THE INTERPLAY OF GENE DOSAGE AND MUTATION TYPE IN EARLY PRION MISFOLDING

Surabhi Mehra, PhD

Postdoctoral Fellow, Watts Lab

Tanz CRND, University of Toronto

Prion diseases: rarely infectious, mostly spontaneous

Prion disease are rare, fatal and incurable neurodegenerative disorders caused by PRIONS-**P**rotease **R**esistant **I**nfectious **O**rgan-specific **N**eurotoxic **S**elf-replicating proteins

Animal prion disorders

Scrapie (Sheep and goats)

- Typical forms
- Atypical or Nor98 scrapie

Bovine Spongiform encephalopathy (BSE) Mad cow disease

- Classical BSE
- Atypical BSE
- BSE in goats

Chronic wasting disease (CWD)

cervids, deer, elk and moose

Human prion disorders

Sporadic (85%)

- Sporadic Creutzfeldt-Jakob Disease (sCJD)
- Variable Protease-Sensitive prionpathy (VSPRS)

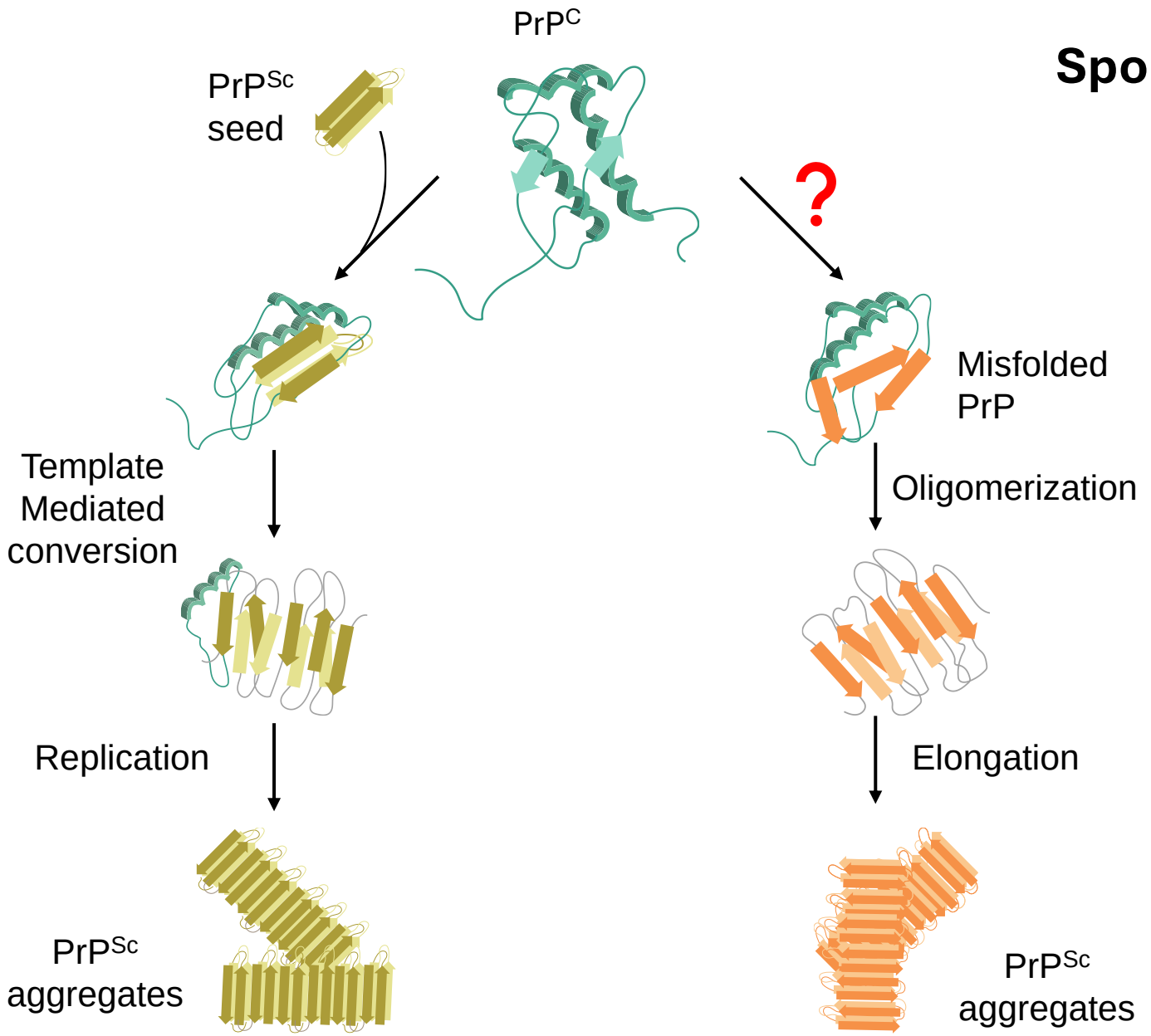
Genetic (10-15%)

- Fatal Familial Insomnia (FFI)
- Gerstmann-Straussler-Scheinker Syndrome (GSS)
- Familial Creutzfeldt-Jakob Disease (fCJD)

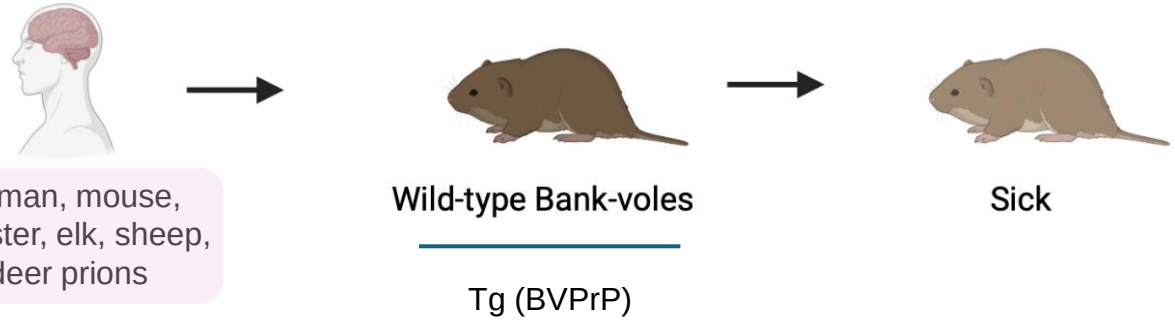
Acquired (<1 %)

Kuru, vCJD, iCJD

Decoding spontaneous prion misfolding

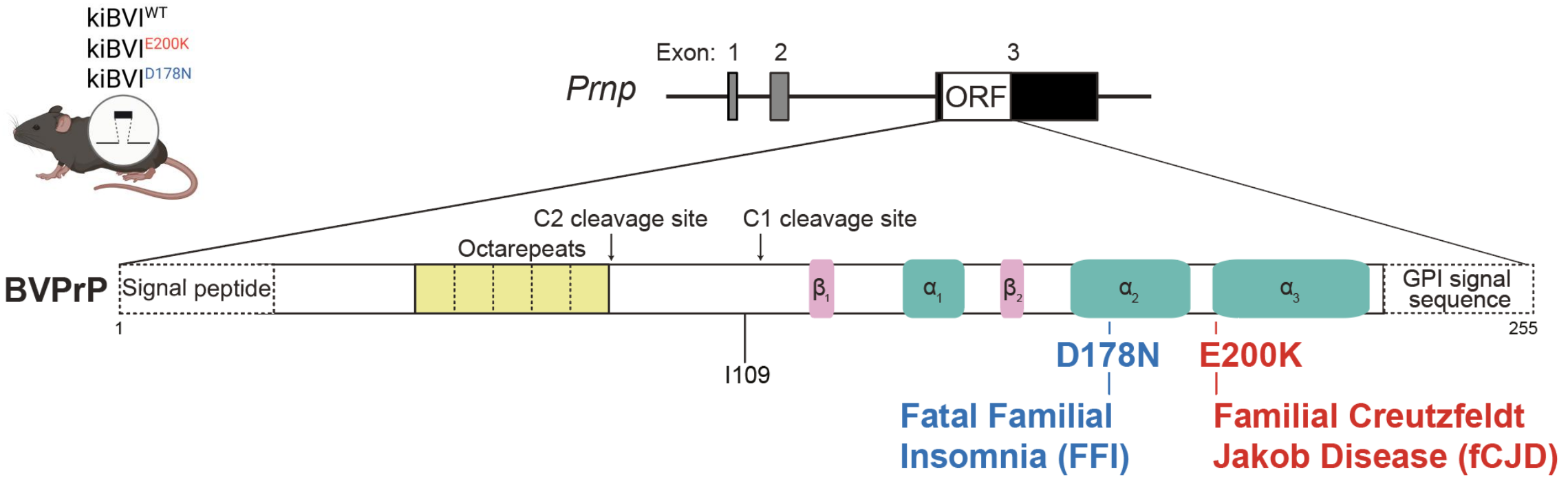


Knocking in bank vole PrP: unlocking spontaneous prion disease



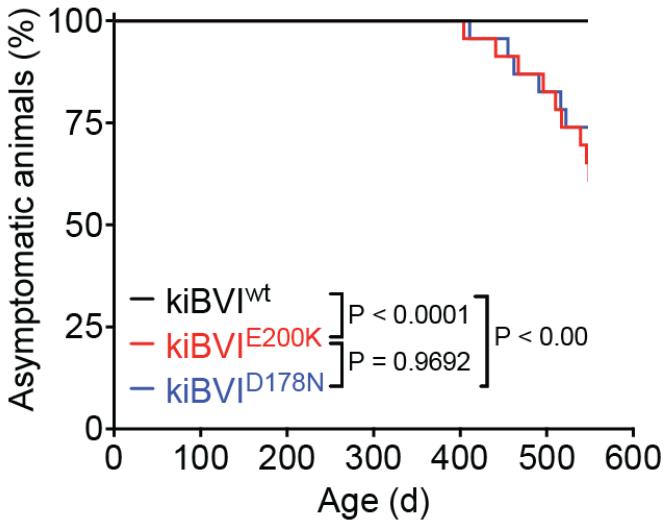
‘No Species Barrier”

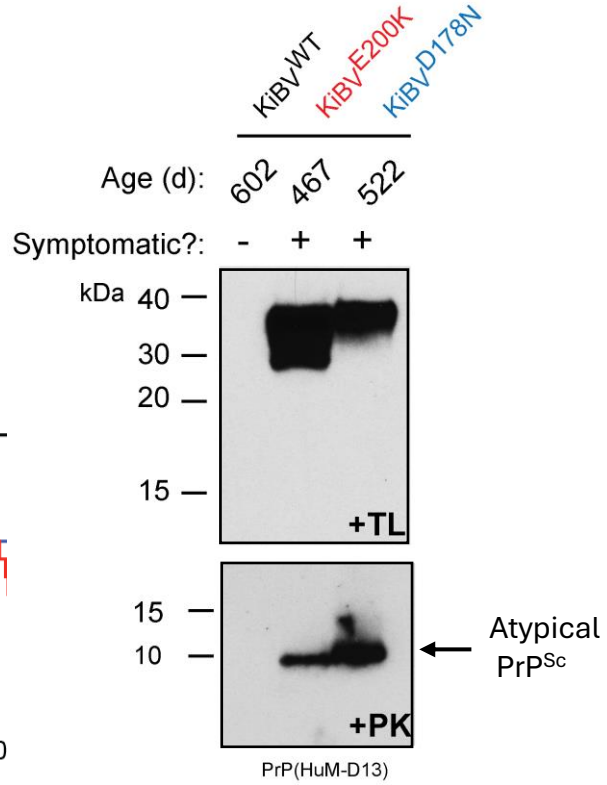
‘High susceptibility to diverse prion types”

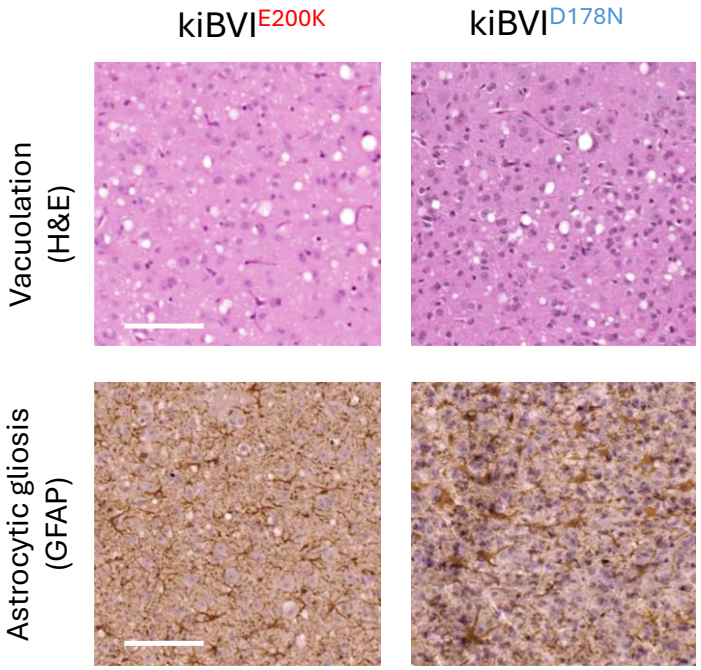


Knocking in bank vole PrP: unlocking spontaneous prion disease

Mutant bank vole PrP knock-in mice (E200K or D178N)







The Journal of Clinical Investigation RESEARCH ARTICLE

Convergent generation of atypical prions in knockin mouse models of genetic prion disease

Surabhi Mehra,[1] Matthew E.C. Bourkas,[1,2] Lech Kaczmarczyk,[3,4] Erica Stuart,[1] Hamza Arshad,[1,2] Jennifer K. Griffin,[1] Kathy L. Frost,[5] Daniel J. Walsh,[6] Surachai Supattapone,[6,7] Stephanie A. Booth,[5,8] Walker S. Jackson,[3,4] and Joel C. Watts[1,2]

[1]Tanz Centre for Research in Neurodegenerative Diseases and [2]Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada. [3]Wallenberg Center for Molecular Medicine, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden. [4]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany. [5]One Health Division, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada. [6]Department of Biochemistry and Cell Biology and [7]Department of Medicine, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire, USA. [8]Department of Medical Microbiology and Infectious Diseases, Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.

Most cases of human prion disease arise due to spontaneous misfolding of WT or mutant prion protein, yet recapitulating this event in animal models has proven challenging. It remains unclear whether spontaneous prion generation can occur within the mouse lifespan in the absence of protein overexpression and how disease-causing mutations affect prion strain properties. To address these issues, we generated knockin mice that express the misfolding-prone bank vole prion protein (BVPrP). While mice expressing WT BVPrP (I109 variant) remained free from neurological disease, a subset of mice expressing BVPrP with mutations (D178N or E200K) causing genetic prion disease developed progressive neurological illness. Brains from spontaneously ill knockin mice contained prion disease–specific neuropathological changes as well as atypical protease-resistant BVPrP. Moreover, brain extracts from spontaneously ill D178N- or E200K-mutant BVPrP–knockin mice exhibited prion seeding activity and transmitted disease to mice expressing WT BVPrP. Surprisingly, the properties of the D178N- and E200K-mutant prions appeared identical before and after transmission, suggesting that both mutations guide the formation of a similar atypical prion strain. These findings imply that knockin mice expressing mutant BVPrP spontaneously develop a bona fide prion disease and that mutations causing prion diseases may share a uniform initial mechanism of action.

Introduction

Human prion diseases such as Creutzfeldt-Jakob disease (CJD) are caused by misfolding of the cellular prion protein (PrP^{C}) into PrP^{Sc}, a pathological conformation that aggregates and deposits in the brain (1). In addition to PrP^{Sc} deposition, the neuropathological hallmarks of prion disease include spongiform degeneration of the brain parenchyma as well as prominent astrocytic gliosis (2). PrP^{C} ease in humans do not manifest due to an infectious etiology. Instead, spontaneous misfolding of PrP^{C} into PrP^{Sc} within the brain is thought to be the initiating event in sporadic prion diseases, such as sporadic CJD (sCJD). Similarly, in genetic prion diseases such as familial CJD (fCJD), fatal familial insomnia (FFI), and Gerstmann-Sträussler-Scheinker disease (GSS), mutations within the *PRNP* gene encoding PrP are believed to promote the

From Findings to Questions

- ❑ Homozygous mutant knock-in mice develop spontaneous prion disease

But ~99 % of human cases of genetic prion diseases are heterozygous !

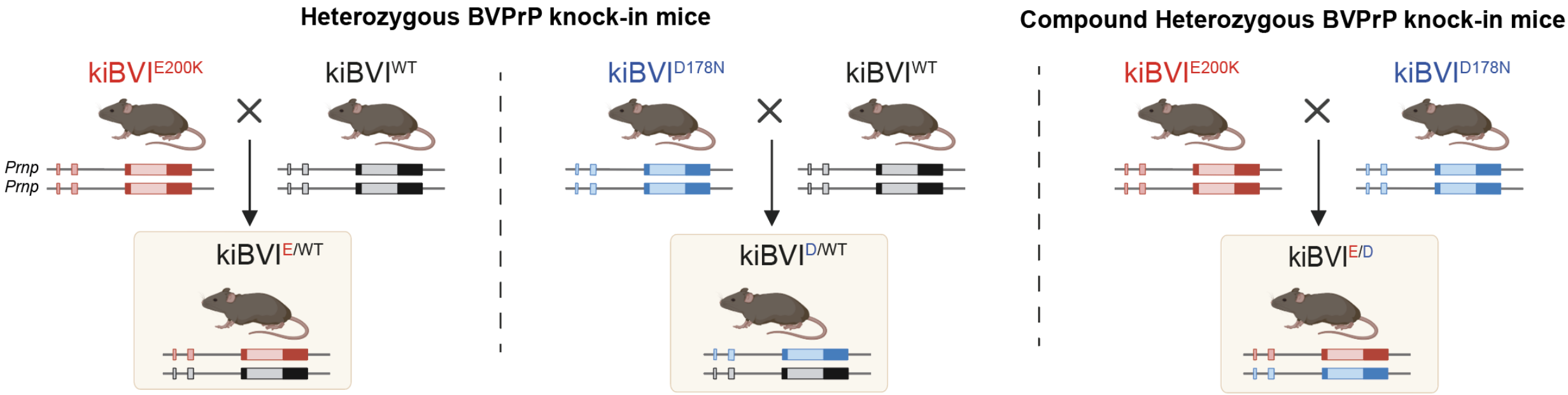
How gene dosage influence the rate of misfolding and disease onset in heterozygous, where only one gene copy is mutated? Could this explain why heterozygous carriers often remain asymptomatic for longer?

- ❑ Distinct mutations, similar prion strains

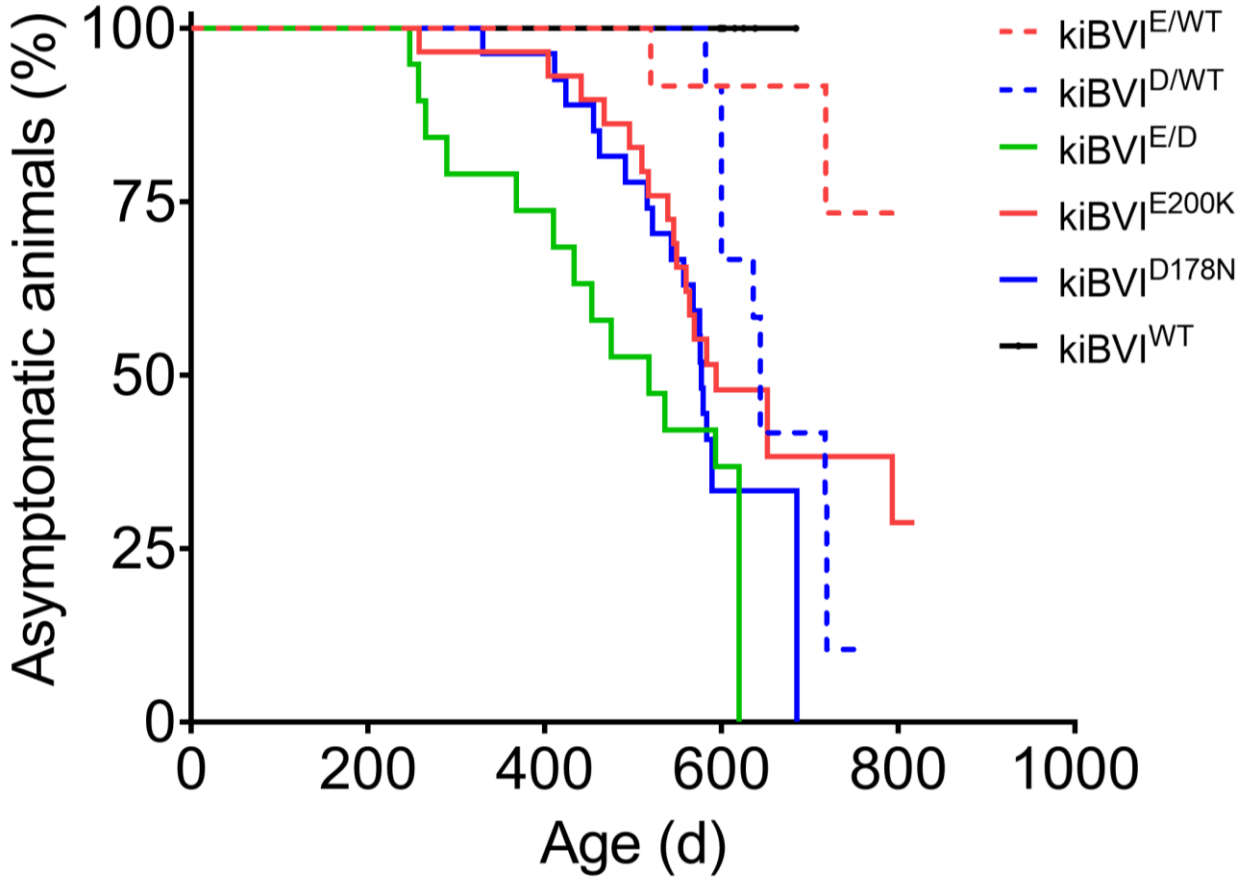
Despite linked to different diseases and distinct biochemical properties, D178N and E200K produced similar atypical prion strains

Does a SHARED early misfolding pathway exist? Do these mutation types specify similar early strain formation, independent of the specific mutation?

Generation of heterozygous knock-in mice



Impact of gene dosage on disease progression and prion accumulation



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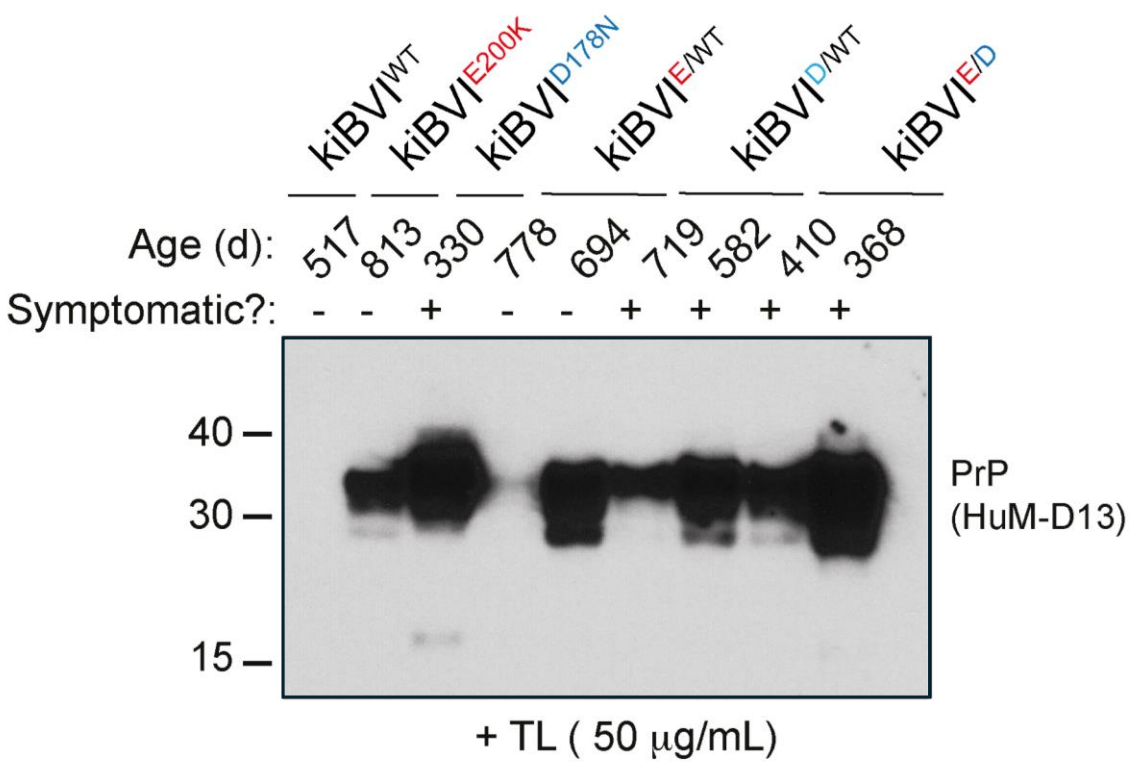

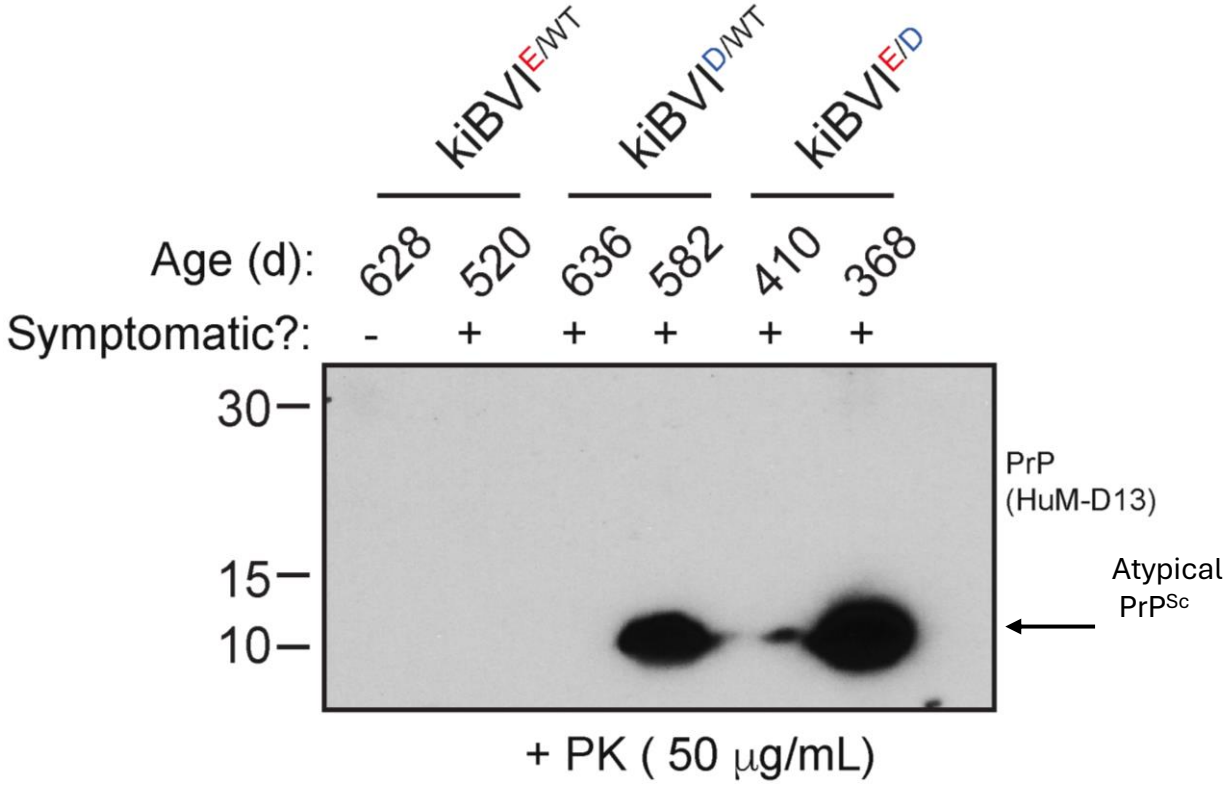

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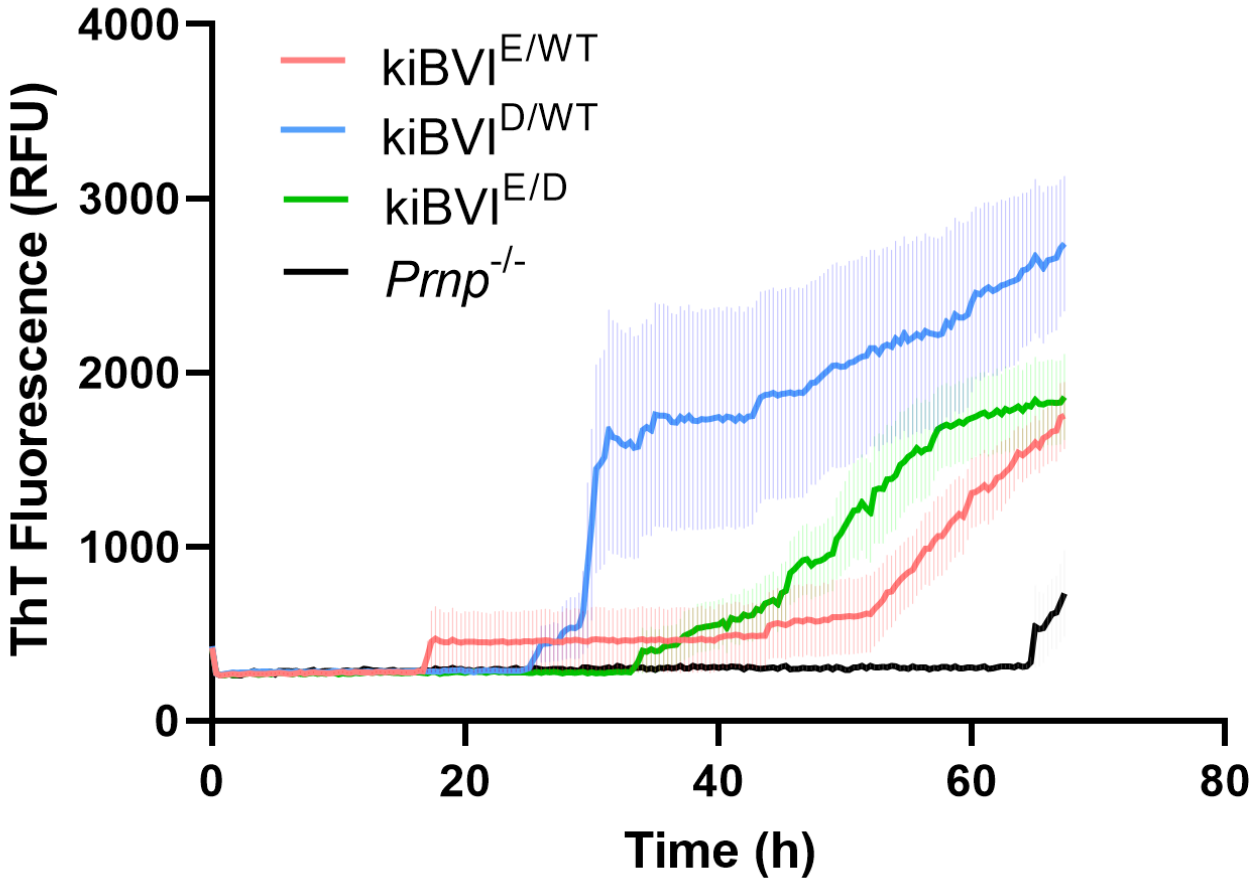


Key takeaways and next steps

- A consistent atypical PrP^{Sc} species forms spontaneously across all mutant lines, indicating **a common unifying mechanism that initiates PrP misfolding.**

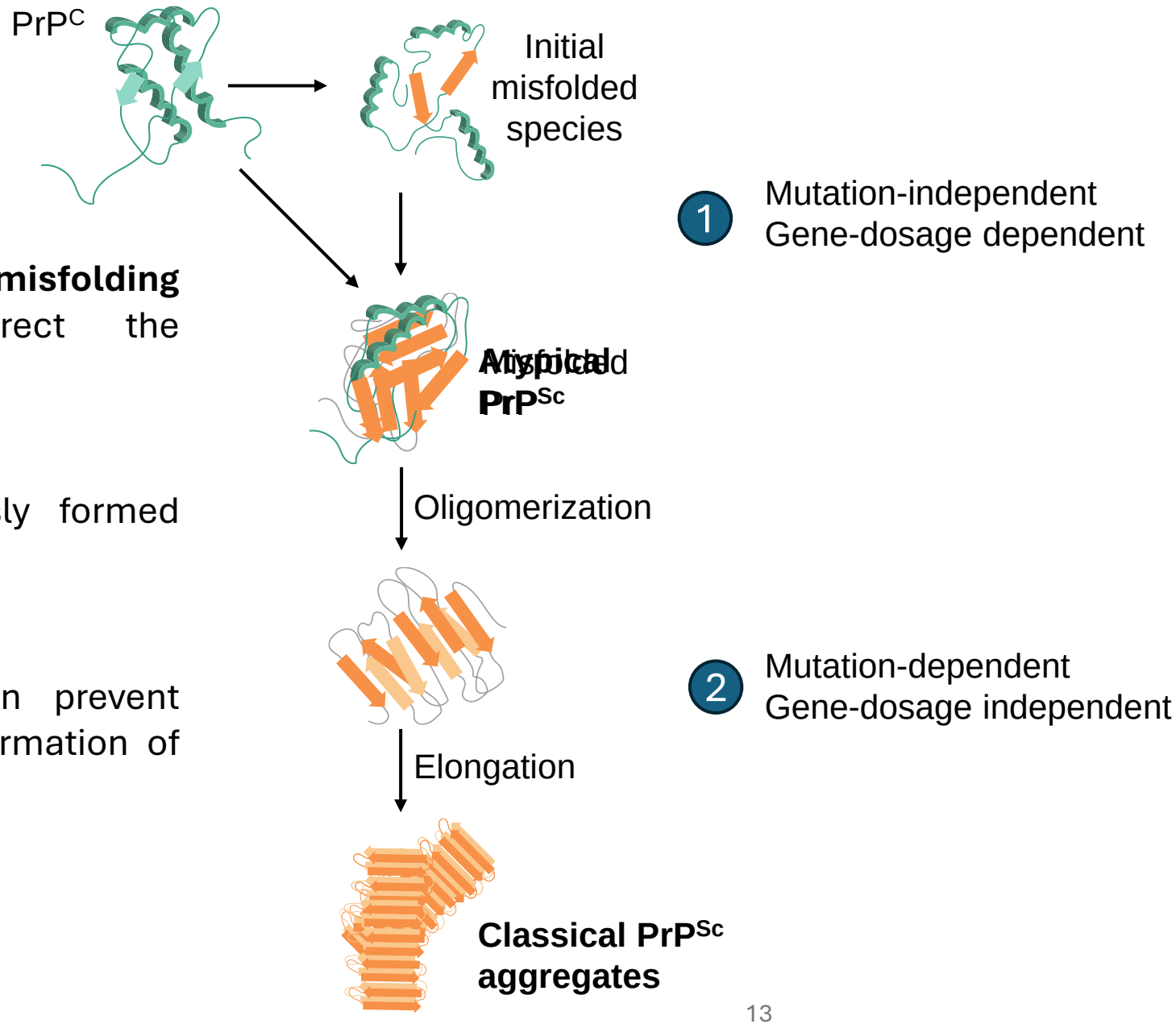
- Slightly accelerated disease progression in the compound heterozygous line suggests that **early prion strain formation occurs independently of mutation type.**

- kiBVI$^{D/WT}$ and kiBVI$^{E/WT}$ lines demonstrate delayed onset of the disease, suggesting that **gene dosage of a misfolding-prone PrP, rather than the specific mutation type**, plays a key role in early prion misfolding.

- Reduced disease penetrance in kiBVI$^{E/WT}$ compared to kiBVI$^{D/WT}$, despite higher E200K protein levels, suggests that **the D178N mutation is inherently more pathogenic than E200K.**

Key takeaways and next steps

- Investigating **mutation-specific PrP misfolding pathways**: how do specific mutations direct the formation of classical PrP^{Sc}.
- Assessing the conversion of spontaneously formed **atypical PrP^{Sc} to Classical PrP^{Sc}**.
- Exploring **therapeutic strategies** that can prevent mutation-specific misfolding or block the formation of atypical PrP^{Sc}.



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