Detection of abnormal prion protein in patients with non-prion disease dementia

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CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.

Supporting Families Affected by Prion Disease

The Jeffrey and Mary Smith Family Foundation

MAJOR QUESTIONS OF THE STUDY

Determine whether:

- Abnormal form of prion protein (PrP) accumulates in the brain of patients with Alzheimer's disease (AD) and frontotemporal dementia (FTD)

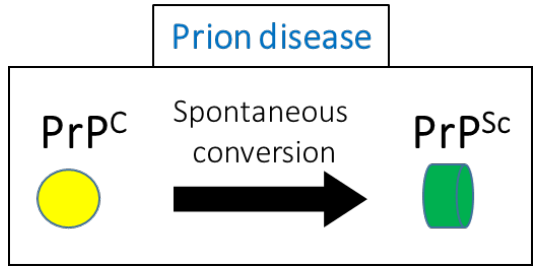
- (1) PrP codon 129 and (2) Apolipoprotein E (ApoE) genotypes as well as (3) age and (4) rate of disease progression affect formation of small PrP aggregates (seeds)

- PrP seeds are present in different brain compartments, or only in specific brain regions (i.e., selective regional vulnerability)

- PrP seeding activity (by RT-QuIC) in AD/FTD resembles that of human sporadic prion diseases

METHODS

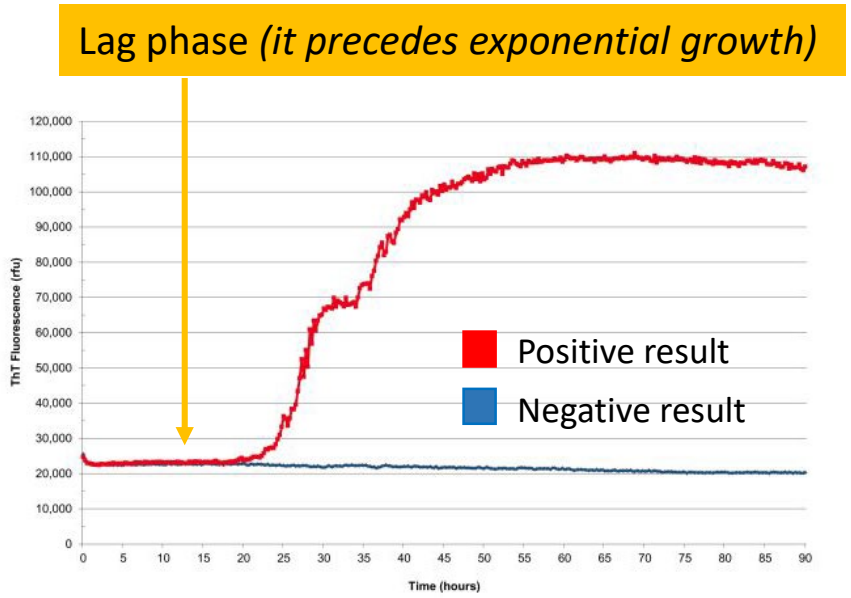
Presence of abnormal PrP (seeds) in BRAIN HOMOGENATES was determined by real-time quaking-induced conversion (RT-QuIC)

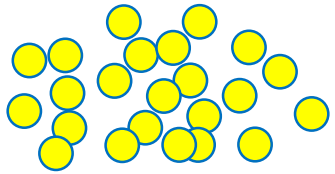


(1) Bacterially-expressed PrP (**substrate**)

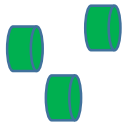
(2) Brain extracts may contain abnormal PrP (**seed**)

(3) Thioflavin T (amyloid stain)





Substrate
(excess)



Seed
(diluted)

Seeded conversion

PrP amyloid fibrils

METHODS

Cases population	N cases	N samples tested	Age at death (years; mean±SD)
DEMENTED			
• Alzheimer's disease (AD)	101	505	80±10
• Frontotemporal dementia (FTD)	9	36	71±11
NON DEMENTED (negative controls)			
• Young subjects	20	57	39±12
• Old subjects	45	150	79±10

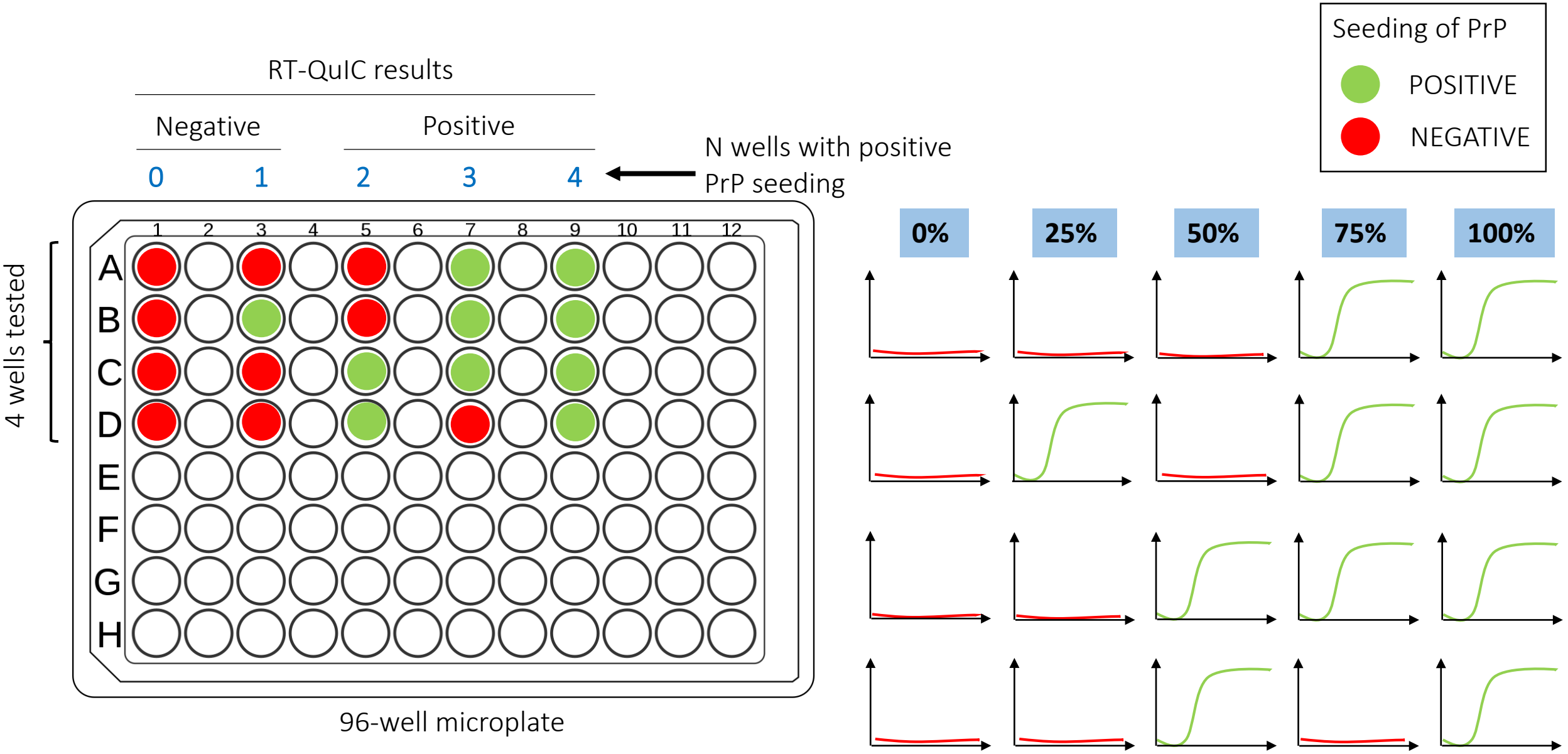
Unknown PrP RT-QuIC outcome

Cases population	N cases	N samples tested	Age at death (years; mean±SD)
DEMENTED			
• Primary age-related tauopathy (PART)	1	27	71
• Metabolic disease (ME)	1	10	68
• Multiple system atrophy (MSA)	1	6	78

Known to give inconsistent PrP RT-QuIC results (NPDPSC)

PRION DISEASE (positive controls)			
• Sporadic Creutzfeldt-Jakob disease (sCJD)	24	35	61±15
• Sporadic fatal insomnia	8	14	48±16
• Variably protease-sensitive prionopathy (VPSPr)	6	6	72±8

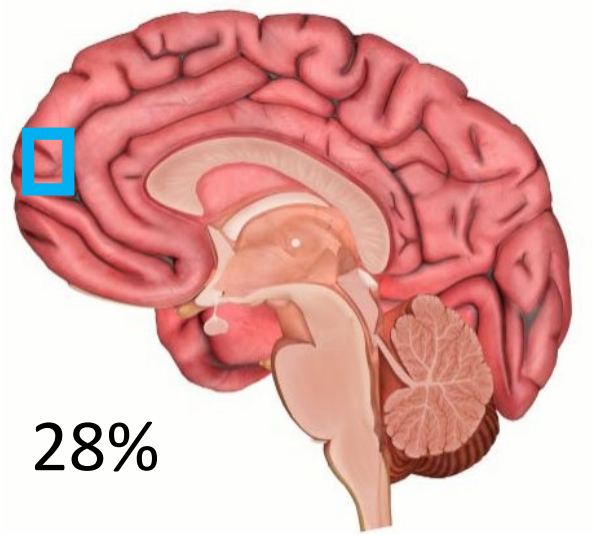
METHODS


RT-QuIC results
Negative
Positive
0
1
2
3
4
N wells with positive PrP seeding
4 wells tested
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
96-well microplate
Seeding of PrP
POSITIVE
NEGATIVE
0%
25%
50%
75%
100%

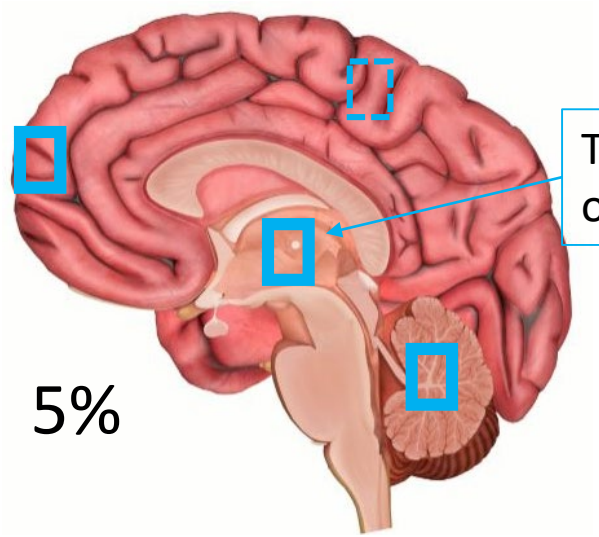
METHODS

Number of brain regions tested

Frontal cortex



28%



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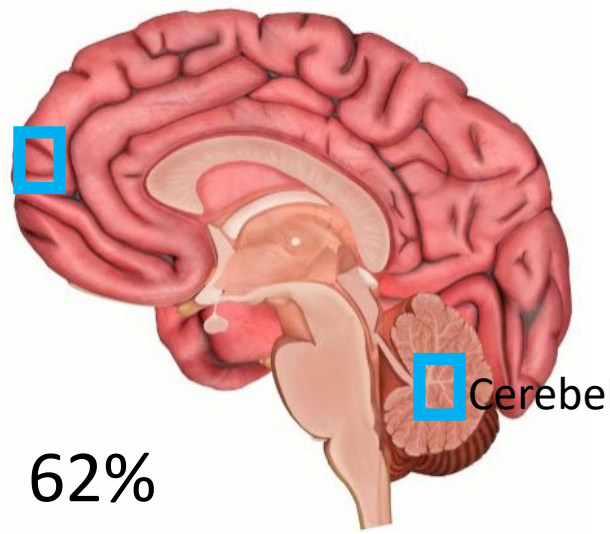
Frontal cortex



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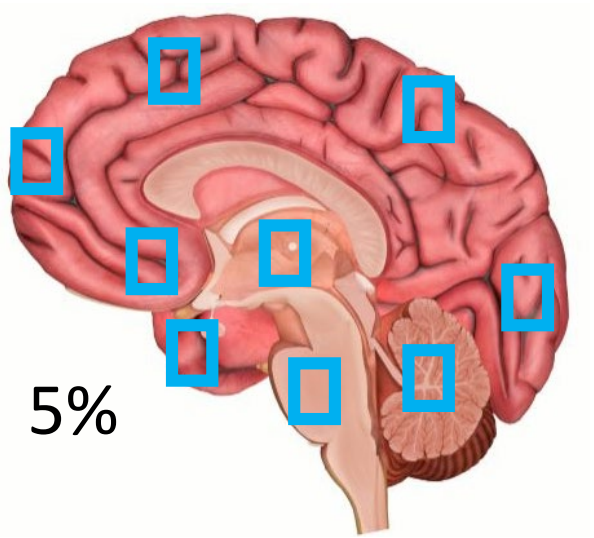


5%

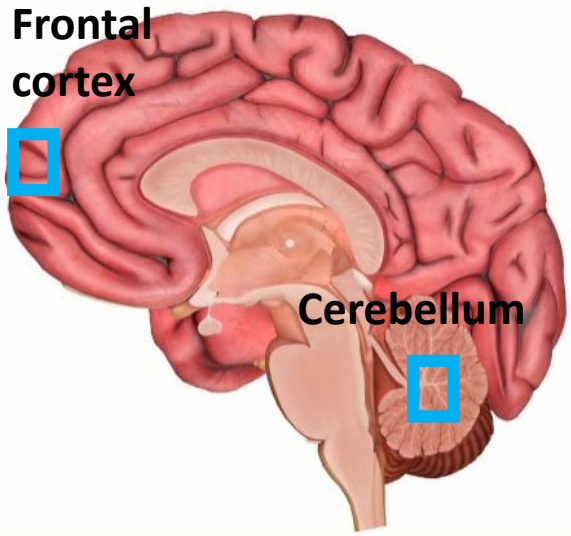
METHODS

	RT-QuIC experiments					
Brain area	1	2	3	4	5	Result
Frontal cx (case 1)	🔴🔴 🔴🔴	🔴🔴 🔴🔴				NEGATIVE
Cerebellum (case 1)	🔴🔴 🔴🔴	🟢🔴 🔴🔴	🟢🔴 🔴🔴			NEGATIVE
Frontal cx (case 2)	🟢🔴 🔴🔴	🔴🔴 🔴🔴	🔴🔴 🔴🟢			NEGATIVE
Cerebellum (case 2)	🔴🔴 🔴🟢	🔴🔴 🔴🔴	🟢🔴 🔴🟢			POSITIVE

Seeding of PrP

🟢 POSITIVE

🔴 NEGATIVE



METHODS

	RT-QuIC experiments					
CASE ID	1	2	3	4	5	Degree positive outcome
3	🟢 🔴 🟢 🔴	🔴 🔴 🔴 🔴	🔴 🟢 🔴 🔴	🔴 🔴 🔴 🔴		LOW
4	🔴 🔴 🔴 🔴	🟢 🟢 🔴 🟢	🔴 🔴 🔴 🔴	🔴 🔴 🔴 🟢		LOW
5	🟢 🟢 🟢 🔴	🔴 🔴 🔴 🔴	🟢 🔴 🟢 🔴	🔴 🔴 🔴 🔴	🔴 🔴 🔴 🔴	MODERATE
6	🟢 🟢 🟢 🔴	🔴 🔴 🔴 🔴	🔴 🔴 🟢 🔴	🟢 🟢 🟢 🟢	🔴 🔴 🔴 🔴	MODERATE
7	🟢 🟢 🟢 🔴	🔴 🔴 🔴 🔴	🔴 🟢 🟢 🔴	🔴 🔴 🔴 🔴	🔴 🟢 🔴 🟢	HIGH
8	🟢 🟢 🟢 🔴	🔴 🔴 🔴 🔴	🟢 🟢 🟢 🟢	🟢 🟢 🔴 🟢	🔴 🟢 🔴 🟢	HIGH
9	🟢 🟢 🟢 🟢	🔴 🟢 🟢 🟢	🟢 🟢 🟢 🟢	🟢 🟢 🟢 🟢	🟢 🟢 🟢 🔴	HIGH

PrP seeding by RT-QuIC

🟢 POSITIVE

🔴 NEGATIVE

METHODS

1. Genetics: PrP codon 129 and ApoE genotyping, mutations on APP, PSEN1, PSEN2 (*MOSTLY COMPLETED*)

2. Disease phenotype (*ONGOING*):
   a) Assess the presence of spongiform degeneration;
   b) Immunohistochemistry (IHC): PrP, amyloid-β, hyperphorylated tau, α-synuclein, and TDP-43;
   c) IHC of frozen tissue (histoblot);
   d) Clinical evaluation of PrP RT-QuIC-positive cases

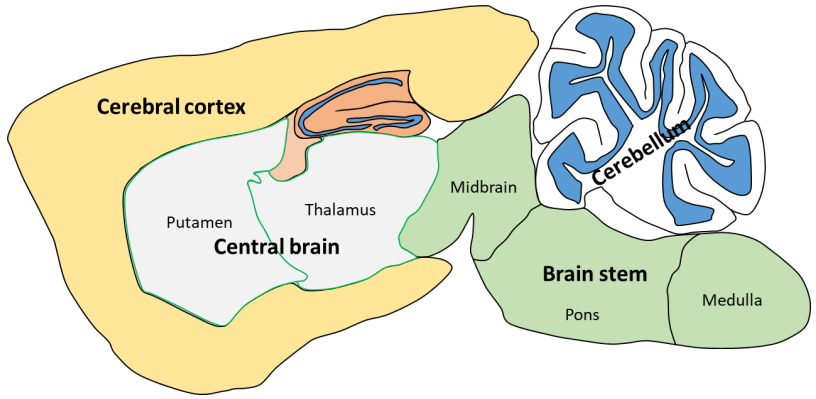
3. Presence of PK-resistant PrP by western blot examination (*COMPLETED*)

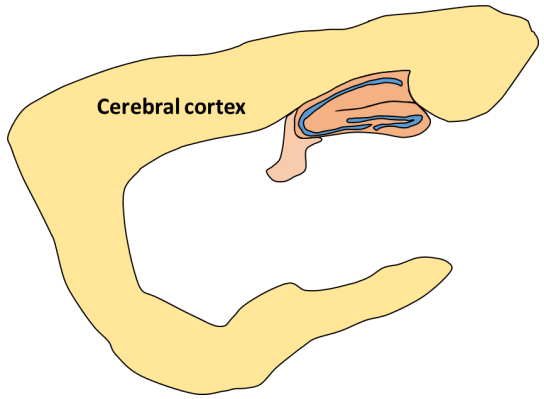
METHODS

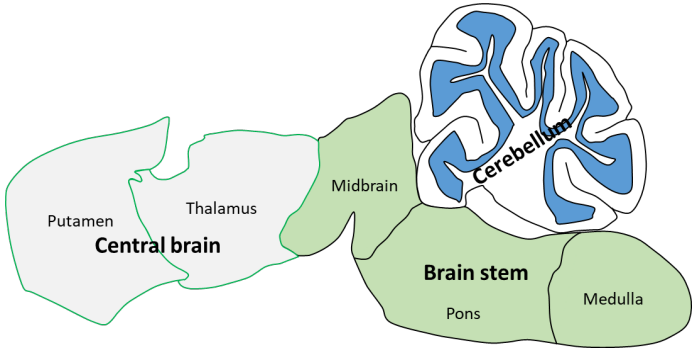
4. Assess PrP seeding activity in transgenic (Tg) mice expressing the human muted APP (Swedish mutation) and human wild type PrP

Tg-APP$_{Swe}$-PrP-129MM

TgAPP$_{Swe}$-PrP-129VV

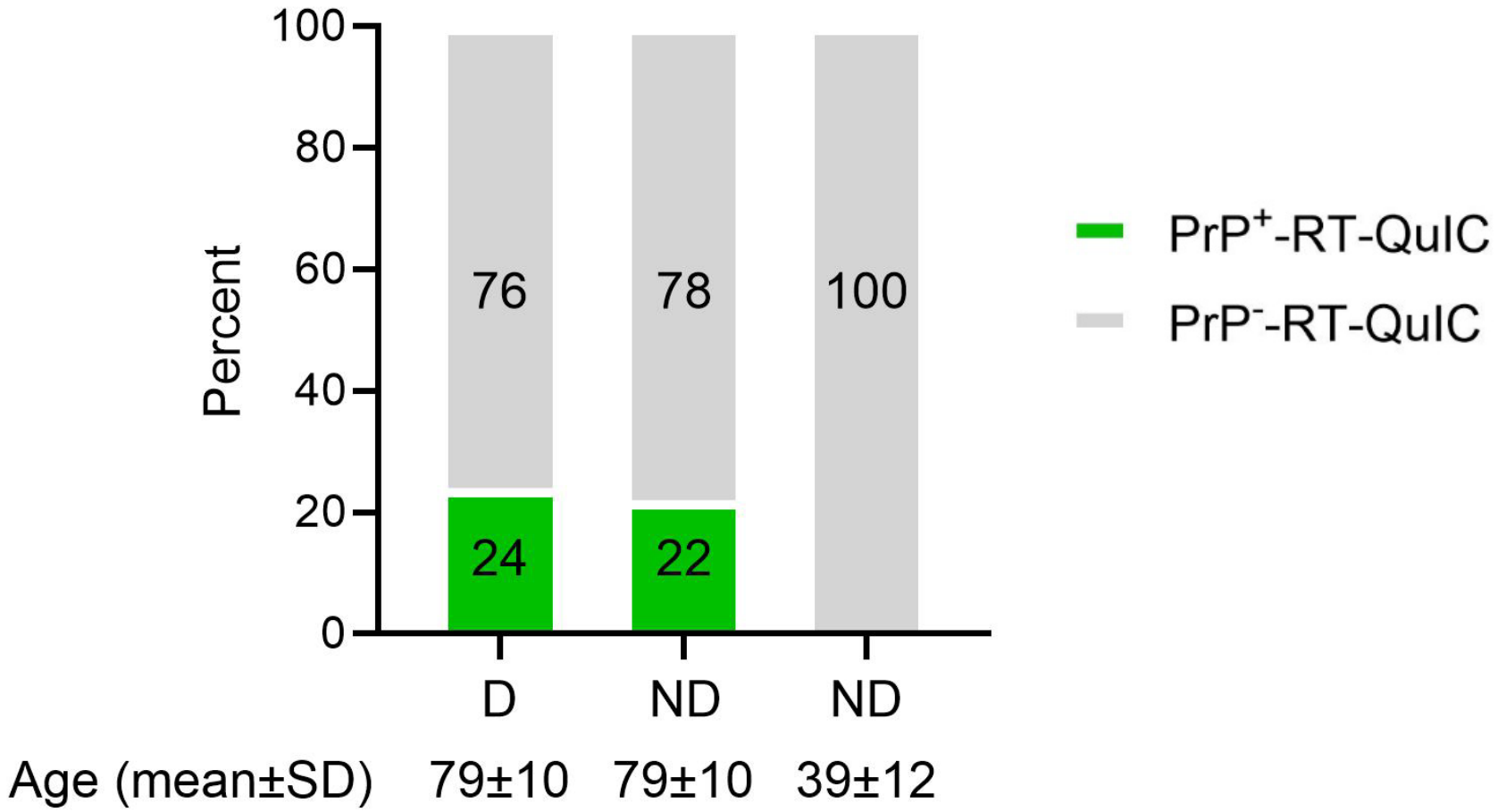






Prevalence PrP^{+}-RT-QuIC

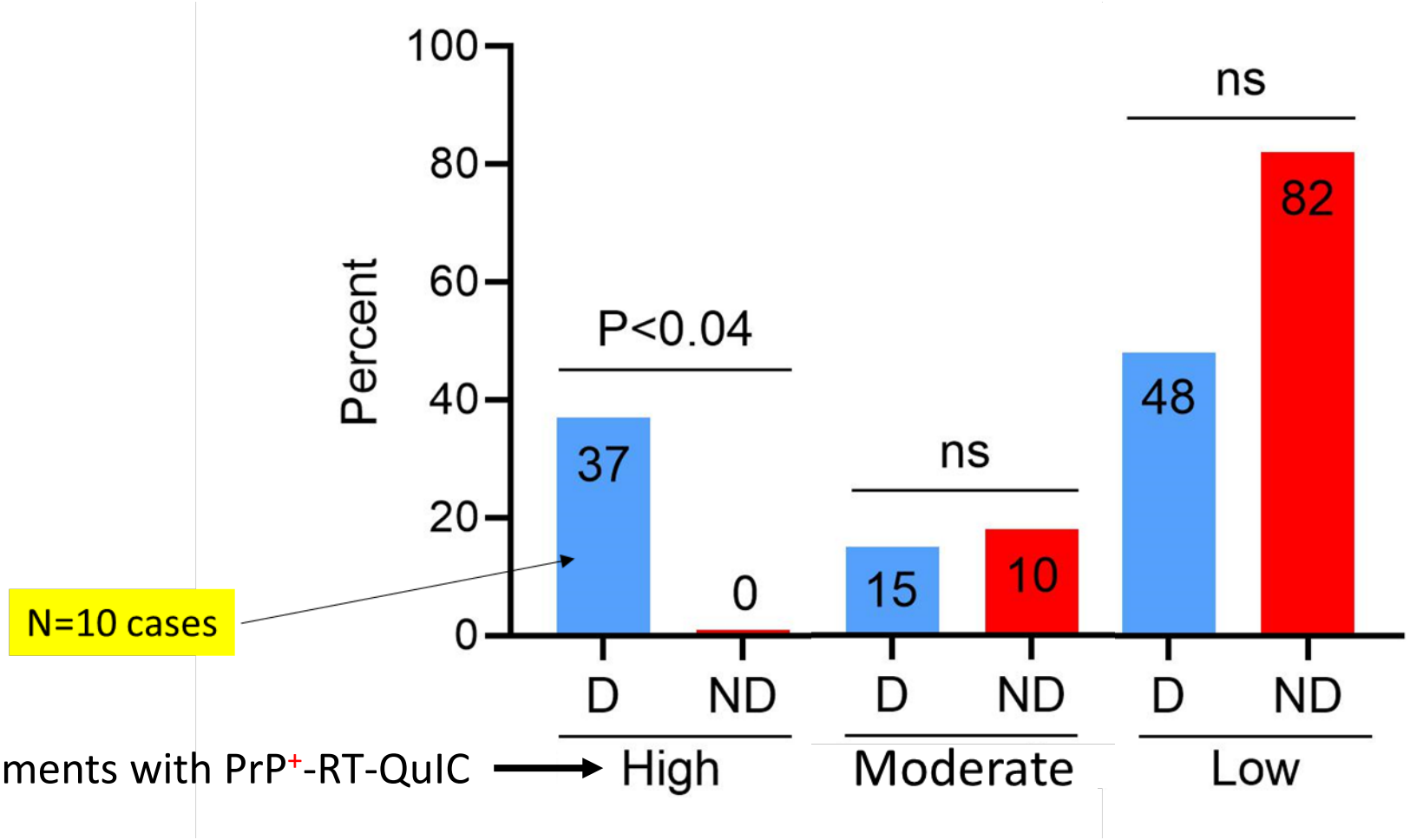
Demented (D) vs. Non-Demented (ND) cases



RESULTS

Prevalence PrP^{+}-RT-QuIC

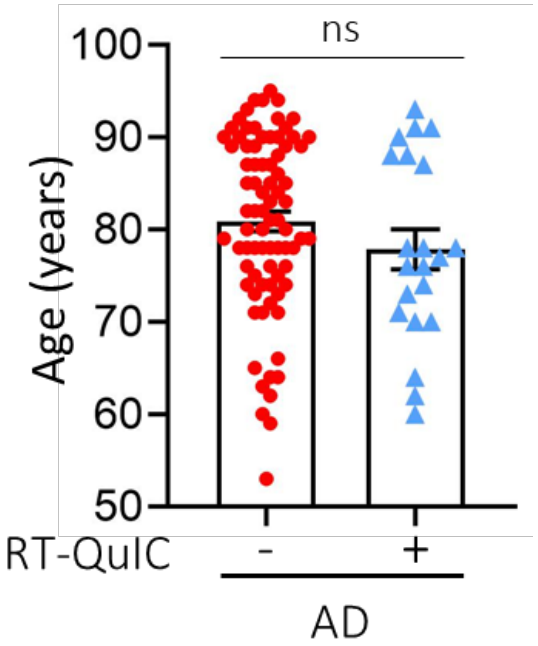
Degree positive outcome: HIGH - MODERATE - LOW

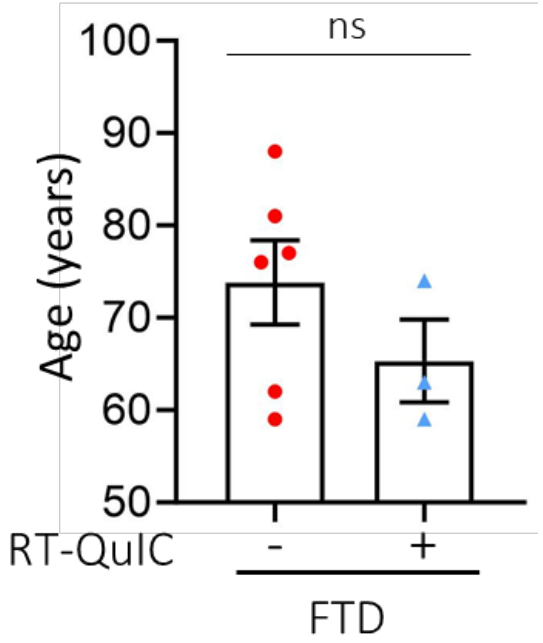


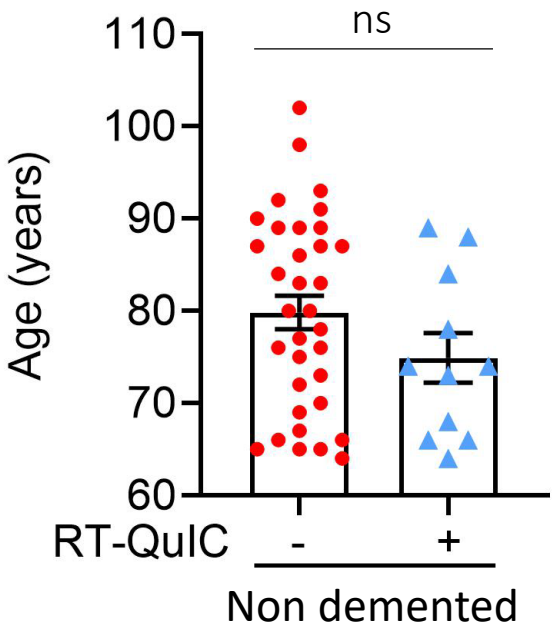
Conclusion: A robust (high) PrP seeding activity is a feature of demented cases only

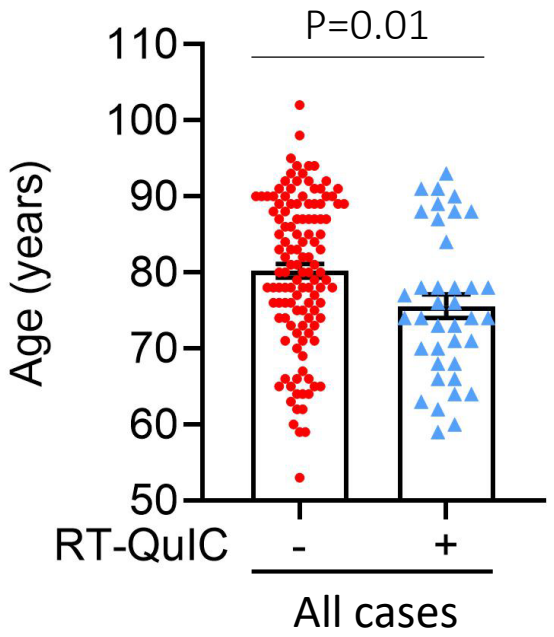
RESULTS

Does age play a role in PrP seeding?



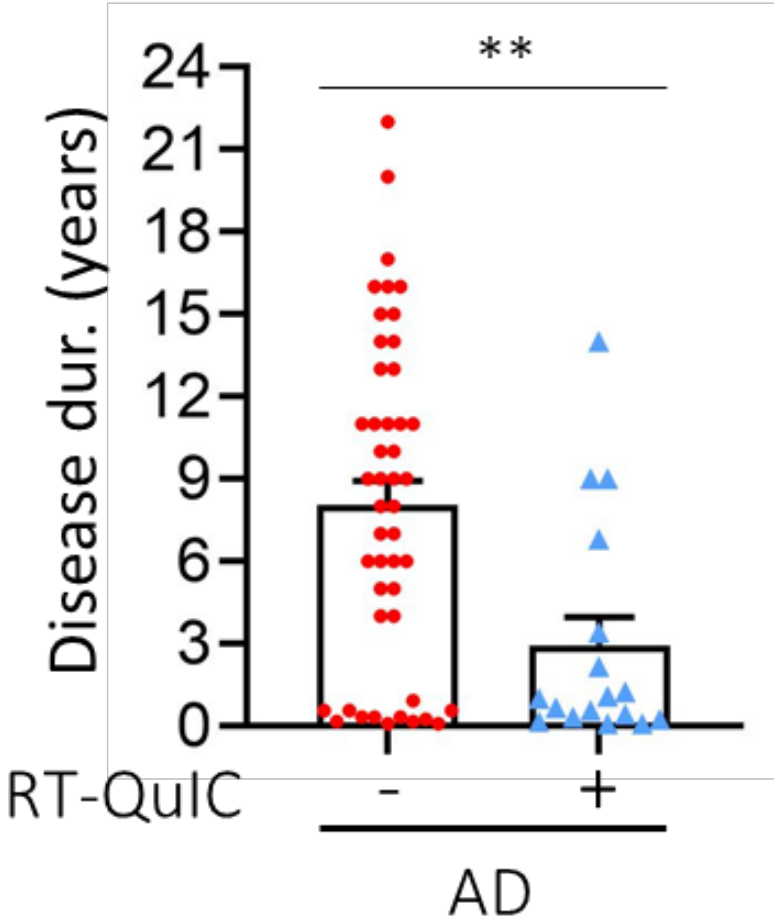






Conclusion: Overall, cases with PrP^{+}-RT-QuIC are younger than cases with PrP^{-}-RT-QuIC

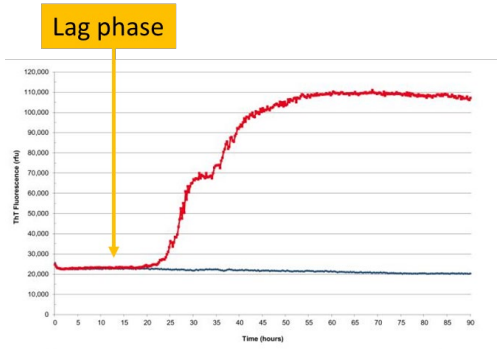
Does the rate of disease progression play a role in PrP seeding?

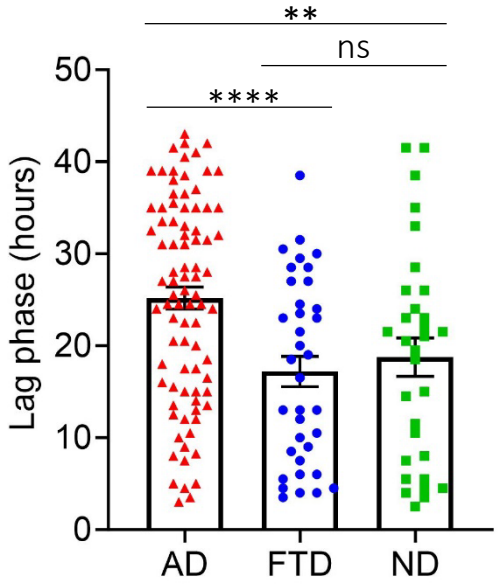


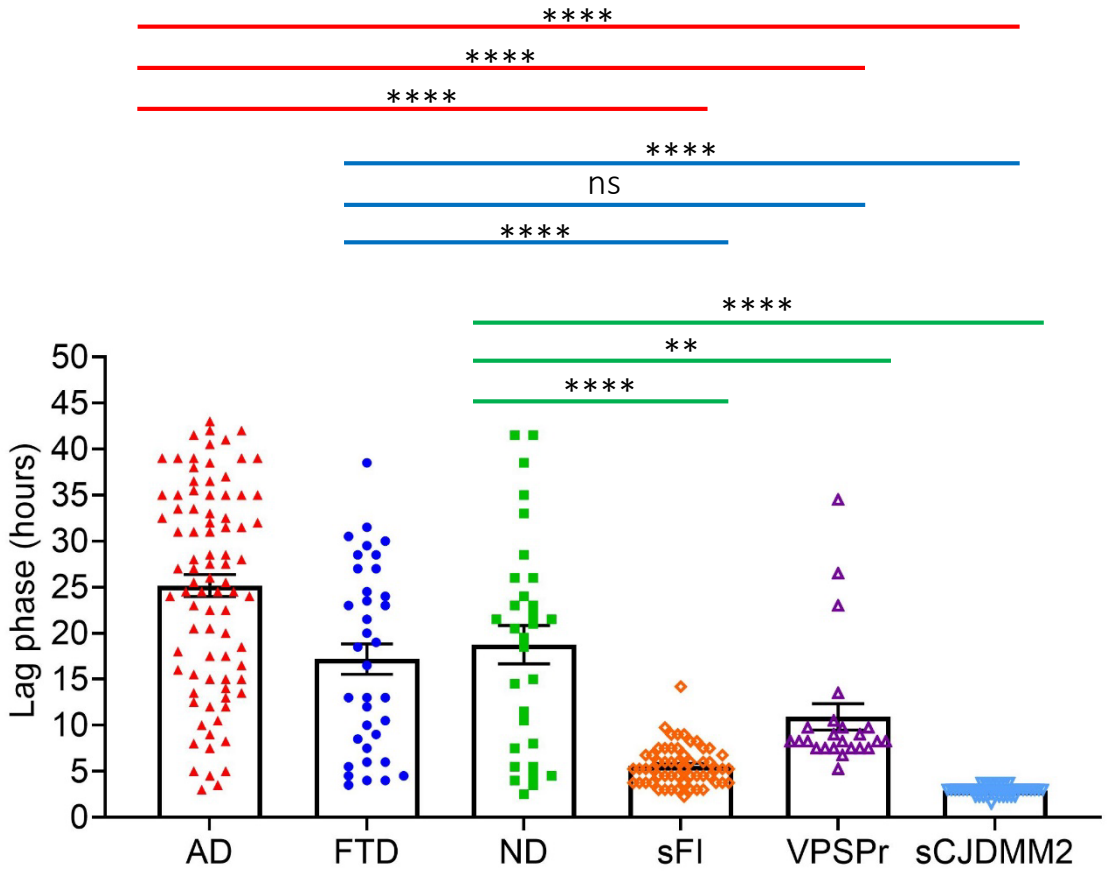
Conclusion: Positive PrP seeding activity is common in AD cases with rapid disease progression

RESULTS

kinetics of PrP seed formation: LAG PHASE



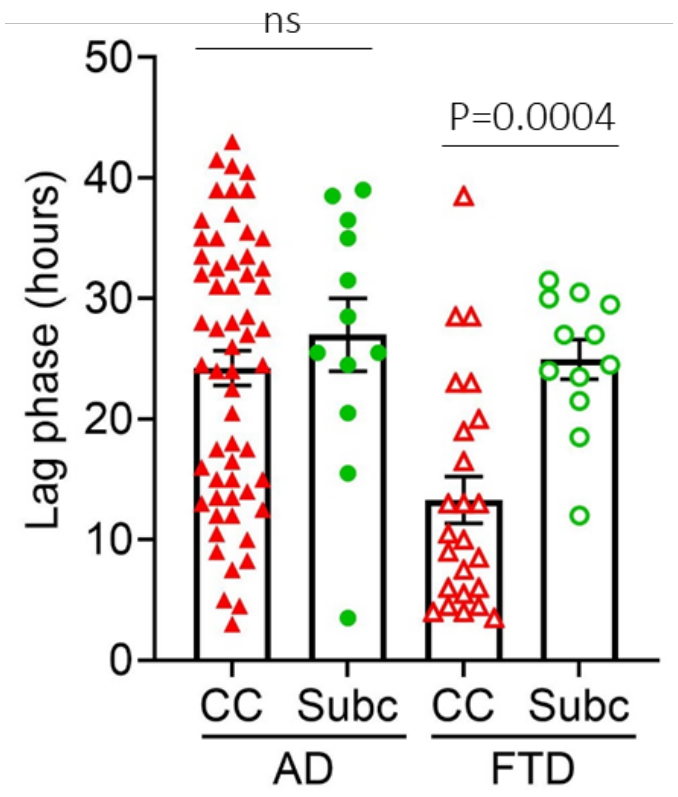


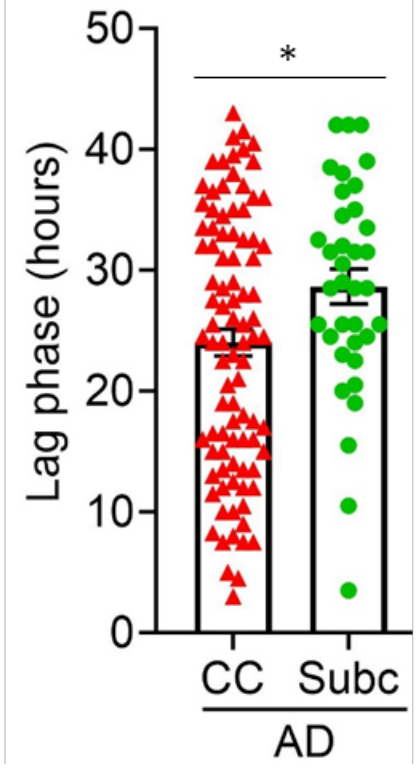


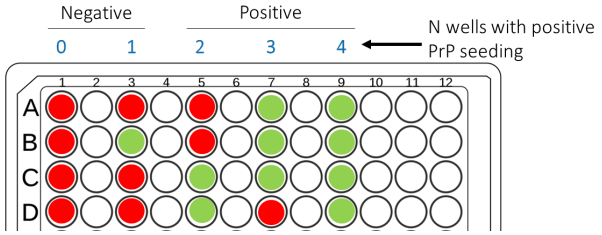
Conclusion: A prolonged lag phase is a feature of demented non-prion disease cases

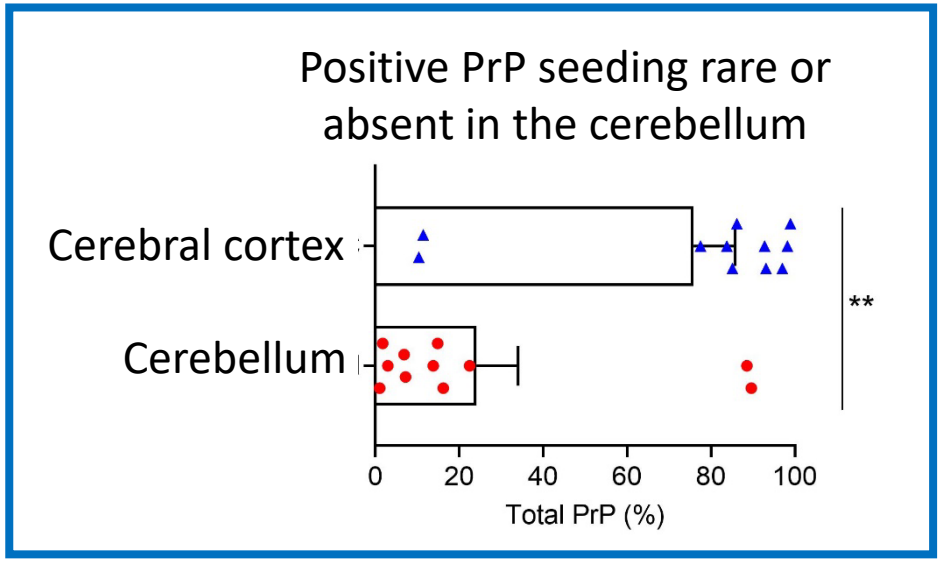
RESULTS

LAG PHASE: cortical vs. subcortical regions







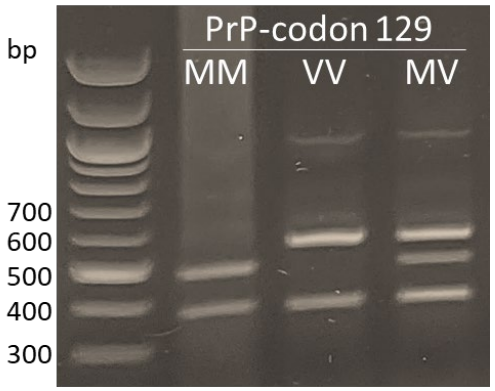


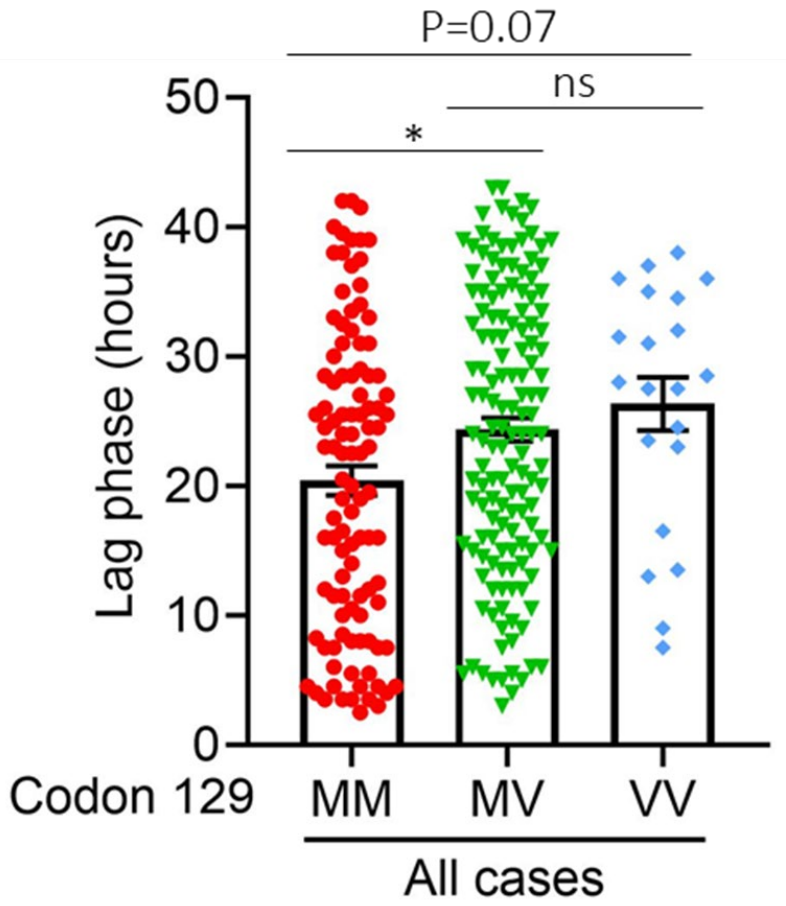
CC: cerebral cortex
Subc: subcortical regions

Conclusion: PrP seeding activity occurs at a faster rate in cortical than subcortical regions. It is rare in the cerebellum

RESULTS

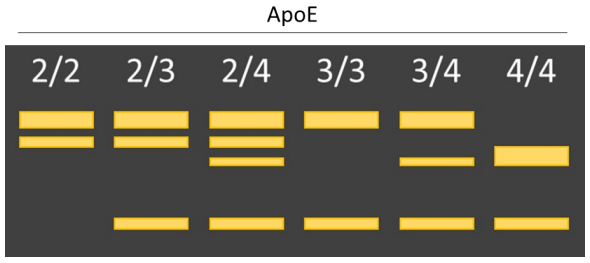
PrP codon 129 genotype



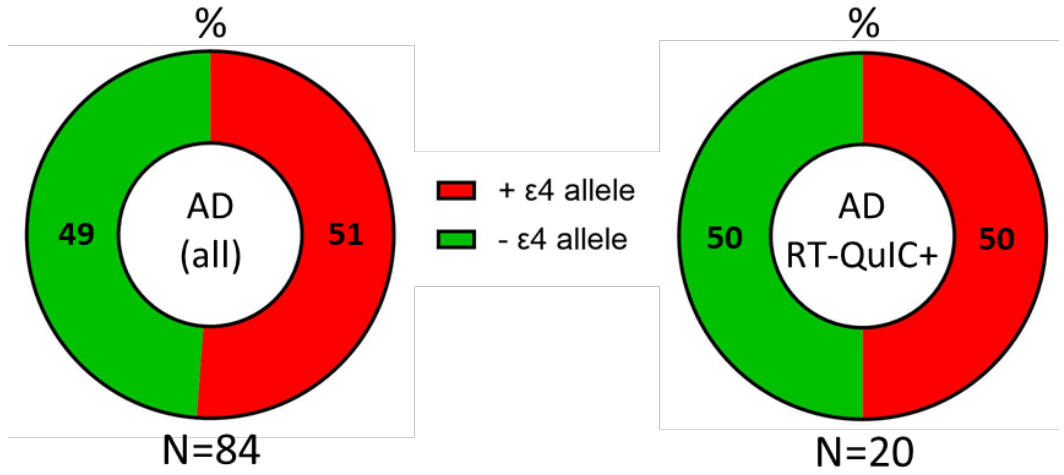


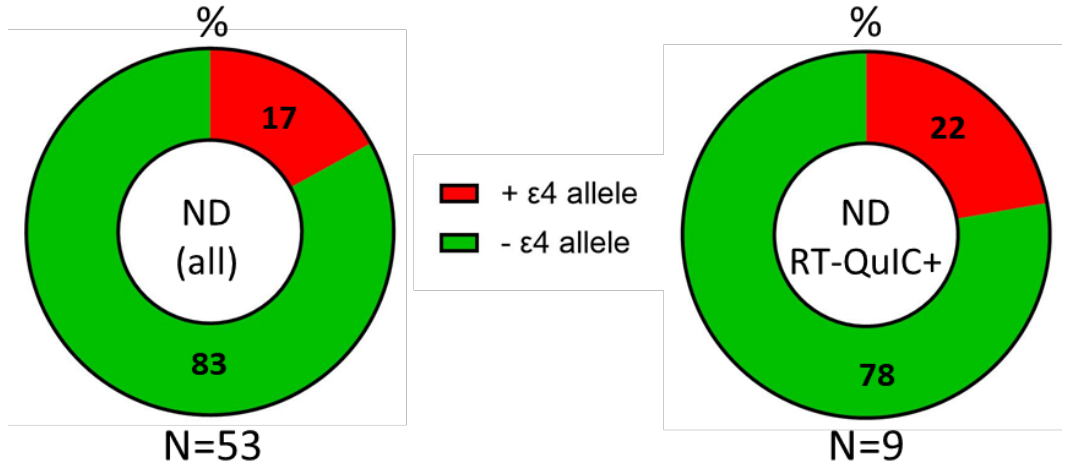
<u>Conclusion</u>: PrP^C to abnormal PrP conversion is most efficient with the 129MM genotype

RESULTS



ApoE genotype

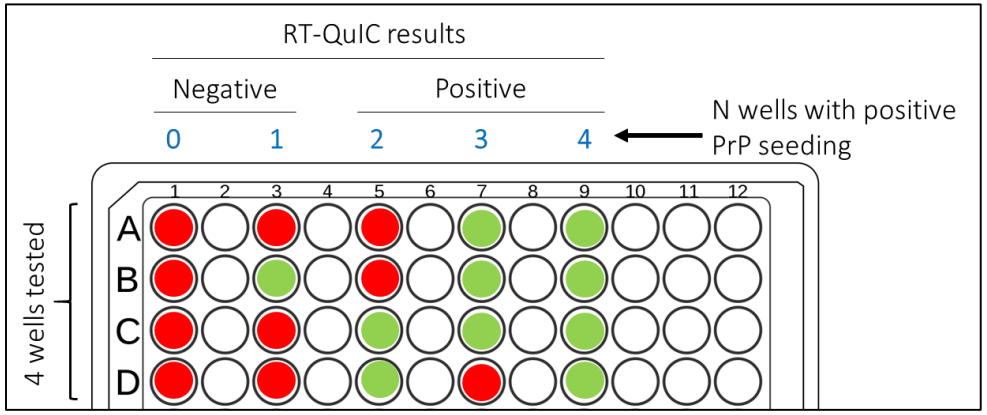


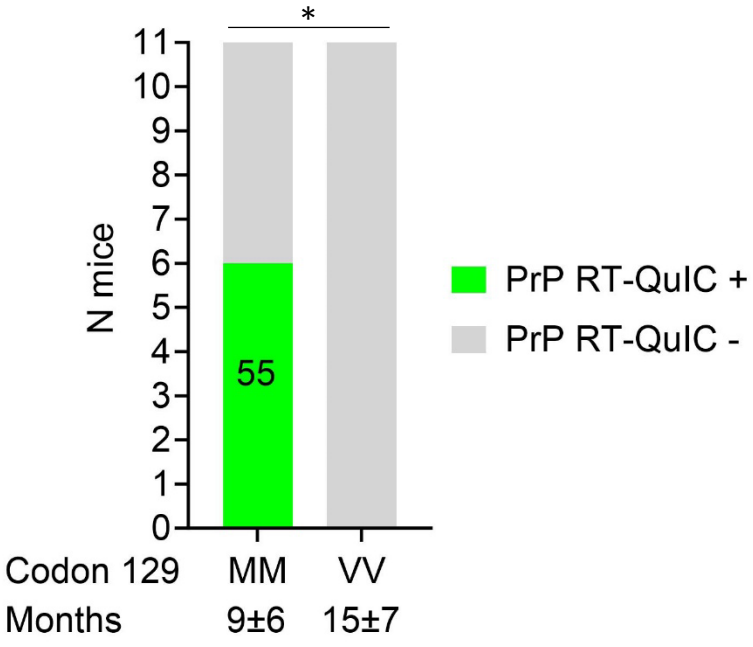


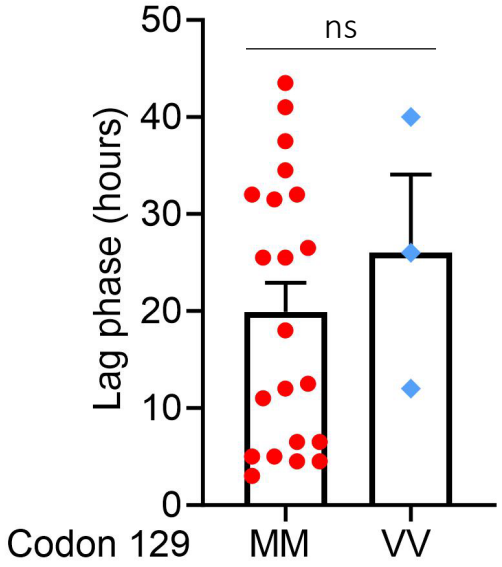
<u>Conclusion</u>: The presence of the ε4 allele does not modify PrP seeding activity

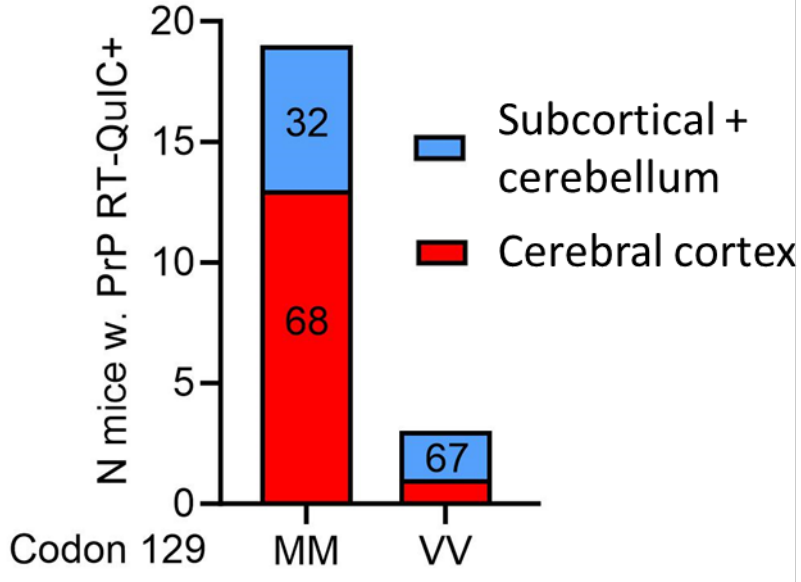
RESULTS

A double transgenic mouse model of AD and prion disease









Conclusion: PrP seeding activity in mice mimics results obtained in human cases

CONCLUSIONS

- A robust PrP seeding activity is found in demented cases but NOT in age-matched non-demented controls. These results point to the deleterious effect of a primary proteinopathy(ies) leading to disease comorbidity

- PrP seeding is AGE-DEPENDENT as young non-demented controls are free of PrP seeds. However, positive PrP seeding activity in the demented cohort is typically seen in younger cases

- AD cases with rapid disease progression (i.e., ≤3 years) and positive PrP seeding were 7 years younger than classical AD cases ($P<0.002$). Thus, age and disease progression, at different levels, may impact PrP seed formation

- Patients with PrP 129MM genotype may be at higher risk for PrP seed formation (human and mouse studies). ApoE has no effect on PrP seeding

CONCLUSIONS

- Shorter lag phases in FTD could be explained by lack/decreased levels of oligomeric amyloid-β interacting with PrP^{C}, or by other unknown mechanisms

- The shortest lag phases in the neocortex may reflect the significantly larger amount of PrP^{C} available for PrP^{C} → PrP^{Sc} conversion

In patients with AD and FTD, the presence of misfolded PrP may represent a <u>VERY EARLY</u> stage of prion disease comorbidity

FUTURE DIRECTIONS: Investigate whether the abnormal PrP detected by RT-QuIC is infectious

ACKNOWLEDGMENTS

CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.

Supporting Families Affected by Prion Disease

The Jeffrey and Mary Smith Family Foundation

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Ensun Cho

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Case Western Reserve University

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