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# Calcium homeostasis modulator 1 (*CALHM1*) polymorphisms in cattle[1]

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**ABSTRACT.-** Yun C.-H. & Jeong B.-H. 2017. **Calcium homeostasis modulator 1 (*CALHM1*) polymorphisms in cattle.** *Pesquisa Veterinária Brasileira 37(6):582-586.* Korea Zoonosis Research Institute, Chonbuk National University, Iksan, 820-120, Hana-ro, Iksan, Jeonbuk 570-390, Republic of Korea. E-mail: bhjeong@jbnu.ac.kr

The calcium homeostasis modulator 1 gene (*CALHM1*), which is located on chromosome 10 in humans and on chromosome 26 in cattle, is a transmembrane glycoprotein that controls the cytosolic calcium concentrations. Altered calcium homeostasis has been associated with several neurodegenerative disorders, including Alzheimer's disease (AD). In a recent study, single nucleotide polymorphisms (SNPs) of *CALHM1* have been associated with sporadic Creutzfeldt-Jakob disease (CJD). The protein sequence of human *CALHM1* shows 93% homology with bovine *CALHM1*. Although SNPs of human *CALHM1* have been correlated with human prion disease, polymorphisms of the bovine *CALHM1* gene have not been reported in cattle thus far. To investigate polymorphisms of the bovine *CALHM1* gene in Korean native cattle, we analyzed the open reading frame (ORF) of this gene in 175 Hanwoo and 141 Holstein cattle. We observed five SNPs: c.219C>T (rs380966453), c.357C>T (rs385969338), and c.869A>G (rs516301908) within the ORF region of two exons; and c.552+92A>G (rs481706737) and c.553-3A>C (rs448524869) in the intron of bovine *CALHM1*. Among the three SNPs that are in the ORF region of bovine *CALHM1*, the genotype and allele frequencies of the c.869A>G (p.His290Arg) and c.219C>T (p.Asn73Asn) SNPs were significantly different between Hanwoo and Holstein cattle (P<0.0001). Haplotype analysis showed that haplotypes *ht*2, *ht3* and *ht*5 were also significantly different in these two cattle breeds. This study provides the first genetic analysis of the bovine *CALHM1* gene in cattle.

INDEX TERMS: CALHM1, calcium homeostasis, single nucleotide polymorphism, cattle, Hanwoo, neurodegenerative disorders.

## INTRODUCTION

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a type of neurodegenerative disorder causing Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and chronic wasting disease (CWD) in deer and elk (Prusiner 1998). BSE is characterized by the accumulation of an abnormal protease-resistant isoform ($PrP^{Sc}$) of the prion protein in the brain, spongiform degeneration, astrocytosis, and neuronal cell loss (Collinge 1997, Prusiner 1997). BSE epidemics are caused by the ingestion of meat and bone meal produced from scrapie-infected sheep or BSE-infected cattle in the United Kingdom (UK) (Wilesmith et al. 1991).

The Hanwoo breed (Han means Korean, and woo means cattle), which originated from crossbreeding between *Bos indicus* and *Bos primigenius*, is widely raised as a beef breed in Korea (Jeong et al. 2005b). In Korea, the majority of dairy cattle are Holstein bred. All 36 BSE cases reported in Japan were diagnosed in 2010 or earlier. Among them, 33 cases were reported in Holstein-Friesian cattle and 3 cases were reported in the Japanese Black (JB) cattle (Msalya et al. 2011). In the UK, most of the diagnosed cases of BSE were in Holstein Friesian dairy cattle (Bradley and Wilesmith 1993). However, BSE has not been reported in Korean native cattle thus far (Lee et al. 2012).

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In previous studies, the polymorphisms in the prion protein gene (*PRNP*) have been known to influence the susceptibility/resistance to prion diseases in humans, cattle, and sheep (Palmer et al. 1991, Belt et al. 1995, Clouscard et al. 1995, Jeong et al. 2004, Jeong et al. 2005a). The *PRNP* gene is located on chromosome 13q17 in cattle (Ryan and Womack 1993). Although polymorphisms associated with BSE susceptibility have not been reported in the open reading frame (ORF) region of bovine *PRNP*, two insertion/deletion (indel) polymorphisms consisting of a 23-bp indel in the promoter region and a 12-bp indel in intron 1 of bovine *PRNP*, have been associated with BSE susceptibility (Sander et al. 2004, Juling et al. 2006). In several recent studies, two polymorphisms (snp 4136 and snp 13861) in non-coding regions of bovine *PRNP* have been correlated with BSE susceptibility in European Holstein cattle (Murdoch et al. 2010a, Murdoch et al. 2010b). Additionally, association studies on genes such as the prion-like protein gene (*PRND*) and shadow of prion protein (*SPRN*) have been performed to identify genetic susceptibility to BSE (Comincini et al. 2001, Balbus et al. 2005, Gurgul et al. 2012).

Calcium homeostasis modulator 1 (*CALHM1*) plays an important role in calcium homeostasis. Altered calcium homeostasis has been linked to several neurodegenerative disorders, including Alzheimer's disease (AD) and prion diseases (Green and LaFerla 2008, Kawamata and Manfredi 2010, Surmeier et al. 2010, Fedrizzi and Carafoli 2011, Giacomello et al. 2011, Peggion et al. 2011). In humans, a previous study suggested that the rs2986017 polymorphism (Pro86Leu) of the human *CALHM1* gene leads to loss of $Ca^{2+}$ permeability and increases amyloid β (Aβ) levels (Dreses-Werringloer et al. 2008). In addition, this single nucleotide polymorphism (SNP) is a genetic risk factor for susceptibility to AD (Dreses-Werringloer et al. 2008, Aqdam et al. 2010, Cui et al. 2010, Lambert et al. 2010, Koppel et al. 2011). However, several studies of the relationship between the rs2986017 polymorphism of human *CALHM1* and increased risk for AD have led to divergent findings (Beecham et al. 2009, Minster et al. 2009, Sleegers et al. 2009, Inoue et al. 2010, Lambert et al. 2010, Nacmias et al. 2010, Feher et al. 2011, Tan et al. 2011). Recently, three polymorphisms and the haplotype frequency of human *CALHM1* have been shown to be associated with sporadic CJD (Calero et al. 2012). These results suggest that human *CALHM1* can play a role in the development of human prion diseases such as sporadic CJD. The *CALHM1* gene is located on chromosome 26 in cattle. Although SNPs of this gene have been associated with human prion disease, no polymorphisms of the bovine *CALHM1* gene, which shares 93% protein sequence identity with human *CALHM1*, have been reported in cattle thus far.

The aim of the present study was to investigate the genotype, allele, and haplotype frequencies of bovine *CALHM1* SNPs in 175 Korean Hanwoo and 141 Holstein cattle.

## MATERIALS AND METHODS

**Genetic analysis.** Blood samples were taken from 175 Hanwoo and 141 Holstein cattle in South Korea. Genomic DNA was isolated from 200μl of blood using the QIAamp DNA blood mini kit (Qiagen, USA) following the manufacturer's instructions.

Polymerase chain reaction (PCR) was performed with the following forward and reverse primers: bovine *CALHM1*-1F (TGTCTCAGCCATGACGTG) and bovine *CALHM1*-1R (ATGGGTCTGTCCACTCAGAT) were designed to amplify a 817 bp products including exon 1 (554 bp) of bovine *CALHM1* gene; bovine *CALHM1*-2F (TCTTTTCCCTAAAGGCCCTG) and bovine *CALHM1*-2R (CCATTTGAGGCGGGAAATTT) were designed to amplify a 743 bp products including ORF region (480 bp) of exon 2. The PCR reagents included 50 pmole of each primer, 5 μl of 10 × *Taq* DNA polymerase buffer, 1 μl of a 10 mM dNTP mixture and 2.5 units of *Taq* DNA polymerase (Promega, USA). The PCR conditions were as follows: denaturing at 94°C for 2 min, followed by 35 cycles of 94°C for 45 sec, 58°C for 45 sec, and 72°C for 1 min 30 sec, and then 1 cycle of 72°C for 10 min for final extension using an S-1000 Thermal Cycler (Bio-Rad Laboratories, USA).

Purification of the PCR products for DNA sequencing was performed using a QIAquick gel extraction kit (Qiagen, USA). The PCR products were directly sequenced with an ABI 3730 automatic sequencer using a *Taq* dideoxy terminator cycle sequencing kit (ABI, USA).

Polymorphism Phenotyping v2 (PolyPhen-2) software was used to predict the possible impact of an amino acid substitution on the non-synonymous SNPs found in this study (http://genetics.bwh.harvard.edu/pph2/).

**Statistical analysis.** Statistical analyses were performed using Statistical Analysis Software (SAS), version 9.3 (SAS Institute Inc., Cary, NC., USA). The differences in genotype or allele frequencies between the groups of Korean native cattle were measured with the $\chi^2$-test or Fisher's exact test. We also examined Lewontin's D' (|D'|) between five SNPs of the *PRNP* gene in Hanwoo and Holstein cattle. The Hardy-Weinberg Equilibrium test and haplotype analysis were carried out with SNP Analyzer 1.2A (http://snp.istech21.com/snpanalyzer/1.2A/).

## RESULTS

The bovine *CALHM1* gene is composed of two exons. To investigate the genotype and allele frequencies of bovine *CALHM1* polymorphisms in Korean native cattle, we screened SNPs within two exons of the bovine *CALHM1* gene through automatic DNA sequencing in the genomic DNA of 175

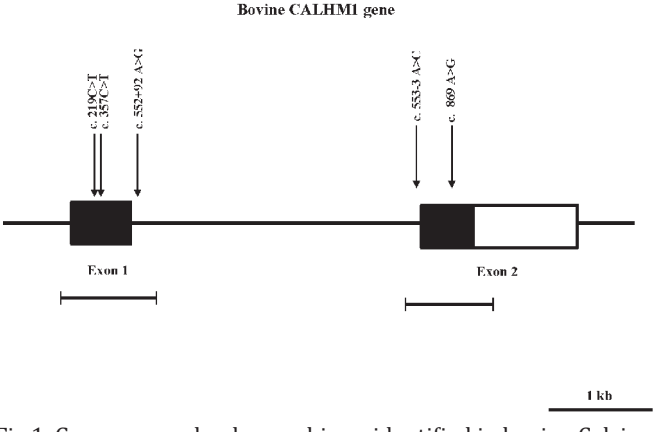


Fig.1. Gene map and polymorphisms identified in bovine Calcium homeostasis modulator 1 gene (*CALHM1*) on chromosome 26. The open reading frame (ORF) within exons was marked by shaded blocks and 3' untranslated region (UTR) by white blocks. Edged horizontal bars indicate the regions sequenced. The words in bold indicate five polymorphisms found in this study.

**Table 1. Genotype and allele frequencies of single nucleotide polymorphisms (SNPs) of the bovine *CALHM1* gene in Korean Holstein and Hanwoo cattle**

| | | Genotype frequency, n (%) | | | *P* value | Allele frequency, n (%) | | *P* value | HWE |
|---|---|---|---|---|---|---|---|---|---|
| c.219C>T | | C/C | C/T | T/T | | C | T | | |
| | Holstein | 51 (51.0) | 36 (36.0) | 13 (13.0) | <0.0001 | 138 (69.0) | 62 (31.0) | <0.0001 | 0.173 |
| | Hanwoo | 175 (100) | 0 (0) | 0 (0) | | 350 (100) | 0 (0) | | 1.0 |
| c.357C>T | | C/C | C/T | T/T | | C | T | | |
| | Holstein | 74 (74.0) | 23 (23.0) | 3 (3.0) | 0.7283 | 171 (85.5) | 29 (14.5) | 0.8055 | 0.636 |
| | Hanwoo | 125 (71.4) | 46 (26.3) | 4 (2.3) | | 296 (84.6) | 54 (15.4) | | 0.251 |
| c.552+92A>G | | A/A | A/G | G/G | | A | G | | |
| | Holstein | 53 (53.0) | 37 (37.0) | 10 (10.0) | 0.0012 | 143 (71.5) | 57 (28.5) | <0.0001 | 0.393 |
| | Hanwoo | 33 (41.3) | 76 (31.4) | 66 (27.3) | | 142 (40.6) | 208 (59.4) | | 0.911 |
| c.553-3C>A | | C/C | C/A | A/A | | C | A | | |
| | Holstein | 141 (100) | 0 (0) | 0 (0) | 0.0037 | 282 (100) | 0 (0) | 0.0039 | 1.0 |
| | Hanwoo | 145 (94.2) | 9 (5.8) | 0 (0) | | 299 (97.1) | 9 (2.9) | | 0.876 |
| c.869A>G | | A/A | A/G | G/G | | A | G | | |
| | Holstein | 141 (100) | 0 (0) | 0 (0) | <0.0001 | 282 (100) | 0 (0) | <0.0001 | 1.0 |
| | Hanwoo | 120 (77.9) | 33 (21.4) | 1 (0.7) | | 273 (88.6) | 35 (11.4) | | 0.519 |

**Table 2. Linkage disequilibrium (LD) among five single nucleotide polymorphisms (SNPs) of the *CALHM1* gene in Hanwoo and Holstein cattle**

| | c.219C>T | c.357C>T | c.552+92A>G | c.553-3A>C | c.869A>G |
|---|---|---|---|---|---|
| c.219C>T | - | 1.0 | 1.0 | 1.0 | 1.0 |
| c.357C>T | - | - | 0.913 | 1.0 | 1.0 |
| c.552+92A>G | - | - | - | 1.0 | 0.703 |
| c.553-3A>C | - | - | - | - | 1.0 |
| c.869A>G | - | - | - | - | - |

Hanwoo and 141 Korean Holstein cattle. We identified a total of 5 SNPs: c.219C>T (p.Asn73Asn; rs380966453) and c.357C>T (p.Leu119Leu; rs385969338) in exon 1; c.869A>G (p.His290Arg; rs516301908) in exon 2; 552+92A>G (rs481706737) and 553-3A>C (rs448524869) in the intron of *CALHM1* (Fig.1). Significant differences in ORF region of bovine *CALHM1* gene between Hanwoo and Holstein cattle were observed in the genotype ($P < 0.0001$) and allele ($P<0.0001$) frequencies of c.219C>T (p.Asn73Asn) and c.869A>G (p.His290Arg) (Table 1). However, there were no significant differences in the genotype ($P = 0.7508$) or allele ($P=0.8051$) frequencies of c.357C>T (p.Leu119Leu) between the two groups (Table 1). In addition, the genotype and allele frequencies of the two SNPs located in the intron were significantly different between Hanwoo and Holstein cattle raised in Korea (Table 1). Moreover, PolyPhen-2 analysis predicted that the c.869A>G (p.His290Arg) SNP was benign with score of 0. The genotype frequencies of all of the identified SNPs followed Hardy-Weinberg equilibrium in Hanwoo and Holstein cattle.

To examine whether there was strong linkage disequilibrium among the 5 SNPs of the bovine *CALHM1* gene in Korean native cattle, linkage disequilibrium (LD) (|D'|) was calculated. All 5 SNPs of the bovine *CALHM1* gene were found to be in strong LD in Korean native cattle with D' values of 0.703-1.0 (Table 2).

Analysis of haplotype frequencies was carried out in Hanwoo and Korean Holstein cattle. As shown in Table 3, there are eight different haplotypes of the bovine *CALHM1* polymorphisms. Among the eight haplotypes, the CCACA (*ht1*) haplotype was observed most frequently (43.18% for Holstein; 40% for Hanwoo). The haplotype frequencies of CCGCA (*ht2*), TCACA (*ht3*), and CCGCG (*ht5*) showed significant differences between the Hanwoo and Korean Holstein cattle.

## DISCUSSION

Polymorphisms of the *PRNP* gene have been shown to play an important role in susceptibility to prion diseases in human and sheep (Palmer et al. 1991, Belt et al. 1995, Clouscard et al. 1995, Jeong et al. 2004, Jeong et al. 2005a). Significant associations of classical BSE susceptibility and bovine *PRNP* genotypes involving a the 23 bp indel polymorphism in the putative promoter region and a 12 bp indel polymorphism within intron 1, have been demonstrated in large samples and various species of cattle (Sander et al. 2004, Juling et al. 2006, Haase et al. 2007, Kashkevich et al. 2007, Muramatsu et al. 2008, Murdoch et al. 2010b). Recently, *PRNP* haplotypes including snp 4136 and snp 13861 have been associated with susceptibility to classical and atypical BSE (Clawson et al. 2008, Murdoch et al. 2010a, Murdoch et al. 2010b).

Several genetic studies have been performed to identify candidate genes related to BSE other than the *PRNP* gene, including *PRND* and *SPRN* (Comincini et al. 2001, Balbus et al. 2005, Gurgul et al. 2012). In humans, the SNPs and the haplotype frequency of the human *CALHM1* gene were shown to be associated with sporadic CJD in a Spanish population (Calero et al. 2012), suggesting that human *CALHM1* plays a role in the development of human prion diseases. The sequence of the bovine *CALHM1* protein shares 93% identity with human *CALHM1*. This result suggests that bovine *CALHM1* could play a role in the pathogenesis of BSE in cattle.

In this study, we analyzed the genotype and allele frequencies of five SNPs, including c.869A>G (p.His290Arg), between Hanwoo and Korean Holstein cattle (Table 1). The genotype and allele frequencies of c.219C>T and c.869A>G showed significant differences between the two groups. Among SNPs found in ORF region of bovine *CALHM1* gene, it is possible that nonsynonymous SNPs are involved in phenotype differences by amino acid substitution of protein. Thus, the predicted damaging effect of c.869A>G

**Table 3. Haplotype frequencies of five polymorphisms of the *CALHM1* gene in Korean Holstein and Hanwoo cattle**

| Haplotypes | c.219C>T | c.357C>T | c.552+92A>G | c.553-3A>C | c.869A>G | Frequency | | P-value |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Holstein | Hanwoo | |
| *ht1* | C | C | A | C | A | 0.4318 (76) | 0.4000 (76) | - |
| *ht2* | C | C | G | C | A | 0.1307 (23) | 0.3105(59) | 0.0012 |
| *ht3* | T | C | A | C | A | 0.3068 (54) | 0 (0) | <0.0001 |
| *ht4* | C | T | G | C | A | 0.1193 (21) | 0.1632 (31) | 0.2307 |
| *ht5* | C | C | G | C | G | 0 (0) | 0.0842 (16) | <0.0001 |
| *ht6* | C | C | A | C | G | 0 (0) | 0.0263 (5) | 0.0592 |
| *ht7* | C | C | G | A | G | 0 (0) | 0.0158 (3) | 0.2454 |
| *ht8* | C | T | A | C | A | 0.0114 (2) | 0 (0) | 0.4968 |

(p.His290Arg) SNP was determined using PolyPhen-2 software program, which was used to predict the effect of the SNPs and mutations on the function of protein with a scale of 0-1 (Adzhubei et al. 2010). However, this SNP has been predicted as benign with a score of 0. Moreover, the CCGCA (*ht2*), TCACA *(ht3*), and CCGCG (*ht5*) haplotype frequencies exhibited significant differences between the Hanwoo and Korean Holstein cattle (Table 3).

Holstein dairy cattle have been reported to develop BSE in many countries including UK, Canada, United States (US), and Japan (Bradley & Wilesmith 1993, Msalya et al. 2011, Dudas et al. 2010, Richt & Hall 2008, Kim and Jeong 2017). However, BSE has never been diagnosed in Korean native cattle, Hanwoo (Lee et al. 2012). These results suggest the possibility that there are significant differences in the genetic distribution of SNPs in certain BSE-related genes including *PRNP*.

In our previous studies, we showed that there is a significant difference in the allele frequency of the 23 bp indel *PRNP* polymorphism between BSE-affected German cattle and Hanwoo (Jeong et al. 2006). In addition, we reported that the T and G allele frequencies at snp 4136 and snp 13861 SNPs of *PRNP*, which have been correlated with BSE resistance, are higher in Hanwoo than in Holstein cattle (Jeong et al. 2013). These results suggest that there is potential difference in BSE susceptibility between Hanwoo and Holstein cattle. Based on data of this study, future studies on polymorphisms of *CALHM1* gene in BSE-affected cattle will be necessary to evaluate the correlation between SNPs and susceptibility to BSE. To our knowledge, the present study provides the first genetic analysis of bovine *CALHM1* in cattle.

**Conflicts of interest statement.-** There are no conflicts of interest.

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