Abnormal Segregation of Alleles and Haplotypes at the Polymorphic Site of the PRNP Gene Within Promoter and Intron 1 Regions in Polish Holstein–Friesian Cattle

Janusz Strychalski · Urszula Czarnik · Tadeusz Zabolewicz

Received: 19 May 2011 / Accepted: 9 December 2011 / Published online: 19 January 2012 © The Author(s) 2012. This article is published with open access at Springerlink.com

Abstract Allele and haplotype segregation at the polymorphic sites within the promoter (23indel) and intron 1 (12indel) regions of the PRNP gene was analyzed in Polish Holstein–Friesian cattle. More 23del/del homozygotes and fewer 23ins/ins homozygotes than expected were observed in the offspring of ♀ 23ins/del × ♂ 23ins/del parents. In the offspring of ♀ 23ins/del × ♀ 23del/del/ del × ♀ 23ins/del parents, a trend toward more 23del/del animals and fewer 23ins/ del animals than expected was noted. At the 12indel polymorphic site, the only trend found was one toward fewer 12ins/ins genotypes and more 12ins/del and 12del/del genotypes than expected in the offspring of ♀ 12ins/del × ♀ 12ins/del parents. An analysis of haplotype segregation revealed more 23del-12del/23del-12del diplotypes and fewer 23ins-12ins/23ins-12ins diplotypes at the significance threshold than expected in the offspring of ♀ 23ins-12ins/23del-12del × ♀ 23ins-12ins/23del-12del parents.

Keywords Allele and haplotype segregation · Indel polymorphisms · Holstein–Friesian cattle · PRNP gene

Introduction

Conformational transformation of the prion protein (PrP) has been found to contribute to transmissible spongiform encephalopathy (TSE) pathogenesis (Prusiner 1998). In mammals the cellular prion protein (PrPc) is found in various cell types, but its biological function remains largely unknown. High protein expression levels are observed in neurons and on the surface of immune system cells, mainly monocytes,
CD8\(^+\) T lymphocytes, and NK cells (Dürig et al. 2000). PrP\(^e\) is required for the maintenance of myelin sheaths around peripheral nerves (Bremer et al. 2010), and it acts as the anti-apoptotic signaling molecule (Premzl et al. 2005). It is believed that PrP\(^e\) may participate in cellular signal transduction to affect the proliferation of T lymphocytes (Hugel et al. 2004; Ballerini et al. 2006).

The prion protein is coded by the \(PRNP\) gene, which is localized within the BTA13 chromosome at position q17 in cattle (Schläpfer et al. 2000). The structure and organization of the bovine \(PRNP\) gene have been well documented (Hills et al. 2001), and numerous mutations have been reported in the gene (Sander et al. 2004; Clawson et al. 2006). Insertion/deletion (indel) polymorphism covering sequence 23 bp (23indel) within the promoter region and sequence 12 bp (12indel) within the intron 1 region of the \(PRNP\) gene may be responsible for the incubation time of and/or the susceptibility to bovine spongiform encephalopathy (BSE) in Holstein–Friesian cattle in Germany (Sander et al. 2004) and Great Britain (Juling et al. 2006). Haplotype 23del-12del has been more frequently observed in the group of cattle affected by BSE. Research has demonstrated that this haplotype also contributes to higher \(PRNP\) expression levels (Sander et al. 2005; Msalya et al. 2010).

Investigations of the genetic structure conditioned by the indel polymorphism within the 23 bp promoter sequence and the 12 bp intron 1 sequence suggest a higher number of del/del homozygous individuals than ins/ins homozygotes in Holstein–Friesian cattle populations (Juling et al. 2006; Nakamitsu et al. 2006; Haase et al. 2007; Brunelle et al. 2008; Czarnik et al. 2011). Within the promoter sequence (23del), the share of del/del animals in the population ranges from 31% in the United States to 62.4% in Japan, whereas the share of ins/ins homozygotes ranges from only 5% in Japan to 18% in the United States. Smaller disproportions are noted in the frequency of opposing homozygotes at the polymorphic site within the intron 1 region (12indel) than in the promoter sequence (23indel). Del/del animals constitute 28.1–37.0% of the population, whereas the frequency of ins/ins homozygotes is reported as 7.2–23.0% (Nakamitsu et al. 2006; Brunelle et al. 2008). The genotype frequency of Polish Holstein–Friesian cattle is characteristic of the breed. At the polymorphic site within the promoter region, del/del animals account for 37% of the population and ins/ins individuals for 12.6%, whereas at the polymorphic site within the intron 1 region, del/del animals make up 25.9% and ins/ins homozygotes 20.6% of the population (Czarnik et al. 2011). This may indicate the presence of unknown factors that inhibit the distribution of the insertion allele, which determines a lower level of \(PRNP\) gene expression in animals with the 23ins-12ins haplotype. Our study set out to verify statistically the segregation of alleles and haplotypes passed down by the parents.

**Materials and Methods**

Allele and haplotype segregation was analyzed in full families comprising 510 heifer and bull calves, aged 1–3 months, that were the offspring of 510 dams with varying 23indel and 12indel genotypes and eight sires. Four of the sires had the
23ins-12ins/23del-12del diplotype (sire 1, 101 individuals; sire 2, 77; sire 3, 42; sire 4, 49), one sire had the 23del-12del/23del-12del diplotype (sire 5, 63 individuals), one sire had the 23ins-12ins/23ins-12ins diplotype (sire 6, 93 individuals), and two sires had the 23del-12ins/23del-12del diplotype (sire 7, 59 individuals; sire 8, 26).

Sires were randomly selected, subject to the availability of a sufficient number of cows and their offspring in large and fully documented herds of Polish Holstein–Friesian cattle. The analyzed sires were the first- or second-generation offspring of the most valuable Holstein–Friesian sires. The randomness of the selection was ensured by qualifying all animals that produced offspring of both sexes from the eight selected sires.

Amplification reactions within polymorphic sites that contained a 23 bp indel in the promoter and a 12 bp indel in intron 1 were carried out by the PCR method, as described by Czarnik et al. (2011). The conformity between the observed and anticipated numbers of offspring produced by different genetic variants (23indel and 12indel) of the mated parent pairs was verified by the chi-square test.

### Results

In the studied group of 510 cows, the heterozygous ins/del genotype was the most frequently observed genotype within the polymorphic sites of the promoter (23 indel) and intron 1 (12 indel) regions (0.500 and 0.545, respectively). Average frequencies were reported for del/del homozygotes (23del/del, 0.386; 12del/del, 0.260), and ins/ins homozygotes had the lowest frequencies (23ins/ins, 0.114; 12ins/ins, 0.194). Allele frequencies were as follows: 23ins, 0.364; 23del, 0.636; 12ins, 0.447; 12del, 0.533 (Table 1).

Allele segregation was analyzed separately at the polymorphic sites within the promoter (23indel; Table 2) and intron 1 (12indel; Table 3) regions. The offspring produced by all possible variants of the mated parent pairs were taken into account.

At the polymorphic site of the promoter region (23indel), significant differences were noted between the observed and expected sizes of genotype groups in the offspring \((n = 134)\) of \(\varrho 23\text{ins/del} \times \varphi 23\text{ins/del}\) parents \((\chi^2 = 5.99, p = 0.0500;\) Table 2). Compared with the expected genotype ratio (25:50:25), a higher proportion of 23del/del homozygotes (33%) and a lower proportion of 23ins/ins homozygotes (18%) were observed. Conformity between the observed and expected numbers of heterozygous animals was preserved.

In the offspring of \(\varrho 23\text{ins/del} \times \varphi 23\text{del/del}\) parents \((n = 106)\) and \(\varphi 23\text{del/del} \times \varrho 23\text{ins/del}\) parents \((n = 77)\), a trend toward more 23del/del animals and

### Table 1  Genotype and allele frequencies of the PRNP gene in Polish Holstein–Friesian dams

| Region              | Number of animals | Genotype frequency | Allele frequency |
|---------------------|-------------------|--------------------|------------------|
|                     |                   | ins/ins ins/del del/del | ins del |
| Promoter indel (23 bp) | 510               | 0.114 0.500 0.386 | 0.364 0.636 |
| Intron 1 indel (12 bp) | 510               | 0.194 0.545 0.260 | 0.447 0.533 |
Table 2  Allele segregation at the polymorphic site within the promoter (23indel) region

| Parental mating variant | Number of offspring | Progeny genotype | Number of animals in progeny genotype group | $\chi^2$ |
|-------------------------|--------------------|-----------------|------------------------------------------|---------|
|                         |                    | 23ins/ins       | Observed 10                              | 0.00    |
|                          |                    | 23ins/ins       | Expected 10                              |         |
| $\delta$ 23ins/ins × ♀ 23ins/ins | 10                 | 23ins/ins      | Observed 23ins/ins 10                    |         |
| $\delta$ 23ins/ins × ♀ 23ins/del | 44                 | 23ins/del      | Expected 23ins/ins 22                    | 1.46    |
| $\delta$ 23ins/ins × ♀ 23del/del | 39                 | 23ins/del      | Observed 23ins/del 39                    | 0.00    |
| $\delta$ 23ins/del × ♀ 23ins/ins | 29                 | 23ins/ins      | Expected 23ins/ins 14.50                 | 0.83    |
| $\delta$ 23ins/del × ♀ 23ins/del | 134                | 23ins/ins      | Observed 23ins/ins 24                    | 5.99*   |
| $\delta$ 23ins/del × ♀ 23del/del | 106                | 23ins/del      | Expected 23ins/del 53.00                 | 3.06    |
| $\delta$ 23del/del × ♀ 23ins/ins | 19                 | 23ins/del      | Observed 23ins/del 19                    | 0.00    |
| $\delta$ 23del/del × ♀ 23ins/del | 77                 | 23ins/del      | Expected 23ins/del 38.50                 | 3.76    |
| $\delta$ 23del/del × ♀ 23del/del | 52                 | 23del/del      | Observed 23del/del 52                    | 0.00    |

* Statistically significant at $p \leq 0.05$

Table 3  Allele segregation at the polymorphic site within the intron 1 (12indel) region

| Parental mating variant | Number of offspring | Progeny genotype | Number of animals in progeny genotype group | $\chi^2$ |
|-------------------------|--------------------|-----------------|------------------------------------------|---------|
|                         |                    | 12ins/ins       | Observed 12ins/ins 14                    | 0.00    |
|                         |                    | 12ins/ins       | Expected 12ins/ins 14                    |         |
| $\delta$ 12ins/ins × ♀ 12ins/ins | 14                 | 12ins/ins      | Observed 12ins/ins 22                    | 1.24    |
| $\delta$ 12ins/ins × ♀ 12ins/del | 52                 | 12ins/del      | Expected 12ins/ins 30                    |         |
| $\delta$ 12ins/ins × ♀ 12del/del | 27                 | 12ins/del      | Observed 12ins/del 27                    | 0.00    |
| $\delta$ 12ins/del × ♀ 12ins/ins | 72                 | 12ins/ins      | Expected 12ins/ins 35                    | 0.06    |
| $\delta$ 12ins/del × ♀ 12ins/del | 194                | 12ins/ins      | Observed 12ins/ins 35                    | 5.04    |
| $\delta$ 12ins/del × ♀ 12del/del | 88                 | 12del/del      | Expected 12del/del 41                    | 0.40    |
| $\delta$ 12del/del × ♀ 12ins/ins | 13                 | 12ins/del      | Observed 12ins/del 13                    | 0.00    |
| $\delta$ 12del/del × ♀ 12ins/del | 32                 | 12ins/del      | Expected 12ins/del 15                    |         |
| $\delta$ 12del/del × ♀ 12del/del | 18                 | 12del/del      | Observed 12del/del 18                    | 0.00    |
fewer 23ins/del animals than expected was noted ($\chi^2 = 3.06$, $p = 0.0802$, and $\chi^2 = 3.76$, $p = 0.0525$, respectively). In the offspring of 23ins/ins × ♀ 23ins/del parents ($n = 44$) and ♀ 23ins/del × ♀ 23ins/ins parents ($n = 29$), more 23ins/ins homozygotes and fewer 23ins/del heterozygotes than expected were observed, but the differences were not statistically significant. Results of low informative value were obtained with respect to the offspring of ♀ 23ins/ins × ♀ 23ins/ins and 23del/del parents, as well as ♀ 23del/del × ♀ 23ins/ins and 23del/del parents.

At the polymorphic site within the intron 1 (12 bp) region, the genotype distribution in the offspring was consistent with the theoretical model in all mating variants (Table 3). A clear trend toward variation between observed and expected values ($\chi^2 = 5.04$, $p = 0.0805$) was reported only in the offspring of ♀ 12ins/del × ♀ 12ins/del parents ($n = 194$). In that group, the share of 12ins/ins animals was smaller (18%) and the shares of 12ins/del (54%) and 12del/del (28%) animals were higher than expected (25:50:25).

In view of the results of allele segregation at the polymorphic sites of the promoter (23 bp) and intron 1 (12 bp) regions, the analysis of haplotype segregation was limited to the most informative mating variants to guarantee the heterogeneity of sires or cows at both polymorphic sites in each variant. The linkage phase between the examined indels in sires was determined by analyzing the number of offspring produced by heterozygous sires and cows with alternative homozygous genotypes at both polymorphic sites of the PRNP gene (Table 4). The group of 62 offspring of ♀ 23ins-12ins/23del-12del × ♀ 23del-12del/23del-12del parents consisted solely of individuals with the diplotypes 23ins-12ins/23del-12del ($n = 27$) and 23del-12del/23del-12del ($n = 35$). The presence of two diplotypes (23ins-12ins/23ins-12ins, $n = 12$; and 23ins-12ins/23del-12del, $n = 17$) was also observed in the 29 offspring of ♀ 23ins-12ins/23del-12del × ♀ 23ins-12ins/23ins-12ins parents. These results point to cis linkage between the examined indels in heterozygous sires.

An analysis of haplotype segregation (Table 4) revealed differences, at the significance threshold ($\chi^2 = 5.82$, $p = 0.0544$), between the observed and expected sizes of genotype groups in the offspring of heterozygous parents (♀ 23ins-12ins/23del-12del × ♀ 23ins-12ins/23del-12del; $n = 102$). The share of 23del-12del/23del-12del homozygotes was higher (34%) and the share of 23ins-12ins/23ins-12ins homozygotes was lower (18%) than expected (25% in both cases). Conformity between the observed (48%) and expected (50%) numbers of heterozygous animals was preserved. The offspring of the remaining mating variants were characterized by a trend toward a smaller share of 23ins-12ins and 23ins-12del haplotypes and a higher share of 23del-12del and 23del-12ins haplotypes.

In the offspring of homozygous sires (23ins-12ins/23ins-12ins and 23del-12del/23del-12del) and two sires with a homozygous genotype at the polymorphic site of the promoter (23 bp) region and a heterozygous genotype at the polymorphic site of the intron 1 (12 bp) region (23del-12ins/23del-12del), randomly mated with heterozygous cows (23ins-12ins/23del-12del), genotype distribution was consistent with the theoretical model (Table 5). The results obtained could not be fully verified, however, owing to a small number of animals.
This experiment was the first documented attempt to analyze allele and haplotype segregation at the polymorphic sites within the promoter (23indel) and intron 1 (12indel) regions of the PRNP gene. Calves aged 1–3 months were studied. The numbers of stillbirths and miscarriages were not analyzed, because relevant data were absent. Compared with the expected values, more 23del/del homozygotes and fewer 23ins/ins homozygotes were observed in the offspring of #23ins/del# parents, whereas the number of heterozygotes was consistent with our expectations. A distinctive trend toward a higher number of 23del/del homozygotes and a lower number of heterozygotes than expected in the offspring of #23del/del# parents and #23del/del# parents was also recorded. At the polymorphic site within the intron 1 region (12 bp), a trend toward fewer 12ins/ins animals and more 12del/del and 12ins/del individuals than expected was observed only in the offspring of #12ins/del# parents and #12del/del# parents. The results of segregation at the polymorphic sites within the promoter (23 bp) and intron 1 (12 bp) regions were translated to the results of haplotype segregation, where a threshold increase in the number of 23del-12del/23del-12del animals and a threshold decrease in the number of 23ins-12ins/23ins-12ins individuals was observed in the offspring of double-heterozygous parents (#23ins-12ins/23del-12del# parents).

| Parental mating variant | Number of offspring | Progeny genotype | Number of animals in progeny genotype group | χ² |
|-------------------------|---------------------|-----------------|------------------------------------------|----|
| Genotype of sire #a     | Genotype of dam     | 23ins-12ins/23del-12del | 62 | 23ins-12ins/23del-12del | 27 | 31.00 | 1.04 |
|                        |                     |                  |                                           | 23del-12del/23del-12del | 35 | 31.00 | |
|                        |                     | 23ins-12ins/23ins-12ins | 29 | 23ins-12ins/23ins-12ins | 12 | 14.50 | |
|                        |                     |                  |                                           | 23ins-12ins/23del-12del | 17 | 14.50 | 0.86 |
|                        |                     | 23ins-12ins/23del-12del | 102 | 23ins-12ins/23ins-12ins | 18 | 25.50 | 5.82* |
|                        |                     |                  |                                           | 23ins-12ins/23del-12del | 49 | 51.00 | |
|                        |                     |                  |                                           | 23del-12del/23del-12del | 35 | 25.50 | |

* Statistically significant at p ≤ 0.05

a Artificial insemination

Discussion

This experiment was the first documented attempt to analyze allele and haplotype segregation at the polymorphic sites within the promoter (23indel) and intron 1 (12indel) regions of the PRNP gene. Calves aged 1–3 months were studied. The numbers of stillbirths and miscarriages were not analyzed, because relevant data were absent. Compared with the expected values, more 23del/del homozygotes and fewer 23ins/ins homozygotes were observed in the offspring of #23ins/del# parents, whereas the number of heterozygotes was consistent with our expectations. A distinctive trend toward a higher number of 23del/del homozygotes and a lower number of heterozygotes than expected in the offspring of #23del/del# parents and #23del/del# parents was also recorded. At the polymorphic site within the intron 1 region (12 bp), a trend toward fewer 12ins/ins animals and more 12del/del and 12ins/del individuals than expected was observed only in the offspring of #12ins/del# parents and #12del/del# parents. The results of segregation at the polymorphic sites within the promoter (23 bp) and intron 1 (12 bp) regions were translated to the results of haplotype segregation, where a threshold increase in the number of 23del-12del/23del-12del animals and a threshold decrease in the number of 23ins-12ins/23ins-12ins individuals was observed in the offspring of double-heterozygous parents (#23ins-12ins/23del-12del# parents).
The analyzed polymorphic sites (23indel and 12indel) of the PRNP gene contain sequences that play a key role in gene expression. It has been demonstrated that the 23del-12del haplotype causes a stronger activation of the promoter than does the 23ins-12ins haplotype, which has the potential to reduce host prion protein expression levels (Sander et al. 2005); thus there is a biological basis for resistance against BSE infections in animals that are homozygous with respect to insertion (Juling et al. 2006; Brunelle et al. 2008). In comparison with 23ins/ins cows, 23del/del cows have been shown to have fewer T lymphocytes with CD2, CD8, and WC1-N2 phenotypes and a higher ratio of T CD4/CD8 lymphocytes, suggesting that the above genotype could lower immune system effectiveness (Kaczmarczyk et al. 2010).

The results obtained in this study are difficult to interpret, since allele and haplotype segregation at the polymorphic site within the promoter (23 bp) and intron 1 (12 bp) regions of the PRNP gene has not been previously analyzed. The only point of reference is the experiment conducted by Walawski and Czarnik (2003), which analyzed the segregation of PRNP alleles in view of the polymorphism determined by a 24 bp insertion/deletion within the open reading frame of exon 3. The investigation was conducted on full-family animals obtained as 10 PRNP 6/6 and 15 PRNP 6/5 AI sire families with 355 cows of various PRNP genotypes and their progenies, in most cases heifers at 1–3 months of age. Based on the research concept presented, the most valuable and fully informative results were
obtained for the ♂ PRNP 6/5 × ♀ PRNP 6/5 parental mating variant. In the common group of 58 calves, the PRNP 6/6 genotype was represented by 26 individuals (44.8%), the 6/5 genotype by 19 individuals (32.8%), and the 5/5 genotype by 13 individuals (22.4%), a drastic departure from the theoretical genotype ratio (25:50:25). The authors theorized that the PRNP locus is linked with an unknown lethal gene that eliminates PRNP 6/5 heterozygotes that are also homozygotes with regard to the hypothetical lethal gene. Alternative linkage forms were proposed in cis and trans systems to explain mutual relationships between the PRNP genotype and the hypothetical locus of the lethal gene (Walawski and Czarnik 2003).

ARR/ARR, ARR/ARQ, and ARR/AHQ sheep have been found to be the most resistant to the fatal disease of scrapie, and ARQ/VRQ and ARR/VRQ sheep the most susceptible (Dawson et al. 2008). Attempts have been made to develop scrapie-resistant flocks by eliminating sensitive genotypes; however, allele segregation has not been studied in sheep to date.

Genotype and allele distribution patterns highly similar to those we found have been observed by others in Holstein–Friesian cattle in Poland and other European countries (Juling et al. 2006; Haase et al. 2007; Czarnik et al. 2011). Therefore, it seems that our findings regarding allele and haplotype segregation in Polish Holstein–Friesian cattle can be extrapolated to other European populations of Holstein–Friesians.

Owing to the limited availability of large groups of offspring fathered by selected sires, in this study allele and haplotype segregations were analyzed mostly as the combined effect of several sires within the same genotype group. Further research involving the offspring of many sires, analyzed individually, is needed to exclude or confirm the effect of single sires. The determination of high-impact sires transmitting the deletion allele at the polymorphic site of the promoter region (23 bp) of the PRNP gene may provide a basis for developing a breeding program aimed at decreasing the frequency of this allele in the cattle population.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

Ballerini C, Gourdain P, Bachy V, Blanchard N, Levavasseur E, Grégoire S, Fontes P, Aucouturier P, Hivroz C, Carnaud C (2006) Functional implication of cellular prion protein in antigen-driven interactions between T cells and dendritic cells. J Immunol 176(12):7254–7262
Bremer J, Baumann F, Tiberi C, Wessig C, Fischer H, Schwarz P, Steele AD, Toyka KV, Nave KA, Weis J, Aguzzi A (2010) Axonal prion protein is required for peripheral myelin maintenance. Nat Neurosci 13(3):310–318
Brunelle BW, Kehrli ME Jr, Stabel JR, Spurlock DM, Hansen LB, Nicholson EM (2008) Allele, genotype, and haplotype data for bovine spongiform encephalopathy-resistance polymorphisms from healthy U.S. Holstein cattle. J Dairy Sci 91(1):338–342
Clawson ML, Heaton MP, Keele JW, Smith TP, Harhay GP, Laegreid WW (2006) Prion gene haplotypes of U.S. cattle. BMC Genet 7:51
Czarnik U, Strychalski J, Zabolewicz T, Pareek CS (2011) Populationwide investigation of two indel polymorphisms at the prion protein gene in Polish Holstein–Friesian cattle. Biochem Genet 49:303–312
Dawson M, Moore RC, Bishop SC (2008) Progress and limits of PrP gene selection policy. Vet Res 39:25–37
Dürig J, Giese A, Schulz-Schaeffer W, Rosenthal C, Schmücker U, Bieschke J, Dühren U, Kretzschmar HA (2000) Differential constitutive and activation-dependent expression of prion protein in human peripheral blood leucocytes. Brit J Haematol 108(3):488–495
Haase B, Doerr MG, Seuberlich T, Drögemüller C, Dolf G, Nicken P, Schiebel K, Ziegler U, Groschup MH, Zubirgen A, Leeb T (2007) PRNP promoter polymorphisms are associated with BSE susceptibility in Swiss and German cattle. BMC Genet 8:15
Hills D, Comincini S, Schlaepfer J, Dolf G, Ferretti L, Williams JL (2001) Complete genomic sequence of the bovine prion gene (PRNP) and polymorphism in its promoter region. Anim Genet 32(4):231–232
Hugel B, Martinez MC, Kunzelmann C, Blättler T, Aguzzi A, Freyssinet JM (2004) Modulation of signal transduction through the cellular prion protein is linked to its incorporation in lipid rafts. Cell Mol Life Sci 61(23):2998–3007
Juling K, Schwarzenbacher H, Williams JL, Fries R (2006) A major genetic component of BSE susceptibility. BMC Biol 4:33
Kaczmarczyk E, Bojarojc´-Nosowicz B, Czarnik U, Strychalski J (2010) Prion protein gene polymorphism and blood lymphocyte profile in cows naturally infected with bovine leukemia virus. Pol J Vet Sci 13(3):415–421
Msalya G, Shimogiri T, Nishitani K, Okamoto S, Kawabe K, Minesawa M, Maeda Y (2010) Indels within promoter and intron 1 of bovine prion protein gene modulate the gene expression levels in the medulla oblongata of two Japanese cattle breeds. Anim Genet 41(2):218–221
Nakamitsu S, Miyazawa T, Horiuichi 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
Premzl M, Delbridge M, Gready JE, Wilson P, Johnson M, Davis J, Kuczek E, Marshall Graves JA (2005) The prion protein gene: identifying regulatory signals using marsupial sequence. Gene 349:121–134
Prusiner SB (1998) Prions. Proc Natl Acad Sci USA 95:13363–13383
Sander P, Hamann H, Pfeifer I, Wemheuer W, Breng 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 5:19–25
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):37408–37414
Schläpfer J, Stahlberger-Saitbekova N, Küffer J, Dolf G (2000) Genetic mapping of the prion protein gene (PRNP) on bovine chromosome 13. J Anim Breed Genet 117:211–216
Walawski K, Czarnik U (2003) Prion protein octapeptide-repeat polymorphism in Polish Black and White cattle. J Appl Genet 44(2):191–195