Chronic wasting disease (CWD) is an emerging prion disease of cervids, affecting free-ranging white-tailed deer (Odocoileus virginianus), mule deer (Odocoileus hemionus), elk (Cervus elaphus canadensis), moose (Alces americanus) (1, 2), and red deer (Cervus elaphus) and sika deer (Cervus nippon) in South Korea (1, 3, 4). Reindeer (Rangifer tarandus), also known as caribou, are susceptible to experimental infection (5).

TSEs are slowly progressive, fatal neurodegenerative disorders for which no effective treatment or vaccine is available. Neuropathological changes include prion protein deposits, spongiform degeneration, neuronal loss, and astrogliosis. These hallmarks are diagnostic for CWD in cervids, scrapie in sheep and goats, bovine spongiform encephalopathy (BSE), as well as kuru, iatrogenic Creutzfeldt-Jakob disease (iCJD), and variant Creutzfeldt-Jakob disease (vCJD) in humans (6–10).

The pathogenesis of TSEs is associated with misfolded prion protein (PrPSc), or PrP\textsuperscript{CWD} for cervid infections), whose ability to propagate, persist, and trigger neuropathology requires the expression of host PRNP-encoded cellular prion protein (PrPC)
biochemical profiles (15–23). Knockout (Prnp) mice are refractory to experimental infection with mouse-adapted scrapie (24).

The difficulty of prion transmission from one species to another is defined as the species barrier, and that between individuals of the same species with different PRNP genotypes is defined as the transmission barrier and is influenced by the primary structure of the recipient’s PrP C (15, 17, 19, 20, 25, 26). This barrier does not necessarily render the host refractory to infection and is impacted by the invading prion strain (20, 21, 26, 27). Prions can exhibit strain diversity. Strains are distinguished on the basis of their host range, clinical presentation, disease progression, and neuropathological and PrP biochemical profiles (28–31). The propagation of prion strains is dependent on both the PRNP genotype of the recipient and the properties of the invading agent (27, 32). For example, sheep expressing the V136-R154-Q171 PrPC (GenBank accession number AJ567988) are most susceptible to classical scrapie, while sheep with distinct PRNP genotypes have reduced susceptibility (33–35). The strain of the agent also plays a role, as sheep expressing A136-R154-R171 PrPC (GenBank accession number AJ567985) or A136-H154-Q171 PrPC (GenBank accession number AJ567983) are susceptible to atypical scrapie, although they are relatively resistant to classical scrapie (31, 36). Similarly, the PrPC primary structure and the invading agent modulate human susceptibility to prion infection; polymorphisms at codon 129 affect susceptibility to vCJD, kuru, and iCJD (20, 21, 26, 27, 37–40), while the G12V mutation renders carriers resistant against kuru (41).

In regions of North America where CWD is enzootic, transmission occurs between cervids expressing heterologous PrP C molecules (PrP C allotypes [18]). Analysis of PRNP allelic frequencies in wild and captive white-tailed deer identified two PrP C polymorphisms, Q95H and G96S, that impact susceptibility to CWD (42–44) (GenBank accession numbers AF156185, AF156184, and AY275711). Homozygous wild-type (wt; Q95 G96) deer are most susceptible to CWD and have relatively short incubation periods. In contrast, deer heterozygous for the S96/wt, H95/wt, and H95/ S96 alleles had extended incubation periods, suggesting that S96-PrP C and H95-PrP C impact CWD prion propagation (45). Miller et al. (46) reported similar observations in experimentally challenged S96/wt and S96/S96 deer when the incubation periods for those deer were compared to those for wt/wt white-tailed deer and mule deer. To further explore the diversity of CWD strains and the consequences of propagation in deer expressing different PrPC primary structures, brain homogenates from CWD-infected white-tailed deer of different PRNP genotypes (wt/wt, S96/wt, H95/wt, or H95/S96 [45]) were inoculated into transgenic (tg) mice expressing the deer wt or S96 allele (47, 48). Our data show that CWD prions passed in deer expressing H95-PrP C have altered transmission properties. The H95/wt and H95/S96 CWD allotypes efficiently triggered prion disease in tg mice with S96-PRNP genotypes, leading to the identification and adaptation of a novel CWD strain. Transmission of first-passage tg60CWD-H95 + prions into tg33 mice resulted in two distinct prion disease phenotypes which resembled those observed after primary passage of H95-PrP heterozygous deer CWD in both tg lines.

**MATERIALS AND METHODS**

**Deer CWD inocula.** Four CWD agents consisting of 10% or 1% (wt/vol) brain homogenates (Bh) in phosphate-buffered saline were used for transmission studies (45). The inocula were designated on the basis of their specific PRNP genotypes. CWD brain homogenates were obtained from orally infected white-tailed deer expressing different PrP C molecules: homozygous Q95 G96 (wt/wt), heterozygous Q95 S96/wt (S96/wt), heterozygous H95 G96/wt (H95/wt), and H95 G96/Q95 S96 (H95/S96) (45). Brain homogenate from an uninfected white-tailed deer was used as a negative control. Frozen sagittal brain halves were homogenized (blended) to 20% (wt/vol) in cold phosphate buffer (1.3 M NaCl, 70 mM Na2HPO4·2H2O, 30 mM NaH2PO4·2H2O, pH 7.4), aliquoted, and stored at −80°C. Subsequently, aliquots were homogenized in a 50-ml syringe by passage through needles of different sizes (18 gauge to 21 gauge).

**Transmission studies in transgenic mice.** Animal studies were conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies with approval from the Health Sciences Animal Care and Use Committee of the University of Alberta Animal Care and Use Committee. Bioassays were performed with transgenic mouse lines expressing the deer wt allele (tg33133/133 and tg60-PrP C allele (S96/wt, S96/S96 and S96/PrP C) genotypes, leading to the identification and adaptation of a novel CWD allotype and the H95/S96 deer CWD allotype were compared using the Mann-Whitney test (P < 0.05). Survival times postinoculation between tg60 mice inoculated with the H95/wt deer CWD allotype and the H95/S96 deer CWD allotype were compared using the Mann-Whitney test (P < 0.05). The statistical analysis of transmission experiments was performed with GraphPad Prism (version 5.04) software.

Isolates derived from tg60 mice infected with the H95/wt or H95/S96 deer CWD allotype (tg60CWD-H95/wt and tg60CWD-H95/S96 isolates, respectively) were used for syngeneic and allogeneic passages. One tg60CWD-H95/wt isolate (10%, wt/vol) was transmitted in tg33 and tg60 mice. A tg60CWD-H95/S96 isolate (10%, 1%, 0.0001%, wt/vol) was also passaged into both tg lines.

**Histopathological analysis.** Brain tissues from at least 5 (range, 5 to 11) tg mice per inoculum group were formalin fixed and paraffin embedded for histopathological analysis. Sagittal brain sections were obtained from 2 to 4 mice in each group of animals receiving each inoculum, and coronal brain sections were obtained from 3 to 7 mice in each group. Six consecutive slides of both sagittal and coronal brain sections (4 coronal sections from each brain) were examined as follows: 2 slides (4 to 6 μm thick) were stained with hematoxylin and eosin (H&E) to evaluate the sections for spongiform degeneration, and the other 4 slides were immunostained for PrP C deposition and glial fibrillary acidic protein (GFAP)-positive astroglia. The sagittal paramedian brain sections were 0.36 to 0.60 mm lateral from the brain midline. All immunostaining experiments included CWD-positive tissue and negative mock-infected control sections. Differences in PrP C deposition patterns could exist in areas of the central nervous system that were not examined. For the purpose of comparison, identification of the structures in H&E-stained slides was performed according to the mouse brain atlas (49).

Lesion profile analysis was performed using coronal brain sections as described previously (50). The lesion profile scores for the first passage in tg33 mice were obtained from 3 to 7 mice per inoculum group. For the CWD-affected tg60 mice, lesion profiles were obtained by scoring 4 mice per inoculum group. The density of spongiform lesions in nine gray matter areas from the brains of prion disease-affected mice were scored by three independent observers in a blind manner. The scores are reported as the mean ± standard deviation.

PrP C deposition was visualized by immunostaining using anti-PrP monoclonal antibody BAR224 or 8G8 (0.2 μg/ml diluted 1:2,000 or 1:100, respectively; Bertin Pharma, formerly Spi-Bio). Briefly, brain slides were pretreated with high-pressure autoclaving (2.1 × 105 Pa) for 30 min in
citric acid (10 mM), pH 6.0, at 121°C, followed by treatment with 98% 
formic acid for 30 min and 4 M guanidine thiocyanate at room 
temperature. Astrogliosis was evaluated by immunostaining of glial fibril-
lar acid protein using an anti-GFAP antibody (0.5 mg/ml diluted 1:1,000; 
BD Biosciences) after hydrated autoclaving for epitope exposure. 
Immunohistochemical detection was achieved with biotinylated sec-
ondary antibodies according to the manufacturer’s instructions (ARK animal 
research kit; Dako). Tissue sections were scanned with a NanoZoomer 
2.0RS scanner (Hamamatsu Photonics) and analyzed using NanoZoomer 
digital pathology software (Hamamatsu Photonics).

Immunoblot analysis. Brain tissues from tg mice were homogenized 
to 10% (wt/vol) in sterile water using disposable syringes and needles of 
decreasing diameters (18 gauge to 21 gauge), aliquoted, and stored at 
−80°C. The brain homogenate protein content was determined using a 
micro-bicinchoninic acid assay kit (Life Technologies). For the proteinase 
digestion reactions, 50 to 70 µg total protein (final sample protein con-
centration, 1 to 1.4 mg/ml) was treated with 150 µg/ml of proteinase K 
(Life Technologies) for 45 min at 37°C. Reactions were terminated by 
boiling the samples in 2.5× Laemmli buffer (150 mM Tris-HCl, pH 6.8, 
0.5% bromophenol blue, 25% glycerol, 5% [wt/vol] SDS, 12.5% β-mer-
captoethanol) at 95°C for 10 min. Samples (10 to 15 µg) were resolved on 
12% NuPAGE bis-Tris gels (Life Technologies) and transferred onto 
polyvinylidene difluoride Immobilon-P membranes (Millipore). The 
membranes were blocked for 1 h at room temperature with 5% (wt/vol) 
nonfat dry milk in Tris-buffered saline containing 0.1% (vol/vol) Tween 
20 (TBST). Detection was performed using primary monoclonal antibody 
BAR224 (0.2 µg/ml diluted 1:10,000 in 5% [wt/vol] nonfat dry milk in 
TBST; Bertin Pharma) or 8G8 (0.2 µg/ml diluted 1:2,000; Bertin Pharma), 
secondary horseradish peroxidase-conjugated goat anti-mouse IgG anti-
body, and chemiluminescent substrate (diluted 1:10,000; Life Technolo-
gies). Images were acquired on X-ray film (Super Rx; Fujifilm). PrP-res 
glycoform ratios were determined using three animals per inoculum 
group; samples were resolved by Western blotting and detected with X-ray 
film. Quantification of PrP-res ratios was performed using ImageJ soft-

FIG 1 Transmission of CWD allotypes into transgenic mice expressing deer wt or S96-PrP C. (A) Susceptibility of tg33 (wt-PrP C) mice to infection with 10% (wt/vol) Bh from deer with CWD. (B) S96-PrP C (tg60) mice developed clinical prion disease only when inoculated with CWD prions derived from deer expressing H95-PrP C. Mice inoculated with wt/wt (open circles) or S96/wt (open squares) CWD prions did not show clinical signs. Symbols with crosses represent animals euthanized due to intercurrent disease. (C and D) Comparison of incubation periods in tg33+/+ and tg33−/− mice. ***, significant differences between groups (the Kruskal-Wallis test with Dunn’s multiple-comparison posttest, P < 0.05). The Mann-Whitney test (P < 0.05) was used to compare the distribution of incubation periods in tg60 mice.
TABLE 1 Prion disease in tg-deer-PRNP mice inoculated with white-tailed deer and tg mouse-passaged CWD prions

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>tg-deer-PRNP mouse line</th>
<th>Bh dose (%</th>
<th>No. of mice positive(^a)/total no. of mice tested</th>
<th>Incubation period range (dpi)</th>
<th>PrP-res type</th>
<th>PrP(^{\text{CWD}}) distribution pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deer CWD wt/wt</td>
<td>tg33</td>
<td>10</td>
<td>22/22</td>
<td>215–310</td>
<td>High MM(^b)</td>
<td>Widespread</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>8/8</td>
<td>256–345</td>
<td>High MM</td>
<td>Widespread</td>
</tr>
<tr>
<td>Deer CWD S96/wt</td>
<td>tg33</td>
<td>10</td>
<td>0/18</td>
<td>&gt;700</td>
<td>Negative</td>
<td>Not determined</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>0/10</td>
<td>&gt;600</td>
<td>Negative</td>
<td>Not determined</td>
</tr>
<tr>
<td>Deer CWD H95/wt</td>
<td>tg33</td>
<td>10</td>
<td>20/20</td>
<td>258–329</td>
<td>High MM</td>
<td>Widespread</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>8/8</td>
<td>225–357</td>
<td>High MM</td>
<td>Widespread</td>
</tr>
<tr>
<td>Deer CWD H95/S96</td>
<td>tg33</td>
<td>10</td>
<td>21/21</td>
<td>242–335</td>
<td>High MM</td>
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<td></td>
<td></td>
<td>1</td>
<td>10/10</td>
<td>316–356</td>
<td>High MM</td>
<td>Widespread</td>
</tr>
<tr>
<td></td>
<td>tg60</td>
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<td>0/21</td>
<td>&gt;700</td>
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<td>Not determined</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>0/10</td>
<td>&gt;600</td>
<td>Negative</td>
<td>Not determined</td>
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<tr>
<td>Deer CWD H95/S96</td>
<td>tg33</td>
<td>10</td>
<td>19/19</td>
<td>394–473</td>
<td>Low MM</td>
<td>Localized</td>
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<td></td>
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<td>10/10</td>
<td>465–608</td>
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<td>Localized</td>
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<tr>
<td>tg60CWD-H95/wt</td>
<td>tg33</td>
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<td>15/15</td>
<td>340–383</td>
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<td>9/9</td>
<td>323–433</td>
<td>High MM</td>
<td>Widespread</td>
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<td>tg60CWD-H95/S96</td>
<td>tg33</td>
<td>10</td>
<td>7/7</td>
<td>373–409</td>
<td>High or low MM</td>
<td>Localized or widespread</td>
</tr>
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<td></td>
<td>1</td>
<td>8/8</td>
<td>397–448</td>
<td>High or low MM</td>
<td>Localized or widespread</td>
</tr>
<tr>
<td></td>
<td>tg33</td>
<td>0.0011</td>
<td>8/8</td>
<td>329–490</td>
<td>High or low MM</td>
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<td></td>
<td></td>
<td>1</td>
<td>18/18</td>
<td>331–369</td>
<td>Low MM</td>
<td>Localized</td>
</tr>
<tr>
<td>Uninfected deer wt/wt</td>
<td>tg33</td>
<td>10</td>
<td>0/6</td>
<td>&gt;560</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>0/5</td>
<td>&gt;560</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

\(^a\) Clinically positive animals.

\(^b\) MM, molecular mass.
abundant in the white matter and the Purkinje cell layers (Fig. 2H). Infection of tg33 mice resulted in more prominent PrP<sub>CWD</sub> accumulation in the corpus callosum (Fig. 2A and B) than that described in other studies (47).

The susceptibility of S96-PrPC<sup>C</sup> (tg60) mice to CWD agent infection was strongly influenced by the invading CWD allotype. All tg60 mice exposed to the H95/wt or H95/S96 CWD agent developed clinical prion disease with similar neuropathologies (Fig. 2I to P and R and 3J to K). The distribution and severity of the neuropathological changes observed in diseased tg60 mice infected with H95<sup>+</sup> deer CWD allotypes followed a consistent lesion pattern (Fig. 2I to P and R and 3J to K). Spongiform degeneration and abnormal S96-PrP<sub>CWD</sub> aggregates were localized in the caudoputamen and the corpus callosum and extended down the septum to the diagonal band nucleus (Fig. 3K). Both vacuolation and PrP<sub>CWD</sub> deposition were of milder intensity in the cerebral cortex and hippocampus than in the other brain areas; however, immunohistochemical staining revealed the presence of small, punctate PrP<sub>CWD</sub> aggregates at higher magnification (Fig. 2K). Pathological changes were more severe in various regions of the thalamus, including the medial-dorsal, ventral-medial, and ventral anterior-lateral thalamic nuclei (Fig. 2I to J, L, and M and 3I) and...
also involved the zona incerta, cerebral peduncle, and subthalamic and hypothalamic nuclei (Fig. 2I to J and 3J). In the midbrain, lesions were localized in the substantia nigra adjacent to the ventral tegmental area and extended to periaqueductal gray and adjacent structures, including the raphe nucleus, mesencephalic reticular formation, and superior cerebellar peduncle (Fig. 2I and 3K). Pathology was also observed in the hindbrain and affected various regions, including the median raphe nucleus and pontine reticular nucleus (Fig. 3K). In the cerebellum, the spongiiform change was the most prominent in the white matter; however, small vacuoles were also observed in the molecular, Purkinje, and granular layers, with the granular layer showing loss of granular neurons (Fig. 2N). PrP<sup>CWD</sup> staining revealed either diffuse deposits (lightly stained) or larger confluent aggregates in the cerebellar nuclei and the granular layer (Fig. 2I and P and 3K).

A few tg60 (S96-PrPC) mice that did not have clinical signs and that were challenged with the wt/wt or S96/wt CWD agents (3/28 and 3/31 mice, respectively) had detectable prion aggregates at >700 dpi, highlighting the low efficacy of these CWD agents for establishing infection in this transgenic line. The accumulation of PrP<sup>CWD</sup> aggregates in these particular mice did not follow the PrP<sup>CWD</sup> distribution patterns described in the other mice.
PrP-res glycotypes in transgenic mice expressing deer PrP<sup>C</sup>. Distinct PrP-res isoforms have been associated with different prion strains (28, 51, 52). PrP-res can vary in their molecular masses, glycoform ratios, and other biochemical properties related to the structural stability of the abnormal PrP conformers (53). These properties have been interpreted to be conformational differences in the structures of the misfolded PrP molecules that carry the information that defines different prion strains (28, 51–53). To compare the PrP-res in mice infected with the different CWD inocula, brain homogenates were digested with proteinase K and analyzed by SDS-PAGE and Western blotting. Lanes M, molecular size markers. PrP-res from tg33 mice had similar molecular masses after enzymatic cleavage (A) and equivalent glycoform ratios (B). S96-PrP-res has a lower molecular mass and was detectable only in brain homogenates derived from tg60 mice infected with H95<sup>+</sup> CWD allotypes. Ul, homogenates fromtg mice inoculated with uninfected deer brain homogenate. PrP-res detection was achieved with anti-PrP monoclonal antibody BAR224.

**FIG 4** Disease-associated PrP-res in tg-deer-PRNP mice inoculated with different CWD allotypes. (A) PrP-res from brains of prion-affected tg33 (wt-PrP<sup>C</sup>) and tg60 (S96-PrP<sup>C</sup>) mice. Brain homogenates were digested with proteinase K (PK) and analyzed by SDS-PAGE and Western blotting. Lanes M, molecular size markers. PrP-res fromtg33 mice had similar molecular masses after enzymatic cleavage (A) and equivalent glycoform ratios (B). S96-PrP-res has a lower molecular mass and was detectable only in brain homogenates derived from tg60 mice infected with H95<sup>+</sup> CWD allotypes. Ul, homogenates fromtg mice inoculated with uninfected deer brain homogenate. PrP-res detection was achieved with anti-PrP monoclonal antibody BAR224.

To evaluate the transmission properties of CWD prions derived from white-tailed deer of four different PRNP genotypes (45), we inoculated transgenic mice expressing deer prion proteins associated with susceptibility (tg33 mice expressing deer wt-PrP<sup>C</sup>) or resistance (tg60 mice expressing deer S96-PrP<sup>C</sup>) to CWD prions (47, 48). Transmission of the H95<sup>+</sup>/H95<sup>+</sup>, H95<sup>+</sup>/S96<sup>+</sup>, and S96<sup>+</sup>/S96<sup>+</sup> allotype combinations resulted in divergent prion disease phenotypes. Inoculation of 10% brain homogenates resulted in extended incubation periods, with some mice developing disease signs and pathology characteristic of tg33 mice infected with the H95<sup>+</sup>/wt deer CWD allotype (Fig. 6A and Table 1). Disease signs and pathological hallmarks were similar to those described during the first passage of deer CWD prions in tg33 mice, characterized by hyperactivity, a widespread distribution of aggregates in the brain, and high-molecular-mass PrP-res (Fig. 6B and C). Transmission of the tg60CWD-H95<sup>+</sup>/S96<sup>+</sup> isolate intotg33 mice resulted in divergent prion disease phenotypes. Inoculation of 10% brain homogenates resulted in extended incubation periods, with some mice developing disease signs and pathology characteristic of tg33 mice infected with the H95<sup>+</sup>/wt deer CWD agents, while others developed disease signs and neuropathology that resembled the disease phenotype described for tg60 mice (Fig. 6). Evaluation of proteinase K-resistant PrP in brain homogenates from affected mice revealed PrP-res glycotypes of distinct molecular masses (Fig. 6B). Passage of 1% and 0.0001% (wt/vol) brain homogenates resulted in further extension of the incubation period and increased the abundance of mice presenting with lethargy (like tg60 mice), accompanied by the accumulation of low-molecular-mass PrP-res and localized deposition of PrP aggregates in brain (Fig. 6).

**DISCUSSION**

To explore the transmission properties of CWD prions derived from white-tailed deer of four different PRNP genotypes (45), we inoculated transgenic mice expressing deer prion proteins associated with susceptibility (tg33 mice expressing deer wt-PrP<sup>C</sup>) or resistance (tg60 mice expressing deer S96-PrP<sup>C</sup>) to CWD prions (47, 48). Transmission of the H95<sup>+</sup>/H95<sup>+</sup> and H95<sup>+</sup>/S96<sup>+</sup> CWD allotype combinations resulted in divergent prion disease phenotypes. Inoculation of 10% brain homogenates resulted in extended incubation periods, with some mice developing disease signs and pathology characteristic of tg33 mice infected with the H95<sup>+</sup>/wt deer CWD allotype (Fig. 6A and Table 1). Disease signs and neurological hallmarks were similar to those described during the first passage of deer CWD prions in tg33 mice, characterized by hyperactivity, a widespread distribution of aggregates in the brain, and high-molecular-mass PrP-res (Fig. 6B and C). Transmission of the tg60CWD-H95<sup>+</sup>/S96<sup>+</sup> isolate intotg33 mice resulted in divergent prion disease phenotypes. Inoculation of 10% brain homogenates resulted in extended incubation periods, with some mice developing disease signs and pathology characteristic of tg33 mice infected with the H95<sup>+</sup>/wt deer CWD agents, while others developed disease signs and neuropathology that resembled the disease phenotype described for tg60 mice (Fig. 6). Evaluation of proteinase K-resistant PrP in brain homogenates from affected mice revealed PrP-res glycotypes of distinct molecular masses (Fig. 6B). Passage of 1% and 0.0001% (wt/vol) brain homogenates resulted in further extension of the incubation period and increased the abundance of mice presenting with lethargy (like tg60 mice), accompanied by the accumulation of low-molecular-mass PrP-res and localized deposition of PrP aggregates in brain (Fig. 6).
guishable from that observed following infection with the wt/wt or S96/wt CWD agents. The ability of H95 CWD agent to cause clinical prion disease in tg60 mice, which have been shown to be resistant to other CWD isolates, indicates that a new strain has emerged (45, 47, 48). Our data show that the passage of CWD (wt/wt pool) through deer with the H95/wt and H95/S96 allotypes resulted in a mixture of at least two CWD strains, distinguishable on the basis of the tg-deer-PRNP genotype in which they were propagated.

Upon first passage into tg33 mice, all deer CWD agents resulted in similar disease signs, PrP-res glyotypes, and neuropathological features, suggesting that expression of wt-PrPC favored the propagation of a CWD strain (prion conformer) common to all inocula. We refer to this agent as “Wisc-1.” Our results show that the passage of CWD (wt/wt pool) through deer with the H95/wt and H95/S96 allotypes resulted in a mixture of at least two CWD strains, distinguishable on the basis of the tg-deer-PRNP genotype in which they were propagated.

We found that inoculation of the H95/S96 CWD agent into tg33 mice resulted in incubation periods significantly different from those obtained by inoculation of CWD prions of the other allotypes. The absence of wt-PrP<sup>C</sup> in this inoculum and, thus, the lack of homologous prion conversion likely contributed to the prolonged incubation period. The presence of more than one prion conformer within this inoculum may result in competition between agents, leading to propagation interference and extension of the incubation periods (56–59).

Incubation periods were not significantly different between tg33 mice infected with the wt/wt, H95/wt or S96/wt CWD agents. Additionally, all tg33 mice presented the same prion disease phenotype irrespective of the CWD inoculum that they received. One possible interpretation for the phenotypic similarities observed between tg33 mice is that the Wisc-1 conformers have an adaptive advantage in hosts (either in deer or in tg mice) expressing wt-PrPC. The differences in incubation periods between the H95/wt CWD allotype- and H95/S96 CWD allotype-infected tg33 mice suggest that the PrP<sup>C</sup> sequence in these deer impacted the proportion of accumulated CWD strains. It has previously been demonstrated in hamster coinfection experiments that the ratio of the strains in a prion mixture influences the emergence of the fastest-replicating or dominant strain (56, 57, 59).

The differential susceptibility to prion infection is modulated by PrP<sup>C</sup> amino acid sequence variability and the invading prion
strain (15, 17, 20, 21, 25, 26, 60). Both natural and experimental infections support the association of S96-PrPC with reduced susceptibility and the slower progression of CWD (3, 42–48, 55). tg60(S96-PrPC) mice were previously shown to be resistant to CWD isolates from different cervid species (47, 48). In our study, tg60 mice inoculated with the wt/wt or S96/wt CWD agents did not present with clinical disease after challenge with passage 1 tg60CWD-H95 + isolates. Passage of tg60CWD-H95/S96 brain homogenates gave rise to different clinical presentations (hyperactivity versus lethargy) resembling the disease phenotypes described for both tg-deer-PRNP lines during the first passage of deer CWD prions. Black symbols, tg33 animals with hyperactive disease presentation, high-molecular-mass PrP-res, and a widespread distribution of brain PrP-res aggregates; orange symbols, tg33 mice with a lethargic presentation, low-molecular-mass PrP-res, and a localized distribution of PrP-res aggregates. (B) PrP-res glycotypes in brains of tg33 mice inoculated with different tg60CWD-H95 + isolates. Infected tg33 mice accumulated different PrP-res types resembling those observed after the first passage of deer CWD prions. Lane M, molecular size markers. (C) Divergent histological phenotypes in tg33 mice infected with tg60CWD-H95/S96 or tg60CWD-H95/wt brain homogenates. Bars, 2.5 mm. Detection of abnormal PrP was performed with anti-PrP monoclonal BAR224.

FIG 6 Allogeneic transmission of tg60 (S96-PrPC) mouse-passaged CWD prions into tg33 mice. (A) Incubation periods of tg33 mice upon challenge with passage 1 tg60CWD-H95 + isolates. Passage of tg60CWD-H95/S96 brain homogenates gave rise to different clinical presentations (hyperactivity versus lethargy) resembling the disease phenotypes described for both tg-deer-PRNP lines during the first passage of deer CWD prions. Black symbols, tg33 animals with hyperactive disease presentation, high-molecular-mass PrP-res, and a widespread distribution of brain PrP-res aggregates; orange symbols, tg33 mice with a lethargic presentation, low-molecular-mass PrP-res, and a localized distribution of PrP-res aggregates. (B) PrP-res glycotypes in brains of tg33 mice inoculated with different tg60CWD-H95 + isolates. Infected tg33 mice accumulated different PrP-res types resembling those observed after the first passage of deer CWD prions. Lane M, molecular size markers. (C) Divergent histological phenotypes in tg33 mice infected with tg60CWD-H95/S96 or tg60CWD-H95/wt brain homogenates. Bars, 2.5 mm. Detection of abnormal PrP was performed with anti-PrP monoclonal BAR224.
homozygous deer (45, 46, 48). Additionally, in areas where CWD is endemic, white-tailed deer with S96-PRNP alleles likely have a fitness advantage over deer with the more susceptible genotypes, and as a result, the resistance allele may become more abundant in the population (64). An increase in the S96-PRNP allele frequency could also affect the potential for the selection of CWD strains able to infect deer with resistant genotypes. Likewise, other PRNP alleles associated with extension of the CWD preclinical phase, such as H95-PRNP, could also be subjected to a disease-driven increase in white-tailed deer populations. Our transmission data show that deer expressing H95-PrP accumulate a CWD strain capable of infecting deer with S96-PRNP genotypes, unlike other CWD agents. An increase in the frequency of H95-PRNP would also increase the likelihood of the emergence of H95- CWD prions. Our data suggest that white-tailed deer expressing different PrPC alleles can accumulate and transmit CWD strain mixtures. CWD epizootics involve multiple factors, including the contagious nature of the agent, host-pathogen interactions, agent strains, and cervid population genetics. Our data indicate that CWD strain emergence is modulated by amino acid polymorphisms in the cervid PrP. CWD transmission between hosts with different PRNP genotypes (65) has the potential to generate and select novel prion conformations. Deer expressing H95-PrP⁺ accumulate CWD prions with different transmission properties, as exemplified by its ability to infect resistant S96-PRNP mice. Finally, our study highlights the importance of characterizing the diversity of CWD strains and their potential for interspecies transmission. Our results demonstrating that H95⁺ deer CWD prions have transmissibility of CWD allotypes.

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