Arch. Tierz., Dummerstorf **50** (2007) 6, 630-631

[1] College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling, Shaanxi 712100, China
[2] Institute of Cellular and Molecular Biology, Xuzhou Normal University, Xuzhou, Jiangsu 221116, China

BAO ZHANG[1], HONG CHEN[1,2], YIKUN GUO[1], LIANGZHI ZHANG[1], LIUSHUAI HUA[1], MIAO ZHAO[1], XIANYONG LAN [1] and CHUZHAO LEI[1]

Five novel single nucleotide polymorphisms (SNPs) of the ghrelin receptor (*GHSR)* gene in cattle (Brief report)

(Fünf neue SNPs im bovinen Ghrelin Rezeptor Gen)

Background: The growth hormone secretagogue receptor (ghrelin receptor, *GHSR*) gene plays an important role in the regulation of food intake and energy homeostasis. The *GHSR* gene lies on human chromosome 3q26 within a quantitative trait locus strongly linked to multiple phenotypes related to obesity and the metabolic syndrome (BAESSLER et al., 2005). In human, the 171T/C SNP mutation is a risk factor for bulimia nervosa (MIYASAKA et al., 2006). The 611 C/A transversion unveils the critical importance of the GHSR-associated constitutive activity, and discloses an unusual pathogenic mechanism of growth failure in humans (PANTEL et al., 2006). By now, no polymorphism of bovine *GHSR* gene is reported. In the present paper, partial 5' untranslated region, coding region and partial 3' untranslated region of *GHSR* were screened to detect the SNPs in Chinese cattle breeds.

Procedures:

Primer sequences:

The primer pairs 1-4 were designed based on bovine *GHSR* gene (GenBank accession No.: LOC514203 and NW_001493715.1).

Exon1	1 F: 5'-CACTCTTTTGCGCCTAACTAA-3';	R: 5'-TCTCGCTGACAAACTGGAAG-3';
	2 F: 5'-TTACCGGCCCTGGAACTTG-3';	R: 5'-CAGCATCTTCACGGTCTGTTTG-3';
Exon2	3 F: 5'-ACTGACGTTCTCTTTCTCATTGT-3';	R: 5'-CCGCTGTACTATGGCTTCTG-3';
	4 F: 5'-AGTACAGCGGAACTTGGGA-3';	R: 5'-ACAGCACTGATCTGGGACC-3';

PCR-SSCP conditions:

The 15 µL PCR amplification contained 50 ng of genomic DNA, 1 µl of each primer (10 pM), 1.50 mM $MgCl_2$, 0.20 mM dNTP, and 0.50 U *Taq* DNA polymerase (TaKaRa, China). The cycling protocol was 5 min at 95°C, 35 cycles of 94°C for 35 s, annealing at 56.3°C, 56.5°C, 63.5°C or 56.5°C corresponding to 4 different primer pairs for 30 s, 72°C for 50 s, with a final extension at 72°C for 10 min. Polymorphism of *GHSR* gene was detected by SSCP in 10% PAGE in constant voltage (200 V) for 2.0-2.5 h after its PCR product was denatured for 10 min at 98°C. The gel was stained with silver nitrate and visualized with 2% NaOH solution (supplied with 0.1% formaldehyde) (PAN et al., 2007). The PCR products from individuals which represented different PCR-SSCP patterns were purified and sequenced.

Results: Five novel SNPs were discovered firstly in *GHSR* gene among 649 unrelated animals which belonged to five cattle breeds in China (Nanyang, 240; Qinchuan, 141; Jiaxian, 133; Chinese Holstein, 61; Jinnan, 30) and an exotic breed (Angus, 44). The discovered SNPs were shown in Table and deposited in GenBank (AccNo.: EU146105-EU146109). According to AccNo.XM_592014, the SNP at nt-7 was in the

5’ untranslated region; the SNPs at nt456 and nt667 were located in exon 1 of the coding sequence, but caused no amino acids exchange. The SNPs at nt456 and nt667 of the *GHSR* gene were always in linkage with GC and AT together, respectively; the SNPs at nt3552 and nt3566 were in the 3’ untranslated region. The five SNPs found in the bovine *GHSR* gene enable to conduct association analyses in order to evaluate these SNP loci as genetic markers for breeding.

Table
SNPs and allele frequencies distribution among six cattle breeds (SNPs und Allelfrequenzen in sechs Rinderrassen)

positions	nt-7	nt456	nt667	nt3552	nt3566
Nanyang cattle	C(0.271)	G(0.889)	C(0.889)	T(0.444)	A(0.722)
	A(0.729)	A(0.111)	T(0.111)	C(0.556)	G(0.278)
Qinchuan cattle	C(0.443)	G(0.855)	C(0.855)	T(0.451)	A(0.774)
	A(0.557)	A(0.145)	T(0.145)	C(0.549)	G(0.226)
Jiaxian cattle	C(0.455)	G(0.891)	C(0.891)	T(0.422)	A(0.678)
	A(0.545)	A(0.109)	T(0.109)	C(0.578)	G(0.322)
Holstein cattle	C(0.159)	G(0.910)	C(0.910)	T(0.497)	A(0.671)
	A(0.841)	A(0.090)	T(0.090)	C(0.503)	G(0.329)
Angus cattle	C(0.442)	G(0.762)	C(0.762)	T(0.440)	A(0.689)
	A(0.558)	A(0.238)	T(0.238)	C(0.560)	G(0.311)
Jinnan cattle	C(0.461)	G(0.903)	C(0.903)	T(0.482)	A(0.777)
	A(0.539)	A(0.097)	T(0.097)	C(0.518)	G(0.223)

The location of the SNP in the sequence XM_592014; the start translation site is “+1”.

Acknowledgements: This study was supported by the National 863 Program of China (No.2006AA10Z197), National Natural Science Foundation of China (No. 30771544), National Key Technology R&D Program (No. 2006BAD01A10-5), Innovative Foundation of Outstanding Talent from Henan Province (No.0521001900), Sustaining Program for Topnotch Persons of Northwest A&F University (No. 01140101), and Natural Science Foundation of Xuzhou Normal University (2003XY234).

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Correspondence to: Hong Chen, chenhong1212@263.net.