Archiv Tierzucht 53 (2010) 3, 372-376, ISSN 0003-9438

Structural features of the 5′ flanking region of the Yak (*Bos grunniens*) growth hormone receptor (*GHR*) gene
(Brief Report)

Strukturelle Merkmale der 5' flankierenden Region des Wachstumshormon-Rezeptor-(*GHR*)-Gens beim Yak (*Bos grunniens*) (Brief Report)

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Background

Yak (*Bos grunniens*) is a species of the *Bovidae* family living on the Qinghai-Tibetan Plateau and its adjacent territories at altitudes from 2 000-5 000 m (LUO *et al.* 2005, MA *et al.* 2009). As a multi-purpose domestic animal, yaks are indispensable to the local animal husbandry development because it can provide life necessities such as meat, milk, fur, velour manufacturing, transportation and manure for fuel to the local herdsmen. There are twelve yak populations numbered around 13.3 million in China and the Bazhou yak is one of Chinese yak populations (WIENER *et al.* 2003). The Growth Hormone Receptor (GHR) gene was identified as a candidate gene affecting key quantitative traits, like growth, milk yield and composition in livestock. At present, only investigations were carried out on the genetic variation in Exon-8, Intron-8 and Exon-10 of the yak *GHR* gene (VARVIO *et al.* 2008), no other information on the yak *GHR* gene is available. The purposes of this study were to

- analyze the structural features of the 5′ flanking region of the yak *GHR* gene
- compare the 5′ flanking region of yak *GHR* gene with that of other species of the *Bovidae* family and
- construct the phylogenetic tree to indicate the evolutionary relationship among them.

Procedures

Primer sequence

GHR-F: 5′-TGC GTG CAC AGC AGC TCA ACC-3′, *GHR-R*: 5′-TAC TAA CCA ATA GTC CGG CAG AC-3′

PCR amplification condition

DNA was isolated from blood samples of 2 Bazhou yaks using phenol-chloroform extraction protocol followed by an ethanol precipitation step. The PCR reaction mixture contained 25-50 ng yak genomic DNA, 10 pM of each primer, 0.50 U *ExTaq* DNA

polymerase (TakaRa, Dalian, China), 10×*ExTaq* Buffer (Mg^{2+} Free), 0.25 mM dNTP, 2.5 mM $MgCl_2$ and ddH_2O in a final volume of 25 µL. The following cycles were applied: 95°C/4 min, followed by 38 cycles at 94°C/1 min, 68.5°C/45 s, 72°C/1 min, and final synthesis at 72°C/5 min.

Cloning, sequencing and sequence analyses

The amplified PCR products were electrophoresed on a 1.0% agarose gel and purified using the DNA Agarose Gel Extraction Kit (Omega) according to the manufacturer's instructions. The purified fragments were cloned into pMD18-T vector (TakaRa, Dalian, China) and subsequently transformed into *Escherichia coli* JM109 (TakaRa, Dalian, China). The identified positive clone was sequenced using an ABI 3730 automated sequencer (Applied Biosystems).

Based on the cattle *GHR* gene sequence available from GenBank (acc. no. U15731), the sequence structure of yak *GHR* gene 5′ flanking region was identified. Searching for repetitive elements and potential transcription factor binding sites in the yak *GHR* gene 5′ flanking region was done using the RepeatMasker online program (version 3.2.8) (http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker) and TFSEARCH online program (version 1.3) (http://mbs.cbrc.jp/research/db/TFSEARCH.html), respectively. The corresponding sequences in GenBank of European bison (*B. bonasus*) (acc. no. AY243962), cattle (*B. taurus*) (acc. no. U15731), sheep (*O. aries*) (acc. no. EF116490), mouflon (*O. musimon*) (acc. no. AY641539), goat (*C. hircus*) (acc. no. AY358031) were used to compare the sequence of yak *GHR* 5′ flanking region with Bioedit 4.8.10 software and refined manually. A phylogenetic tree was constructed based on the Kimura's 2-parameter model using Mega 4.0 by UPGMA method and the confidence of probability of each branch was assessed using bootstrap of 1 000 replications.

Results

The 888 bp of yak *GHR* gene Exon 1A (205 bp), P1 promotor region (511 bp) and its partial flanking sequence were sequenced (Figure A1). The sequence was deposited in the GenBank database (acc. no. EF202183). The percentage of simple repeats was 5.63% and no SINEs, LINEs, LTR elements or DNA elements were found, although one $(TG)_{25}$ microsatellite DNA was found firstly in yak *GHR* gene P1 promotor region. There are 37 and 20 potential high-scoring binding sites for transcription factors (threshold: 85.0 point), including C/EBP, HFH-2, HNF-3b, SP1,C/EBPb, and Oct-1 etc, were detected in yak *GHR* gene P1 promotor region and its partial flanking sequence, respectively.

In the P1 promotor region, the sequence similarities between yak and sheep, mouflon, goat, cattle and European bison was 85%, 85%, 89%, 96%, 96%, respectively, while that for Exon 1A was 94%, 95%, 94%, 97%, 98%, respectively (Figure A2).

The molecular phylogenetic tree among 6 species of *Bovidae* constructed by the UPGMA method based on the sequences of P1 promotor and Exon 1A of the *GHR* gene (Figure A3), placed the three *Bovinae* species on one branch and the three *Caprinae* species on the other. The yak and European bison were joined first, followed by the cattle, and then the *Caprinae* species, including the sheep, mouflons and the goat. The result of

phylogenetic clustering was not only identical to the taxonomy, but also to the phylogenetic clustering using the mitochondrial DNA of yak and other species of *Bovidae* (GUO *et al.* 2006, LI *et al.* 2006).

Acknowledgements

We thank Ya Zhuo who helped us for sample collection. This study was supported by the Young and Middle-aged Scientific Research Fund Project of Qinghai University (no. 2008-QN-11), the National Natural Science Foundation of China (no. 30860213/C120209) and the International Scientific and Technological Cooperation project of National Science and Technology Ministry (no. 2008DFA31100).

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Appendix

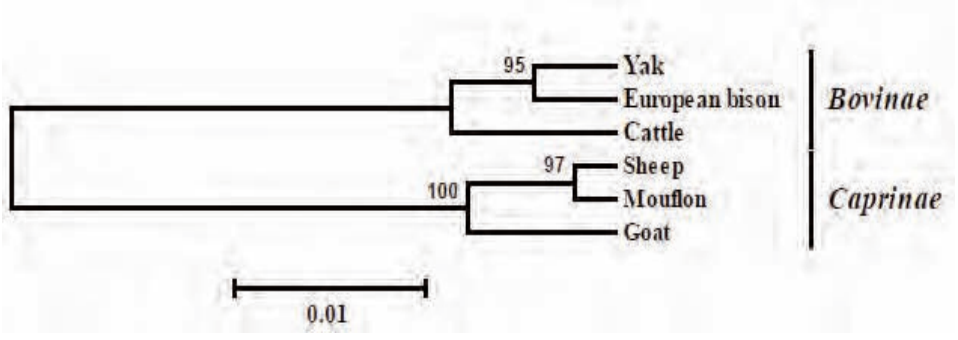

Figure A3
Phylogenetic tree among 6 species of *Bovidae* family constructed by the UPGMA method based on the sequences of P1 promotor and Exon 1A of the *GHR* gene

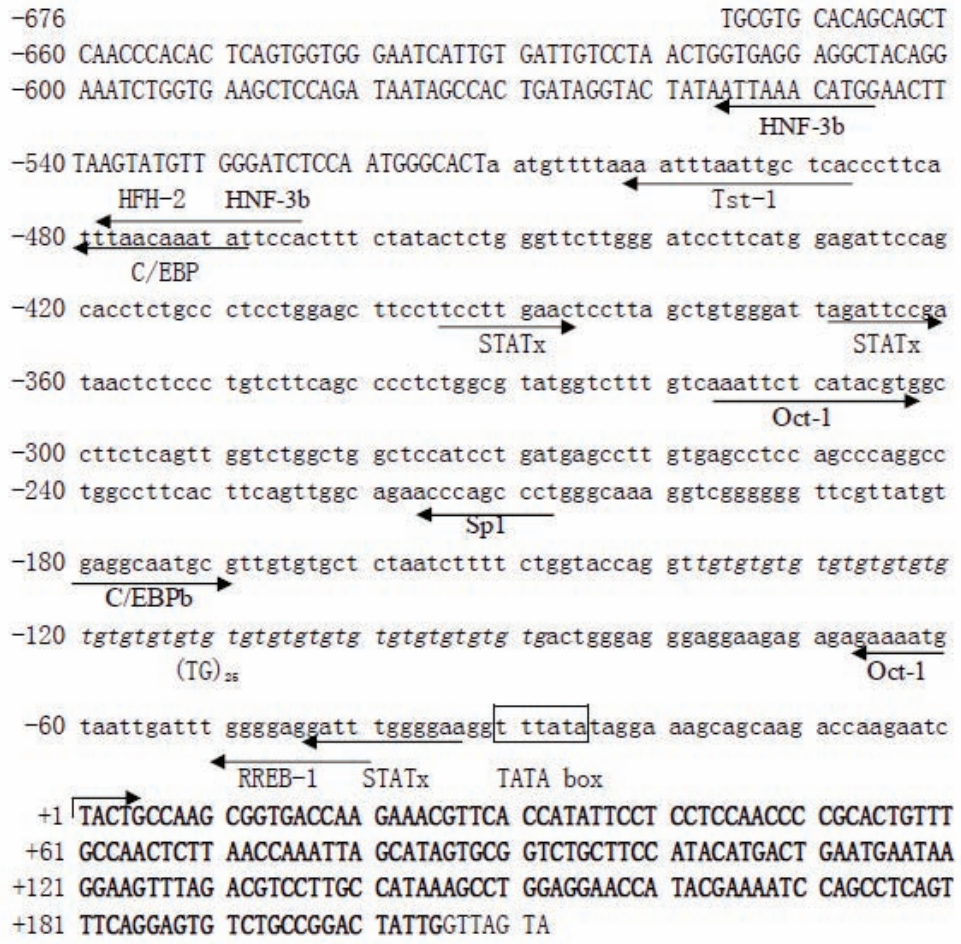

Figure A1
Nucleotide sequence of the 5′ flanking region of the yak *GHR* gene

The promoter P1 is presented in small letters. The Exon 1A is presented in capital and bold letters. The transcription start site is indicated by an arrow and defined as +1. Nucleotide markers are marked on the left side. Upstream nucleotides have negative numbers. Partial potential transcription factor binding sites showed and lined with the arrow noting the orientation. The TATA box is boxed. The $(TG)_{25}$ microsatellite DNA is indicated by italic small letters.

Partial sequence-specific transcription factor, Abbreviation: HNF-3b, hepatic nuclear factor 3 beta; Tst-1, POU-factor; HFH-2, fork head domain factor 2; C/EBP, enhancer binding protein; STATx, signal transducers and activators of transcription; Oct-1, octamer-binding factor 1; Sp1, stimulating protein 1; C/EBPb, enhancer binding protein beta; RREB-1, Ras-responsive element binding protein 1

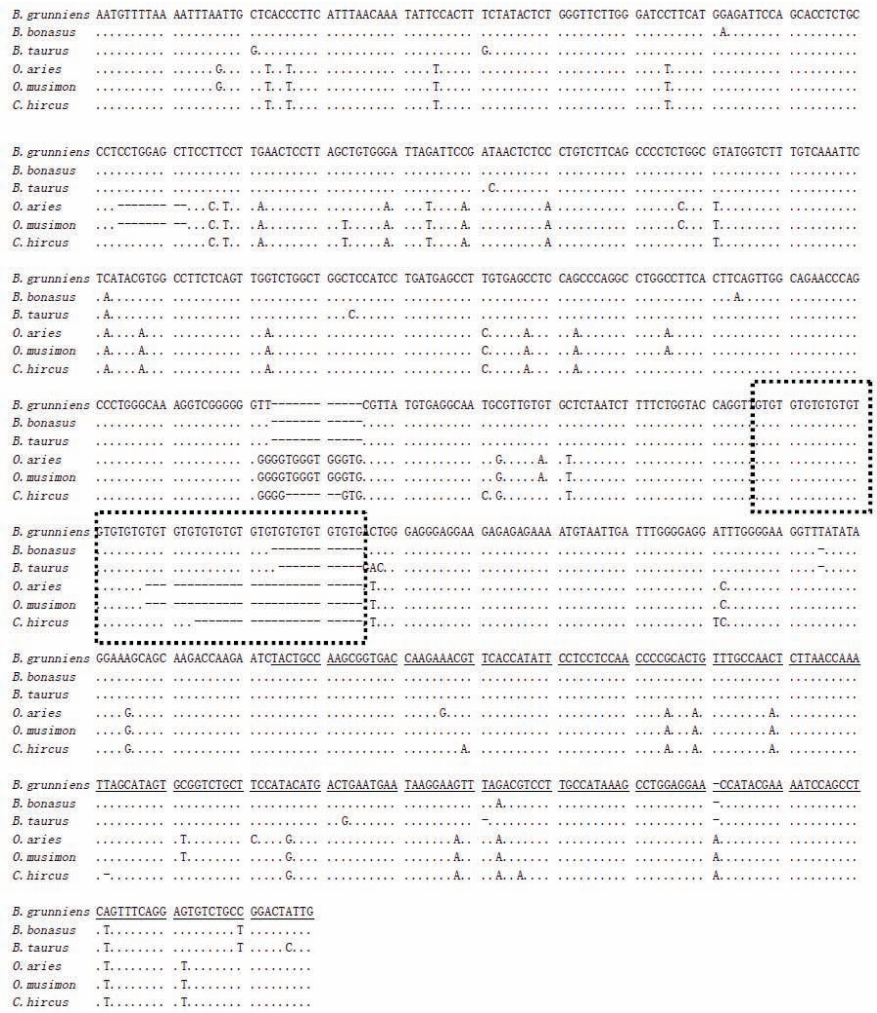

Figure A2
Alignment of the *GHR* promoter P1 and Exon 1A sequences among yak (*B. grunniens*), European bison (*B. bonasus*), cattle (*B. taurus*), sheep (*O. aries*), mouflon (*O. musimon*) and goat (*C. hircus*)

.: Indicates sequence identity with that of the yak, –: Indicates either insertion or deletion
Exon 1A sequence is underlined and the 5′ sequence to Exon 1A is promoter P1. The position of microsatellite loci in 6 species of *Bovidae* family is boxed.

Received 7 January 2010, accepted 21 June 2010.

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