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LACK OF GERMLINE MUTATION AT CODON 211 OF THE PRION PROTEIN GENE *(PRNP)* IN KOREAN NATIVE CATTLE – SHORT COMMUNICATION

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Bovine prion diseases are composed of two types of bovine spongiform encephalopathy (BSE), classical BSE and atypical BSE. Recent studies have identified one case of atypical BSE with an E211K mutation. E211K is homologous to the human E200K mutation, which is related to familial Creutzfeldt-Jakob disease (CJD), one of the familial forms of human prion diseases. To date, familial forms of prion diseases have not been reported in non-human animals. Because the familial forms of human prion diseases account for more than 10% of all human prion disease cases, the detection of the E211K mutation in healthy cattle is very important for verifying the role of this mutation as a familial form of BSE. To detect putative mutations related to familial BSE, specifically E211K in Korean native cattle (Hanwoo) and Korean dairy cattle (Holstein), we performed direct sequencing targeting codon 211 and the adjacent regions of the bovine prion protein *(PRNP)* gene in 384 Hanwoo and 152 Holstein cattle. We did not find the E211K mutation in any of the Korean cattle. Although we did not find the E211K mutation in Korean native cattle, E211K is a postulated mutation; therefore, further screening in other countries and larger samples is highly desirable.

Key words: Bovine, prion protein gene, codon 211, mutation

Transmissible spongiform encephalopathies (TSEs), also called prion diseases, are characterised by the accumulation of an abnormal infectious form of the prion protein in humans and animals (Prusiner, 1998). Human prion diseases can occur spontaneously, be inherited, or be acquired. The inherited forms of human prion diseases include familial Creutzfeldt-Jakob disease (CJD), Gerstmann–Sträussler–Scheinker disease (GSS), and fatal familial insomnia (FFI), which are caused by germline mutations in the prion protein gene *(PRNP)*. To date, more than 30 mutations of *PRNP* in humans have been observed in the open reading frame (ORF) of *PRNP* (Jeong and Kim, 2014). Among them, muta-

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tion at codon 200 of *PRNP* is the most common mutation in European populations with genetic prion diseases, and it is strongly associated with familial forms of human prion disease such as familial CJD (Kovacs et al., 2005).

The major form of bovine prion diseases is classical bovine spongiform encephalopathy (BSE). It has been assumed that classical BSE was caused by feed that was made from scrapie-affected sheep (Wilesmith et al., 1988). In contrast, very few cases of sporadic forms of BSE have been reported in comparison to classical BSE. Animals affected by sporadic BSE, also called atypical BSE, have been reported in several countries; however, their aetiology has not been fully explained so far (Guldimann et al., 2012; Orge et al., 2015).

Recent studies have indicated that one case of atypical BSE, a putative inherited form of BSE, is related to a *PRNP* ORF mutation. The mutation, located in codon 211 of bovine *PRNP,* is in a region homologous with codon 200 of human *PRNP*. Both codon 200 in humans and codon 211 in cattle, encode the same amino acid, Glu (E), and the mutated amino acids are identical (Lys, K). Atypical BSE in an animal with the E211K mutation was first reported in 2006, in the US. Nicholson et al. (2008) performed sequence analysis of both the 2006 US atypical animal and its 2-year-old offspring. Both of them were heterozygous for the E211K mutation. These results suggest that this allele, which is associated with the familial form of atypical BSE, can be heritable (Nicholson et al., 2008).

In this context, we investigated the presence of a point mutation at codon 211 of the *PRNP* gene in the genomic DNAs of 536 Korean native cattle. Blood samples were taken from 384 Hanwoo and 152 Holstein cattle in South Korea. Genomic DNA was extracted from 200 µl of whole blood using QIAamp® DNA Blood Mini Kit (QIAGEN, Valencia, CA, USA). Polymerase chain reaction (PCR) was performed with a sense primer (5'-CACATAGGCAGTTGGATCCTGG-3') and an antisense primer (5'-GGTACTGGGTAATGCACATTTGCTC-3'). After purification using the QIAquick® Gel Extraction Kit (QIAGEN), the PCR products were directly sequenced with an ABI 3730 Capillary Electrophoresis Sequencer (Applied Biosystems, Foster City, CA, USA).

The E211K mutation of bovine *PRNP* was not found in 1072 chromosomes of Korean Hanwoo and Holstein cattle (Table 1).

Table 1

Genotype and allele frequencies at codon 211 of the prion protein gene in Korean Hanwoo and Holstein cattle

Sample	Total, n	Genotype frequency (%)		Allele frequency (%)	
		Glu/Glu	Glu/Lys	Glu	Lys
Hanwoo	384	384 (100)	0 (0)	768 (100)	0 (0)
Holstein	152	152 (100)	0 (0)	304 (100)	0 (0)
Total	536	536 (100)	0 (0)	1072 (100)	0 (0)

BSE is classified into two types, classical BSE and atypical BSE. The first identification of TSE in cattle was classical BSE. Classical BSE was probably caused mainly by widespread prion-contaminated meat and bone meal (MBM) (Bradley and Wilesmith, 1993). The clinical signs of BSE may include tremors, ataxia, aggressive behaviour, apprehension, oversensitivity to stimuli, PrP^{Sc} aggregation, and spongiform vacuolation of the brain (Novakofski et al., 2005). There have been several polymorphism studies in the *PRNP* ORF (Zhang et al., 2004; Jeong et al., 2005), and unlike human CJD, no direct relationship between classical BSE and *PRNP* ORF polymorphisms has been identified. Animals affected by classical BSE show a significantly higher frequency of the 23 bp del/del genotype in the putative promoter region of *PRNP* (Jeong et al., 2006). In addition, a 12-bp indel polymorphism within intron 1 of *PRNP* has been related to classical BSE (Haase et al., 2007; Gurgul et al., 2012*a*). However, several studies have failed to find a correlation between 12-bp indel polymorphisms and classical BSE (Hresko et al., 2009; Vernerova et al., 2014). Furthermore, SNP 4136 and 13861 in the noncoding region of *PRNP* have been shown to confer susceptibility to classical BSE (Murdoch et al., 2010; Jeong et al., 2013).

Another form of BSE, atypical BSE, is composed of two types, H-type and L-type. L-type BSE is also called bovine amyloidotic spongiform encephalopathy (BASE). Cattle affected by H-type BSE possess a higher molecular mass of unglycosylated PrP^{res} compared to those affected by classical BSE (Biacabe et al., 2004). However, cattle affected by L-type BSE contain a lower molecular mass of unglycosylated PrP^{res} than those affected by classical BSE (Casalone et al., 2004). Generally, animals affected by either form of atypical BSE are older than those affected by classical BSE. In a recent study of Polish cattle, all of the cattle affected by atypical BSE were homozygous for a 23-bp deletion at the transcription factor binding site of *PRNP,* and they showed higher prevalence of a 12-bp deletion allele (Gurgul et al., 2012*b*). Furthermore, *PRNP* gene haplotypes are associated with both forms of atypical BSE (Clawson et al., 2008).

Among animals affected by H-type BSE, a novel form of BSE with mutation E211K has recently attracted attention due to the existence of a familial form in non-human animals. The E211K mutation of bovine *PRNP* is homologous to the E200K mutation in human *PRNP*, which is associated with a familial form of CJD. Nicholson et al. (2008) reported that the 211K allele, a mutated form of codon 211, could be heritable. In addition, the authors performed intracranial inoculation of 2006 US H-type BSE with the K211 allele to a calf with the K211 allele. The calf showed severe clinical signs of BSE in a bioassay after 9.8 months. That is a shorter incubation period than has been observed for average H-type atypical BSE (Balkema-Buschmann et al., 2011). In addition, immunoblot analysis showed distinct molecular features distinguished from typical H-type BSE (Greenlee et al., 2012). Although two types of atypical BSE, H-type and L-type, did not transmit to three lines of human prion transgenic mice (HuMM, HuMV,

HuVV) (Wilson et al., 2012; Wilson et al., 2013), the transmissibility to humans of this novel type of H-type atypical BSE, which contains the E211K mutation, has not been assessed thus far.

Based on previous reports, we investigated the E211K mutation in 384 Hanwoo and 152 Holstein cattle by direct sequencing. However, we did not identify the K allele in Korean cattle. This result indicates that the germline mutation at codon 211 of the bovine *PRNP* gene was not found in Korean native cattle. There is little research that focuses on E211K (Heaton et al., 2008; Zhao et al., 2010), and only few cases have been reported so far. Hence, further screening of E211K mutation in other countries is highly desirable in the future. If the offspring of US cattle affected by atypical BSE in 2006 show the clinical signs of BSE and detection of PrP^{Sc} in the brain, a familial form of BSE will be verified for the first time in bovines. In addition, like in a prior study on the A117V mutation of *PRNP*, which is associated with GSS, the use of knock-in transgenic mice may help to determine the existence of a familial form of BSE in cattle (Yang et al., 2009). Furthermore, bioassays using E211K-mutant atypical BSE brain homogenate in human *PRNP* transgenic mice can be used to assess the risk of transmission to humans.

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