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Experimental Chronic Wasting Disease in wild type VM mice

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Yoon-Hee Lee(1)*#, Hyun-Joo Sohn(1)#, Min-Jeong Kim(1), Hyo-Jin Kim(1), Kyung-Je Park(1), Won-Yong Lee(1), Eun-Im Yun(1), Dong-Seob Tark(1), Young-Pyo Choi(1), In-Soo Cho(1) and Aru Balachandran(2)

(1) OIE Reference Laboratory for CWD, Animal, Plant and Fisheries Quarantine and Inspection Agency, Ministry for Food, Agriculture, Forestry and Fisheries, Anyang 430-757, Republic of Korea

(2) National and OIE Reference Laboratory for Scrapie and CWD, Ottawa Laboratory Fallowfield, Ottawa, Canadian Food Inspection Agency, Ottawa, Ontario, Canada

*Correspondence to Yoon-Hee Lee, Animal, Plant and Fisheries Quarantine and Inspection Agency, Ministry for Food, Agriculture, Forestry and Fisheries, Anyang 430-757, Republic of Korea
e.mail: lyhee74@korea.kr (Tel: 82-31-467-1879, Fax: 82-31-467-1830)

#These two authors contributed equally to this work
ABSTRACT

Chronic wasting disease (CWD) is a naturally occurring prion disease in North American deer (*Odocoileus species*), Rocky mountain elk (*Cervus elaphus nelsoni*) and moose (*Alces alces*). The disease was first confirmed in the Republic of Korea in 2001, and subsequent cases were diagnosed in 2004, 2005 and 2010. The experimental host range of CWD includes ferrets, several species of voles, white-footed mice, deer mice and Syrian golden hamsters. In addition, CWD was transmitted to the transgenic mouse over-expressing elk or deer prion protein efficiently, but not to wild type mouse.

Here, we report the experimental transmission of elk CWD to conventional VM/Dk mice reaching 100% attack rate after second passage. The CWD-prion-affected wild type mice will be a useful model for future CWD studies.

**KEY WORDS:** CWD, experimental transmission, Republic of Korea, wild type VM mice
Chronic wasting disease (CWD) has been recognized as an important prion disease in North American deer and Rocky mountain elk [16]. This disease was confirmed only in elk in the Republic of Korea in 2001, 2004 and 2005 [9, 14]. Additional CWD cases were observed in red deer, sika deer, and crossbred sika and red deer in 2010 (unpublished data). A CWD-susceptible rodent animal model is crucial in order to study CWD pathogenesis, prevention schemes and therapeutics, and conduct strain characterization and discrimination from other animal prion diseases. Bovine Spongiform Encephalopathy (BSE) and Scrapie have been easily transmitted to wild type mice, and these diseases were well characterized using these in vivo models [4]. The transmission potential of CWD to wild type mice is unknown. The VM/Dk is one line of wild type mice that is susceptible to both BSE and Scrapie. In the present study, a pool of brain homogenate from two elk confirmed with CWD in 2001 (E190Y+229Y) was intracranially inoculated into a group of VM mice and serially passaged. The results are compared with the previous transmission study with the same inocula in transgenic mice over-expressing elk prion protein (referred as TgElk mice here-after) [10, 11]. All procedures involving mice were approved by Animal Ethics Committee (AEC), Animal, Plant and Fisheries Quarantine and Inspection Agency (QIA) under the Animal Protection Act of 1991. The VM mice were sourced from
Animal Health and Veterinary Laboratories Agency (AHVLA, New Haw, Addlestone, Surrey, UK). Six-week-old VM mice were inoculated intracranially with 20 μl of a 10% (w/v) elk CWD for the primary transmission and with 1% (w/v) VM mice brains for the secondary passage. Inoculua for the secondary passage was chosen based on the strongest intensity by western blotting (WB) analysis with anticipated the short incubation period proving efficient transmission (Table 1). Inoculated mice were monitored daily and clinically assessed once a week. Clinical parameters were markedly affected gait, generalized tremors and convulsions, rough coat, hunched back and loss of weight and condition. When the animals were terminally ill, they were euthanized and necropsied. Half of the brain was immediately frozen for WB, and the other half was fixed in 10% formalin for Hematoxylin & Eosin (H&E) and PrP immunohistochemistry (IHC). The summary of the incubation period is presented in Table 1. IHC was conducted as described previously, with minor modifications [14]. Primary antibody used was a polyclonal antibody S1 to avoid non-specific staining. Rabbit antiserum S1 was raised against peptide sequence CTHGQWNKPSKPKNMK from amino acids 106-122 of the bovine prion protein (PrP) by the QIA. WB was conducted as described previously, with minor modifications, using polyclonal antibody S1 as the primary antibody [5]. The attack rate was 4.3% (1 out of 23) in the
primary transmission, but dramatically increased to 100% (10 out of 10) in the secondary passage. The incubation period was 657 and 355±30 dpi, respectively. This is in contrast to, the outcome with the same inoculum which resulted in an incubation period of 150±12 dpi, marking 100% attack rate (4 out of 4) in the primary passage in Tg Elk mice (Table 1). The WB profile including the banding patterns and the position of unglycosylated band after PNGase treatment of VM-adapted CWD and TgElk-adapted CWD after primary and secondary passages were identical (Fig. 1A, B). However, in IHC, focal immune-labeling (Fig. 2A) with minimal vacuolation was observed in VM mice after secondary passage, whilst widespread scrapie associated prion protein (PrP\textsuperscript{Sc}) with severe vacuolation in TgElk mice (Fig. 2B). IHC was not conducted on positive VM mice at primary passage due to brain tissues being autolysed and unsuitable for microscopic examination. Unlike in TgElk mice (Fig. 2D), PrP\textsuperscript{Sc} accumulation was also observed in the spleen of VM mice (Fig. 2C).

Animal model studies have demonstrated shortening of incubation period and a variable biochemical profile following several passages. Mule deer CWD when intracranially inoculated to ferrets (Mustela putorius furo) resulted in an incubation period of 17-21 months on primary passage and 5 months by the third passage. The same study reported, ferret-passaged mule deer CWD was readily transmissible to
Syrian golden hamsters (*Mesocricetus auratus*) [2]. Another study by Sigurdson *et al* using mule deer CWD revealed that intracranially inoculated ferrets showed typical predominant diglycosylated PrP\(^{Sc}\) in Western blotting after 15-20 months of primary passage, and shortened to 5 months in the secondary passage. An altered pattern of PrP\(^{Sc}\) deposition in the brain by IHC may be suggestive of adaptation to a new, modified prion strain as seen in the present study. Sequence comparison between ferret and mule deer PrP genes revealed 91% sequence identity, and it was speculated that observed differences in three amino acid sequence between the host cellular prion protein (PrP\(^{C}\)) and agent PrP\(^{Sc}\) molecules suffice to prevent the formation of new PrP\(^{Sc}\) [13]. In a later study, elk, mule deer and white-tailed deer CWD isolates were successfully transmitted to Syrian golden hamsters after serial passage. Incubation periods rapidly stabilized, with isolates having either shorter (85-89 days in case of mule deer CWD) or longer (408-544 days in case of elk CWD) mean incubation periods [12]. White-tailed deer CWD was also transmitted to Meadow voles (*Microtus pennsylvanicus*), red-backed voles (*Myodes gapperi*), deer mice (*Peromyscus maniculatus*) and white-footed mice (*Peromyscus leucopus*) successfully. These species are epidemic-sympatric rodents in North America. The glycoform profile showed no conspicuous differences between the recipient rodent species [6].
addition, elk CWD was readily transmitted to European bank voles (*Myodes glareolus*), resulting short (109II: 185-190 dpi) or long (109MM: 260-280 dpi) survival times depending on the PRNP polymorphism at Codon 109 of the inocula [1]. Bruce *et al* reported that mule deer CWD transmitted to wild type VM mice poorly, with incubation period longer than 500 days. Vacuolar degeneration was minimal, and perivascular PrP amyloid was the only significant immunohistochemical feature [3]. In this study, the transmissibility of elk CWD was similar to a previous report. In addition, we confirmed that the subsequent passage generated the fully mouse-adapted CWD prions. Recently, in an attempt to generate efficient transgenic line of mice, chimeric elk/mouse prion protein has been used. Chimeric elk/mouse transgenes encode the N-terminus of ElkPrP up to residue Y168 and the C-terminus of mouse PrP beyond residue 169. Between codons 169 and 219, six residues distinguish MoPrP from ElkPrP: S^{169}N^{-173}I^{-183}V^{202}V^{214}K^{219}. It was reported that Tg(Elk3M, SNIVVK) mice were less susceptible to elk CWD, but became readily infectious following two passages in Tg(ElkPrP^{+/+}) mice [15]. In the present study, we were able to transmit elk CWD to wild type VM mice without genetic engineering after two passages marking 100% attack rate. In addition, VM adapted CWD share common biochemical features with elk CWD. Immuno-labeling features of VM adapted CWD were different from those
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of TgElk adapted CWD in the brain and spleen. In case of the spleen, it could be explained by the low PrP\textsuperscript{C} expression level in TgElk mice (data not shown). Similar to the features of mule deer CWD in VM mice [3], minimal vacuolation was observed in the brain. However, perivascular PrP plaques were not a significant finding possibly due to less severe brain pathology and degeneration owing to host characteristics as observed in elk compared to mule deer CWD [17]. In conclusion, we experimentally transmitted elk CWD to include the wild type VM mice, an animal model that can serve as a useful representation for future CWD experimental studies.

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Fig. 1. WB results of the elk CWD, elk CWD in VM mouse and elk CWD in TgElk mouse (A) Primary passage, (B) Secondary passage

Fig. 2. (A) Brain; VM case 2011_d0(300 dpi), Focal accumulation of PrP<sup>Sc</sup> in the brain(X40, *), (B) Brain; TgElk case 2009_h25(167 dpi), Extensive accumulation of PrP<sup>Sc</sup> in the brain(X40, *), (C) Spleen; VM case 2011_d0(300 dpi), Strong intensity of immunolabeling in the spleen(X200, *), (D) Spleen; TgElk case 2009_h20(152 dpi), No immunolabeling in the spleen(X200), Immunlabeling with S1 (A, C) and F99/97.6.1(B, D) and hematoxylin counterstain
1, 4 : E190Y+229Y
2, 5 : E190Y+229Y in VM mouse (1st IC)
3, 6 : E190Y+229Y in Tg Elk mouse (1st IC)

Fig 1 A

1, 4 : E190Y+229Y
2, 5 : E190Y+229Y in VM mouse (2nd IC)
3, 6 : E190Y+229Y in Tg Elk mouse (2nd IC)

Fig 1 B
Table 1. Incubation period of VM mice and TgElk mice intracranially inoculated Korean CWD prions

<table>
<thead>
<tr>
<th>Mice</th>
<th>1st passage</th>
<th>2nd passage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of positive</td>
<td>Incubation period</td>
</tr>
<tr>
<td>VM mice</td>
<td>1/23</td>
<td>657±30</td>
</tr>
<tr>
<td>TgElk mice</td>
<td>4/4</td>
<td>150±123</td>
</tr>
</tbody>
</table>

1) A pool of 2 CWD cases in 2001 (E190Y+229Y) was intracranially inoculated
2) mean±standard deviation (days)
3) The brain sample of diseased mice (133 days of incubation period) was used for subsequent passage