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Bovine spongiform encephalopathy (BSE) associated polymorphisms of the prion-like protein gene (PRND) in Korean dairy cattle and Hanwoo

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Bovine spongiform encephalopathy (BSE) involves insertion/deletion (in/del) polymorphisms in the prion protein gene (*PRNP*) promoter region that are associated with vulnerability to disease progression. Recently, a second member of the prion gene family, prion-like protein gene (*PRND*), has been reported to show the *PRND* R132Q polymorphism, which is associated with the susceptibility to BSE in German Fleckvieh breeds. The objective of this study was to examine the genotype, allele, and haplotype frequencies of *PRND* gene in Korean cattle and evaluate their susceptibility to BSE. We did this in 277 Korean native cattle (Hanwoo) and 124 Korean dairy cattle (Holstein) by direct sequencing and compared the R132Q genotype frequency between BSE-affected German cattle and Korean cattle. The results indicated a total of 5 single nucleotide polymorphisms (SNPs) including *PRND* c.149G > A (p.50Arg > His; R50H), *PRND* c.285C > T (C4819T), *PRND* c.395G > A (p.132Arg > Gln; R132Q) and *PRND* c.528T > A (T5063A) in the open reading frame (ORF) and c.602C > G in the 3′ untranslated region (UTR) of exon 2 in Korean Holstein and Hanwoo cattle. Except for c.149G > A, the remaining 4 SNPs showed significantly different genotype and allele frequencies between the Korean Holstein and Hanwoo ($P < 0{\cdot}01$). There were no significant differences in genotype distribution of c.395G > A SNP between BSE-affected German and Korean Holstein cattle ($P = 0{\cdot}6778$), but a significant difference was detected between BSE-affected German cattle and Hanwoo cattle ($P = 0{\cdot}0028$). The results suggest that Hanwoo cattle may possess a relatively more BSE-resistant genotype than Korean Holstein cattle.

Keywords: Dairy cattle, single nucleotide polymorphism, BSE, prion-like gene, prion protein gene.

Prion disease in cattle was called bovine spongiform encephalopathy (BSE) and was first recognised in the United Kingdom in 1986 (Bradley et al. 2006). BSE shares common features with Creutzfeldt–Jakob disease (CJD) showing scrapie like spongiform vacuolation of brain tissue and accumulation of the scrapie form of prion protein, PrP^{Sc} (Wood et al. 1997; Aguzzi & Heikenwalder, 2006). In humans and sheep, prion protein gene (*PRNP)* acts as a major genetic factor in prion diseases. Met/Met genotype of human *PRNP* codon 129 is susceptible to CJD in human, and haplotypes of ovine *PRNP* codons 136, 154 and 171 are significantly associated with susceptibility to scrapie in sheep (Hunter et al. 1997; Jeong et al. 2005b; Groschup et al. 2007; Jeong & Kim, 2014). Similarly, BSE-affected cattle show higher distributions of 23 bp deletion in the *PRNP* promoter region and 12 bp deletion in the *PRNP* intron region than healthy cattle (Jeong et al. 2006; Haase et al. 2007). Recent studies suggested that single nucleotide polymorphism (SNP) 4136 and 13 861 in the non-coding region of bovine *PRNP* gene are related to BSE susceptibility (Murdoch et al. 2010a, b; Jeong et al. 2013). However, the frequency of *PRNP* Met/Met genotype in Korean population is approximately three times higher than that in British population, but the incidence of sporadic CJD is somewhat similar (Brandel et al. 2003; Nurmi et al. 2003; Jeong et al. 2005b; Jeong & Kim, 2014). In addition, the same *PRNP* gene transgenic mouse has different disease incubation times (Lloyd et al. 2001), indicating that other factors besides the *PRNP* gene may contribute to the progression of prion diseases.

In recent studies, human prion diseases showed association with prion-like protein gene (*PRND)* polymorphisms. Among several polymorphisms, T174M polymorphism in

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Table 1. Genotype and allele frequencies of *PRND* polymorphisms in Korean Hanwoo and Holstein cattle

	Genotype frequency, *n* (%)			*P* value	Allele frequency, *n* (%)		*P* value	HWE
c.149G > A	GG	GA	AA		G	A		
Holstein	104 (83·9)	20 (16·1)	0 (0)	1·0	228 (91·9)	20 (8·1)	1·0	0·353
Hanwoo	231 (83·4)	46 (16·6)	0 (0)		508 (91·7)	46 (8·3)		0·132
c.285C > T	CC	CT	TT		C	T		
Holstein	115 (92·7)	8 (6·5)	1 (0·8)	<0·001	238 (96·0)	10 (4·0)	<0·001	0·033
Hanwoo	126 (45·5)	124 (44·8)	27 (9·7)		376 (67·9)	178 (32·1)		0·660
c.395G > A	GG	GA	AA		G	A		
Holstein	8 (6·4)	42 (33·9)	74 (59·7)	<0·001	58 (23·4)	190 (76·6)	<0·001	0·479
Hanwoo	50 (18·1)	151 (54·5)	76 (27·4)		251 (45·3)	303 (54·7)		0·096
c.528T > A	TT	TA	AA		T	A		
Holstein	115 (92·7)	8 (6·5)	1 (0·8)	<0·001	238 (96·0)	10 (4·0)	<0·001	0·033
Hanwoo	127 (45·8)	123 (44·4)	27 (9·8)		377 (68·1)	177 (31·9)		0·725
c.602C > G	CC	CG	GG		C	G		
Holstein	9 (7·2)	42 (33·9)	73 (58·9)	<0·001	60 (24·2)	188 (75·8)	<0·001	0·561
Hanwoo	58 (21·0)	143 (51·6)	76 (27·4)		259 (46·8)	295 (53·2)		0·539

the coding region and polymorphisms at the 3′ untranslated region (UTR) are significantly related to human prion diseases (Mead et al. 2000; Peoc'h et al. 2000; Croes et al. 2004; Jeong et al. 2005a). In addition, codon 26 of *PRND* gene is related with susceptibility to scrapie in sheep (Mesquita et al. 2010). Two studies in cattle have identified relationships between BSE and *PRND* genotype. The *PRND* genotype differs significantly between healthy cattle and BSE cattle of the Fleckvieh breed in Germany (Balbus et al. 2005). UK cattle also showed different haplotype distribution between healthy cattle and BSE cattle (Comincini et al. 2001).

The purpose of this study was to assess the susceptibility of Korean native cattle (Hanwoo) and Korean dairy cattle (Holstein) to BSE. Thus, we investigated the *PRND* genotype, allele and haplotype frequencies of SNPs in 124 Korean Holstein and 277 Hanwoo and compared the distribution of *PRND* genotype between Korean cattle and BSE cattle from the previous study.

Material and methods

Genetic analysis

Peripheral blood samples from 277 Hanwoo and 124 Holstein cattle in South Korea were obtained in ethylenediaminetetraacetic acid (EDTA) tubes. Genomic DNA was extracted from 200 µl peripheral blood sample using DNA blood mini kit (Qiagen, USA) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was carried out with the following forward and reverse primers: Bovine PRND-F (GAGACTCAGAACTCCACTGA) and Bovine PRND-R (TGCTCTTTGGTACCTTCAGA). The genomic DNA sequence of *PRND* gene was obtained from GenBank (Gene ID: 281426) and PCR primers were designed to amplify the open reading frame (ORF) of the gene. Each reaction mixture contained 50 pmole of each primer, 5 µl of 10 × Taq DNA polymerase buffer, 1 µl of 10 mM dNTP mixture, 2·5 units of Taq DNA polymerase (Promega, USA) and nuclease-free water to a total volume of 25 µl. The PCR cycling parameters were as follows: denaturing at 95 °C for 2 min, followed by 35 cycles of 95 °C for 20 s, 59 °C for 40 s, and 72 °C for 2 min, and then 1 cycle of 72 °C for 10 min for final extension using an S-1000 Thermal Cycler (Bio-Rad Laboratories, USA). The PCR products for automatic DNA sequencing were prepared using a gel extraction kit (Qiagen, USA). Purified PCR products were directly sequenced with an ABI 3730 Capillary Sequencer (ABI, USA).

Statistical analysis

All statistical analyses were calculated using Statistical Analysis Software version 9.3 (SAS Institute, Cary, NC, USA). We also examined Lewontin's D' ($|D'|$) between five SNPs of *PRND* gene in Hanwoo and Holstein cattle. Hardy-Weinberg Equilibrium (HWE) test and haplotype analysis were performed using SNP Analyser TM 2.0 (http://snp.istech.info/istech/board/ detail_snpa2.jsp). The susceptibility to BSE was compared across genotype using the Chi-square test. P value < 0·05 was considered as statistically significant.

Results

The bovine *PRND* gene is composed of two exons. To examine the genotype and allele frequencies of *PRND* SNPs in Korean Holstein and Hanwoo cattle, we investigated SNPs within exon 2 including ORF of bovine *PRND* gene by direct sequencing of the genomic DNA of 277 Hanwoo and 124 Holstein cattle. A total of five SNPs were detected including *PRND* 149G > A (p.50Arg > His; R50H), *PRND* c.285C > T (C4819T), *PRND* c.395G > A (p.132Arg > Gln; R132Q) and *PRND* c.528T > A (T5063A) in the ORF and c.602C > G in the 3′ UTR of exon 2. The differences of genotype and allele frequencies of the five *PRND* polymorphisms are described in Table 1. The

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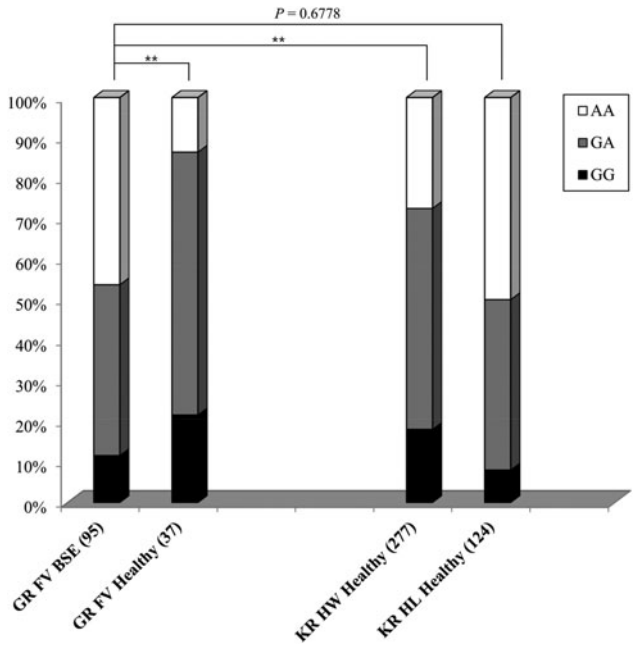

Fig. 1. Genotype comparison of c.395G > A (p.132 Arg > Gln; R132Q) polymorphism of prion-like protein gene (*PRND*) in BSE-affected German cattle† and Korean cattle. GR FV BSE: BSE-affected German Fleckvieh cattle; GR FV Healthy: Healthy German Fleckvieh cattle; KR HW Healthy: Healthy Korean native cattle (Hanwoo); KR HL Healthy: Healthy Korean dairy cattle (Holstein). Parentheses indicate the sample numbers of cattle. Asterisks indicate statistically significant differences of genotype distribution between BSE-affected cattle and Healthy cattle. $^{**}P < 0{\cdot}001$. †Data from Balbus et al. (2005), used with permission: online Supplementary File.

genotype frequencies of all five SNPs followed HWE in the Hanwoo. In the Korean Holstein, two SNPs, c.285C > T and c.528T > A were not in HWE ($P = 0{\cdot}033$), the other three SNPs were in HWE.

To evaluate the susceptibility to BSE in Korean Holstein and Hanwoo cattle, we compared the genotype frequency of *PRND* c.395G > A (p.132 Arg > Gln; R132Q) polymorphism between BSE-affected German cattle (data from Balbus et al. 2005, used with permission: online Supplementary File) and Korean cattle (Fig. 1). A significant difference in genotype distribution of c.395G > A SNP was previously shown between BSE-affected and healthy German Fleckvieh cattle (Balbus et al. 2005). However, there were no significant differences in genotype distribution of this SNP between BSE-affected German cattle and Korean Holstein cattle ($P = 0{\cdot}6778$). A significant difference in genotype distribution was detected between BSE-German cattle and Hanwoo cattle ($P = 0{\cdot}0028$).

To examine whether there was a strong linkage disequilibrium (LD) among the five SNPs, LD coefficient ($|D'|$) was calculated in the SNPs of Korean Holstein and Hanwoo cattle. The results indicated a weak LD for c.149G > A with c.285C > T, c.395G > A, c.528T > A and c.602C > G. The remaining 4 SNPs showed a strong LD with D' value 0·970–1·0 (Table 2).

Analysis of haplotype frequency was carried out for Korean Holstein and Hanwoo cattle. As shown in Table 3, among the six haplotypes, GCATG haplotype was observed more frequently (51·2% for the Hanwoo; 70·7% for the Korean Holstein). The haplotype frequencies of GCATG, GTGAC, GCGTC, ATGAC and ACATG revealed substantial differences between Korean Holstein and Hanwoo cattle.

Discussion

PRNP is considered a major genetic factor of several prion diseases including scrapie, CJD and BSE. A significant difference in insertion/deletion (in/del) genotype frequencies of bovine *PRNP* gene has been found in the promoter and intron regions between BSE-affected cattle and healthy cattle. These two polymorphisms are associated with transcription factor binding site of RP58 and SP1; in addition, follow-up studies confirm that the polymorphisms are related to the expression level of cellular prion protein (PrP^C). Since prion expression level is associated with the incubation period of disease onset, it is concluded that these two polymorphisms are associated with BSE progression (Sander et al. 2005). In addition, a previous study identified the *PRNP* ORF mutation, E211K, which is potentially associated with the familial form of BSE (Nicholson et al. 2008). This mutation, located in codon 211 of the bovine *PRNP* gene, is in a region homologous with codon 200 of human *PRNP*. Since the major form of familial CJD is caused by the E200K mutation (Jeong & Kim, 2014; Cohen et al. 2015), E211K mutation in cattle can act as the genetic factor of the putative inherited form of BSE. Several attempts to identify this mutation in the germline have been unsuccessful thus far (Heaton et al. 2008; Zhao et al. 2010; Kim & Jeong, 2017).

Recent studies have focused on the *PRND* gene, paralogue of *PRNP*. *PRND* is located 25·9 kb downstream of *PRNP* and encodes the prion-like protein, doppel. Doppel, an N-terminal truncated form of the PrP^C is composed of 178 amino acids (Golaniska et al. 2004). Because of biochemical and structural similarity with *PRNP,* several *PRND* SNPs were investigated and analysed for association with BSE. Comincini and Balbus conducted a BSE case-control study on the *PRND* gene in British and German cattle. In British Friesian cattle, the R50H N110N R132R genotype frequency was different between BSE-affected cattle and healthy cattle (Comincini et al. 2001). In German Fleckvieh cattle, the distribution of genotype C4815T and R132Q was significantly different between BSE-affected cattle and healthy cattle (Balbus et al. 2005). Among several *PRND* polymorphisms, we are interested in the nonsynonymous polymorphism, R132Q, since this polymorphism can cause conformational change of the peptide and shows statistically significant association with BSE. We tried to evaluate the susceptibility to BSE in Korean Holstein

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Table 2. Linkage Disequilibrium (LD) among five single nucleotide polymorphisms (SNPs) of *PRND* gene in Korean Hanwoo and Holstein cattle

	c.149G > A	c.285C > T	c.395G > A	c.528T > A	c.602C > G
c.149G > A	–	0·506	0·400	0·508	0·385
c.285C > T	–	–	0·970	0·993	0·979
c.395G > A	–	–	–	0·980	0·995
c.528T > A	–	–	–	–	1·0
c.602C > G	–	–	–	–	–

Table 3. Haplotype frequency of five *PRND* polymorphisms in Korean Hanwoo and Holstein cattle

Haplotype	Hanwoo ($n = 554$)	Holstein ($n = 246$)	*P*-value
GCATG	284 (0·512)	174 (0·707)	0·009
GTGAC	138 (0·249)	3 (0·012)	<0·001
GCGTC	74 (0·134)	49 (0·199)	0·049
ATGAC	38 (0·068)	5 (0·020)	0·008
ACATG	8 (0·015)	14 (0·058)	0·001
GCATC	8 (0·015)	0 (0)	0·115
Others†	4 (0·007)	1 (0·004)	1·0

†Others contain rare haplotypes with frequency <0·001.

and Hanwoo cattle by comparing the genotype distribution of R132Q. Hanwoo cattle showed statistically different genotype distribution as compared to BSE-affected cattle ($P < 0·05$), whereas Korean Holstein cattle showed similar genotype distribution with BSE-affected cattle ($P > 0·05$) (Fig. 1). This implies that the Hanwoo possesses a relatively more BSE-resistant genotype than the Korean Holstein. Our previous study on the *PRNP* gene showed that the resistant allele distribution of 23 bp in/del polymorphism of the promoter region in Hanwoo cattle was higher than that in Korean Holstein cattle (Jeong et al. 2006). These results suggested that the Hanwoo possess more resistant genotypes in two major members of the prion gene family, *PRNP* and *PRND*. However, the SNP studies did not show the identical results in cattle of all breeds, and was performed in relatively limited sample groups. Therefore, these SNPs should be assessed in a large BSE-affected group and various cattle breeds in the future.

PRND polymorphisms showed a propensity toward susceptibility to various types of prion diseases in several species (Comincini et al. 2001; Croes et al. 2004; Balbus et al. 2005; Jeong et al. 2005a; Mesquita et al. 2010). In addition, doppel was localised in the dystrophic neurites of senile plaques in Alzheimer's disease (AD) (Ferrer et al. 2004), and *PRND* polymorphism at the 3′ UTR was associated with several phenotypes such as increased cumulative behavioural load and an elevated risk for delusions, anxiety, agitation/aggression, apathy and irritability/emotional ability in AD patients (Flirski et al. 2012). Moreover, the ectopic expression of doppel was toxic in neuronal cells but not in spermatogenic cells (Qin et al. 2013). Thus, further investigation on the association between doppel and neurodegenerative diseases is needed in the future.

In conclusion, the analysis of the bovine *PRND* polymorphisms in Korean cattle revealed that the genotype frequency of *PRND* c.395G > A (p.132 Arg > Gln; R132Q) in Hanwoo was significantly different from that in Korean Holstein and from that previously reported in BSE-affected German cattle. It suggests that Hanwoo have a genotype that is relatively resistant to BSE. Our data will help to predict the BSE susceptibility based on the SNP of the bovine *PRND* gene in Korean cattle.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0022029917000814.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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