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Polymorphisms in the Prion Protein Gene, Associated with Chronic Wasting Disease, in the Korean Water Deer (*Hydropotes inermis argyropus*)

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Abstract

Prion diseases are fatal neurodegenerative diseases that affect humans and non-human mammals. Different alleles of the prion protein gene (*PRNP*) of humans and sheep are associated with varying susceptibilities to transmissible spongiform encephalopathies. Chronic wasting disease (CWD) is a prion disease of cervids, and polymorphisms at specific codons in the *PRNP* gene are associated with this disease. To assess the susceptibility of free-ranging deer to CWD, polymorphisms in *PRNP* were examined in the Korean water deer (*Hydropotes inermis argyropus*), focusing on codons that are potentially associated with CWD susceptibility (95, 96, 116, 132, 225, and 226). CWD surveillance was conducted by an antigen ELISA of tissue samples from 545 Korean water deer collected in eight provinces of the Republic of Korea. No prion protein associated with CWD was detected in any of the samples. These results suggest that *PRNP* of the Korean water deer shows low variation and the species has not been infected with CWD.

Keywords: Chronic wasting disease; *Hydropotes inermis argyropus*; *PRNP*; Surveillance

Introduction

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of lethal neuronal disorders presenting as the degeneration of neurons. They include Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattle, scrapie in sheep and goats, and chronic wasting disease (CWD) in cervids [1].

CWD affects species in the family Cervidae. It has been reported in the USA [2] and Canada [3] in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and rocky mountain elk (*Cervus elaphus nelsoni*). More recently, the first European CWD infection was reported in Norway in a free-ranging reindeer (*Rangifer tarandus tarandus*) [4].

CWD attacks either captive or wild animals [5]. Among all TSEs, CWD is the most efficiently transmitted and can reach 30% transmissibility in wildlife. Although not entirely understood, it is hypothesized that horizontal transmission is particularly efficient, via direct contact with body secretions [6], excreta [7], and decomposed carcasses [8]. Its prevalence among captive animals can reach approximately 80% because they are kept in restricted areas (research facilities and breeding farms) where the exchange of fluids is constant [6].

Korean CWD was introduced by elk imported from Canada in 1997. CWD outbreaks in farmed animals were reported in 2001, 2004, 2005, 2010, and 2016 in the Republic of Korea. The Korean water deer is the dominant species of wild deer in Korea, with approximately 620 thousand heads (8.0 heads/100 ha) [9].

The development of CWD is commonly associated with polymorphisms in exon 3 of the prion protein gene *PRNP*. Susceptibility and resistance to TSEs follow genetic patterns based on *PRNP* alleles [10]. Genetic analyses show that susceptibility to CWD in white-tailed deer is strongly influenced by polymorphisms at codons 95 and 96 [11,12]. In addition, in white-tailed deer, alleles encoding glycine at codon 116 and lysine at codon 226 are putatively associated with CWD resistance [13,14]. In elk, variation at PrP codon 132 significantly influences susceptibility to CWD [15].

The goal of this study was to examine CWD susceptibility in the Korean water deer (*Hydropotes inermis argyropus*) based on polymorphisms in the *PRNP* gene at sites associated with the development of CWD in North American deer and to evaluate CWD occurrence in wild Korean water deer in 2014-2016.

Materials and Methods

All Korean water deer tissue samples were obtained from Korean wildlife rescue centres in eight provinces and the National Institute of Environmental Research (NIER). For CWD surveillance, tissue samples (obex, retropharyngeal lymph nodes, and tonsils) from the heads of 545 Korean water deer were collected from 2014 to 2016. CWD was evaluated by antigen ELISA using the HerdCheck® BSE-Scrapie Ag Kit (IDEXX, Westbrook, ME, USA) according to the methods recommended by the manufacturer (short protocol).

For the *PRNP* analysis, DNA was extracted from the obex samples obtained from 545 deer using automated DNA extraction instruments (Maxwell® RSC; Promega, Madison, WI, USA).

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To identify polymorphisms in *PRNP*, a primer set of CF70 (5′-TGCAAGAAGCGACCAAAACCT-3′) and CF729 (5′-CACAGGAGGGGAGGAGGAGAAGAGGAT-3′) was designed based on the *PRNP* sequence of the North American elk (GenBank accession no. EU082291) to amplify a 659 bp fragment from exon 3 of the gene. Polymerase chain reaction (PCR) conditions were as follows: initial denaturation at 95°C for 15 min, amplification using the HotStar Taq® Master Mix Kit (Qiagen, Gaithersburg, MD, USA) for 30 cycles at 94°C (30 sec), 60°C (60 sec), and 72°C (90 sec), and a final extension at 72°C (15 min).

Among the PCR products, 155 were used to sequence exon 3 of *PRNP*. Sequencing was conducted at Macrogen Inc. (www.macrogen.com) (Seoul, Korea) using BigDye Terminator Cycle Sequencing conditions on an ABI 3730 xl Automatic Sequencer (Applied Biosystems, Foster City, CA, USA). Sequences (n=155) were aligned using BioEdit version 5.0.9 [16] and used as queries for BLASTn searches against the GenBank database to verify that they belonged to exon 3 of *PRNP*. Amino acid translations were obtained using BioEdit, and a BLASTx search was performed against the GenBank database for additional confirmation. Polymorphisms in *PRNP* were detected base-by-base by eye or using ABI Variant Reporter v1.1.0, focusing on known polymorphisms related to CWD susceptibility. The polymorphisms most commonly associated with CWD occur at codons 95, 96, 132, and 225 [11,12,15,17]. In addition, codons 116 and 226, where putative CWD-resistant polymorphisms (116G and 226K) have recently been identified, were examined [13]. Codons 77 and 100, for which single-nucleotide polymorphisms (SNPs) have been observed in the Korean water deer, were also analyzed [18].

Results

For CWD surveillance, 545 heads of Korean water deer were collected from eight provinces, i.e., Gangwon (250 heads), Gyeonggi (54 heads), Gyeongnam (72 heads), Gyeongbuk (33 heads), Jeonnam (44 heads), Jeonbuk (12 heads), Chungnam (43 heads), and Chungbuk (45 heads), in 2014-2016, and the obex, retropharyngeal lymph node, and tonsil samples were tested for CWD. All samples were CWD-negative (Table 1).

In an analysis of *PRNP* from 155 Korean water deer, 16 silent mutations were identified. SNPs were found at codons 38 (GGG>GGA), 42 (CCG>CCA), 69 (GGT>GGG), 70 (CAG>CAA), 71 (CCC>CCT), 74 (GGT>GGA), 77 (GGA>GGT), 85 (GGT>GGA), 87 (CCC>CCA), 113 (AAG>AAA), 124 (GTA>GTG), 128 (CTC>CTT), 141 (CTC>CTT), 145 (GGC>GGG), 146 (AAT>AAC), and 206 (ATC>ATT). Sequence variants other than those at codon 141 were found in 1-5 deer, indicating a relatively low frequency in only a few animals. The variants at codon 141 were identified in 29 deer, indicating an allele frequency of 18.7% (Table 2).

Exon 3 of *PRNP* from 155 Korean water deer exhibited very low diversity, and a BLASTx search showed that the amino acid sequences were very similar or even identical to those of homologs in elk (identity, 98.4-99.2%), mule deer (identity, 97.7-98.8%), and white-tailed deer (identity, 98.8-99.6%). The 95Q, 132M, and 225S alleles, which are related to CWD susceptibility, were homozygous. However, 18.7% of the deer showed a change at codon 96, from 96GG to 96DD, and only one deer was confirmed to have the heterozygous 96GD genotype. Putative CWD-resistant 116G and 226K alleles were not identified in the investigated Korean water deer, all of which had the 116AA and 226QQ genotypes [19].

A SNP at 77GG occurs in the Korean water deer; all of the deer examined had the GG homozygous genotype at codon 77, and a silent mutation of GGA>GGT was observed in only three deer. At codon 100, amino acid variants, such as 100NN (75.3%), 100SS (6.5%), and 100NS (18.1%), were observed. In addition, codon 187 was identified as valine in all deer examined. One deer had the 170DG heterozygous genotype at codon 170, while the remaining deer had 170DD (Tables 2 and 3).

Discussion

In Korea, CWD was first reported in captive elks in 2001; it was introduced by CWD-infected elks imported from Canada in 1997. Since then, additional CWD outbreaks have been reported in 2004, 2005, 2010, and 2016. Owing to recurrent CWD outbreaks in farmed deer, CWD surveillance in Korean water deer, the dominant species of wild deer, has been carried out since 2014. In this study, no evidence of CWD infection in Korean water deer was found during a 3 year period.

As confirmed in this study, tests for CWD infection in Korean water deer are required for accurate evaluation. However, considering that 95Q, 96G, 132M, and 225S (known CWD susceptibility-related genotypes) exhibited high frequencies [11,12,15,17] and that 116G and 226K (predicted to be CWD resistance-related genotypes in white-tail deer [13,14]) were not detected in the Korean wild deer population, additional CWD outbreaks are still likely to occur in captive cervids and wild animals.

Seo et al. [20] reported that PrP^{CWD} in CWD-infected elks, red deer, and sika deer could be *in vitro* converted to PrP^{C} in Korean water deer by protein misfolding cyclic amplification with Teflon beads, suggesting the potential for CWD infection in Korean water deer, as well.

Although CWD propagation in Korean water deer was not

Province (No. of heads)	2014	2015	2016	CWD test results	
				Negative	Positive
Gangwon (250)	131	91	28	250	0
Gyeonggi (54)	23	20	11	54	0
Gyeongnam (72)	29	30	13	72	0
Gyeongbuk (33)	17	8	8	33	0
Jeonnam (44)	10	33	1	44	0
Jeonbuk (12)	11	-	1	12	0
Chungnam (43)	14	16	13	43	0
Chungbuk (45)	8	18	11	45	0
Total (545)	243	216	86	545	0

Table 1: Results of the CWD test for Korean water deer in eight provinces in 2014-2016.

Codon	Allele	Allele frequency (%)	Codon	Allele	Allele frequency (%)
38 (Glycine)	GGG	96.1	87 (Proline)	CCC	98.1
	GGA	3.9		CCA	1.9
42 (Proline)	CCG	99.4	113 (Lysine)	AAG	99.4
	CCA	0.6		AAA	0.6
69 (Glycine)	GGT	98.1	124 (Valine)	GTA	98.1
	GGG	1.9		GTG	1.9
70 (Glutamine)	CAG	98.1	128 (Leucine)	CTC	98.1
	CAA	1.9		CTT	1.9
71 (Proline)	CCC	98.1	141 (Leucine)	CTC	81.3
	CCT	1.9		CTT	18.7
74 (Glycine)	GGT	98.1	145 (Glycine)	GGC	95.5
	GGA	1.9		GGG	4.5
77 (Glycine)	GGA	98.1	146 (Asparagine)	AAT	98.1
	GGT	1.9		AAC	1.9
85 (Glycine)	GGT	98.1	206 (Isoleucine)	ATC	98.1
	GGA	1.9		ATT	1.9

Table 2: Analysis of SNPs in *PRNP* of the Korean water deer (n=155).

No. of heads (n=155)	Genotype frequency (%)	Codon									
		77	95	96	100	116	132	170	187	225	226
Reference gene[1]	-	GG	QQ	GG	NN	AA	MM	DD	AA	SS	QQ
Reference gene[2]	-	GG	QQ	GG	SS	AA	MM	DD	AA	SS	QQ
Reference gene[3]	-	GG	QQ	GG	NN	AA	MM	DD	VV	SS	QQ
1	0.6	GG	QQ	GD	NN	AA	MM	DD	VV	SS	QQ
1	0.6	GG	QQ	DD	NN	AA	MM	DD	VV	SS	QQ
28	18.1	GG	QQ	DD	NS	AA	MM	DD	VV	SS	QQ
10	6.5	GG	QQ	GG	SS	AA	MM	DD	VV	SS	QQ
1	0.6	GG	QQ	GG	NN	AA	MM	DG	VV	SS	QQ
114	73.5	GG	QQ	GG	NN	AA	MM	DD	VV	SS	QQ

[1]GenBank accession: DQ358969.1; [2]GenBank accession: EF192237.1; [3]GenBank accession: KU680312.1

Table 3: Analysis of amino acid polymorphisms in *PRNP* of the Korean water deer.

observed during this 3 year surveillance period, farmed species, such as elks, red deer, and sika deer, show high sequence similarity in the *PRNP* gene [14]. *PRNP* of the Korean water deer examined in this study also had a homology of approximately 98% or greater with *PRNP* in the genomes of these three species; thus, the possibility of CWD infection in Korean water deer cannot be excluded.

CWD has a high risk of mutual transmission between farmed deer and wild deer due to environmental contamination caused by the release of pathogens through blood, saliva, and faeces [21]. In Korea, where recurrent CWD outbreaks occur in farmed deer, CWD propagation in wild deer has not been confirmed to date. Therefore, the eradication of this disease is believed to be possible with highly sensitive diagnostic techniques and strong surveillance. For this, continuous monitoring of CWD infection and investigations of *PRNP* polymorphisms are necessary.

In this study, the Korean water deer had amino acid residues Q, G, N, A, M, D, V, S, and Q at positions 95, 96, 100, 116, 132, 170, 187, 225, and 226 of PrP. Previously unreported *PRNP* alleles at codon 96 (DD, GD) were also identified. Some genotypic differences from the reference gene were observed, such as 96DD and the heterozygous genotype 100NS. To determine the relationship between these genotypes and CWD susceptibility or resistance, continued infection studies and monitoring of PRNP polymorphisms in wild deer are needed.

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