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The prion protein gene polymorphisms associated with bovine spongiform encephalopathy susceptibility differ significantly between cattle and buffalo

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ABSTRACT

Prion protein, encoded by the prion protein gene (*PRNP*), plays a crucial role in the pathogenesis of transmissible spongiform encephalopathies (TSEs). Several polymorphisms within the *PRNP* are known to be associated with influencing bovine spongiform encephalopathy (BSE) susceptibility in cattle, namely two insertion/deletion (indel) polymorphisms (a 23-bp indel in the putative promoter and a 12-bp indel in intron 1), the number of octapeptide repeats (octarepeats) present in coding sequence (CDS) and amino acid polymorphisms. The domestic buffaloes, *Bubalus bubalis*, are a ruminant involved in various aspects of agriculture. It is of interest to ask whether the *PRNP* polymorphisms differ between cattle and buffalo. In this study, we analyzed the previously reported polymorphisms associated with BSE susceptibility in Chinese buffalo breeds, and compared these polymorphisms in cattle with BSE, healthy cattle and buffalo by pooling data from the literature. Our analysis revealed three significant findings in buffalo: 1) extraordinarily low deletion allele frequencies of the 23- and 12-bp indel polymorphisms; 2) significantly low allelic frequencies of six octarepeats in CDS and 3) the presence of S4R, A16V, P54S, G108S, V123M, S154N and F257L substitutions in buffalo CDSs. Sequence alignments comparing the buffalo coding sequence to other species were analyzed using the McDonald–Kreitman test to reveal five groups (*Bison bonasus*, *Bos indicus*, *Bos gaurus*, *Boselaphus tragocamelus*, *Syncerus caffer caffer*) with significantly divergent non-synonymous substitutions from buffalo, suggesting potential divergence of buffalo *PRNP* and others. To the best of our knowledge this is the first study of *PRNP* polymorphisms associated with BSE susceptibility in Chinese buffalo. Our findings have provided evidence that buffaloes have a unique genetic background in the *PRNP* gene in comparison with cattle.

1. Introduction

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a group of invariably fatal neurodegenerative diseases detected in a wide range of species, such as bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and Creutzfeldt–Jakob disease (CJD) in humans. It has been reported that the host-encoded cellular prion protein (PrP^C), which is encoded by the prion protein gene (*PRNP*), plays a central role in prion disease due to an inability to develop the disease or for the disease agent to replicate when PrP^C is lacking (Brandner et al., 1996). Moreover, low *PRNP* expression effectively reduces TSE susceptibility (Manson et al., 1994), whereas high expression is associated with increased susceptibility and a shortened incubation time prior to disease development (Westaway et al., 1991). All TSEs are characterized by PrP^C undergoing structural isomerization to an infectious form, termed PrP^{Sc}, that is pathologically accumulated (Prusiner, 1998). Structurally, PrP^C possesses a flexible unstructured N-terminus and a globular C-terminus composed of three α-helices and two β-sheet secondary motifs, whereas PrP^{Sc} is rich in β-sheet secondary motifs (Pan et al., 1993).

Uniquely, the etiology of prion diseases can be described as inherited (familial), sporadic (unknown cause) or acquired (transmission of an infectious agent). Sequence variants of *PRNP* are the sole cause of inherited prion diseases, with reportedly at least 30 different mutations known to cause human prion diseases (Lloyd et al., 2011). Strong genetic susceptibility to both inherited and acquired human prion diseases has also been clearly demonstrated (Lloyd et al., 2011). Moreover, polymorphisms in codons 136, 154, and 171 of the *PRNP* gene in sheep are known to be highly related to scrapie susceptibility (Belt et al., 1995).

BSE has been outstandingly visible among prion diseases due to human infections causing variant CJD (vCJD) (Ward et al., 2006). To date BSE cases have occurred worldwide in nearly 0.2 million cattle (http://www.oie.int/en/animal-health-in-the-world/bse-specific-data/

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). Three factors of bovine *PRNP* influencing BSE susceptibility include: 1) two insertion/deletion (indel) polymorphisms, a 23-bp indel in the putative promoter and a 12-bp indel in intron 1; 2) the number of octapeptide repeats (octarepeats) present in the coding sequences (CDSs); and 3) amino acid polymorphisms (Brunelle et al., 2008a). Reportedly, the 23-bp deletion (D_{23}) and the 12-bp deletion (D_{12}) have been associated with *PRNP* expression (Sander et al., 2005; Msalya et al., 2011) and BSE susceptibility (Sander et al., 2004; Haase et al., 2007; Juling et al., 2006). Previous studies have also revealed that insertional mutations can lead to the expansion of the octarepeat domain of PrP and can be directly linked to prion disease (Stevens et al., 2009). Lastly, an amino acid change from glutamic acid to lysine (E211K), which is homologous to the most common pathogenic mutation in humans (E200K) leading to Creutzfeldt–Jakob disease, was identified in one atypical BSE animal (Heaton et al., 2008) and its only known living offspring (Nicholson et al., 2008). The domestic buffalo, *Bubalus bubalis*, has been an integral part of livestock agriculture in Asia for over 5000 years producing draft power, milk, meat and hides (Nanda and Nakao, 2003). We were curious to know if there are genetic differences in *PRNP* polymorphisms associated with BSE susceptibility between buffalo and cattle. In this regard, previous studies have revealed that Anatolian, Pakistani, Indonesian and Thai buffalo breeds display significant frequency differences in the two *PRNP* indel polymorphisms compared to cattle (Oztabak et al., 2009; Imran et al., 2012; Uchida et al., 2014). Additionally, our recent study has revealed several significant differences in the shadow prion protein gene (*SPRN*), a possible regulatory factor in prion diseases, between cattle and buffalo (Zhao et al., 2012). While it is known that China has a huge variety of buffalo genetic resources (Borghese and Mazzi, 2005), little research on the genetic susceptibility of Chinese buffalo to BSE has been reported.

In the present study, we analyzed the frequency of polymorphisms associated with BSE susceptibility in Chinese buffalo, and compared the frequency of these polymorphisms in cattle with BSE, healthy cattle and buffalo by pooling data from the literature.

2. Materials and methods

2.1. Animals and samples

Animal care and use of protocol for all individuals involved in the study were approved by the Ethics and Experimental Animal Committee of Kunming Institute of Zoology, Chinese Academy of Sciences. A total of 334 blood samples including 312 buffaloes (*B. bubalis*) covering 8 different breeds (Dehong, Guangnan, Guangxi, Guangdong, Chongqing, Guizhou, Fuan and Hainan) and 22 yaks (*Bos grunniens*) sampled from Yunnan, Sichuan, Gansu, Tibet and Qinghai provinces were used. Total genomic DNA was isolated according to a conventional proteinase K and phenol/chloroform extraction method.

2.2. Indel and octarepeat polymorphisms analysis

The *PRNP* gene regions containing 23- and 12-bp indel polymorphisms and octarepeat polymorphisms were analyzed in a total of 312 buffalo samples. These regions were amplified via polymerase chain reaction (PCR) and genotyped as previously described (Zhao et al., 2010). The genotype, allele and haplotype frequencies were estimated by direct gene counting, followed by comparative analysis within these polymorphisms by pooling data from the literature. With the exception of our observed animals (312 buffaloes), the remaining samples were obtained from the literature, with citations as follows: *healthy cattle*: Asian native cattle (Shimogiri et al., 2010); Brazil cattle (Galvão et al., 2012; Kerber et al., 2008; Kues et al., 2006); Chinese cattle (Qin et al., 2011; Zhao et al., 2010; Zhu et al., 2011); Croatia cattle (Premzl et al., 2000); European cattle (Haase et al., 2007; Juling et al., 2006; Kashkevich et al., 2007; Murdoch et al., 2010; Sander et al., 2004; Saunders et al., 2007; Schläpfer et al., 1999); Indonesian cattle (Uchida et al., 2014); Japanese cattle (Msalya et al., 2009; Nakamitsu et al., 2006); Korean cattle (Choi et al., 2012; Jeong et al., 2005, 2006; Kim et al., 2009); Pakistani cattle (Imran et al., 2012); Polish cattle (Czarnik et al., 2007, 2011; Gurgul et al., 2012a, 2012b; Walawski and Czarnik, 2003); Thai cattle (Uchida et al., 2014); Turkish cattle (Un et al., 2008); US cattle (Brunelle et al., 2008a, 2008b; Clawson et al., 2006; Seabury et al., 2004); Vietnamese cattle (Muramatsu et al., 2008; Uchida et al., 2014); *BSE case cattle*: BSE-classical German and UK, US imported cattle (Brunelle et al., 2007); European Holstein BSE cattle (Murdoch et al., 2010); German BSE cattle (Kashkevich et al., 2007; Sander et al., 2004); Japan BSE cattle (Muramatsu et al., 2008; Nakamitsu et al., 2006); Polish BSE cattle (Gurgul et al., 2012a, 2012b); Swiss and German BSE cattle (Haase et al., 2007); UK and German BSE holstein (Juling et al., 2006); UK BARB BSE case (Saunders et al., 2007); *Buffalo*: Anatolian buffalo (Oztabak et al., 2009); Indonesian buffalo (Uchida et al., 2014); Pakistani buffalo (Imran et al., 2012) and Thai buffalo (Uchida et al., 2014).

Differences in genotype and allele frequency distributions among Chinese buffalo breeds were examined, in addition to examining genotype, allele and haplotype frequency differences between BSE-infected cattle and the examined Chinese buffalo using the Chi-squared test in SPSS 11.5 (SPSS Inc.). Comparison of each allele or genotype frequency distribution was examined using Fisher's exact test in SPSS 11.5.

2.3. Coding region resequencing

The entire coding region of the *PRNP* gene was amplified and sequenced in 133 buffaloes (32 Dehong buffaloes, 26 Guangnan buffaloes, 10 Guangxi buffaloes, 27 Guangdong buffaloes, 14 Chongqing buffaloes, 10 Guizhou buffaloes, 11 Fuan buffaloes, and 3 Hainan buffaloes) and 22 Chinese yaks, as described in our previous study (Zhao et al., 2010). Resulting *PRNP* CDS sequences of 13 haplotypes were inserted into GenBank under accession numbers: KC137622 to KC137634 (buffalo) and KC137635 to KC137647 (yak).

2.4. The samples for coding sequence analysis

In this study, 3 *Bison bison*, 2 *Bison bonasus*, 6 *Bos frontalis*, 2 *Bos gaurus*, 5 *B. grunniens*, 125 *Bos indicus*, 4 *Bos javanicus*, 2 *Bos primigenius*, 848 *Bos taurus*, 2 *Boselaphus tragocamelus*, 5 *B. bubalis*, 2 *Bubalus depressicornis*, 2 *Syncerus caffer*, 2 *Syncerus caffer nanus*, 2 *Tragelaphus angasii*, 2 *Tragelaphus imberbis*, 1 *Tragelaphus oryx*, 1 *Tragelaphus spekii*, and 5 *Tragelaphus strepsiceros* were used for analysis. In addition, we re-sequenced 22 *B. grunniens* (this study), 133 *B. bubalis* (this study) and 50 *B. taurus* (Zhao et al., 2010) *PRNP* coding sequences. For coding sequence analysis, a buffalo *PRNP* coding sequence was searched against the BLASTN NR nucleotide database in NCBI with 100% coverage and >90% identity. After excluding short sequences and sequences containing unknown bases (N), a total of 1226 animals were used for analysis. Haplotypes were constructed using the PHASE program (Stephens et al., 2001; Stephens and Donnelly, 2003). The McDonald–Kreitman test was used to compare the coding sequence divergence between buffalo and other species (McDonald and Kreitman, 1991). A median-joining network for the inference of haplotype genealogy was constructed using NETWORK 4.5 (Bandelt et al., 1999).

3. Results

3.1. 23- and 12-bp indel polymorphisms

To investigate the distributions of 23- and 12-bp indel polymorphisms in Chinese buffalo, a total of 312 animals were genotyped, with allele, genotype and haplotype frequencies shown in Table 1. Extraordinarily low frequencies were found for the D_{23} allele (0.050) and the D_{23}/D_{23} genotype (0.026) in the 23-bp indel polymorphism site. The D_{12}/D_{12} genotype was undetected in the 12-bp indel polymorphism

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