czar@uwm.edu.pl

Polymorphisms of two indels at the PRNP gene in Polish Holstein-Friesian cattle

CZARNIK U., STRYCHALSKI J., ZABOLEWICZ T., KAMIŃSKI S.
Department of Animal Genetics, University of Warmia and Mazury, Olsztyn, Poland

Two polymorphisms located in 5` - flanking region of prion protein gene (PRNP) are thought to be associated with resistance to classical BSE disease: a 23-bp sequence in the promoter region that contains a binding site for the repressor protein RP58, and a 12-bp sequence in intron 1 region that has a binding site for the transcription factor SP1. The aim of this study was to determine allele, genotype and haplotype frequencies in both PRNP polymorphic sites in groups of cattle differentiated by milk performance level. 837 animals were investigated: 186 AI bulls used in artificial insemination in the years 2005-2007, 105 young bulls (aged 3 months, born in the years 2005 - 2006), 96 cows qualified as sire-mothers and 450 randomly selected cows from large commercial herds located in different regions of Poland. Insertion/deletion (indel) polymorphisms at the PRNP gene were determined by PCR (Sander et al. 2004). The amplified sequences included 2 fragments of the PRNP gene (GenBank accession No. AJ 298878), located within the promoter (between positions 47784 and 47883) and within intron 1 (between positions 49686 and 49777).

Differences between the observed and expected frequency of the PRNP genotypes as well as the accordance of genotype, allele and haplotype frequencies between the experimental groups and German Holstein BSE-affected and clinically healthy cattle were verified using the chi-square test. Haplotype frequencies were deduced from the genotypic data using an expectation-maximization (EM) algorithm, as implemented in the Arlequin v.3 package.

The tested population of Polish Holstein-Friesian (HF) cattle has a typical genetic structure for the HF breed, with insertion/deletion (indel) polymorphism in the promoter sequence and in the intron sequence of the PRNP gene. The heterozygous genotype (23 bp indel – 0.504, 12 bp indel – 0.535) was most frequent at both polymorphic loci, followed by del/del homozygotes (23 bp indel – 0.370, 12 bp, indel – 0.259) and ins/ins homozygotes (23 bp indel – 0.126, 12 bp indel – 0.206).

A comparative analysis of Polish HF with German Holstein BSE-affected cattle (Juling et al. 2006) revealed significant differences in genotype frequencies at the polymorphic locus of intron 1. Comparison of Polish HF cattle with German Holstein BSE-affected cattle showed a substantially lower frequency of del/del homozygotes and a higher frequency of ins/ins homozygotes and ins/del heterozygotes (*P* = 0.0038).

No significant differences were observed between the Polish HF and German Holstein BSE-affected cattle population at the polymorphic locus of the promoter. Among the analyzed animals, the most profound differences were noted in the group of AI bulls, and the calculated *P*-value (0.0951) was very close to the *P*-value reported for German Holstein BSE-affected and control cattle (0.0987).

Interesting results were obtained with respect to allele frequencies. Significant differences were found in both regions of the PRNP sequence between German BSE-affected Holsteins and Polish HF. These differences were more significant at the polymorphic locus of intron 1 (*P* = 0.0028) than in the region of the promoter sequence (*P* = 0.0407). The results reported for both investigated regions of the PRNP sequence confirmed the similarity of allele distribution patterns between Polish HF cattle and German Holstein controls.

Haplotype composition is formed randomly, and it constitutes an exact representation of the number of genotypes within each indel. The presence of four haplotypes was observed in the analyzed groups of young bulls, sire-mothers and cows from commercial herds, while three linkages were identified in the group of breeding bulls. The tested population sample and particular groups of animals were marked by the highest frequency of the 23del-12del haplotype (0.512), a considerably lower frequency of the 23ins-12ins linkage (0.363) and a very low frequency of the 23del-12ins haplotype (0.111). The 23ins-12del linkage was observed sporadically (0.014), mainly in cows from commercial herds (0.022).

A comparison of Polish HF and German Holstein BSE-affected cattle revealed a lower frequency of the 23del-12del haplotype and higher frequencies of the 23ins-12ins and 23del-12ins haplotypes (*P* = 0.0019) among Polish HF cattle, indicating differences between the examined groups of animals. The most significant differences were reported in respect of the group of randomly tested cows from commercial herds (*P* = 0.0005) and the group of breeding bulls (*P* = 0.0148).

The obtained results showed no significant differences in the genetic structure of the investigated groups of Polish HF cattle at various stages of selection for milk performance traits.

The genetic equilibrium of the tested population was maintained. However, the low *P*-values (23 bp indel – *P* = 0.1096, 12 bp indel - *P* = 0.1038) are indicative of certain selection preferences manifested in a lower frequency of ins/ins homozygotes and a higher frequency of ins/del heterozygotes.

Conclusions

Polish HF cattle are characterized by a genetic structure typical of the HF breed, which is similar to that of healthy German Holstein and significantly different from that of BSE-affected Holstein cattle. Significant differences in the frequencies of alleles, haplotypes and genotype frequency in the region of the intron 1 sequence were noted at both polymorphic loci of the PRNP gene, allowing to differentiate between the analyzed groups of animals. The stage of selection had no effect on differences in the genetic structure of the tested cattle populations.

Reference

Juling K., Schwarzenbacher H., Williams J.L., Fries R.: *A major genetic component of BSE susceptibility*. BMC Biol 2006, 4, 33.

Sander P., Hamann H., Pfeifer I., Wemheuer W., Breng B., Groschup M.H., Ziegler U., Distl O., Leeb T.: *Analysis of sequence variability of the bovine prion protein gene (PRNP) in German cattle breeds*. Neurogenetics 2004, 5, 19-25.