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Cross-species transmission of CWD prions

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ABSTRACT. Prions cause fatal neurodegenerative diseases in humans and animals and can be transmitted zoonotically. Chronic wasting disease (CWD) is a highly transmissible prion disease of wild deer and elk that affects cervids over extensive regions of the United States and Canada. The risk of cross-species CWD transmission has been experimentally evaluated in a wide array of mammals, including non-human primates and mouse models expressing human cellular prion protein. Here we review the determinants of cross-species CWD transmission, and propose a model that may explain a structural barrier for CWD transmission to humans.

KEYWORDS. chronic wasting disease, CJD, neurodegeneration, prion, zoonotic, amyloid

CHRONIC WASTING DISEASE OF CERVIDS

Chronic wasting disease (CWD) is the only known prion disorder affecting free-ranging wildlife, including deer, elk, and moose, and has spread extensively throughout North America, occurring in 23 US states and 2 Canadian provinces.[1,2] CWD prions are highly infectious and readily transmitted among cervids, leading to remarkably high prevalences that can exceed 90% in captive deer.[3] Humans, wildlife, and domestic species such as cattle and sheep are likely exposed to CWD through consumption of prion-infected animals or grazing on prion-contaminated pastures.

Within an individual animal, CWD prions are extraordinarily widespread and accumulate in neural and non-neural tissues and body fluids, including brain and spinal cord fat, pancreas, adrenal gland, heart, peripheral nerves, lymph nodes, saliva, blood, and skeletal muscle, many of which are ingested by humans and other animals.[4-9] Venison consumption is common; more than 60% of Americans have eaten deer or elk meat,[10] and known human exposures to CWD-infected venison have occurred in New York[11] and in Wisconsin, where hundreds of people have eaten CWD-infected cervids (E. Belay, personal commun). As CWD-infected animals gain access to new areas through migration or animal transport, human and animal exposure to CWD prions will likely increase. Here we review species susceptibility to CWD infection as well as new models to study CWD species barriers.

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CROSS-SPECIES CWD PRION TRANSMISSION

Prions transmitted into a different species typically result in few infections and prolonged incubation periods due to a transmission barrier.[12] Transmission barriers are caused by amino acid sequence differences between the host cellular prion protein, PrP^{C}, and the misfolded, aggregated conformation, PrP^{Sc}.[13-15] The conformation of PrP^{Sc} also plays a role in species barriers. For example, CWD prions are not transmissible to mice expressing human PrP^{C}, yet are efficiently transmitted to mice expressing cervid PrP^{C}, demonstrating the importance of amino acid sequence in susceptibility.[16,17] Interestingly, transgenic mice expressing human PrP^{C} are more susceptible to human sporadic Creutzfeldt-Jakob disease (sCJD) prions than to variant CJD (vCJD) prions.[18] vCJD prions transmit readily to wild type mice, indicating that PrP^{Sc} conformation also impacts prion transmission.[18] Bank vole PrP^{C} has been touted as a universal acceptor as it is efficiently converted by diverse human and animal prions despite sequence differences between bank vole PrP^{C} and the infectious PrP^{Sc}.[19-21]

Many species have been experimentally exposed to CWD prions by intracerebral or oral routes of inoculation, including rodents, mustelids, felids, and ruminants. Oral inoculation with CWD led to prion disease in cervids and squirrel monkeys, whereas 5 additional species resisted oral CWD challenge (Table 1).[22-24] However, intracerebral CWD inoculation caused prion infection in voles, hamsters, ferrets, sheep, cats, mink, and cattle, with variable attack rates.[25-33] Wild type mice and raccoons resisted CWD prion infection (Table 1).[16,34,35] An extensive study of CWD susceptibility in transgenic mice expressing ovine and bovine PrP^{C} revealed no mice with prion disease, supporting the strong barrier to CWD infection observed in sheep and cattle.[36]

The ability of CWD prions to convert PrP^{C} from 12 mammalian species was evaluated in vitro by protein misfolding cyclic amplification (PMCA).[37] Efficient CWD conversion strongly correlated with *Prnp* encoding PrP^{C} with an asparagine (N) at position 170, similar to cervid PrP. CWD converted PrP^{C} from 5 of 5 species having N170 (Syrian, Chinese, and Armenian hamsters, prairie vole, *Peromyscus* mouse), but only 1 of 7 species having serine (S) at position 170 (ferret).[37] CWD did not convert PrP^{C} from mink, *Mus* mouse, cat, coyote, macaque, or transgenic mice expressing human PrP^{C}, suggesting a lower risk of CWD infection for species having a *Prnp* gene encoding S170. Interestingly, prions from CWD-infected prairie voles (N170) converted PrP^{C} from several species that express S170 (coyotes, cats and mink), consistent with PrP^{Sc} conformation playing an important role in conversion.[37] Collectively, these studies suggest that few species would be orally susceptible to CWD following prion ingestion, and that an asparagine at position 170 of PrP^{C} is a risk factor for CWD infection.

ASSESSING PRIMATE SUSCEPTIBILITY TO CWD INFECTION

To gain insight into human susceptibility to CWD, squirrel monkeys and cynomolgus macaques were challenged with CWD prions and showed surprising results, in that squirrel monkeys were highly susceptible to CWD by either intracerebral[22] or oral exposure routes,[22,23] whereas macaques resisted CWD prion infection, even after intracerebral injection.[23] A comparison of the PrP amino acid sequences of the squirrel monkey and macaque shows that both primates express S170, in contrast to the N170 expressed by deer. However, 2 intriguing amino acid differences in the N-terminus (positions 100 and 108) of squirrel monkeys and macaques may impact the CWD barrier. Nevertheless, the underlying structural mechanism that explains the profound differences in CWD susceptibility remains unresolved, and the CWD susceptibility of squirrel monkeys is not likely predictive for that of humans.

To further assess human susceptibility to CWD, 4 laboratories performed an intracerebral CWD challenge of transgenic mice expressing human PrP.[17,36,38,39] Mice either

TABLE 1. Species susceptibility to CWD infection following experimental exposure via the intracerebral (IC) or oral (PO) routes of exposure.

Species	Amino acid - position 170*	CWD – IC†	CWD – PO	References
Elk	N	ND	+++	54, 55
Fallow deer	N	+++	ND	56
Mule deer	N	ND	+++	57
White-tailed deer	N	+++	+++	7, 58
Reindeer	N	ND	++	59
Reeves' muntjac deer	N	ND	+++	60
Red deer	N	ND	+++	61
Shira's moose	N	ND	+++	62
Squirrel monkey	S	+++	+++	22-24
Macaque	S	0	0	23,24
Tg(humanPrP) (129V)	S	0	ND	38,39
Tg(humanPrP) (129M)	S	0	ND	17,36,38,39
Tg(humanPrP) (129MV)	S	0	ND	39
Sheep	S	+‡	ND	27
Tg(ovine PrP)	S	0	ND	36
Cattle	S	++	0	26
Tg(bovine PrP)	S	0	ND	36
Raccoon	S	0	ND	34
Ferret	S	+++	0	31,32
Mink	S	++	0	35
Cat	S	++	0	63
Chinese hamster	N	++	ND	33
Armenian hamster	N	+	ND	33
Syrian golden hamster	N	+	ND	33
Djungarian hamster	N	0	ND	33
Siberian hamster	N	+	ND	33
Tg(hamsterPrP)	N	++	ND	33
Prairie vole	N	+++	ND	30
Meadow vole	N	+++	ND	29
Red-backed vole	S/N	+++	ND	29
Bank vole	N	+++	ND	19
Deer mouse	N	++	ND	29
White-footed mouse	N	++	ND	29
C57/BL6 mouse	S	0	ND	33
Tg(mousePrP)-overexpressing PrP	S	+, +++	ND	36,64

*Human numbering.

†+++: 75–100%, ++: 25–74%, +: 0–24% to terminal prion disease.

‡ARQ/VRQ only.

overexpressed or expressed endogenous levels of human PrP. All human codon 129 polymorphisms were represented (129MM, VV, or MV). Mice invariably resisted deer and elk CWD infection, as not a single animal developed clinical disease or PrP^{Sc} deposits in the brain, suggesting a strong barrier for CWD conversion of human PrP. This result is consistent with in vitro conversion experiments, which also indicate a strong barrier for CWD conversion of human PrP^{C}.[40,41]

STRUCTURAL DETERMINANTS OF CWD SUSCEPTIBILITY

The structural underpinnings of PrP sequence differences associated with prion resistance are unclear, however recent findings from our lab and others have revealed the importance of key interacting segments for CWD conversion. Mammalian PrP^{C} consists of approximately 210 amino acids, with an unstructured N-terminus and a globular

C-terminal domain composed of 3 α-helices and a short anti-parallel β-sheet.[42] One region of structural diversity is the β2-α2 loop (residues 165–175), which shows either a disordered, or a well-defined conformation by NMR spectroscopy.[43] For example, elk and bank vole PrP^C show a well-defined loop, whereas human and mouse PrP^C show a disordered loop.[42-46] To determine how the β2-α2 loop conformation impacts species barriers, mice were engineered to express mouse PrP^C with the elk β2-α2 loop, which required the S170N and N174T substitutions. These two substitutions change the loop from disordered (mouse) to well-defined (elk).[43] The resulting Tg($MoPrP^{S170N,N174T}$) mice were highly susceptible to CWD infection compared to mice expressing wild type mouse PrP^C.[47] Further studies utilizing mice with a well-defined loop due to a different substitution, D167S, showed that the loop conformation had no effect on CWD susceptibility,[48] as the mice had identical barriers as WT mice. Taken together, these results suggest that the 165–175 sequence similarity between cervid and host PrP, and not the secondary structure, governs CWD susceptibility.

Tamguney et al. generated an extensive series of transgenic mice expressing chimeric elk/mouse PrP^C sequences to determine the key residues involved in CWD conversion, with a focus on the C-terminus of PrP from 170 to 220 (human numbering)[49] as prior studies had shown the importance of this region.[50] Mice that expressed elk/mouse chimeric PrP^C having the mouse β2-α2 loop sequence (170S, 170N) showed barriers to CWD infection with attack rates ranging from 0–57%. In contrast, 6 of 7 lines having the elk N170 residue showed attack rates of 100%, further illustrating the importance of the loop segment for conversion of CWD.[49]

INVESTIGATING THE CWD-HUMAN SPECIES BARRIER

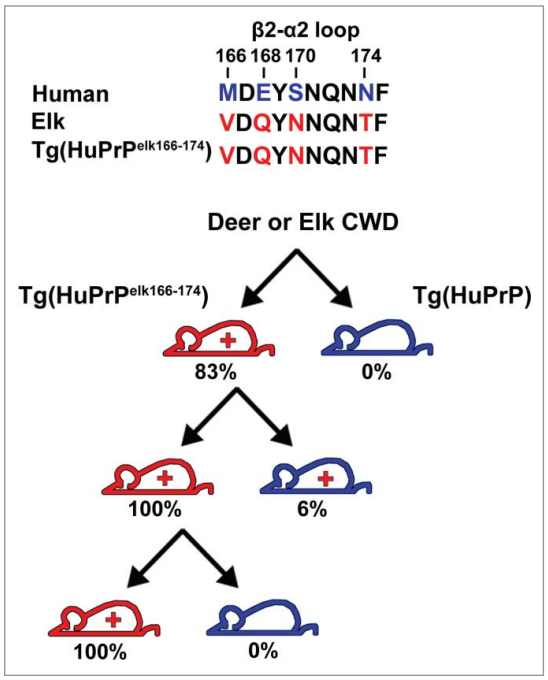
The elk β2-α2 loop promoted CWD conversion of mouse PrP, suggesting that the loop sequence may serve as a gatekeeper for CWD conversion in the context of mammalian PrP^C. To investigate this possibility, transgenic mice expressing human PrP with the elk β2-α2 loop sequence [Tg($HuPrP^{elk166-174}$)] were exposed to deer and elk CWD prions. As previously observed, mice expressing human PrP [Tg(HuPrP) mice] resisted CWD infection. However, [Tg($HuPrP^{elk166-174}$)] mice expressing human/elk chimeric PrP were highly susceptible to CWD prions. Further passage of CWD-infected Tg($HuPrP^{elk166-174}$) brain transmitted the prion infection to all Tg($HuPrP^{elk166-174}$) mice, yet to only 1 of 17 Tg(HuPrP) mice, indicating a significant barrier for prion transmission from Tg($HuPrP^{elk166-174}$) to Tg(HuPrP) mice, even though the PrP sequences differed by only 4 amino acid residues (Fig. 1). Interestingly, the elk β2-α2 loop sequence in human PrP created a barrier to sCJD infection, as the Tg($HuPrP^{elk166-174}$) mice were infected with human CJD prions after a moderate delay as compared to the Tg(HuPrP) mice.

STERIC ZIPPER MODELS MAY EXPLAIN SPECIES BARRIERS

Solving the molecular mechanism underlying cross-species prion conversion has been challenging due to the lack of high resolution structures for prions. Sequence similarity between PrP^C and PrP^{Sc} facilitates cross-species prion conversion, suggesting that the packing of amino acid side chains may play an important role in determining susceptibility to prion conversion (Fig. 2). A potential mechanism for PrP^C conversion is suggested by high resolution crystallography of microcrystals formed from amyloidogenic segments of fibril-forming proteins. The microcrystals are composed of β-sheets arranged parallel to the fibril axis, with complementary side chains from adjacent sheets interdigitating to form a dry "steric zipper" interface.[51]

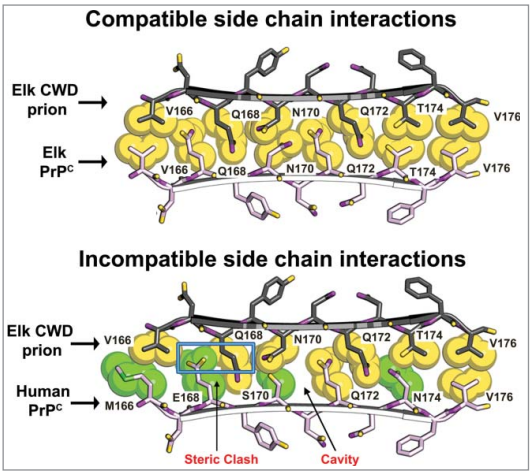
Several isolated PrP segments form microcrystals with steric zipper interfaces, including residues 170–175 from the β2-α2 loop. Interestingly, the 170–175 segment (SNQNNF in humans and mice, NNQNTF in deer and elk) forms distinct steric zipper structures in humans and mice as compared to deer and elk.

FIGURE 1. Investigating the structural determinants of the CWD-human transmission barrier. The human and elk β2-α2 loop amino acid sequences differ at 4 positions: 166, 168, 170, and 174 (top). Transgenic mice expressing full-length human PrP^C (blue) or human PrP^C with the elk β2-α2 loop (red) were inoculated intracerebrally with CWD prions. Although mice expressing human PrP^C did not develop disease, mice expressing the human-elk loop PrP^C [Tg(HuPrP$^{elk166-174}$)] were susceptible to CWD infection (83%). Inoculation of brain from a CWD-infected Tg(HuPrP$^{elk166-174}$) mouse into additional transgenic mice transmitted the disease to all Tg(HuPrP$^{elk166-174}$) mice, but to only 1 of 17 Tg(HuPrP) mice.



These differences in zipper structures offer an explanation for how a few human residues can inhibit conversion by CWD. Modeling the human and elk 165–175 segment reveals poor interdigitation of the human and cervid amino acid side chains at positions 168 and 170 that leads to gaps and steric clashes expected to destabilize the zipper (Fig. 2). Thus, PrP^C and PrP^{Sc} side chain interactions at the 165–175 segment may inhibit the stable incorporation of the PrP^C monomer into a growing fibril. One assumption in this model is that the interacting

FIGURE 2. Structural models of elk and human side chain packing within the β2-α2 loop may explain CWD transmission barriers. Atomic space-filling models of amino acid side chains within the β2-α2 loop of PrP were modeled as a parallel β-sheet.[53] In this model, the CWD PrP^{Sc} and cervid PrP^C (top pair) interdigitate in a steric zipper. In contrast, the CWD PrP^{Sc} and human PrP^C (bottom pair) interaction generates a steric clash (blue rectangle) and a cavity (arrow) that would be incompatible with zipper formation and may explain why CWD does not convert human PrP^C. Amino acids common to both cervids and humans are yellow; human-specific residues are green.



165–175 segment is exposed in both PrP^C and PrP^{Sc}.

In support of the steric zipper model for CWD conversion, in vitro conversion experiments using human PrP^C with elk substitutions revealed that human PrP^C with the elk E168Q and S170N substitutions was converted as efficiently as full length cervid PrP^C.[53] Surprisingly, human PrP^C with the elk E168Q, S170N, and N174T substitutions was converted poorly, revealing that the *human* N174 residue had bolstered CWD conversion. These findings indicate that in some cases, PrP sequence mismatches between the infectious prion and the host PrP^C promote cross-species conversion. These results also suggest a basis for the high susceptibility of voles to CWD infection, as the bank vole PrP sequence includes Q168, N170, and N174.

CONCLUSIONS

CWD has spread rapidly within the United States over the past decade. With the increased exposure of wildlife and other species to CWD, predicting prion infection risk has become more important and will enable a more targeted species surveillance as well as management of potential CWD reservoirs, such as wild voles. Utilizing a structural model of PrP^{C}-PrP^{Sc} interaction may facilitate those predictions.

Although the secondary structure of the β2-α2 loop varies depending on the sequence of the loop or the tightly interacting third helix, the secondary structure has not correlated with susceptibility to CWD prion conversion.[48,52,53] Instead, the amino acid sequence of the β2-α2 loop has an important role in promoting CWD conversion of PrP^{C} from other species. However, as the ferret and the squirrel monkey are highly susceptible to CWD infection, and neither has a β2-α2 loop that matches elk, it is clear that other PrP segments also interact during CWD conversion. Additionally, how segments interact in the context of full length PrP, as well as how potential hetero-zippers that could be accommodated in the new models of PrP^{Sc} structure should be considered. Future studies to define PrP^{C} : PrP^{Sc} interaction sites will help to refine the list of species most at risk for CWD infection.

ABBREVIATIONS

CWD	chronic wasting disease
PrP^{C}	cellular prion protein
PrP^{Sc}	misfolded, aggregated prion protein

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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