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Association of polymorphisms of the *GHRHR* gene with growth traits in cattle (Brief report)

(Assoziation von Polymorphisme des *GHRHR* mit Wachstumsmerkmalen beim Rind)

Background: The hypothalamic hormone, growth hormone-releasing hormone (GHRH), is the principal stimulator of pituitary growth hormone (GH) synthesis and secretion. Its pituitary receptor is well characterized as a member of the superfamily of G protein-coupled receptors (MAYO et al., 1992). GHRH and its receptor provide important functions in the regulation of the GH axis and in the development and proliferation of pituitary somatotropes (LIN-SU et al., 2002). By now, polymorphisms of bovine *GHRHR* were not reported. In the present experiment the 5' flanking region, the coding region and partially introns of *GHRHR* were screened to detect the SNPs in the predominant cattle breeds of China.

Procedures:

Primer sequences:

Based on bovine *GHRHR* (GenBank accession. No. NW_001494911), 5 pairs of primers were designed to amplify the 5' flanking region, exon 1-6 and partially introns.

- | | |
|--------------------------------|-----------------------------|
| 1: 5'-accgcaggtgtcaggagttc-3' | 5'-aggggacagtggaaggaacg-3' |
| 2: 5'-cattctaccaccaccaagga-3' | 5'-ccattattctccctccctcc-3' |
| 3: 5'-acaaacatccgtgacgagcct-3' | 5'-ccagaaaggaaggtcccagaa-3' |
| 4: 5'-tcaggttggtgctgtggatg-3' | 5'-gctccgttgctgagattcctt-3' |
| 5: 5'-catcctgggtgcttctgaag-3' | 5'-acgccaccctcttcaccag-3' |

PCR-SSCP conditions:

The 15 µL polymerase chain reaction (PCR) contained 50 ng of genomic bovine DNA, 0.2 µM of each primer, dNTPs (0.2 mM), MgCl₂ (1.5 mM), and 0.50 U Taq DNA polymerase (TaKaRa, Dalian, China). The cycling protocol was 4 min at 95 °C, 35 cycles of 94 °C for 45 s, annealing at 67 °C, 62 °C, 62 °C, 62 °C or 67 °C corresponding to 5 different primer pairs for 45 s, 72 °C for 1 min, with a final extension at 72 °C for 10 min. The single stranded conformation polymorphisms (SSCP) were analyzed according to previous description (PAN et al., 2007). The PCR products from individuals which represented different PCR-SSCP patterns were purified and sequenced.

Results: Ten SNPs were detected in the 5' flanking region, intron 3, exon 4 and intron 7 in 475 unrelated cattle of three cattle breeds in China (Nanyang cattle, 219; Qinchuan cattle, 114; Jiaxian cattle, 142). The discovered sequences of SNPs were deposited in GenBank (accession No.: EU152114, EU152115, EU152116, EU152117, EU152118, EU152119, EU152120, EU152121, EU152122, EU152123). Compared with the sequence of *GHRHR* (NW_001494911), the G>A mutation at nt265678 in the

5' flanking region of *GHRHR* was detected in three breeds with similar frequency. The G>T mutation at nt261155 (exon 4) did not cause amino acid variation. The five mutations at nt258908, nt258914, nt258975, nt258988, and nt259000 (intron 7) were discovered in Nanyang and Qinchuan cattle. The three mutations at nt261285, nt261309, and nt261338 (intron 3) were in linkage disequilibrium with CCA always together and TAG always together. Therefore, the association of mutations at intron 3 with average daily gain and body weight of 99 individuals from Nanyang cattle were analyzed together (Table 1). The genotypes were named AA, AB and BB. Fixed effects of genotype and age were included as independent variables in the linear model. The result indicated that three linked mutations in *GHRHR* gene were significantly associated with body weight of 12 months and average daily gain of 12 months ($P<0.05$). The individuals with genotype AA had higher average daily gain and body weight than individuals with genotype AB. While the differences between the individuals with genotype BB and the individuals with genotype AA and AB were not significant. So, three linked mutations in *GHRHR* gene have effect on growth traits in bovine. This result provoked the *GHRHR* gene as important candidate gene.

Table 1

Least square mean (means \pm standard error of means) of average daily gain and body weight for the alternative *GHRHR* genotypes (Least square means der durchschnittlichen täglichen Gewichtszunahme und des Körpergewichts für den *GHRHR*-Genotyp)

Traits	Average daily gain of 12 months (kg)	Body weight of 12 months (kg)
AA (53)	0.370 \pm 0.016*	228.241 \pm 3.145*
BB (14)	0.346 \pm 0.031	221.786 \pm 6.176
AB (31)	0.315 \pm 0.021*	217.194 \pm 4.150*

* data in the same column differ significantly ($P<0.05$)

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