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Detection of Bovine Spongiform Encephalopathy-Related Prion Protein Gene Promoter Polymorphisms in Local Turkish Cattle

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Abstract Polymorphisms in open reading frames of the prion protein gene (*PRNP*) have been shown to be associated with prion disease susceptibility in humans, sheep, and mice. Studies in recent years have demonstrated a similar effect of *PRNP* promoter and intron-1 polymorphisms on bovine spongiform encephalopathy (BSE) susceptibility in cattle. In this study, the deletion/insertion (indel) polymorphisms of the bovine *PRNP* gene within the promoter sequence (23 bp) and intron 1 (12 bp) were analyzed in local Turkish cattle. For this, 150 animals belonging to three different local breeds—the South Anatolian red, the East Anatolian red, and the Turkish gray—were tested using DNA purification and polymerase chain reaction. The ins allele in the 12 bp indel, which is associated with low susceptibility to BSE, showed a high frequency in all three breeds. The low-susceptibility allele of the 23-bp indel was identified in Turkish gray cattle with a frequency of 0.80. Results of the study have shown that local Turkish cattle might have an important genetic value for selection against BSE.

Keywords Local Turkish cattle · *PRNP* · Promoter · Indel polymorphism · Genotypes

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Introduction

Modifications in the prion protein cause Creutzfeldt-Jakob Disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, wasting disease in deer and elk, feline spongiform encephalopathy, and scrapie disease in sheep and goats (Prusiner 1998; Hopp et al. 2001). In humans and some mammalian species, polymorphisms within the *PRNP* gene have been shown to influence disease susceptibility and pathologies (Vaccari et al. 2001). For example, polymorphism in codon 129 is highly correlated with kuru and CJD in humans. All CJD patients have been shown to be homozygous for methionine in codon 129, and heterozygotes at the same codon appear most resistant to kuru (Mead et al. 2003; Peden et al. 2004).

Polymorphisms in codons 136, 154, and 171 of the *PRNP* gene in sheep are known to be highly related to the degree of susceptibility to scrapie (Vaccari et al. 2001; Tranulis 2002; Tongue et al. 2004). In cattle, many studies were conducted in order to find such a relationship between BSE and polymorphisms in the cattle genome (Goldmann et al. 1991; Neibergs et al. 1994; Hunter et al. 1996; Walawski and Czarnik 2003; Heaton et al. 2003). Studies on BSE-affected animals in Germany and the USA demonstrated the effect of *PRNP* promoter polymorphisms on BSE susceptibility in cattle (Sander et al. 2004; Seabury et al. 2004). Further studies revealed the effects of a 23-bp insertion-deletion (indel) polymorphism located 1.6 kbp upstream of exon 1, and a 12-bp indel within intron 1 on BSE susceptibility in cattle (Sander et al. 2005; Juling et al. 2006; Haase et al. 2007). Although it is clear that cattle with the −/−23 bp promoter genotype and the −/− 12 bp Intron 1 genotype have both been significantly associated with BSE, there is no consensus on which genotype is most related to BSE (Sander et al. 2004, 2005; Juling et al. 2006; Kashkevich et al. 2007). Moreover, indel polymorphisms that influence classical BSE susceptibility seem to be not applicable to other transmissible spongiform encephalopathies in cattle (Brunelle et al. 2007).

So far, the frequency of polymorphism in the *PRNP* gene promoter region has been determined in some cattle in Asia (Nakamitsu et al. 2006; Jeong et al. 2006), Europe (Sander et al. 2004; Juling et al. 2006; Kashkevich et al. 2007; Czarnik et al. 2007), and America (Seabury et al. 2004; Kerber et al. 2007). The number of local cattle breeds in Turkey has been decreasing because of their low productivity for milk and meat. On the other hand, their high resistance to diseases and parasites is valued in different platforms (Bakır and Kaygısız 2003; Kaymakçi and Koçak 2004). Nevertheless, little has been done to investigate the molecular genetic structure of the local Turkish cattle breeds. The aim of this study was to identify the deletion/insertion polymorphisms of the prion protein gene (*PRNP*) within the promoter sequence (23 bp) and intron 1 (12 bp) in Turkish South Anatolian red, East Anatolian red, and Turkish gray cattle.

Materials and Methods

For this study, DNA from 50 unrelated South Anatolian red, 50 East Anatolian red, and 50 Turkish gray cattle was analyzed using DNA isolation and PCR methods. In

selection of the animals, care was taken not to include animals that were parentally related so they were representing their own breed characteristics.

Animals

South Anatolian cattle are raised in South Anatolia, with some varieties being raised in Syria, Israel, and Egypt. The cattle are bred in extensive and poor feeding and housing conditions. East Anatolian red cattle are reared in the east of Turkey, bred at high altitudes and in extreme winter conditions. Like other local Turkish cattle breeds, they can tolerate climate changes, poor feeding, parasites, and epidemic diseases. Turkish gray cattle are raised in the west part of Turkey (in the Marmara, Trachea, and Ege regions). They can tolerate climate changes, poor feeding, and parasite infestation, and they are able to live and reproduce in wild conditions without human help (Bakır and Kaygısız 2003; Kaymakçi and Koçak 2004).

For this study, South Anatolian red cattle were supplied from the Southeast Anatolian region of Turkey, East Anatolian red cattle came from East Anatolia, and the Turkish gray cattle were from the Marmara region. Blood samples were taken in 2 ml sterilized tubes with EDTA. The genomic DNA extraction procedure from whole blood samples used the standard salt-out method (Miller et al. 1998). Care was taken to select unrelated animals from each breed.

PCR

Amplification reactions of polymorphic fragments of the *PRNP* gene promoter were carried out by the PCR method, using primers described in the literature (Juling et al. 2006). PCR was carried out in a final volume of 25 µl containing 1 U *Taq* DNA polymerase (Fermantas Life Sciences, Canada), 2.5 µl 10× PCR buffer (750 mM Tris–HCl, pH 8.0, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 1.5 mM $MgCl_2$, 50–100 ng genomic DNA, 100 µM dNTP (Fermantas Life Sciences, Canada), and 10 pmol each primer. The thermal cycling program was as follows: 95°C for 5 min; 32 cycles of 94°C for 45 s, 58°C for 45 s, 72°C for 45 s; and a final extension at 72°C for 7 min.

PCR product electrophoresis was conducted on a 3% agarose gel. Electrophoresis of the PCR product resulted in either a 191 bp (+) or 168 bp (−) fragment for indel 23. Similarly, the PCR product for indel 12 resulted in either a 215 bp (+) or a 203 bp (−) fragment.

Statistical Analysis

Direct counting was used to estimate genotype and allele frequencies of *PRNP* promoter variants. The chi-square test (χ^2) was used to check whether the populations were in Hardy–Weinberg equilibrium using PopGene32 software (Yeh et al. 2000). Haplotype frequencies were derived from the genotypic data using the program package Arlequin version 3 (Excoffier et al. 2005).

Results

Frequencies of the alleles and genotypes investigated in the three local Turkish cattle breeds are presented in Table 1.

Allele Frequency

In the 23-bp indel polymorphism of the *PRNP* promoter, the frequency of the del (−) allele was higher than the ins (+) allele in both Anatolian red breeds. In contrast, the frequency of the + allele was higher (0.62) and the frequency of the − allele lower (0.38) in Turkish gray cattle. Overall, the more frequent allele in the 23-bp indel polymorphism was the − allele in the South Anatolian cattle (frequency = 0.64), followed by the + allele in the Turkish gray cattle (0.62) and the − allele in the East Anatolian cattle (0.60). In the 12-bp indel polymorphism of the *PRNP* intron-1, the + allele was more frequent in all analyzed breeds (Turkish gray 0.80, East Anatolian 0.72, and South Anatolian cattle 0.69).

Genotype Frequency

After electrophoresis of the PCR products of the *PRNP* gene comprising the 23-bp indel within the promoter region, three genotypes were identified: homozygous for the insertion (+/+), heterozygous for the indel (+/−), and homozygous for the deletion (−/−). Similarly, three genotypes were identified in intron-1 (12 bp indel) of the *PRNP* gene: homozygous for the insertion (+/+), heterozygous for the indel (+/−), and homozygous for the deletion (−/−). In the 23-bp indel polymorphism of the *PRNP* promoter, the more frequent genotype was observed to be heterozygous (+/−) in breed animals, with a frequency of 0.60. The frequency of the homozygous genotype (+/+) was the same in both Anatolian red breeds (0.10). In the 12-bp indel polymorphism of the *PRNP* intron-1, the more frequent genotype was homozygous (+/+), with a frequency of 0.66 in the Turkish gray breed, whereas the lowest frequency (0.10) observed in the East Anatolian breed was for the homozygous (−/−) genotype.

Table 1 Frequency of *PRNP* promoter alleles and genotypes in local Turkish cattle

Locus	Breed	*n*	Allele		Genotype		
			+	−	+/+	+/−	−/−
Promoter	South Anatolian Red	50	0.36	0.64	0.10	0.52	0.38
23-bp indel	East Anatolian Red	50	0.40	0.60	0.10	0.60	0.30
	Gray	50	0.62	0.38	0.46	0.32	0.22
Intron 1	South Anatolian Red	50	0.69	0.31	0.52	0.34	0.14
12-bp indel	East Anatolian Red	50	0.72	0.28	0.54	0.36	0.10
	Gray	50	0.80	0.20	0.66	0.28	0.06

Table 2 Frequency of 23- and 12-bp indel *PRNP* haplotypes in local Turkish cattle

Breed	*n*	Haplotype		
		del23/del12	in12/del23	in23/in12
South Anatolian Red	50	0.245	0.460	0.295
East Anatolian Red	50	0.195	0.470	0.335
Gray	50	0.175	0.205	0.620

Haplotype Frequency

Haplotype frequencies investigated in the three local Turkish cattle breeds are presented in Table 2. Three different haplotypes of the promoter 23-bp indel polymorphism and the intron-1 indel polymorphisms (del23/del12, in12/del23, and in23/in12) were investigated in the three breeds. The haplotype in23/del12 was not observed in investigated animals. The most frequent haplotype was in23/in12, identified in the Turkish gray breed (frequency 0.62), followed by in12/del23 in the East (0.470) and South (0.460) Anatolian reds.

Discussion

The main purpose of animal husbandry is the production of milk and meat for human consumption. Thus, animals that produce more meat and milk are favored to be retained, whereas animals with low productivity are eliminated. This approach could cause loss of genes associated with low susceptibility to diseases or parasites and with adaptation to a given environmental condition. The numbers of local Turkish cattle breeds have decreased dramatically because they have not been retained for production. As a result, the South and East Anatolian red and Turkish gray cattle were reported to be endangered breeds (Ertuğrul et al. 2000). Molecular genetic studies (Loftus et al. 1999; Troy et al. 2001; Bruford et al. 2003) and archeological findings (Bruford and Towsend 2004) demonstrated that one of the domestication centers was the Near East, close to the eastern part of Anatolia. There is a strong possibility that local Turkish breeds represent the ancestors of domestic cattle in the world. Therefore, conservation and protection of these breeds against infectious diseases such as BSE may play a crucial role in the further use of these genetic resources.

Prion diseases such as scrapie in sheep, BSE in cattle, and CJD in humans are caused by modifications in the prion protein. Pathogens that infect multiple species can jump species boundaries and affect threatened species, as is the case with the prion diseases (McCallum and Dobson 1995; Daszak et al. 2000). There is no therapeutic treatment for BSE in cattle or for prion diseases in other mammals. Therefore, genetic selection is a unique method for eradicating BSE in the cattle population. Originally the 23-bp promoter polymorphism was found to be significantly associated with occurrence of BSE (Sander et al. 2004). Later, the 23-bp promoter indel was further considered to be most relevant to BSE (Sander

et al. 2005). Recent reports, however, indicate that statistically and biologically the 12-bp indel is more relevant to BSE status in cattle (Juling et al. 2006; Kashkevich et al. 2007). At this time, there is no consensus regarding which of the indel polymorphisms is more closely associated with the occurrence of BSE. Nevertheless, it is clear that both indel polymorphisms are strongly associated with BSE in cattle. Local Turkish cattle breeds presented 69% (South Anatolian red), 72% (East Anatolian red), and 80% (gray) of the 12-bp insertion allele. Compared with allele frequencies of other cattle breeds (Table 3), local Turkish cattle, together with the German Brown (Juling et al. 2006) and the Braunvieh (Kashkevich et al. 2007), present a high frequency of the low-susceptibility 12-bp allele. Several breeds with greater 12-bp indel allele frequencies are given by Seabury et al. (2004), including Brahman ($n = 4$, frequency $= 1$), Brown Swiss ($n = 4$, frequency $= 1$), and Nelore ($n = 8$, frequency $= 1$). The number of animals considered in this study is insufficient to provide reliable figures for these breeds.

Although BSE has so far not been identified in Turkey (Kahraman et al. 2007), care should be taken to avoid infection among local Turkish cattle. Compared with prion gene polymorphisms related to scrapie in sheep, there is a gap in the knowledge of prion gene polymorphisms related to BSE susceptibility. Further studies in different cattle breeds utilizing larger sample sizes will be useful in determining the genetic disposition of BSE.

Table 3 Comparison of *PRNP* promoter indel allele frequency among cattle breeds

Breed	Indel allele				Reference
	23 bp del	23 bp in	12 bp del	12 bp in	
German cattle	0.57	0.43	0.51	0.49	Sander et al. (2004)
German Holstein	0.62	0.38	0.53	0.47	Juling et al. (2006)
German Brown	0.35	0.65	0.14	0.86	Juling et al. (2006)
Fleckvieh	0.68	0.32	0.62	0.38	Juling et al. (2006)
Japanese Holstein	0.79	0.21	0.74	0.26	Nakamitsu et al. (2006)
Japanese Brown	0.59	0.41	0.57	0.43	Nakamitsu et al. (2006)
Korean Hanwoo	0.60	0.40	0.56	0.44	Jeong et al. (2006)
Holstein (Korean)	0.30	0.70	0.61	0.39	Jeong et al. (2006)
Polish Holstein-Friesian	0.63	0.37	0.54	0.46	Czarnik et al. (2007)
Aberdeen Angus	0.73	0.27	0.56	0.44	Kerber et al. (2007)
Charolais	0.68	0.32	0.58	0.42	Kerber et al. (2007)
Franqueiro	0.64	0.36	0.33	0.67	Kerber et al. (2007)
German Holstein	0.58	0.42	0.53	0.47	Kashkevich et al. (2007)
Braunvieh	0.40	0.60	0.16	0.84	Kashkevich et al. (2007)
Fleckvieh	0.71	0.29	0.64	0.36	Kashkevich et al. (2007)
Turkish Gray	0.38	0.62	0.20	0.80	Present study
South Anatolian Red	0.64	0.36	0.31	0.69	Present study
East Anatolian Red	0.60	0.40	0.28	0.72	Present study

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