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## **Five novel single nucleotide polymorphisms (SNPs) of the prophet of PIT1 (*PROPI*) gene in bovine**

(Brief report)

(Fünf neue Polymorphismen im bovinen *PROPI* Gen)

**Background:** The prophet of Pit1 (*PROPI*) gene encodes a paired class homeodomain transcription factor of 226 amino acids and is organized of 3 exons. *PROPI* is necessary for the specification, differentiation and proliferation of cells. Its function is essential for anterior pituitary organogenesis, and heritable mutations in this gene are associated with combined pituitary hormone deficiency (CPHD) in human patients and animals (SAVAGE et al., 2003; CARVALHO et al., 2006). To date, no polymorphisms of the bovine *PROPI* gene were described. In the present experiment, the 5' flanking region, the coding region and partial introns of bovine *PROPI* were scanned for single nucleotide polymorphism (SNPs) in five cattle breeds of China.

### **Procedures:**

#### *Primer sequences:*

Based on bovine *PROPI* (Acc. No. AF453512), 3 pairs of primers were designed to amplify the 5' flanking region, exon 1-3 and partial introns.

Exon 1, Forward: 5'- ATCAAGATACCAGGCATAGAGG-3';

Exon 1, Reverse: 5'- CCCAAAGATTCACTCACCC -3';

Exon 2, Forward: 5' -ATGTGGTCTGGGATGGATG-3',

Exon 2, Reverse: 5'- CTGGTGAAGTTTGGGTTAG -3';

Exon 3, Forward: 5'- CTGATGCGGCTCTTCTTC -3';

Exon 3, Reverse: 5'- ACTTTAGTTCCAGGACTTTGG -3'.

#### *PCR conditions:*

The 15  $\mu$ L PCR amplification contained 50 ng of genomic DNA, 10 pM of each primer, dNTPs (0.2 mM), MgCl<sub>2</sub> (1.5 mM), and 0.50 U *Taq* DNA polymerase (MBI). The cycling protocol was 5 min at 95°C, 35 cycles of 94°C for 30 s, annealing at 61.8°C, 52.9°C or 61.3°C corresponding to 3 different primer pairs for 30 s, 72°C for 1 min, with a final extension at 72°C for 10 min. Polymorphism of *PROPI* was detected by SSCP in 10% PAGE in constant voltage (180V) for 1.5-2.5 h after its PCR product was denatured 10 min at 98°C. The gel was stained with silver nitrate and visualized with 2% NaOH solution (supplied with 0.1% formaldehyde) (LAN et al., 2006). The eighteen PCR fragments from different SSCP patterns in five breeds were amplified by the pair of primers (Exon 1), subcloned and sequenced in both directions.

**Results:** Five SNPs were found in 5' flanking region, exon 1 and intron 1 of the *PROPI* gene in 606 unrelated cattle which belonged to five cattle breeds in China

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(Nanyang cattle, 220; Qinchuan cattle, 136; Jiaxian cattle, 144; Holstein, 61; Angus, 45). The distributions of these alleles were showed in the Table. These discovered SNPs were deposited in GenBank (AccNo.:EF518217, EF518218, EF518219, EF518220, EF518221, EF518222, EF518223, EF518224, EF518225, EF518226, EF518227, EF518228, EF518229, EF518230, EF518231, EF518232, EF518233, EF518234). According to Acc.No.AF453512, the alleles of nt349 and nt381 were located in the 5' flanking region (40 and 8 nt upstream of start codon, respectively); the allele of nt457 was located in nt69 of exon 1 and resulted in a silent mutation; the alleles of nt508 and nt511 were located at nt11 and nt14 of intron 1, respectively. At nt508 and nt511, allele A was discovered at low frequencies in Nanyang and Qinchuan and Jiaxian cattle, but was not found in Holstein and Angus cattle. Previous reports showed that mutations in 5' flanking region of candidate genes as well as silent mutations in coding regions and other polymorphisms without obvious functional relevance are useful for evaluation of association with production traits (DYBUS et al., 2006; JEDRZEJCZAK et al., 2006; LAN et al., 2006). Hence, the above described SNPs of *PROPI* will contribute to conducting association analysis and evaluating them as genetic markers for animal breeding and genetics.

Table  
SNPs and allele frequencies of *PROPI* among five cattle populations

positions	nt349		nt381		nt457		nt508		nt511	
	G	A	T	A	T	C	C	A	T	A
Nanyang cattle	0.951	0.049	0.734	0.266	0.734	0.266	0.814	0.186	0.966	0.034
Qinchuan cattle	0.918	0.082	0.631	0.369	0.631	0.369	0.951	0.049	0.975	0.025
Jiaxian cattle	0.969	0.031	0.724	0.276	0.724	0.276	0.806	0.194	0.969	0.031
Holstein cattle	0.967	0.033	0.533	0.467	0.533	0.467	1.000	0.000	1.000	0.000
Angus cattle	0.889	0.111	0.611	0.389	0.611	0.389	1.000	0.000	1.000	0.000

Note: The location of the SNPs in the sequence AF453512

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