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Polymorphism in *GHRH* gene and its association with growth traits in Chinese native cattle

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ABSTRACT

Growth hormone-releasing hormone (GHRH) is secreted by the hypothalamus and stimulates growth hormone (GH) released from the pituitary. Mutations detected in *GHRH* gene showed associations with animal production traits. The purpose of this study was to investigate the association of the *GHRH* gene with growth traits in Chinese native cattle. PCR-SSCP and sequencing were used to detect mutations of the *GHRH* gene in this study. One novel mutation 4251nt (C > T) was found and the frequencies of C allele were 0.8778 and 0.8476 for Qinchuan and Nanyang cattle, respectively. Body weight with the CT genotype was significantly higher ($P < 0.05$ or $P < 0.01$) than those with CC genotype for different growth periods (6, 12, 18, and 24 months old) in Nanyang cattle. Our findings suggested that polymorphism in bovine *GHRH* might be one of the important genetic factors to influence body weight.

1. Introduction

Genes involved in regulating the expression of growth factors might be interesting as candidate markers for livestock's performance traits. Linear growth in animals was mainly regulated by GH (McDowell et al., 1995), a polypeptide hormone produced by the somatotroph cells of the pituitary gland. The GH played an important role in lactation and also was essential for postnatal growth and general metabolism (Cheong et al., 2006). However, the synthesis and secretion of GH was highly affected by hypothalamic hormones, including GHRH, GH secretagogue (GHS), and somatostatin (SS) (Karen and Majnrajch, 2002). Meanwhile, GHRH was secreted by the hypothalamus and acted on the pituitary stimulating the production and releasing GH (Andrew et al., 2001; Kiaris et al., 2005). Scholars revealed that targeted ablation of the *GHRH* gene showed isolated GH deficiency and anterior pituitary hypoplasia in mice (Alba et al., 2005). Evidences also demonstrated that GHRH not only played an important role in releasing GH, but also regulated the rate of cell proliferation and acted in neuroendocrine (Andrew et al., 2001; Hippokratis et al., 2003; Kiaris et al., 2005).

In cattle, the *GHRH* gene consists of five exons and locates on chromosome 13 (Barendse et al., 1994). Association between *GHRH* gene with an increased milk yield was confirmed by Baile and Buonomo (Baile and Buonomo, 1987), who found that administering this hormone increased the metabolic activity of mammary gland cells. Amplification of 297-bp fragment of *GHRH* gene followed by digestion with restriction enzyme *Hae*III had detected the existence of rare genotype (7.7%) called AA genotype. This genotype had also been demonstrated to improve both fat percentage and fat yield in Limousine cattle. However, calves with AA genotype were shorter than AB and BB individuals in regards to height at sacrum and height at withers (Dybus et al. 2003). Cheong et al. (2006) detected −4241A > T polymorphisms in 5′UTR of *GHRH* gene might be one of the important genetic factors that influence carcass yield in beef cattle. Furthermore, another SNP in *GHRH* gene and its effects were also found in pigs. A 455-bp fragment spanning exon 3 of the *GHRH* gene digested with *Alu*I restriction enzyme revealed a SNP that related to dressing percentage, meat percent, back fat thickness over the shoulder and meat share (%) in pig carcass (Kuryl et al., 2000; Pierzchala et al., 2003). Soon after, Franco et al. (2005) also found that the same SNP was associated with the average daily gain ($P = 0.0001$) and expected progeny differences for fat thickness ($P = 0.0004$) in Landrace pigs. Taken together, these results suggested that *GHRH* gene was an excellent candidate gene for growth-related traits in livestock.

Our tested populations, Nanyang and Qinchuan cattle are two of outstanding indigenous breeds in China. The distribution of Nanyang breed is in Henan province, while Qinchuan breed rear in Shaanxi province. These breeds are famously used in crossbreeding because of their excellent meat quality, adaptability, and physical features. Here, the purpose of this study was to detect SNP of *GHRH* gene in these excellent native Chinese cattle breeds and analyzed

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the results of the study on association with growth traits in Nanyang breed.

2. Materials and methods

2.1. Animals

Blood samples were obtained from 240 individuals (without genetic relationships) of two native Chinese cattle breeds: Qinchuan ($n = 135$) and Nanyang cattle ($n = 105$). Records of growth traits and body sizes for different growth periods (6, 12, 18, and 24 months old) in Nanyang female cattle were collected for statistical analysis. DNA samples were extracted from leucocytes and tissues according to the standard procedures (Joseph Sambrook, 2001).

2.2. Primers and genotype determination by PCR-SSCP

Based on the bovine *GHRH* gene sequence (GenBank accession number.AF242855), one pair of primers were designed to amplify part of intron 1, exon 2, intron 2 and part of exon 3 of the *GHRH* gene. Forward primer (5′-GAATGCCCTGTAGGAAGCG-3′) and reverse primer (5′-CATCTGCGTACCGTGGAAT-3′) were used to amplify a 699-bp (3972nt-4670nt) fragment. The PCR reaction system of 25 μL volume contained: 50 ng genomic DNA, 10 pmol each primer, 5 μL buffer (including $MgCl_2$), 200 μM dNTPs, and 1.0 U Taq DNA polymerase (MBI). Amplification was programmed for an initial 5 min, at 95 °C, followed by 34 cycles of 94 °C for 30 s, 55 °C annealing for 40 s, and 72 °C for 40 s, and a final extension at 72 °C for 10 min. The PCR-SSCP method was used to scan mutation within the amplified region. Aliquots of 4 μL PCR products were mixed with 6 μL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene–cyanole, and 0.025% bromophenol blue), heated at 98 °C for 10 min, and immediately chilled on ice. Denatured DNA was subjected to 10% PAGE in 1 × TBE buffer and constant voltage 10–12.5 V/cm for 14 h at a constant temperature of 4 °C. The gel was stained with 0.1% silver nitrate. After electrophoresis, the PCR products of different electrophoresis patterns were sequenced and polymorphism of the *GHRH* gene was analyzed.

2.3. Statistical analysis

Gene frequencies were determined by direct counting. Population genetic indexes, such as He (gene heterozygosity), Ho (gene homozygosity), Ne (effective allele numbers) and PIC (polymorphism information content) were calculated according to Nei's and Botstein's methods (Nei and Roychoudhury, 1974; Botstein et al., 1980), respectively. The formulas were as follows:

$$Ho = \sum_{i=1}^{m} p_i^2 \qquad He = 1 - \sum_{i=1}^{m} p_i^2 \qquad Ne = 1 / \sum_{i=1}^{m} p_i^2$$

$$PIC = 1 - \sum_{i=1}^{m} p_i^2 - \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} 2 p_i^2 p_j^2$$

("P_i, P_j' are the frequency of the i and j allele, "m" is the number of allele).

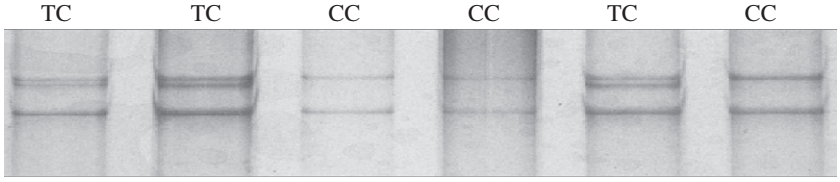
The linear model was applied to analyze the association of the variation of *GHRH* gene with growth traits in Nanyang female cattle using the software of SPSS 13.0. Following model for the PCR-SSCP marker effect was used for analysis:

$$Y_{ij} = \mu + age_i + marker_j + e_{ij}$$

where Y_{ij} is the phenotype of the animal, μ is the mean of the population, age_i is the age effect, $marker_j$ is the marked genotype effect, e_{ij} is the stochastic error. Statistical significance of differences between genotypes was evaluated with *t*-test.

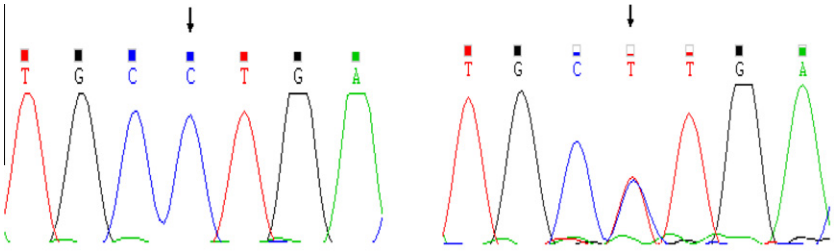
3. Results

In present study, the 699 bp PCR product including part of intron 1, exon 2, intron 2 and part of exon 3 of *GHRH* gene in Qinchuan and Nanyang cattle was amplified. Thereafter, two unique SSCP banding patterns (CC/CT) were detected after PCR-SSCP analysis (Fig. 1).



Note: TC and CC were two genotypes detected by PCR-SSCP in *GHRH* gene.

Fig. 1. PCR-SSCP patterns of bovine *GHRH* gene.



Note: Sequencing maps of the SNP 4251nt (C>T) in intron1 from different genotypes of the bovine *GHRH* gene.

Fig. 2. The sequencing 1 maps of the novel SNP in the bovine *GHRH* gene.