Refined transgenetic approaches to investigate selective adaptation of human prions during intracranial and peripheral transmissions

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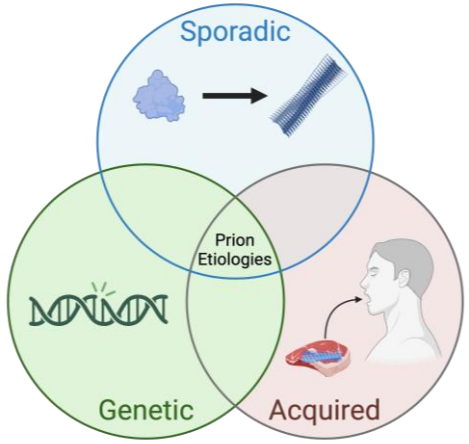
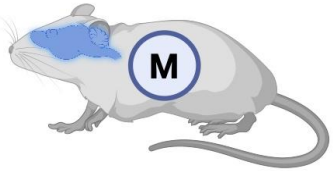
Knowledge gaps in human prion disease research this project seeks to address

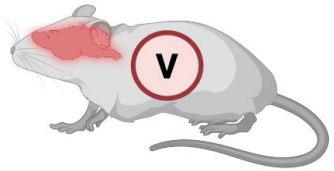


While most cases of human prion disease have a sporadic origin, infectious, iatrogenic transmission of human prions remains a potential risk to human health

Traditional transgenic mice



Tg(Hu-M129)
~16x Wt

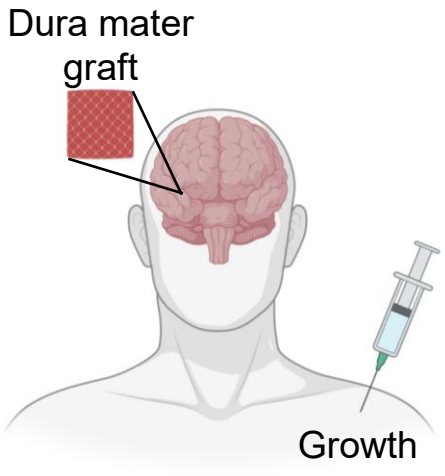


Tg(Hu-V129)
~2x Wt

<u>Pros</u>:
- Can express any prion protein of interest
- Easy to study prion transmission

<u>Cons</u>:
- Hard to control level of expression
- Expression often limited to CNS
- Hard to study affects of heterozygosity (MV129)

Clinical and pathological features of iatrogenic Creutzfeldt-Jakob (iCJD) disease can differ depending on whether exposure occurs in the central nervous system (CNS) or in the periphery



Knowing how exposure route and genotype affects human prion disease pathogenesis is critical gap in knowledge

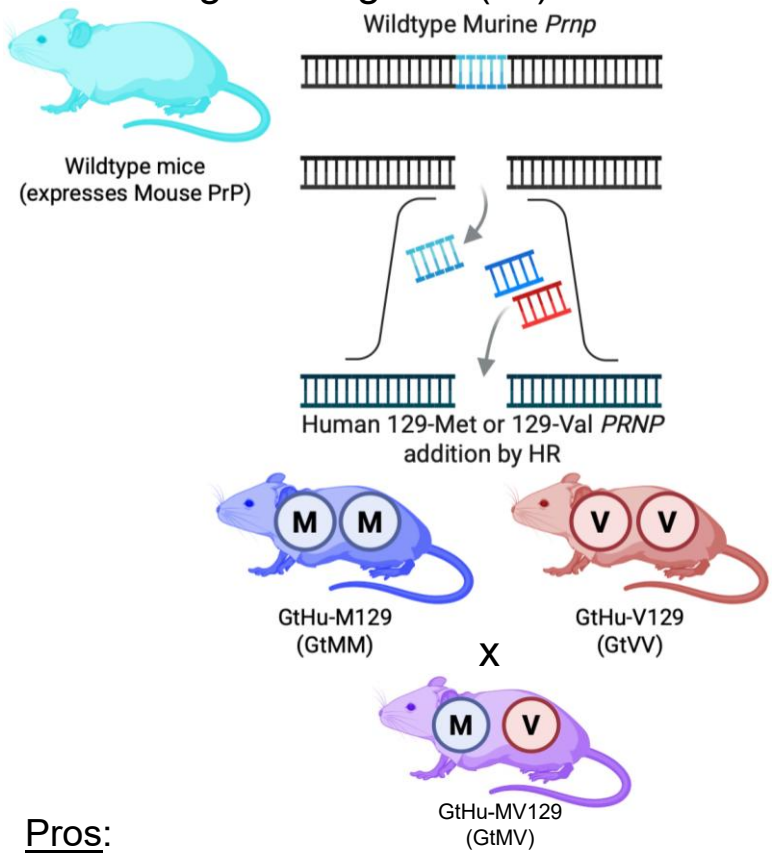
To fill this gap, we need models of human prion disease that improve upon and minimize the downsides of traditional transgenic mouse models

Our goal is to generate an improved mouse model for studying human prion disease

All images in presentation made with BioRender

An improved model to study human prion disease: our approach

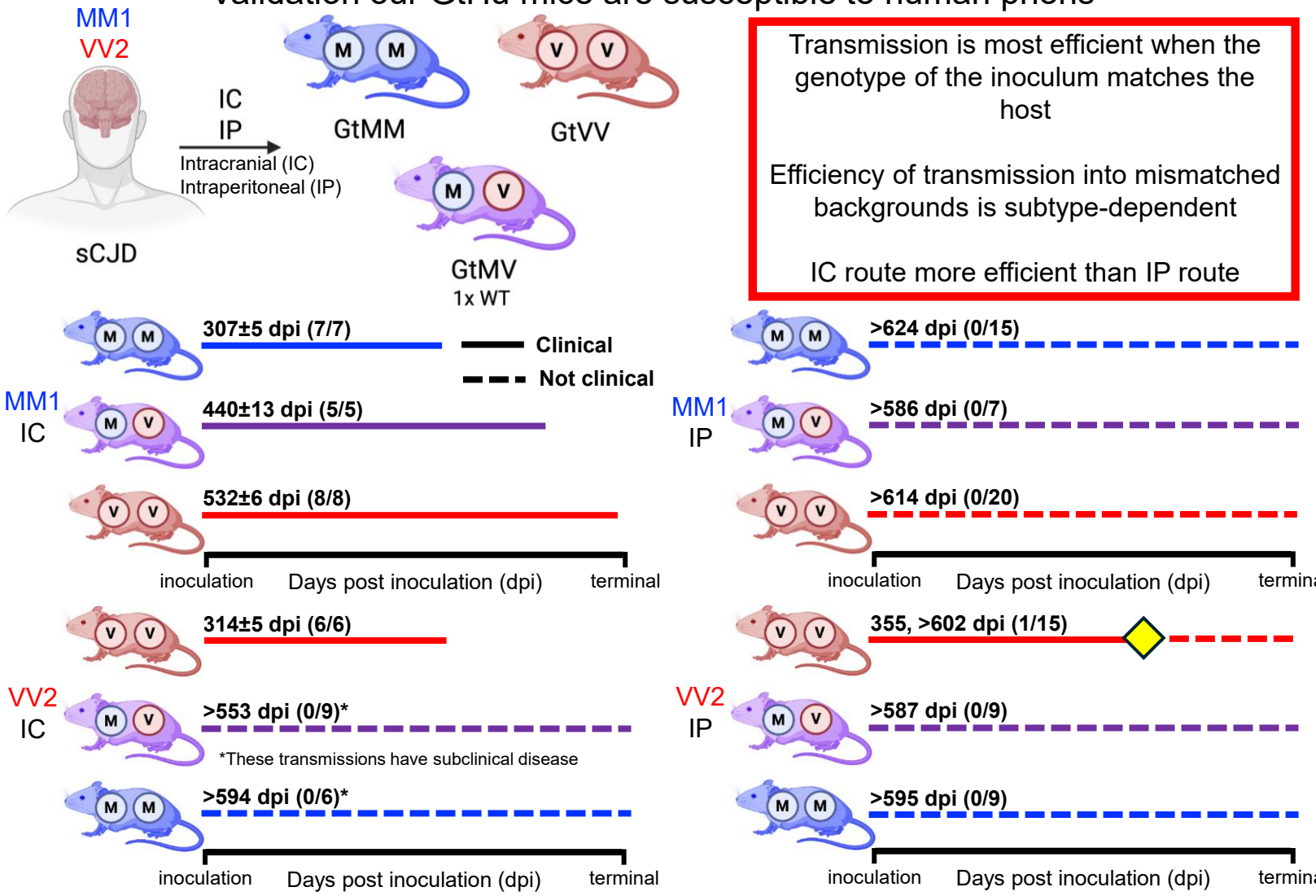
Our gene-targeted (Gt) mice



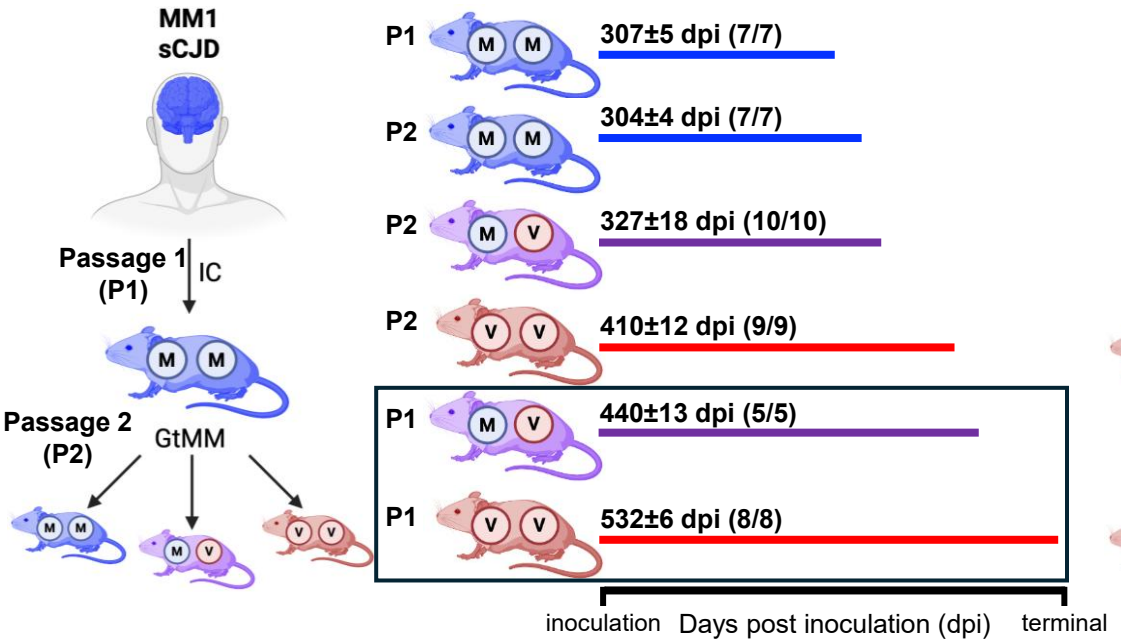
Pros:

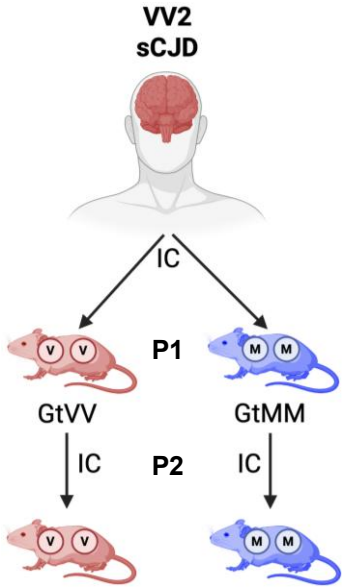
- PrP expression level matches Wt mice
- Expression not limited to CNS
- Can breed GtMM and GtVV mice to generate GtMV mice and study affects of heterozygosity on prion pathogenesis

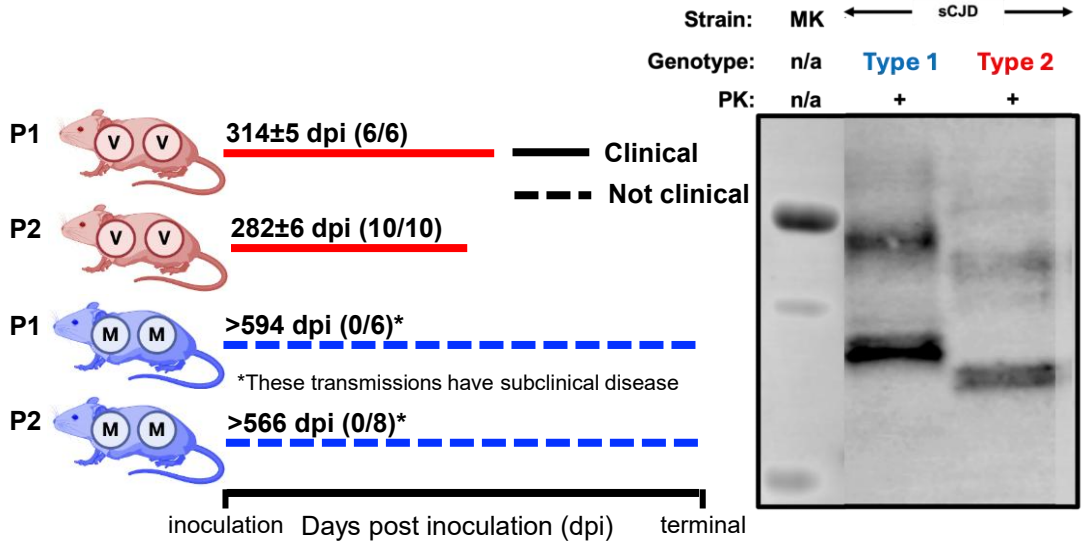
Validation our GtHu mice are susceptible to human prions

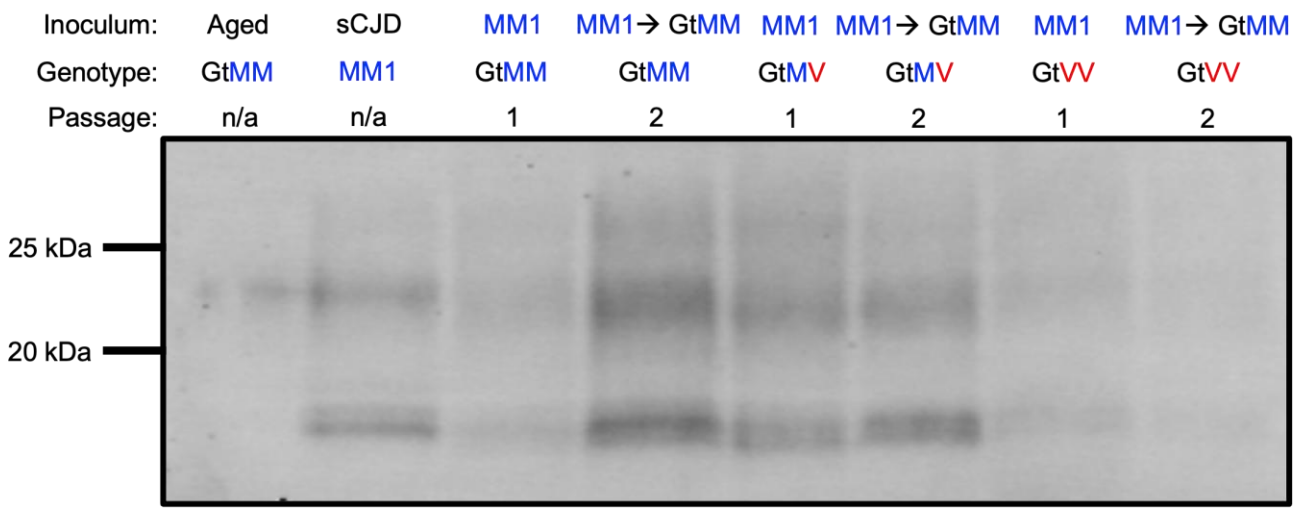


Selective adaptation of sCJD upon serial transmission

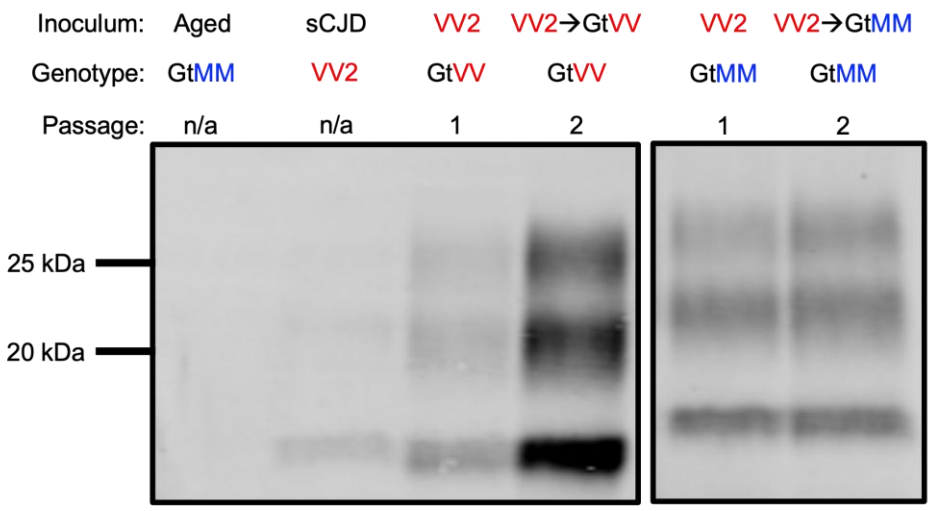








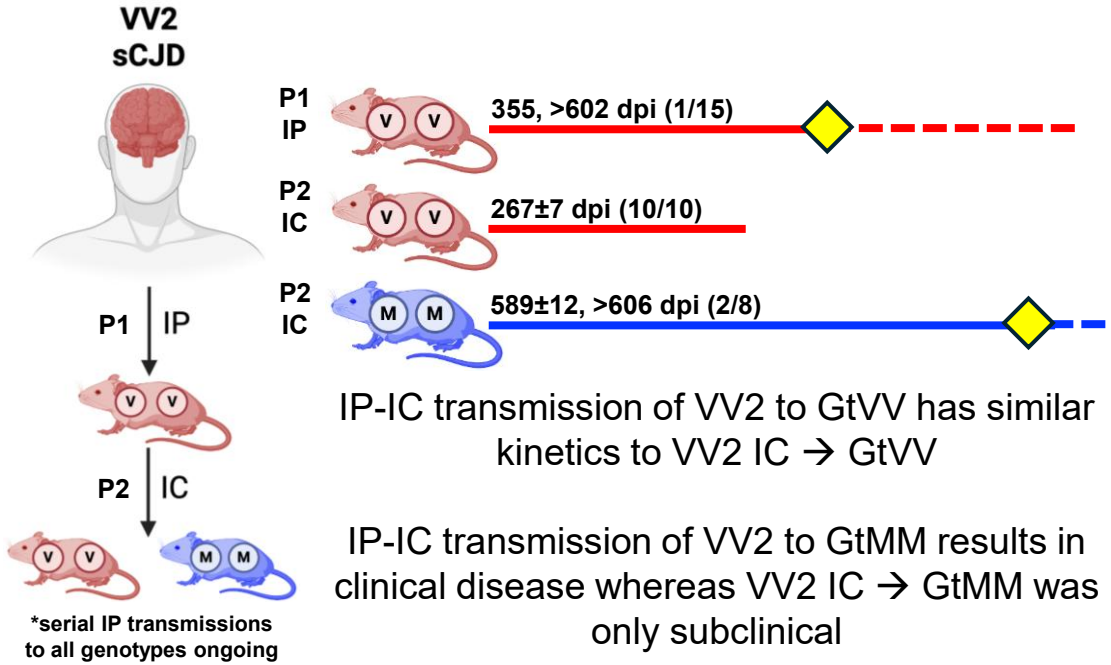
Type 1 (T1) migration pattern is maintained in all genotypes



Type 2 (T2) migration pattern is maintained in GtVV mice but switches to Type 1 in GtMM mice

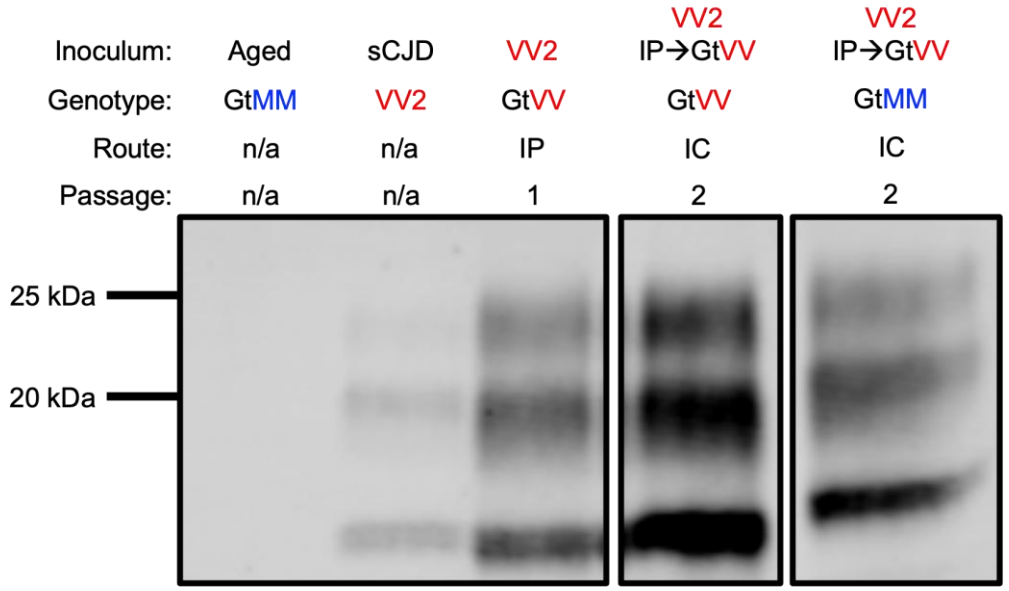
WB panels cropped from same blot

Selective adaptation of sCJD upon serial transmission



IP-IC transmission of VV2 to GtVV has similar kinetics to VV2 IC → GtVV

IP-IC transmission of VV2 to GtMM results in clinical disease whereas VV2 IC → GtMM was only subclinical



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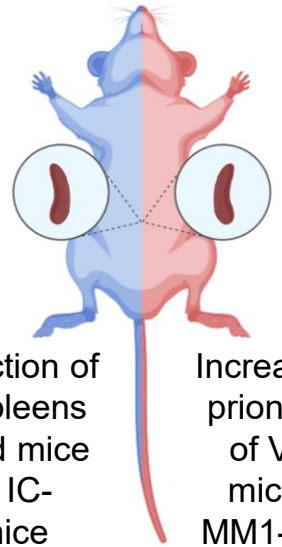
Type 2 migration pattern is maintained in GtVV mice but switches to Type 1 in GtMM mice

IC MM1

P1: 0/21 (0%)

IP MM1

P1: 0/11 (0%)



IC VV2

P1: 1/21 (4.8%)

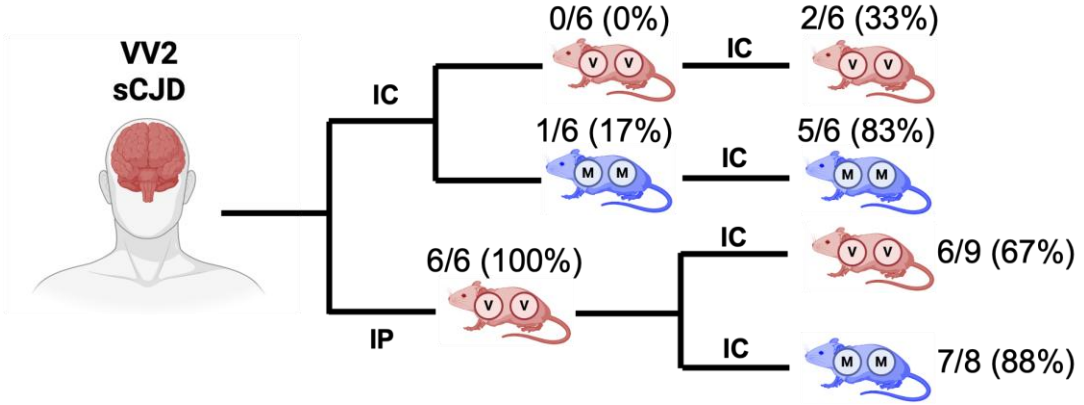
IP VV2

P1: 7/14 (50%)

Increased detection of prions in the spleens of IP-inoculated mice compared to IC-inoculated mice

Increased detection of prions in the spleens of VV2-inoculated mice compared to MM1-inoculated mice

Shifts in spleen positivity during serial passage



Serial transmission of VV2 sCJD by IC-IC and IP-IC routes results in increased detection of prions in the spleen

Increased peripheralization of prions in like (GtVV) and unlike (GtMM) backgrounds by IC-IC and IP-IC routes

Discussion and ongoing/future work

- Gene-targeted mice expressing the human prion protein (GtHu) successfully model human prion disease
  - ”Like into like” transmissions of sCJD maintain clinical and biochemical properties of isolate
  - sCJD is a brain-adapted disease; IC transmission route >> IP transmission route

- GtHu mice highlight factors affecting infectious human prion transmission and adaptation
  - Development of **clinical** prion disease is highly sCJD subtype and host genotype dependent
  - M129 PrP preferentially selects for T1 prions, even when infecting sCJD is T2
  - Transmission of sCJD by peripheral routes can alter infectivity
  - Peripheralization of sCJD prions is inoculation route and sCJD subtype dependent

- GtHu mice can be used as a model to better identify and study infectious human prion transmission risks
  - Subclinical and non-clinical GtHu mice can still have detectable prions in the CNS and periphery, respectively, which can increase risk of horizontal transmission if prions remain undetected

- Peripheralization of sCJD in GtHu mice gives us the opportunity to use this model to further study acquired forms of human prions by clinically-relevant routes
  - Transmission of sCJD by intramuscular (IM) and retro-orbital (RO) routes are ongoing
  - Transmission of dura mater and human growth hormone iCJD by IC, IP, IM, and RO routes are ongoing to evaluate how pathogenesis differs from sCJD in our GtHu mice

- Future work includes assessing peripheralization of human prions in other tissues (e.g., muscle, intestine) and transmitting positive peripheral tissue

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The individuals and their families that donate tissue to turn their loss into hope for others

Summary and Future Directions

- Gene-targeted mice expressing wild type levels of the human prion protein (HuPrP) are an improved model to study human prion disease because this model allows us to:
  - Assess how sCJD disease outcomes are affected by the amino acid present at residue 129 of HuPrP (MM129, MV129, VV129)
  - Study how disease course of various subtypes of sCJD changes based on if transmission is direct to the brain or indirect via the periphery
  - Identify in which tissues different subtypes/strains of human prions are detected outside of the CNS
  - Investigate how properties of sCJD subtypes evolve during serial transmission
- Transmission of sCJD and iCJD by clinically relevant routes are ongoing

- Future directions
  - Serially transmit positive peripheral tissue
  - Assess how GtHu mice challenged with iCJD compares to sCJD in our model