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Study of SNP 775C>T polymorphism within the bovine ITGB2 gene in Polish Black-and-White cattle and in local breeds of cattle

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ABSTRACT: The present study addresses the characteristics of the frequency and segregation of alleles determining the SNP 775C>T polymorphism within the bovine ITGB2 gene in the Black-and-White cattle population as well as in two endemic breeds of Polish Red and Polish White-Back cattle population qualified to the international programme of genetic resource diversity preservation in farm animals. The SNP 775C>T polymorphism revealed three amplified restriction fragments of 31 bp, 77 bp and 108 bp, forming three genotypes CC (31 bp, 77 bp), CT (31 bp, 77 bp, 108 bp) and TT (108 bp). A group of randomly selected Black-and-White cows was characterized by a negligible percentage of homozygous genotypes TT (5.1%) and by a prevailing percentage of heterozygous CT (60.1%) and homozygous CC (34.8%). In contrast, the between breeds analysis revealed that cows involved in the programme of genetic diversity preservation had a high prevalence of homozygotes CC (Polish Red – 55.9%, Polish White-Back – 60.0%), and a low percentage of homozygotes TT (Polish Red – 8.8%, Polish White-Back – 5.7%) in comparison with the Black-and-White cattle population. The degree of homozygosity in groups of Polish White-Back cows (65.7%) and Polish Red cows (64.7%) was remarkably higher than that of the Black-and-White cows (39.9%).

Keywords: BLAD carriers; point mutation; lethal genes; SNP 775 C>T; polymorphism
acterize the frequency of alleles determining the SNP 775C>T polymorphism within the bovine ITGB2 gene in Black-and-White cattle as well as in endemic populations of Polish Red and Polish White Back cattle involved in an international programme of preserving the sources of genetic variability in farm animals.

MATERIAL AND METHODS

Animals

The study was carried out on 49 Black-and-White bulls and randomly selected population of Black-and-White cows (n = 129), Polish Red cows (n = 34) and Polish White Back cows (n = 35).

Laboratory procedure

DNA was isolated from peripheral blood and sperm with the use of a Wizard genomic DNA purification kit (Promega, USA), following the producer’s instructions. The number, purity, and quality of DNA preparations were controlled spectrophotometrically (GeneQuant, Pharmacia, USA) and electrophoretically on 1% agarose gel.

The SNP 775C>T polymorphism within the bovine ITGB2 gene was determined by the PCR−RFLP method following the procedure elaborated by Czarnik et al. (2004). The amplification of a CD18 gene fragment, 108 bp in length, was performed using 25 μl of a reaction mixture containing: 1.25 μl buffer 20 × Master AmpTM Tfl Buffer (Epicentre Technology) (500mM KCl, 100mM TRIS–HCl pH 9.0 1% TRITON X–100), 1.5 μl of nucleotide solution dNTP (dATP, dGTP, dCTP, dTTP), each at a concentration of 2mM, 1.0 μl primer solution at a concentration of 100 pM/μl: forward primer (5’ GAG GAA ATC GGC TGG CGC AAT G 3’), reverse primer (5’ GTCA TTT GGG GTT GAG GAT G3’), 2.0 μl of a 25mM solution of MgCl2, 0.5U polymerase Tfl Master AmpTM (Epicentre Technology) at a concentration of 1 U/μl, 3.0μl 10 × Master AmpTM PCR enhancer, 1 μl DNA at a concentration of 100 ng/ml, H2O up to a volume of 25 μl.

The PCR was carried out in a Rapidcycler, Idaho Technology, USA, in the “touch down” thermal programme (Don et al., 1991). The specificity of the PCR product was confirmed by sequencing performed at the DNA sequencing laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, using an automatic ABI PRISM™ 377 sequencer (Applied Biosystems). Positively verified PCR products were subjected to digestion with a restriction enzyme Fnu 4HI (BioLabs, UK). The polymorphism of restriction fragments was analyzed electrophoretically on 3% agarose gel (Certified™ Low Range Ultra Agarose, Bio-Rad) against the molecular weight marker PhiX 174 digested with endonuclease Hae III. The results were recorded using the Fluor S™ Multimager system (BIO-RAD).

Statistics

The results were compiled taking into account the frequency of CC, CT, and TT genotypes for SNP 775C > T polymorphism in the examined population. Differences between the genotypes and allele frequencies, genetic equilibrium were verified using the chi-square test.

RESULTS AND DISCUSSIONS

The SNP 775C>T polymorphism within the bovine ITGB2 gene was determined by subjecting a PCR product to the activity of endonuclease Fnu 4HI (BioLabs, UK). The substitution of cytosine with thymine at the position 775 cDNA resulted in a loss of recognition site for Fnu 4HI reductase (Figure 1).

In the analyzed population, three size variants of restriction fragments were identified, namely: 108 bp, 77 bp, and 31 bp. An analysis of the localization of migration bands of the restriction fragments enabled to identify three genotypes of “silent mutation C>T”. The genotype CC represents the occurrence of two bands of 77 bp and 31 bp, genotype CT represents three restriction fragment bands of 108 bp, 77 bp and 31 bp, and genotype TT represents one band of 108 bp in length.

The results of SNP 775C>T polymorphism within the bovine ITGB2 gene in the domestic population of Black-and-White cattle as well as herds of the Polish Red and Polish White-Back breeds included in the programme of genetic variability preservation are presented in Table 1.

The results indicate that the group of randomly examined Black-and-White cows had a negligible number of homozygous genotypes TT (0.051) and
a prevailing number of heterozygous CT (0.601) and homozygous CC (0.348). The frequency of
genotypes and alleles in these cows was similar to
that reported previously on the macro-population
scale for the Black-and-White breed (Czarnik et
al., 2004). The dam population of Polish Red and
White-Back cattle demonstrated a completely dif-
ferent genetic structure. In both cases, the most
numerous group was that of homozygotes CC
(0.559 in Polish Red cattle, 0.600 in Polish White-
Back cattle), whereas intermediate values were
reported for the group of heterozygotes (0.353 in
Polish Red cattle, 0.343 in Polish White-Back cat-
tle), and the lowest values for the group of ho-
mozygotes TT (0.088 in Polish Red cattle, 0.057 in
Polish White-Back cattle). As a consequence, the
degree of homozygosity appeared to be low in
Black-and-White cattle (0.399) and remarkably
higher in herds of Polish Red (0.647) and Polish
White-Back cattle (0.657). The observed regular-
ity is likely to result from the specificity of dam
herd reproduction in preservation breeding, i.e.
reproduction with a small number of bulls.

The differences in the frequency of genotypes and
alleles of SNP 775C>T within the bovine ITGB2
gene in bulls and cows of the Black-and-White breed
are presented Table 2. The results revealed that the
group of sires had a relatively high frequency of ho-
mozygotes CC (0.490), whereas the group of cows
demonstrated considerable prevalence in the fre-
quency of heterozygotes CT (0.651). In both male
and female groups, there was a single case of the
occurrence of homozygous TT animal (0.041 in
bulls, 0.054 in cows). Consequently, the frequency
of allele C in bulls (0.725) was higher compared to
that of the cows (0.620).

Table 1. Frequency of genotypes and alleles of SNP 775C>T within the bovine ITGB2 gene in Black-and-White, Polish Red, and Polish White-Back breeds

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Number of animals</th>
<th>Genotype frequency</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>Black-and-White</td>
<td>n = 178</td>
<td>0.348</td>
<td>0.601</td>
</tr>
<tr>
<td>Polish Red</td>
<td>n = 34</td>
<td>0.559</td>
<td>0.353</td>
</tr>
<tr>
<td>Polish White-Back</td>
<td>n = 35</td>
<td>0.600</td>
<td>0.343</td>
</tr>
</tbody>
</table>

Table 2. Frequency of genotypes and alleles of SNP 775C>T within the bovine ITGB2 gene in bulls and cows of the Black-and-White breed

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Number of animals</th>
<th>Genotype frequency</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>Bulls</td>
<td>n = 49</td>
<td>0.490</td>
<td>0.469</td>
</tr>
<tr>
<td>Cows</td>
<td>n = 129</td>
<td>0.295</td>
<td>0.651</td>
</tr>
</tbody>
</table>
The results of a genetic equilibrium analysis are illustrated in Table 3. The Black-and-White cattle had a deviation from the equilibrium, manifested by an increasing frequency of heterozygotes $CT$ and a diminished frequency of homozygotes $TT$ (a highly statistically significant difference), whereas the groups of cows representing the Polish Red and Polish White-Back breeds demonstrated an almost absolute conformity between the observed and expected numbers of genotype groups $CC$, $CT$ and $TT$.

An analysis of the genetic equilibrium for SNP $775C>T$ polymorphism within the bovine $ITGB2$ gene in the male and female Black-and-White cattle population (Table 4) indicates that the selected group of bulls had a statistically verified conformity of the observed and expected numbers of genotypic groups $CC$, $CT$ and $TT$, whereas the randomly tested group of cows, representing a random population of Black-and-White cattle, demonstrated a distinct deviation from the state of equilibrium, manifested in an increased number of heterozygotes $CT$ and a decreased number of homozygous genotypes (a highly statistically significant difference) for genotype $TT$.

The results indicate that the effect of breeding selection of male reproducers was not linked with the specificity of genetic equilibrium diversification, determined by the SNP $775C>T$ polymorphism within the bovine $ITGB2$ gene. The higher number of heterozygous animals reported in the group of cows provides initial data confirming the selective prevalence of animals determined by a non-additive effect of natural selection.

**CONCLUSIONS**

SNP $775C>T$ polymorphism within the bovine $ITGB2$ gene in randomly examined Black-and-White breed revealed the highest frequencies of $CT$ genotypes, however, the Polish Red and White-Back breeds had the highest frequencies of $CC$. The genotype and allele frequencies of SNP $775C>T$ within the bovine $ITGB2$ gene in the sire and dam population of Black-and-White cattle showed a high frequency of $C$ allele in both groups, $CC$ in sire population and $CT$ in dam population. Furthermore, the SNP $775C>T$ polymorphism revealed genetic disequilibrium for Black-and-White cattle, as well as in both male and female population groups and genetic equilibrium for Polish Red and Polish White-Back breeds.
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