Supporting Online Material for

Prion Strain Mutation Determined by Prion Protein Conformational Compatibility and Primary Structure

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Supporting Online Material

Materials and Methods

Transgenic mice and inocula Transgenic mice expressing deer or elk PrP coding sequences, referred to as Tg(CerPrP)1536$^{+/-}$ and Tg(CerPrP-E226)5037$^{+/-}$ respectively, have been described previously (*S1, S2*). All transmitted isolates in this study originated from deer and elk expressing wild type *PRNP* coding sequences. The 03W1755 elk used in PMCA studies was heterozygous (M/L) at codon 132. Ten % (w/v) homogenates, in phosphate buffered saline (PBS) lacking calcium and magnesium ions, of cervid and mouse brains were prepared by repeated extrusion through an 18 gauge followed by a 21 gauge syringe needle.

Determination of Incubation Periods Groups of anesthetized mice were inoculated intracerebrally with 30 μl of 1 % (w/v) brain extracts prepared and diluted in PBS, or 1 % v/v of the final PMCA product diluted in PBS. Groups of mice were monitored thrice weekly for the development of prion disease. Following a relatively non-specific prodromal phase, early definitive and progressive clinical signs included stimulation-induced hyperexcitability, and flattened posture, culminating in profound ataxia toward the endpoint of disease. CWD-affected mice were rarely kyphotic and maintained a deep pain reflex at end stage. Inoculated mice were diagnosed with prion disease following the progressive development of at least three clinical signs, the time from inoculation to the onset of definitive and subsequently progressive clinical signs being referred to as the incubation time.

Analysis of PrP Animals whose death was obviously imminent were euthanized and their brains taken for biochemical and histopathological studies. For PrP analysis in brain extracts, total protein content from 10 % brain homogenates prepared in PBS was determined by bicinchoninic acid assay (Pierce Biotechnology Inc., Rockford, IL). Brain extracts were either untreated or treated with 40 μg/ml PK for one hour at 37^{o}C in the presence of 2 % sarkosyl and the reaction was terminated with 4 mM phenyl methyl sulfonyl fluoride. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, electrophoretically transferred to PVDF-FL membranes (Millipore, Billerica, MA), which were probed with anti-PrP mAbs followed by horse radish peroxidase-conjugated sheep anti-mouse IgG, developed using ECL-plus detection (Amersham), and analyzed using an FLA-5000 scanner (Fuji). To determine the relative values of CerPrP^{Sc} glycoforms, band intensities were analyzed by densitometry of Western blots using the FLA-5000 scanner.

For histoblot analysis, mice exhibiting neurological dysfunction were humanely killed and their brains immediately frozen on dry ice. Ten μm thick cryostat sections were transferred to nitrocellulose as previously described (*S3*). Histoblots were immunostained with mAb 6H4 followed by alkaline phosphatase-conjugated sheep anti-mouse secondary antibody. Images were captured with a Nikon SMZ1000 microscope with Photometrics Coolsnap CF digital imager and processed with MetaMorph software.

PrP^{Sc} in brain homogenates of terminally sick mice was also analyzed by conformational stability assay (*S4-S7*). The relative amounts of bands

representing PK resistant CerPrP^{Sc} were analyzed by densitometry of Western blots using the FLA-5000 scanner. The sigmoidal dose-response was plotted using a four-parameter algorithm and non-linear least square fit. The Gdn.HCl concentration required to denature 50% of CerPrP^{Sc} is denoted as the $(Gdn.HCl)_{1/2}$ value.

For histopathological studies, brains were dissected rapidly after sacrifice of the animal and immersion fixed in 10% buffered formalin. Tissues were embedded in paraffin and 8 μm thick coronal microtome sections were mounted onto positively charged glass slides. Analysis of PrP in the brains of mice by IHC was performed as previously described (*S8*) using anti-PrP mAb 6H4 as primary antibody, and IgG_1 biotinylated goat anti-mouse secondary antibody (Southern Biotech). Following inactivation of endogenous peroxidases by incubation in 3% H_2O_2 in methanol, peroxidase immunohistochemistry was used to evaluate the extent of reactive astrocytic gliosis using antibodies to glial fibrillary acidic protein. Detection was with Vectastain ABC reagents and slides were developed with diaminobenzidine. Digitized images for figures were obtained by light microscopy using a Nikon Eclipse E600 microscope equipped with a Nikon DMX 1200F digital camera.

Neuropathological Lesion Profiling Paraffin-embedded mouse brains were sectioned coronally to areas corresponding to the five levels of the brain that contained the mouse brain regions of interest. Brain sections were stained with hematoxylin and eosin. Images of the each of the brain regions were captured using a Photometrics Cool Snap digital camera and a Nikon Eclipse E600

microscope. The extent of vacuolar degeneration in the cerebral grey matter was assessed using a semi-quantitative method for discriminating prion strains (*S9*). The numbers of vacuoles per field were manually counted.

Text

In addition to intrinsic strain characteristics, time to onset of disease is dependent on prion titers (*S10*). Both effects were evident in these studies. Preparations containing low CWD prions titers produce longer incubation times in Tg(CerPrP)1536$^{+/-}$ mice than higher titer isolates (*S2*). Prolonged incubation times resulting from primary transmission of low titer CWD isolates generally shorten on second passage in syngeneic hosts, while strain-related incubation time properties are expected to persist during serial transmission. To begin to distinguish the effects of strain and titer on the variable incubation times observed during primary transmissions, we performed serial transmissions in Tg(CerPrP)1536$^{+/-}$ mice (Table S1). Titer-related reduction in mean incubation time was a feature of many of serially passaged isolates (Table S1). Generally, on second passage, there was less variance of incubation times for both strains, an effect that was also likely to be related to more consistent prion titers. Factors affecting CWD prion titers include the stage of disease in affected deer and elk, the neuroanotomical locations from which prions were isolated, and possible effects of post-mortem interval.

The most extreme effects of titer were observed during transmission of the 012-22012 elk isolate. Despite a 387 d incubation time, CWD1 neuropathology

was registered in the only mouse available for analysis following transmission of elk isolate 012-22012 (Fig. 1A); 3 of 8 inoculated mice in this cohort did not develop disease (Table S1 and Fig. 1A). The protracted time to onset of disease and the less than 100 % attack rate on primary passage suggests that the titer of CWD1 prions in this elk isolate was close to the endpoint of sensitivity of the bioassay. Consistent with this notion, serial passage of 012-22012 prions from the brain of a second diseased mouse with a 380 d incubation time, produced a rapid mean incubation time of 208 ± 4 d in 8 inoculated mice and CWD1 neuropathology in all analyzed mice (n = 5) (Fig. 2A and Table S1).

Table S1: Transmission of CWD prions to Tg(CerPrP)1536$^{+/-}$ mice

		Incubation time, mean days ± SD (n/n_0)	
Inoculum	**Origin**	**First**	**Second**
Elk			
012-22012	Colorado	384 ± 3.3 (5/8)	208 ± 3.5 (8/8)
012-09442	Colorado	208 ± 16.9 (8/8)	307 ± 25.3 (6/6)
02-0306	Saskatchewan	225 ± 8.3 (7/7)	238 ± 38.1 (7/7)
12389	Wyoming	230 ± 24.4 (8/8)	
001-44720	Colorado	231 ± 13.7 (7/7)	248 ± 37.5 (8/8)
7378-47	Wyoming	235 ± 5.5 (8/8)	230 ± 28.6 (7/7)
001-403022	Colorado	271 ± 35.9 (8/8)	235 ± 38.1 (8/8)
04-0306	Saskatchewan	281 ± 14.9 (7/7)	211 ± 7.5 (7/7)
CWD pool	Alberta	293 ± 30.9 (6/6)	
01-0306	Saskatchewan	322 ± 25.3 (8/8)	274 ± 29.3 (8/8)
03-0306	Saskatchewan	335 ± 12.6 (7/7)	226 ± 45.9 (9/9)
Mule deer			
8481	Wyoming	173 ± 3.8 (7/7)	217 ± 28.8 (7/7)
978-24384	Colorado	211 ± 22.5 (7/7)	229 ± 26.8 (5/5)
D10	Colorado	228 ± 28.9 (15/15)	217 ± 28.3 (8/8)
D92	Colorado	232 ± 48.8 (15/15)	244 ± 46.6 (7/7)
9179	Wyoming	239 ± 64.6 (7/7)	216 ± 32.0 (7/7)
989-09147	Colorado	250 ± 6.5 (8/8)	325 ± 36.0 (5/5)
W97	Colorado	254 ± 27.1 (5/5)	226 ± 43.9 (7/7)
8905	Wyoming	259 ± 63.3 (8/8)	238 ± 28.6 (8/8)
Db99	Colorado	259 ± 11.2 (7/7)	246 ± 15.8 (4/4)
7138	Wyoming	260 ± 46.6 (7/7)	216 ± 22.9 (7/7)
CWD Pool	Colorado	264 ± 9.3 (7/7)	207 ± 6.0 (6/6)
33968	Colorado	278 ± 27.0 (6/6)	239 ± 19.5 (8/8)
H92	Colorado	283 ± 20.4 (6/6)	259 ± 44.6 (8/8)
04-22412	Wyoming	284 ± 54.4 (6/6)	
001-39647	Colorado	289 ± 7.9 (5/5)	217 ± 47.1 (8/8)
V92	Colorado	310 ± 30.5 (7/7)	288 ± 16.0 (8/8)
Whitetail deer			
	Wisconsin	200 ± 19.2 (6/6)[2]	206 ± 3.7 (8/8)
PMCA			
Elk 03W1755	Wyoming/Texas[3]	446 ± 22.6 (5/5)	
Deer 04-22412	Wyoming/Texas[3]	264 ± 73.1 (6/6)	
Total CWD1[4]		212 ± 34.1 (n = 64)	206 ± 11.9 (n = 68)
Total CWD2[4]		306 ± 48.5 (n = 78)	286 ± 22.1 (n = 46)
Saline		410 – 597 (0/7)	
None		421 – 490 (0/7)	

[1] The number of mice developing prion disease (n), divided by the number inoculated (n_0) is shown in parentheses. Mice dying of causes unrelated to prion disease were excluded.

[2] PrP^{Sc} in this sample was precipitated with sodium phosphotungstate prior to inoculation.

[3] Samples originated from Wyoming elk and deer; PMCA was accomplished in Texas.
[4] Mean incubation times for primary transmission of naturally-occurring and PMCA-generated CWD prions were determined in 64 neuropathologically confirmed mice with the CWD1 pattern, and 78 neuropathologically confirmed mice with the CWD2 pattern. Mean incubation times for secondary transmissions of CWD prions were determined in 68 neuropathologically confirmed mice with the CWD1 pattern, and 46 neuropathologically confirmed mice with the CWD2 pattern. For both primary and secondary passages, incubation times of CWD1 and CWD were different ($p < 0.0001$).

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