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World Federation for Hospital Sterilisation Sciences

Alpha-Synuclein Seeds of Parkinson´s Disease: Transmissible Biological Agents with Prion-Exceeding Resistance to Steam Sterilization

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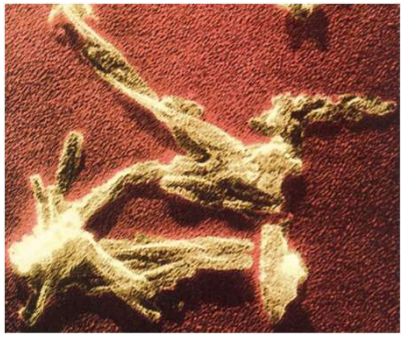
An Unconventional Biological Principle of Infection

Prions - Proteinaceous infectious particles

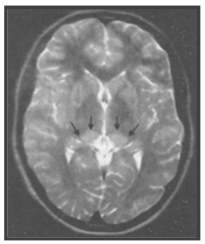
- Unconventional pathogens that fundamentally differ from bacteria, viruses, fungi, parasites or viroids.
- Devoid of nucleic acid genome.
- Cause and transmit fatal neurodegenerative brain diseases: Transmissible spongiform encephalopathies (TSEs), or prion diseases.



Prion rods

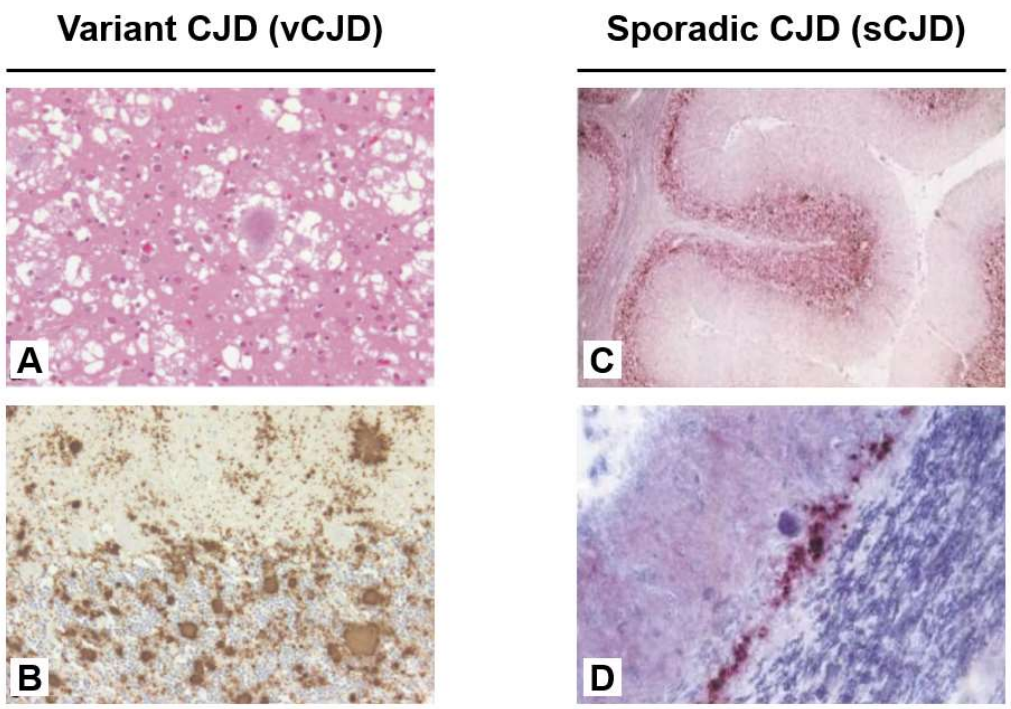
Prion Diseases of Animals and Humans

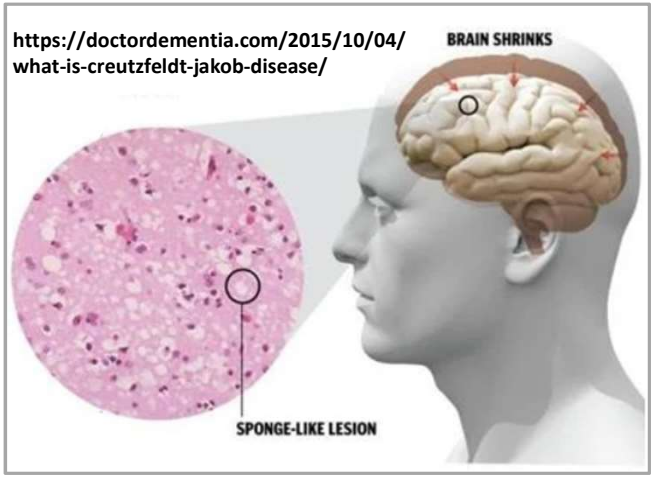
- **Scrapie**
  Sheep and goat
- **Bovine Spongiform Encephalopathy (BSE)**
  Cattle
- **Chronic Wasting Disease (CWD)**
  Deer and elk
- **Creutzfeldt-Jakob Disease (CJD)**
  Human
- **Variant Creutzfeldt-Jakob Disease (vCJD)**
  Human



Pulvinar sign in vCJD
(MRI)

Cerebral Vacuolation and Deposition of Pathological Prion Protein (PrP) in Patients with Disease



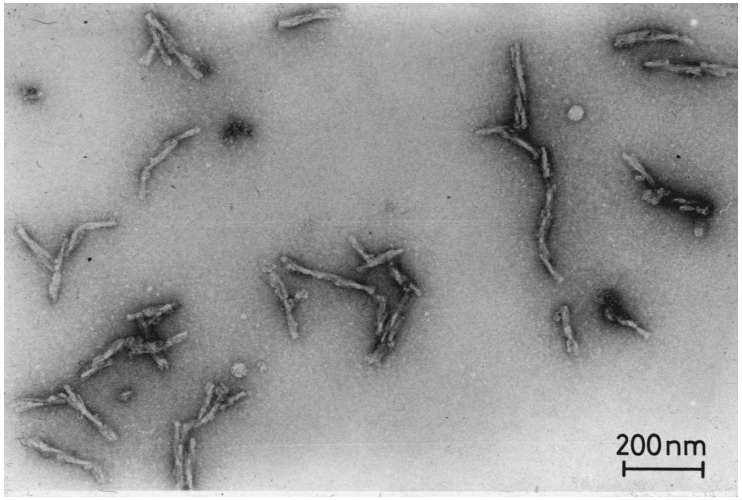


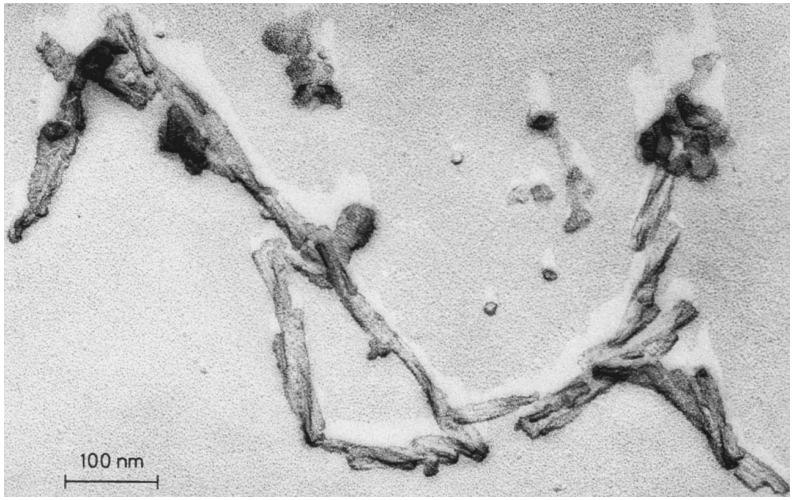
Pathological prion protein deposits in the brain of patients with vCJD (A, B) or sporadic CJD of type MM1 (C) or VV2 (D). A: haematoxylin-eosin staining; B-D: anti-PrP immunostaining.

A, B: Ironside et al., 2002, APMIS, 110: 79-87; C, D: Kretzschmar & Parchi, 2006. In: Prions in Animals and Humans: 287-314

Fibrillar Ultrastructure of TSE-Associated PrP Deposits

Scrapie-associated fibrils / Prion rods

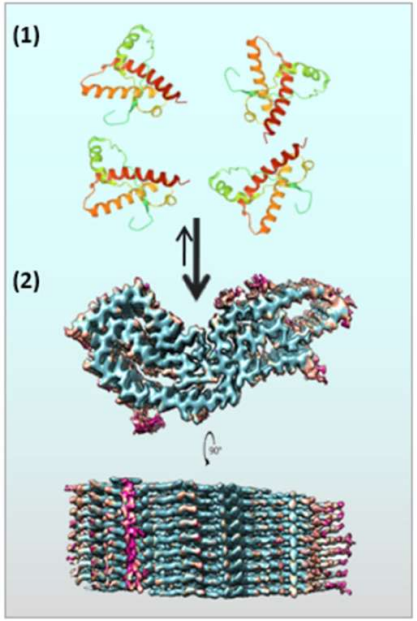




Electron micrographs: M. Özel & H. Diringer, RKI

The Prion Concept of TSEs

- TSE-associated pathological prion protein species (PrP^{TSE}) are formed by misfolding and aggregation of host-encoded, cellular prion protein (PrP^{C}).
- The abnormal three-dimensional structure of PrP^{TSE} confers infectious properties, thereby creating self-propagating proteinaceous infectious particles, or prions.



(1) Baral et al. 2015, J Struct Biol 192:37-47;
(2) Kraus et al. 2021, bioRxiv,https://doi.org/10.1101/2021.02.14.431014

TSEs and Parkinson's Disease (PD): Cerebral Proteopathies with Fundamental Communalities

Cerebral Proteopathies

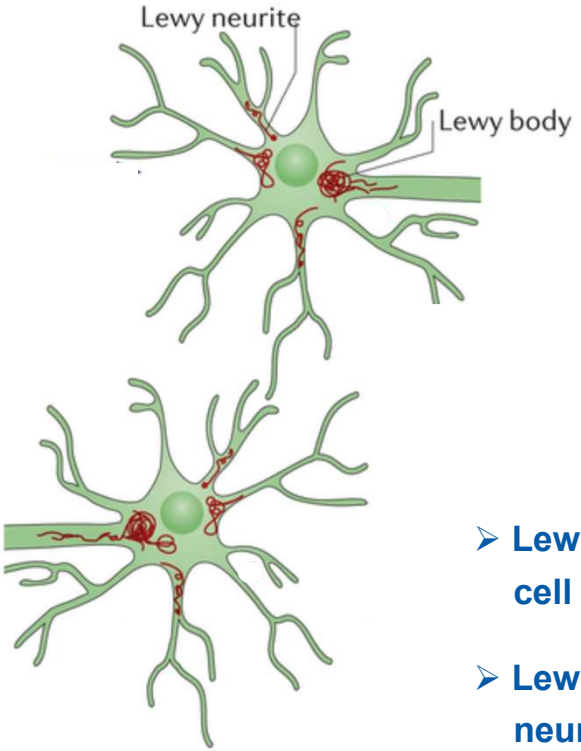
Neurodegenerative diseases characterized by disturbances of protein processing and degradation, and aggregation of misfolded protein particles in nerve cells and glia.

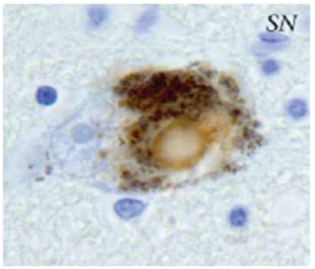
Jellinger, 2005,
J Neurol Neurochir Psychiatr, 6: 9-18

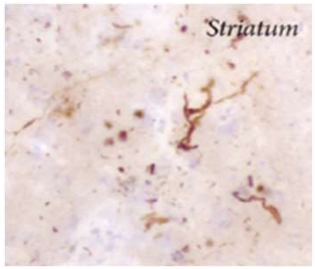
Disease	Major Protein
Alzheimer's Disease	β-peptide (Aβ) 4R, 3R tau
Cerebral Aβ Angiopathy	β-peptide (Aβ)
Multiple System Tauopathy (familial)	4 R tau
Progressive Supranuclear Palsy	4 R tau
Corticobasal Degeneration	4 R tau
Pick's Disease	3 R tau
Diffuse Lewy Body Disease	α-synuclein
Parkinson's Disease	α-synuclein
Multiple System Atrophy	α-synuclein
Amyotrophic Lateral Sclerosis (ALS)	α-synuclein
Familial ALS	SOD1 mutants
Triplet Repeat Disorders (HD, etc.)	polyglutamine inserts
Prion diseases (CJD, etc.)	prion protein
Familial British Dementia	ABri
Familial Danish Dementia	ADan
Familial Encephalopathy w/ Neuroserpin Inclusion Bodies (FENIB)	neuroserpin
Familial Cerebral Hemorrhage w/ Amyloidosis (Icelandic)	cystatin C
Familial Amyloidotic Neuropathy	transthyretin

Walker & Levine, 2000, Mol Neurobiol, 21: 83-95

Cerebral Deposition of Pathologically Aggregated α-Synuclein (αSyn) in PD: Lewy Bodies and Lewy Neurites



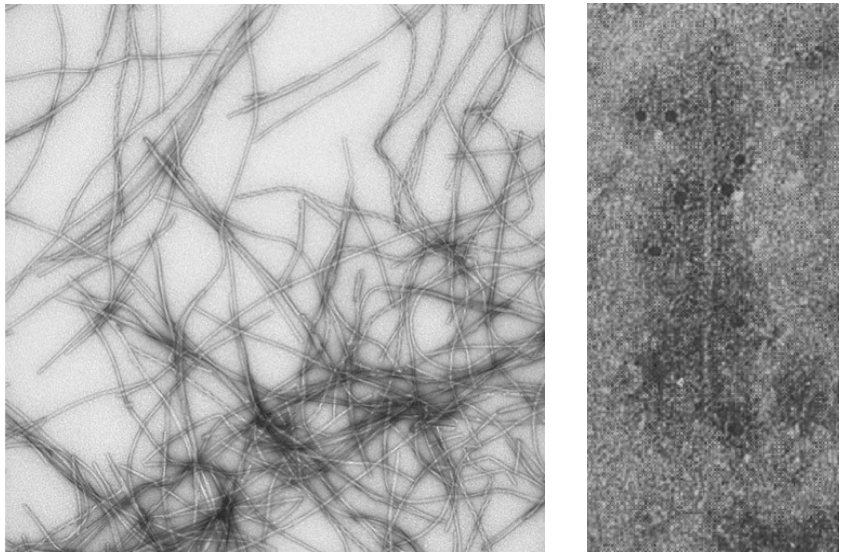




- **Lewy bodies: Spherical inclusions in the cell body of neurons (e. g. in the SN)**
- **Lewy neurites: Abnormal neurites in diseased neurons, containing granular material**

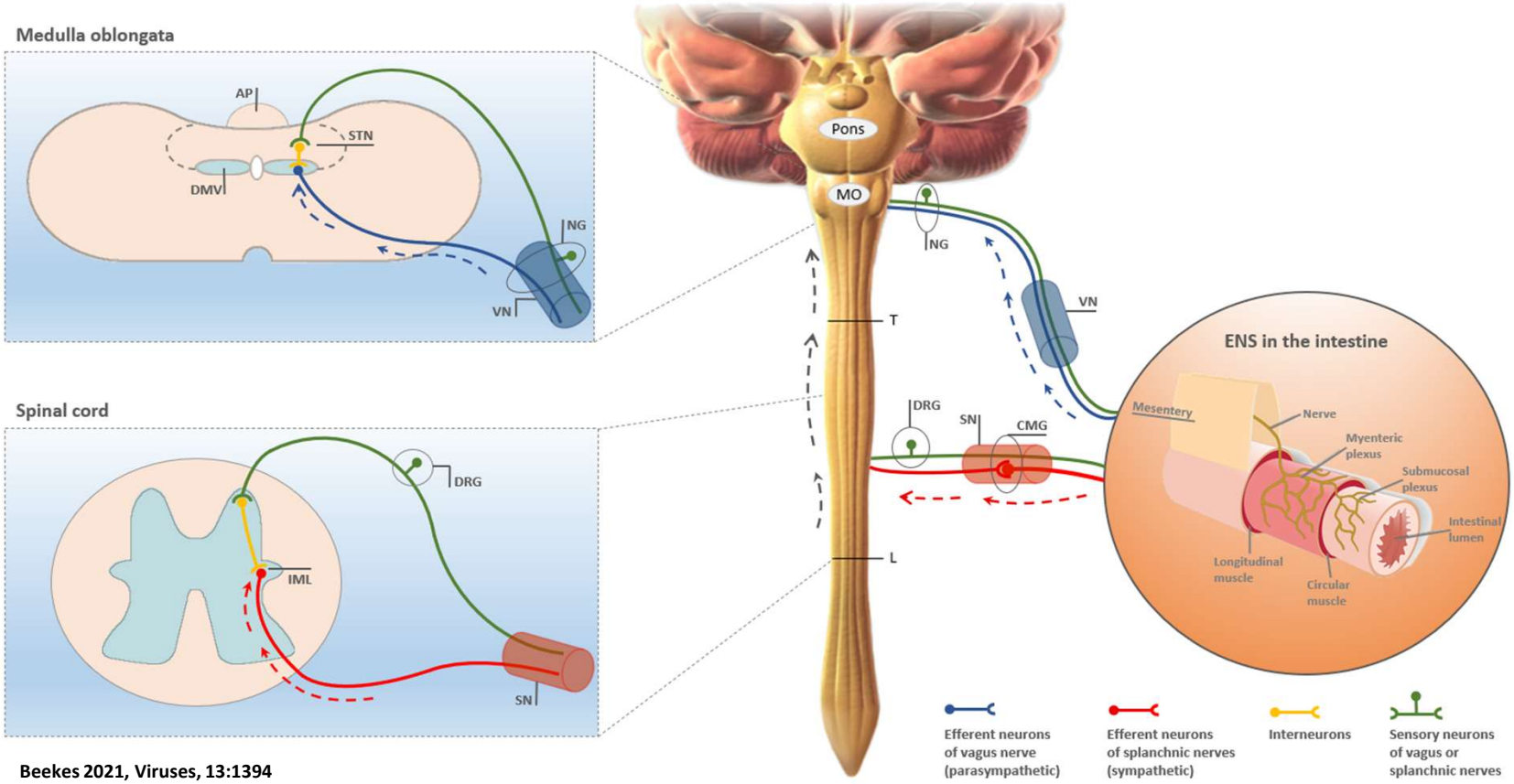
Fibrillar Ultrastructure of PD-Associated αSyn Deposits

Fiber-like morphology of pathological protein deposits in PD formed by misfolding and aggregation of host-encoded αSyn



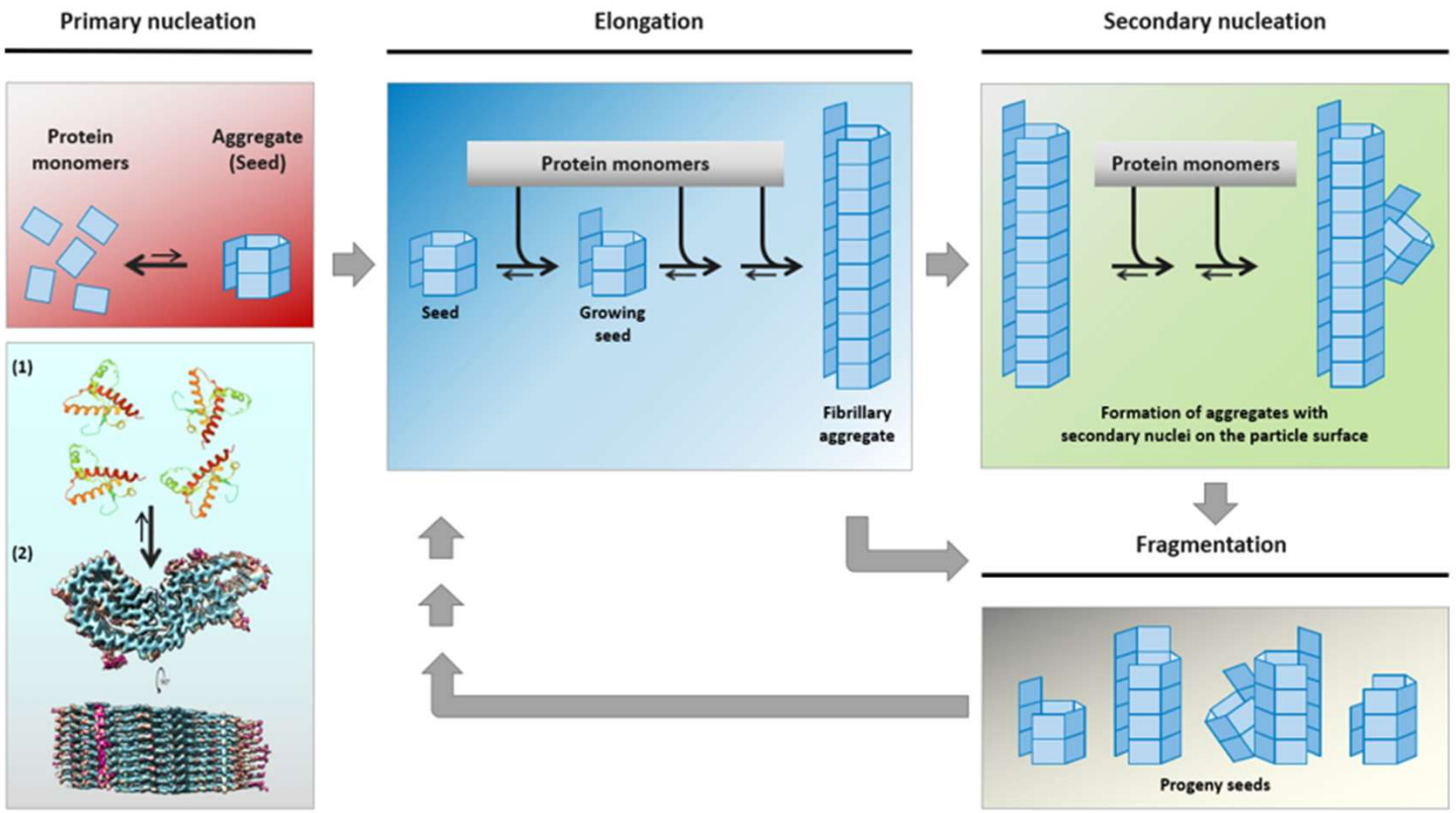
Similar Proposed Pathways of Proteopathic Spread

from the GI-Tract to the CNS in PD and



Beekes 2021, Viruses, 13:1394

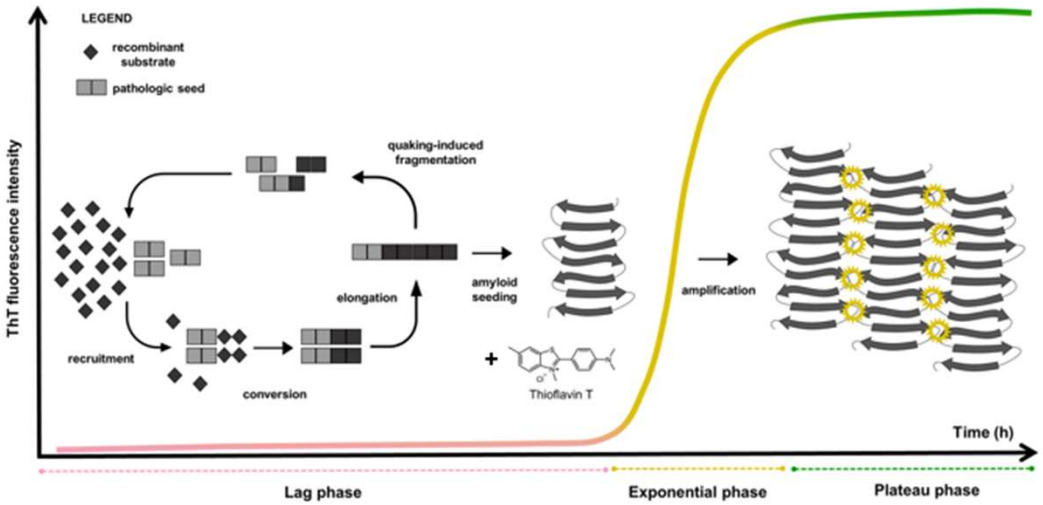
Similar Mechanism of Nucleation-Dependent Protein Aggregation in Prion Diseases and PD



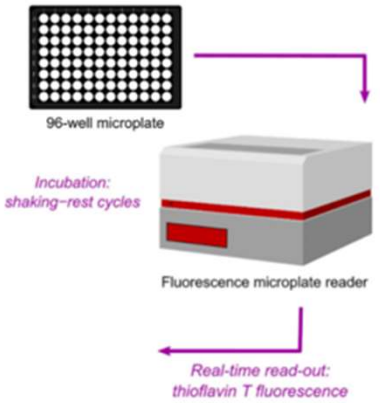
Beekes 2021, Viruses, 13:1394
(1) Baral et al. 2015, J Struct Biol 192:37-47; (2) Kraus et al. 2021, bioRxiv,https://doi.org/ 10.1101/2021.02.14.431014

In vitro Detection and Quantification of PrP- or αSyn-Seeding Activity by Real-Time Quaking-Induced Conversion (RT-QuIC)

Concept of RT-QuIC



Technical Implementation



Reproduced with modifications from:
Candelise et al., 2020, Acta Neuropathol Commun, 8: 117
Ascari et al., 2020, Front Bioeng Biotechnol, 8: 585896

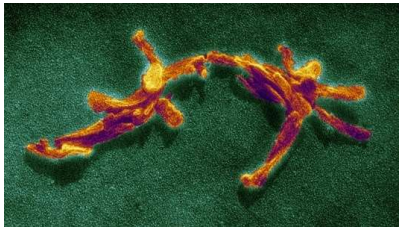
"Prion-like" Phenomena in Parkinson´s Disease

- PD and (peroral) TSEs show striking similarities with regard to the
  - formation
  - replication
  - and neuroanatomical spread

  of their disease-associated αSyn or PrP seeds, respectively.

- The PrP seeds (prions) of TSEs are infectious and able to transmit disease.

- To what extent are transmissible prion diseases exemplary for PD?



Prion rods

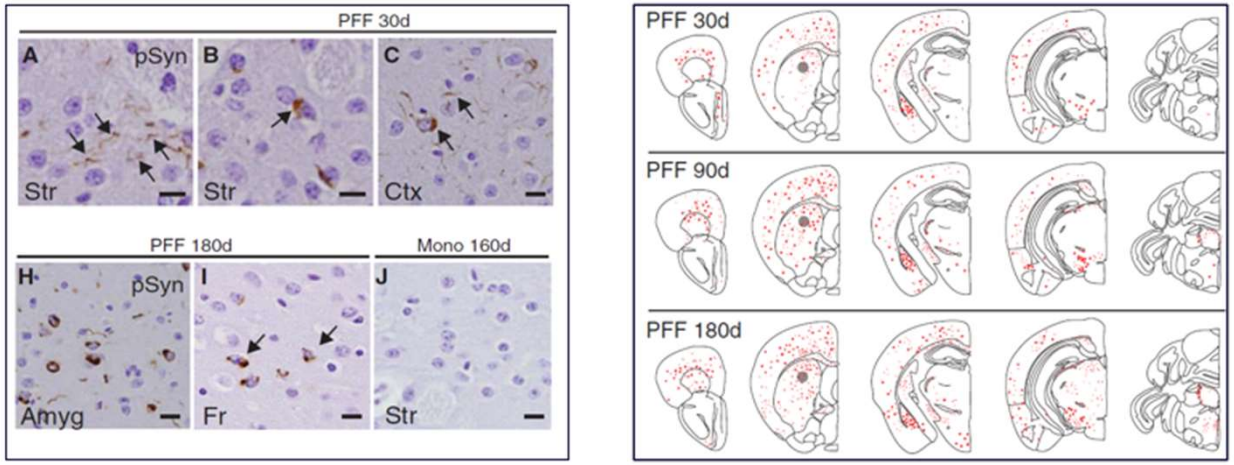
Epidemiological Data on the Transmissibility of PD

- To date, there is no epidemiological evidence that PD is a transmissible disease entity.
- The interpretation of epidemiologic study results, however, on highly prevalent multifactorial diseases with decades of preclinical development is complex.
- If subgroups of PD cases have an infectious cause, this may be difficult to detect epidemiologically.

Transmission Studies with non-PD αSyn Seeds in Mice (I)

- **Synucleopathic Seeding in the Brain of wild-type Mice after Intracerebral Injection of Pre-Formed non-PD αSyn Fibrils**

Cerebral Deposition of pathologically aggregated αSyn in recipient mice



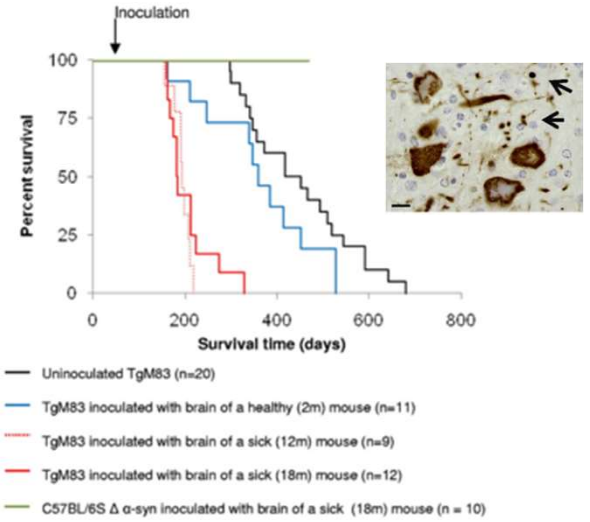
Luk et al., 2012, Science, 338: 949-953.

Transmission Studies with non-PD αSyn Seeds in Mice (II)

- **Acceleration of Disease Development by Synucleopathic Inocula in a Transgenic Mouse Model for Familial PD**

Survival times of transgenic TgM83$^{+/+}$ mice expressing human αSyn with the A53T mutation of familial PD after exposure to synucleopathic inocula

Brain extracts from old TgM83$^{+/+}$ mice with clinical motor disease were used as synucleopathic inocula and injected intracerebrally into young TgM83 mice.



Mougenot et al., 2012, Neurobiol Aging, 33, 2225-2228

Initial Transmission Studies with αSyn Seeds from PD Patients in Mice

Study Parameter	Recasens et al., 2014	Prusiner et al., 2015
Animal model	Wt C57BL/6	TgM83$^{+/-}$
Inoculum	Lewy body extracts	Brain tissue homogenates
Mode of inoculation	Injection into substantia nigra or striatum	Injection into right parietal lobe
Incubation time after injection	4 months - 17 months	> 360 days
Cerebral αSyn pathology	Diffuse αSyn accumulations in neuronal cytoplasm, partly phosphorylated at Ser 129 and resistant to Proteinase K	Not detected
Neuronal damage	Progessive nigrostriatal neurodegeneration	Not reported
Motor or other impairments	Impaired motor coordination in pole test	Not detected
	Ann Neurol, 75: 351-362	PNAS, 112: E5308-5317

RKI-Transmission Study with αSyn Seeds from PD Patients in Mice

Acta Neuropathologica
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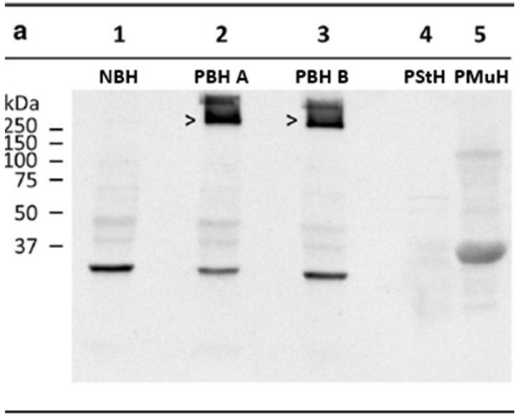
ORIGINAL PAPER

Transmissible α-synuclein seeding activity in brain and stomach of patients with Parkinson's disease

Achim Thomzig[1] · Katja Wagenführ[1,2] · Phillip Pinder[1] · Marion Joncic[1] · Walter J. Schulz-Schaeffer[3] · Michael Beekes[1]

Western blot detection of pathological αSyn aggregates in PD brain homogenates



Titration of αSyn seeding activity in PD brain homogenates by RT-QuIC*

Log_{10} dilution	None			
	NBH_0 Donor 1	NBH_0 Donor 2	PBH_0 Patient A	PBH_0 Patient B
-1	13/129[a]	6/52	10/10	10/10
-2	-	-	-	-
-3	-	-	10/10	10/10
-4	-	-	9/10	10/10
-5	-	-	10/10	10/10
-6	-	-	5/10	8/10
-7	-	-	5/10	2[b]/10
-8	-	-	3[b]/10	1[b]/10
-9	-	-	0/10	0/10

*Pinder et al., 2021, J Hosp Inf, 108: 25-32

- **Animal model:** TgM83$^{+/-}$ mice
- **Inoculum:** Brain tissue (caudate nucleus) homogenate from PD patients A & B
- **Titre:** 10^{10} SD_{50}/g for both PD patients
- **Mode of inoculation:** Intracerebral injection into right hemisphere
- **Incubation time:** Up to 612 dpi

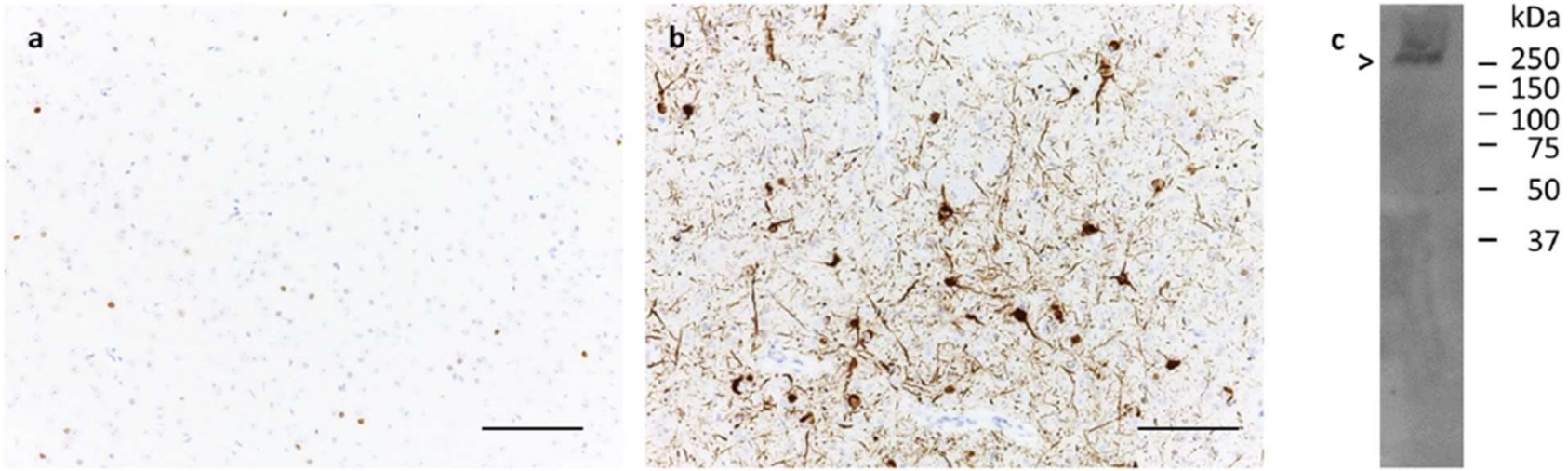
Cerebral αSyn Deposition in TgM83$^{+/-}$ Mice Injected with Brain Homogenate from a WT- or Sick TgM83$^{+/+}$ Mouse

Murine NBH

TgM83$^{+/+}$ BH

Spheroid-like αSyn immunostaining

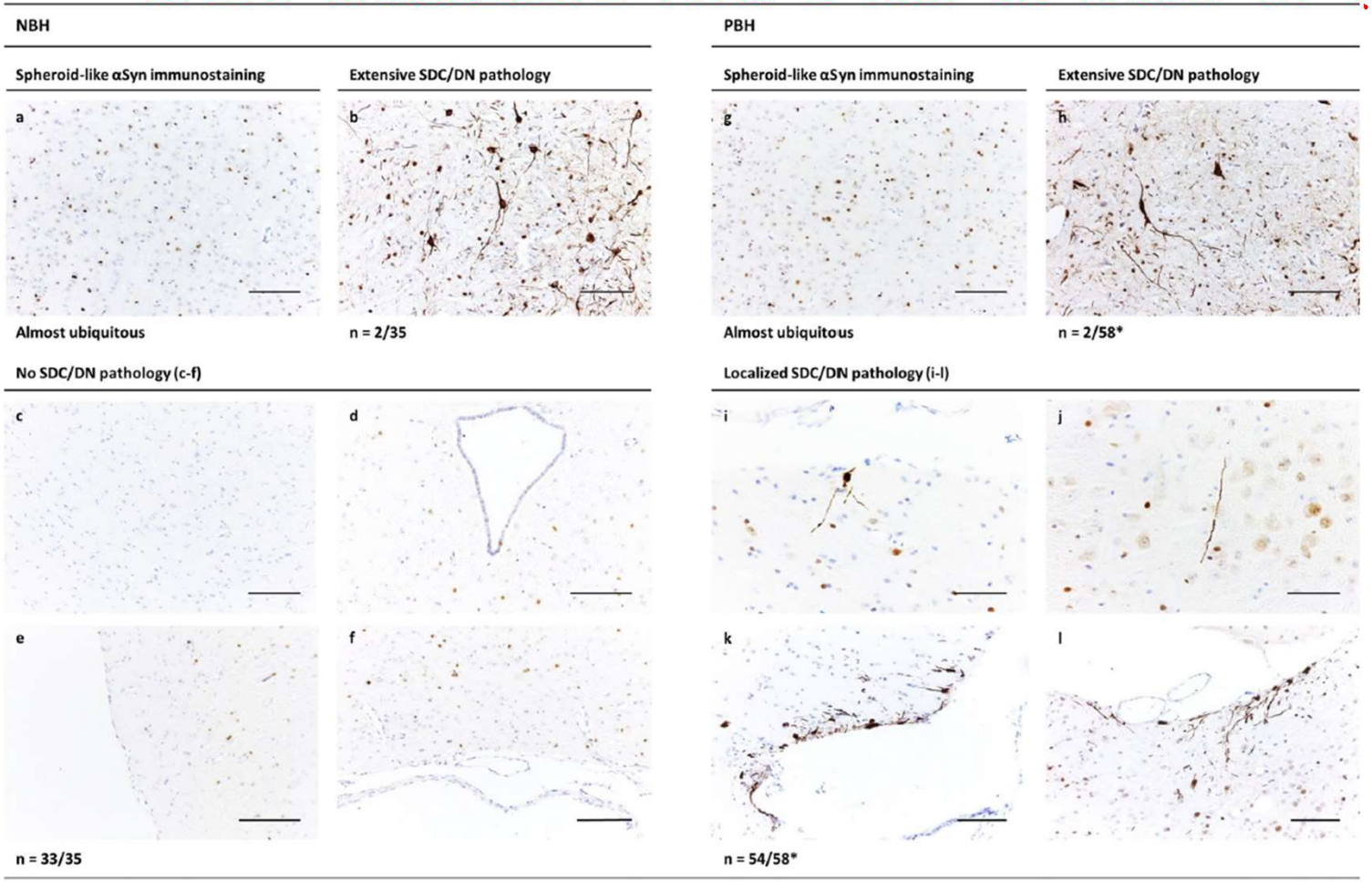
Extensive SDC/DN pathology



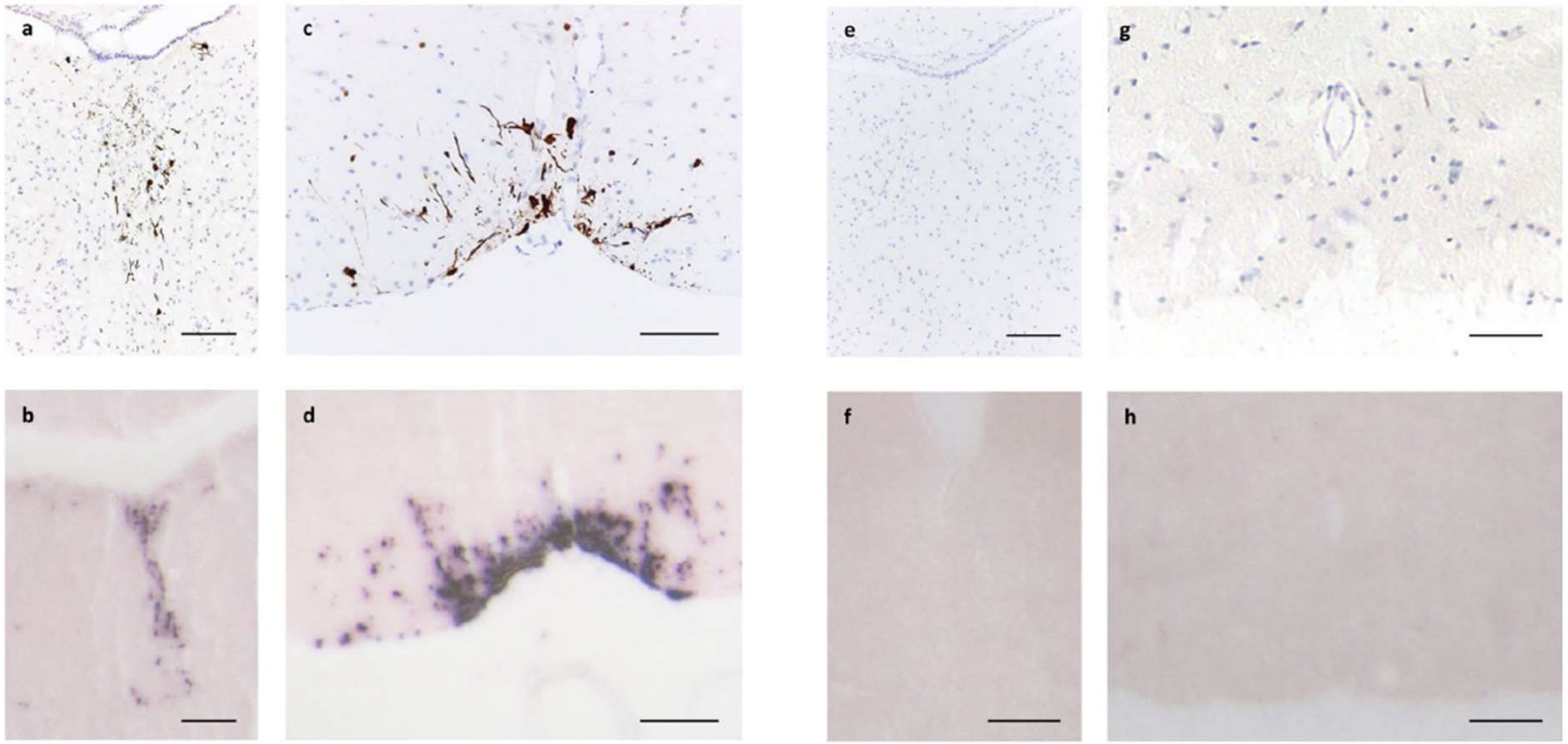
n = 5/5

n = 5/5

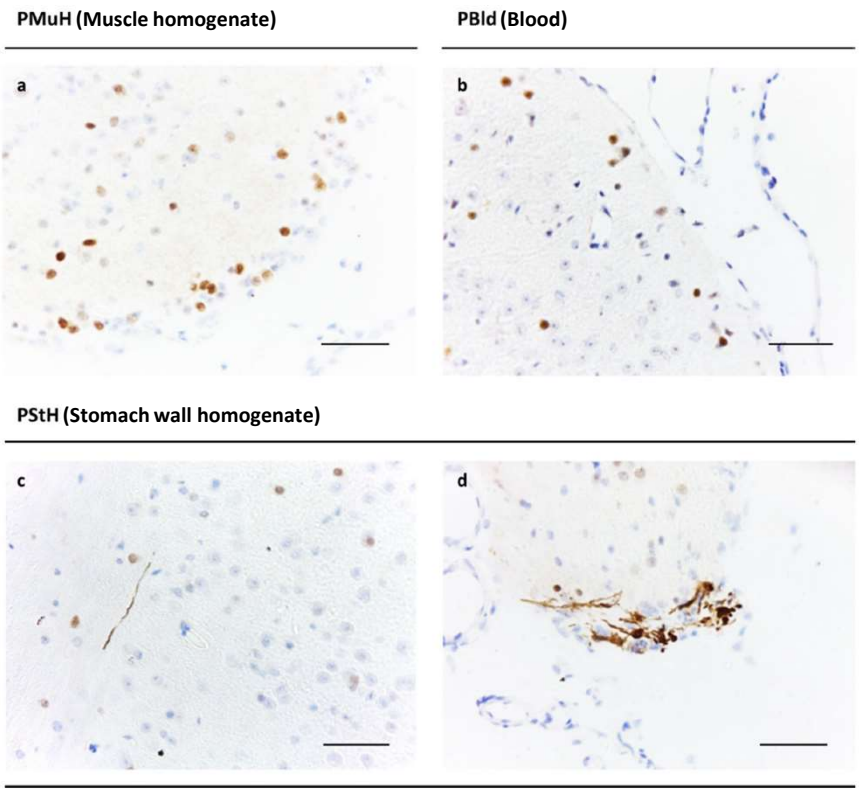
Cerebral αSyn Deposition in TgM83$^{+/-}$ Mice Injected with Brain Homogenate from a non-PD Donor or PD



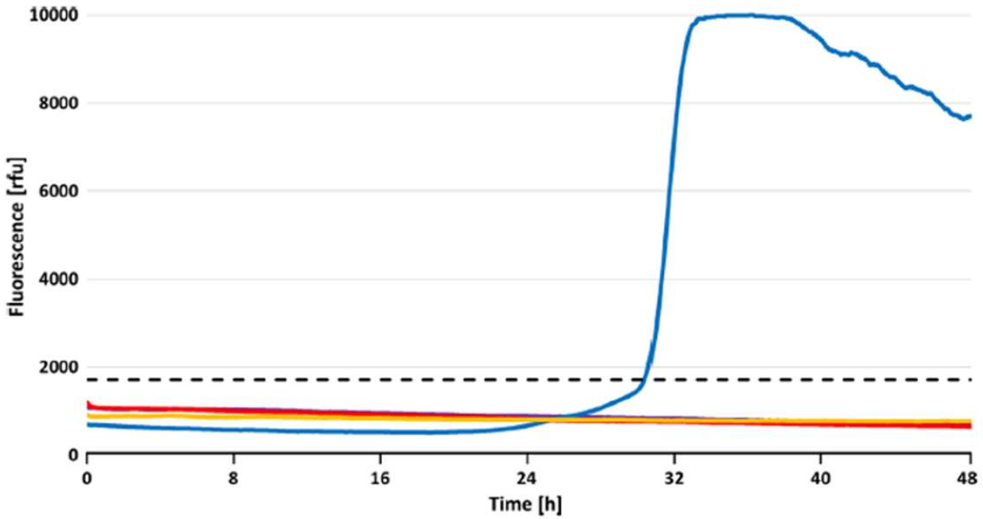
Consistent IHC and PET Blot Detection of Cerebral αSyn Deposits in PD-Challenged TgM83$^{+/-}$ Mice



Analysis of Cerebral αSyn Pathology in TgM83$^{+/-}$ Mice Injected with Peripheral Tissue or Blood of a PD Patient



Detection of αSyn Seeding Activity in Stomach Wall Tissue from a PD Patient by RT-QuIC



Conc. [%]	PStH		PStH (65 °C)	NStH (65 °C)
	+NBH	+PBH	+NBH	+NBH
10	0/10	0/10	0/10	0/5
1	10/10	10/10	10/10	0/10

Colour code of samples: Violet, 10% (w/v) PStH + 10% (w/v) NBH; red, 10% (w/v) NStH + 10% (w/v) NBH; blue, 1% (w/v) PStH + 10% (w/v) NBH; yellow, 1% (w/v) NStH + 10% (w/v) NBH.

Statistical Analysis of Localized SDC/DN Pathology Detection in the Brain of TgM83$^{+/-}$ Test Mice

Sex of mice	Inoculum	*N*	Incubation period		Age		Localized SDC/ DN pathology[a]	*P*
			Median	Mean	Median	Mean		
Male	NBH	13	572	552	621	601	0/13	n. a
	PBH A	15	580	582	627	630	15/15	<0.00001*
	PBH B	11	571	546	623	598	10/11	<0.00001*
	PStH A	7	546	542	599	595	3/7	0.0307*
Female	NBH	20	570	563	621	614	0/20	n. a
	PBH A	15	572	545	620	592	15/15	<0.00001*
	PBH B	15	570	560	620	612	14/15	<0.00001*
	PStH A	7	546	541	599	595	4/7	0.002*
Male and female	NBH	33	570	559	621	608	0/33	n. a
	PBH A	30	572	563	622	611	30/30	<0.00001*
	PBH B	26	570	554	620	606	24/26	<0.00001*
	PStH A	14	546	542	599	595	7/14	0.0001*

Clinical Examination and Readout of TgM83$^{+/-}$ Test Mice

- Daily health assessment based on a clinical score sheet.
- Up to 612 dpi no evidence for higher incidence or faster onset of neurological or other health impairments in TgM83$^{+/-}$ test mice than in negative control animals.

Clinical sign	*Qualifier*	Score	*year = 20….* Date						
Appearance:									
Body weight loss (compared to initial weight)	Up to 10% 10 to 20% 20 to 35%	1 2 3							
Coat condition	Slightly unkempt Lack of grooming Marked/prolonged piloerection	1 2 3							
Body function:									
Bladder incontinence	Evidence of some loss of control (small amount of urine in nest) More pronounced "leaking" of urine Incontinence	1 2 3							
Bladder retention	Palpated but will empty on handling More effort required to empty bladder Unable to urinate without assistance; signs of discomfort/distress during or after manual emptying	1 2 3							
Tail tone	Diminished lifting or curling of tail Loss of tone in distal half of tail Loss of tone in entire tail	1 2 3							
Respiration (rapid, slow or deep breathing)	Slight alteration Moderate alteration Marked alteration	1 2 3							
Interaction with environment:									
Nest condition	Slightly disorganised Some attempt at nest building but disorganised No nest	1 2 3							
Social behaviour	Reduced interaction with other animals Significantly reduced interaction, passive	2 3							
Position and movement:									
Side resting position	Present	3							
Righting time	Slow to right when placed on back Marked difficulty in righting Inability to right within 5 seconds	1 2 3							
Paresis	Slow forelimb abduction when placed on back Reduced forelimb abduction No forelimb abduction	1 2 3							
Gait	Clumsy Dragging one hind limb Dragging two hind limbs	1 2 3							
Paralysis	Present	3							
Other observations: (scores to be established)	…… ……								
Total daily score:									
Actions taken:	Who was consulted, what has been done								

Exemplary score sheet template

Fentener van Vlissingen et al., 2015, Lab Animals 49: 267-283

Conclusions from TgM83$^{+/-}$ Transmission Study at RKI

- Our study in TgM83$^{+/-}$ mice substantiated that experimentally transmitted αSyn seeds of PD, including those from the stomach wall, are able to propagate in new mammalian hosts.
- The detected stimulation of αSyn pathology was not accompanied by apparent neurological symptoms or other overt health impairments.
- The consequences of the observed αSyn seed propagation and potential safeguards need to be further investigated.

Conceivable risk scenario

Transfer of seeding-active αSyn particles of PD could accelerate genetically predisposed forms of synucleopathies or confer health impairments below the threshold of overtly visible symtoms.

Reduction of PD-Associated αSyn Seeding Activity by Steam Sterilization at 134°C as Determined by RT-QuIC

Parameter [Units]	Steam sterilization at 134°C					
	None		5 min		90 min	
	PBH_0 'A'	PBH_0 'B'	PBH_5 'A'	PBH_5 'B'	PBH_{90} 'A'	PBH_{90} 'B'
Titre of seeding activity [SD_{50}/g]	$10^{10.1}$	$10^{10.0}$	$10^{7.7}$	$10^{7.9}$	$10^{7.8}$	$10^{7.8}$
Reduction factor of seeding activity [$logs_{10}$]	-	-	2.4	2.1	2.3	2.2

Pinder et al., 2021, J Hosp Inf, 108: 25-32

Prion-Exceeding Resistance of PD-Associated αSyn Seeds to Steam Sterilization at 134°C

- Decontamination studies testing several disinfectants or physical processes on 263K scrapie and vCJD brain- or BSE spinal cord homogenates revealed a strong correlation between seeding activity (PMCA) and animal bioassay results.

  [Pritzkow et al., 2011, PLoS One, 6:e20384; Yoshioka et al., 2013, Vet Res, 9:134; Belondrade et al., 2016, PLoS One, 11:e0146833; Belondrade et al., 2020, mSpehre, 5:e00649-19]

- European and French Medicine agencies encourage or request the use of both human prions and highly sensitive cell-free prion amplification assays to quantify prion inactivation efficacy.

  [ANSM, 2018; EMA, 2018; Moudjou et al., 2020, Front Bioeng Biotechnol, 8:591024]

Type of seeds	Sample material	Duration of steam sterilization at 134°C [min]	Reduction factors of seeding activity [$logs_{10}$]
PD-associated αSyn particles	Brain Homogenates (BH)	5, 90	2.1 - 2.4
vCJD prions	BH	5, 18	7.5*, 8.2*
	Dried BH on steel wires	20	> 7.0[a]
CJD prions (MM2)	BH	5, 18	8.9*, 9.0*
263K scrapie prions	BH	5, 18	9.5*, 9.7*
	Dried BH on steel wires	5	≈ 5.0
127S scrapie prions	Dried BH on steel wires	20	> 6.0[a]

[a] Detection limit of the assay
* Schwenke et al., 2022, J Hosp Inf (in press; DOI: https://doi.org/10.1016/j.jhin.2022.08.014)
References for other indicated reduction factors provided in: Pinder et al., 2021, J Hosp Inf, 108: 25-32

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Overall Conclusion

- PD-associated αSyn seeds show properties of transmissible replicative agents and prion-exceeding resistance to steam sterilization.
- Health risks possibly emanating from iatrogenic transfers of PD-associated αSyn seeds, as well as the similarities and differences between such seeds and prions need to be further investigated.
- For the time being possible contaminations of seeding-active αSyn aggregates should ideally be thoroughly removed or inactivated when reprocessing medical devices.

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Ms. Safak Bayram
Ms. Annette Dietrich
Ms. Stefanie Redlich

Prof. Dr. Walter Schulz-Schaeffer

An additional "R" to the 3Rs

Remembering the animals
Iliff (2002). ILAR Journal 43: 38-47.

Bundesministerium für Gesundheit

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