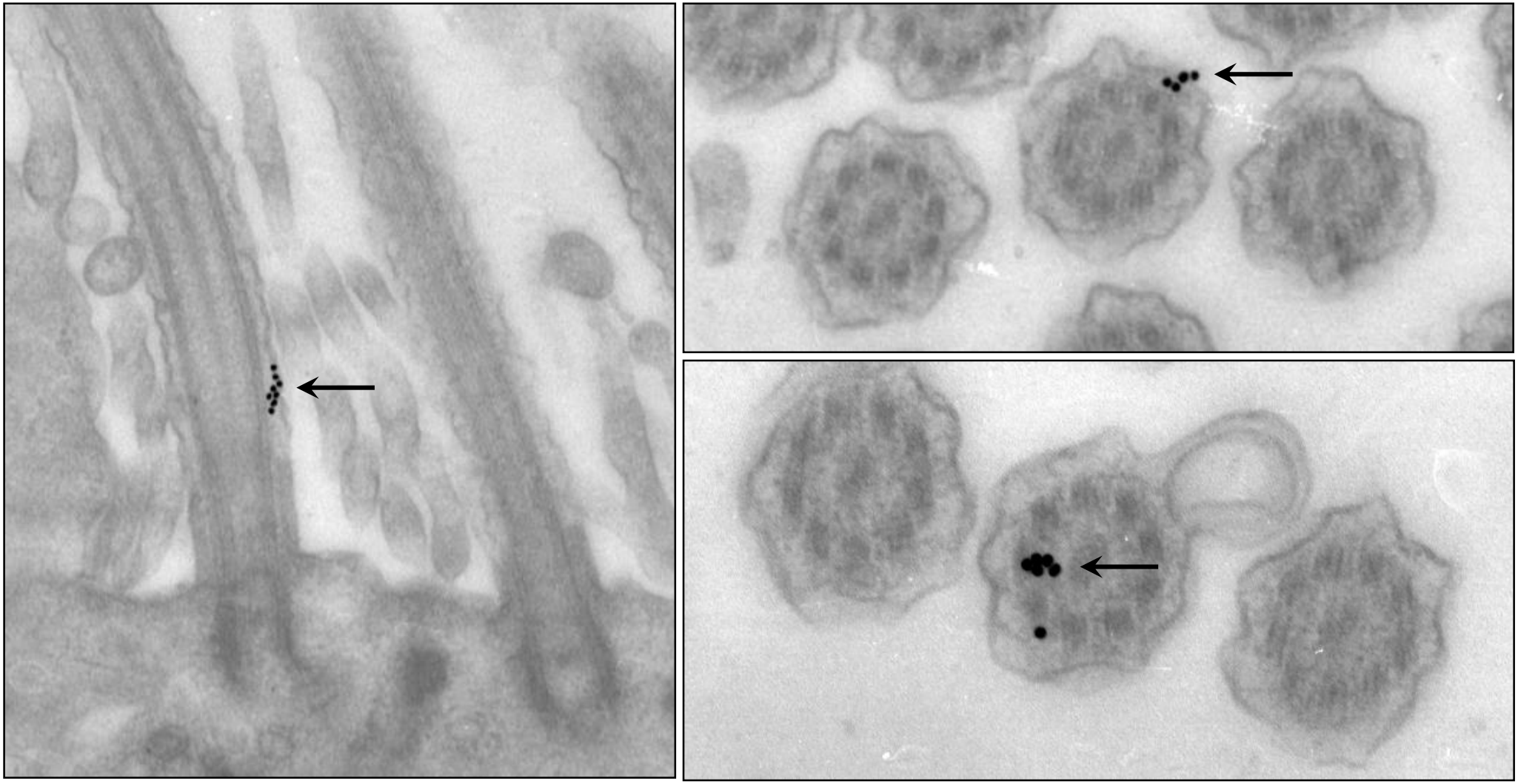
PrP^{Sc} deposition in olfactory neuroepithelium



Olfactory Mucosa NaPTA treated (from 100mg)



Ultrastructural Deposition of PrP^{CJD}

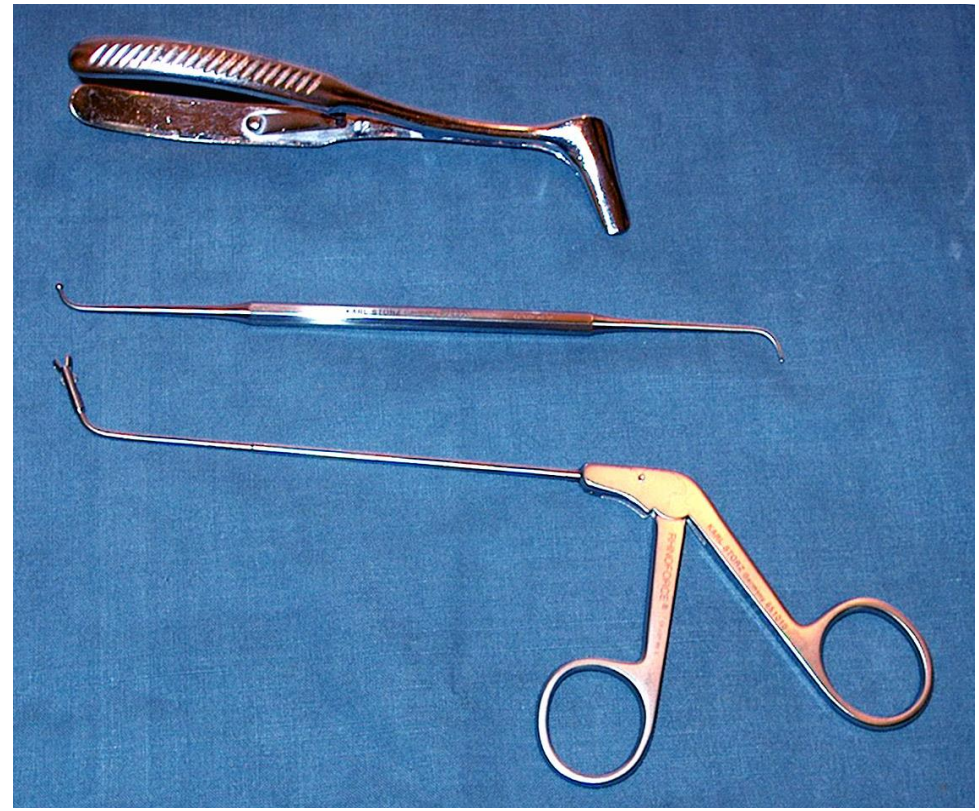


Olfactory Mucosa Biopsy: Surgical Instruments

Prion Detection in Olfactory Biopsy of Sporadic Creutzfeldt–Jakob Disease

Massimo Tabaton, MD,[1] Salvatore Monaco, MD,[2] Maria Paola Cordone, MD,[3] Monica Colucci, MD,[1] Giorgio Giaccone, MD,[4] Fabrizio Tagliavini, MD,[4] and Gianluigi Zanusso, MD, PhD[2]

Annals of Neurology, 2004



Case # 1

Female 59 years old

August 2002: left alien hand syndrome, MCI

September 2002: Extrapyramidal signs;

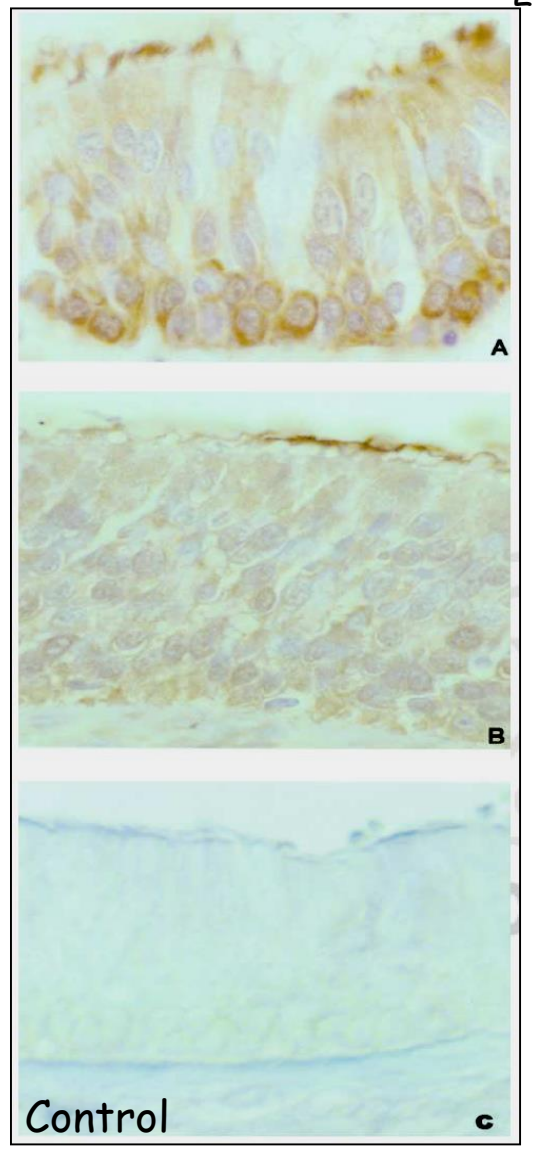
October: akinetic mutism and myoclonus; EEG: sporadic PSWCs;

CSF: 14-3-3 positive;

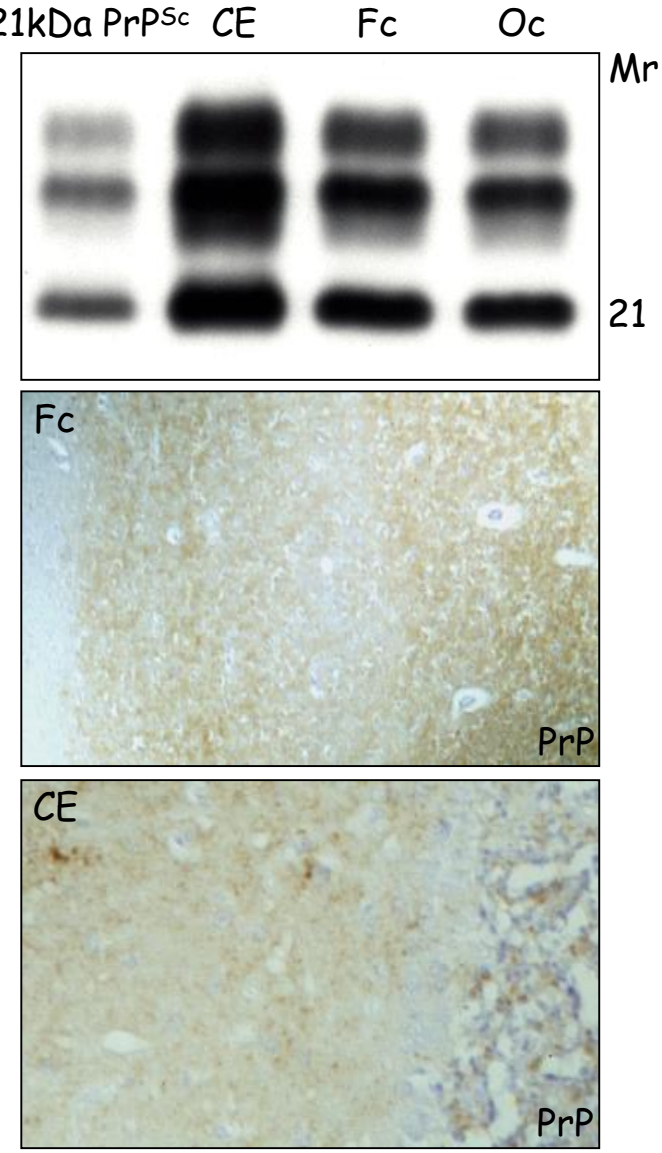
PRNP : No mutations, Met/Met at codon 129. Nasal biopsy was performed.

February 2003: exitus.

Olfactory Mucosa Biopsy



Definite sCJD Diagnosis



RT-QuIC

A Novel Diagnostic test for CJD

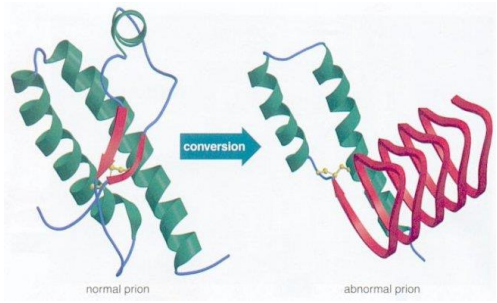
nature medicine

2011

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

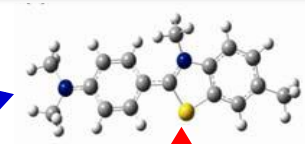
Ryuichiro Atarashi[1,2], Katsuya Satoh[1], Kazunori Sano[1,3], Takayuki Fuse[1], Naohiro Yamaguchi[1], Daisuke Ishibashi[1], Takehiro Matsubara[1], Takehiro Nakagaki[1], Hitoki Yamanaka[4], Susumu Shirabe[5], Masahito Yamada[6], Hidehiro Mizusawa[7], Tetsuyuki Kitamoto[8], Genevieve Klug[9], Amelia McGlade[9],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

Plate-based fluorescence detection of PrP^{res}-seeded rPrP amyloid ("Real-time QuIC": *Atarashi 2011*)



Sample

Binding sites of ThT in amiloid fibrils



Recombinant Prion protein

PrPres

PrP fibrils

96 well plate

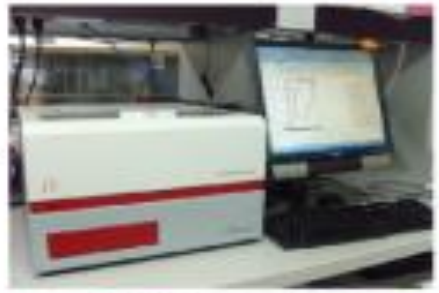
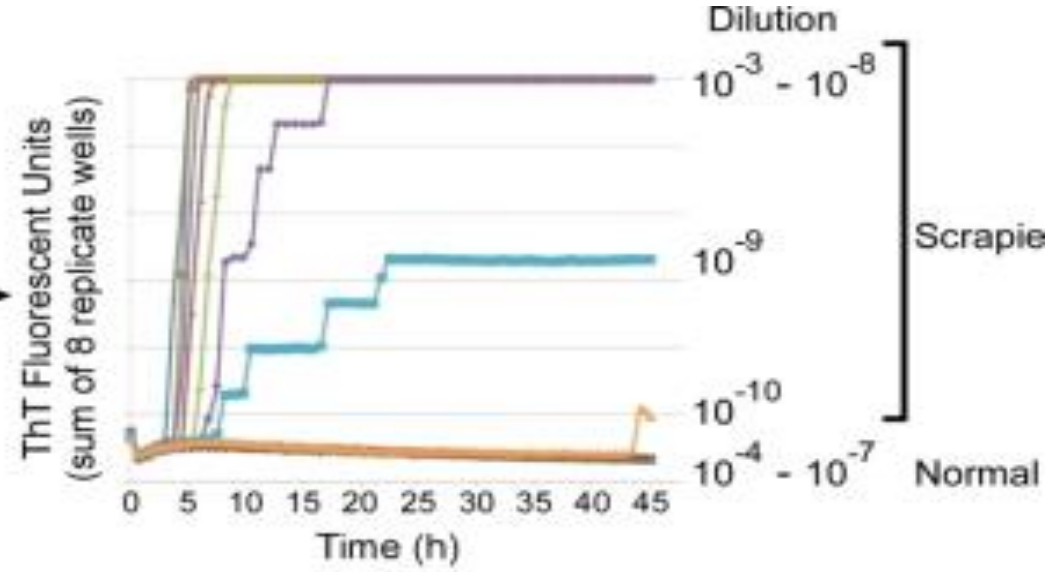


Plate reader



Real Time QuIC (RTQ)

To couple a 100% specific and sensitive test (RT-QuIC)
to a gently procedure for sampling olfactory mucosa
(Brushing Nasale)

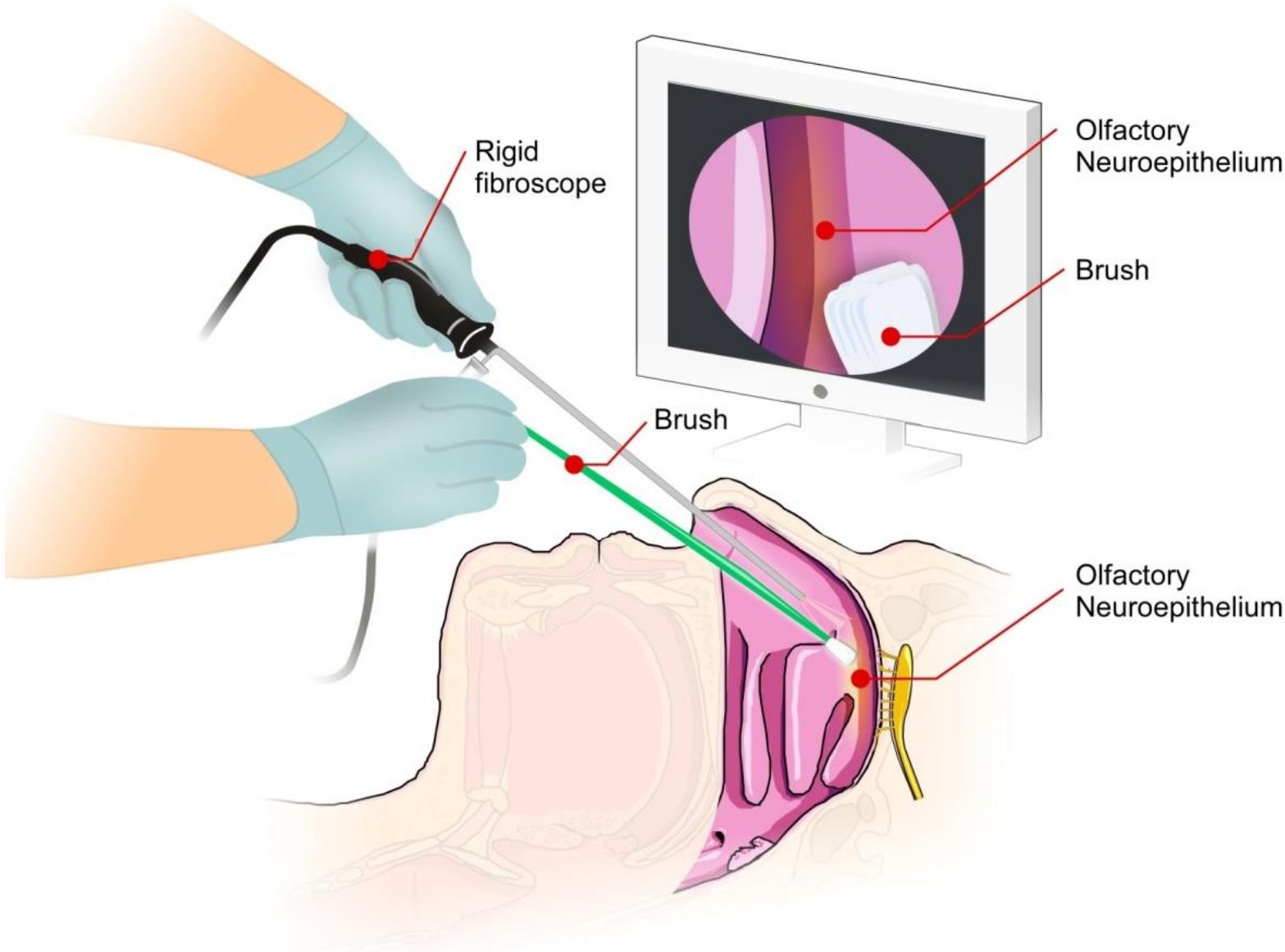
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

A Test for Creutzfeldt–Jakob Disease Using Nasal Brushings

Christina D. Orrú, Ph.D., Matilde Bongianni, Ph.D., Giovanni Tonoli, M.D., Sergio Ferrari, M.D., Andrew G. Hughson, M.S., Bradley R. Groveman, Ph.D., Michele Fiorini, Ph.D., Maurizio Pocchiari, M.D., Salvatore Monaco, M.D., Byron Caughey, Ph.D., and Gianluigi Zanusso, M.D., Ph.D.

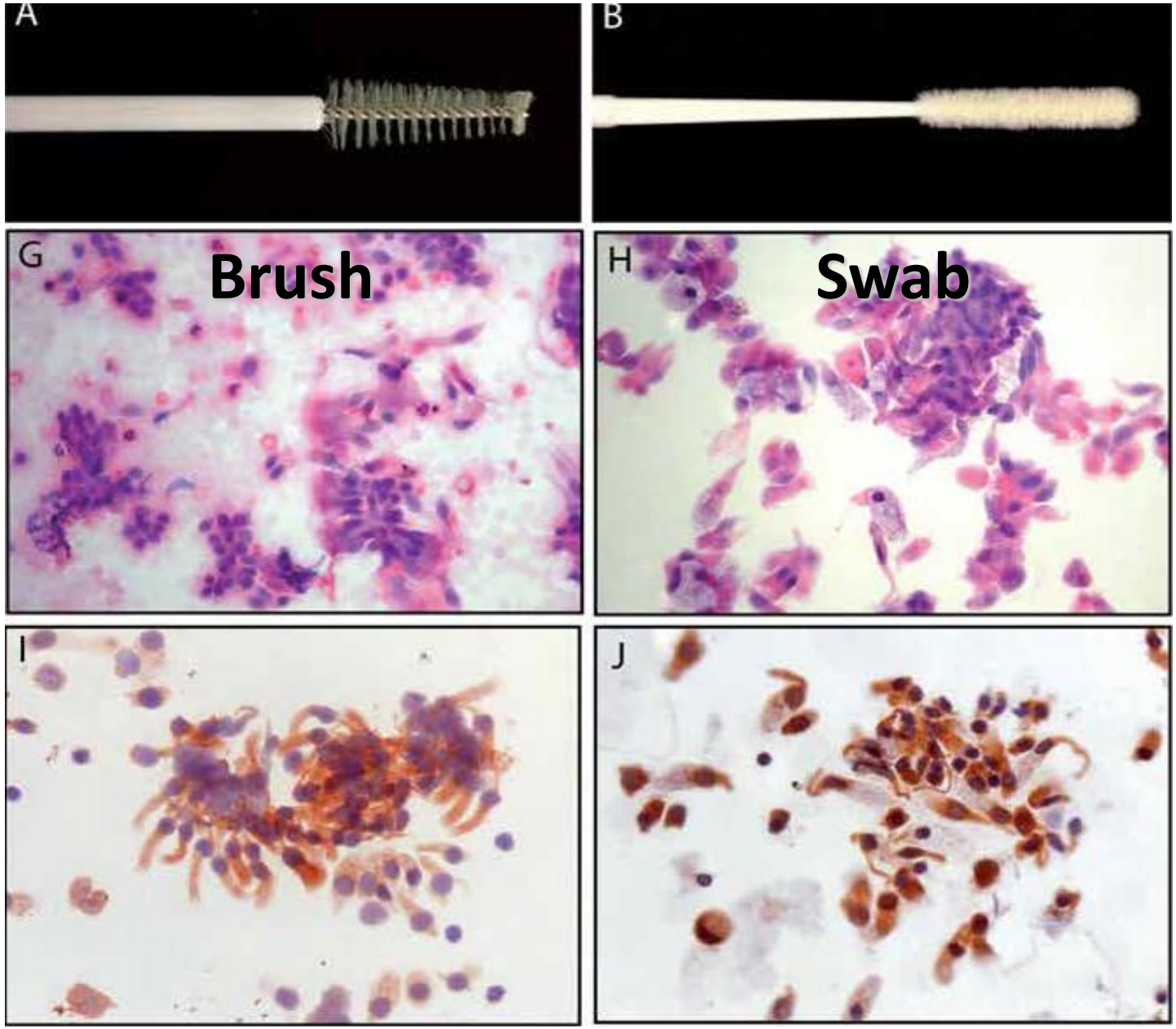
Nasal Brushing Procedure



Nasal Brushing Procedure

The beginning of Collaboration with Santina Castriciano

Olfactory mucosa cytological analysis following cyto-brush or flocked swab sample collection



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

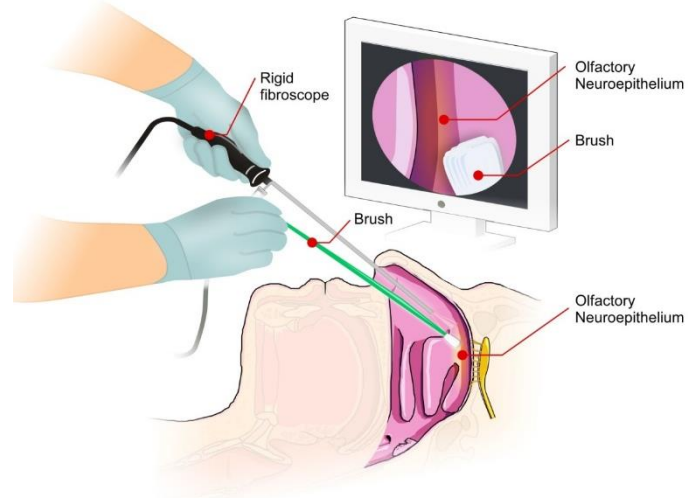


Figure 1. Olfactory Mucosa (OM) Sample Collection and Results of Real-Time Quaking-Induced Conversion (RT-QuIC) Assays

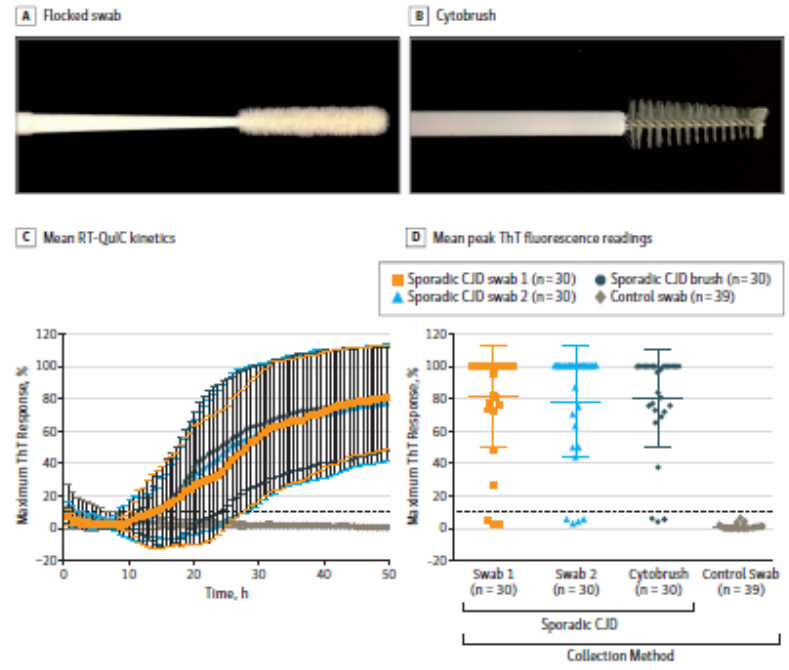


Table 2. Clinical Data and Results of RT-QuIC Analyses of CSF and OM Samples Collected From Patients With Probable, Possible, or Suspected CJD

CJD Diagnosis at Time of Sampling Sorted by Final Diagnosis	CSF				OM				Overall Outcome, No. of Patients With Positive Finding/No. Tested (Accuracy, %)
	Time From Disease Onset to Spinal Tap, Mean (SD), mo	No. of Patients With Positive Finding/Tested			Time From Disease Onset to Sample, Mean (SD), mo	No. of Patients With Positive Finding/Tested			
		RT-QuIC Results		Final Outcome		Sampling Method		Final Outcome	
		PQ	IQ			Swab	Brush		
Probable CJD (n = 51)									
Definite CJD (n = 21)[a]	2.4 (1.2)	14/18	4/6	18/20	2.6 (1.2)	20/20	15/15	21/21	21/21 (100)
Probable CJD (n = 25)[b]	5.9 (5.3)	14/22	10/10	23/23	8.2 (8.1)	21/23	15/16	24/25	25/25 (100)
Genetic CJD (n = 4)	3.5 (2.4)	3/4	1/1	4/4	4.8 (4.5)	3/3	3/3	4/4	4/4 (100)
Non-CJD (n = 1)	4	NP	0/1	0/1	4	0/1	0/1	0/1	0/1 (100)
Possible CJD (n = 24)									
Definite CJD (n = 9)[a]	5.6 (5.9)	5/6	3/4	8/9	5.8 (5.9)	7/7	5/6	9/9	9/9 (100)
Probable CJD (n = 6)[b]	7.0 (4.0)	3/4	2/2	5/5	8.3 (3.8)	5/6	3/5	5/6	6/6 (100)
Genetic CJD (n = 2)	4.0 (4.3)	1/2	0/1	1/2	12.0 (7.1)	1/2	0/1	1/2	1/2 (50)
Non-CJD (n = 7)	3.7 (3.0)	NP	0/7	0/7	4.0 (3.2)	0/7	0/7	0/7	0/7 (100)
Suspected CJD (n = 11)									
GSS (n = 2)	12	0/1	NP	0/1	31.0 (26.9)	1/2	1/1	1/2	1/2 (50)
Non-CJD (n = 9)	8.7 (9.1)	NP	0/9	0/9	8.1 (9.1)	0/9	0/9	0/9	0/9 (100)

Abbreviations: CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; GSS, Gerstmann-Sträussler-Scheinker syndrome; IQ, improved QuIC; NP, not performed; OM, olfactory mucosa; PQ, previous QuIC; RT-QuIC, real-time quaking-induced conversion.

[a] In 1 patient, spinal tap was not performed; 5 samples were tested with IQ only.

[b] In 3 patients, spinal tap was not performed; 3 samples were tested with IQ only.

Exp. conditions: PQ-CSF Rec Hamster PrP FL at 42°C

IQ-CSF Rec Hamster PrP 90-231 at 55°C

EU Diagnostic Criteria for Surveillance of sporadic CJD

from 1 January 2017

➢ Definite CJD

Neuropathologically or immunohistochemically or biochemically confirmed

Clinical signs

I Rapidly progressive cognitive impairment

II
- A. Myoclonus
- B. Cerebellar or Visual problems
- C. Pyramidal or Extrapyramidal features
- D. Akinetic Mutism

Tests

- ✓ PSWCs in EEG
- ✓ 14.3.3 detection in the CSF and RT-QuIC assay in the CSF or other tissues
- ✓ High signal in caudate/putamen on MRI scan or at least two cortical regions either on DWI or FLAIR

➢ Probable CJD

I + Two out of II and at least one test positive

Progressive neurological syndrome and positive RT-QuIC in the CSF or other tissues

➢ Possible CJD

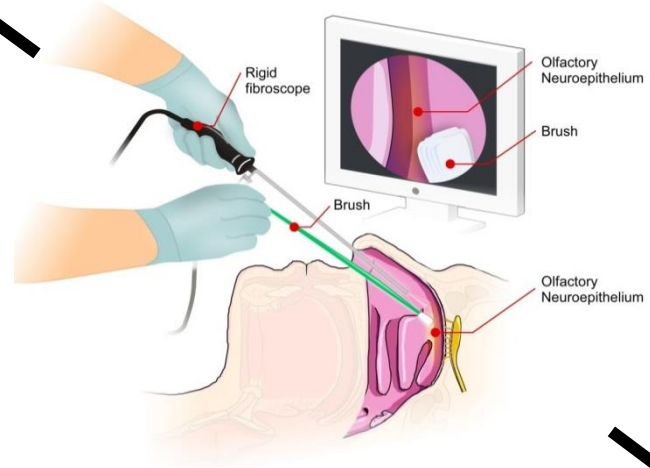
I + Two of II and duration less than two years

Training is not Required

RT-QuIC Positive in sCJD, gCJD, GSS and FFI

High Diagnostic Sensitivity and Specificity

Short Non invasive No costs



Safety Precautions Should be considered

No complications Linked to the Procedure

Therapy Indipendent

3 Log Higher RT-QuIC Seeding Activity Compare to CSF

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RESEARCH ARTICLE

Transmission of CJD from nasal brushings but not spinal fluid or RT-QuIC product

Gregory J. Raymond[1], Brent Race[1], Christina D. Orrú[1], Lynne D. Raymond[1], Matilde Bongianni[2], Michele Fiorini[2], Bradley R. Groveman[1], Sergio Ferrari[2], Luca Sacchetto[3], Andrew G. Hughson[1], Salvatore Monaco[2], Maurizio Pocchiari[4], Gianluigi Zanusso[2] & Byron Caughey[1]

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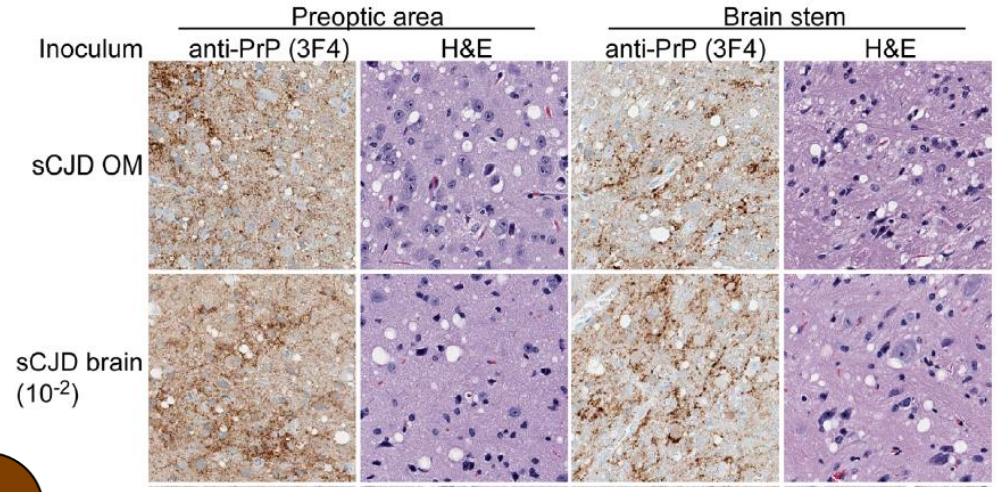
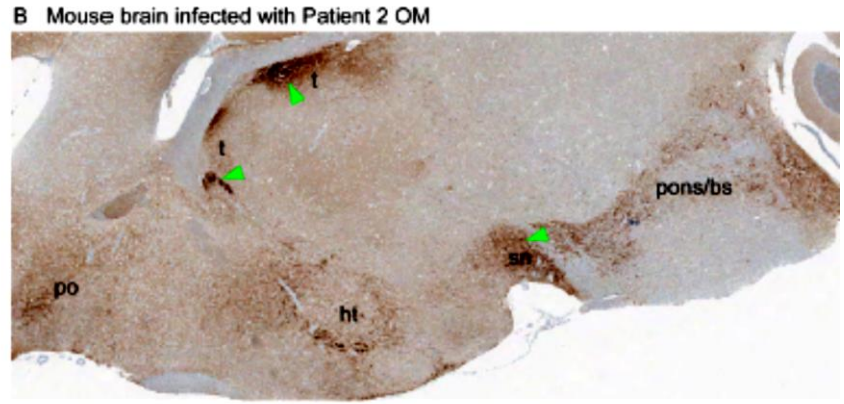
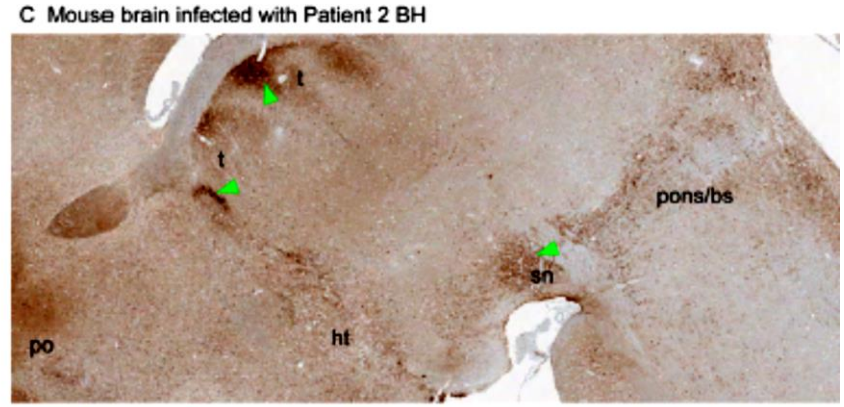
OM transmission to TgHu129MM (i.c. inoculation)

Table 1. 1st and 2nd passages of sCJD OM samples in humanized Tg66 mice.

1st passages				
Donor	Inoculum dilution	Clinical prion disease (+/total)	Survival time dpi (mean +/- SD)	Neuropathology and PrP IHC (+/total tested)
CJD Patient 2	Neat	1/6	Pos: 331 Neg: 531 +/- 0	1/6[1]
	10^{-1}	0/5	605 +/- 4	0/5
	10^{-2}	0/5	607 +/- 0	0/5
	10^{-3}	0/5	573 +/- 69	NT
CJD Patient 8	Neat	0/6	483 +/- 42	0/6
	10^{-1}	0/6	632 +/- 51	0/4
	10^{-2}	0/5	638 +/- 42	NT
	10^{-3}	0/6	676 +/- 16	NT
	Conc'd pool	0/12	317[3], 379 (n = 11)	0/12
CJD Patient 11	Conc'd pool	2/4[2]	Pos: 308,303 Neg/Pos: 267[4] Neg: 379 Acute: 1 (n = 7)	3/3[1]
Non-CJD	Neat	0/5	493 +/-40	0/4
Uninoculated	not applicable	0/8	317 (n = 4) 378 (n = 4)	0/5







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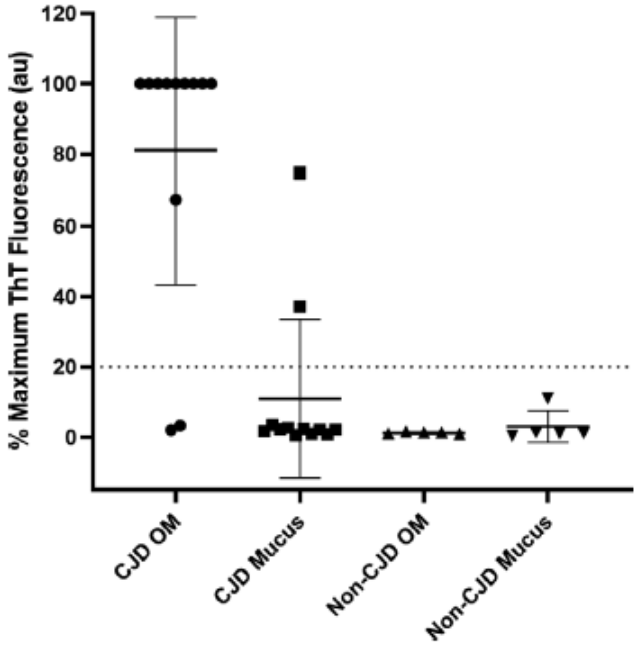
Experimental transmission to TgHu129MM (i.c. inoculation)

Nasal Mucus and OM RT-QuIC



Cerebrospinal Fluid

Table 3. Inoculation of sCJD CSF samples into Tg66 mice.

Donor	Inoculum dilution	Clinical prion disease (+/total)	Survival time dpi (mean +/- SD)	Neuropathology and PrP IHC (+/total tested)	RT-QuIC (+/ total tested)
sCJD Patient 1	1:1	0/6	556 +/-56	0/5	0/5
	1:20	0/6	616 +/-64	NT	0/4
sCJD Patient 2	1:1	0/6	547 +/-74	NT	0/4
	1:20	0/5	657 +/-40	NT	0/3
sCJD Patient 8	1:1	0/5	528 +/-89	0/2	0/4
	1:20	0/5	587 +/-13	NT	0/2
Non-CJD	1:1	0/6	550+/-73	0/3	0/5

NT: not tested.

RT-QuIC Products

Table 4. Inoculation of sCJD-seeded RT-QuIC products into Tg66 mice.

RT-QuIC product inoculum	Total PrP inoculated	Clinical prion disease (+/total)	Survival time dpi (mean +/- SD)	Atypical neuropathology and PrP IHC[1] (+/total tested)	RT-QuIC (+/ total tested)
CJD Patient 2-seeded	5 µg	0/5	644 +/- 7	4/4	2/2
	0.5 µg	0/6	554 +/-104	2/3	NT
CJD Patient 8-seeded	5 µg	0/5	560 +/- 62	2/2	1/1
	0.5 µg	0/6	624 +/-53	6/6	3/3
Control Patient-seeded	2.5 µg	0/4	526 +/-36	0/4	0/4

ANNALS of Clinical and Translational Neurology
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RESEARCH ARTICLE

Ring trial of 2nd generation RT-QuIC diagnostic tests for sporadic CJD

Christina D. Orrú[1,a], Bradley R. Groveman[1,a], Aaron Foutz[2], Matilde Bongianni[3], Franco Cardone[4], Neil McKenzie[5], Audrey Culeux[6], Anna Poleggi[4], Katarina Grznarova[6], Daniela Perra[3], Michele Fiorini[3], Xiaoqin Liu[2], Anna Ladogana[4], Marco Sbriccoli[4], Andrew G. Hughson[1], Stéphane Haïk[6], Alison J. Green[5], Michael D. Geschwind[7], Maurizio Pocchiari[4], Jiri G. Safar[2], Gianluigi Zanusso[3] & Byron Caughey[1]

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[7]Department of Neurology, Memory and Aging Center, University of California San Francisco, San Francisco, California

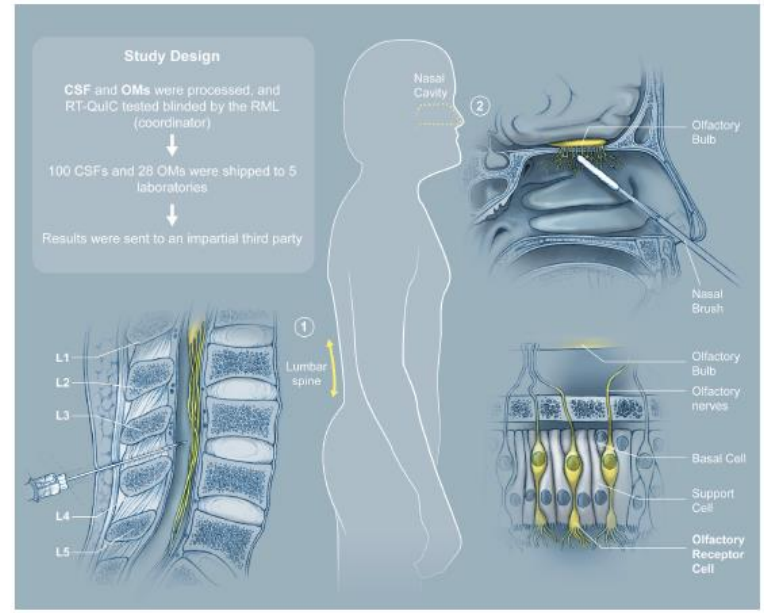


Figure 1. Cerebrospinal fluid and olfactory mucosa ring trial study design.

Table 3. Overall concordance of CSF testing for each testing laboratory

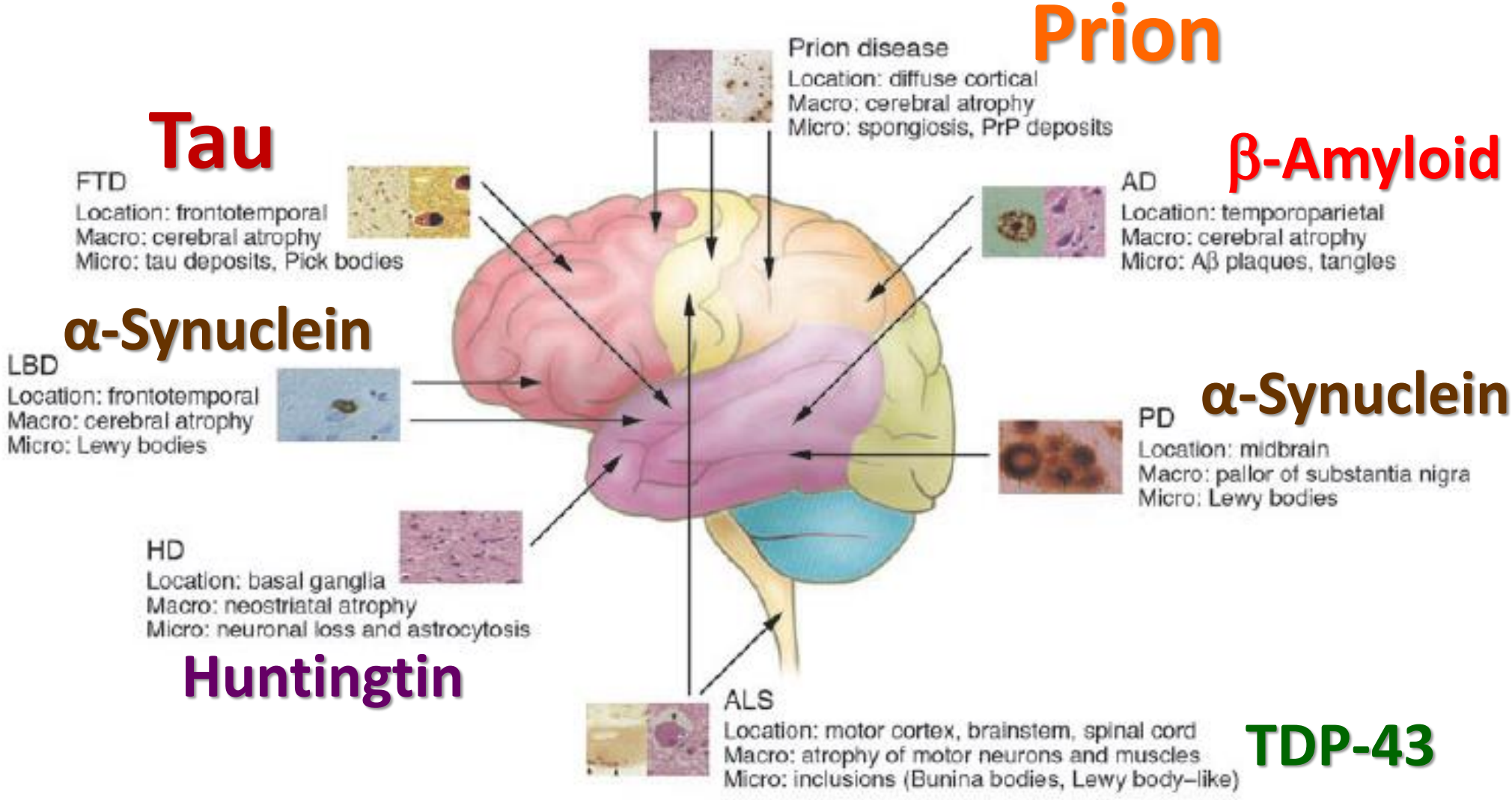
	RML	UV	NPDPSC	ISS	UE	SU	
CJD	100% (55/55)	96% (53/55)	100% (55/55)	98% (54/55)	98% (54/55)	96% (53/55)	
Non-CJD	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	Total Concordance
Overall	**100% (100/100)**	**98% (98/100)**	**100/100 (100%)**	**99% (99/100)**	**99% (99/100)**	**98% (98/100)**	**99% (594/600)**

Table 4. Concordance of olfactory mucosa testing by testing laboratory

	RML	UV	NPDPSC	ISS	UE	SU	
CJD	100% (9/9)	89% (8/9)	100% (9/9)	89% (8/9)	100% (9/9)	100% (9/9)	
Non-CJD	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	95% (18/19)	Total Concordance
Overall	**100% (28/28)**	**96% (17/28)**	**100% (28/28)**	**96% (27/28)**	**100% (28/28)**	**96% (27/28)**	**98% (165/168)**

Olfactory System Involvement in Neurodegeneration

Prionoids

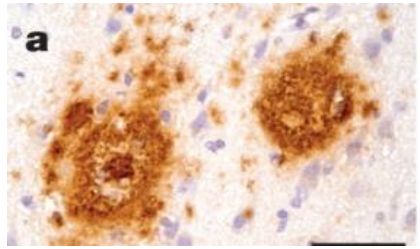

Prion
Prion disease
Location: diffuse cortical
Macro: cerebral atrophy
Micro: spongiosis, PrP deposits
Tau
FTD
Location: frontotemporal
Macro: cerebral atrophy
Micro: tau deposits, Pick bodies
β-Amyloid
AD
Location: temporoparietal
Macro: cerebral atrophy
Micro: Aβ plaques, tangles
α-Synuclein
LBD
Location: frontotemporal
Macro: cerebral atrophy
Micro: Lewy bodies
α-Synuclein
PD
Location: midbrain
Macro: pallor of substantia nigra
Micro: Lewy bodies
HD
Location: basal ganglia
Macro: neostriatal atrophy
Micro: neuronal loss and astrocytosis
Huntingtin
ALS
Location: motor cortex, brainstem, spinal cord
Macro: atrophy of motor neurons and muscles
Micro: inclusions (Bunina bodies, Lewy body–like)
TDP-43

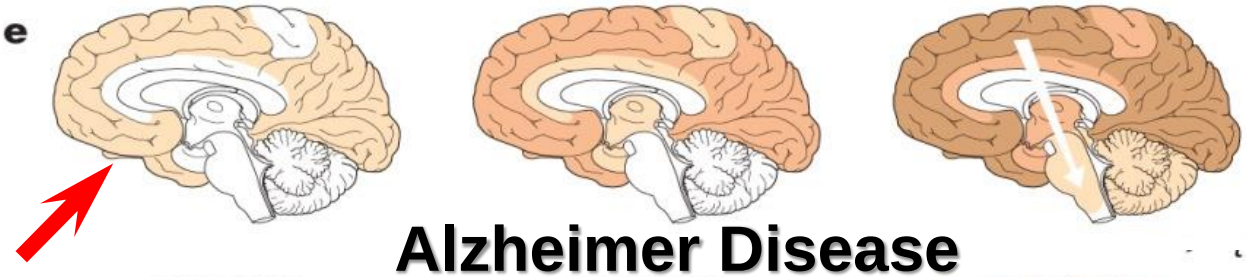
Misfolded Proteins and Disease Phenotypes

Alpha-Synucleinopathies	Parkinson Disease Mutisystem Atrophy Lewy Body Dementia PAF
Tauopathies	FTLD Progressive Sopranuclear Palsy Corticobasal Degeneration
β-pathies	Alzheimer Disease
TDP43-pathies	ALS-FTD and FTD

Early Involvement of Olfactory System in Neurodegenerative Disorders

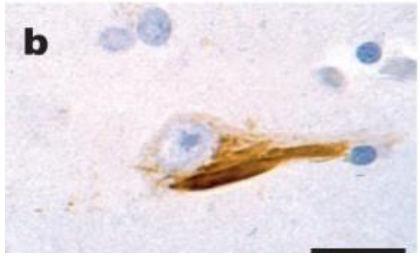
β-Amyloid

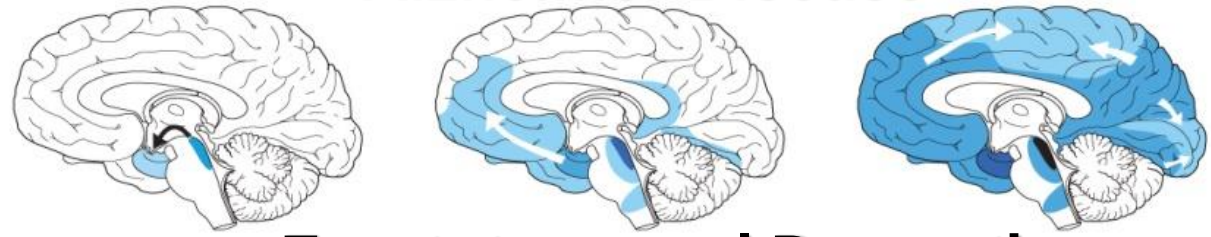




Alzheimer Disease

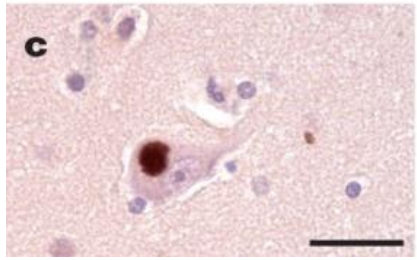
Tau

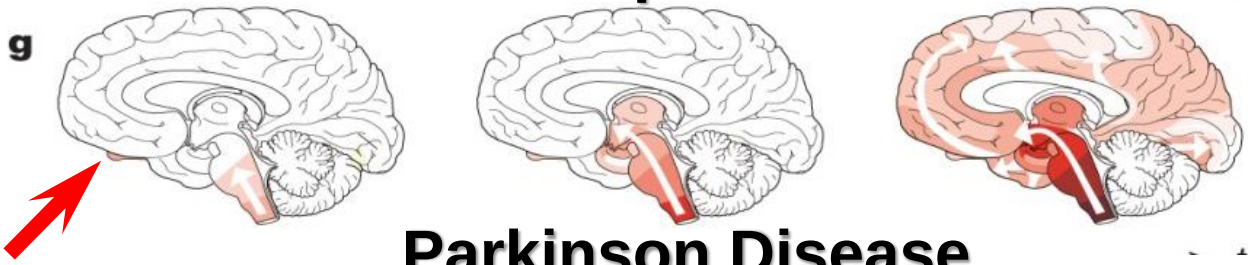




Frontotemporal Dementia

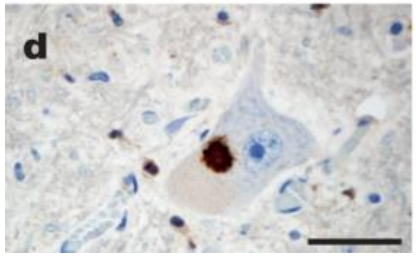
Alpha-Synuclein





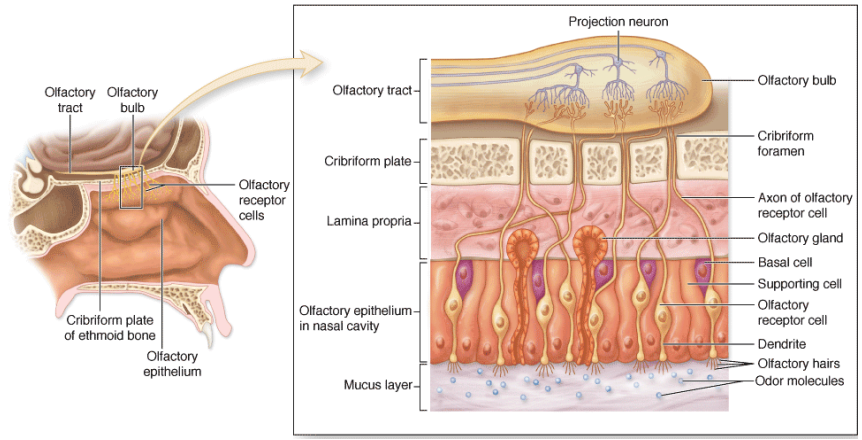
Parkinson Disease

TDP43



ALS FTD

Olfactory System in Neurodegenerative Disorders

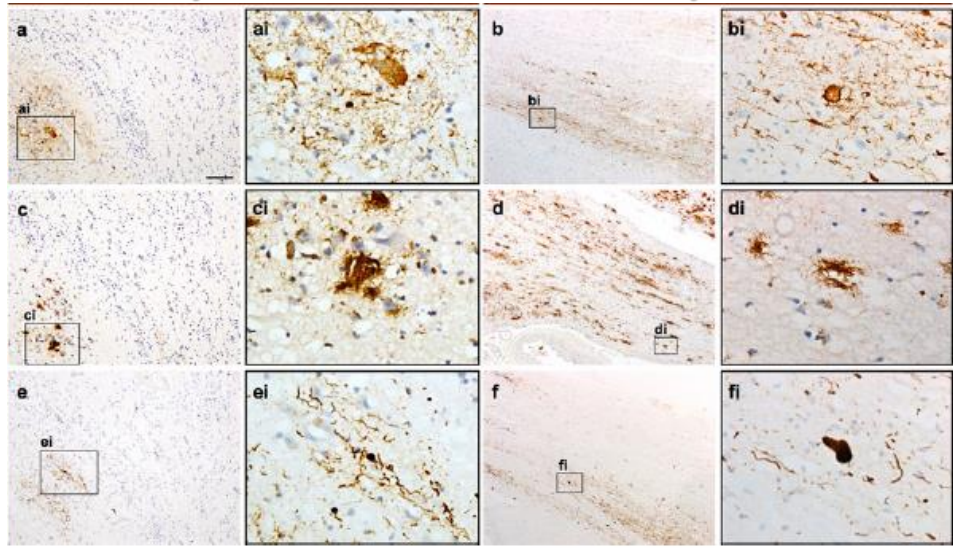


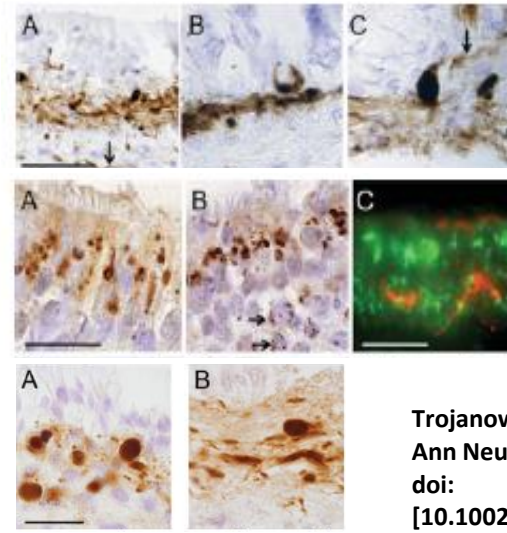
Olfactory Bulb | **Olfactory Tract** | **Olfactory Neuro-epithelium**

Alzheimer Disease
β-amyloid

Fronto-temporal Dementia
Tau

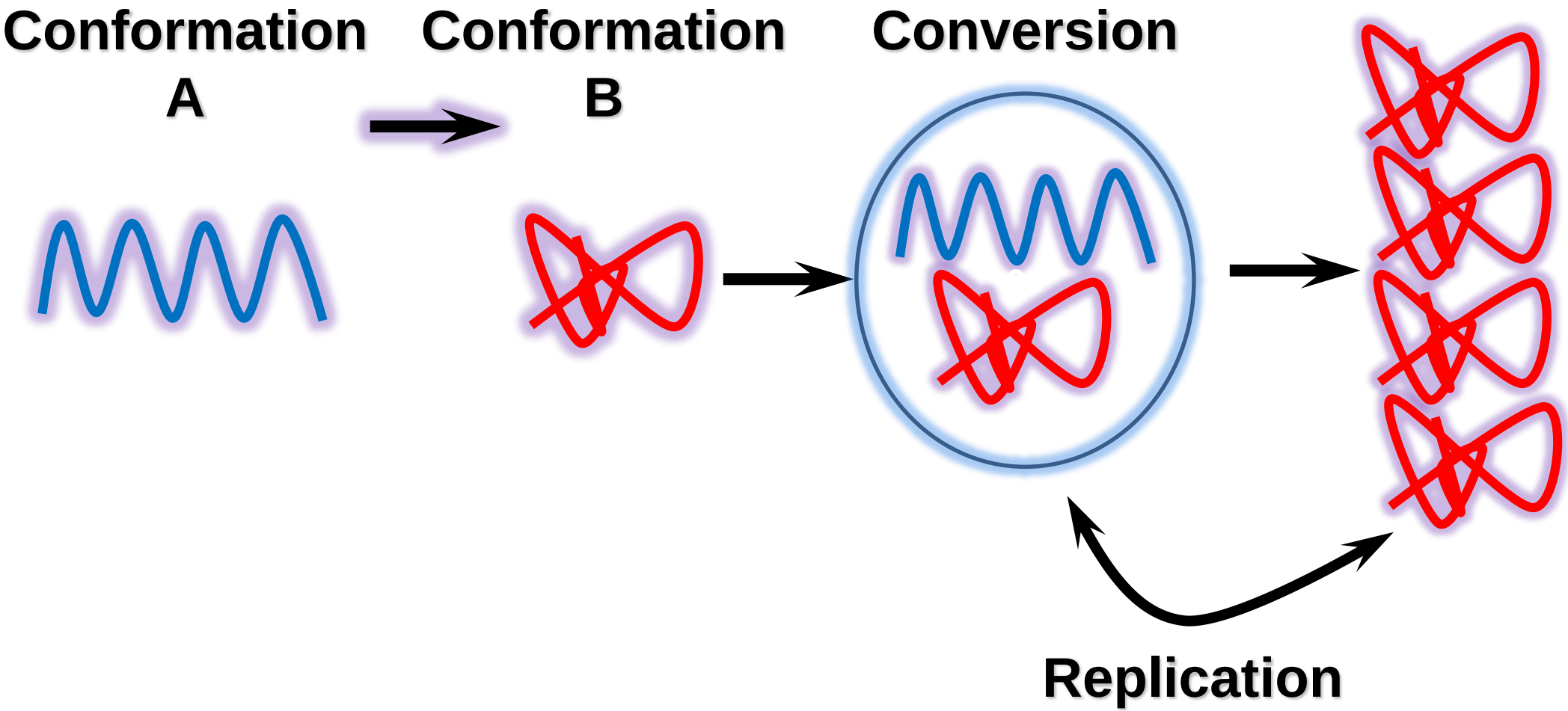
Parkinson Disease
a-Synuclein



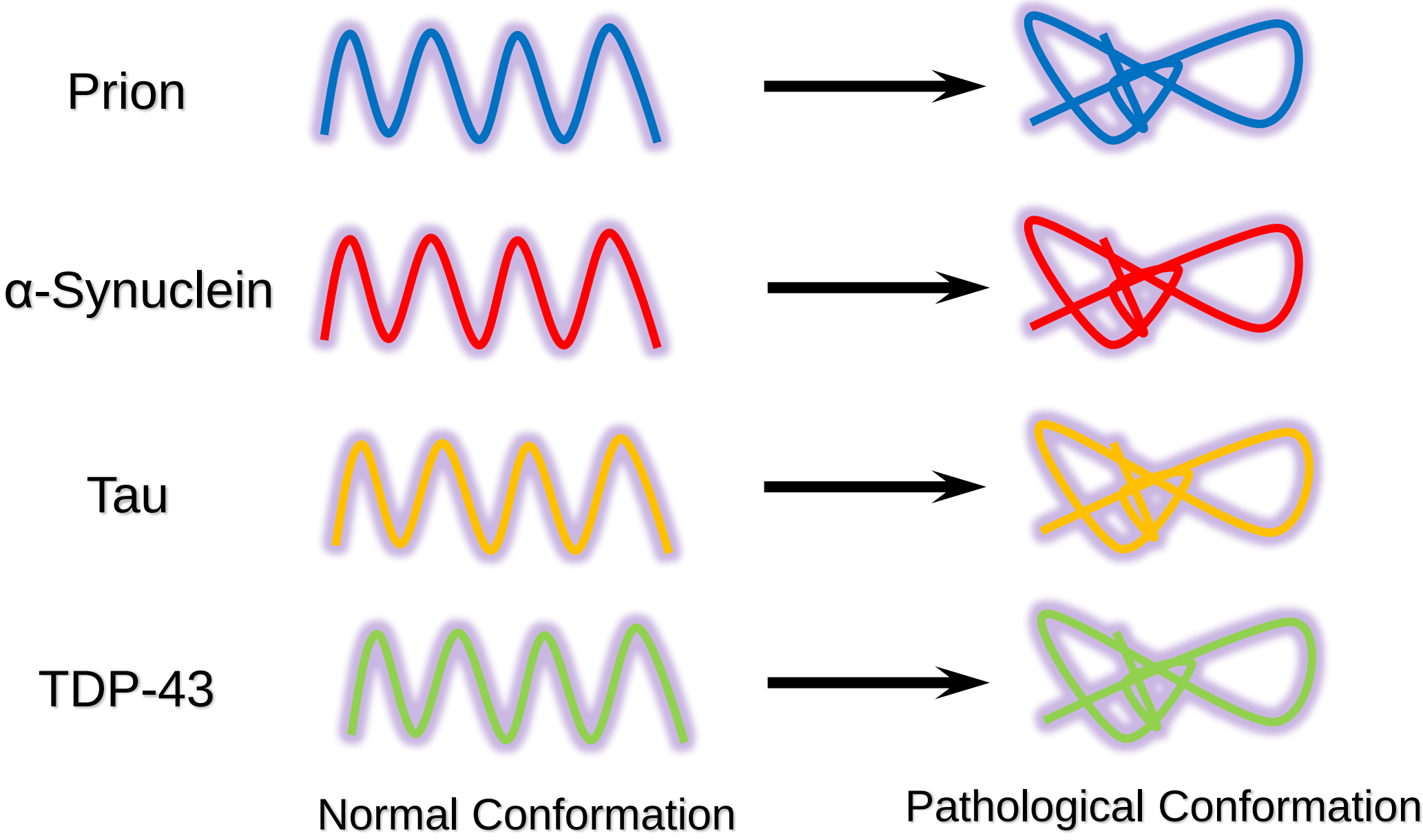


Trojanowski J.Q.
Ann Neurol. 2011 Apr;
doi:
[10.1002/ana.21910]

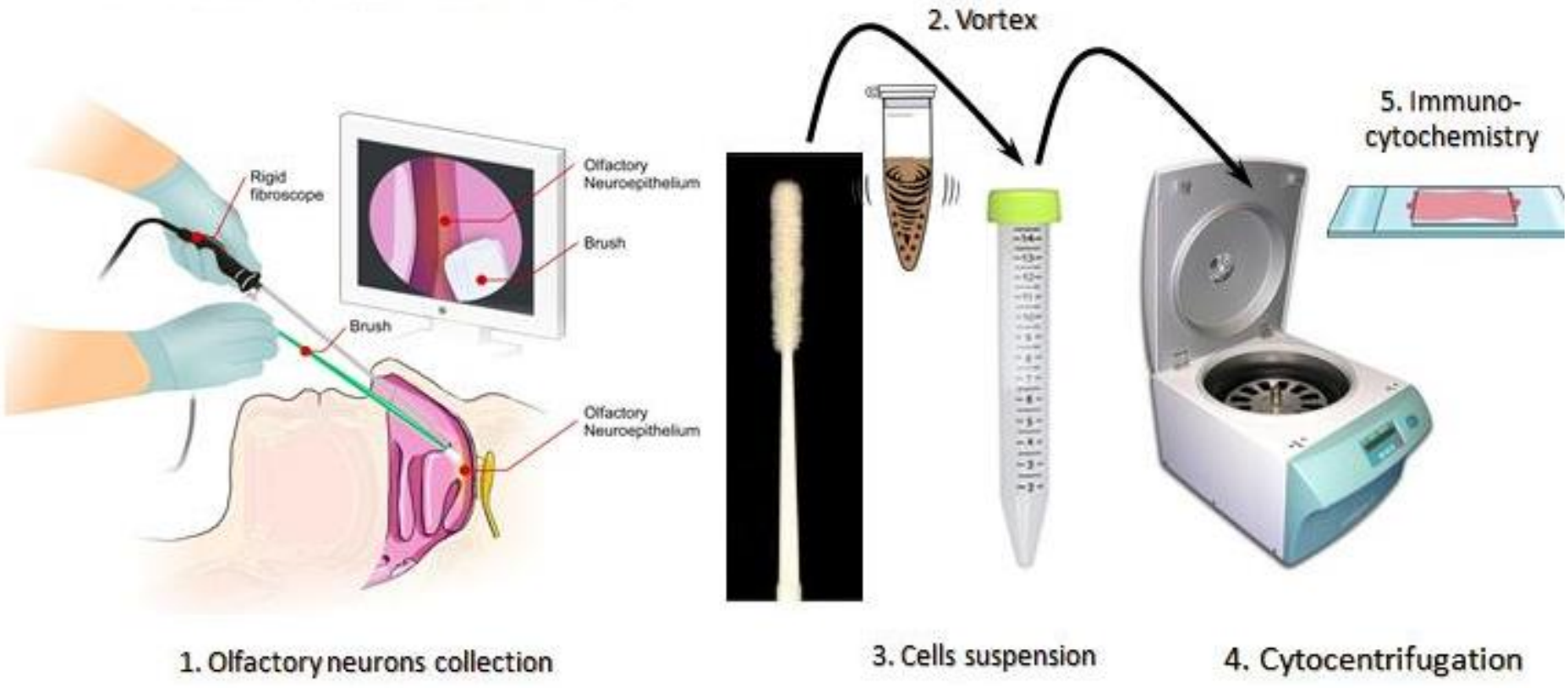
Prion-like Propagation



Conformational Changes and Neurodegeneration



Nasal Swabbing Sample Processing



Neurodegeneration-Associated Proteins in Human Olfactory Neurons Collected by Nasal Brushing

frontiers in Neuroscience

ORIGINAL RESEARCH
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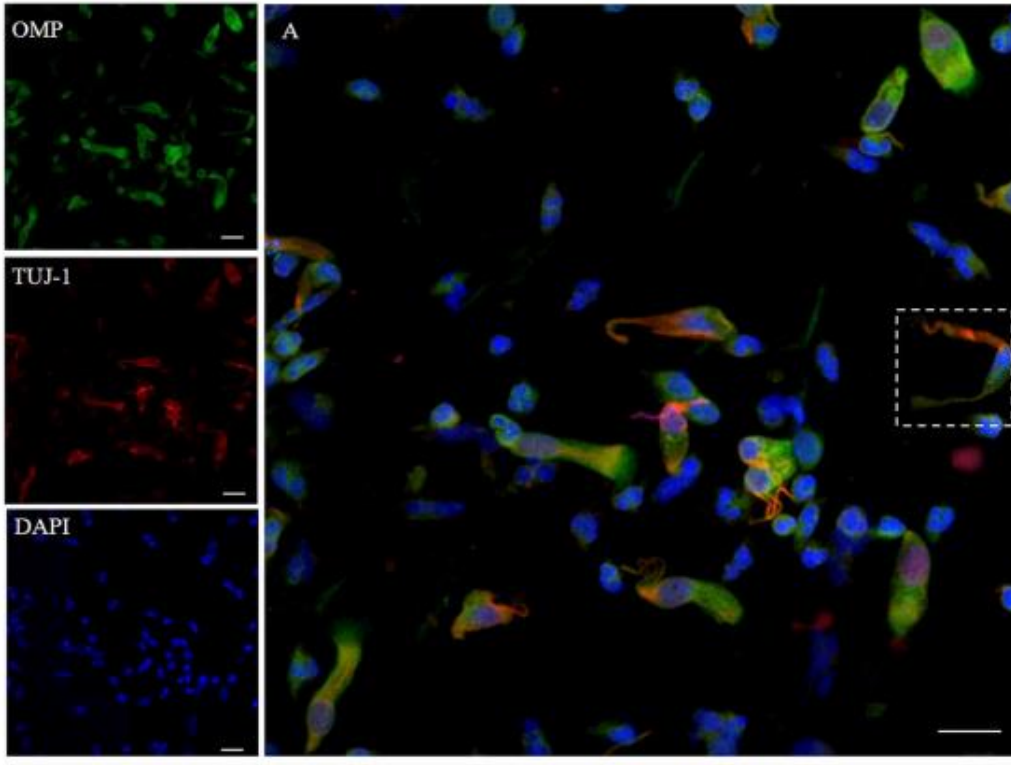
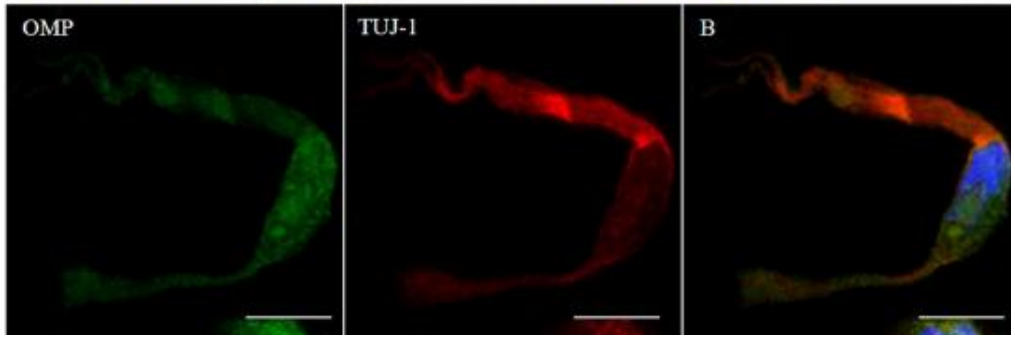
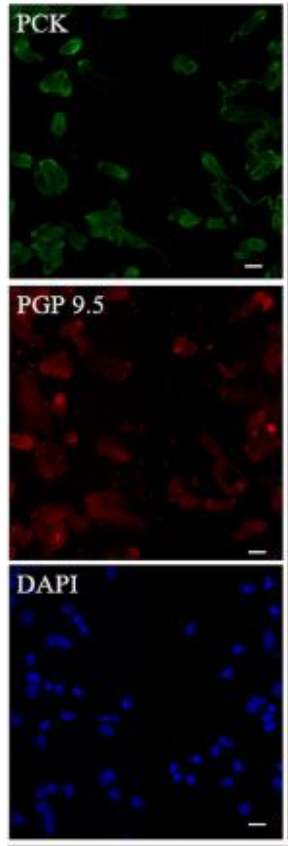
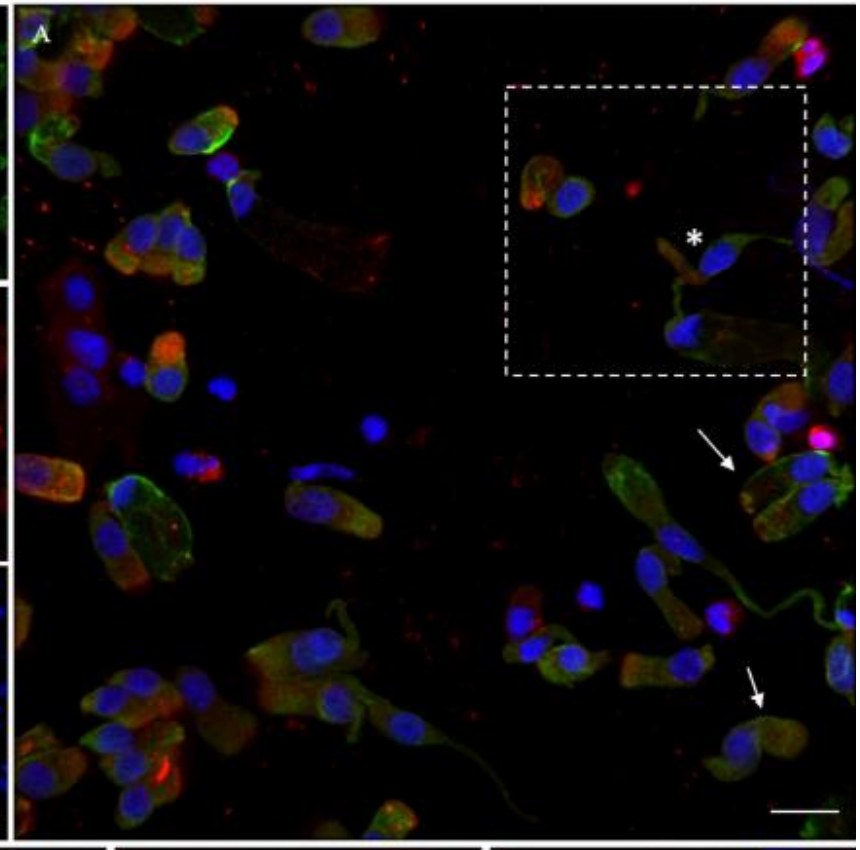
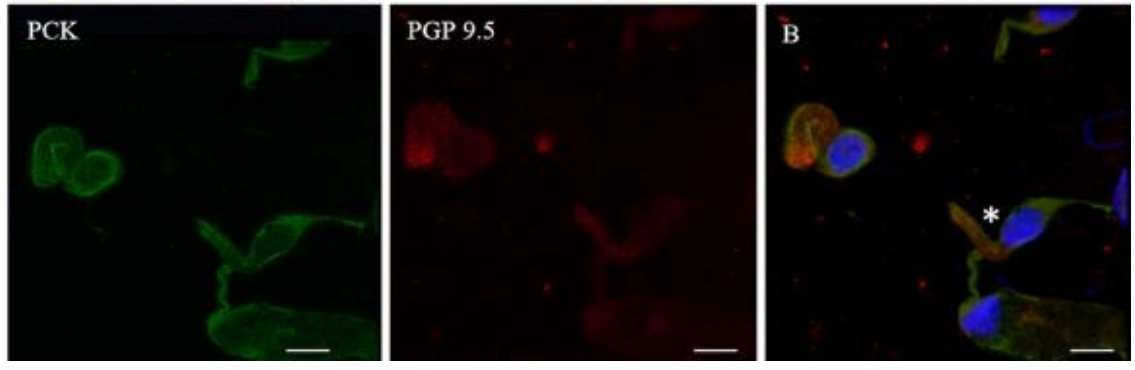


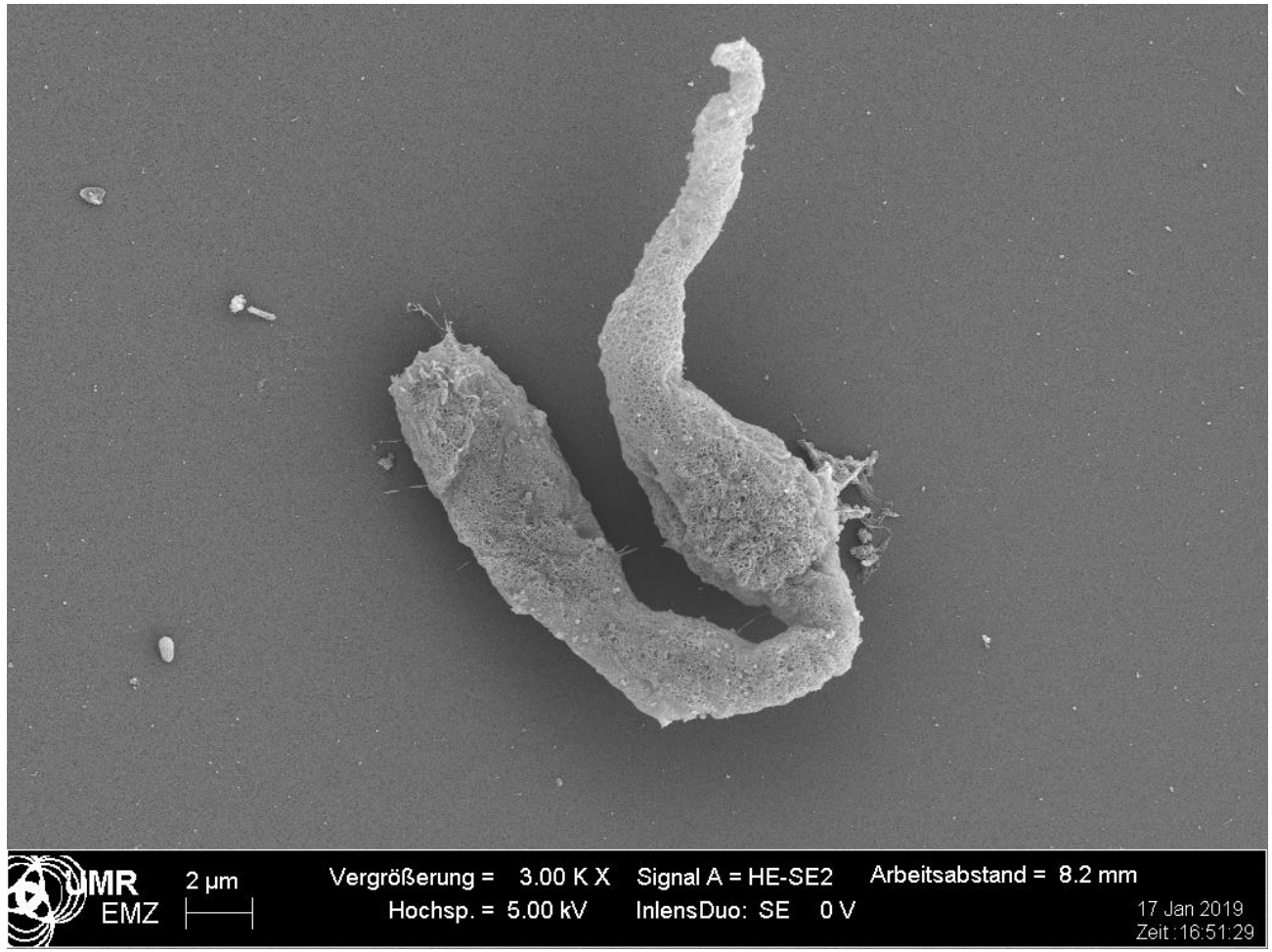








Olfactory neurons



Lorenzo Brozzetti Ph.D student UNIVR
2019

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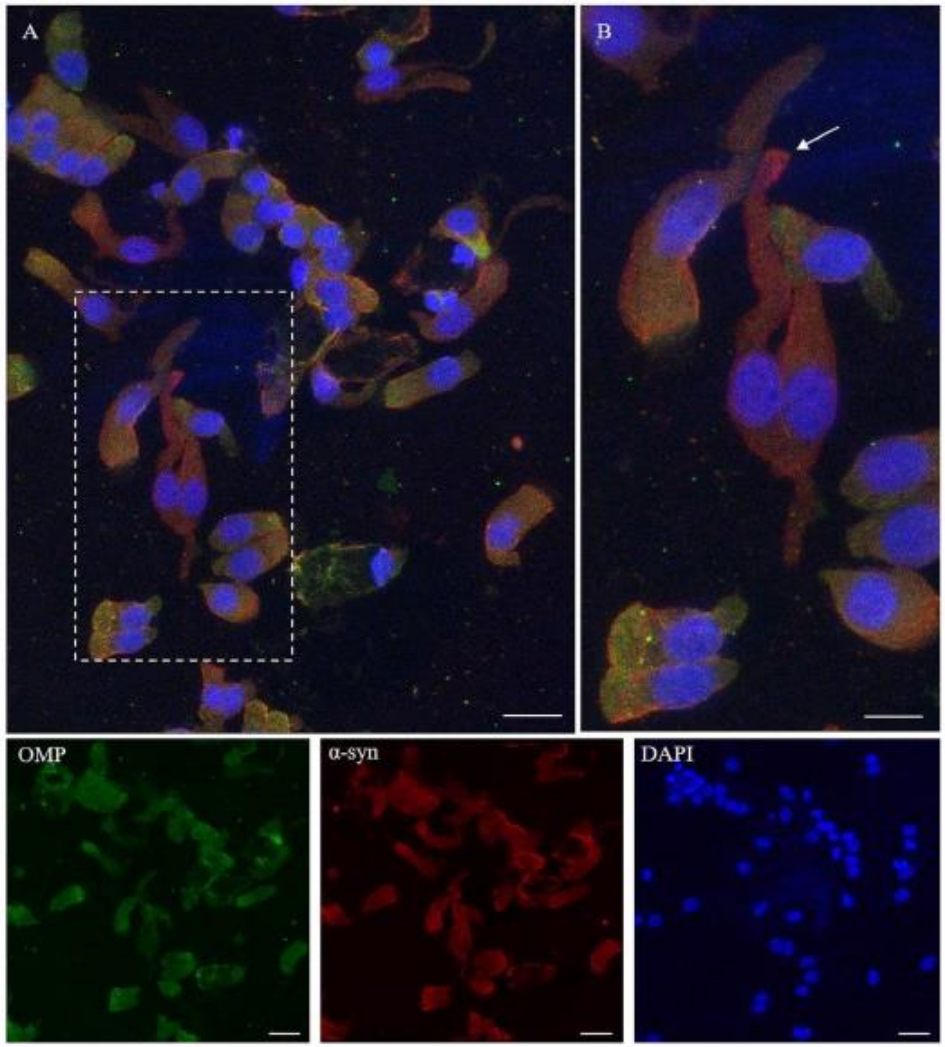
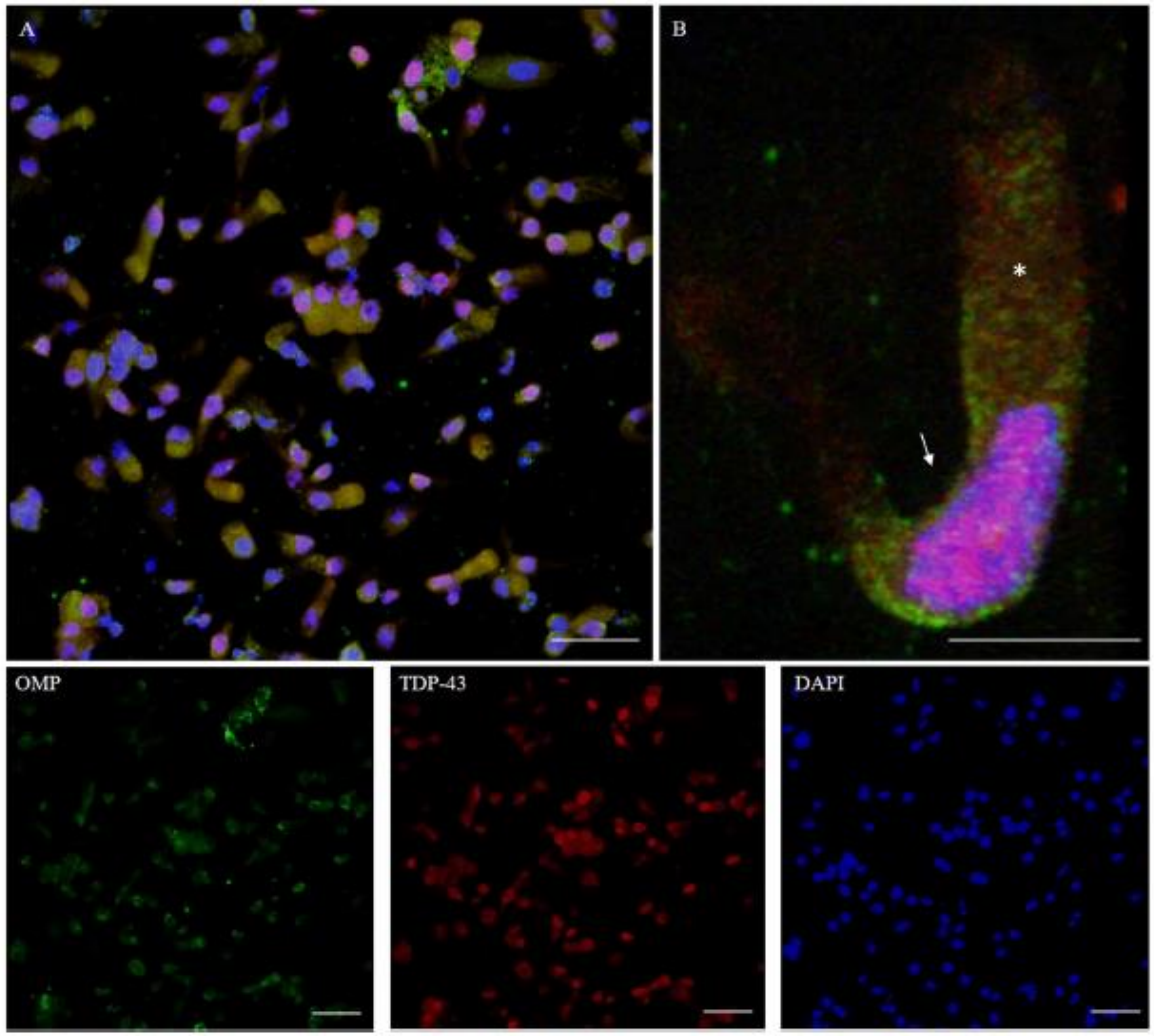
Normal Alpha-Synuclein



Normal TDP-43



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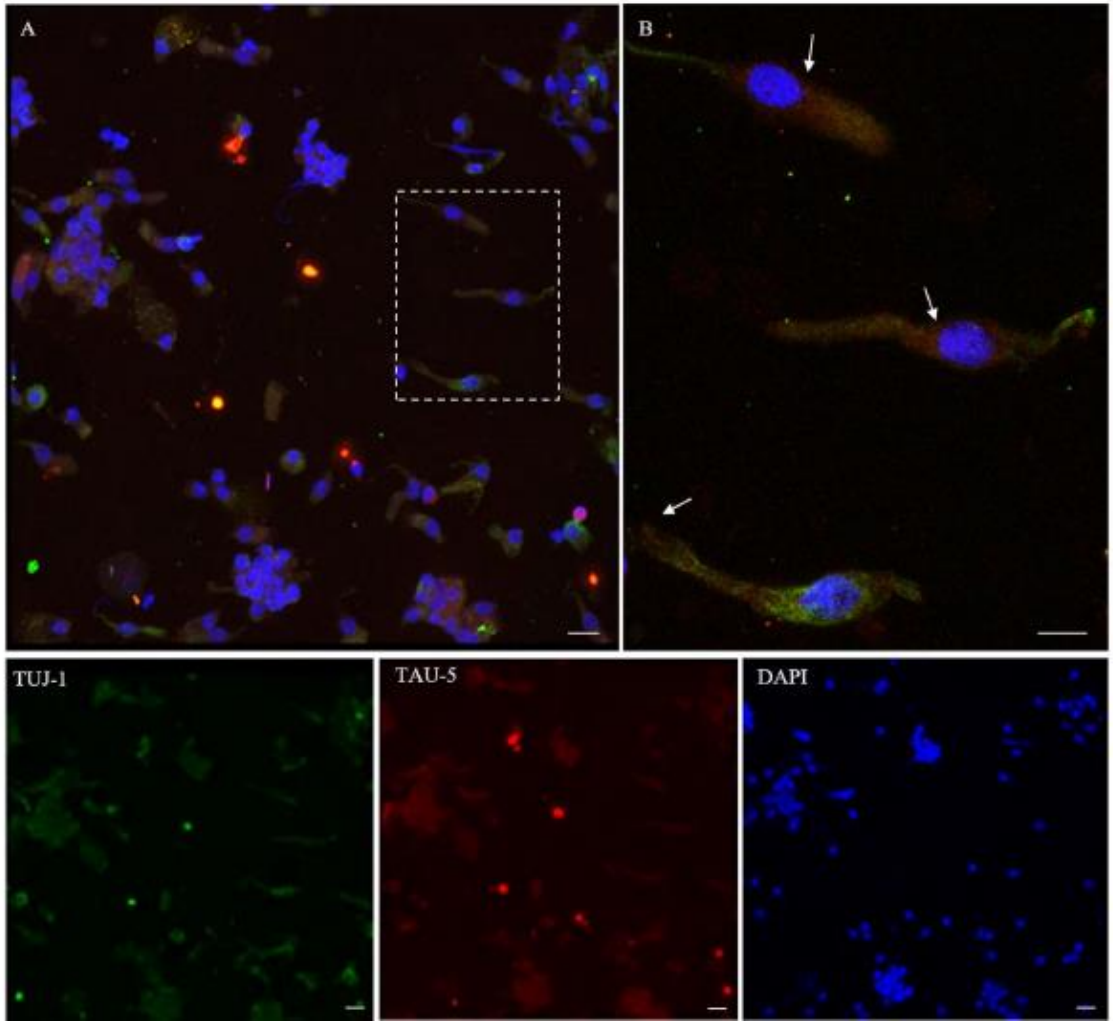
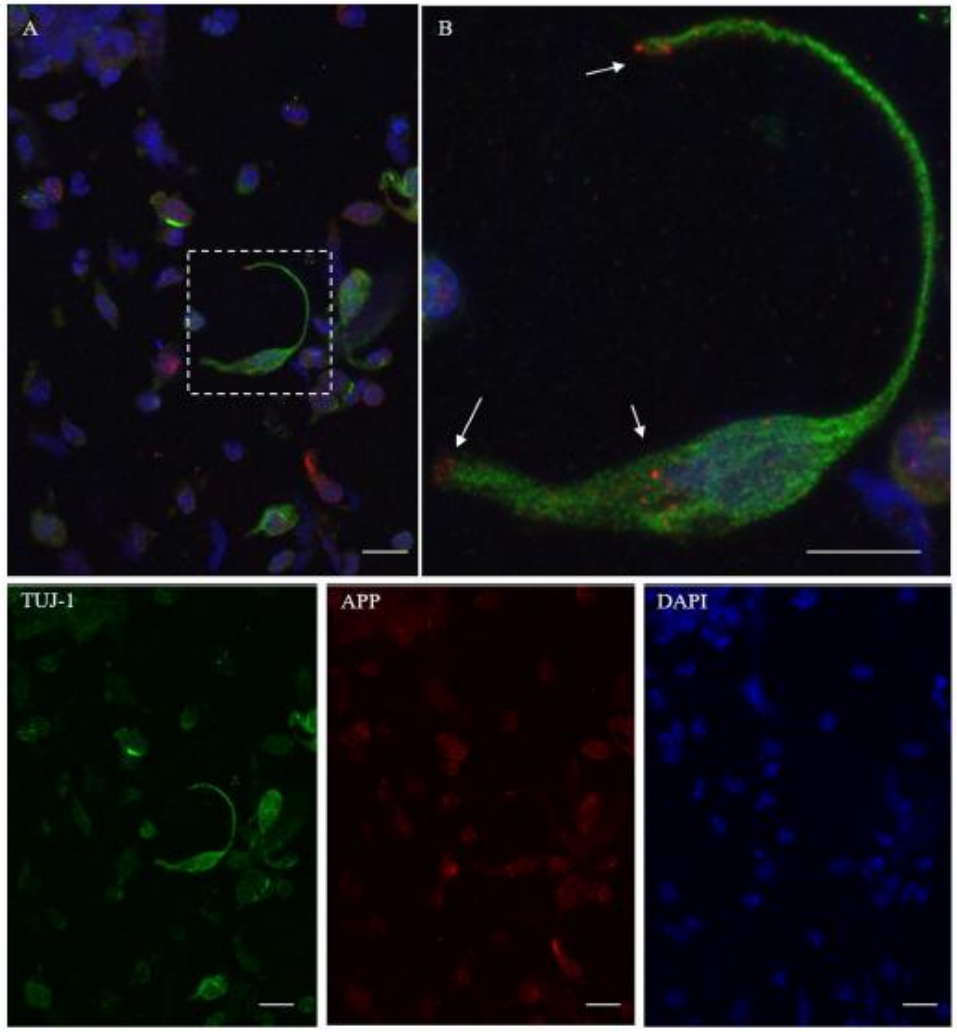
Normal Tau Protein



Normal APP



Misfolded Proteins and Disease Phenotypes

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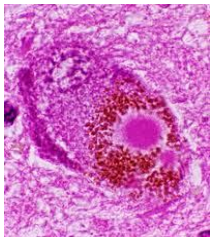
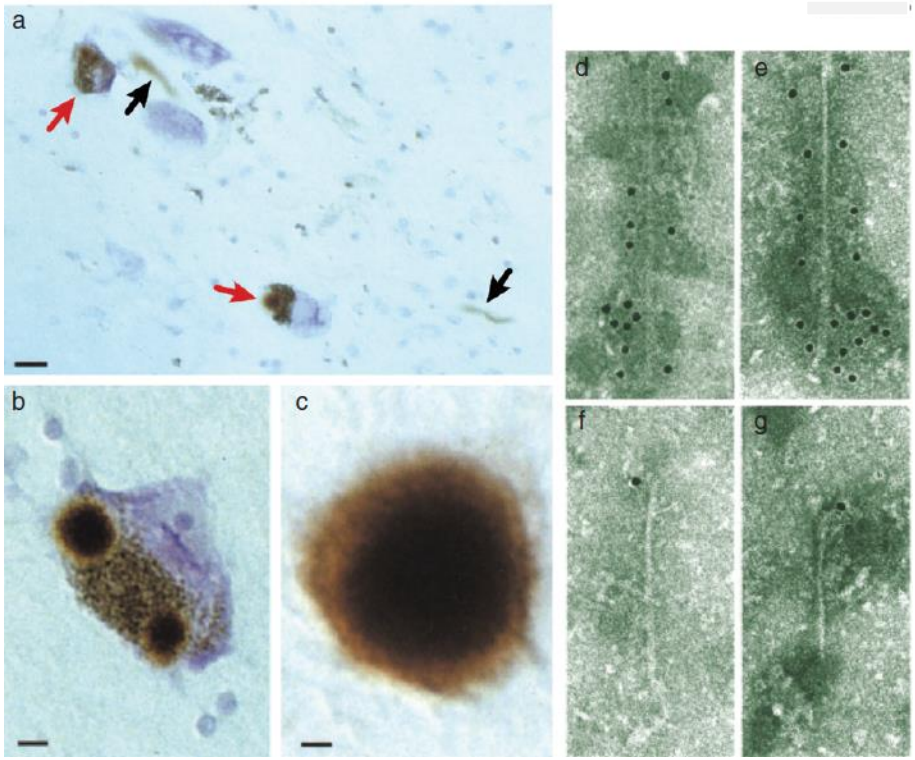
β-pathies	Alzheimer Disease
TDP43-pathies	ALS-FTD and FTD



Alfa-Synucleino-pathies

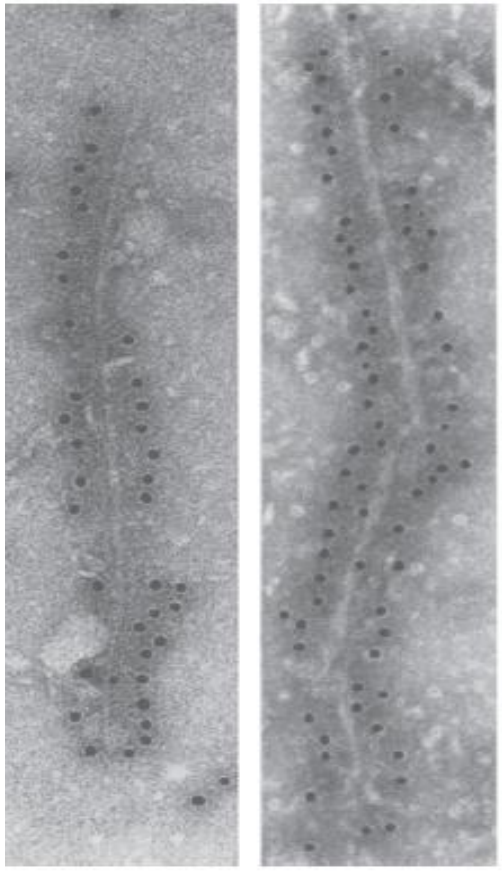
Parkinson Disease



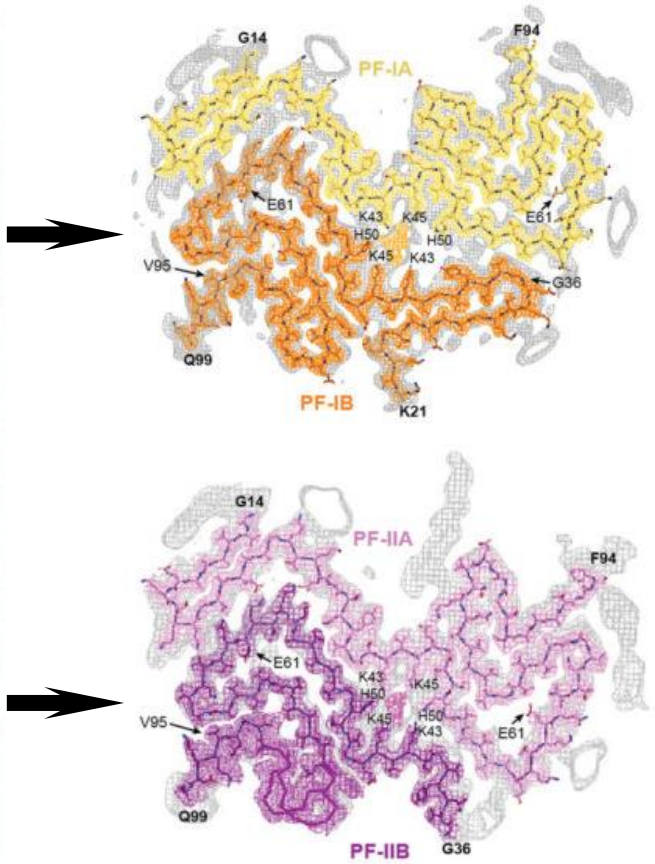
M. Goedert et al. / The Synucleinopathies: Twenty Years On

Dementia with Lewy Bodies

Multisystem Atrophy



Cryo-EM Structure of MSA a-Synuclein Fibrils



Schweighauser M., et a
Nature 2020

Biomarkers in α-Synucleinopathies

- **Clinical core**

Supporting Investigations:

- **MRI**
- **^{123}I-ioflupane single-photon emission CT (Da Tscan)**
- **PET or SPECT for myocardial sympathetic denervation**

No Radiotracers are available for α-Synuclein

CSF Biomarkers or other Tissues

- **Biomarkers of disease progression**
- **Diagnostic value**
- **Prognostic value**

Multiorgan α-synuclein deposits in Parkinson's disease

Postmortem

Stellate ganglion

Paravertebral sympathetic Ganglia

Vagus nerve

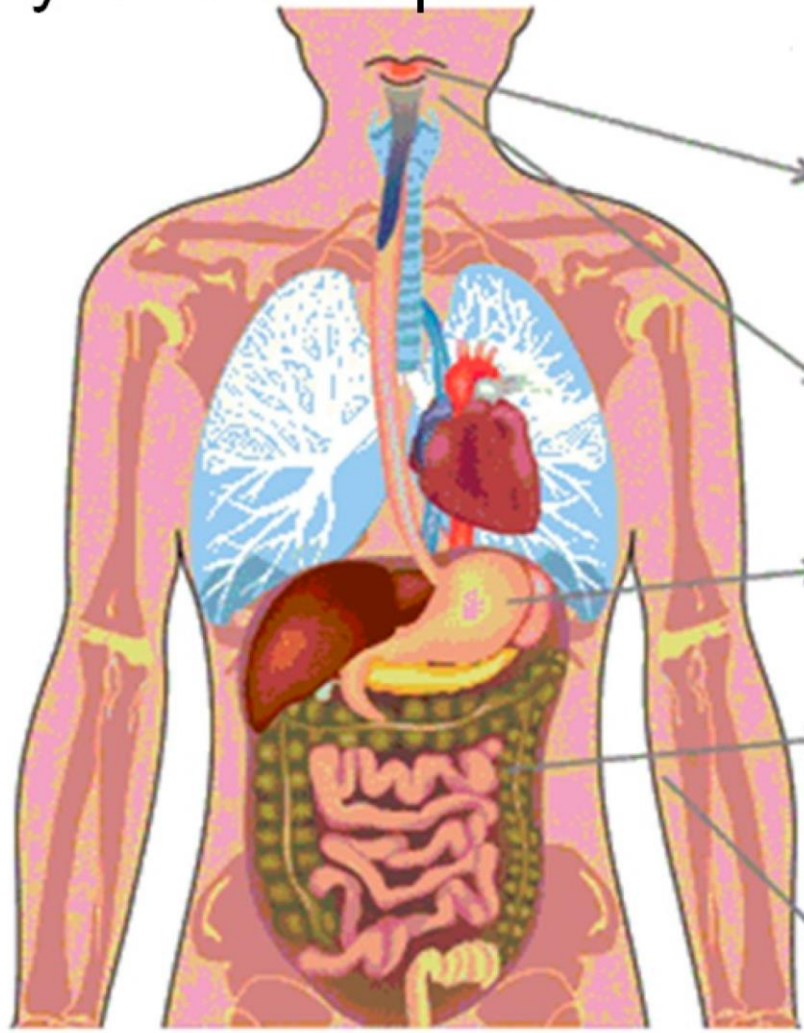
Epicardial plexus

Mesenteric sympathetic ganglia

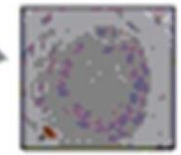
Enteric nervous system

Adrenal gland

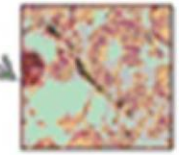
Genitourinary tract



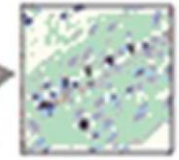
Living patients



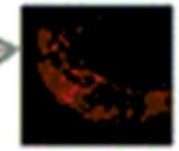
Minor salivary glands



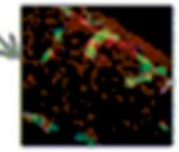
Submandbular gland



Stomach



Colon



Skin

RESEARCH ARTICLE

α-Synuclein Real-Time Quaking-Induced Conversion in the Submandibular Glands of Parkinson's Disease Patients

Sireesha Manne, DVM, PhD,[1] Naveen Kondru, DVM, PhD,[1] Huajun Jin, PhD,[1] Vellareddy Anantharam, PhD,[1] Xuemei Huang, MD, PhD,[2] Arthi Kanthasamy, PhD,[1] and Anumantha G. Kanthasamy, PhD[1*]

[1]*Department of Biomedical Sciences, Parkinson's Disorder Research Program, Iowa Center for Advanced Neurotoxicology, Iowa State University, Ames, Iowa, USA*

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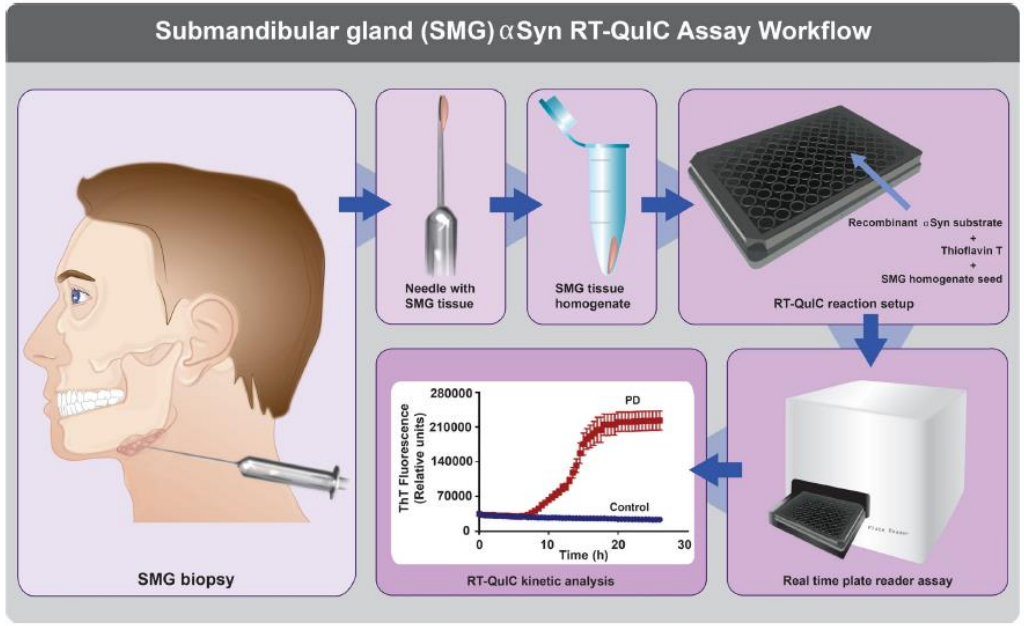
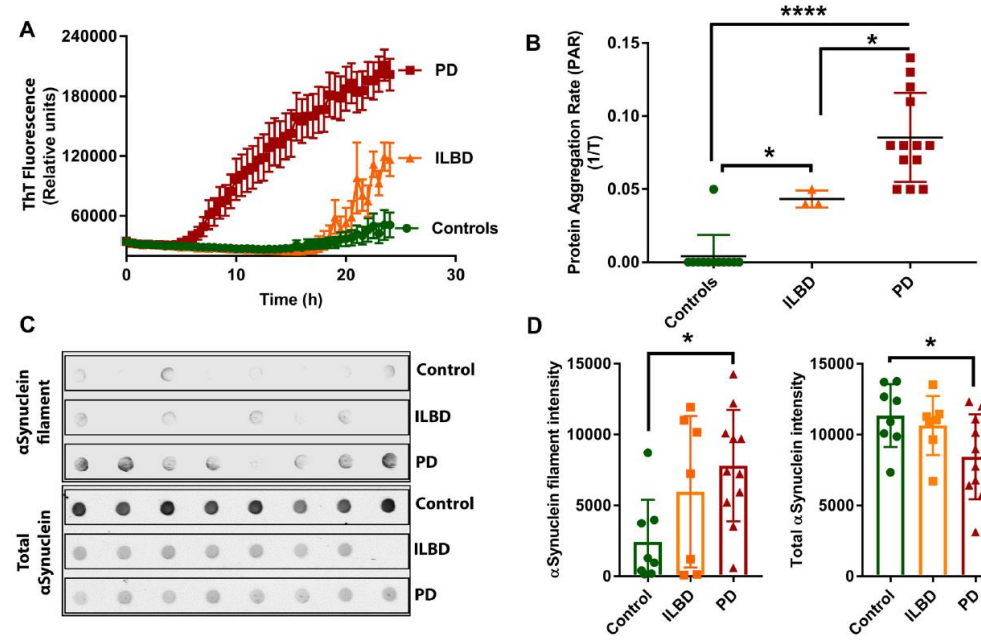
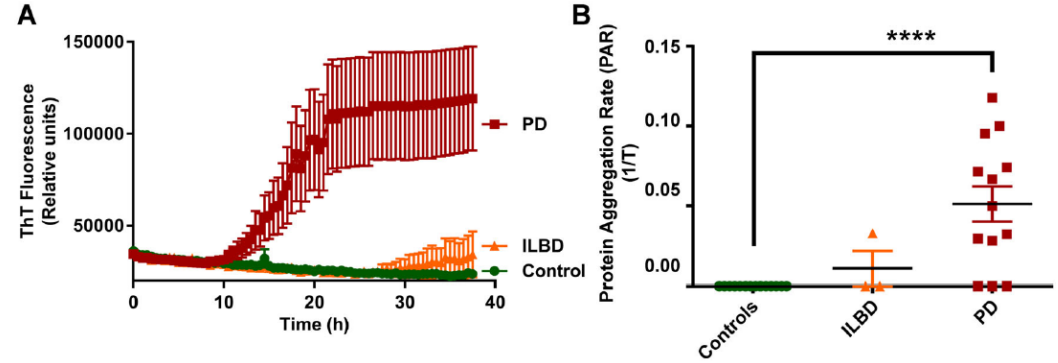
Received: 29 July 2019; **Revised:** 8 October 2019; **Accepted:** 11 October 2019



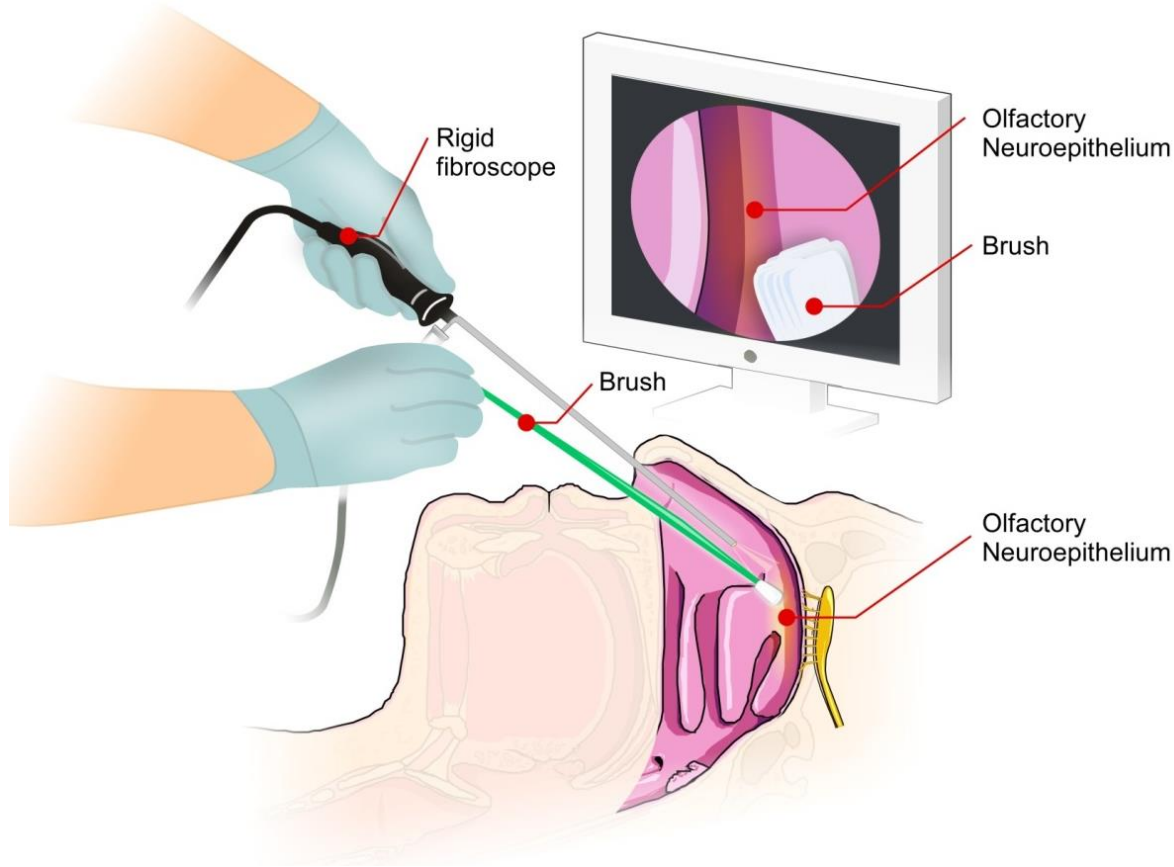


Fresh tissue homogenates



Formalin fixed sections

Nasal Swabbing in α-Synucleinopathies



doi:10.1093/braincomms/fcab045 BRAIN COMMUNICATIONS 2021: Page 1 of 11 | 1

BRAIN COMMUNICATIONS

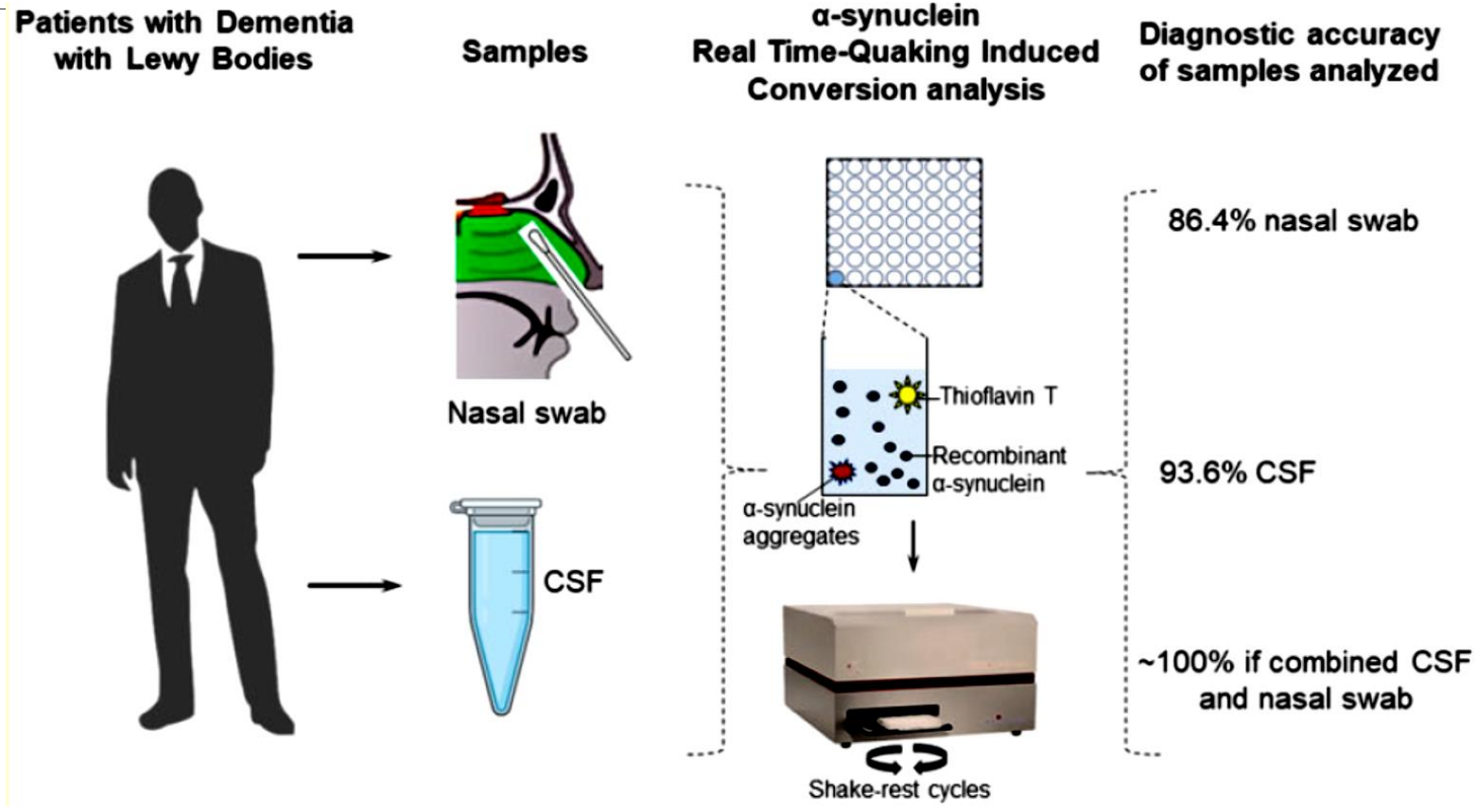
Alpha-synuclein seeds in olfactory mucosa and cerebrospinal fluid of patients with dementia with Lewy bodies

Daniela Perra,[1,*] Matilde Bongianni,[1,*] Giovanni Novi,[2] Francesco Janes,[3] Valentina Bessi,[4] Stefano Capaldi,[5] Luca Sacchetto,[6] Matteo Tagliapietra,[1] Guido Schenone,[2] Silvia Morbelli,[2,7] Michele Fiorini,[1] Tatiana Cattaruzza,[8] Giulia Mazzon,[8] Christina D. Orrù,[9] Mauro Catalan,[8] Paola Polverino,[8] Andrea Bernardini,[3] Gaia Pellitteri,[3] Mariarosa Valente,[3] Claudio Bertolotti,[8] Benedetta Nacmias,[10,11] Giandomenico Maggiore,[12] Tiziana Cavallaro,[1] Paolo Manganotti,[8] Gianluigi Gigli,[3] Salvatore Monaco,[1] Flavio Nobili[2,13] and Gianluigi Zanusso[1]

Table 3 Patients with olfactory mucosa only and both olfactory mucosa and cerebrospinal fluid samples analysed by α-syn RT-QuIC

Clinical diagnosis	OM only (33)	Patients with both OM and CSF (16)			
	OM positive	OM and CSF positive	OM positive and CSF negative	OM negative and CSF positive	OM negative and CSF negative
DLB-Group (43)	**24/27**	**11/16**	**–**	**5**	**–**
Probable DLB (32)	19/22	8/10	–	2	–
DLB/AD mixed dementia (6)	–	3/6	–	3	–
Prodromal DLB (5)	5/5	–	–	–	–
Non-α-syn NDs (38)	**0/6**	**0/32**	**3**	**3**	**26**
CJD (10)	–	0/10	–	–	10
AD (10)	–	0/10	1[a]	1[a]	8
PSP (8)	0/2	0/6	1[a]	1[a]	4
CBS (1)	–	0/1	–	–	1
FTD (3)	–	0/3	–	1	2
Others (6)	0/4	0/2	1	–	1

[a]This positive sample does not belong to the same patient.
Rows in bold text indicate the totals for the DLB group and non-α-syn NDs patients.



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https://doi.org/10.1186/s40035-019-0164-x

Translational
Neurodegeneration

RESEARCH Open Access

Efficient RT-QuIC seeding activity for α-synuclein in olfactory mucosa samples of patients with Parkinson's disease and multiple system atrophy

Chiara Maria Giulia De Luca[1†], Antonio Emanuele Elia[2†], Sara Maria Portaleone[3], Federico Angelo Cazzaniga[1], Martina Rossi[4], Edoardo Bistaffa[1], Elena De Cecco[4], Joanna Narkiewicz[4], Giulia Salzano[4], Olga Carletta[1], Luigi Romito[2], Grazia Devigili[2], Paola Soliveri[2], Pietro Tiraboschi[1], Giuseppe Legname[4], Fabrizio Tagliavini[5], Roberto Eleopra[2], Giorgio Giaccone[1] and Fabio Moda[1*]

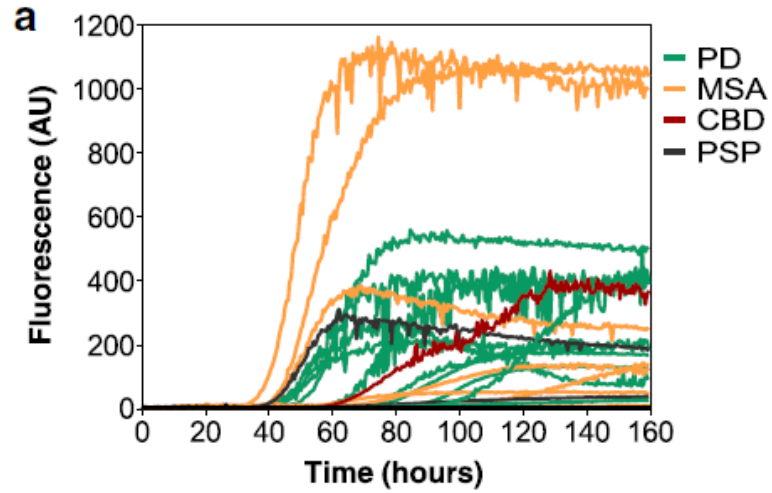
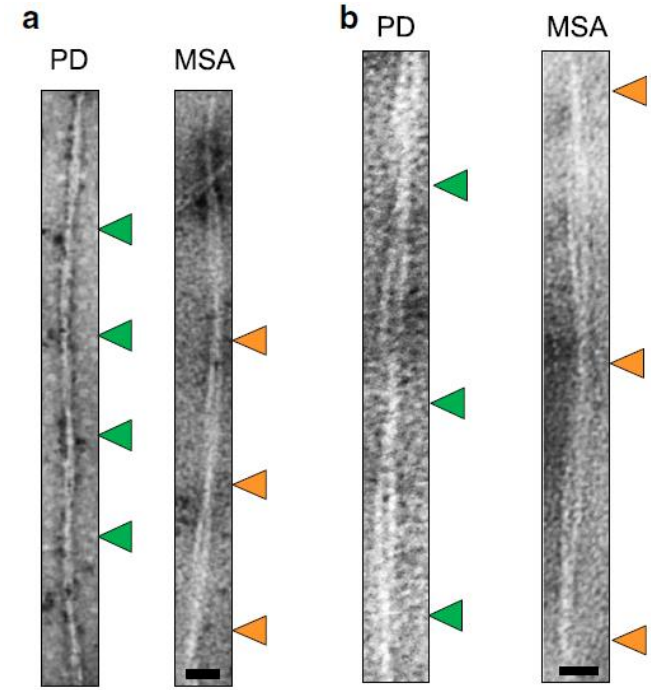


Table 1 Clinical data and OM/RT-QuIC results of all patients included in the study

	PD	MSA	CBD	PSP
Clinical criteria (ref.)	[18, 61]	[33]	[62]	[39]
Number of patients	18	11	6	12
Age at time of evaluation (years)	64.2 ± 7.8	62.3 ± 9.2	63.3 ± 10.6	68.3 ± 7.0
Age at disease onset (years)	52.4 ± 6.1	56.5 ± 9.5	60.2 ± 10.9	64.3 ± 8.2
Disease duration (years)	10.1 ± 5.1	5.8 ± 3.4	3.2 ± 1.6	4.0 ± 3.6
Gender (F/M)	8/10	5/6	4/2	5/7
Frequency of symptoms (%)				
• Rigid akinetic parkinsonism	100	90.1	83.3	91.7
• Tremor	88.9	81.8	50	8.3
• Ataxia	0	90.1	50	91.7
• Apraxia	0	0	100	33.3
• Delusions	16.7	9.1	0	8.3
• Dementia	11.1	0	16.7	58.3
• Psychiatric disorders	33.3	45.5	33.3	50
• REM behavioural disorder	55.6	63.6	0	0
• Autonomic impairment	83.3	100	33.3	16.7
RT-QuIC seeding activity for α-synuclein (% in total patients)	10 (56%)	9 (82%)	1 (16%)	2 (16%)

Values of continuous variables are presented as mean ± standard deviation (SD)



Article

Discriminating α-synuclein strains in Parkinson's disease and multiple system atrophy

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Mohammad Shahnawaz[1], Abhisek Mukherjee[1], Sandra Pritzkow[1,6], Nicolas Mendez[1,6], Prakruti Rabadia[1], Xiangan Liu[2], Bo Hu[2], Ann Schmeichel[2], Wolfgang Singer[2], Gang Wu[4], Ah-Lim Tsai[4], Hamid Shirani[5], K. Peter R. Nilsson[5], Phillip A. Low[3] & Claudio Soto[1]*

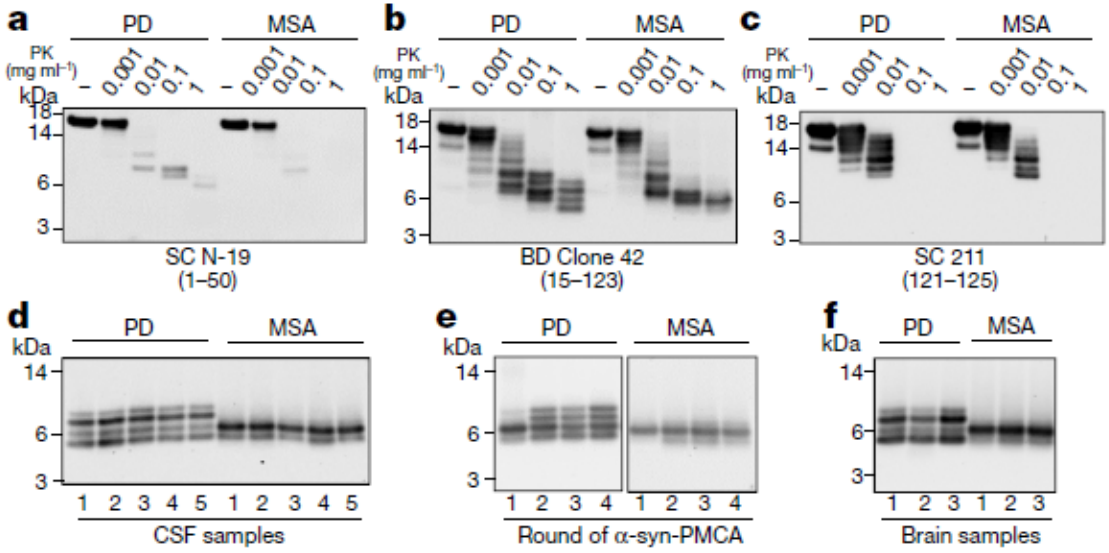


Fig. 2 | Protease resistance and epitope mapping of α-syn aggregates derived from the CSF or the brain of patients with PD or patients with MSA.

CSF from 43 patients with PD and 43 with MSA-C and MSA-P

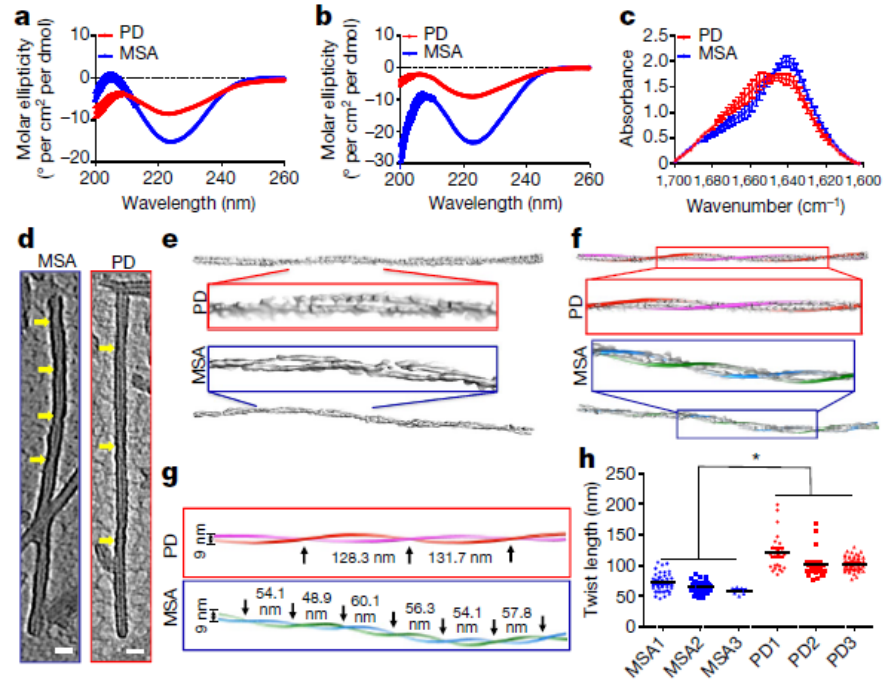


Fig. 3 | Structural differences between α-syn aggregates derived from patients with PD or patients with MSA. a, Circular dichroism spectra of α-syn aggregates from the CSF of patients with PD (red) or patients with MSA (blue),

RT-QuIC in α-Synucleinopathies

Optimal RT-QuIC Experimental Conditions

Molecular Diagnosis of α-synucleinopathy
During Clinical Disease or
During a prodromal stage

Diagnostic Tissues
CSF and Body Fluids
Tissue biopsy
Olfactory Mucosa

Distinguishing α-Synucleinopathies

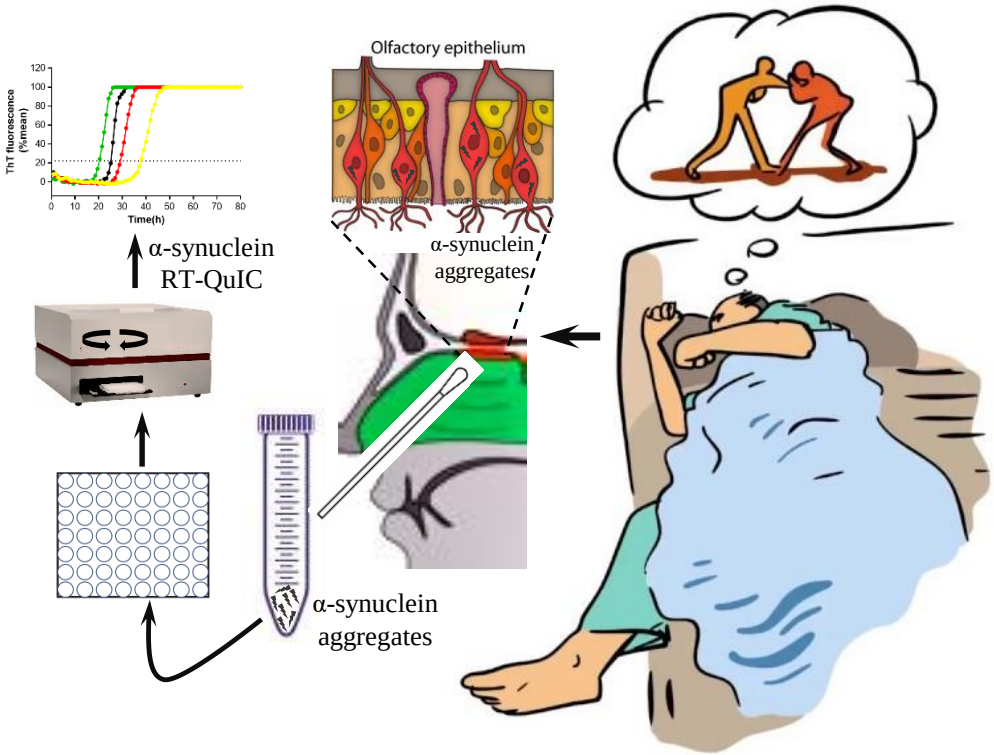
Is it possible Pre-clinical Diagnosis?

doi:10.1093/brain/awab005 BRAIN 2021: 144; 1118–1126 | 1118

BRAIN
A JOURNAL OF NEUROLOGY

Alpha-synuclein seeds in olfactory mucosa of patients with isolated REM sleep behaviour disorder

Ambra Stefani,[1] Alex Iranzo,[2] Evi Holzknecht,[1] Daniela Perra,[3] Matilde Bongianni,[3] Carles Gaig,[2] Beatrice Heim,[1] Monica Serradell,[2] Luca Sacchetto,[4] Alicia Garrido,[2] Stefano Capaldi,[5] Almudena Sánchez-Gómez,[2] Maria Paola Cecchini,[6] Sara Mariotto,[3] Sergio Ferrari,[3] Michele Fiorini,[3] Joachim Schmutzhard,[7] Pietro Cocchiara,[3] Isabel Vilaseca,[8] Lorenzo Brozzetti,[3] Salvatore Monaco,[3] M. Jose Marti,[2] Klaus Seppi,[1] Eduardo Tolosa,[2] Joan Santamaria,[2] Birgit Högl,[1] Werner Poewe[1,2] and Gianluigi Zanusso[3] for the SINBAR (Sleep Innsbruck Barcelona) group



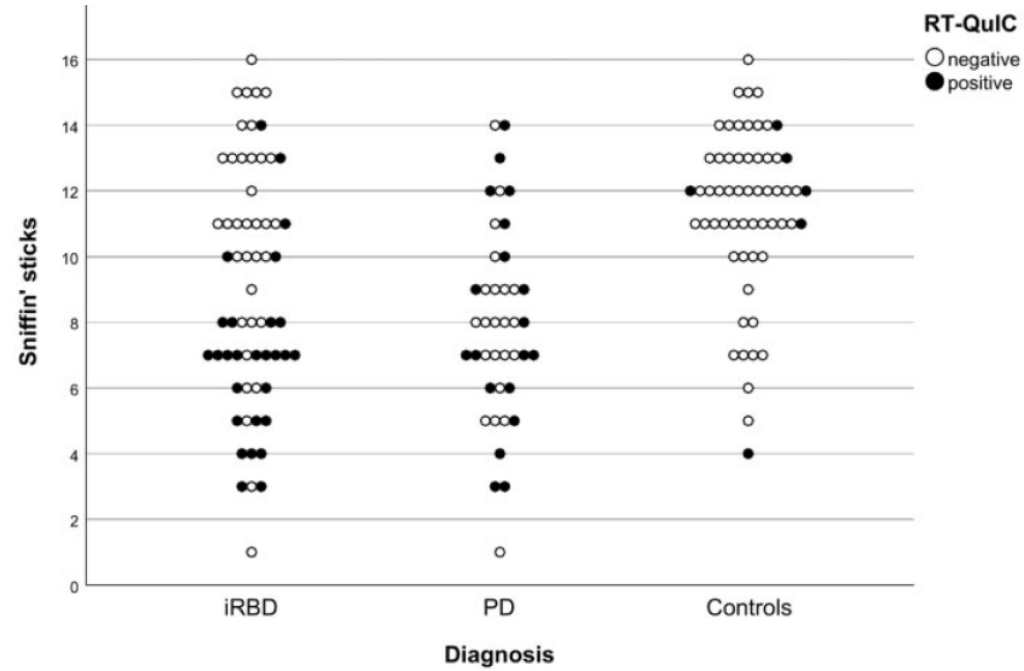


Table 2 Demographic and clinical characteristics of the isolated RBD group (n = 63) and of the Parkinson's disease group (n = 41)

	Isolated RBD			Parkinson's disease		
	α-syn-positive n = 28 (44.4%)	α-syn-negative n = 35 (55.6%)	P-value	α-syn-positive n = 19 (46.3%)	α-syn-negative n = 22 (53.7%)	P-value
Age, years	72 (67–74.8)	67 (60–74)	0.059	70 (65–75)	71 (63.8–76.3)	0.704
Age at diagnosis, years	67 (63–68.8)	60 (56–67)	**0.004**	64 (57–68)	64 (58–71)	0.708
Age at onset, years	62 (59–67.5)	53 (45–61)	**0.011**	64 (56–68)	63 (57–69)	0.936
Disease duration, years	4.5 (1.3–8)	6 (2–10)	0.176	7 (1–12)	5 (2–9)	0.934
Sex, n (%)			0.170			0.703
Female	6 (21.4)	3 (8.6)		3 (15.8)	5 (22.7)	
Male	22 (78.6)	32 (91.4)		16 (84.2)	17 (77.3)	
MDS-UPDRS III score	4 (1–7)	4 (2–7)	0.692	21 (13–30)	21 (12–28)	0.937
SCOPA-AUT score	18 (11–29)	13 (8–21)	0.133	15 (10–20)	19 (8–26)	0.432
MoCA score	27 (24–28)	27 (24–28)	0.737	27 (23–28)	27 (24–28)	0.926
Sniffin' Sticks score	7 (5–8)	11 (8–13)	**<0.001**	7 (6–11)	8 (7–9)	0.968
Olfactory dysfunction, n (%)	22 (78.6)	8 (22.9)	**<0.001**	11 (57.9)	8 (36.4)	0.757
Hoehn and Yahr stage	n.a.	n.a.	n.a.	2 (2–2)	2 (2–2)	n.a.
Rigidity, n (%)	n.a.	n.a.	n.a.	13 (100)	21 (95.5)	1.000
Rest tremor, n (%)	n.a.	n.a.	n.a.	7 (53.8)	12 (54.5)	0.536

Data are shown as median (IQR) or n (%). iRBD = isolated RBD; MDS-UPDRS III = Movement Disorders Society Unified Parkinson's Disease Rating Scale, part III; MoCA = Montreal Cognitive Assessment; n.a. = not applicable/available; SCOPA-AUT = Scales for Outcomes in Parkinson's Disease-Autonomic Dysfunction. P-values were calculated using the Mann-Whitney U-test.

HIV-1 detection in the olfactory mucosa of HIV-1-infected participants

Luca Bertero[a,*], Sarah Beth Joseph[b,*], Mattia Trunfio[c], Tiziano Allice[d], Sebastiano Catera[e], Daniele Imperiale[f], Paola Cassoni[a], Laura Pesci Kincer[b], Veronica Pirriatore[c], Valeria Ghisetti[d], Enrica Amasio[e], Gianluigi Zanusso[g], Stefano Bonora[c], Giovanni Di Perri[c] and Andrea Calcagno[c]

AIDS 2019, **33**:665–674

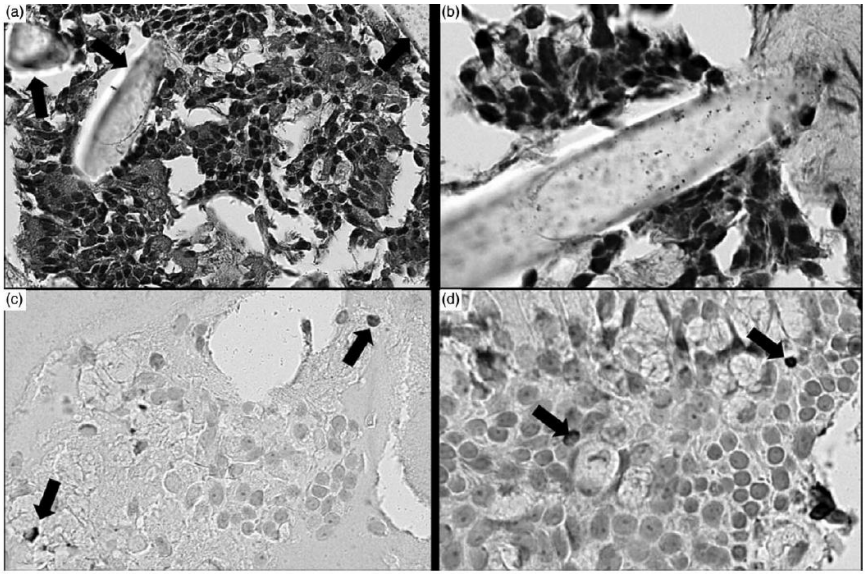


Fig. 1. Immunohistochemistry of the olfactory mucosa sample from subject #12. (a) Low power H&E image showing olfactory mucosa cells intermixed with brush bristles (arrows). (b) Anti-OMP immunohistochemistry showing a strong (+++) and diffuse staining. (c and d) Anti-$CD4^+$ and anti-$CD8^+$ immunohistochemistry, respectively, showing few positive cells (arrows) mixed with olfactory mucosa cells. H&E, hemotoxylin and eosin; OMP, olfactory marker protein.

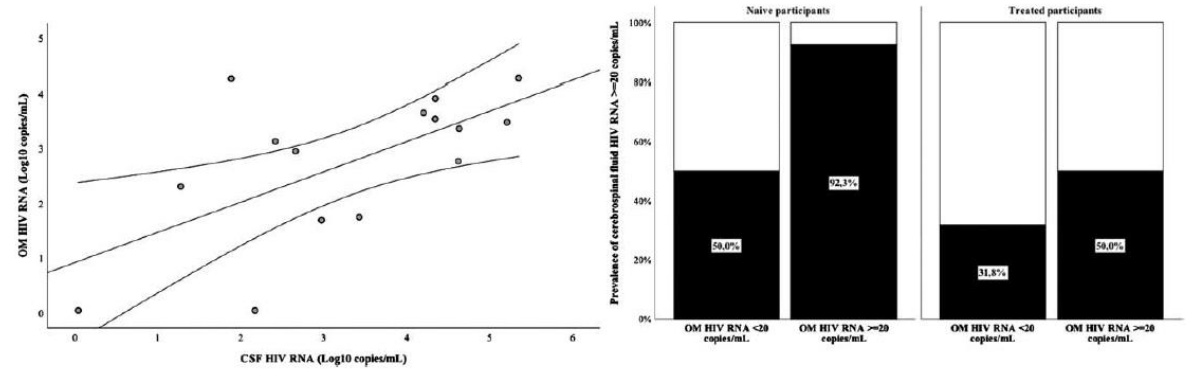
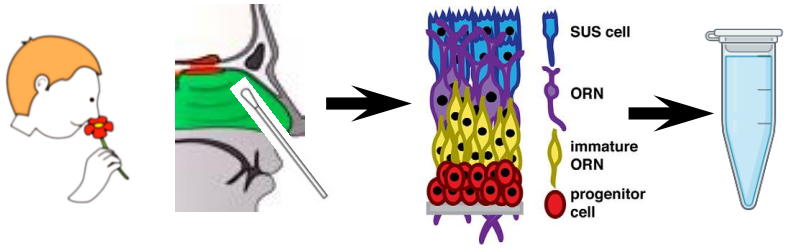


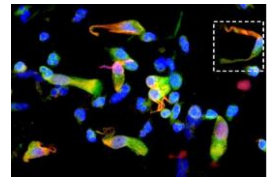
Fig. 2. HIV-RNA in the olfactory mucosa and in the cerebrospinal fluid of HIV-positive participants. The scatter-dot graph (left) represents the linear direct correlation between OM and CSF HIV-RNA values in naïve patients (Spearman's rho = 0.534, $P = 0.040$). The stacked bar plot (right) represents the prevalence of CSF HIV-RNA above 20 copies/ml according to the detection of OM HIV-RNA above 20 copies/ml (stratified as naïve participants on the left and treated ones on the right). CSF, cerebrospinal fluid; OM, olfactory mucosa.

Olfactory Swabbing Application In Neurodegeneration

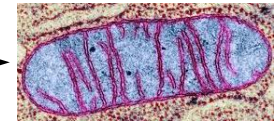
Nasal swabbing

Cell preparation



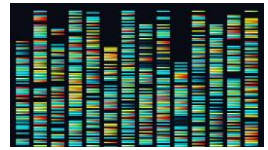


Neurodegeneration Associated Protein Deposits



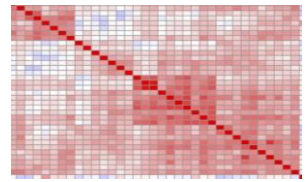
Identification of cell organelles

Proteomics



Genomics

RT-QuIC and Detection of Pathological forms



Transcriptomics

- Molecular Diagnosis
- Prognostic information
- Therapy

- Early pathological changes
- Intracellular processing of pathological changes of disease associated normal proteins


Premio Aspen Roma 23 Ottobre 2019
Roma, 23 ottobre 2019

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