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Eleven single nucleotide polymorphisms (SNPs) were found at coding region of hypocretin receptor1 (*HCRTR1)* gene in cattle

(Brief report)

(Elf SNPs in der kodierenden Region des bovinen Hypokretin Rezeptor 1 (*HCRTR1*))

Background: The hypocretin receptor1 (*HCRTR1*) gene encodes a Orexin A receptor (SAKURAI et al., 1998). It belongs to the class I subfamily within the superfamily of G-coupled receptors and is coupled to Ca^{2+} mobilization. Via HCRTR1, Orexin A is involved in the control of feeding, sleep-wakefulness, neuroendocrine homeostasis and autonomic regulation (VOISIN et al., 2003; TAKESHI SAKURAI, 1999). These characters are important in animal production. In human, a 408 isoleucine to valine mutation in *HCRTR1* showed significant association with polydipsic–hyponatremic schizophrenia (MEERABUX et al., 2005). By now, no polymorphisms of the bovine *HCRTR1* gene were reported.

Procedures:

Primer sequences:

exon1, F: 5' AGCCTGGGATGCCCTTCA 3';
R: 5' ACACGGCCAGGCACACCAGTGTGTTGCCCA 3'(T_m=60℃)

exon2, F: 5' TGTGCCTGGCCGTGTGG 3'; R: 5' GACACGGCCTGTAGATAGGGGAT 3'(T_m=63℃)

exon3, F: 5' TCTACAGGCCGTGTCTGTGTCC3'; R: 5' GAGATCATCAGCCCAGCGTTCAT3'(T_m=63℃)

exon4, F:5' TGGGCTGATGATCTCTACCCCAAGAT3'; R:5'AGGGATCTGGCGGCCCCAG 3'(T_m=64℃)

exon5, F:5' CGCCAGATCCCTGGCACCACG3';
R: 5' AAACACCCTCTTGAGGACGTTGAGGACA 3'(T_m=64℃)

exon6, F: 5' CTCAAGAGGGTGTTTGGGATGTTC 3';
R: 5' TTGCCACTGAGGAAGTTGTAGATGAT(T_m=62℃)

exon7, F: 5' CCTCAGTGGCAAGTTCCGGGA 3'; R: 5' TCAGGGCAGCACCGTGGT 3'(T_m=62℃)

In order to develop polymorphic genetic markers for cattle breeding, SNPs were screened in 352 unrelated cattle from three major cattle breeds in China (Nanyang cattle, 122; Qinchuan cattle, 120; Jiaxian cattle, 110) at whole coding region of *HCRTR1* gene. Seven pairs of PCR primers for amplifying seven exons of *HCRTR1* gene were designed according to sequence of *HCRTR1* (AccNo.XM_864404). Polymerase chain reaction (PCR) amplifications were performed in 20μl volume containing 50 ng DNA template, 0.20 mM dNTP, 2.5 mM $MgCl_2$, and 0.5 U *Taq* DNA polymerase (TaKaRa, Dalian, China). The PCR protocol was 94 °C for 5 min followed by 35 cycles of 94°C for 30 s, annealing for 30 s, and 72 °C for 30 s and a final extension at 72°C for 10 min. PCR products were analyzed for single-stranded conformation polymorphisms (SSCP). Aliquots of 5 μl of the PCR products were mixed with 5 μl of the denaturing solution, heated for 10 min at 98°C then chilled on ice. Denatured DNA was subjected to PAGE in TBE buffer and constant voltage (150V) for 1 h. The gel was stained with silver nitrate and visualized with 2% NaOH solution (supplied with 0.1% formaldehyde). The PCR products from individuals

which represented different PCR-SSCP patterns were purified and sequenced by an ABI 377 sequencer in both directions.

Results:

Eleven SNPs were discovered in exons 1, 2, 3 of *HCRTR1* gene among three cattle populations (Table). These SNP loci were found. The discovered SNPs were deposited in GenBank (AccNo.: DQ981401, DQ986909, DQ986910, DQ986911, DQ986912, DQ986913, DQ986914, DQ901743, DQ986915, DQ986916, DQ901742). The alleles at 384, 420 and 423 were in linkage disequilibrium with GTC always together and CCT always together. Things were the same to the SNP loci at 690 and 714. GG were always together, and AA were always together in an individual. At 690 and 714, allele A was discovered at very low frequencies in Nanyang and Jiaxian cattle, but was not found in Qinchuan cattle. The variation at four SNP loci caused amino acid mutation 322: Val to lle; 481: Trp to Arg; 631: Arg to Trp; 736: Arg to Trp, respectively. While variation at the other 7 SNP loci were silent mutations. The SNPs found at the coding region of the *HCRTR1* gene enable to conduct association analyses in order to evaluate these SNP loci as genetic markers for breeding.

Table
SNPs and allele frequencies distributing among three cattle populations

Fragment	Position[1]	Nanyang cattle		Qinchuan cattle		Jiaxian cattle	
		Frequencies		Frequencies		Frequencies	
Exon1	322 bp	A(0.5430)	G(0.4570)	A(0.7210)	G (0.2790)	A(0.9080)	G (0.0920)
	384 bp	G(0.8525)	C(0.1475)	G(0.7881)	C(0.2119)	G(0.8545)	C(0.1455)
	420 bp	T(0.8525)	C(0.1475)	T(0.7881)	C(0.2119)	T(0.8545)	C(0.1455)
Exon2	423 bp	C(0.8525)	T(0.1475)	C(0.7881)	T(0.2119)	C(0.8545)	T(0.1455)
	481 bp	T(0.9221)	A(0.0779)	T(0.9407)	A(0.0593)	T(0.7545)	A(0.2455)
	510 bp	C(0.7254)	A(0.2746)	C(0.7712)	A(0.2288)	C(0.8909)	A(0.1091)
	627 bp	T(0.9526)	C(0.0474)	T(0.9297)	C(0.0703)	T(0.7931)	C(0.2069)
	631 bp	C(0.9158)	T(0.0842)	C(0.9766)	T(0.0234)	C(0.9397)	T(0.0603)
Exon3	690 bp	G(0.9895)	A(0.0105)	G(1.0000)	A(0.0000)	G(0.9828)	A(0.0172)
	714 bp	G(0.9895)	A(0.0105)	G(1.0000)	A(0.0000)	G(0.9828)	A(0.0172)
	736 bp	C(0.7789)	T(0.2211)	C(0.9062)	T(0.0938)	C(0.8621)	T(0.1379)

[1]The location of the SNP in the sequence XM_864404

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