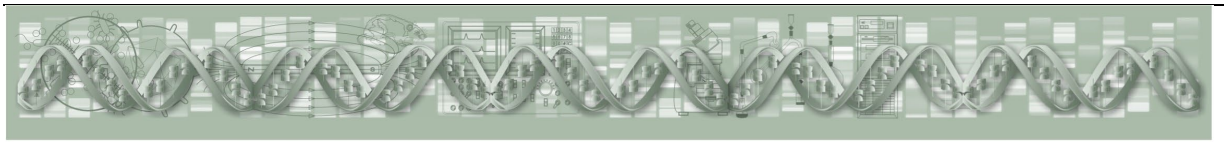

DISTRIBUTION OF THE PRION PROTEIN GENE 23-BP INDEL POLYMORPHISM IN JERSEY CATTLE IN TURKEY

Melih Sercan Ustaoglu[1*], Serbulent Yigit[2,3] , Muhammed Taha Kaya [4]

[1] Suluova Vocational High School, Amasya University, Amasya, Turkiye
[2] Department of Genetics, Faculty of Veterinary, Ondokuz Mayis University, Samsun, Turkey
[3] Department of Medical Biology, Ggraduate Institute, Ondokuz Mayis University, Samsun, Turkey
[4] Department of Pathology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkiye.
[*] Corresponding author e-mail: sercan.ustaoglu@amasya.edu.tr

Abstract

Bovine spongiform encephalopathy (BSE) is a prion disease that is always fatal in cattle and is considered an important risk factor for human health. Genetic polymorphisms that alter prion proteins may be associated with susceptibility or resistance to infectious spongiform encephalopathy. Therefore, we investigated the distribution of the 23 bp indel variant in the prion protein (*PRNP*) gene in Jersey cattle in Turkey. A total of 95 unrelated Jersey cattle (79 of reproductive age and 16 of non-reproductive age) from a private farm in Izmir were included in the study. Genomic DNA was obtained from the milk of reproductive-age cattle and the saliva of non-reproductive-age cattle. A 23-bp indel polymorphism in the *PRNP* gene promoter region was genotyped by polymerase chain reaction (PCR) analysis. The three genotypes of the PRNP 23-bp indel variant were classified as follows: I/I (223 bp), I/D (both 223 and 200 bp fragments), and D/D (200 bp).. The frequencies of the I/I, I/D, and D/D genotypes of the *PRNP* 23-bp indel variant in cattle were 22 (23.16%), 48 (50.53%), and 25 (26.32%). We then examined genotype and allele distribution according to service period. No significant difference was detected in terms of *PRNP* gene 23 bp-indel variant genotype and allele distribution in the groups created according to the service period ($p>0.05$**).** Although the *PRNP* gene 23 bp-indel variant genotype and allele distribution in jersey-type cattle in Turkey did not differ according to service period, our results may benefit the understanding of the genetic characteristics of the *PRNP* gene in cattle breeds.

Keywords: Bovine spongiform encephalopathy, prion protein, variant, PCR

Introduction

Prion diseases, also called *transmissible spongiform encephalopathies* (TSEs), are a group of neurodegenerative diseases that also affect both mammals and humans (Maghsood et al. 2011). This disease primarily targets the central nervous system (CNS) and is presented with a range of neuropathological symptoms. Common TSE pathologies consist of spongy changes, neuronal loss, glial cell activation, and, most importantly, the accumulation of amyloid aggregates (Murdoch et al. 2015). With the accumulation of this abnormal prion protein in tissues, Creutzfeldt-Jakob disease (CJD) and Gerstmann-Straussler-Scheinker syndrome in humans, bovine spongiform encephalopathy (BSE) in cattle, scrapie in small ruminants, and chronic wasting disease in deer occur (Vaccari et al. 2009). Prion diseases may arise through acquired transmission, in accordance with inherited genetic risk, or through sporadic origins. Acquired prion diseases in cattle are usually transmitted by oral exposure to infectious prions.

Humans can also be transmitted through contaminated human products or surgical instruments. This is known as iatrogenic contagion (Cali et al. 2015).

Prion protein (PRNP), a glycoprotein consisting of four alpha helical chains, belongs to the group of protective proteins, suggesting that it may play an important role in an organism. Being part of the surface membrane indicates that it is involved in signal transmission between cells (Rzewucka-Wójcik et al. 2013,). The bovine *PRNP* gene is localized at q17 on chromosome BTA13. The structure and organization of this gene have been determined (Czarnik et al. 2007). *PRNP* plays a role in infectious BSEs. Resistance to prion diseases in a wide variety of mammalian species is affected by polymorphisms in the *PRNP* gene (Ün et al. 2008). In their study of 7 German cattle breeds, Sander et al. showed that the insertion/deletion (I/D) polymorphism in the promoter region (23 base-pairs (bp) and 12 bp in intron 1 was different between clinically healthy and infected animals (Sander et al. 2004). These indels are thought to affect the binding sites of transcription factors and may affect the expression of this protein (Sander et all, 2005). A 23-bp I/D located in the PRNP promoter contains a binding site for the RP58 repressor protein (Yang et al. 2018).

The Jersey breed has higher efficiency in converting feed into milk. It costs less than other dairy cattle, as they reach a productive age between 2 and 10 months before other dairy cattle (Ahmad et al. 2007).

Therefore, in this study, we aimed to investigate the distribution of a 23-bp indel variant in the Prion protein (*PRNP*) gene in Jersey cattle in Turkey.

Materials and Methods

Study population

A total of 95 unrelated Jersey cattle from a private farm in Izmir, Turkey, were included in the study. Seventy-nine of the cattle were of reproductive age, and 16 of them were not. The cattle were fed on a farm under the same environmental conditions and with the same feed.

Genotyping

Genomic DNA was obtained from the milk of reproductive-age cattle and the saliva of non-reproductive age cows using a commercial kit. Qualitative and quantitative analysis of the isolated DNA was performed using the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). *The PRNP* 23-bp indel genotype distribution was determined by the polymerase chain reaction (PCR) method. Forward (5′- GTGCCAGCCATGTAAGTG-3′) and reverse (5′-CCTATTCTGGCTATTGTTGC-3') primers were used for amplification, with initial denaturation at 5 min at 95°C; 2 cycles of 94°C for 30 s, annealing from 68°C to 52° by 2°C decrease for 30 s, respectively; 72°C for 30 s; 30 cycles of 94°C for 30 s, 50° The 25 µL PCR amplification volume contained 50 ng of genomic DNA, 0.5 µmol/L of each primer, 1 × buffer (including 1.5 mmol/L MgCl2), 200 µmol/L dNTPs (dATP, dTTP, dCTP, and dGTP), and 0.625 units of Taq DNA polymerase (Thermo Fisher Scientific, USA). PCR products were identified by electrophoresis on a 2% agarose gel stained with ethidium bromide. To check the results, 10% of randomly selected samples were reevaluated, and a 100% match was found.

STRING analysis

In molecular biology, the STRING database, a biological database and web resource, describes functional interactions between proteins in a cell (https://string-db.org/).

Statistical analysis

The Statistical Package for Social Sciences (SPSS) software version 20.0 for Windows was used to analyze the data (SPSS Inc., Chicago, IL). The mean and standard deviation were used to show the continuous quantitative variables. The *PRNP* overall genotype distribution was compared with the chi-square (χ^2) test, and the allele and genotype distributions were compared with Fisher's exact test. The p-values smaller than 0.05 were considered statistically significant.

Results

In this study, the PRNP 23-bp indel polymorphism was investigated in 95 cattle populations. Baseline demographic features of the groups are shown in Table 1.

Table 1. Baseline demographic features of the cattle

	n: 95 (%)
Reproductive age	
Yes	79 (83.16)
No	16 (16.8)
Age (years)	
<1	6 (6.32)
1	24 (25.26)
2	7 (07.36)
3	58 (61.05)
Service period (days)	
<90	55 (57.89)
90-180	24 (25.26)
>180	16 (16.84)

The three genotypes of the PRNP 23-bp I/D variant were classified as follows: I/I (223 bp), I/D (both 223 and 200 bp fragments) and D/D(200bp). The frequencies of the I/I, I/D, and D/D genotypes of the *PRNP* 23-bp I/D variant in cattle were 22 (23.16%), 48 (50.53%), and 25 (26.32%). The allele distribution was I allele 92 (48.42%) and D allele 98 (51.58%). The data are presented in Table 2.

Table 2. Genotype distribution and allele frequencies of *PRNP* 23-bp indel variant

***PRNP* 23-bp indel**	**n:95 (%)**
Genotypes	
I/I	22 (23.16)
I/D	48 (50.53)
D/D	25 (26.32).
Alleles	
I	92 (48.42)
D	98 (51.58)

We then examined genotype and allele distribution according to service period. Results are shown in Table 3. No significant difference was detected in terms of *PRNP* gene 23 bp-indel variant genotype and allele distribution in the groups created according to the service period (*p>0.05*).

Table 3. Genotype and allele distribution *PRNP* gene 23bp-indel variant according to service period

	Service period			
***PRNP*-23bp-indel**	**<90 n=55 (%)**	**90-180 day n=24 (%)**	**≥181 n=16 (%)**	**P**
Genotypes				
I/I	11 (20.00)	5 (20.83)	6 (37.50)	>0.05
I/D	30 (54.55)	12 (50.00)	6 (37.50)	
D/D	14 (25.45)	7 (29.17)	4 (25.00)	

Alleles				
I	52 (47.27)	22 (45.83)	18 (56.25)	>0.05
D	58 (52.73)	26 (54.17)	14 (43.75)	

STRING analysis

Analyzing the PRNP protein with the STRING database, we predicted the functional partners of the protein with high confidence and found them as follows: Tubulin alpha-1D (TUBA1), Tubulin beta-1 chain (TUBB1), Tubulin beta-3 chain (TUBB3), Tubulin beta-6 chain (TUBB6), Tubulin beta-5 chain (TUBB), Tubulin beta-4B chain (TUBB4B), Tubulin beta-2B chain (TUBB2B), Tubulin beta-2a chain isoform x1 (TUBB2A), Tubulin beta-4B chain (TUBB4B), and Tubulin beta-4A chain (TUBB4A).

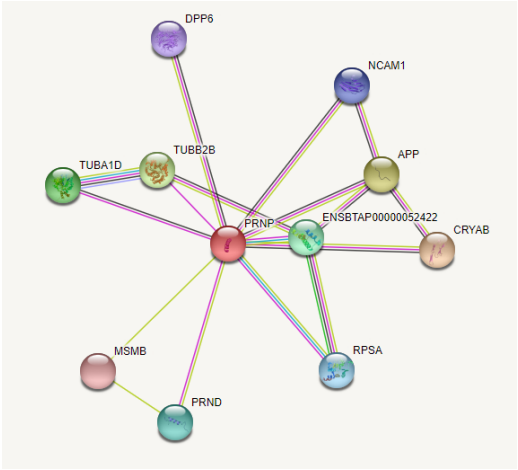

Figure 1. Interactions of Bos taurus PRNP protein, according to STRING database

Discussion

Frequent outbreaks of Prion disease are always fatal and incurable. Therefore, it is a major concern for animal and human health. Classical human prion diseases initially present with memory loss, behavioral changes, and communication problems. Subsequently, the imbalance and ataxia are accompanied by rapidly progressive dementia. Similarly, cattle show progressive neurological and behavioral changes (increased irritability, aggression, and anxiety), altered gait or movement (tremors, weakness, and hind leg ataxia), and weight loss (Murdoch, 2015). Factors controlling interspecific and intraspecific prion transmission are partially understood. Therefore, over the past few decades, there has been much interest and effort in research into understanding prion diseases, their etiology, contagion, and causes.

Prion diseases such as BSE in cattle, scrapie in sheep, and CJD in humans are caused by changes in the PRNP protein. The PRNP protein is a polypeptide that differs slightly between species. Pathogens infecting more than one species can cross species boundaries and affect threatened species, as with prion diseases (Ün et al. 2008). There is no appropriate treatment for BSE in cattle or prion diseases in other mammals. Therefore, it seems appropriate to use genetic selection to eliminate BSE in the cattle population.

The *PRNP* gene consists of three exons and two introns. In exon 3, which is the longest exon, and has an open reading frame (ORF) of 795 bp (Strychalski et al. 2011), one study showed that Japanese black cattle carrying the homozygous del genotype had higher mRNA levels in the medulla oblongata (Msalya et al. 2011). I/D allele frequencies were found to be different in studies conducted with different cattle breeds. The 23del-12del haplotype has been predicted to be associated with an increased risk of BSE. This haplotype is more common in healthy

Holstein-Friesian cattle (Brunelle et al. 2008). The Polish study found a significant association between *PRNP* indel polymorphisms (23 and 12 bp indels) and the susceptibility of Polish Holstein-Friesian cattle to classical BSE. Del variants of both polymorphisms were associated with increased susceptibility, whereas ins variants were found to be protective against BSE (Gurgul et al. 2012). Zhu et al. studied the polymorphism frequencies of two indels (23-bp and 12-bp) in four main cattle breeds (Hereford, Simmental, Black Angus, and Mongolian) from Northern China (Zhu et al. 2011). The del genotype and allele frequency of 23 and 12-bp indels were lower in Mongolian cattle, whereas the ins genotype and allele frequencies were higher than in the other three cattle breeds. In Mongolian cattle, 23-bp ins / 12-bp ins were the main haplotypes, while 23-bp del / 12-bp del were the main haplotypes in Hereford, Simmental, and Black Angus cattle. These results indicated that Mongolian cattle may be more resistant to BSE than the other three cattle breeds due to their relatively low del genotypes and allelic frequencies of 23- and 12-bp indel polymorphisms. The del allele was more common in German cattle, German Holstein, Fleckvieh, Japanese Holstein, and Korean Hanwoo breeds (Ün et al. 2008, Juling et al. 2006, Jeong et al. 2006). Un et al. examined three local cattle breeds of Turkey (South Anatolian Red, East Anatolian Red, and Turkish Grey), del allele frequency was higher in both Anatolian Red breeds, while the frequency of the ins allele (0.62) was higher than the del allele (0.38) in Turkish Gray cattle (Ün et al. 2008). The allele of 23 bp was higher in German Brown, Holstein (Korean), and Braunvieh cattle (Juling et al. 2006, Jeong et al. 2006, Kashkevich et al. 2006). In a study conducted in different races in Turkey, the highest del/del genotype frequency in the promoter region of PRNP was found in East Anatolian Red and Southern Anatolian Red, followed by Turkish Grey. But Anatolian Black and Zavot breeds showed low frequencies.

In the present study, we studied the *PRNP* gene's 23-bp indel variant in Jersey cattle in Turkey. When we examined the *PRNP* 23-bp indel genotype distribution in 95 Jersey cattle, it was the most common I/D genotype (50.53%). Then, we examined the genotype distribution according to the service period. Although the relationship between *PRNP* genotypes and milk yield is not clear, breeding TSE-resistant breeds will not result in a reduction in economically important reproductive traits. There was no significant difference between genotype distribution and service period ($p>0.05$).

Conclusions

Genetic polymorphisms can predict some diseases' susceptibility. We analyzed *PRNP* 23-bp indel variant genotype distribution and allele frequency in Jersey cattle breeds in Turkey. However, species-specific differences must be taken into account when analyzing such data. To further evaluate the association between BSE and this variant, larger sample sizes and studies of different breeds are required.

Author Contributions: All authors have contributed equally to this work.

References

Ahmad B, Khan S, Manan A. 2007. Production and Reproduction Performance of Jersey Cattle at Cattle Breeding and Dairy Farm Harichand Charsadda NWFP. Journal of Agrıcultural and Biological Science, 2(1).

Brunelle BW, Kehrli ME Jr, Stabel JR, Spurlock DM, Hansen LB, Nicholson EM. 2008. Short communication: Allele, genotype, and haplotype data for bovine spongiform encephalopathy-resistance polymorphisms from healthy US Holstein cattle. J Dairy Sci. 91(1): 338-342.

Cali I, Miller CJ, Parisi JE, Geschwind MD, Gambetti P, Schonberger LB. 2015. Distinct pathological phenotypes of Creutzfeldt-Jakob disease in recipients of prion-contaminated growth hormone. Acta Neuropathol Commun. 3(1): 37.

Czarnik U, Zabolewicz T, Strychalski J, Grzybowski G, Bogusz M, Walawski K. 2007. Deletion/insertion polymorphism of the prion protein gene (PRNP) in Polish Holstein-Friesian cattle. J Appl Genet. 48(1): 69-71.
Gurgul A, Czarnik U, Larska M, Polak MP, Strychalski J, Słota E. 2012. Polymorphism of the prion protein gene (PRNP) in Polish cattle affected by classical bovine spongiform encephalopathy. Mol Biol Rep. 39(5): 5211-5217.
Jeong BH, Lee YJ, Kim NH, Carp RI, Kim YS. 2006. Genotype distribution of the prion protein gene (PRNP) promoter polymorhisms in Korean cattle. Genome, 49:1539-1544.
Juling K, Schwarzenbacher H, Williams JL, Fries R. 2006. A major genetic component of BSE susceptibility. BMC Biol. 4: 33.
Kashkevich K Humeny A, Ziegler U, Groschup MH, Nicken P, Leeb T, Fischer C, Becker CM, Schiebel K. 2007. Functional relevance of DNA polymorphisms within the promoter region of the prion protein gene and their association to BSE infection. FASEB J. 21(7): 1547-1555.
Maghsoodi MS, Miraei-Ashtiani SR, Banabazi MH, Yeganeh HM. 2011. Polymorphism Of Prion Protein Gene (Prnp) In Iranian Holstein And Two Local Cattle Populations (Golpayegani And Sistani) Of Iran. Iranıan Journal of Biotechnology, 9(2): 115.
Msalya G, Shimogiri T, Ohno S, Okamoto S, Kawabe K, Minezawa M, Maeda Y. 2011. Evaluation of PRNP expression based on genotypes and alleles of two indel loci in the medulla oblongata of Japanese Black and Japanese Brown cattle. PLoS One, 6(5): e18787.
Murdoch BM, Murdoch GK. 2015.Genetics of Prion Disease in Cattle. Bioinform Biol Insights, 9(Suppl 4): 1-10.
Nakamitsu S, Miyazava T, Horuichi M, Onoe S, Ohoba Y, Kitagawa H, Ishiguro N. 2006. Sequence variation of bovine prion protein gene in Japanese cattle (Holstein and Japanese Black). J Vet Med Sci. 68(1): 27-33.
Rzewucka-Wójcik E, Frost A, Jedrzejczak M, Zaborski D, Pilarczyk P. 2013. The PRNP ins/del and octapeptide repeat polymorphisms in Jersey cattle and their associations. Journal of Applied Animal Research, 41(2): 244-248.
Şahin İ, Bulut Z, Kurar E, Özşensoy Y, Doğan M, Nizamlıoğlu M. 2017. Investigation of DGAT1 and PRNP gene polymorphism. Eurasian J Vet Sci. 33(1): 20-25.
Sander P, Hamann H, Drögemüller C, Kashkevich K, Schiebel K, Leeb T. 2005. Bovine prion protein gene (PRNP) promoter polymorphisms modulate PRNP expression and may be responsible for differences in bovine spongiform encephalopathy susceptibility. J Biol Chem. 280(45): 37408-14.
Sander P, Hamann H, Pfeiffer I, Wemheuer W, Brenig B, Groschup MH, Ziegler U, Distl O, Leeb T. 2004. Analysis of sequence variability of the bovine prion protein gene (PRNP) in German cattle breeds. Neurogenetics, 5(1): 19-25.
Strychalski J, Czarnik U, Pierzchala M, Pareek CS. 2011, Relationship between the insertion/deletion polymorphism within the promoter and the intron 1 sequence of the PrnP gene and milk performance traits in cattle. Czech J Anim. Sci. 56 (4): 151-156.
Ün C, Oztabak K, Özdemir N, Tesfaye D, Mengi A, Schellander K. 2008. Detection of bovine spongiform encephalopathyrelated prion protein gene promoter polymorphisms in local Turkish cattle. Biochem Genet. 46: 820-827.
Vaccari G, Panagiotidis CH, Acin C, Peletto S, Barillet F, Acutis P, Bossers A, Langeveld J, van Keulen L, Sklaviadis T, Badiola JJ, Andreéoletti O, Groschup MH, Agrimi U, Foster J, Goldmann. 2009. State-of-the-art review of goat TSE in the European Union, with special emphasis on PRNP genetics and epidemiology Vet Res. 40(5): 48.
Yang Q, Zhang S, Liu L, Lei C, Qi X, Lin F, et al. 2018. The evaluation of 23-bp and 12-bp insertion/deletion within the PRNP gene and their effects on growth traits in healthy Chinese native cattle breed. Journal of Applied Animal Research, 46(1): 505-511.s

Zhu XY, Feng FY, Xue SY, Hou T, Liu HR. 2011. Bovine spongiform encephalopathy associated insertion/deletion polymorphisms of the prion protein gene in the four beef cattle breeds from North China. Genome, 54(10): 805-11.