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Chronic Wasting Disease
Prion Strain Emergence and Host Range Expansion

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Human and mouse prion proteins share a structural motif that regulates resistance to common chronic wasting disease (CWD) prion strains. Successful transmission of an emergent strain of CWD prion, H951, into mice resulted in infection. Thus, emergent CWD prion strains may have higher zoonotic potential than common strains.

Chronic wasting disease (CWD) is a contagious prion disease of cervids that is spreading globally. CWD is enzootic in multiple cervid species, including deer and elk; the major foci of disease are Colorado/Wyoming (USA), Wisconsin/Illinois (USA), and Alberta/Saskatchewan (Canada). CWD is also present in captive cervids in South Korea and wild reindeer and moose in Norway (https://www.nwhc.usgs.gov/images/cwd/cwd_map.jpg). CWD results from the conformational transformation of the host-encoded cellular prion protein (PrPc) into protease-resistant, detergent-insoluble, β-sheet rich, amyloidogenic conformers, termed prions (PrPcWD). Within their conformation, prion strains encipher the information that directs the templated misfolding and aggregation of PrPc molecules into additional prions (1).

Although the sequence homology of PrP among mammals is high, the ability of particular prion strains to cause disease in different species is determined by the conformational compatibility between a given strain and the host PrPc (2). We

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previously identified 2 strains of CWD prion in white-tailed deer (3), Wisc-1 and H95⁺; these strains exhibit distinct biological properties in deer and transgenic cervidized mice. To ascertain the host range of different strains from cervids, we inoculated CWD prions isolated from experimentally infected deer with different PRNP genotypes (Q95G96 [wild type (wt)], S96/wt, H95/wt, and H95/S96) and from elk (CWD2 strain) into hamsters and mice. All isolates have been successfully transmitted into transgenic mice expressing wt cervid PrP and contain high titers of CWD prions (3).

Mice inoculated with H95⁻ CWD prions succumbed to clinical disease at 575 ± 47 or 692 ± 9 days, depending on the H95⁻ isolate (Table). Mice inoculated with Wisc-1 or elk CWD or uninfected deer homogenates were euthanized at day 708 after infection with no signs of prion disease. Clinical signs of H95⁻ CWD in C57Bl/6 mice included ataxia, lethargy, tail rigidity, and dermatitis. Protease-resistant PrP⁹⁵wt was present in all mice infected with H95⁻ prions and was not detected in mice infected with Wisc-1 or CWD2 (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/9/16-1474-Techapp1.pdf).

In contrast to mice, hamsters succumbed to clinical disease when inoculated with Wisc-1 CWD prions but were less susceptible to H95⁻ CWD prions (Table). Clinical signs of CWD in hamsters began with lethargy and, upon arousal, retrocollis; as the disease progressed, lethargy declined with increased dystonic movement including ataxia and tremors. Hyperesthesia was not observed. Subclinical disease (no clinical signs but PrP-res positive by Western blot) was observed in a subset of hamsters (online Technical Appendix).

Successful interspecies prion transmission at the molecular level depends on the compatibility of the invading prion conformers and structural determinants imposed by host PrP. One structural motif is the loop region between β sheet 2 and α helix 2 of PrP⁺ at aa 170–174 (online Technical Appendix). Host species containing PrP⁺ molecules with a flexible β2-α2 loop (mice and humans) are hypothesized to be incompatible with prions derived from species containing a rigid loop (deer and elk) (4,5). Previous attempts to transmit CWD to mice have failed (6,7). Our data show that prions from a prototypic rigid-loop species (deer) can transmit to a flexible-loop species (mice). The transmission is strain dependent. H95⁺ overrides the conformational restriction imposed by the mouse PrP flexible loop that Wisc-1 and CWD2 cannot overcome, suggesting that the invading prion strain is a dominant contributor to the species/transmission barrier. How the N terminal amino acid polymorphism (Q95H) affects the conformation of PrP, altering the deer-to-mouse transmission barrier, is unknown. Further structural studies may clarify the effect of N terminal residues on β2-α2 loop rigidity.

Transmission of H95⁺ CWD prions to mice further confirms the value of specifying strain when defining species barriers. Experimental transmission of CWD prions into macaques and transgenic mice expressing human PrP suggests a considerable transmission barrier to CWD prions (although squirrel monkeys are susceptible), and human prion protein is converted inefficiently in vitro (8,9). Successful infection of a flexible-loop species (mice) with H95⁺ CWD raises concerns for the potential pathogenicity of H95⁺ prions to other flexible-loop species. Transmission studies with Wisc-1 and H95⁺ in transgenic humanized and bovinized mice are ongoing.

The increasing prevalence of CWD indicates selection for cervids with resistance alleles, such as S96 and H95. Genetic resistance to a given prion strain selects for the emergence of novel prion strains with altered properties such as H95⁺ and Nor98 (3,10). The iterative transmission of CWD prions to cervids with protective alleles of PrP⁺ and the consequent emergence of new CWD prion strains highlights the dynamics of the CWD panzootic and the value of characterizing the host range of emergent CWD prion strains.

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<th>Table. Results of CWD prion inoculation into rodents*</th>
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* Mice infected with CWD prions were observed for up to 708 d; hamsters infected with white-tailed deer and elk CWD prions were observed for 659 and 726 d, respectively. Control mice and hamsters were inoculated with brain homogenates from CWD-negative wt/wt deer. CWD, chronic wasting disease; NA, not applicable; PrP-res+, positive for proteinase-K–resistant prion protein; wt, wild type.
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Dr. Herbst is a research associate and Dr. Duque Velásquez is a postdoctoral fellow at the University of Alberta. Their primary research interest is the mechanism(s) of pathogenicity underlying neurodegeneration, as exemplified by prion diseases in animals and humans.

References

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We report rabies virus transmission among solid organ transplantation recipients in Changsha, China, in 2016. Two recipients were confirmed to have rabies and died. Our findings suggest that more attention should be paid to the possibility of rabies virus transmission through organ transplantation for clinical and public health reasons.

In 2016, Zhou et al. reported a case of rabies virus transmission in China that was probably a result of organ transplantation (1). We report on rabies transmission that occurred among solid organ transplant recipients in Changsha, China, during December 2015–January 2016.

In November 2015, the donor, a previously healthy boy, showed development of fever, insomnia, and agitation. On day 6 of infection, these symptoms progressed, and he was sent to a healthcare center. At this time, he experienced weakness, no desire to drink water, poor appetite, and panic. One day later, he began vomiting, and was admitted to a local hospital (hospital A), where he exhibited anemophilia, convulsions, limb rigidity, and hypersalivation. The patient was moved to hospital B (days 7–14) in Changsha. At admission, some examination findings indicated a possibility of viral encephalitis (online Technical Appendix, Table 1, https://wwwnc.cdc.gov/EID/article/23/9/16-1704-TechnicalAppendix.pdf). Subhypothermia hibernation therapy and assisted ventilation were administered within 72 hours of admission, and the patient’s vital signs became stable. On day 10, hyponatremia was observed, and on day 11, the patient again became febrile and tachycardic, with hypertensive abdominal distention and alimentary tract hemorrhage. On day 13, viral encephalitis was diagnosed, and rabies was suspected. However, rabies virus antibody tests performed on serum samples by using ELISA yielded negative results.

On day 14, the patient was transferred to hospital C, where he became comatose and was declared brain dead. Permission was granted for organ donation, because no specific pathogen had been detected and China’s organ

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Technical Appendix

Additional Data

Brain homogenates from all mice (experimentally infected animals and uninfected controls) were analyzed for the presence of PrP-res. Proteinase K-resistant PrP was detected in all mice infected with H95+ prions (Figure 1) demonstrating that H95+ is 100% penetrant. PrP-res was not, however, detected in mice infected with the Wisc-1 strain from wt/wt or S96/wt deer or CWD2 from elk.

A similar analysis of brain homogenates from hamsters was also performed. Wisc-1 CWD was preferentially transmitted to hamsters upon primary passage (Figure 2). Wt/wt CWD caused clinical disease or subclinical accumulation of PrP-res in exposed hamsters. Transmission of S96/wt and H95/wt CWD prions resulted, primarily, in subclinical disease. Inoculation of elk CWD prions resulted in clinical disease in two out of five hamsters and subclinical disease in one animal. The H95/S96 isolate transmitted inefficiently with only one of eight hamsters having subclinical disease. This subclinical infection may be due to Wisc-1 in the H95/S96 deer isolate. Hamsters inoculated with uninfected deer brain homogenate did not show signs of prion disease nor accumulate PrP-res.

Secondary structure of host PrP influences the ability of a strain to propagate in a new host. Based on NMR studies, it has been hypothesized that amino acid variation at residues 170-175 (Figure 3) in PrP\textsuperscript{C} results in a loop structure between beta-sheet 2 and alpha-helix 2 (\(\beta2-\alpha2\)) whose flexibility influences the outcome of interspecies transmissions (1–7). Mice and humans share a flexible loop sequence (SNQNN) whereas deer and elk possess a rigid loop (NNQNT). Multiple attempts to transmit CWD prions to mice have failed (5–11). The transmission of prions occurs more readily when the loop structures match, e.g. BSE transmits to humans and mice (12,13). Experimental alteration of the mouse flexible loop to a rigid loop facilitated CWD
transmission (4,7). Our data suggests emergent CWD strain H95+ can overcome the conformational restriction imposed by the loop.

**Methods and Materials**

This study was conducted in accordance with the guidelines of the Canadian Council on Animal Care. The protocols used were approved by the Institutional Animal Care and Use Committees at the University of Alberta.

Brain homogenates were prepared from clinically-affected white-tailed deer (*Odocoileus virginianus*) of defined genotypes (14,15) or as a pool from three captive CWD-positive 132M/M elk (*Cervus canadensis*) (16,17), a kind gift from Catherine Graham. Four different white-tailed deer CWD isolates were used, one isolate was derived from a deer homozygous for the most common *PRNP* gene (wt = Q95G96) and three heterozygous deer (H95/wt, 96S/wt and H95/S96) expressing *PRNP* allele variants. These isolates have been extensively characterized in transgenic mice (15). Weanling C57Bl/6 mice were intracerebrally inoculated with 30 µl of 1% brain homogenate. Weanling Syrian Golden hamsters (*Mesocricetus auratus*) were intracerebrally inoculated with 50 µl of 10% brain homogenates (elk CWD was passaged at 1% brain homogenate). Mock-infected controls received brain homogenate from unaffected, PrP-res negative deer. Mice and hamsters were monitored for onset of clinical signs and disease progression. Individual incubation periods are expressed as the number of days post-inoculation (dpi) and were calculated from the time that the mice were inoculated until the time that clinical disease was established. Experiments were terminated at 708 dpi (mice) and 659 dpi (hamsters); all remaining animals were euthanized and assayed for PrP-res.

Brains were collected from mice and hamsters, flash frozen and stored at -80 until analysis. Tissues were homogenized to 10% (wt/vol) in sterile water with a tissue disruptor (Omniprep) and disposable homogenization tubes and beads. PrP-res was detected following proteinase K digestion and immunoblotting as previously described (15). Blots were probed with antibodies SAF83 (mice; Cayman Chemical, Michigan) or 3F4 (hamsters; a kind gift from Richard Rubenstein) monoclonal antibodies.
References


Figure 1. Representative western blot analysis of PrP-res on primary passage of CWD in mice. Each lane is an individual mouse brain homogenate sample treated with 50 µg/ml of proteinase K; equivalent volumes were loaded in each well. Only mice infected with H95+ isolates accumulated PK-resistant PrP. UI is a brain homogenate from a mouse inoculated with CWD negative wt/wt deer brain homogenate. Blots were probed with monoclonal antibody SAF83 at a 1:10,000 dilution. An asterisk denotes a subclinical mouse.

Figure 2. Representative western blot analysis of PrP-res on primary passage of CWD prions into Syrian golden hamsters. A. Brain homogenates from each passage line (10 µl of 10% brain homogenate) were treated with 100 µg/ml of proteinase K and 10 µl of each digested sample was loaded. One of 8 hamsters inoculated with H95/S96 CWD accumulated PK-resistant PrP. UI is a brain homogenate from a mock-infected hamster. Blots were probed with monoclonal antibody 3F4 at a 1:10,000 dilution. Asterisks denote subclinically affected hamsters.
Figure 3. Alignment of PrP amino acid sequences from humans, cervids and rodents. Numbering is based on the human sequence. Deer polymorphisms at 95, 96 (human residues 91, 92 respectively) and residues forming the β2-α2 loop are boxed.