

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)

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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 AGCTGG 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 caused 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
Genotypenverteilung und Allelfrequenzen vom Exon 1 des GLI2 Gens beim Rind

Breeds	Observed Genotypes			Total	Allele Frequencies	
	TT	TC	CC		T	C
NY	158	25	24	207	0.824	0.176
JX	66	14	30	110	0.664	0.336
QC	255	32	0	287	0.944	0.056

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
Assoziation des Exon 1 SNP von GLI2 mit dem Körpergewicht beim Nanyang Rind

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

^{a,b} different superscripts within the same line differ significantly at $P<0.05$, SE standard error of means, BW body weight

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