RESEARCH NOTE

A national survey on the allelic, genotypic, and haplotypic distribution of *PRNP* insertion and deletion polymorphisms in Korean cattle

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Introduction

Transmissible spongiform encephalopathies (TSEs), also referred to as prion diseases, are fatal diseases which gradually destroy the brain tissues with a characteristic 'sponge-like' appearance. They include Kuru and Creuzfeldt-Jakob disease (CJD) in humans, scrapie in sheep and goats, and bovine spongiform encephalopathy (BSE) in cattle. The PrP^{Sc} generated from the endogenous cellular prion protein (PrP^{c}) by a posttranslational modification, is the pathogenic agent and its accumulation causes the prion diseases (Prusiner 1991). What these diseases make more hazardous is that the pathogenic agent is transmissible across species. The BSE, resulting from cattle ingesting scrapie-infected or BSE-infected meat and bone meal, is also implicated in development of variant CJD (vCJD) in humans through consumption of beef from BSE-affected cattle (Hill *et al.* 1997). For this reason, BSE poses a serious threat to humans as well as cattle.

Prion diseases may be present not only as sporadic or infectious disorders, but also as genetic illnesses (Prusiner 1991). The genetic resistance to prion diseases is believed to be an important factor in preventing disease recurrence (Goldmann 2008). Previous research indicates that the *PRNP* gene encoding the prion protein is present in all vertebrates (Premzl and Gamulin 2007; Kim *et al.* 2008), and that its polymorphisms were associated with susceptibility of prion diseases in humans (Palmer *et al.* 1991), sheep (Belt *et al.* 1995), goat (Billinis *et al.* 2002), deer (O'Rourke *et al.* 2004) and mice (Westaway *et al.* 1994). Much effort has been devoted to establish the association between *PRNP* and BSE susceptibility in cattle over the last decade (Premzl *et al.* 2000; Walawski and Czarnik 2003). Recently, Sander *et al.* (2004) reported the association of BSE susceptibility with insertion/deletion polymorphisms (indels) within a putative promoter as well as intron 1 of this gene in German cattle.

As of now, BSE-affected animals have not been found in Korea. Nevertheless, there has been a great deal of concern about potential occurrence of BSE in Korean cattle (*Bos taurus coreanae*), because Korea has had close and frequent contacts with animal industries in USA, Canada, and Japan where many cases of BSE outbreaks were reported. To date, genotypic frequencies for BSE-related gene of Korean cattle were rarely reported, and the frequencies were observed only using samples in a specific location (Jeong *et al.* 2006). The objective of this study is to examine sequence variants of the *PRNP* gene in cattle from all over South Korea.

Materials and methods

Animals

We collected 437 blood samples from Korean cattle throughout South Korea, which includes six provinces as shown in figure 1. From each farm, three or lesser cattle were sampled and made certain that the selected cattle were genetically independent to avoid genetic relationship that could be caused by artificial insemination.

Genotyping

Genomic DNA was isolated from the blood samples using QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturers' instructions. Then, the DNA samples were genotyped for the promoter 23-bp indel, intron 1 12-bp indel, and exon 3-octapeptide-repeat polymorphisms by polymerase chain reaction (PCR) as described by Sander *et al.* (2004).

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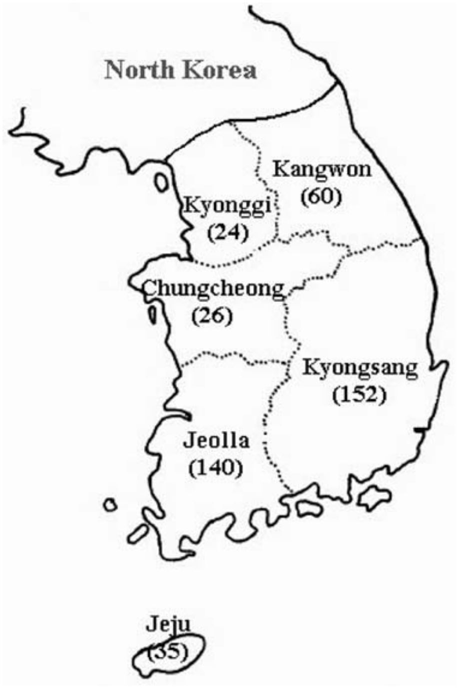

Figure 1. Geographical distribution of Korean cattle used in the current study. The number in parenthesis indicates number of cattle sampled from each province in South Korea.

Statistical evaluation

The *PRNP* allelic and genotypic differences among the cattle populations were evaluated by the G-based exact test utilizing the program GENEPOP 3.4 (http://genepop.curtin.edu.au). The maximum-likelihood estimates of haplotype frequencies employing expectation-maximization (EM) algorithm were obtained with the Arlequin program version 2.0 (University of Geneva, Geneva, Switzerland, http://lgb.unigene.ch/arlequin). Haplotype distributions were compared among populations using the likelihood ratio test using SAS Release 9.1 (SAS Institute, Cary, USA).

Results

Allelic and genotypic frequencies

The frequencies of alleles and genotypes for three *PRNP* polymorphisms in Korean cattle were obtained (table 1) and compared with those of healthy and BSE-affected cattle reported in Sander *et al*. (2004). In allelic and genotypic distributions of 23-bp and 12-bp indel polymorphisms, similar patterns were observed between Korean cattle and healthy German cattle ($P > 0.05$). On the other hand, the frequencies of Korean cattle were discovered to be significantly different ($P < 0.05$) from those of BSE-affected German cattle.

Table 1. Distribution of alleles and genotypes of *PRNP* polymorphisms in Korean cattle.

		Allele		Genotype			*P* value[a]			
							German cattle			
	n	I	D	II	ID	DD	Healthy	BSE	US cattle	Korean cattle
23-bp indel										
German cattle[b]										
Healthy	48	0.43	0.57	0.21	0.44	0.35	–	**0.030**	**0.032**	0.829
BSE	43	0.27	0.73	0.05	0.44	0.51	**0.033**	–	0.587	**0.002**
US cattle[c]	132	0.30	0.70	0.14	0.32	0.54	0.052	0.616	–	**< 0.001**
Korean cattle	437	387	487	86	215	136	0.830	**0.002**	**< 0.001**	–
12-bp indel										
German cattle[b]										
Healthy	48	0.49	0.51	0.21	0.56	0.23	–	**0.034**	1.000	0.449
BSE	43	0.33	0.67	0.09	0.47	0.44	**0.028**	–	**0.009**	0.040
US cattle[c]	132	0.49	0.51	0.32	0.35	0.33	1.000	**0.018**	–	0.117
Korean cattle	437	388	486	91	206	140	0.456	**0.044**	0.203	–
24-bp indel[d]										
German cattle[b]										
Healthy	48	0.95	0.05	0.90	0.10	0.00	–	1.000	–	**< 0.001**
BSE	43	0.95	0.05	0.91	0.09	0.00	1.000	–	–	**< 0.001**
Korean cattle	437	1.00	0.00	1.00	0.00	0.00	**< 0.001**	**< 0.001**	–	–

I, insertion; D, deletion. [a]*P*-value was obtained from G-based exact test for all pairs of healthy German cattle, BSE-affected German cattle, US cattle, and Korean cattle. The values for allelic and genotypic differences are presented above and below diagonal for each indel, respectively. Bold face indicates the significance with $P < 0.05$. [b]Data for healthy and BSE-affected German cattle reported in the study of Sander *et al.* (2004). [c]Data for US cattle reported in the study of Seabury *et al.* (2004). [d]Octapeptide repeat polymorphism (I, 6-repeat allele; D, 5-repeat allele).

Additional comparison with US cattle also showed a significant differences ($P < 0.01$) in the allelic and genotypic distributions of 23-bp indel polymorphism between Korean and US cattle (table 2), whereas no significant difference ($P > 0.05$) was observed between US cattle and BSE-affected German cattle (Seabury *et al*. 2004).

The polymorphism at octapeptide-repeat region was not found in any of the 437 Korean cattle, and as a result, its distribution differed significantly ($P < 0.01$) from those of both the healthy and BSE-affected German cattle.

Allelic and genotypic frequencies by province

Further analyses of the allelic and genotypic frequencies for 23-bp and 12-bp indel polymorphisms were conducted with the data partitioned by province (see table 2). Any geographical difference in *PRNP* polymorphisms of Korean cattle was not observed in the two variants ($P > 0.05$). Regardless of the province, the D allele at the locus of 23-bp indel was shown to be significantly less frequent in healthy Korean cattle than in BSE-affected German cattle and in healthy US cattle ($P < 0.05$). The significance of genotypic distribution in the locus was also observed ($P < 0.05$). Furthermore, the frequencies of D allele (0.48–0.57) were less in healthy Korean cattle nationwide, except for Jeju Island, than in healthy German cattle, although the difference was not statistically significant. On the other hand, the D allele in 12-bp indel polymorphism was less frequent in healthy Korean cattle than in BSE-affected German cattle and the differences were significant ($P < 0.05$) for some provinces.

Haplotypic frequency

Haplotypic frequencies for the three *PRNP* polymorphisms in Korean cattle were compared to those with German cattle calculated from the data of Sander *et al*. (2004). The distribution of haplotype in Korean cattle differed largely ($P < 0.001$) from those of healthy German cattle as well as BSE-affected German cattle (table 3). The haplotype DI5 is absent in Korean cattle, whereas ID6 is absent in German cattle. Most cattle in the analysis had DD6 or II6 (95% for Korean cattle and 94% for German cattle). Each of these common haplotypes showed the differences ($P < 0.05$) between Korean (0.48 − 0.59 for DD6 and 0.39 − 0.50 for II6) and BSE-affected German cattle (0.67 for DD6 and 0.27 for II6).

Discussion

Significant associations of the bovine *PRNP* gene with BSE susceptibility were recently discovered in German cattle (Sander *et al*. 2004). The *PRNP* indel polymorphisms of 23 bp in promoter and 12 bp in intron 1 were the only two loci that had associations with susceptibility to BSE of German cattle. These polymorphisms contained binding sites of RB58 and SP1 transcription factors, respectively. Differential expression of the gene by the polymorphisms were also observed in the subsequent study of Sander *et al*. (2005). This prompted our nationwide investigation of its allelic, genotypic, and haplotypic distribution of Korean cattle, which is the first nationwide genetic survey for the *PRNP* variants. In the current study, the allele and genotype distributions of the two indel polymorphisms in Korean cattle also differed from those in BSE-affected German cattle.

Table 2. Allelic and genotypic distribution of indel polymorphisms of *PRNP* gene in Korean cattle by province.

		Allele frequency		Genotype frequency		
Province	*n*	I	D	II	ID	DD
Promoter 23-bp indel						
Kyonggi	24	0.48	0.52	0.25	0.46	0.29
Chungcheong	26	0.52	0.48	0.31	0.42	0.27
Kangwon	60	0.43	0.57	0.17	0.53	0.30
Jeolla	140	0.43	0.57	0.18	0.50	0.32
Jeju	35	0.40	0.60	0.17	0.46	0.37
Combined	437	0.44	0.56	0.20	0.49	0.31
Intron 1 12-bp indel						
Kyonggi	24	0.40	0.60	0.17	0.46	0.38
Chungcheong	26	0.50	0.50	0.31	0.38	0.31
Kangwon	60	0.43	0.57	0.18	0.50	0.32
Kyongsang	152	0.46	0.54	0.22	0.47	0.30
Jeolla	140	0.44	0.56	0.19	0.49	0.32
Jeju	35	0.41	0.59	0.20	0.43	0.37
Combined	437	0.44	0.56	0.21	0.47	0.32

I, insertion; D, deletion.

Table 3. Estimates of haplotype frequencies for *PRNP* sequence variants in Korean cattle.

	Haplotype frequency[a]					P value[b]	
						German cattle	
	DD6	II6	D16	D15	ID6	Healthy	BSE
German cattle							
Healthy	0.51	0.43	0.01	0.05	0.00	–	0.142
BSE	0.67	0.27	0.01	0.05	0.00	0.142	–
Korean cattle							
Kyonggi	0.52	0.40	0.00	0.00	0.08	**0.008**	**0.004**
Chungcheong	0.48	0.50	0.00	0.00	0.02	0.102	**0.009**
Kangwon	0.53	0.39	0.04	0.00	0.04	**0.003**	**0.001**
Kyongsang	0.51	0.42	0.04	0.00	0.03	**< 0.001**	**< 0.001**
Jeolla	0.56	0.43	0.01	0.00	0.00	**0.006**	**0.001**
Jeju	0.59	0.40	0.01	0.00	0.00	0.111	0.062
Combined	0.53	0.42	0.02	0.00	0.02	**< 0.001**	**< 0.001**

[a]For German cattle, haplotype frequencies were calculated on the basis of genotype frequencies obtained in the study of Sander *et al.* (2004). For Korean cattle, haplotype frequencies were obtained by maximum likelihood estimation using Arlequin program. [b]Likelihood ratio test was utilized to compare the haplotypic distributions of Korean cattle with those of healthy and BSE-affected German cattle. Bold face indicates the significance with $P < 0.05$.

The 23-bp and 12-bp deletion alleles which had associations with a higher susceptibility to the BSE in German cattle were both found to be less frequent in Korean cattle than in BSE-affected German cattle. This was proven to be robust because of a consistently larger deletion frequency in BSE-affected German cattle than any of the corresponding frequencies resulted from the data partitioned by province with Korean cattle.

The other locus analysed in this study is the octapeptide-repeat polymorphism with three alleles (5, 6 and 7 copies) in the open reading frame of the *PRNP* gene (Goldmann *et al*. 1991; Premzl *et al*. 2000; Walawski and Czarnik 2003). Unfortunately, this locus is monomorphic in Korean cattle without 5 and 7 copies of the octapeptide repeat in this study. Although its small heterozygosities in cattle has restricted the association study of the locus with incidence of BSE, its potential possibility of association with BSE should not be negligible. The octapeptide-repeat locus is the region where the copper binds to *PRNP*, and the associations of its deletion/insertion with prion diseases were reported in human (Owen *et al*. 1992) and in transgenic mice (Chiesa *et al*. 2000). The allelic and genotypic distributions of the octapeptide-repeat polymorphism in Korean cattle differed from those of German cattle, both with and without BSE ($P < 0.001$). This implied that the deletion, i.e. 5-octapeptide repeats, might be more susceptible to BSE. This did not concur with the finding of Sander *et al*. (2004) where the frequencies of the 5-octapeptide repeat allele from healthy and BSE German cattle were both 0.05. We could not exclude the possibility of a false negative due to the small statistical power in their study.

The haplotype analysis with the three polymorphisms revealed that two common haplotypes, DD6 and II6, and two rare haplotypes, DI6 and ID6, were found in Korean cattle. This was due to a strong linkage disequilibrium ($D' = 0.92$) between 23-bp and 12-bp indel polymorphisms and the lack of octapeptide-repeat polymorphism. Interestingly, all German cattle with 5-octapeptide repeats had DI haplotype of 23-bp indel and 12-bp indel. The German cattle with the haplotype DI5 were much more frequent than those with the haplotype DI6. This showed a strong linkage between 5-octapeptide-repeat allele and DI haplotype of 23-bp and 12-bp indel loci. Of course, the haplotype DI5 was not found in Korean cattle from null of octapeptide-repeat polymorphism. On the other hand, the haplotype ID6 was observed only in Korean cattle. Such dissimilarity taken together with distinctions of common haplotypes resulted in a significant difference ($P < 0.001$) in the haplotype distribution between Korean and German cattle.

In the current study, we estimated haplotypic frequencies utilizing genotypic data for healthy and BSE-affected German cattle reported in Sander *et al*. (2004) and further analysed a susceptibility of the haplotypes to BSE. The haplotype analysis revealed that haplotype DD6 is associated with a higher risk of BSE (OR = 1.99; 95% CI = 1.09–3.63), and II6 with a lower risk (OR = 0.49; 95% CI = 0.26–0.92). The frequencies of these haplotypes estimated in Korean cattle were corresponding to those in healthy German cattle ($P > 0.05$), but not to those in BSE-affected German cattle ($P < 0.05$).

We could not evaluate the susceptibility of Korean cattle to BSE because comparison with other foreign populations might produce ambiguous effects confounded with environmental effects and/or other genetic effects. Granted that the 23-bp and 12-bp deletion alleles were associated with a higher susceptibility to the BSE as found in Sander *et al.* (2004), our results showed that the genetic distributions for Korean cattle had similar patterns to those for healthy population across the regions. With respect to these two indel variants, Korean cattle might not be any more susceptible to BSE than other populations. Additionally, the current study discusses a potential association between octapeptide-repeat locus and BSE with a reduced susceptibility of Korean cattle. Elucidation of clear regulatory effects of the sequence variants on promoter, intron 1, and exon 3 would warrant a convincing conclusion. Also, cumulative data for such nationwide genetic evaluation in the countries both with and without BSE-affected cattle would be required for more clear and accurate genetic epidemiology for the sequence variants.

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