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Single-nucleotide polymorphisms in prion protein gene of the Korean subspecies of Chinese water deer (*Hydropotes inermis argyropus*)

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Abstract : Susceptibility to chronic wasting disease (CWD) in cervid species has been associated with polymorphisms in the prion protein gene (*PRNP*). The single nucleotide polymorphisms (SNPs) were found in the *PRNP* of the Korean subspecies of Chinese water deer via analyses of the DNA sequences obtained from 34 individual deer. Two SNPs were detected at codons 77 and 100. One SNP at codon 77 encoding Glycine was determined to be a silent mutation but the other SNP detected at codon 100 induced an amino acid change, from Asparagine to Serine. The prion protein (PrP) amino acid sequence of the water deer showed 98.8-99.2% homology with those of American elk, white-tailed deer and mule deer. The PrP of the water deer contained amino acid residues closely related with CWD-susceptibility. This study is the first to describe genetic variations in the *PRNP* of the Korean subspecies of Chinese water deer.

Keywords : CWD, *PRNP*, SNP, susceptibility, water deer

Transmissible spongiform encephalopathies (TSE) or prion diseases are major concerns for public health in humans. Chronic wasting disease (CWD) is a unique prion disease, which affects both free-ranging and captive cervid species [9, 12]. Both CWD and scrapie can be transmitted horizontally in the excreta, unlike other prion diseases [8]. Susceptibility to CWD is significantly associated with the polymorphic genotypes of the prion protein gene (*PRNP*) of cervid species, including the mule deer, white-tailed deer, and Rocky Mountain elk [3, 5, 7, 10]. The association between TSE susceptibility and the *PRNP* genotype was observed in vCJD patients and scrapie-infected sheep [2, 4]. In this study, we have characterized the single-nucleotide polymorphisms (SNPs) in the *PRNP* of the Korean subspecies of Chinese water deer.

Blood and muscle samples were obtained from 34 Korean subspecies of Chinese water deer. 7 blood and 10 muscle samples of captive water deer were kindly provided by the Seoul Grand Park Zoo (Korea). The Korean Association for Bird Protection (Korea) provided 17 muscle samples of free-ranging water deer. The *PRNP* was amplified using genomic DNA as a template and PCR primers designed from the DNA sequence of American elk (GenBank accession no. AF016227). The primers were composed of the following sequences; forward primer 5'-ATG GTG AAA AGC CAC ATA GGC-3' and reverse primer 5'-CTA TCC TAC TAT GAG AAA AAT G-3'. PCR was conducted in a total volume of 50 μl at the following conditions; an initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, an extension at 72°C for 60 sec, and a final extension step for 5 min at 72°C. The 771 bp of PCR product was then purified using a PCR purification kit (Bioneer, Korea) and the DNA sequence was determined using an automatic DNA sequencer with the same primers as those used for PCR amplification. In addition, the

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amplified *PRNP* DNA was cloned into the TOPO TA cloning vector (Invitrogen, USA) in accordance with the manufacturer's instructions. The *PRNP* DNA sequence identified from a correct clone was submitted to the GenBank database (Accession no. DQ358969).

Two single-nucleotide polymorphisms (SNPs) were identified in the *PRNP* of the Korean subspecies of Chinese water deer, at codons 77 (GGA/GGT) and 100 (AAT/AGT). The allele sequences were reported to GenBank database (Accession no. EF192236, EF192237). The first SNP at codon 77 encoding Glycine was determined to be a silent mutation with no amino acid change. However, the second SNP was identified at the second position nucleotide of codon 100, and that polymorphism was shown to induce an amino acid change from Asparagine to Serine. At codon 77, 29.41 and 50% of the water deer harbored homozygous GGA/GGA and GGT/GGT genotypes, respectively. In contrast, 20.59% of the water deer tested harbored the heterozygous GGA/GGT genotype. The allelic frequencies at codon 77 were 39.71 and 60.29% for A and T, respectively (Table 1). At codon 100, 44.12 and 26.47% of the water deer had the homozygous AAT/AAT genotype for Asparagine and the AGT/AGT genotype for Serine, respectively. However, 29.41% of the water deer harbored the heterozygous AAT/AGT genotype for Asparagine/Serine. The allelic frequencies at codon 100 were 58.82 and 41.18% for A and G, respectively (Table 1). The PrP amino acid sequence of the water deer demonstrated 98.8, 98.8 and 99.2%

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elk     MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGGW  60
mule    MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGGW  60
white   MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGGW  60
water   MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGGW  60

                                              **                *
elk     GQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGTHSQWNKPSKPKTNMKHVAGAAA  120
mule    GQPHXGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQXGTHSQWNKPSKPKTNMKHVXGAAA  120
white   GQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGTHSQWNKPSKPKTNMKHVAGAAA  120
water   GQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGTHNQWNKPSKPKTNMKHVAGAAA  120

                    *
elk     AGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYYRENMYRYPNQVYYRPVDQYNNQNTFVH  180
mule    AGAVVGGLGGYMLGSAMXRPLIHFGNDYEDXYYRENMYRYPNQVYYRPVDQYNNQNTFVH  180
white   AGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYYRENMYRYPNQVYYRPVDQYNNQNTFVH  180
water   AGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYYRENMYRYPNQVYYRPVDQYNNQNTFVH  180

                                                        **
elk     DCVNITVKQHTVTTTTKGENFTETDIKMMERVVEQMCITQYQRESEAYYQRGASVILFSS  240
mule    DCVNITVKQHTVTTTTKGENFTETDIKMMERVVEQMCITQYQRESQAYYQRGASVILFSS  240
white   DCVNITVKQHTVTTTTKGENFTETDIKMMERVVEQMCITQYQRESQAYYQRGASVILFSS  240
water   DCVNITAKQHTVTTTTKGENFTETDIKMMERVVEQMCITQYQRESQAYYQRGASVILFSS  240

elk     PPVILLISFLIFLIVG  256
mule    PPVILLISFLIFLIVG  256
white   PPVILLISFLIFLIVG  256
water   PPVILLISFLIFLIVG  256
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Fig. 1. Analysis of amino acid sequence of water deer PrP. Amino acid sequences of the PrPs of American elk (*Cervus elaphus nelson*, AF016227), mule deer (*Odocoileus hemionus*, AF009181), and white-tailed deer (*Odocoileus virginianus*, AY275711) were compared with that of water deer. Amino acids at 95, 96, 116, 132, 225, and 226 residues marked by asterisks are known to be closely related with chronic wasting disease-susceptibility.

Table 1. Analysis of single nucleotide polymorphisms in the *PRNP* of the Korean subspecies of Chinese water deer (n = 34)

Polymorphism	Genotype frequency, n (%)			Allele frequency (%)	
	A/A	A/T	T/T	A	T
Codon 77	10 (29.41)	7 (20.59)	17 (50.00)	39.71	60.29
Polymorphism	Genotype frequency, n (%)			Allele frequency (%)	
	A/A	A/G	G/G	A	G
Codon 100	15 (44.12)	10 (29.41)	9 (26.47)	58.82	41.18

Table 2. Percent homology of water deer PrP to those of American elk, white-tailed deer, and mule deer in their amino acid sequences (nucleotide sequences)

	American elk	White-tailed deer	Mule deer
Water deer	98.8 (98.0)	98.8 (97.0)	99.2 (98.0)

*Gene accession numbers used; water deer (DQ358969) American elk (*Cervus elaphus nelsoni*, AF016227), white-tailed deer (*Odocoileus virginianus*, AY275711) and mule deer (*Odocoileus hemionus*, AF009181)

homology with those of American elk, white-tailed deer and mule deer, respectively (Table 2). The nucleotide sequences of the water deer *PRNP* also showed 97.0-98.0% identity with them.

The susceptibility of elk, white-tailed deer, and mule deer to CWD is closely associated with their *PRNP* genotypes. It has been well-established that elk harboring the homozygous M^{132} [3, 10], white-tailed deer with the wild type allele $Q^{95}G^{96}A^{116}Q^{226}$ [6, 7, 11] and mule deer with the allele S^{225} are susceptible to CWD [1, 5]. The water deer had amino acid residues Q, G, A, M, S and Q at 95, 96, 116, 132, 225 and 226 positions of PrP (Fig. 1). This directly indicated that the *PRNP* of the Korean subspecies of Chinese water deer have amino acid sequences commonly determined in the CWD-susceptible elk and deer. Elk with LL^{132}, mule deer with SF^{225} and white-tailed deer with GS^{96} genotypes evidenced reduced susceptibility to CWD or longer incubation time in cases of infection with CWD [3, 5, 7]. We have generated transgenic mice expressing the PrP of the Korean subspecies of Chinese water deer that will be used to examine the susceptibility to CWD in a further study.

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