Prion strain properties are dictated by route of inoculation

Glenn Telling
Prion Research Center, Colorado State University

Mechanisms of Neurodegeneration in Human Prion Diseases and their Intersection with AD/ADRD
November 12, 2024

CJD FOUNDATION

NIH National Institutes of Health

PRC prion research center

Abrogating the species barrier to human prions in transgenic mice expressing human PrP

Cell, Vol. 83, 79–90, October 6, 1995, Copyright © 1995 by Cell Press

Prion Propagation in Mice Expressing Human and Chimeric PrP Transgenes Implicates the Interaction of Cellular PrP with Another Protein

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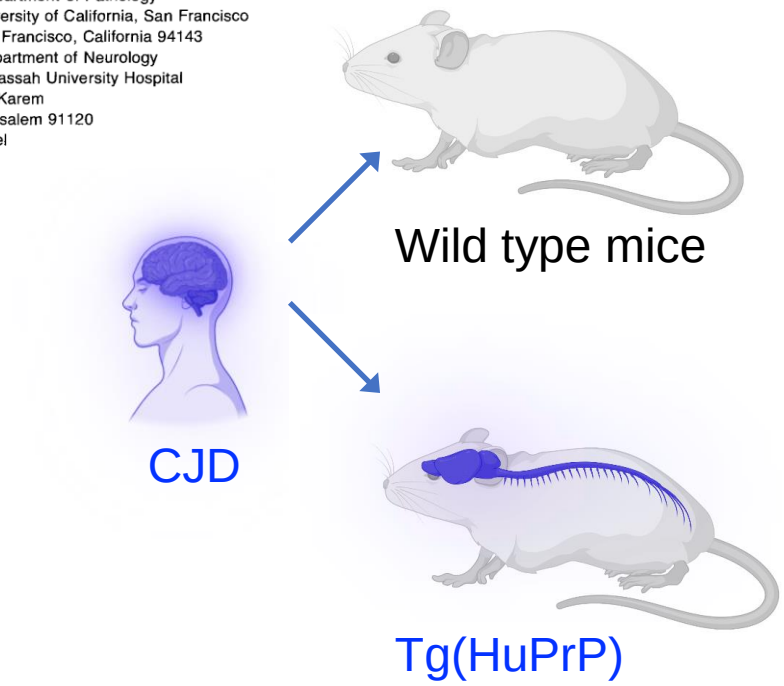
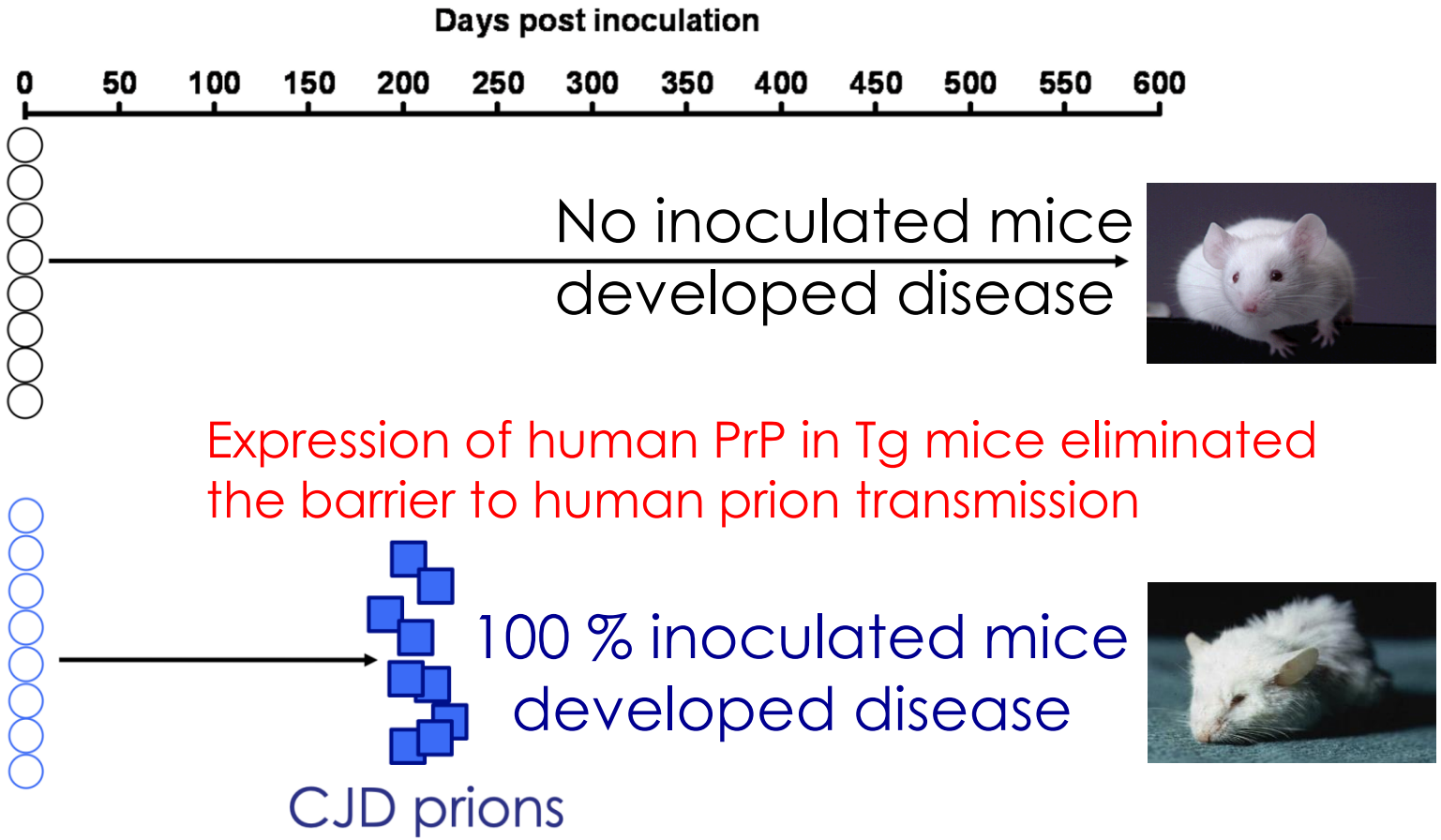
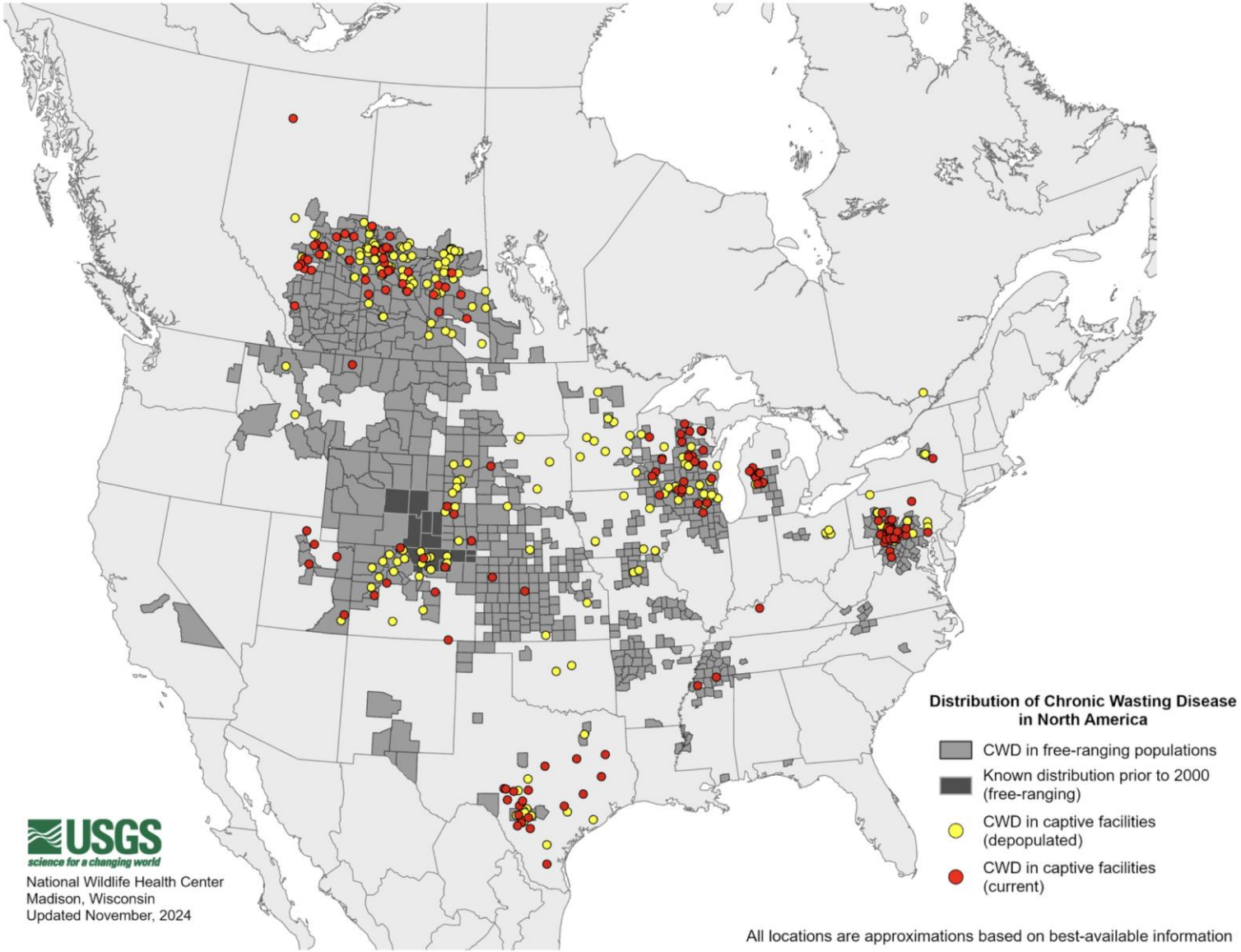
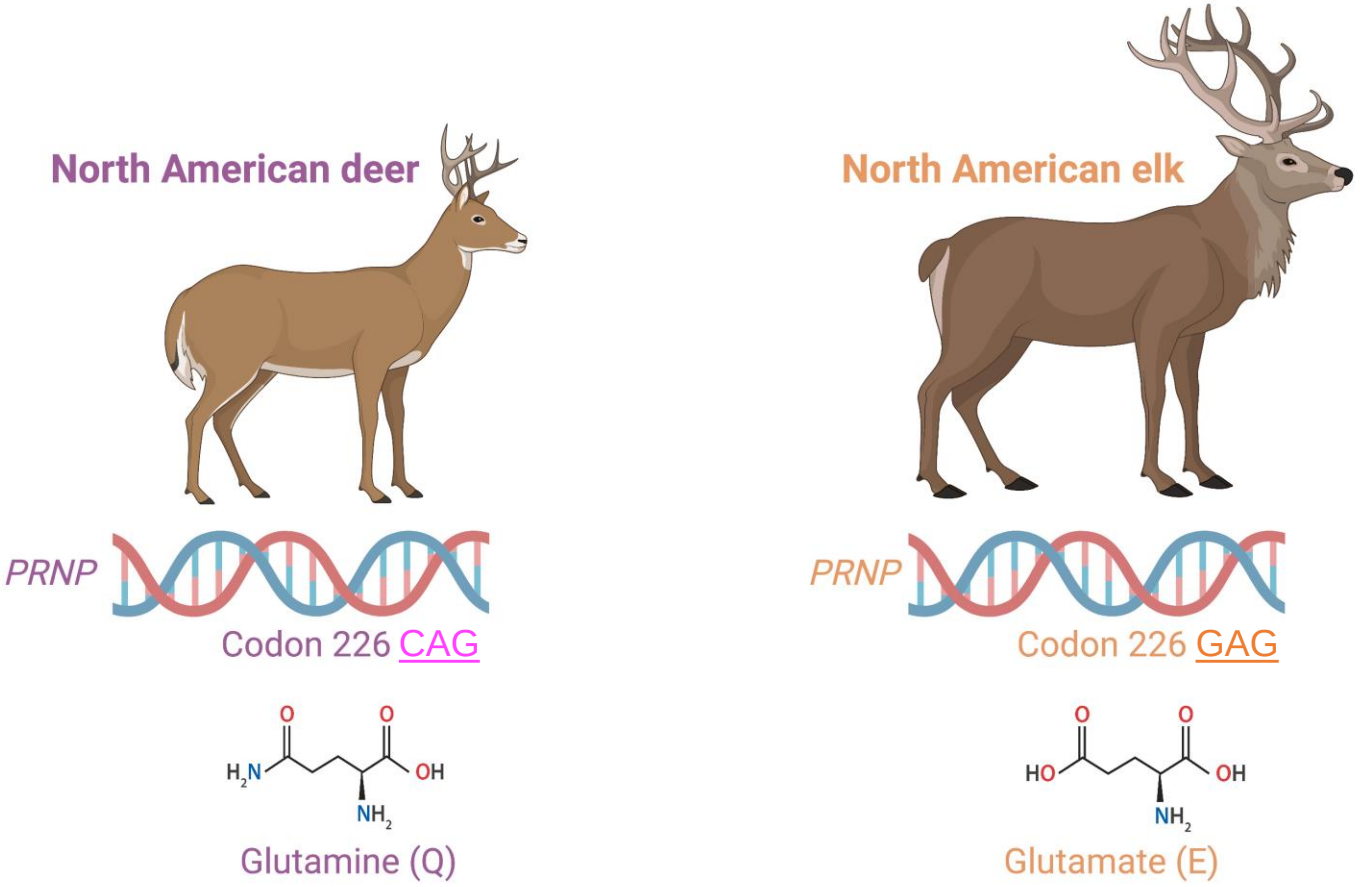




Epidemic spread of CWD in North America

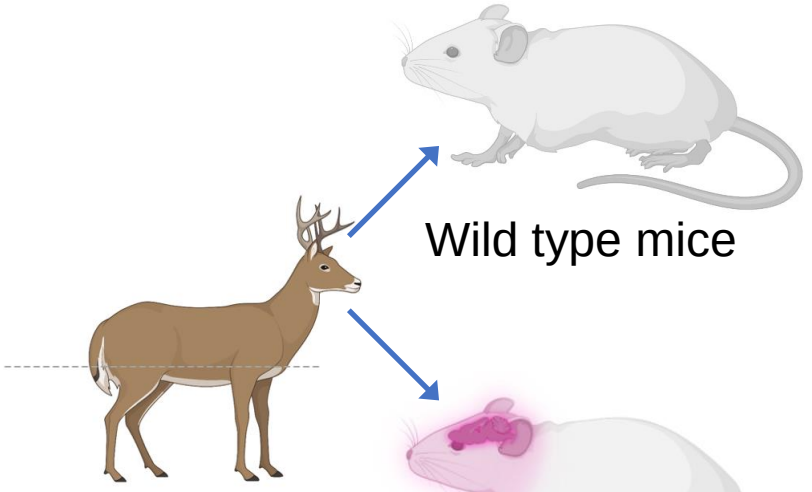


North American deer and elk differ at PrP residue 226



Abrogating the species barrier to CWD prions in transgenic mice expressing deer or elk PrP

Species barrier to CWD transmission

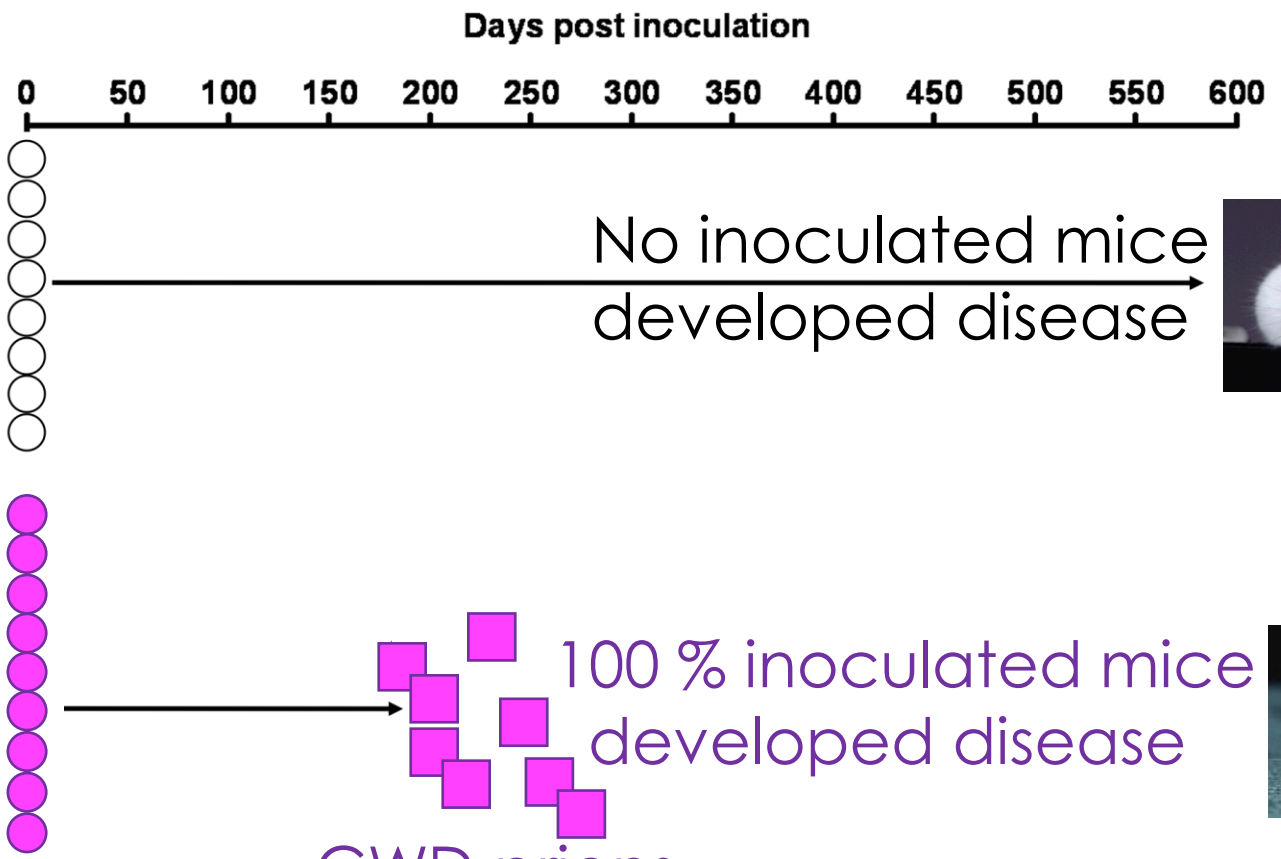


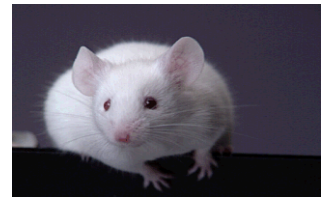
Wild type mice

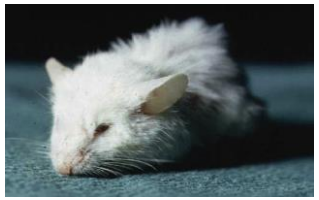
Tg(DeerPrP)

Tg(ElkPrP)

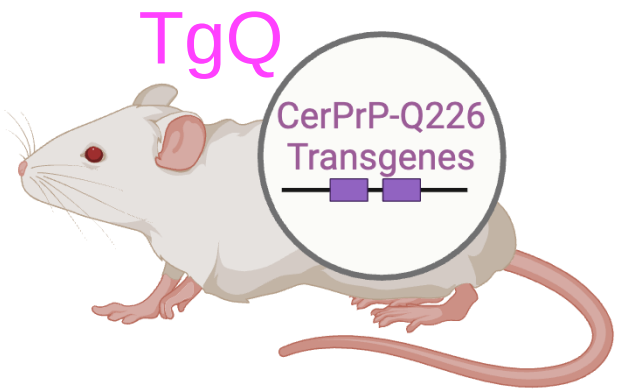
No barrier to CWD transmission

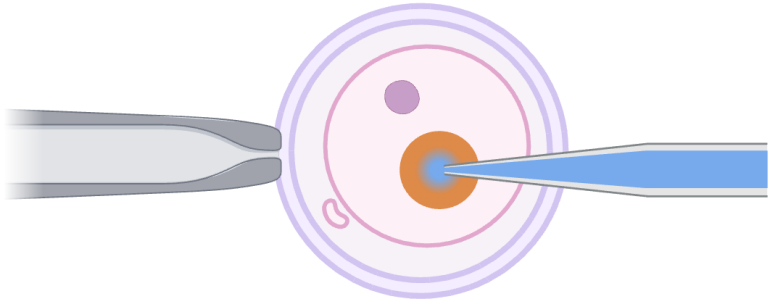


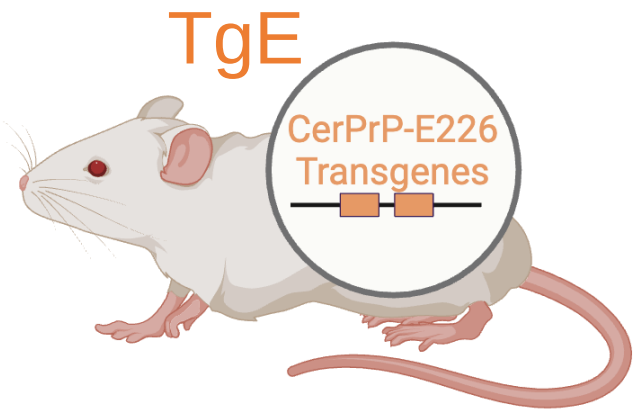




Drawbacks of conventional transgenic mice





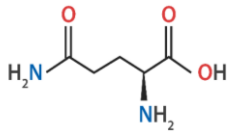


- ~ five-fold PrP overexpression in the CNS of TgQ and TgE mice
- Uncontrolled transgene copy number and chromosomal integration
- TgQ and TgE mice – different expression vectors
- Challenging to assess the effects of 226E/Q heterozygosity
- Uncertain peripheral pathogenesis

Targeted expression of deer and elk PrP to *Prnp*

Deer PrP
GtQ(FVB)

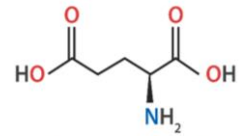
Codon 226



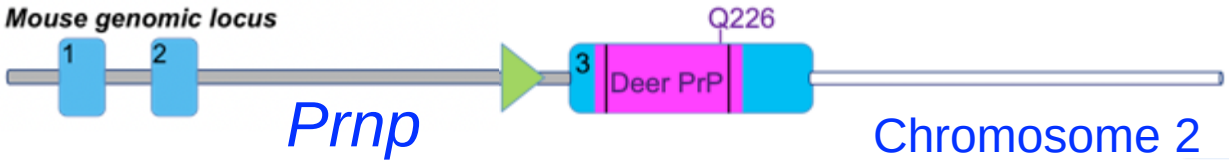
Glutamine (Q)

Elk PrP
GtE(FVB)

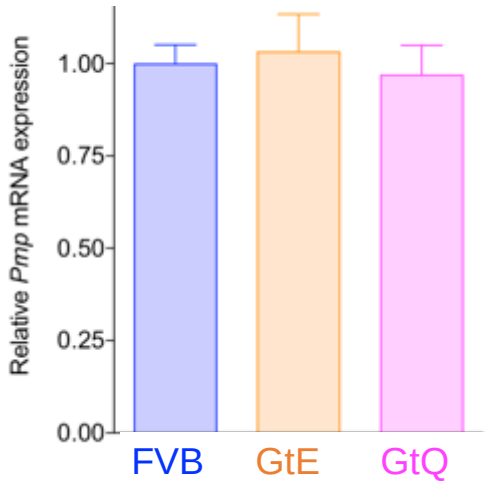
Codon 226



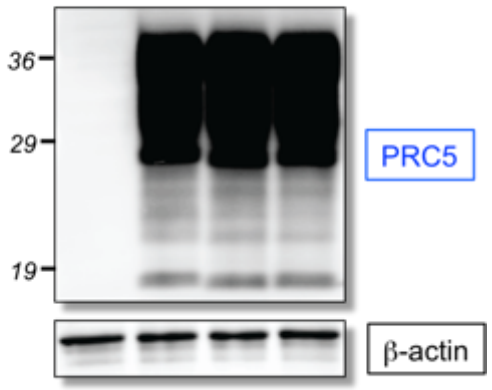
Glutamate (E)



CNS mRNA



CNS PrP

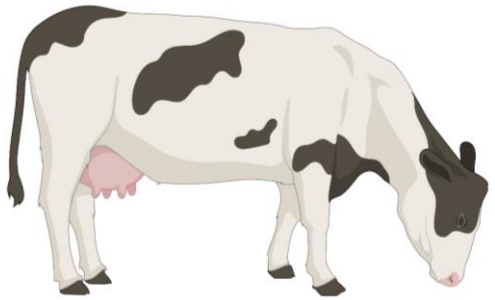


Prnp$^{0/0}$ FVB GtE GtQ

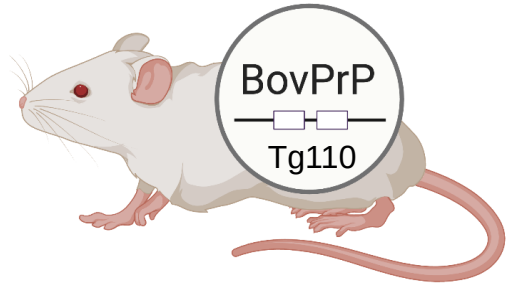
Bian et al., *PNAS*, 2019

Previous knock-in mouse models failed to efficiently eliminate prion transmission barriers

cBSE

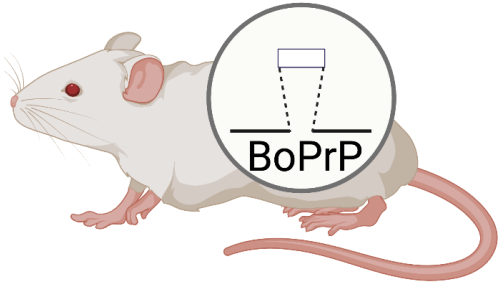


Cf. also CJD



311 ± 17 (10/10)

Scott et al., Proc. Natl. Acad. Sci. U.S.A. 94, 14279–14284 (1997)
Buschmann et a, M. H. Groschup,l., J. Infect. Dis. 192, 934–942 (2005)
Castilla et al., Arch. Virol. 148, 677–691 (2003)



551 ± 47 (15/22)

Bishop et al., Lancet Neurol. 5, 393–398 (2006)

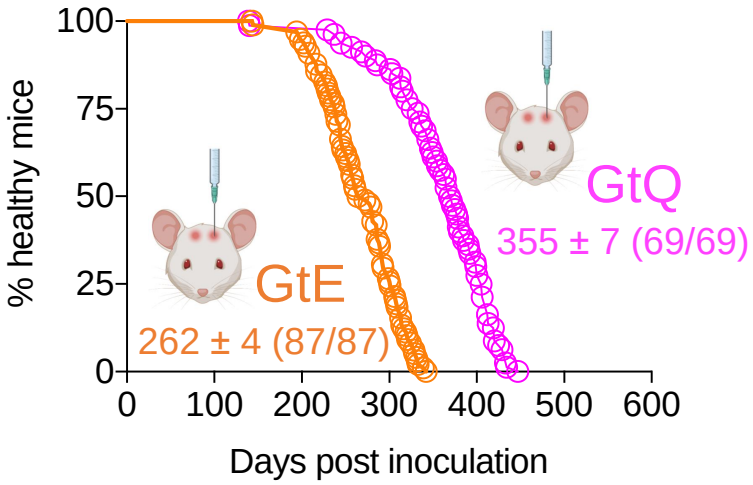
302 – 335 d (all mice)

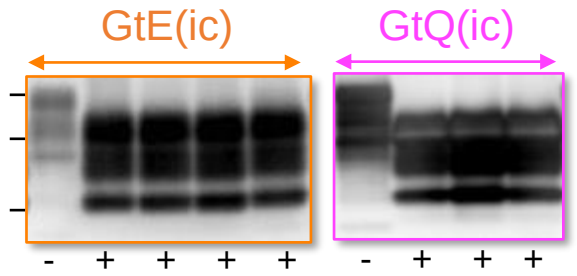
Bruce et al., Nature 389, 498–501 (1997)

CONCLUSION - Over-expression of transgenes encoding foreign PrP was required to confer susceptibility to foreign prions within the lifespan of mice

Residue 226 controls CWD prion disease kinetics in Gt mice

- Gt mice are susceptible to CWD prions from North American deer and elk
- Disease is ~ 26 % faster in intracerebrally-challenged GtE compared to GtQ mice

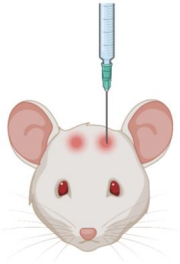




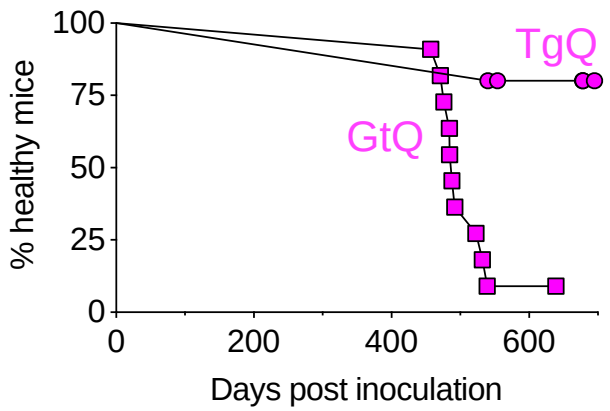
- We ascribe this effect to residue 226 since GtE and GtQ mice are otherwise syngeneic

Bian et al., *PNAS*, 2019

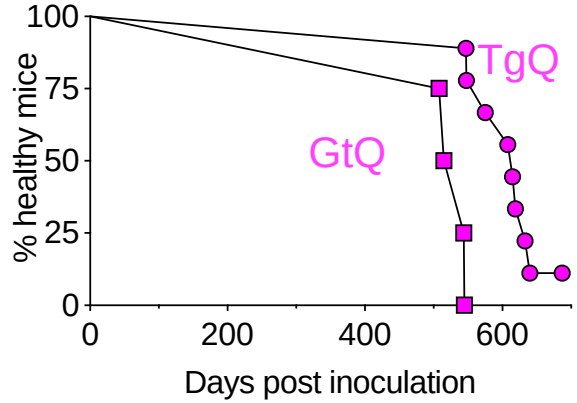
More efficient transmission of particular CWD prion strains in Gt mice than over-expressing Tg mice



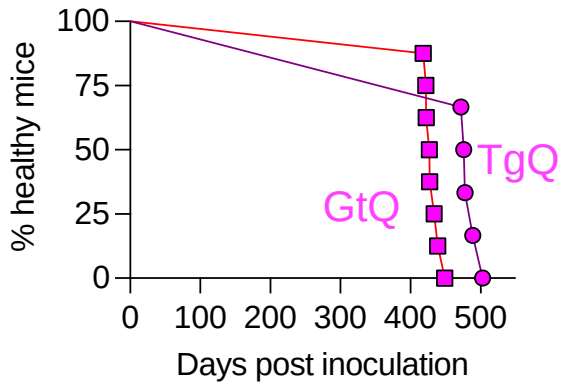
Norway Reindeer 1 (R-NO1)



Norway Reindeer 2 (R-NO2)

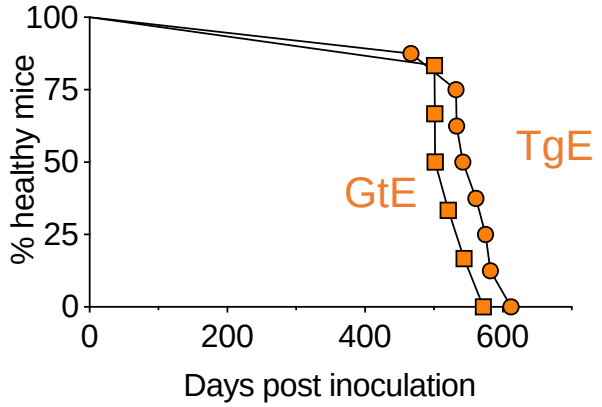


Norway Reindeer 3 (R-NO3)

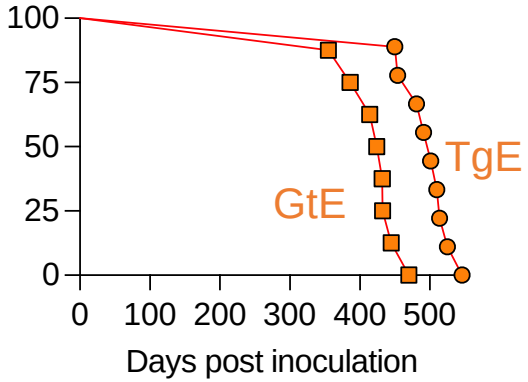


Consistently more rapid and efficient disease onset of Norwegian reindeer CWD in Gt mice compared to Tg mice

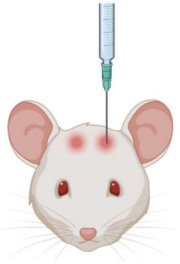
Norway Reindeer 2 (R-NO2)



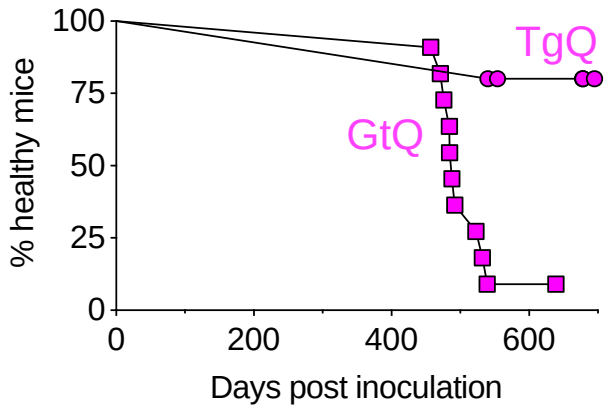
Norway Reindeer 3 (R-NO3)



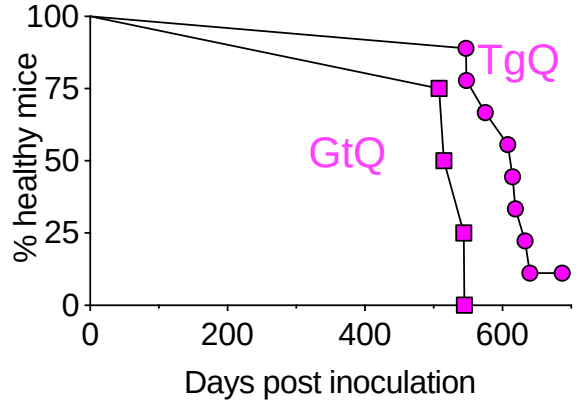
More efficient transmission of particular CWD prion strains in Gt mice than over-expressing Tg mice



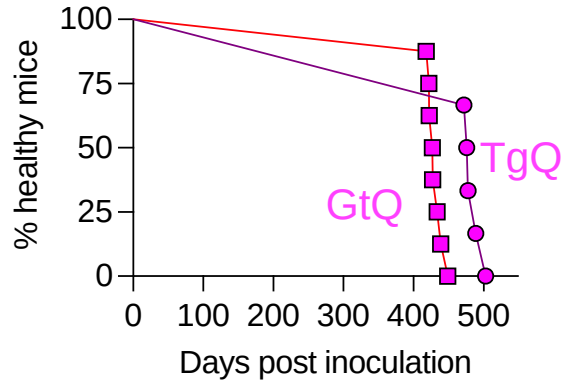
Norway Reindeer 1 (R-NO1)



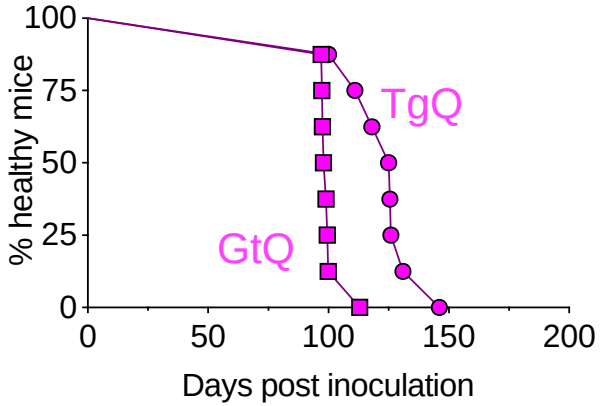
Norway Reindeer 2 (R-NO2)



Norway Reindeer 3 (R-NO3)



Sweden Moose (M-SW2)



Accelerated disease onset of Swedish moose CWD in GtQ mice compared to TgQ mice

PNAS RESEARCH ARTICLE | BIOCHEMISTRY OPEN ACCESS

Heterozygosity for cervid S138N polymorphism results in subclinical CWD in gene-targeted mice and progressive inhibition of prion conversion

2023

Maria I. Arifin[a], Lech Kaczmarczyk[b], Doris Zeng[a], Samia Hannaoui[a], Chi Lee[a], Sheng Chun Chang[a], Gordon Mitchell[c], Debbie McKenzie[d], Michael Beekes[e], Walker Jackson[b], and Sabine Gilch[a,1]

Edited by Reed Wickner, NIH, Bethesda, MD; received December 12, 2022; accepted March 6, 2023

Arifin et al. 2023	*Prnp*.Cer.Wt	488 ± 26 (7/7)
	(DeerPrP)	457 ± 16 (8/8)

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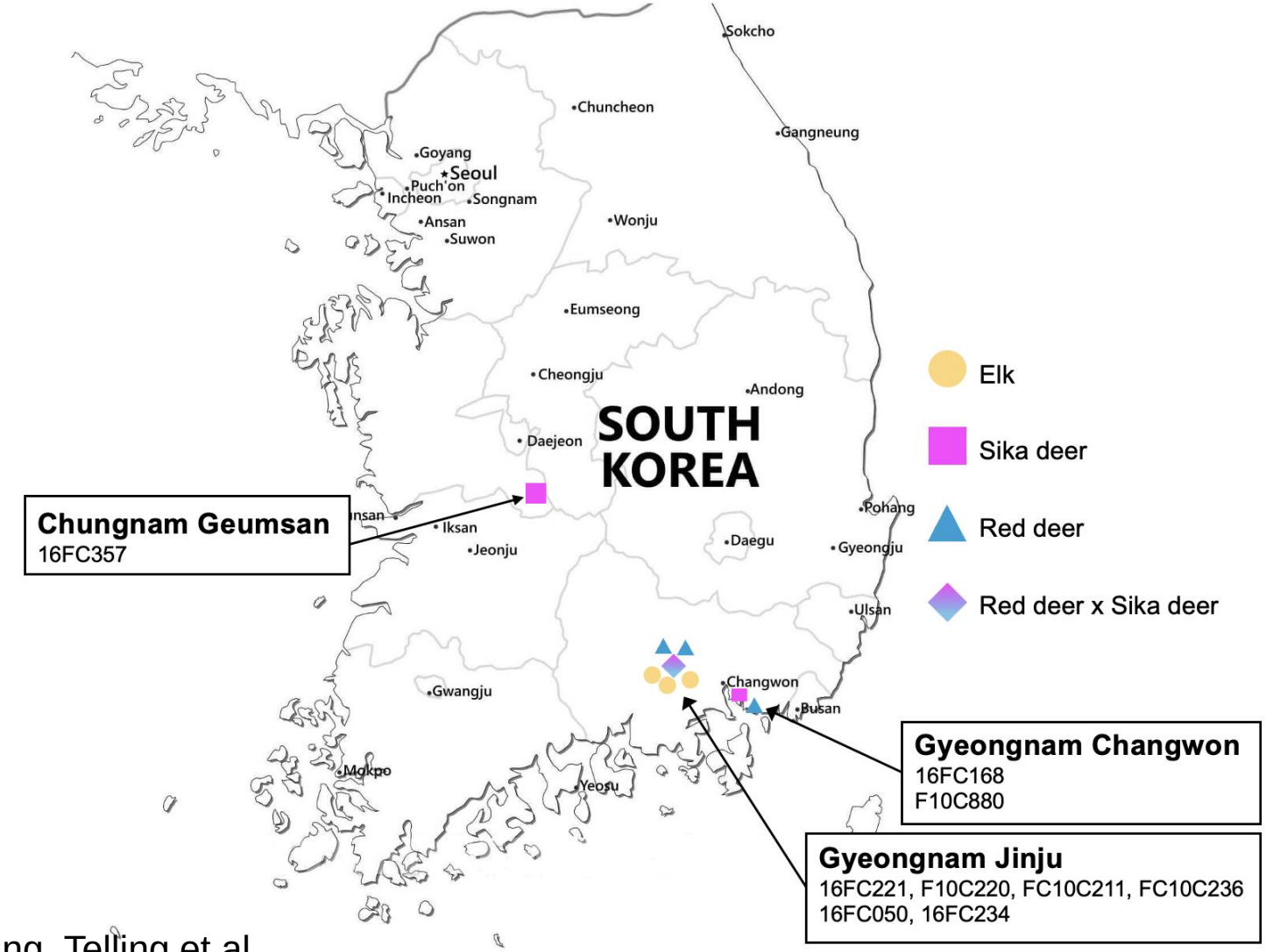
	GtE (FVB)	225 ± 16 (10/10)
	GtQ (C57BL/10)	397 ± 16 (7/7)
	GtE (C57BL/10)	223 ± 9 (10/10)

Assessing the strain properties of CWD prions from South Korea using Gt mice



CWD appears to have been exported to S. Korea from N. America in the late 1990's.

Are the strain properties Korean CWD comparable with N. American CWD, or have they evolved by passage in different hosts?

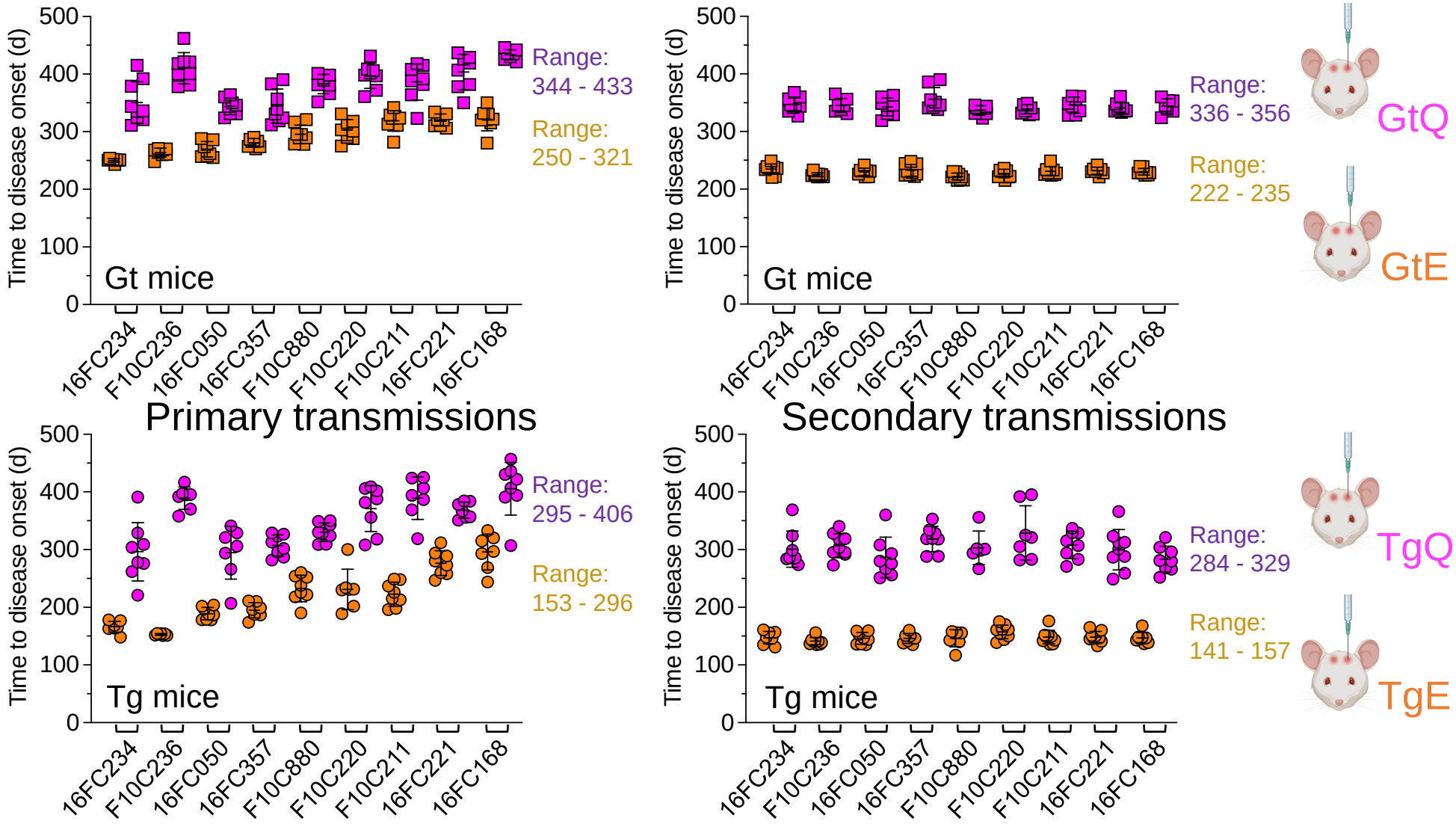
Sohn, Kang, Telling et al.

CWD isolates from South Korea

Species[1]	Cervid ID	Polymorphism at 226 codon
Elk	F10C220(B)	Q/Q
	F10C211(B)	E/E
	16FC050(B)	E/Q
Cross	F10C236(B)	E/E
Red deer	F10C880(B)	Q/Q
	16FC357(B)	E/Q
Sika deer	16FC168(B)	Q/Q
	16FC221(B)	E/Q
	16FC234(B)	Q/Q

Sohn, Kang, Telling et al.

Transmissions to Gt mice reveal strain identity between Korean and North American CWD prions



Sohn, Kang, Telling et al.

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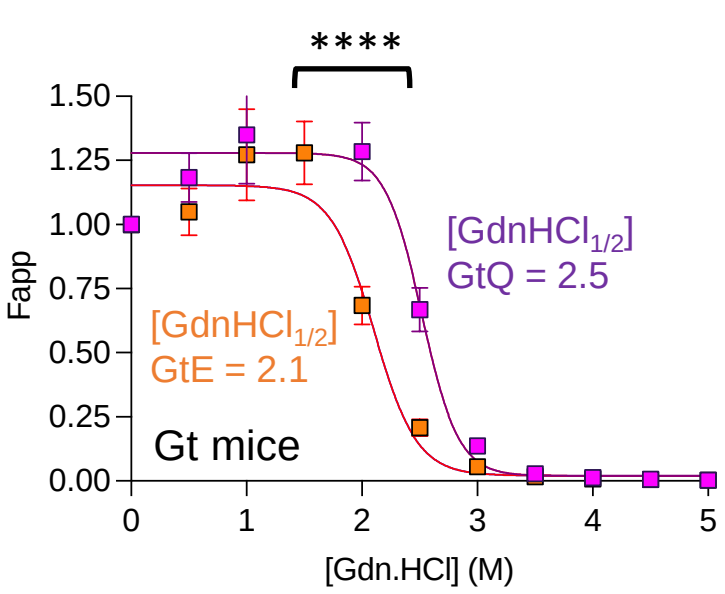
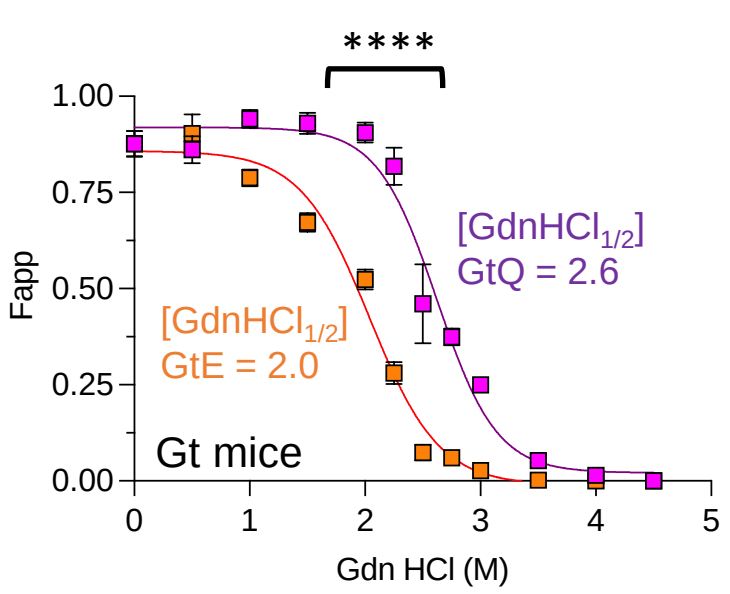
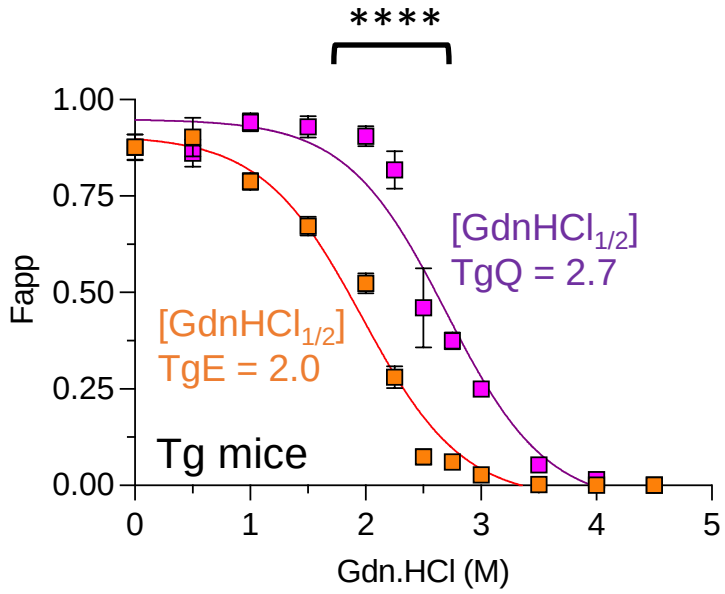




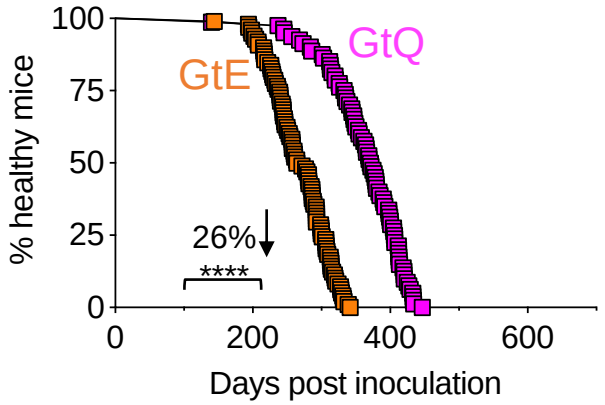


N. American CWD prions

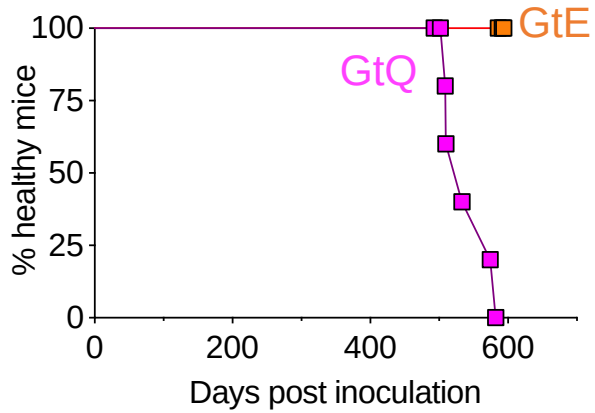
S. Korean CWD prions

Sohn, Kang, Telling et al.

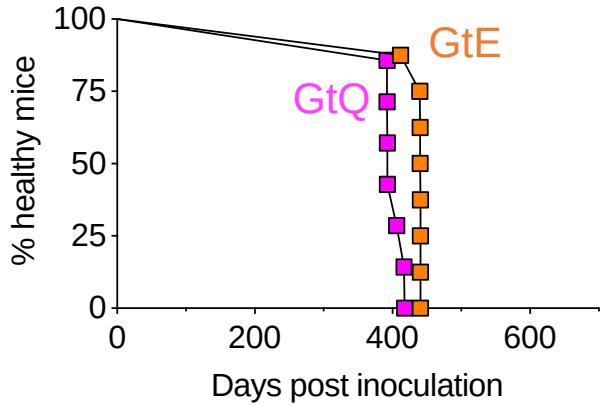
Transmissions to Gt mice reveal strain differences between Nordic and North American CWD



N. America/S. Korea CWD - Transmits both to GtE and GtQ. Disease faster in GtE.



Norway/Finland moose CWD – Transmits only to GtQ.



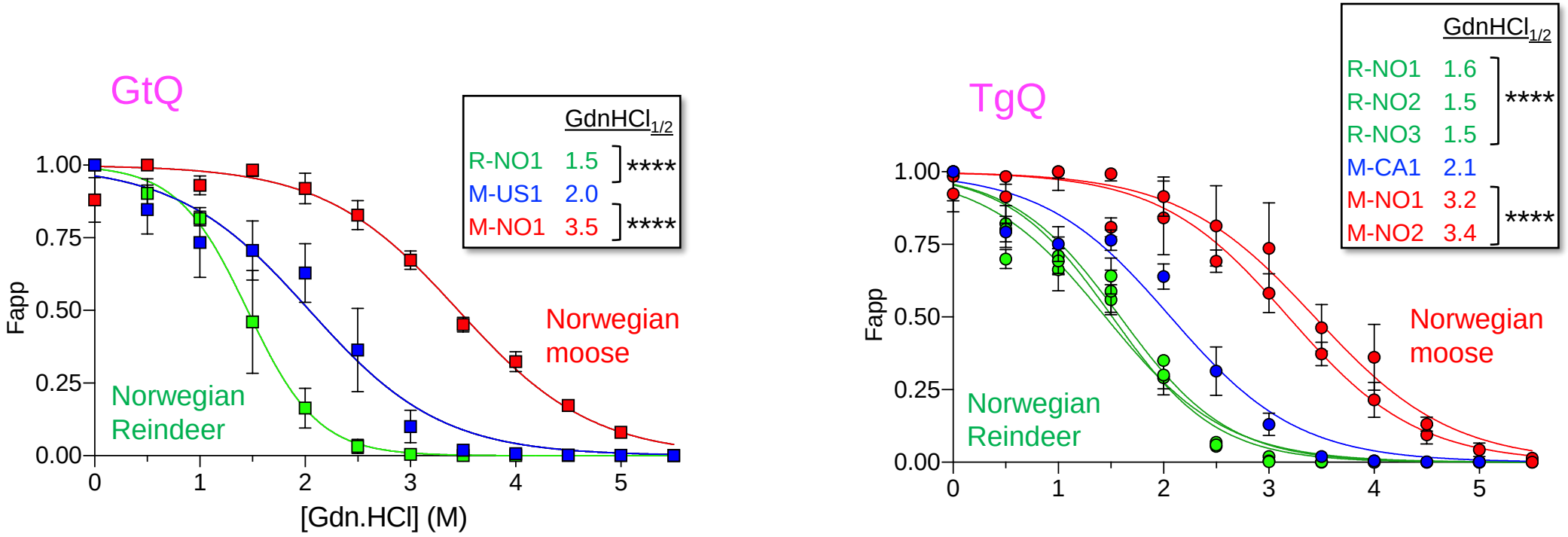
Norwegian reindeer CWD - equal incubation times in GtE and GtQ.

Sun, J. et al. Novel Prion Strain as Cause of Chronic Wasting Disease in a Moose, Finland. *Emer. Inf. Dis.*, *29(2)*: 323-332, 2023

Bian, J. et al. Adaptive selection of a prion strain conformer corresponding to established North American CWD during propagation of novel emergent Norwegian strains in mice expressing elk or deer prion protein. *PLoS Path*, *17(7)*: e1009748, 2021

Bian, J. et al. Primary structural differences at residue 226 of deer and elk PrP dictate selection of distinct CWD prion strains in gene-targeted mice. *Proc. Natl. Acad. Sci. USA*, *116(25)*:12478-12487, 2019

Transmissions to Gt mice reveal strain differences between Nordic and North American CWD



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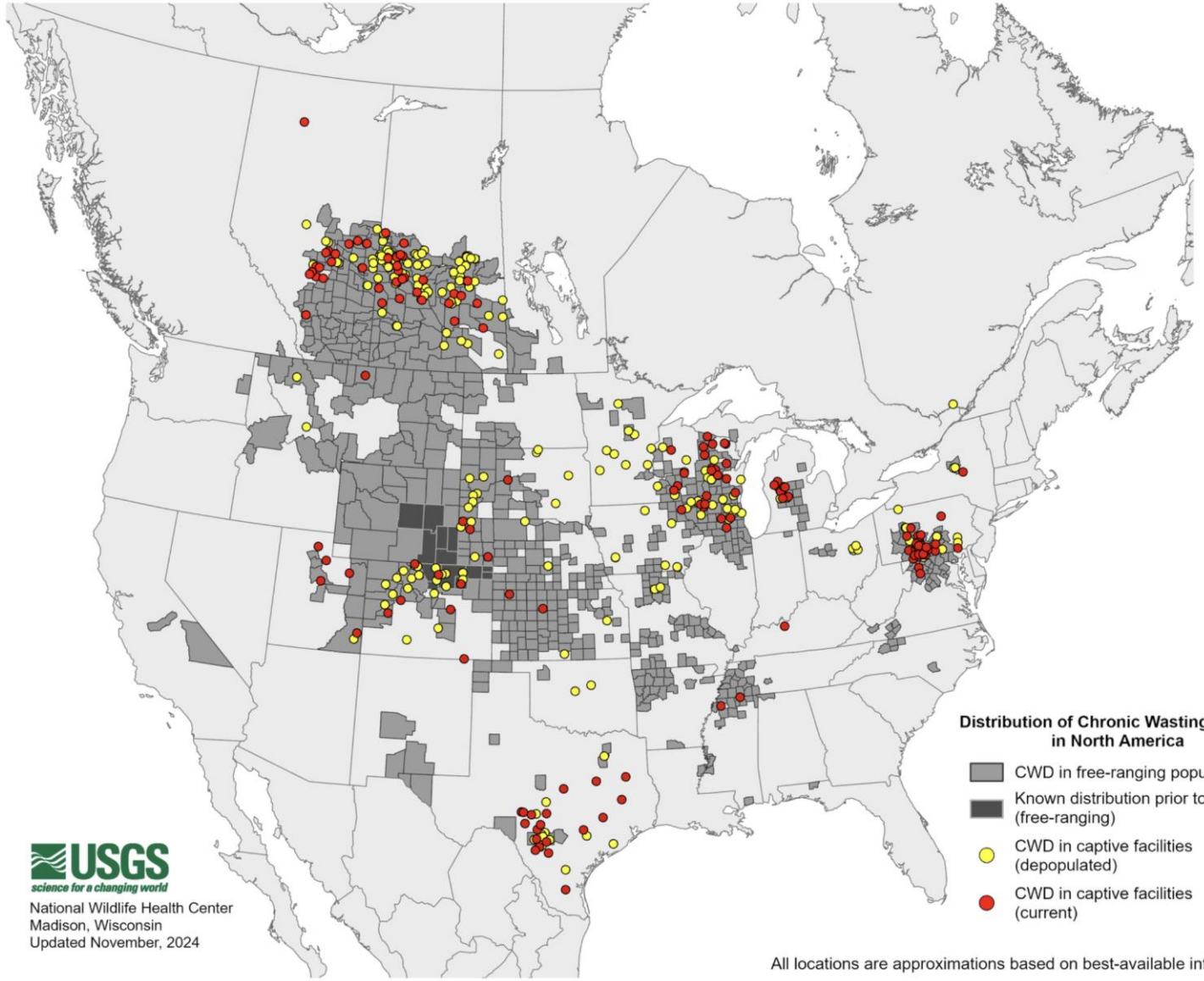
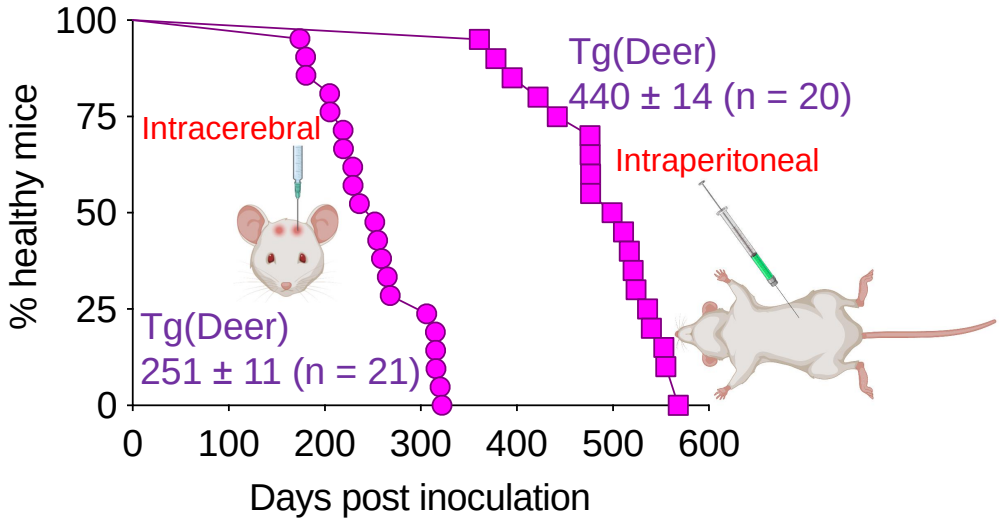
Summary - 1

- North American deer and elk differ at PrP residue 226.
- Gt mice expressing E-PrP and Q-PrP respond better to certain CWD prion strains than their over-expressing Tg counterparts.
- Previously-generated knock-in mice designed to be susceptible to human or bovine prions respond comparatively poorly.
- Residue 226 controls the responses of Gt mice to North American and Nordic CWD prions.
- The strain properties of Nordic CWD prions in reindeer, moose, and red deer are distinct from those of North American CWD.
- The strain properties of Korean and North American CWD prions are concordant.

Naturally contagious CWD transmission linked to efficient prion propagation by peripheral routes

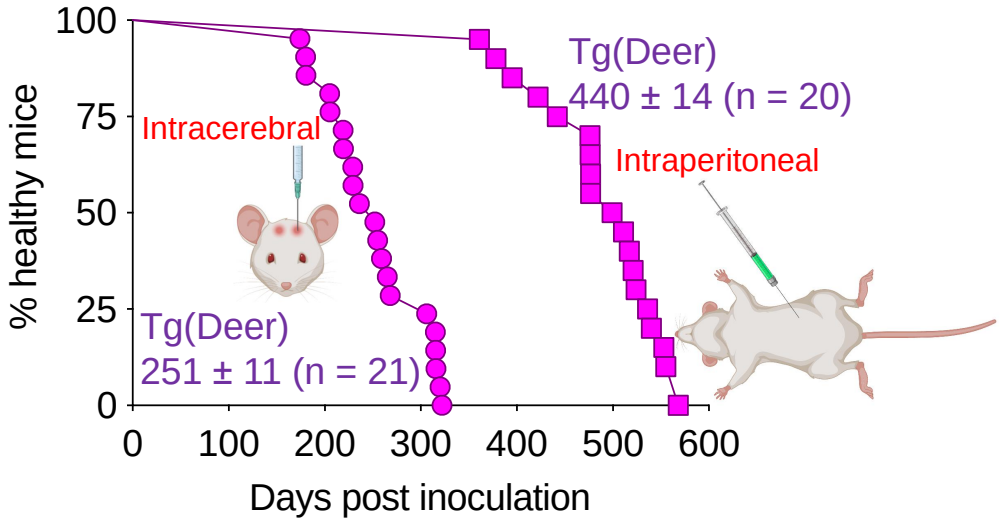


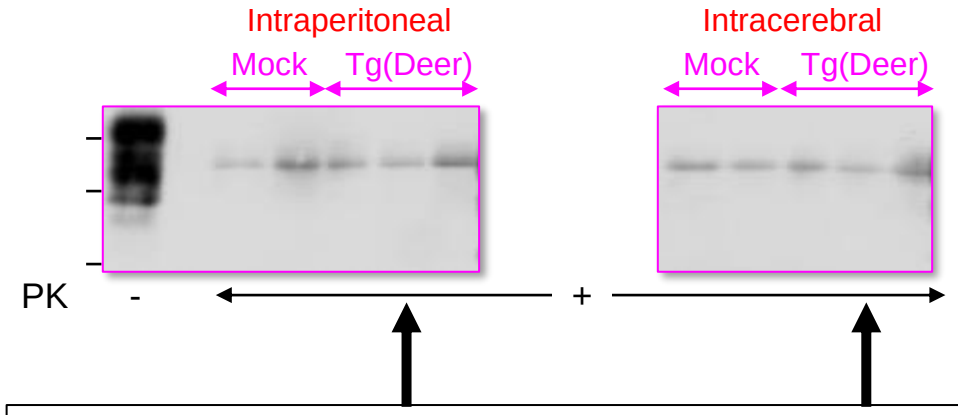
CWD prions are inefficiently transmitted by peripheral routes of exposure in Tg mice

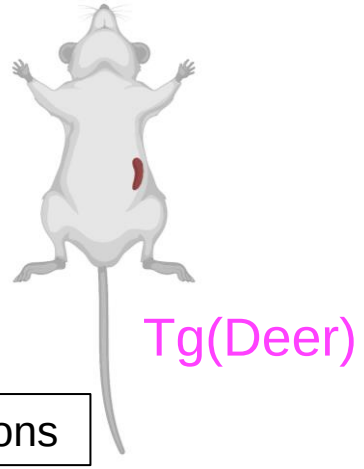


Intraperitoneally-challenged Tg(Deer) mice
have significantly longer incubation times
than their intracerebrally-challenged counterparts

CWD prions are inefficiently transmitted by peripheral routes of exposure in Tg mice

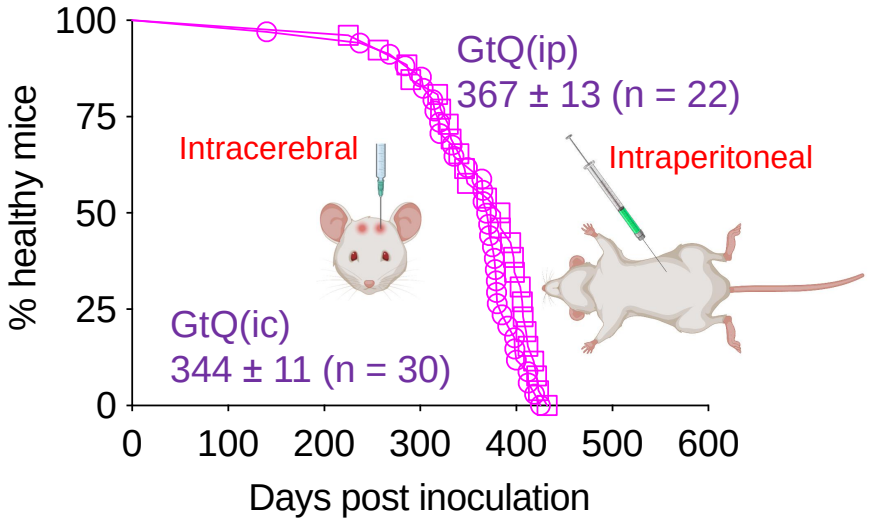






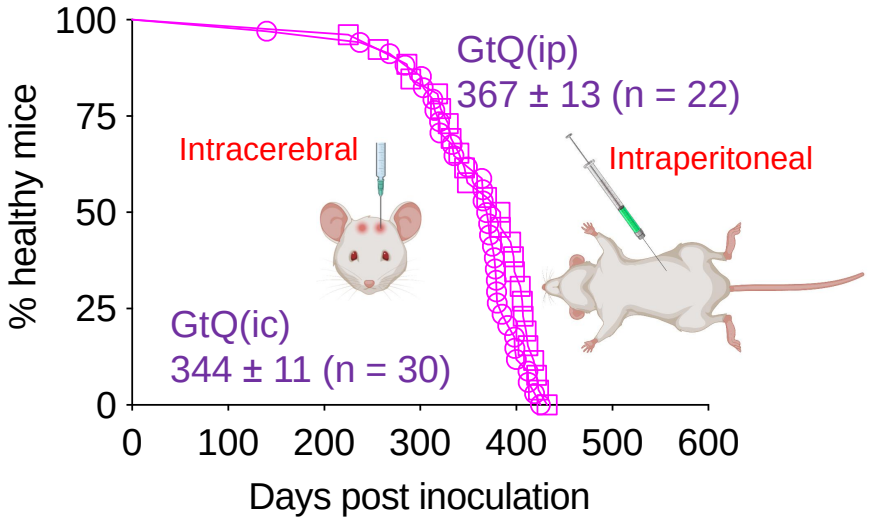
Spleens of diseased Tg mice fail to accumulate CWD prions

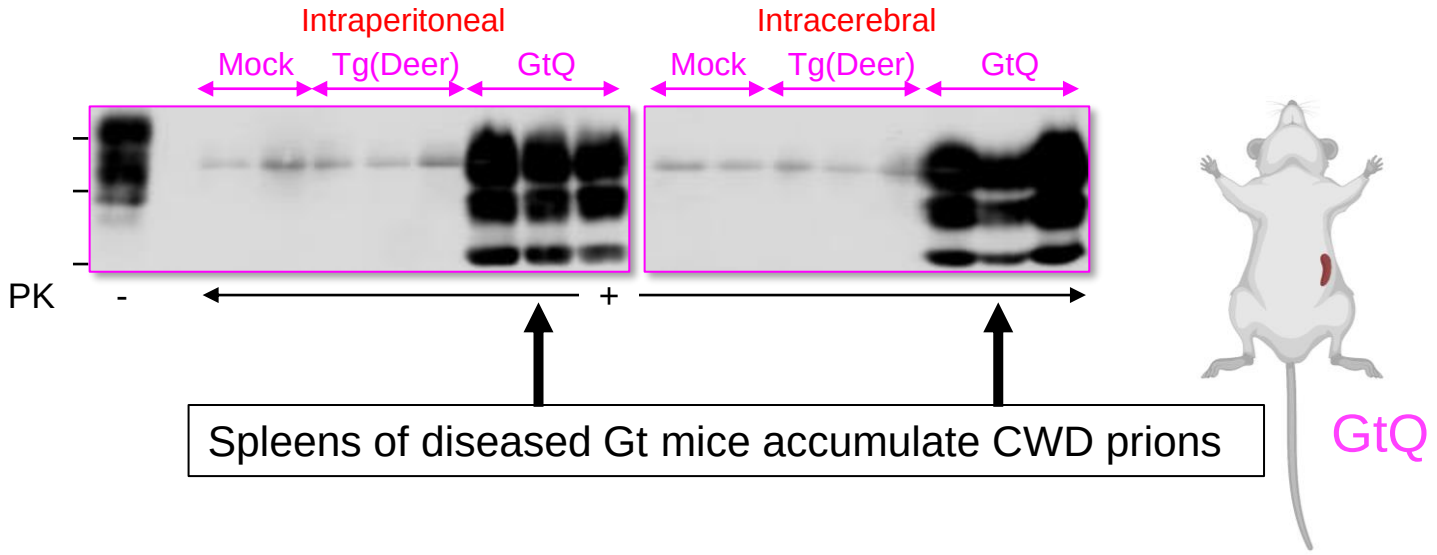
CWD prions are efficiently transmitted by peripheral routes of exposure in Gt mice



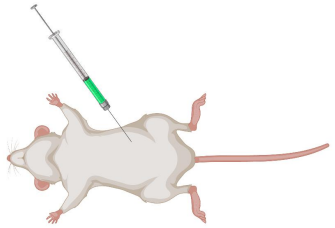
By contrast, intracerebrally and intraperitoneally challenged GtQ mice have indistinguishable incubation times

CWD prions are efficiently transmitted by peripheral routes of exposure in Gt mice

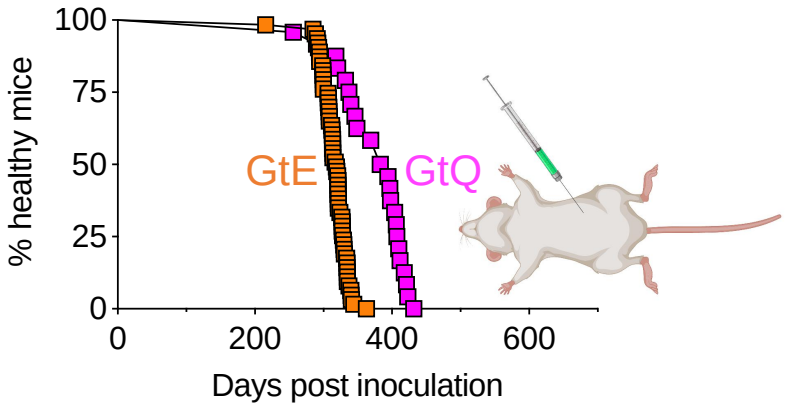




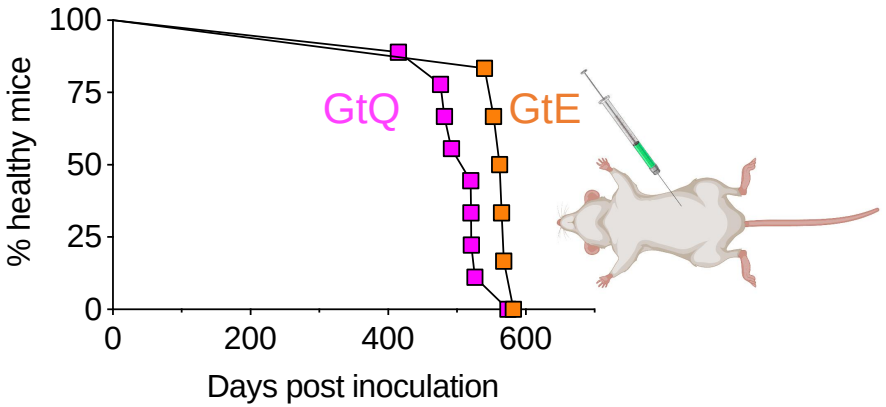
Assessing the lymphotropic properties of CWD strains in Gt mice



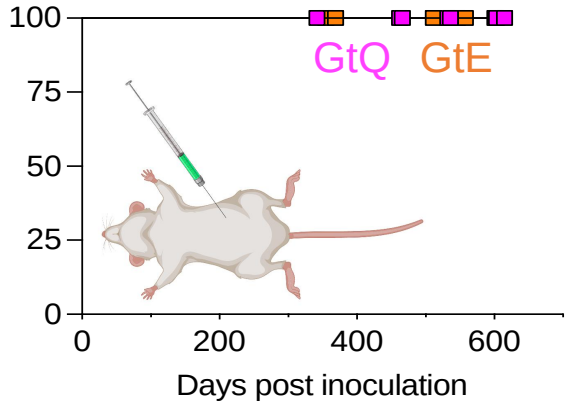
North American CWD



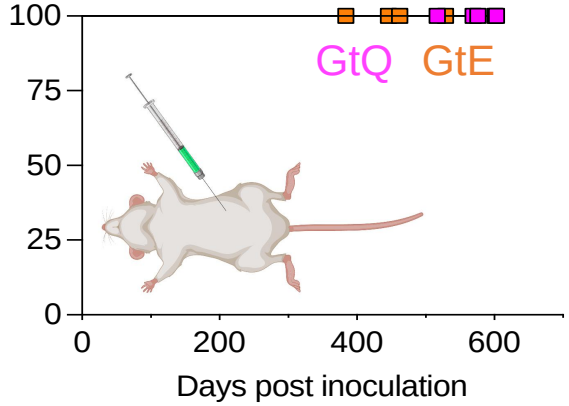
Norway Reindeer 2 (R-NO2)



Norway Moose 1 (M-NO1)

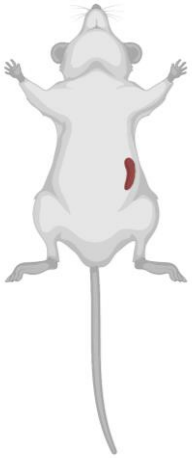


Norway Moose 2 (M-NO2)



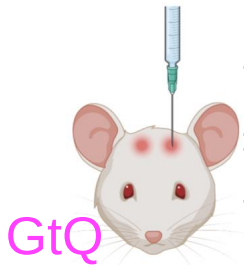
Norwegian moose CWD prions fail to cause disease following intraperitoneal challenge of GtQ or GtE mice

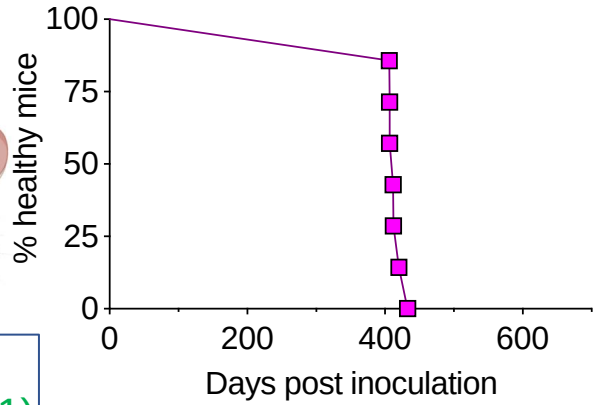
Assessing the lymphotropic properties of CWD strains in Gt mice



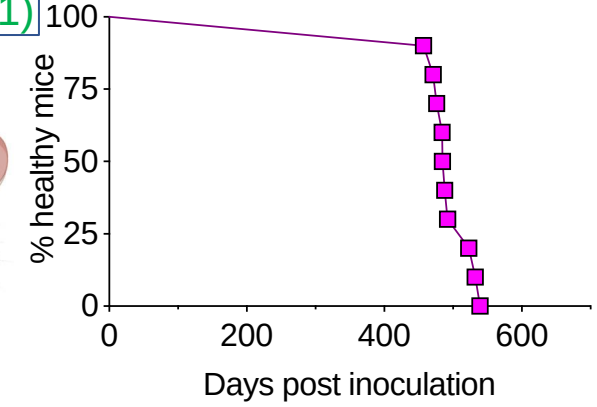
Splenic prions in diseased Gt mice inoculated with North American CWD prions and Norwegian reindeer CWD prions

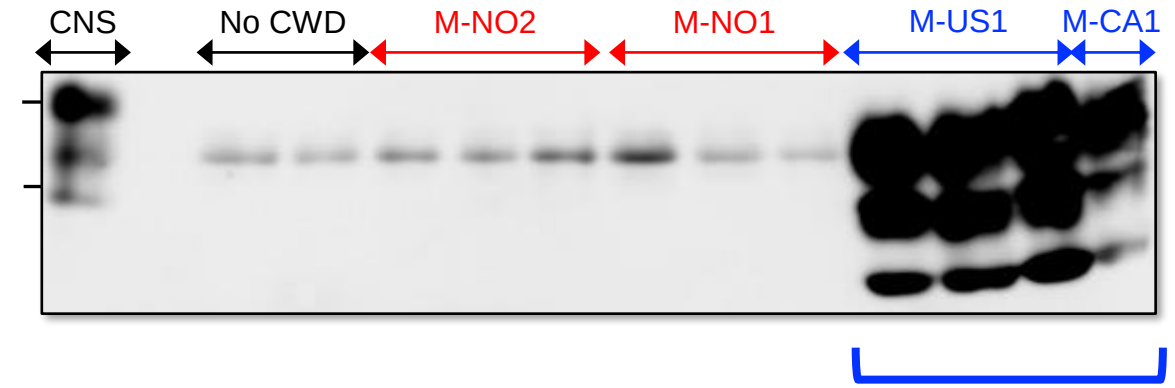
US Moose 1 (M-US1)

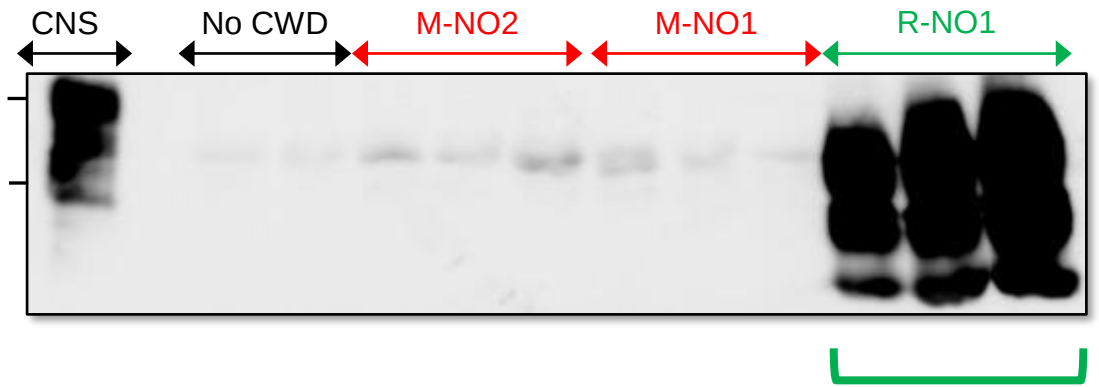




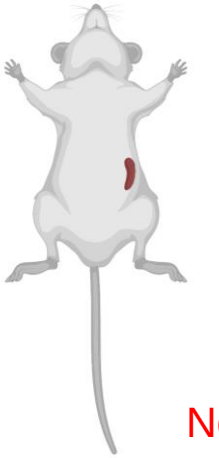
Norwegian reindeer (R-NO1)



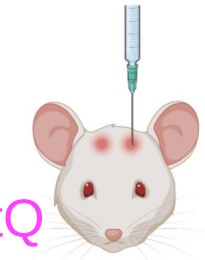




Assessing the lymphotropic properties of CWD strains in Gt mice

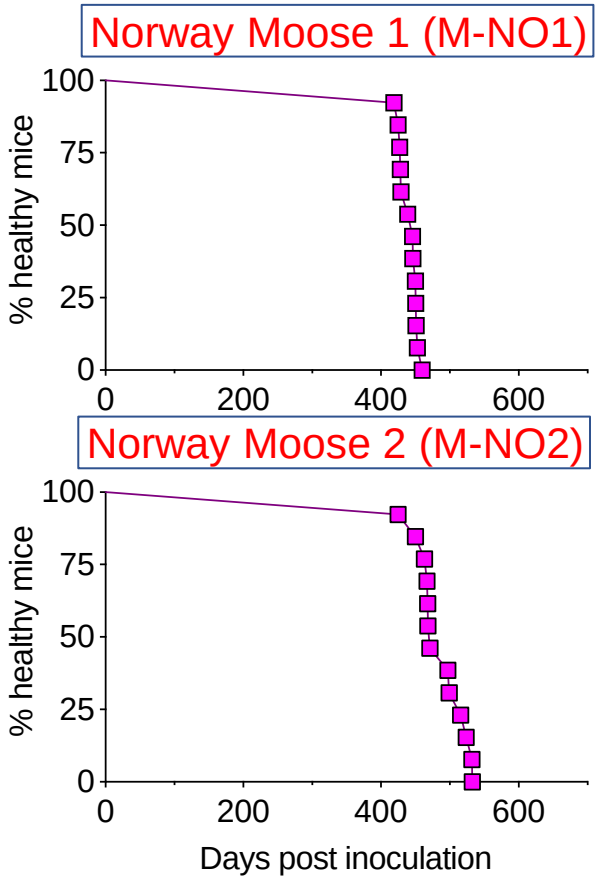


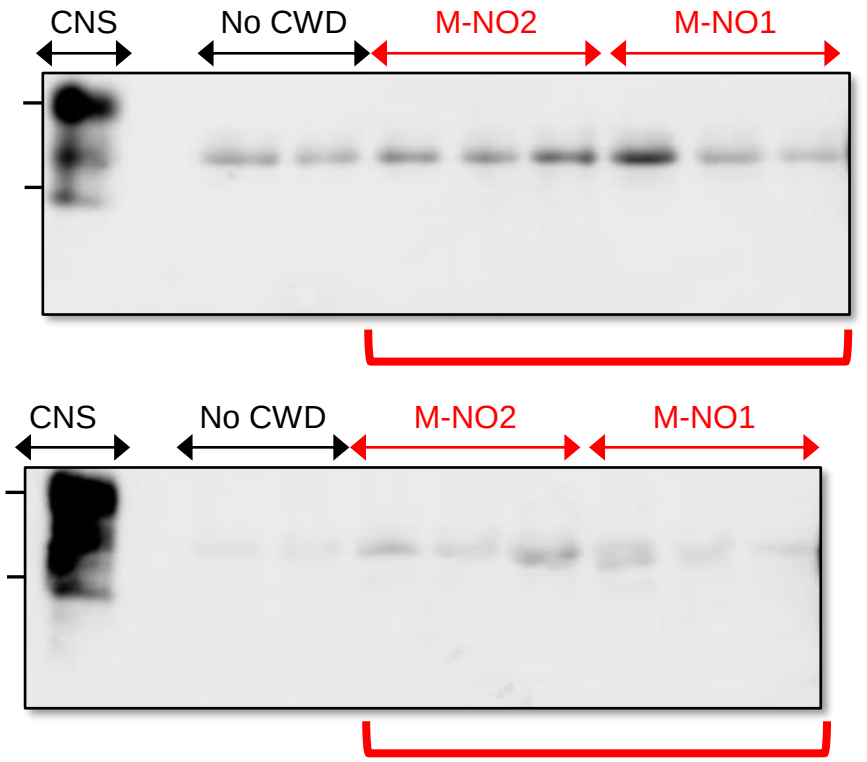
Norwegian Moose CWD



GtQ

No prions in the spleens of diseased GtQ mice inoculated with Norwegian moose CWD prions



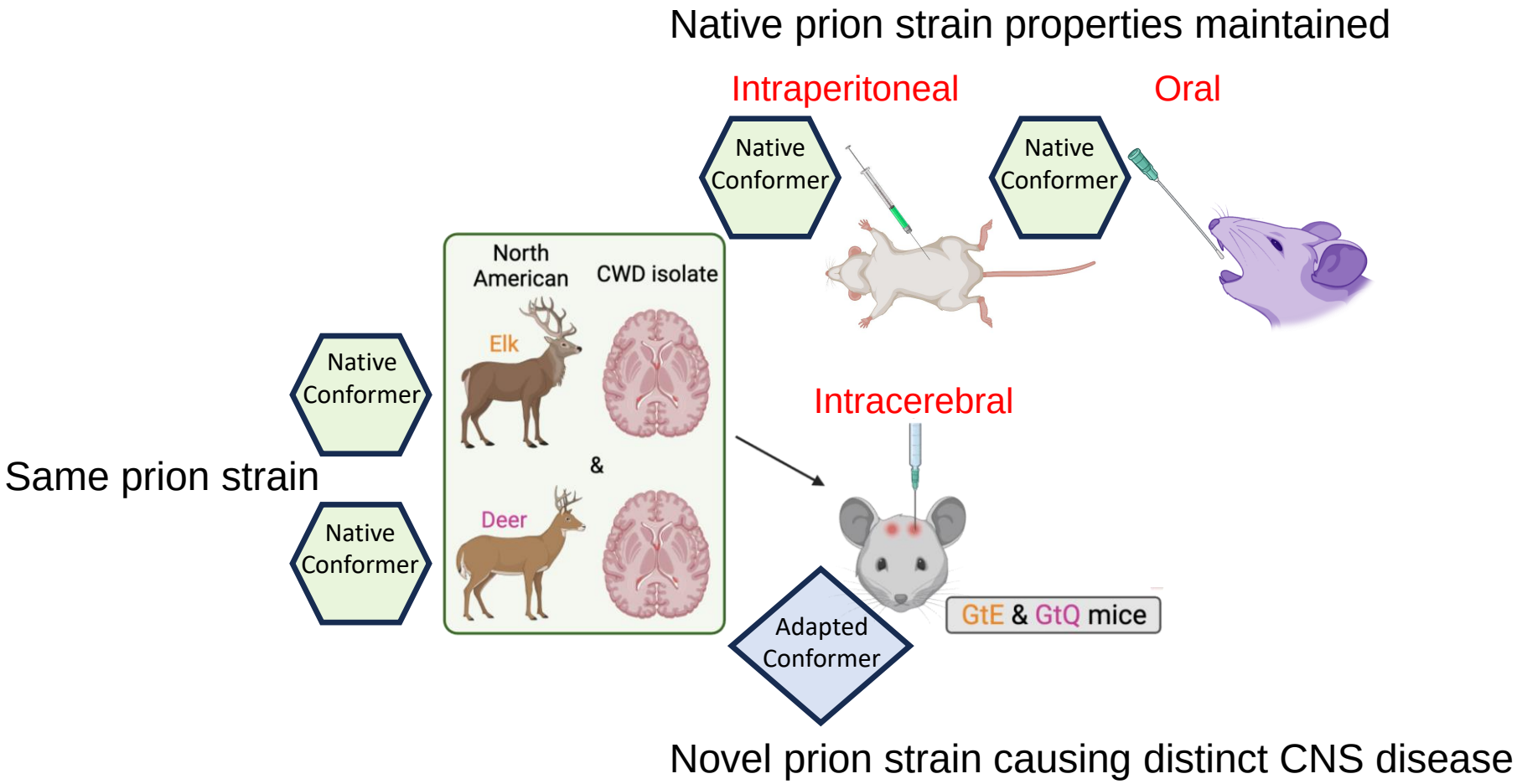


GtQ

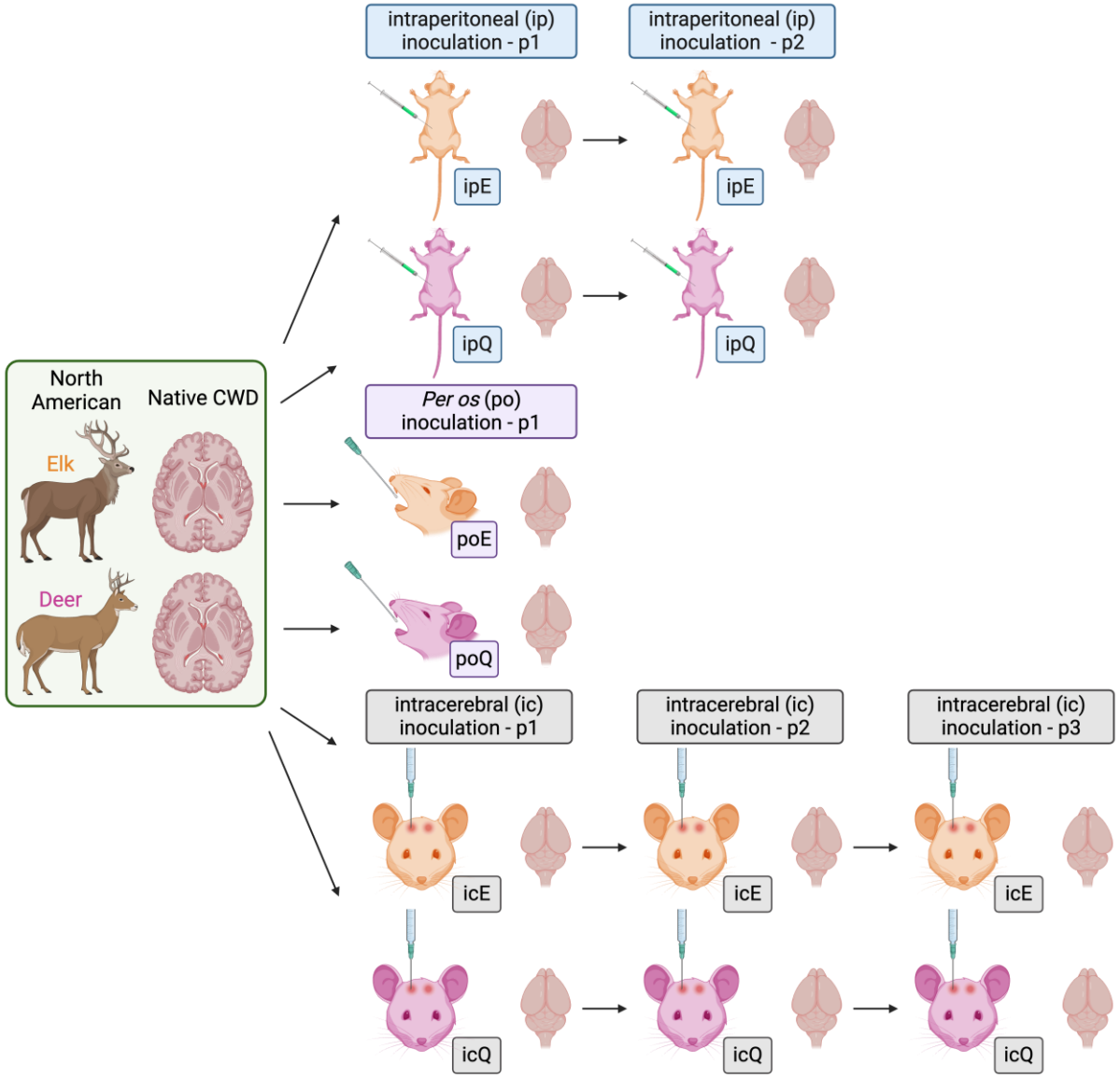
Summary - 2

- North American CWD prions are inefficiently transmitted by peripheral routes of exposure in Tg mice.
- Inoculated Tg mice fail to accumulate CWD prions in the spleen.
- North American CWD prions are efficiently transmitted by peripheral routes of exposure in Gt mice.
- North American CWD prions and Norwegian reindeer CWD prions are lymphotropic.
- Norwegian moose CWD prions are non-lymphotropic.
- Gt mice are susceptible to peripheral challenges with lymphotropic CWD strains.
- Peripheral challenges of Gt with non-lymphotropic strains fails to cause disease.

Propagation of different strains during peripheral and intracerebral transmissions of CWD to Gt mice



How does route of CWD prion exposure impact CNS dysfunction and strain propagation in Gt mice?



Analysis of the strain properties of the resulting CNS prions

ipE prions

ipQ prions

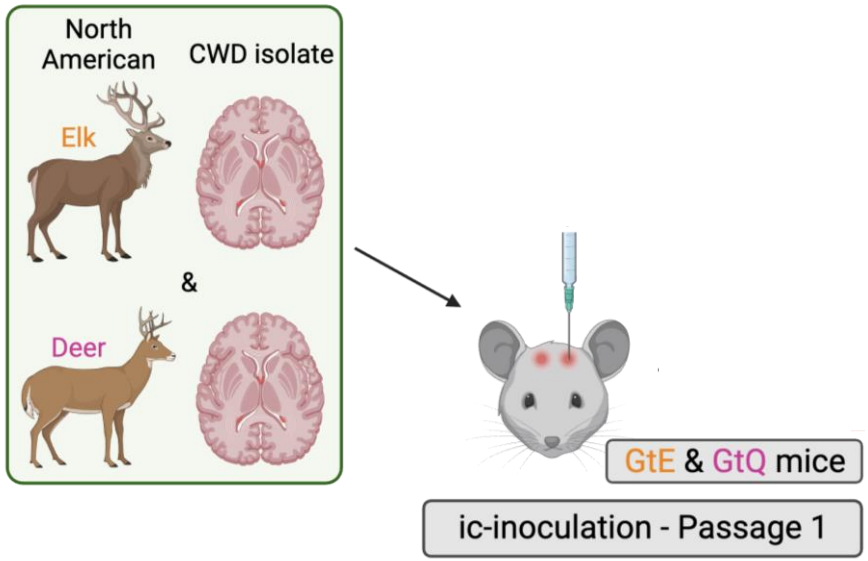
poE prions

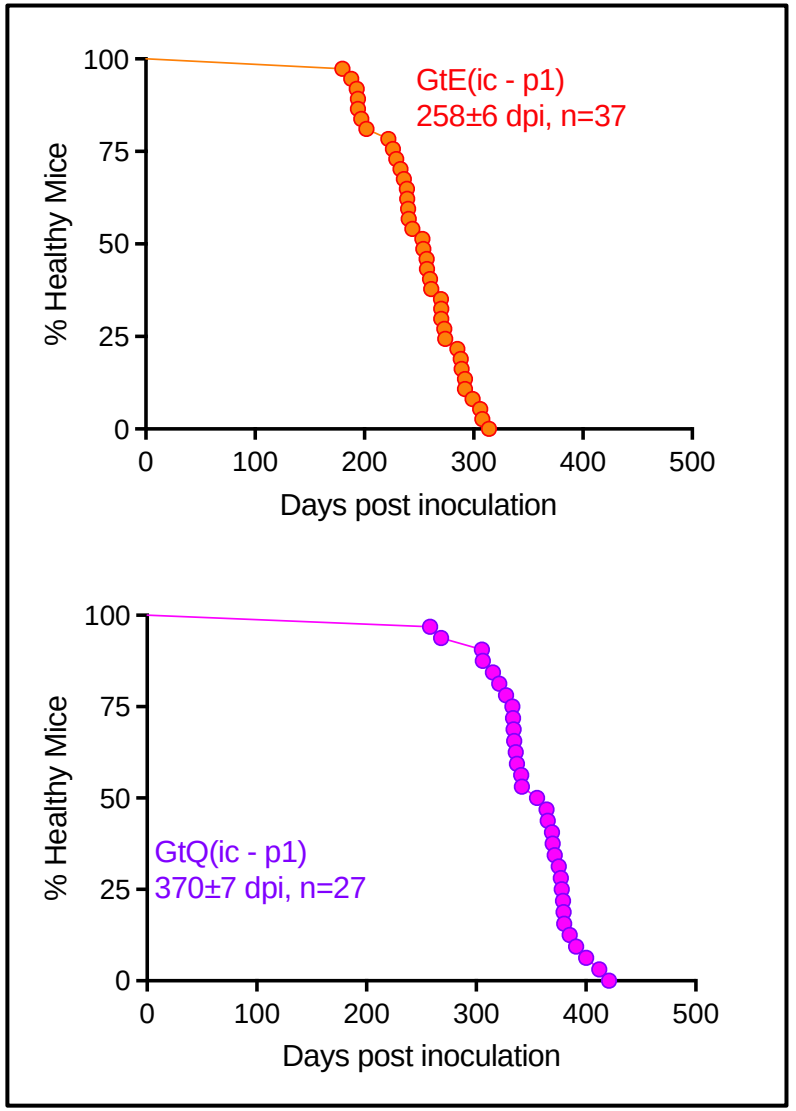
poQ prions

icE prions

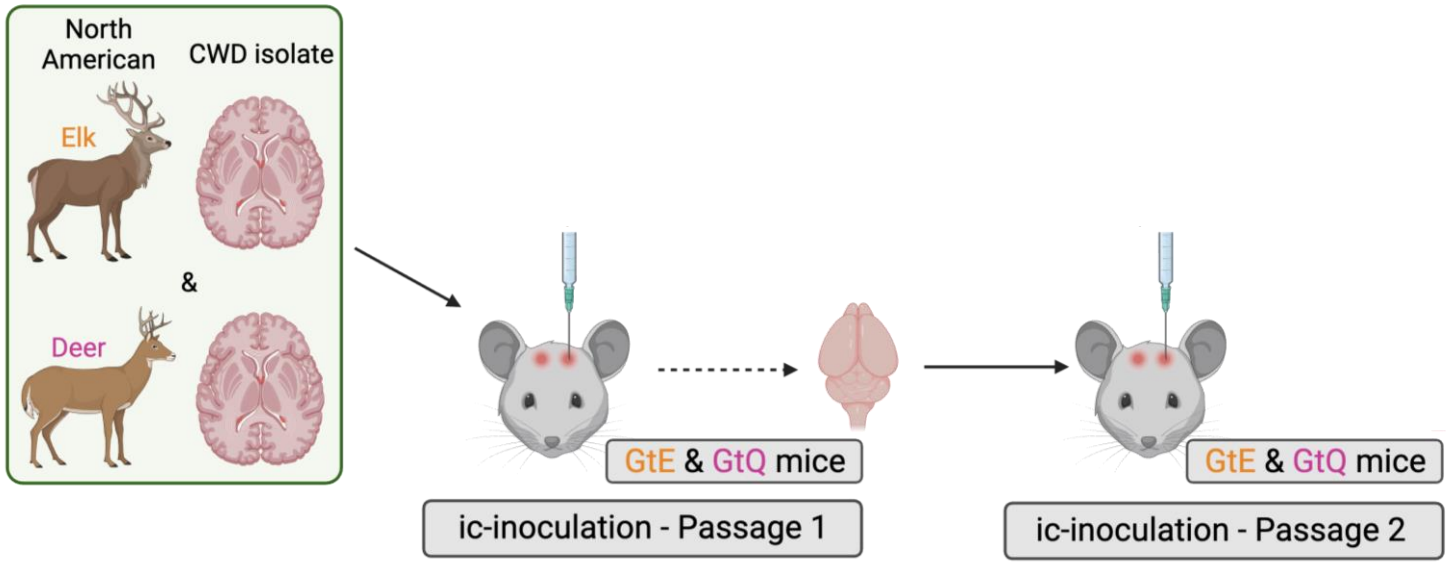
icQ prions

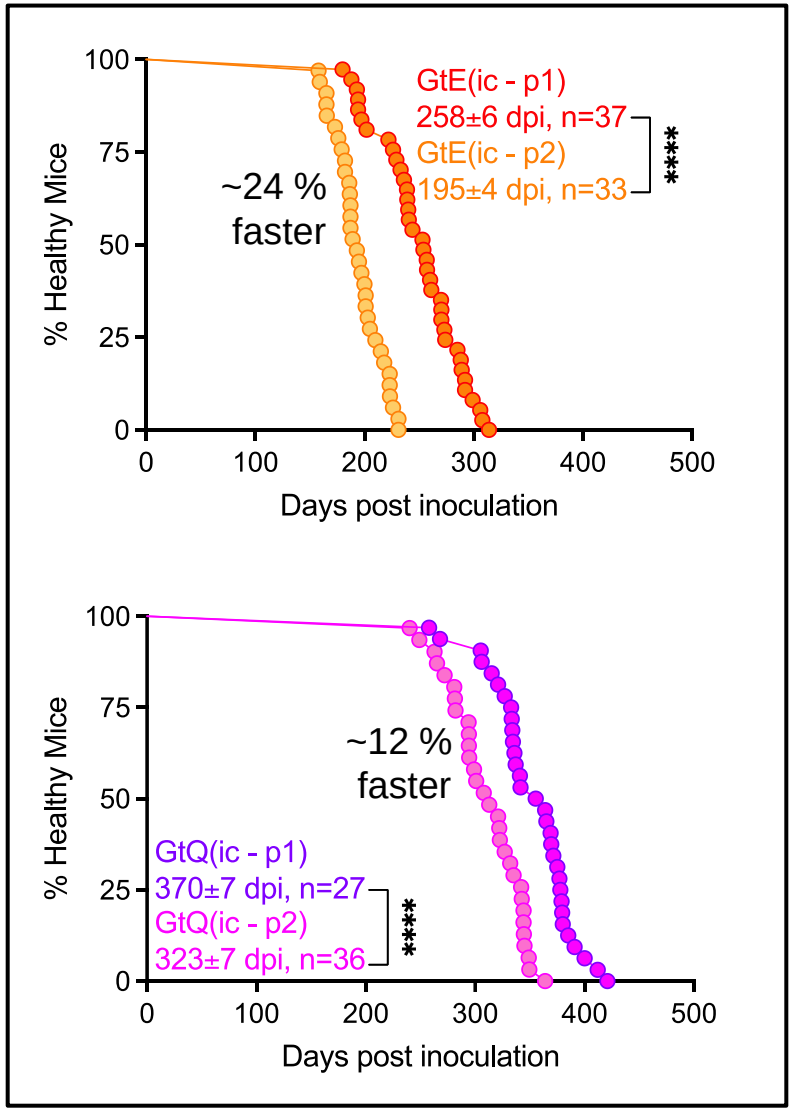
Altered CWD prion replication kinetics during iterative intracerebral transmissions in Gt mice





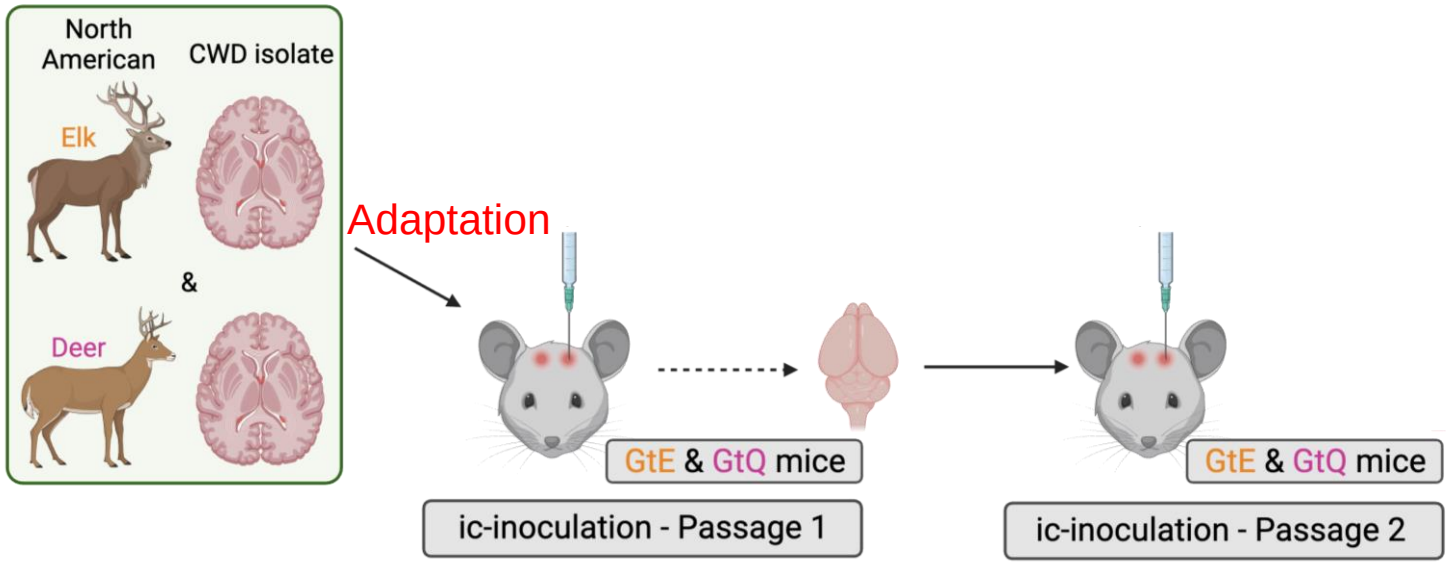
Altered CWD prion replication kinetics during iterative intracerebral transmissions in Gt mice

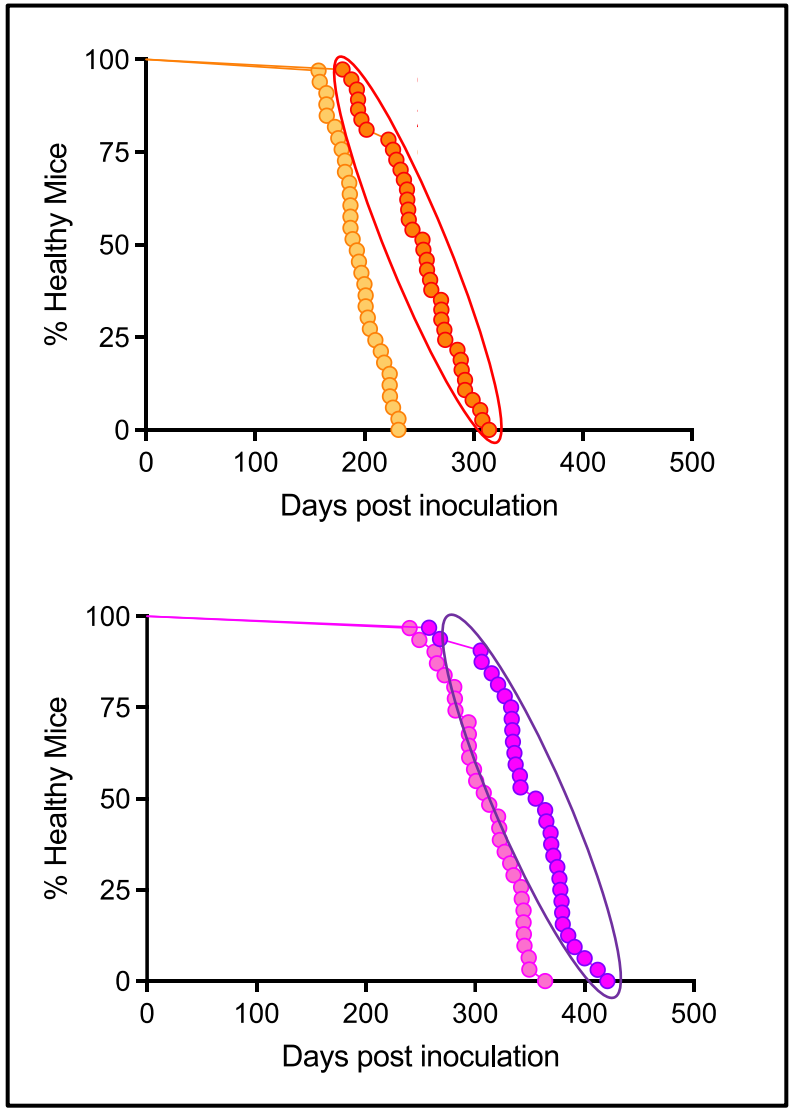




Altered CWD prion replication kinetics during iterative intracerebral transmissions in Gt mice

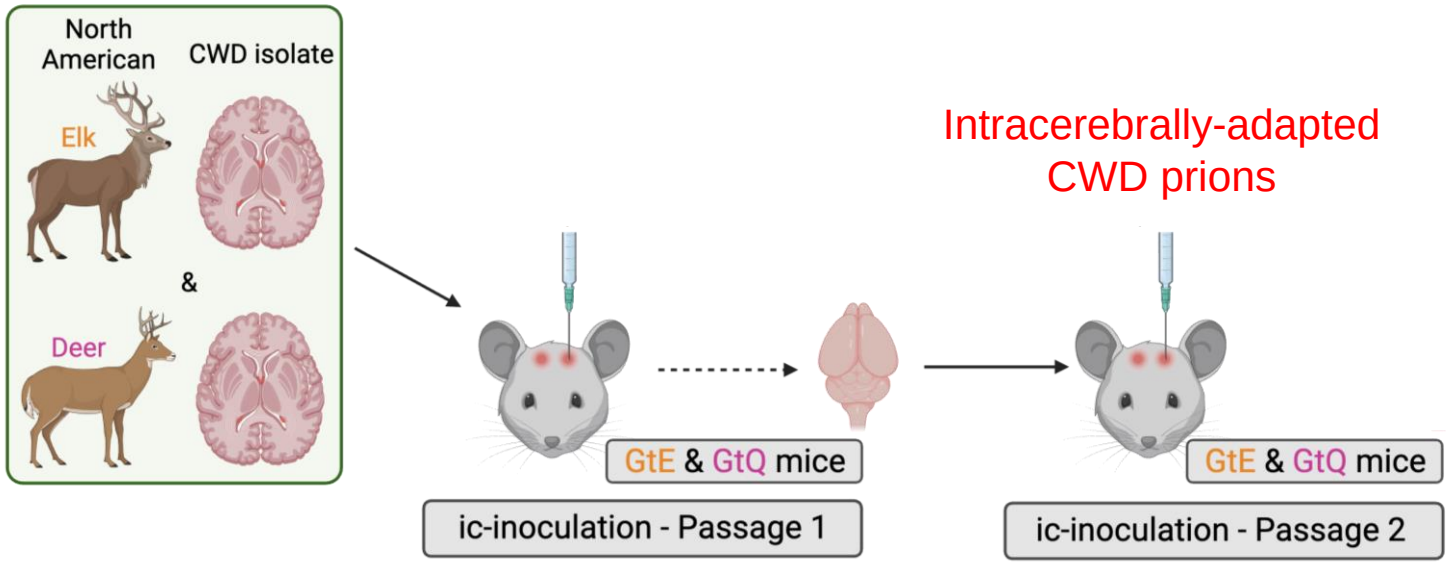
Adaptation of native CWD prions on primary intracerebral passages

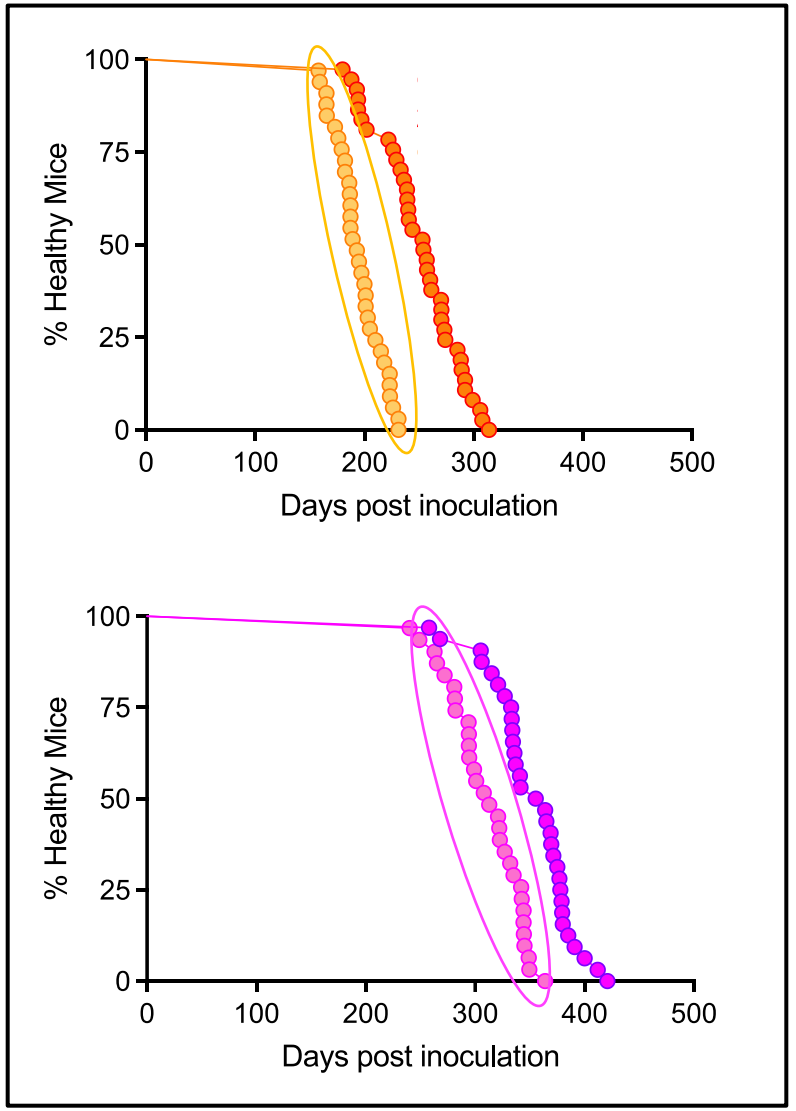




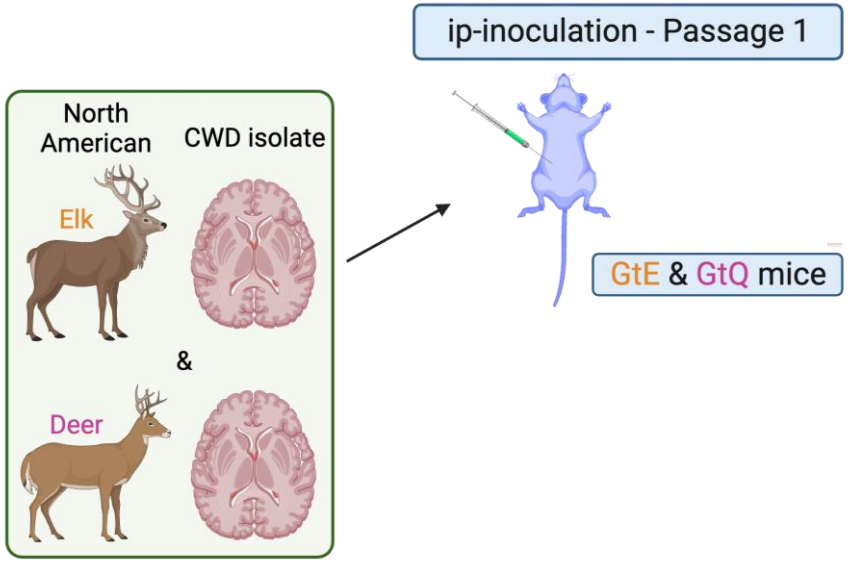
Altered CWD prion replication kinetics during iterative intracerebral transmissions in Gt mice

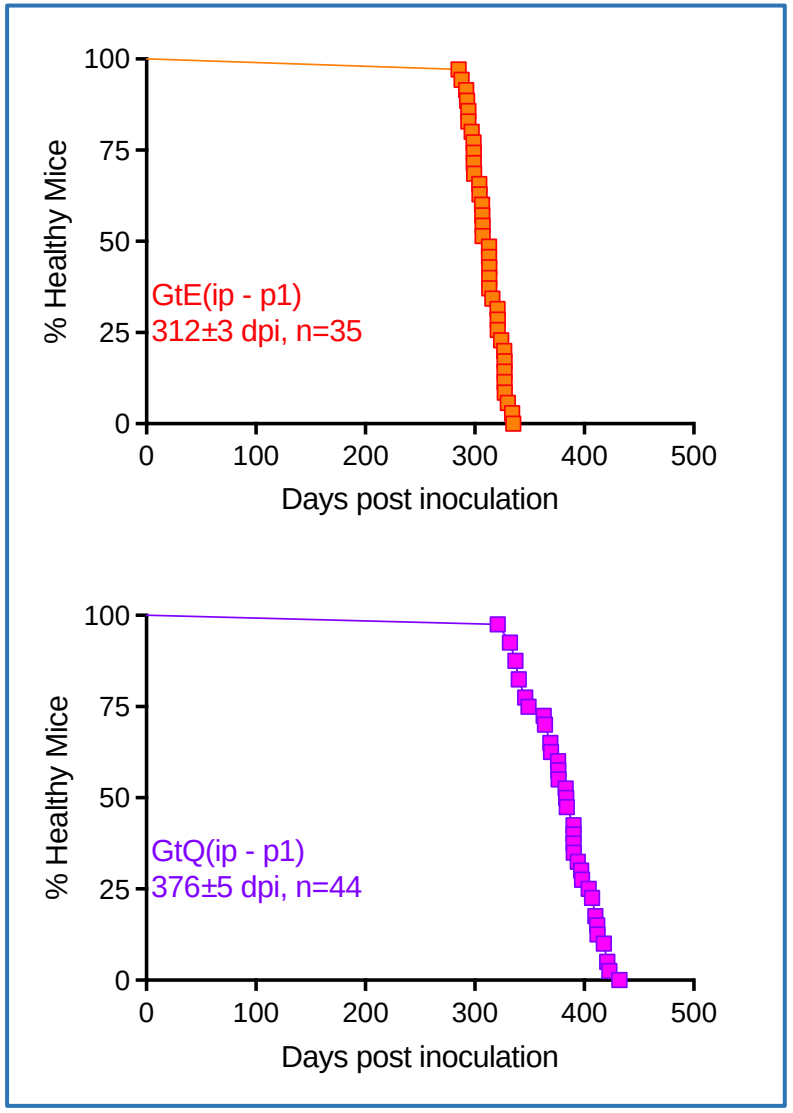
Adaptation of native CWD prions on primary intracerebral passages produces intracerebrally-optimized strains with more rapid kinetics on second passages



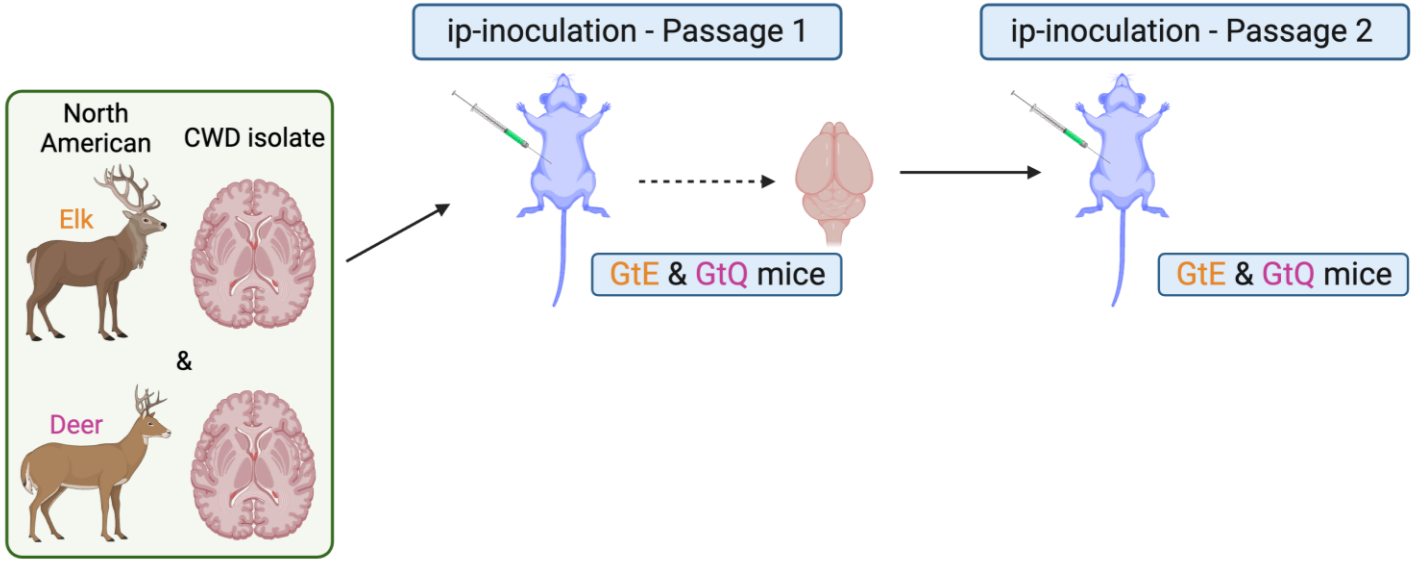


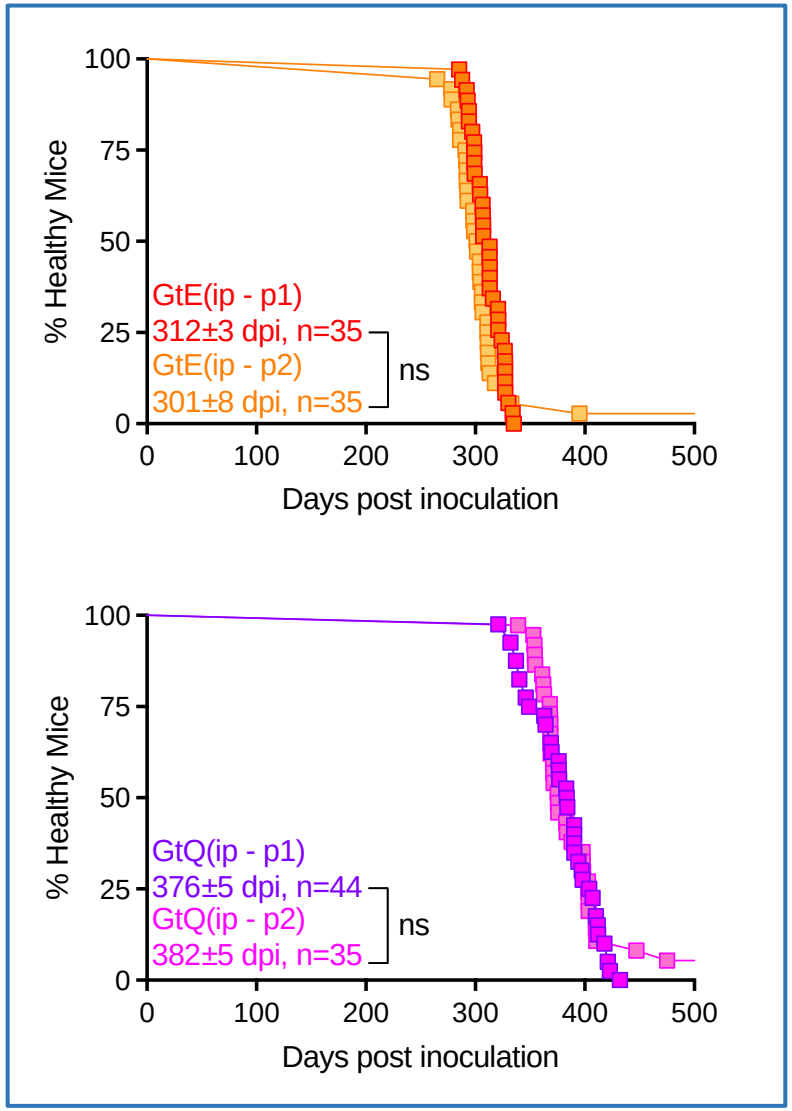
Unchanging CWD prion replication kinetics during iterative peripheral transmissions in Gt mice



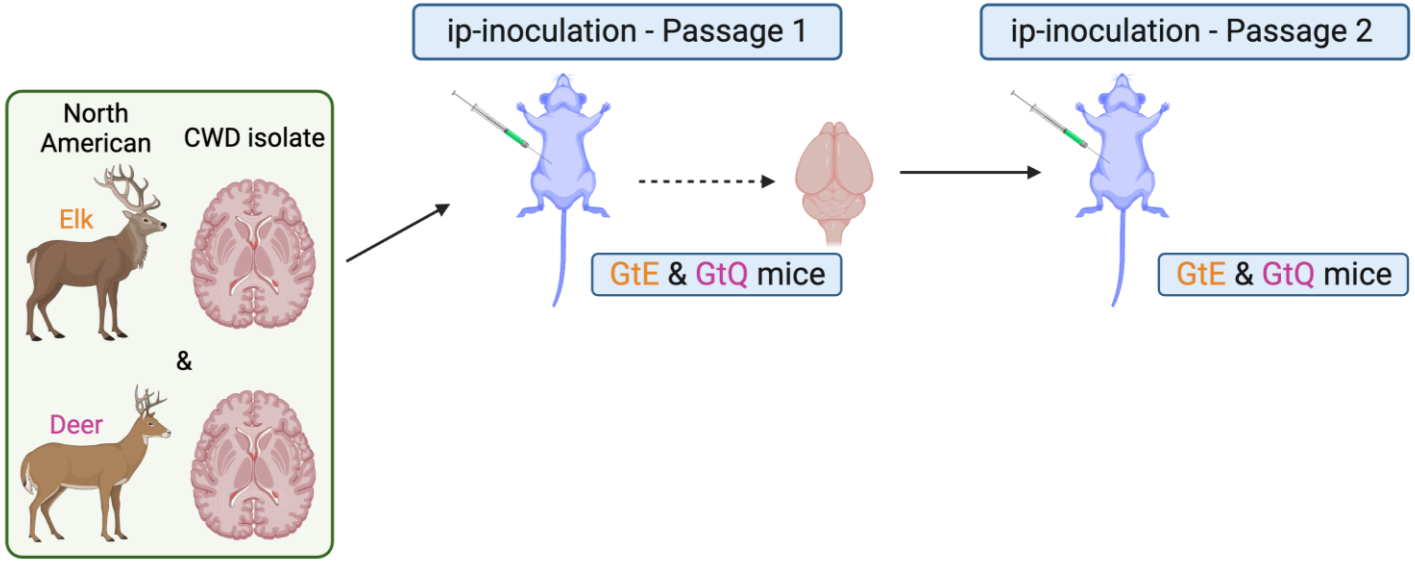


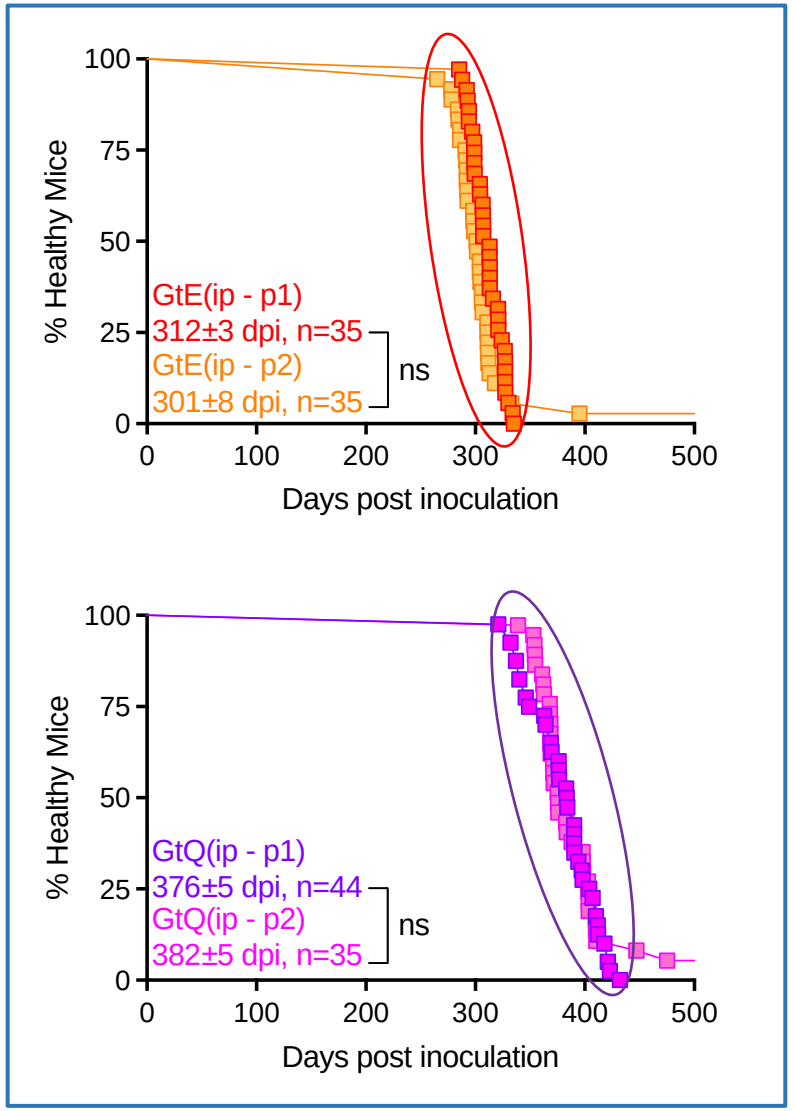
Unchanging CWD prion replication kinetics during iterative peripheral transmissions in Gt mice





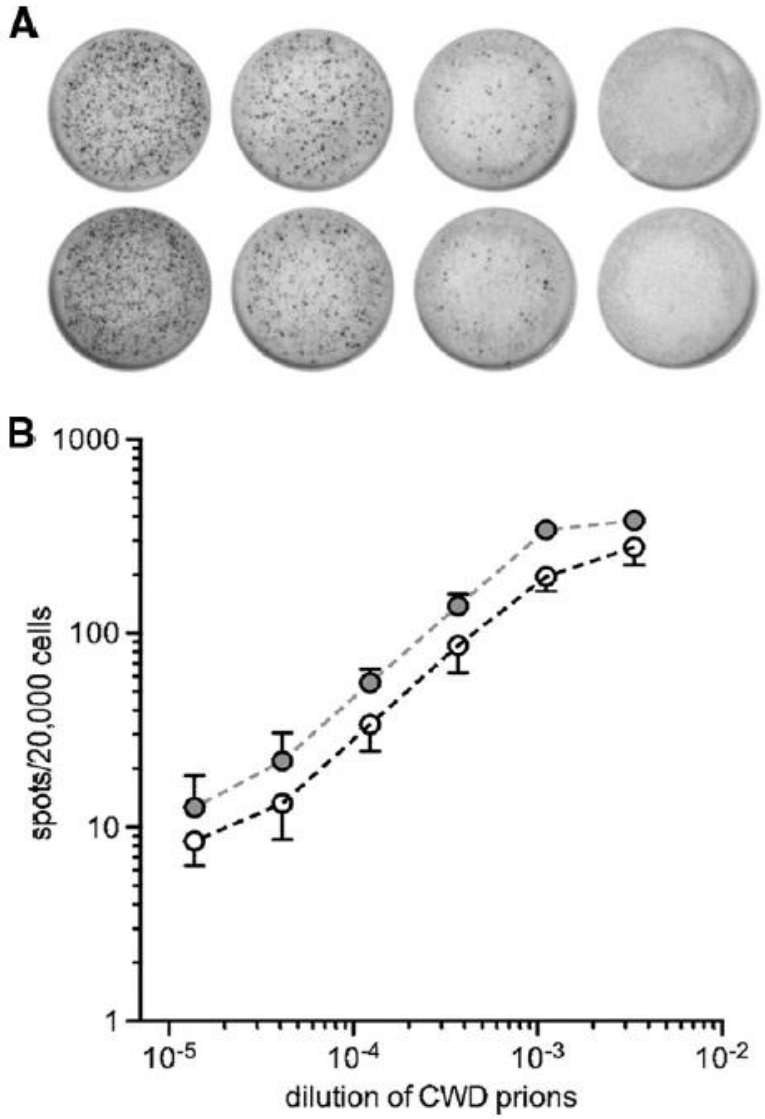
Unchanging CWD prion replication kinetics during iterative peripheral transmissions in Gt mice





The invariant incubation times on first and second intraperitoneal passages indicates that native elk and deer CWD prions are fully adapted for propagation by peripheral transmission in GtE and GtQ mice

Quantifying prion levels in the CNS of intraperitoneally- and intracerebrally-challenged Gt mice



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0022-538X/10/$12.00 doi:10.1128/JVI.00633-10

Cell-Based Quantification of Chronic Wasting Disease Prions[▽†]

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Catherine Graham,[4] and Glenn Telling[1,2,3*]

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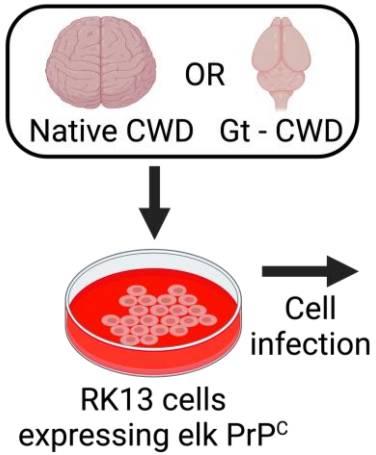
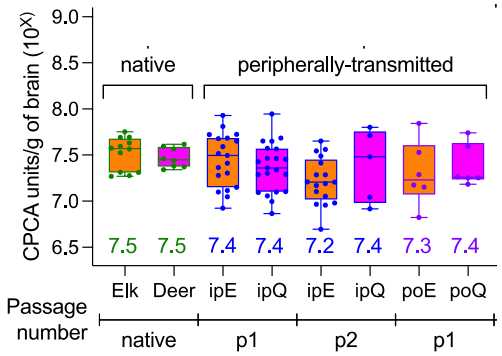
To address whether the accelerated kinetics during intracerebral transmissions were associated with changes in the levels of prion replication, we used the cervid prion cell assay to compare titers of native elk and deer CWD prions with those of icE and icQ prions.

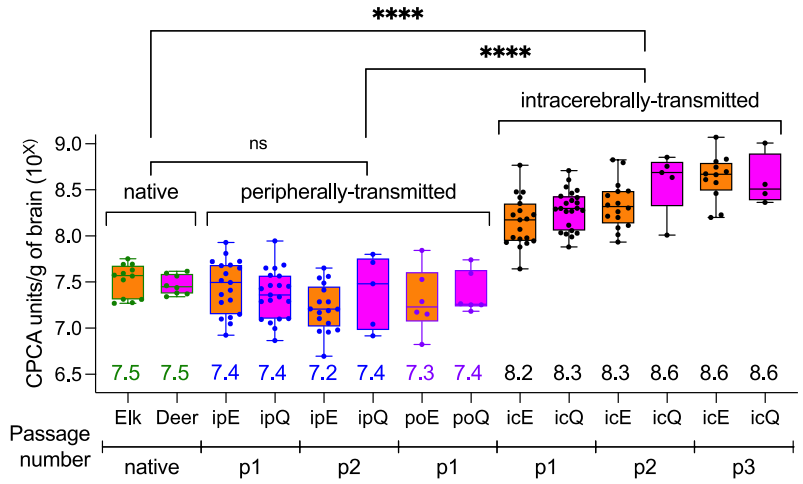


Different prion levels in the CNS of intraperitoneally- and intracerebrally-challenged Gt mice



ipE and ipQ prion titers were equivalent to native CWD titers

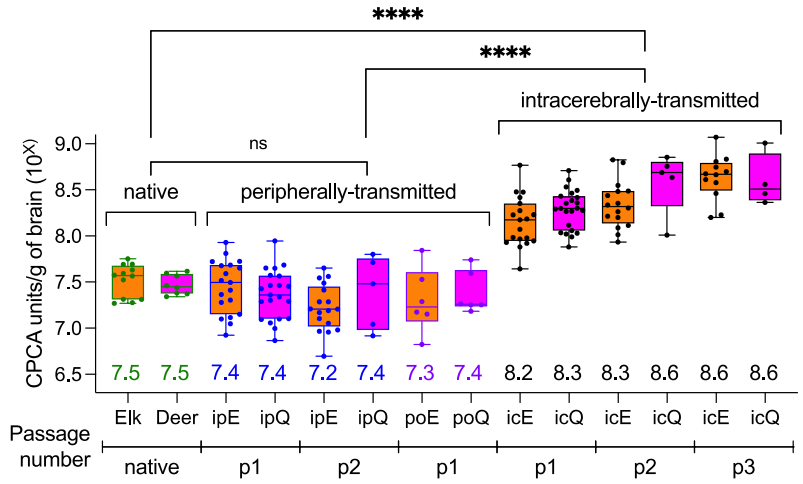
Different prion levels in the CNS of intraperitoneally- and intracerebrally-challenged Gt mice



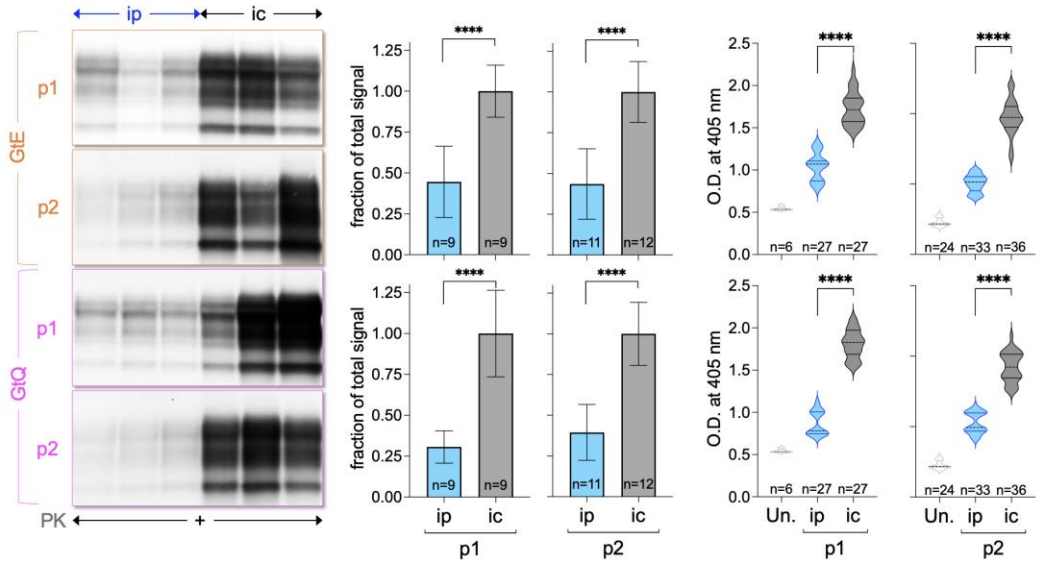
ipE and ipQ prion titers were equivalent to native CWD titers

icE and icQ prion titers were ~ 10-fold higher than native CWD titers

Different prion levels in the CNS of intraperitoneally- and intracerebrally-challenged Gt mice

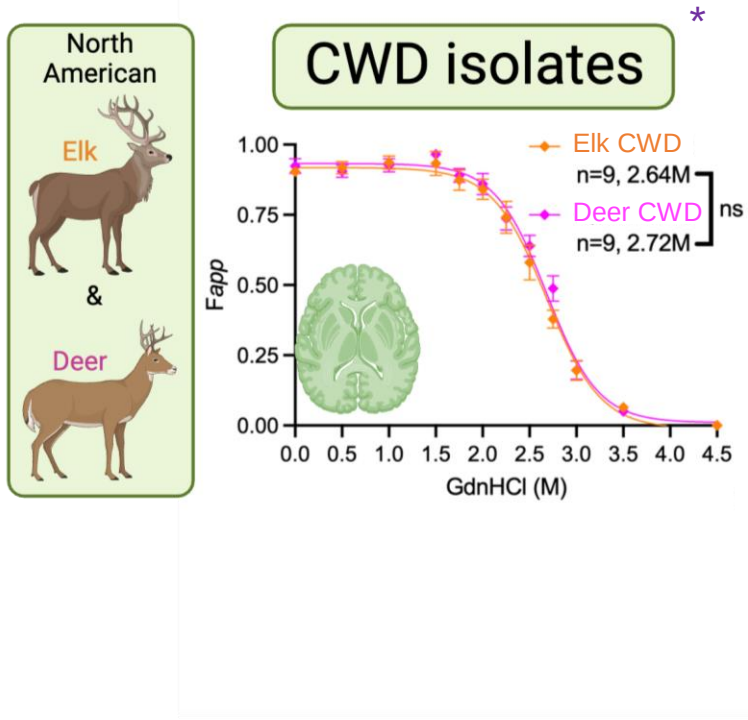


ipE and ipQ prion titers were equivalent to native CWD titers
icE and icQ prion titers were ~ 10-fold higher than native CWD titers



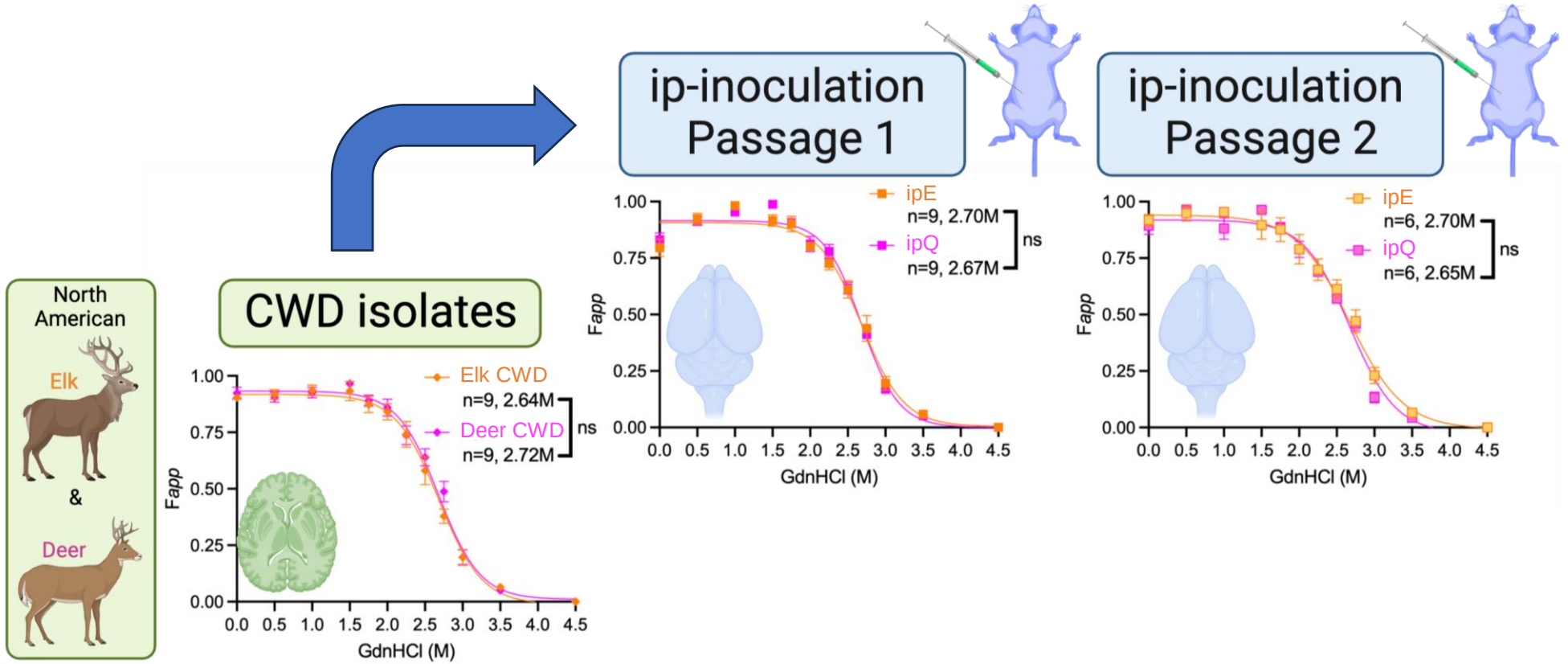
Western blotting and prion-specific ELISA confirmed increased prion levels in the CNS of intracerebrally- compared to intraperitoneally-challenged Gt mice

Convergent conformations of native CWD prions in the CNS of elk and deer



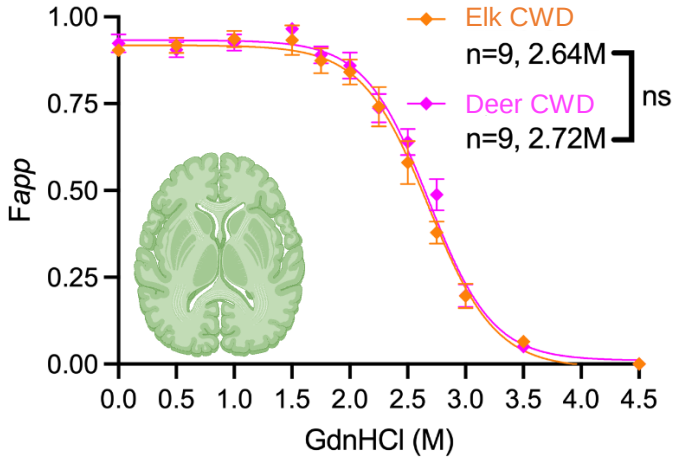
*3 biological replicates,
with 3 technical replicates

ipE and ipQ prions maintain the convergent conformational properties of elk and deer CWD prions

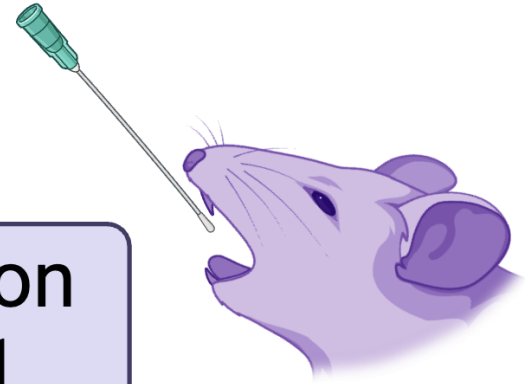


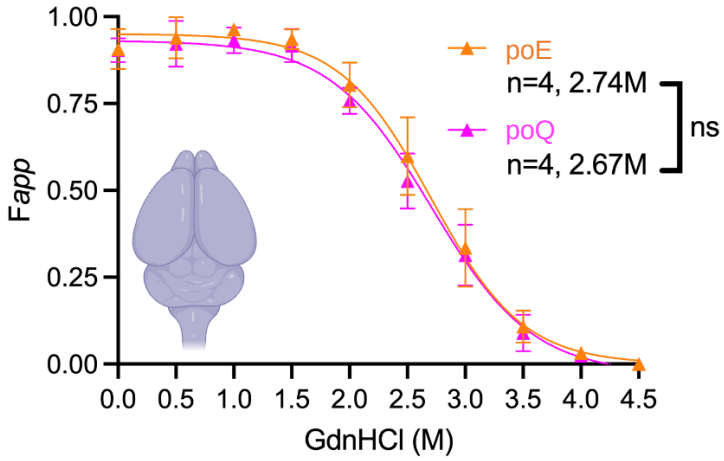
poE and poQ prions also maintain the convergent conformational properties of elk and deer CWD prions

CWD isolates

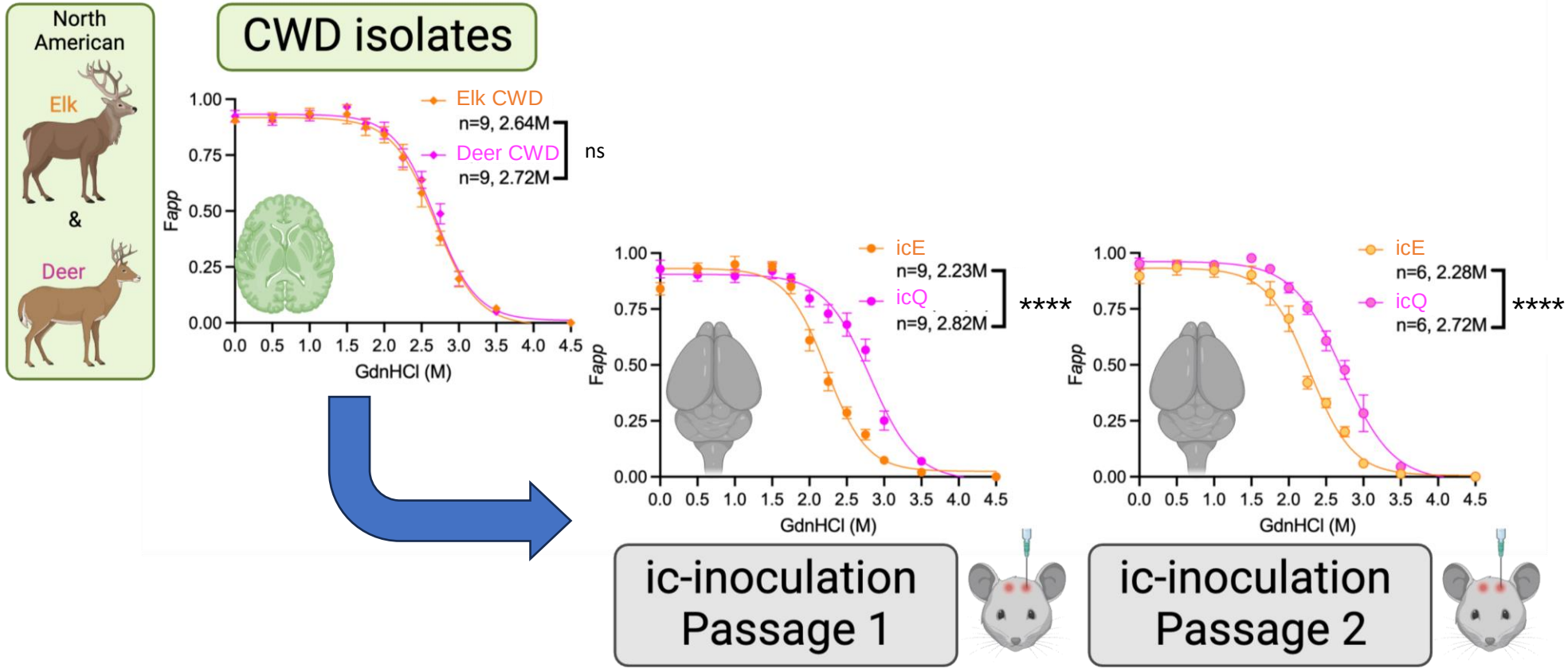


po-inoculation Passage 1

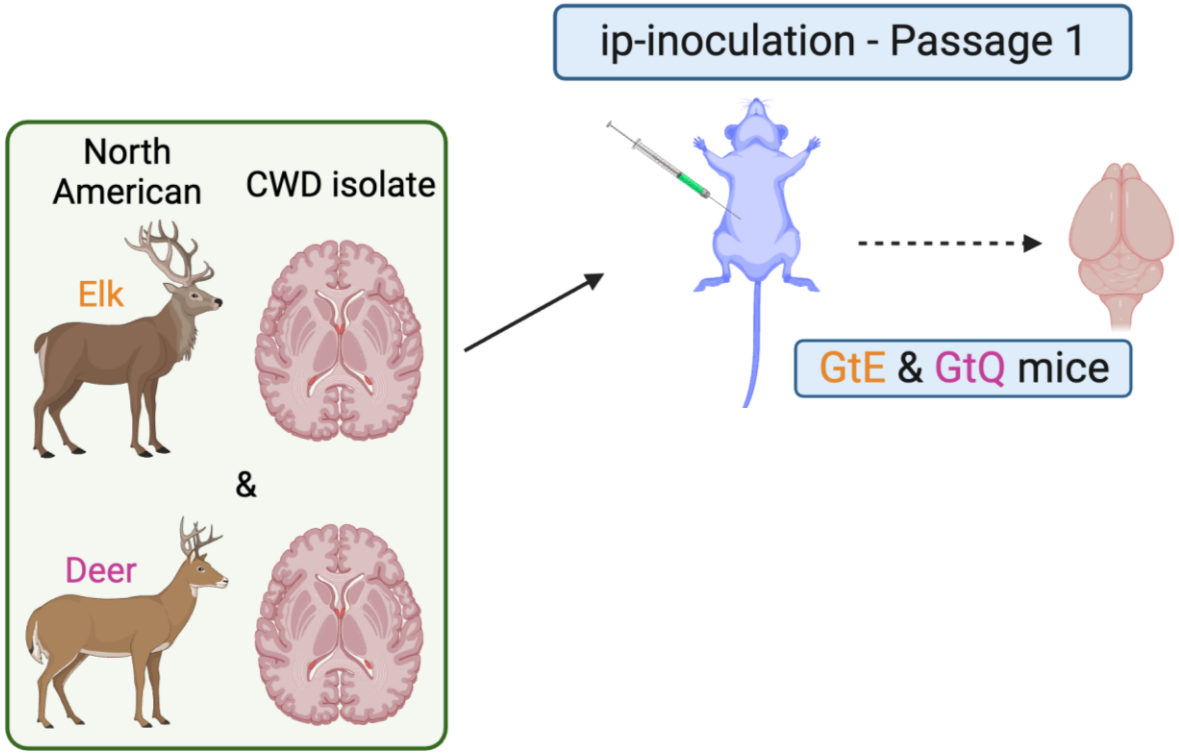


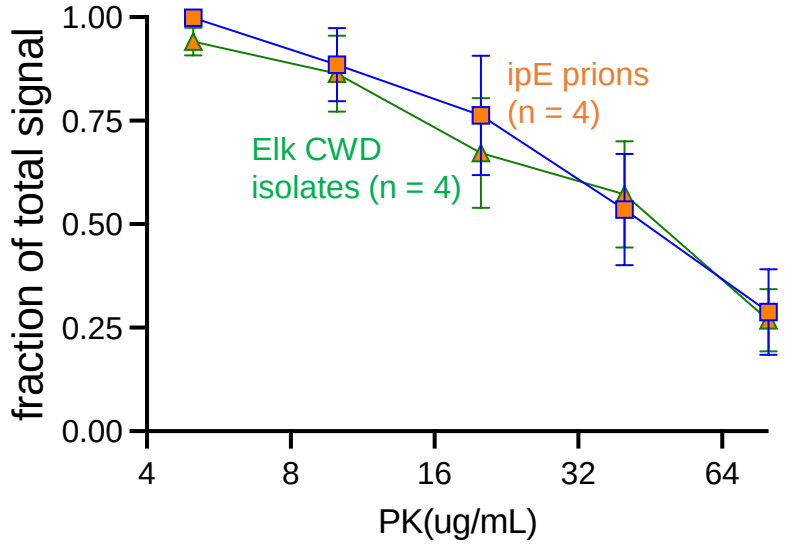


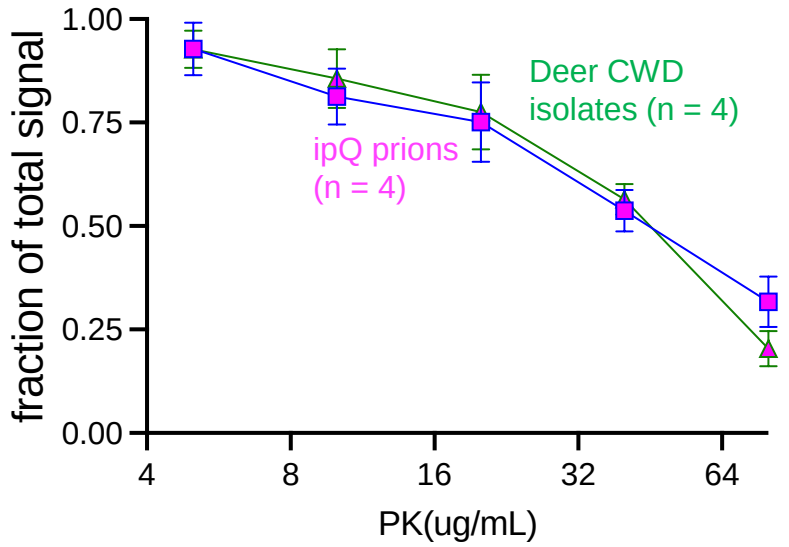
Intracerebral transmissions of CWD prions produce icE and icQ prions with divergent conformations



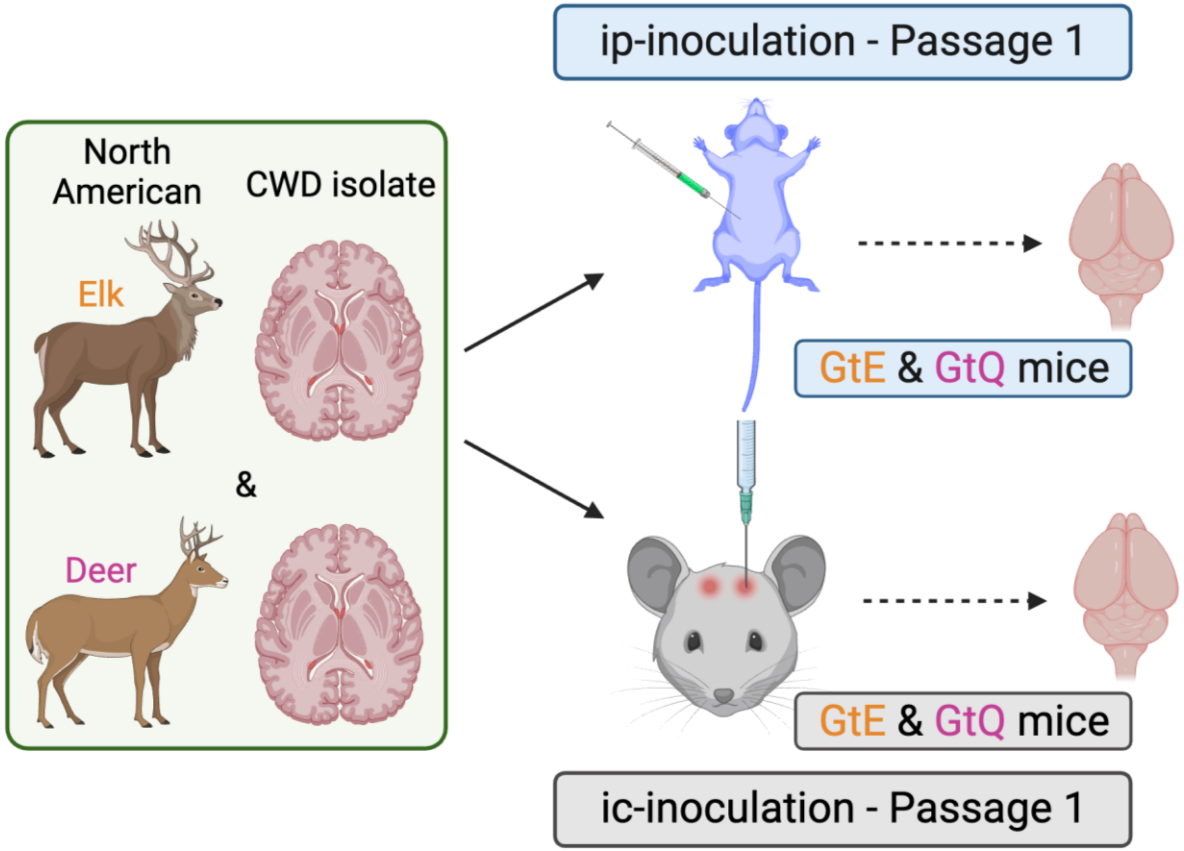
ipE and ipQ prions have protease-sensitivities equivalent to elk and deer CWD prions

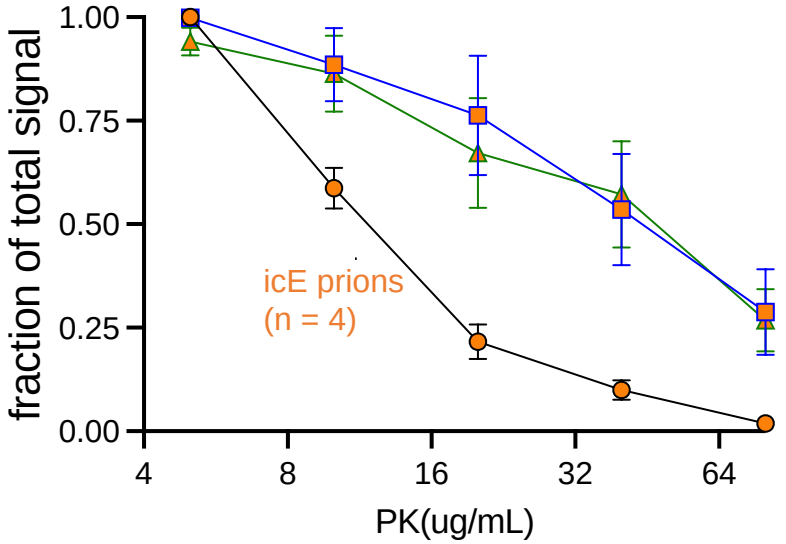


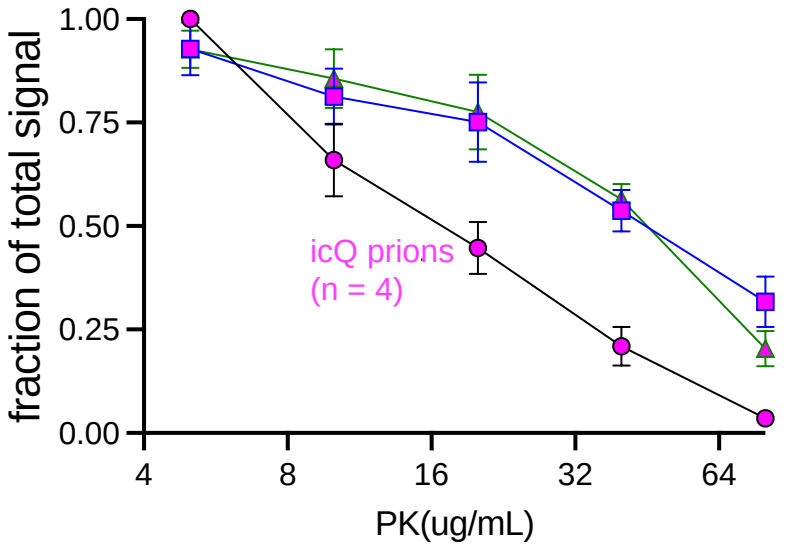




Protease-sensitivities of icE and icQ prions differ from native elk and deer CWD prions







How does route of CWD prion exposure impact CNS dysfunction and strain propagation in Gt mice?

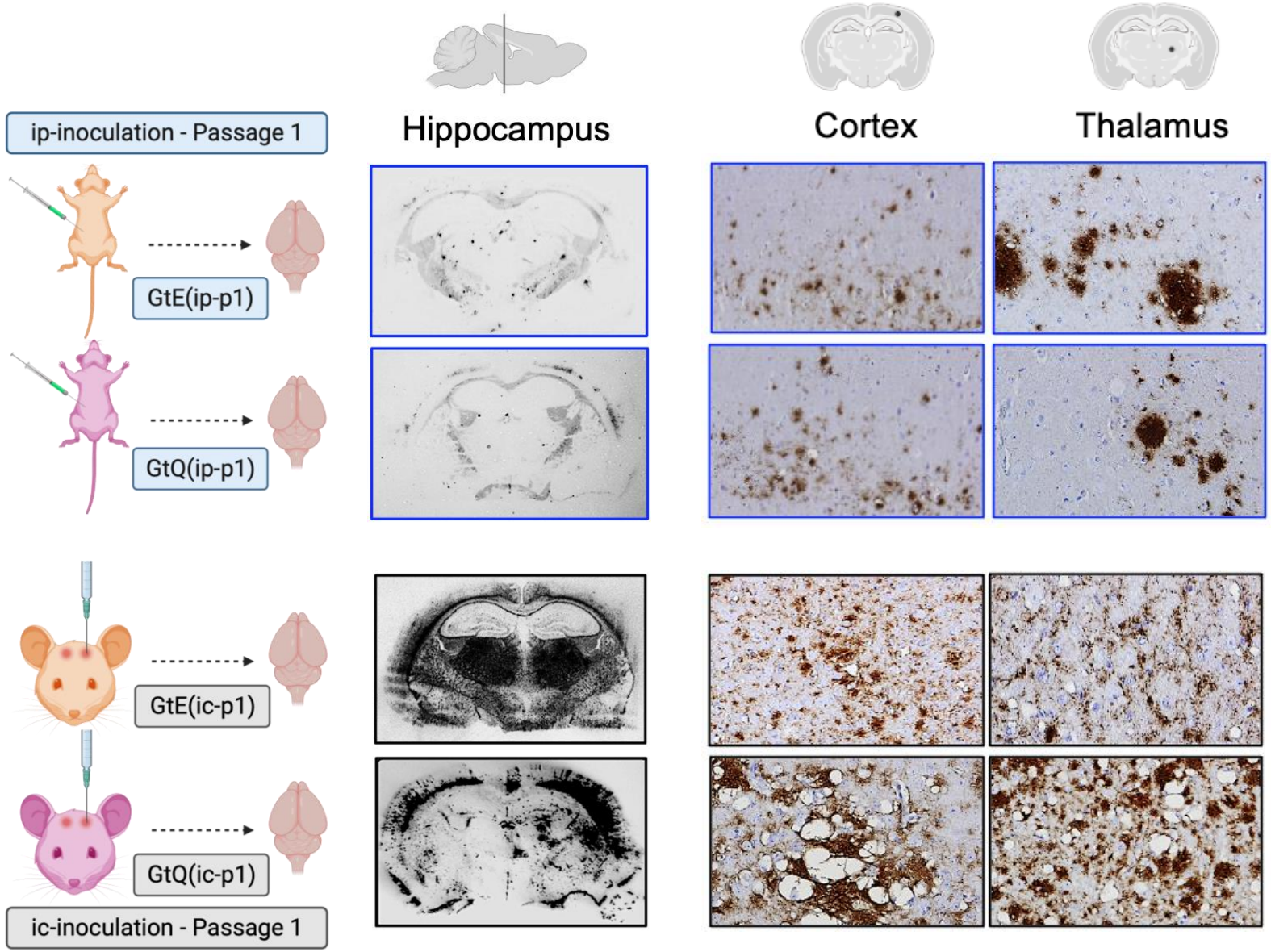
- The conformations and protease sensitivities of elk and deer CWD prions are indistinguishable
- These biochemical properties remain unchanged during iterative oral and intraperitoneal transmissions in Gt mice
- Conclusion - native CWD prions are adapted for peripheral propagation in Gt mice

How does route of CWD prion exposure impact CNS dysfunction and strain propagation in Gt mice?

- The invariant biochemical properties of native elk and deer CWD prions change upon intracerebral transmissions to GtE and GtQ mice to produce novel CNS prions with divergent conformations and biochemical properties

- Unlike peripheral transmissions, intracerebral transmissions require adaptation of native CWD strain properties

Distinct CNS abnormalities in intraperitoneally- and intracerebrally-challenged Gt mice

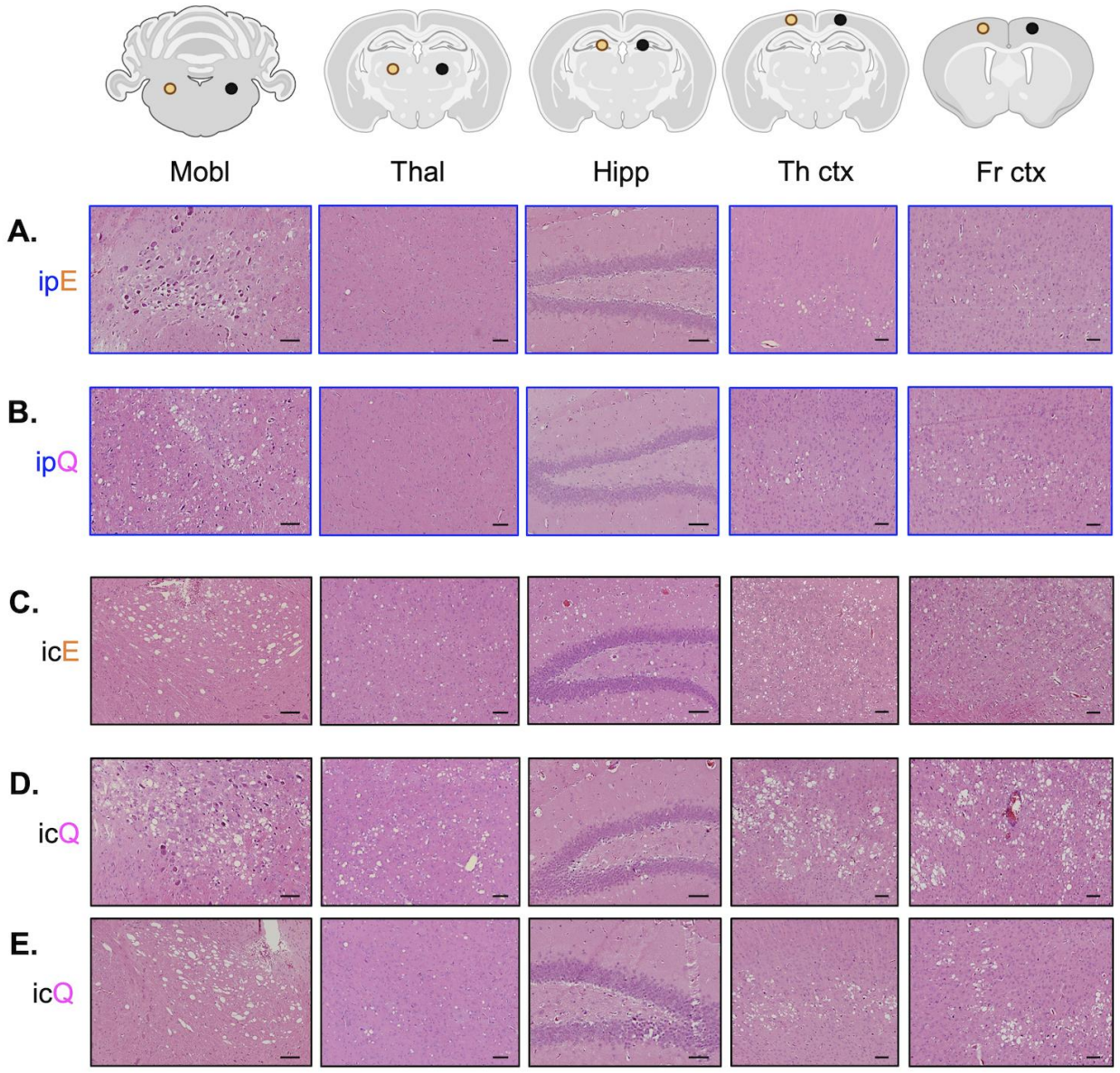


Intraperitoneal transmissions

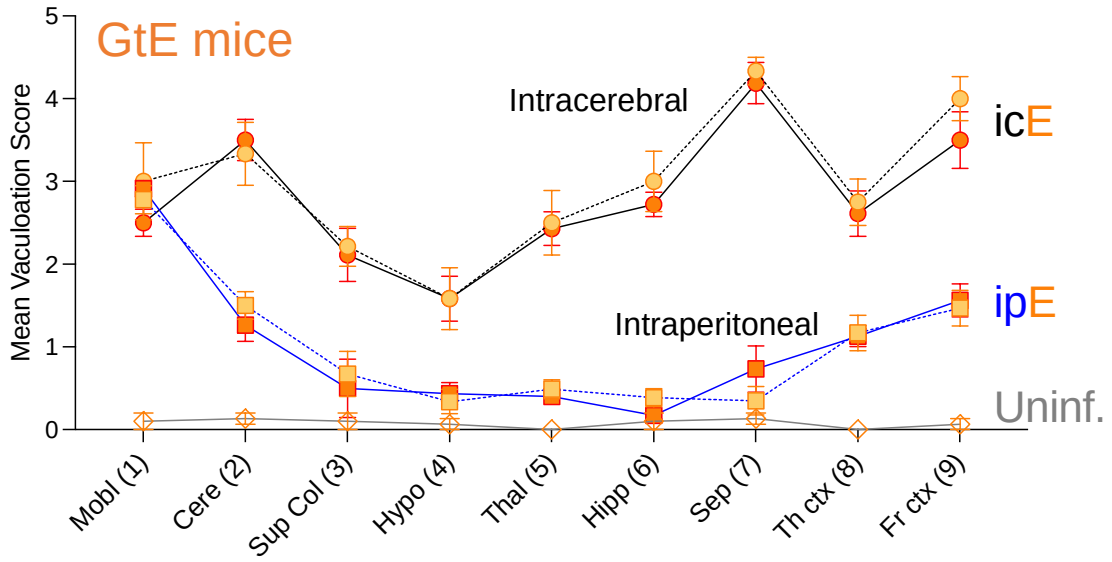
Intracerebral transmissions

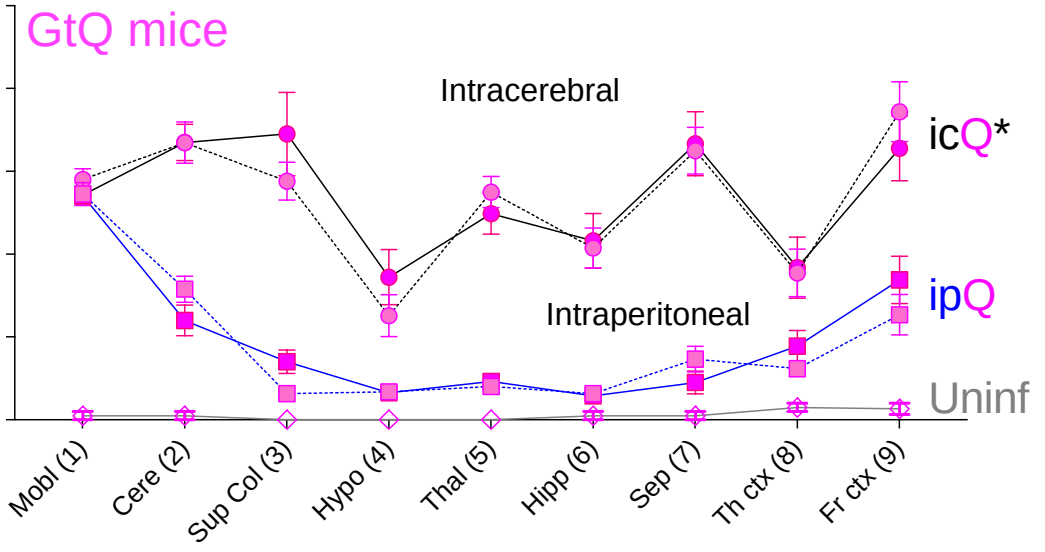
20x magnification

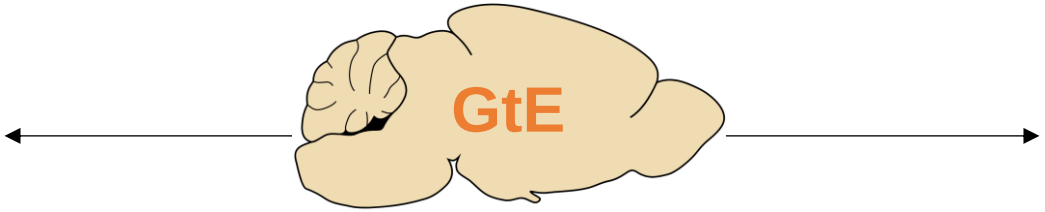
Distinct CNS abnormalities in intraperitoneally- and intracerebrally-challenged Gt mice

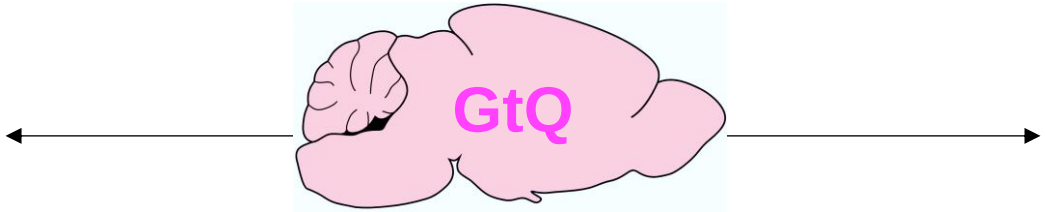


Distinct CNS abnormalities in intraperitoneally- and intracerebrally-challenged Gt mice





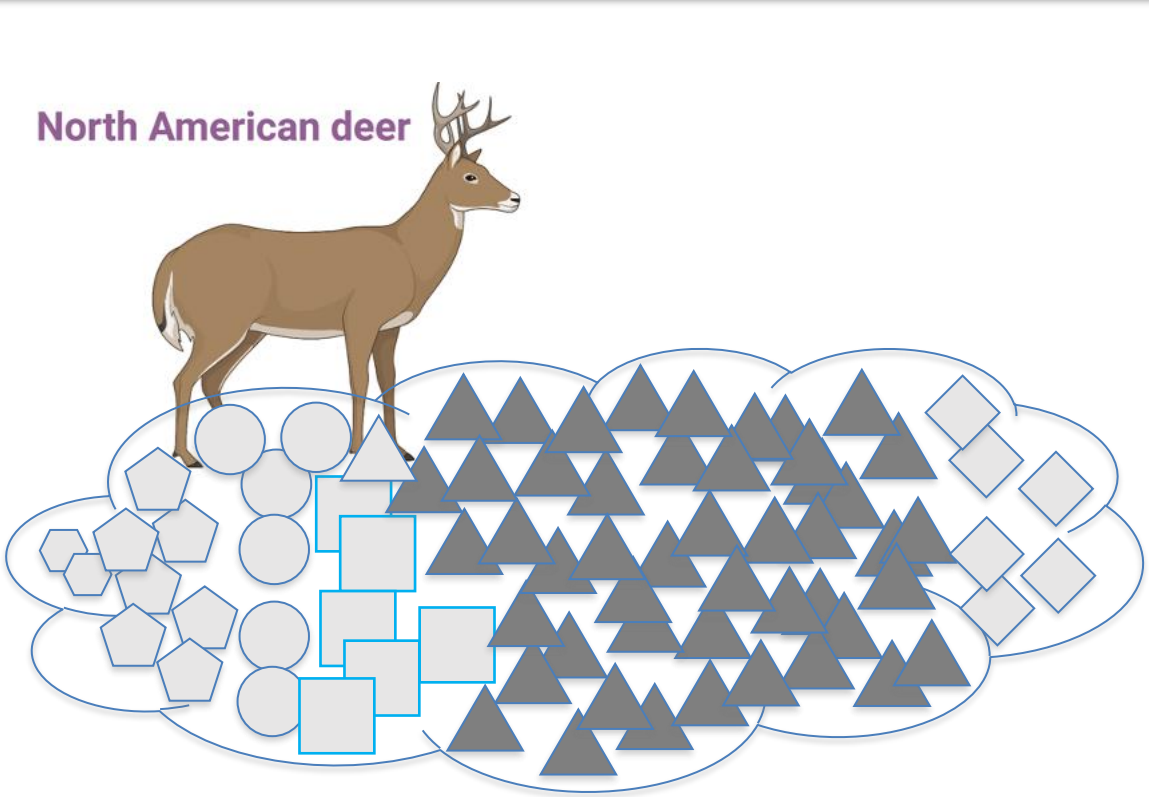


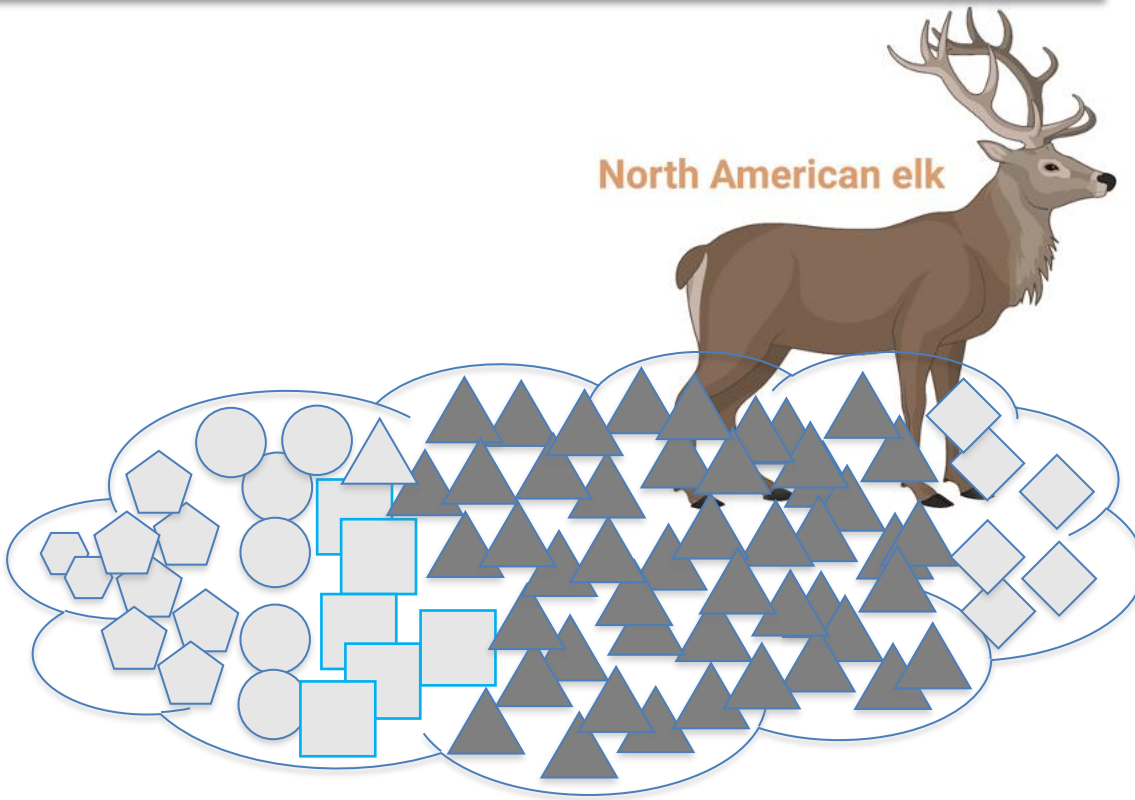


Conclusions

- Different strains are selectively propagated during peripheral and intracerebral transmissions of CWD in Gt mice.

Working hypothesis

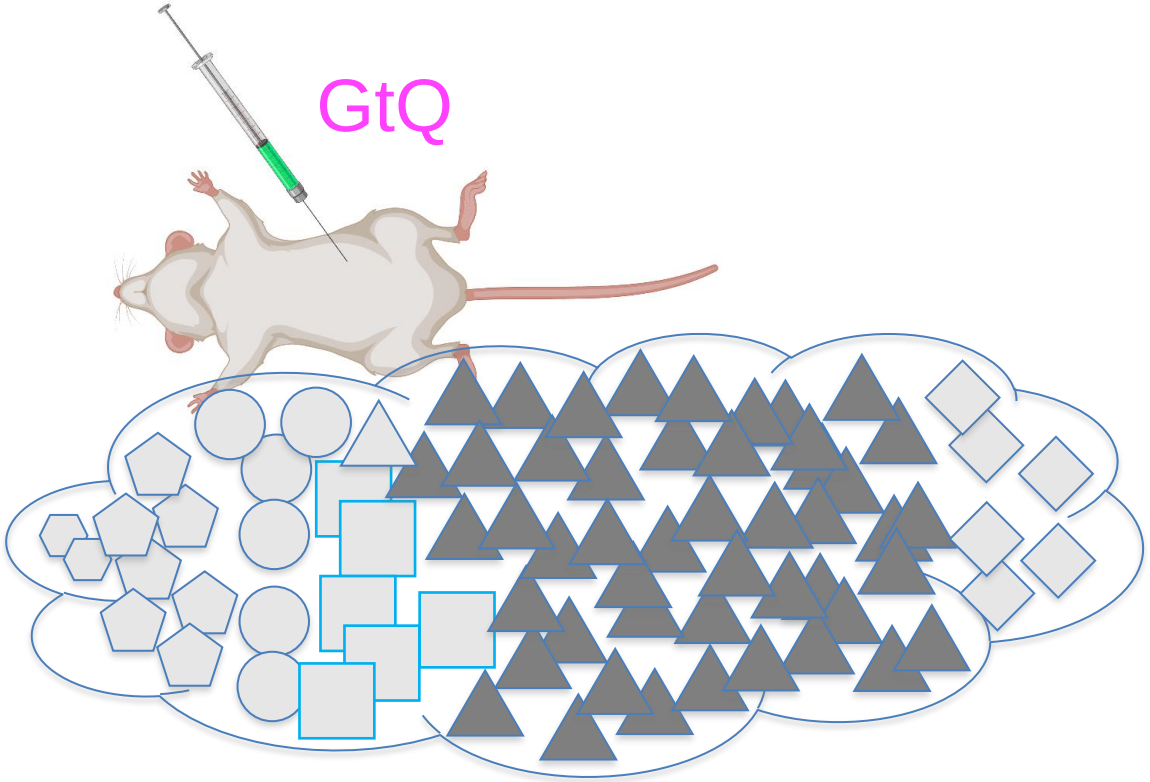


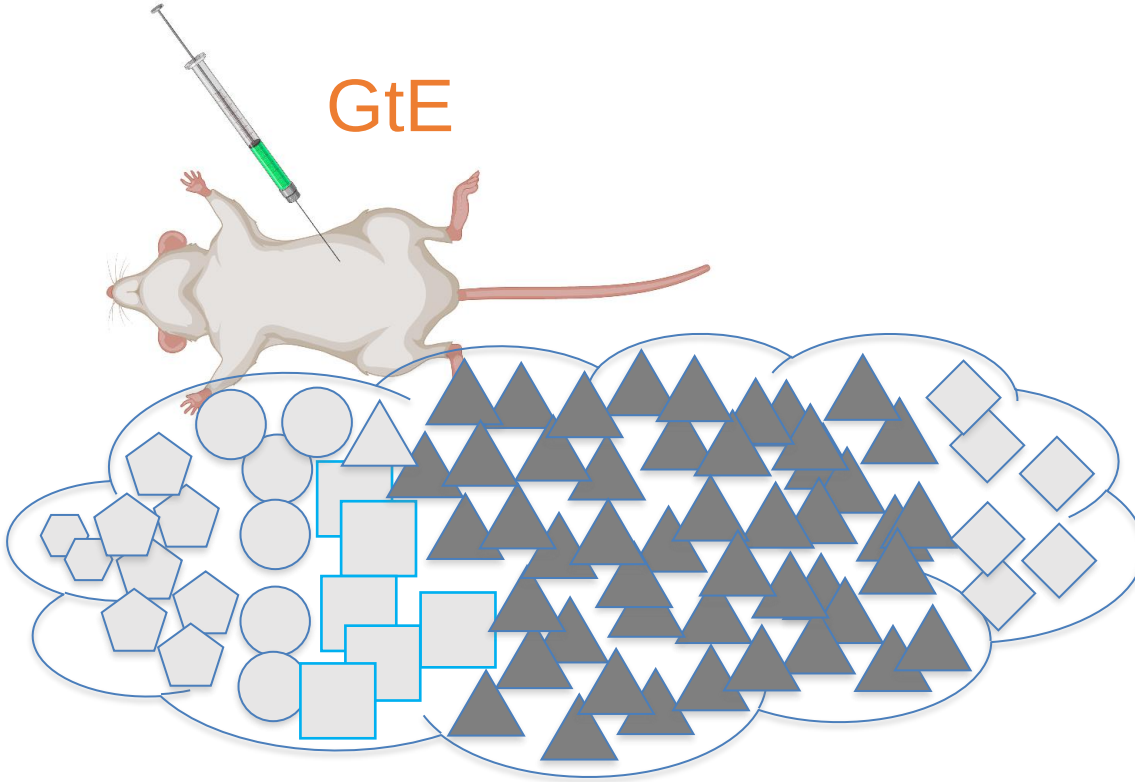


Native elk and deer CWD prion preparations comprise an ensemble of quasispecies conformations.

Elk and deer CWD strains are represented by the same dominant prion conformer

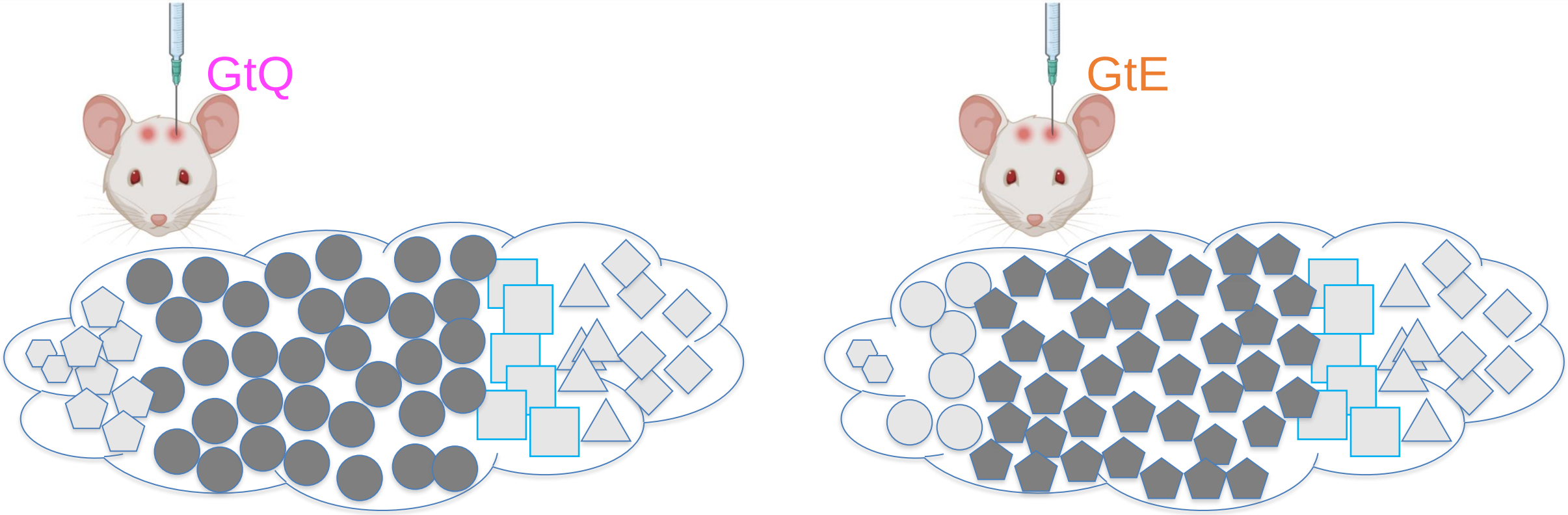
Working hypothesis





Peripheral transmissions to GtQ and GtE mice preserve this conformational equilibrium and maintain the natural strain properties of elk and deer CWD prions

Working hypothesis



Intracerebral transmissions requires adaptation leading to the selective propagation of different conformers in the CNS of GtQ and GtE mice

Conclusions

- Different strains are selectively propagated during peripheral and intracerebral transmissions of CWD in Gt mice.

- Involvement of tissue-specific factors during strain selection.

- We speculate that peripheral transmissions preserve the features of CWD prions because this is their natural mode of transmission.

- Since it is an artificial mode of transmission, intracerebral propagation requires strain adaptation resulting in divergent, distinctly different strains.

- Our findings illustrate the importance of experimental strain characterizations using hosts that not only abrogate species barriers but also accurately recapitulate natural transmission routes of native strains.

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