A novel mutation of the GLI2 gene associated with body weight in bovine (Bos taurus) (Brief Report)

Eine neue Mutation des GLI2 Gens assoziiert mit dem Körpergewicht bei Rindern (Bos taurus) (Brief Report)

XINLEI WANG¹, XIANYONG LAN¹, XINSHENG LAI¹, KEYI WANG¹, HUI YU¹, MOU WANG¹, YIKUN GUO¹, CHUZHAO LEI¹ and HONG CHEN¹,²

¹College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling, People’s Republic of China, ²Institute of Cellular and Molecular Biology, Xuzhou Normal University, Xuzhou, Jiangsu, People’s Republic of China

Background

During pituitary gland development, the actions of transcription factors control the development of the hormone-producing cell types. Defects in transcription factor genes, including PIT1/POU1F1, PROP1, GLI2, HESX1, LHX3, and LHX4, are associated with combined pituitary hormone deficiency (CPHD) (SAVAGE et al. 2007). Removal of the mouse GLI2 gene by targeted disruption leads to an embryonic lethal phenotype with defects in early brain and spinal cord development, which include absence of the floor plate (MATISE et al. 1998). At present, no polymorphisms of GLI2 gene have been reported in bovine. In the present paper, partial 5’ flanking region, coding region and partially introns of GLI2 were screened to detect the SNPs in Chinese cattle breeds.

Procedures

Primer sequences

The primer pairs 1-2 were designed based on bovine GLI2 gene (GenBank acc. no. NW_001494637).

1  Exon 1  F: 5’ TTG GTG CTT CCT AGC TGG GTC 3’  R: 5’ CAA TTT GGG AGG GCT GGA C 3’
2  Exon 5  F: 5’ CGG CTT GGC TTG GAT GTT C 3’  R: 5’ GTC TTG TCT TTC TCC TGG GAT GG 3’

PCR-SSCP method

50 ng DNA template, 0.20 mM dNTP, 2.5 mM MgCl₂, and 0.5 U Taq DNA polymerase (MBI). The PCR was performed using the following program: 94°C for 5 min followed by 35 cycles of 94°C for 30 s, annealing for 35 s, and 72°C for 40 s and a final extension at 72°C for 10 min. PCR products were electrophoresed on 2% agarose gels using 1×TBE buffer, containing 200 ng/mL ethidium bromide. Aliquots of 5 μL PCR products were mixed with 5 μL denaturing solution, heated for 10 min at 98°C and chilled on ice. Denatured DNA was subjected to PAGE (80×73×0.75 mm) in 1×TBE buffer and constant voltage (160 V) for 2.5-3.0 h. The gel was stained with 0.1% silver nitrate (LAN et al. 2007). The PCR products from individuals which represented different PCR-SSCP patterns were purified and sequenced.
Results

604 individuals belonging to three Chinese cattle breeds (Nanyang, \( n=207 \); Qinchuan, \( n=287 \); Jiaxian, \( n=110 \)) were used in this study. Three unique SSCP banding patterns were detected in the exon 1 locus after PCR-SSCP analysis, the genotypes were named TT, TC and CC (Table 1). No polymorphism was detected in the region of exon 5. DNA sequencing analysis showed, in the exon 1 locus, a novel SNP was revealed in exon 1 (GenBank acc. no. FJ215663). Compared with the sequence of \( GLI2 \) (GenBank acc. no. NW_001494637), the T>C mutation at nt1597927 in the coding region of \( GLI2 \), but casued no amino acids exchange. Growth traits were analyzed in Nanyang cattle at 6, 12, 18, and 24 months old. Body weight at 12 months, individuals with TT genotype was significantly higher than individuals with CC genotype (\( P<0.05 \))(Table 2). No other statistically significant differences were observed between the TT, TC, and CC genotypes of the breed concerning growth traits (\( P>0.05 \)). The SNP found in the bovine \( GLI2 \) gene enable to conduct association analyses in order to evaluate the SNP locus as genetic markers for breeding. The result provoked the \( GLI2 \) gene as important candidate gene.

Table 1
Genotype distribution and allele frequencies at the bovine \( GLI2 \) gene exon 1

<table>
<thead>
<tr>
<th>Breeds</th>
<th>TT</th>
<th>TC</th>
<th>CC</th>
<th>Total</th>
<th>Allele Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>NY</td>
<td>158</td>
<td>25</td>
<td>24</td>
<td>207</td>
<td>0.824 0.176</td>
</tr>
<tr>
<td>JX</td>
<td>66</td>
<td>14</td>
<td>30</td>
<td>110</td>
<td>0.664 0.336</td>
</tr>
<tr>
<td>QC</td>
<td>255</td>
<td>32</td>
<td>0</td>
<td>287</td>
<td>0.944 0.056</td>
</tr>
</tbody>
</table>

NY Nanyang breed, QC Qinchuan breed, JX Jiaxian breed

Table 2
Association of genotypes at the exon 1 locus of the \( GLI2 \) gene with growth traits in Nanyang cattle

<table>
<thead>
<tr>
<th>Ages</th>
<th>Growth Traits</th>
<th>TT (Mean±SE)</th>
<th>Genotypes at ( GLI2 ) gene</th>
<th>CC (Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>birth</td>
<td>BW, kg</td>
<td>30.042±0.308</td>
<td>30.500±0.749</td>
<td>29.318±0.553</td>
</tr>
<tr>
<td>6 months</td>
<td>BW, kg</td>
<td>161.873±2.245</td>
<td>159.333±5.462</td>
<td>155.591±4.034</td>
</tr>
<tr>
<td>12 months</td>
<td>BW, kg</td>
<td>226.014±2.685</td>
<td>224.583±6.531(^{ab})</td>
<td>213.545±4.823(^b)</td>
</tr>
<tr>
<td>18 months</td>
<td>BW, kg</td>
<td>301.577±3.654</td>
<td>287.833±8.889</td>
<td>293.682±6.565</td>
</tr>
<tr>
<td>24 months</td>
<td>BW, kg</td>
<td>370.437±4.736</td>
<td>358.417±11.519</td>
<td>365.545±8.508</td>
</tr>
</tbody>
</table>

\(^{ab}\) different superscripts within the same line differ significantly at \( P<0.05 \), SE standard error of means, BW body weight

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Corresponding author:
Prof. Dr. HONG CHEN
email: chenhong1212@263.net

College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, People’s Republic of China, Institute of Cellular and Molecular Biology, Xuzhou Normal University, Xuzhou, Jiangsu 221116, People’s Republic of China