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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

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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 parents and ♂ 23del/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 (PrP^c) 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,

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$CD8^+$ T lymphocytes, and NK cells (Dürig et al. 2000). PrP^c 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^c 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 ♂ 23ins/del × ♀ 23ins/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 ♂ 23ins/del × ♀ 23del/del parents ($n = 106$) and ♂ 23del/del × ♀ 23ins/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		χ^2
			Observed	Expected	
♂ 23ins/ins × ♀ 23ins/ins	10	23ins/ins	10	10.00	0.00
♂ 23ins/ins × ♀ 23ins/del	44	23ins/del	18	22.00	1.46
		23ins/ins	26	22.00	
♂ 23ins/ins × ♀ 23del/del	39	23ins/del	39	39.00	0.00
♂ 23ins/del × ♀ 23ins/ins	29	23ins/ins	12	14.50	0.83
		23ins/del	17	14.50	
♂ 23ins/del × ♀ 23ins/del	134	23ins/ins	24	33.50	5.99*
		23ins/del	66	67.00	
		23del/del	44	33.50	
♂ 23ins/del × ♀ 23del/del	106	23ins/del	44	53.00	3.06
		23del/del	62	53.00	
♂ 23del/del × ♀ 23ins/ins	19	23ins/del	19	19.00	0.00
♂ 23del/del × ♀ 23ins/del	77	23ins/del	30	38.50	3.76
		23del/del	47	38.50	
♂ 23del/del × ♀ 23del/del	52	23del/del	52	52.00	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		χ^2
			Observed	Expected	
♂ 12ins/ins × ♀ 12ins/ins	14	12ins/ins	14	14.00	0.00
♂ 12ins/ins × ♀ 12ins/del	52	12ins/ins	22	26.00	1.24
		12ins/del	30	26.00	
♂ 12ins/ins × ♀ 12del/del	27	12ins/del	27	27.00	0.00
♂ 12ins/del × ♀ 12ins/ins	72	12ins/ins	35	36.00	0.06
		12ins/del	37	36.00	
♂ 12ins/del × ♀ 12ins/del	194	12ins/ins	35	48.50	5.04
		12ins/del	105	97.00	
		12del/del	54	48.50	
♂ 12ins/del × ♀ 12del/del	88	12ins/del	41	44.00	0.40
		12del/del	47	44.00	
♂ 12del/del × ♀ 12ins/ins	13	12ins/del	13	13.00	0.00
♂ 12del/del × ♀ 12ins/del	32	12ins/del	15	16.00	
		12del/del	17	16.00	
♂ 12del/del × ♀ 12del/del	18	12del/del	18	18.00	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.

Table 4 Haplotype segregation at the polymorphic sites within the promoter (23indel) and intron 1 (12indel) regions in the offspring of 23ins-12ins/23del-12del sires

Parental mating variant		Number of offspring	Progeny genotype	Number of animals in progeny genotype group		χ^2
Genotype of sire[a]	Genotype of dam			Observed	Expected	
23ins-12ins/ 23del-12del	23del-12del/ 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 $p \leq 0.05$

[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 × ♀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 ♂ 23ins/del × ♀ 23del/del parents and ♂ 23del/del × ♀ 23ins/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 × ♀ 12ins/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 × ♀ 23ins-12ins/23del-12del).

Table 5 Haplotype segregation at the polymorphic sites within the promoter (23indel) and intron 1 (12indel) regions in the offspring of homozygous and 23del-12ins/23del-12del sires

Parental mating variant		Number of offspring	Progeny genotype	Number of animals in progeny genotype group		χ^2
Genotype of sire[a]	Genotype of dam			Observed	Expected	
23ins-12ins/ 23ins-12ins	23ins-12ins/ 23del-12del	34	23ins-12ins/ 23ins-12ins	14	17.00	1.06
			23ins-12ins/ 23del-12del	20	17.00	
23del-12del/ 23del-12del	23ins-12ins/ 23del-12del	21	23ins-12ins/ 23del-12del	10	10.50	0.48
			23del-12del/ 23del-12del	11	10.50	
23del-12ins/ 23del-12del	23ins-12ins/ 23del-12del	38	23del-12del/ 23ins-12ins	8	9.50	4.32
			23del-12ins/ 23ins-12ins	5	9.50	
			23del-12ins/ 23del-12del	12	9.50	
			23del-12del/ 23del-12del	13	9.50	

[a] Artificial insemination

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.

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