



Supporting Online Material for

Prions in Skeletal Muscles of Deer with Chronic Wasting Disease

Rachel C. Angers, Shawn R. Browning, Tanya S. Seward, Christina J. Sigurdson,
Michael W. Miller, Edward A. Hoover, Glenn C. Telling§

§To whom correspondence should be addressed: E-mail: gtell2@uky.edu

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This PDF file includes:

Materials and Methods
Fig. S1

Supporting Online Materials

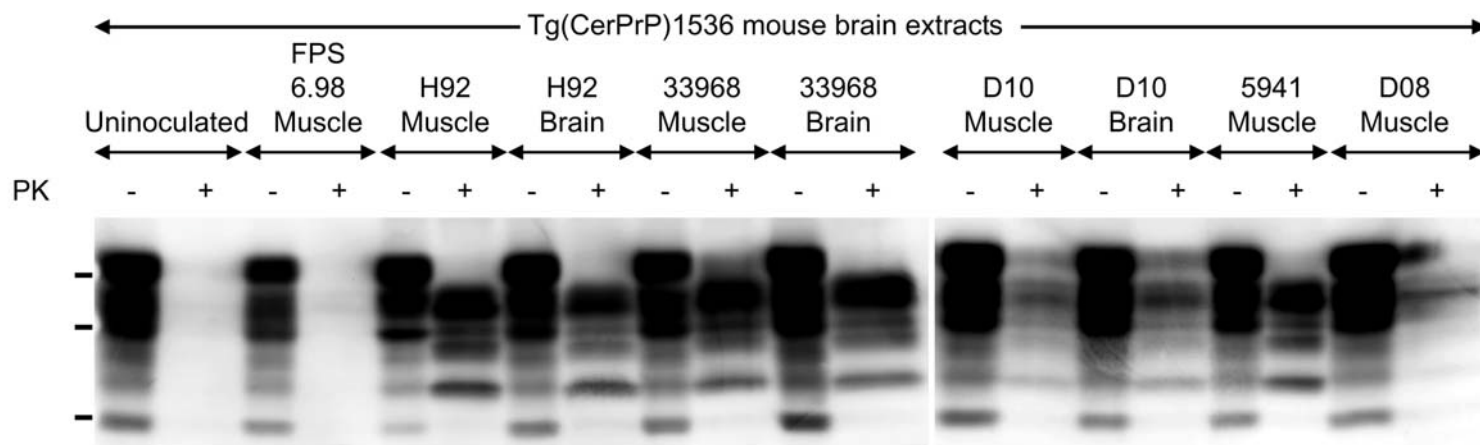
Materials and Methods

Homogenates of semitendinosus/semimembranosus muscle (10% w/v in phosphate buffered saline) were prepared from five emaciated and somnolent mule deer, naturally infected with CWD at the Colorado Division of Wildlife, Wildlife Research Center. These deer were identified as D10, D08, 33968, H92, and 5941. CWD infection was confirmed in all cases by the presence of histologic lesions in the brain including spongiform degeneration of the perikaryon, the immunohistochemical detection of disease-associated PrP in brain and tonsil, or by immunoblotting of protease-resistant, disease associated PrP (CerPrP^{Sc}). Semitendinosus/semimembranosus muscle was also obtained from two asymptomatic, mock inoculated deer, referred to as FPS 6.68 and 9.98, that originated from a CWD non-endemic area and which were held indoors at Colorado State University from ten days of age. These control deer were confirmed negative for CWD by histopathological and immunohistochemical analysis of brain tissue at autopsy. The utmost care was taken to avoid inclusion of obvious nervous tissue when muscle biopsies were prepared and to ensure that contamination of skeletal muscle samples with CNS tissue did not occur. Fresh, single-use instruments were used to collect each sample biopsy and a central piece from each sample was prepared with fresh, disposable instruments to further isolate muscle tissue for inoculum preparation. Brain samples for transmission were prepared separately from muscle as additional insurance against cross contamination.

Groups of anesthetized Tg(CerPrP)1536 mice were inoculated intracerebrally with 30 μ l of 1 % skeletal muscle or brain extracts prepared in phosphate buffered saline (PBS). Inoculated Tg(CerPrP) mice were diagnosed with prion disease following the progressive development of at least three neurologic symptoms including truncal ataxia, 'plastic' tail, loss of extensor reflex, difficulty righting, and slowed movement. The time from inoculation to the onset of clinical signs is referred to as the incubation time.

For PrP analysis in brain extracts of Tg(CerPrP)1536 mice, 10 % homogenates prepared in PBS were either untreated (-) or treated (+) with 40 μ g/ml proteinase K (PK) for one hour at 37°C in the presence of 2% sarkosyl. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, analyzed by immunoblotting using anti PrP monoclonal antibody 6H4 (Prionics AG, Switzerland), incubated with appropriate secondary antibody, developed using ECL-plus detection (Amersham), and analyzed using a FLA-5000 scanner (Fuji).

Fig. S1



PrP in brain extracts from representative Tg(CerPrP)1536 mice receiving muscle or CNS tissue inocula from CWD-affected or CWD-negative deer. Extracts were either treated (+) or untreated (-) with proteinase K (PK) as indicated. The positions of protein molecular weight markers at 21.3, 28.7, 33.5 kDa (from bottom to top) are shown to the left of the immunoblot.