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Six novel coding SNPs of the nucleophosmin 1 (*NPM1*) gene and their associations with growth traits in bovine
(Brief Report)

Sechs SNPs in kodierende Abschnitten des Nukleophosmin 1 (*NPM1*) Gens und ihre Assoziation mit Wachstumsmerkmalen beim Rind (Brief Report)

YONGZHEN HUANG[1], ENPING ZHANG[1], ZHUANJIAN LI[1], JING WANG[1], YONGTAO HUAI[1], LIANG MA[1], FUYING CHEN[1], XIANYONG LAN[1], CHUZHAO LEI[1], JUQIANG WANG[3], XINGTANG FANG[2] and HONG CHEN[1]

[1]College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling, Shaanxi, People's Republic of China, [2]Institute of Cellular and Molecular Biology, Xuzhou Normal University, Xuzhou, Jiangsu, People's Republic of China, [3]Research Center of Cattle Engineering Technology in Henan; Zhengzhou Henan, People's Republic of China

Background

The Nucleophosmin 1 (*NPM1*) gene encodes a multifunctional nucleolar phosphoprotein that has crucial roles in the control of different aspects of cell growth and homeostasis, such as ribosome biogenesis, centrosome duplication, cell cycle progression, apoptosis and cell differentiation (GRISENDI *et al.* 2006, NAOE *et al.* 2006). As mutants of *NPM1* gene impact protein synthesis, *NPM1* is an essential protein in mouse development and cell growth (MAGGI *et al.* 2008). The bovine *NPM1* gene contains one exon and locates at chromosome 9. In previous work, the 12-bp deletion was detected in bovine *NPM1* gene coding region. (HUANG *et al.* 2010). In this study, the coding region of bovine *NPM1* gene has been scanned by PCR-SSCP, DNA sequencing and forced PCR-RFLP methods for SNPs in 1 032 individuals belonging to four Chinese cattle breeds. Association of six mutations of *NPM1* gene with growth traits was analyzed.

Procedures

Primer sequences and conditions:

Two pairs of primers P1 and P2 were designed based on bovine *NPM1* gene (GenBank acc. no. NC_007307).

P1 P1-F1: 5′-GCT TCT CTC CCA CAT AAG-3′ (nt75-92)
P1-R1: 5′-CTT CAA CCG TAA GAC CAC-3′ (nt400-417)
P1-R2: 5′-TAC AGA AAT GAA ATA AGA CG-3′ (nt1027-1046)

P2 P2-F1: 5′-CAC CAC CTG TGG TCT TAC-3′ (nt392-409)
P2-R1: 5′-CTT CCT CAT CAA AAT CGT-3′ (nt650-667).

One pair of primers was designed to amplify a 972 bp fragment (checked by DNA sequencing; primers: P1-F1/P1-R2) contain the CDS region (nt109-993) of the bovine *NPM1* gene.

The 15μL PCR amplification contained 30 ng of genomic DNA, 0.20 mM dNTP, 2.5 mM $MgCl_2$, and 0.5 U *Taq* DNA polymerase (MBI). The PCR was performed using the following program: 94°C for 3 min followed by 34 cycles of 94°C for 30 s, annealing at 56.5°C and 56°C corresponding two different pairs of primers for 30 s, 72°C for 30 s and a final extension at 72°C for 10 min.

PCR-SSCP, DNA sequencing and forced PCR-RFLP

Aliquots of 5 μL PCR products were mixed with 5 μL denaturing solution, heated for 10 min at 98°C and chilled on ice. Denatured DNA was subjected to 12% PAGE in constant voltage (200 V) for 2.5~3.0 h. The gel was stained with 0.1% silver nitrate (WANG *et al.* 2009).

P1-F1/P1-R1 and P2-F1/P2-R1 provide two overlapping PCR fragments used to detect polymorphisms by PCR-SSCP. The PCR fragments from different SSCP patterns were selected for sequencing.

Two novel PCR-RFLPs were designed to detect for these polymorphisms. The primers sequences used were:
P1-F2: 5′TGA TGA AAA TGA GCA CCA GAT AT 3′ (*EcoR*V, GAT^ATC; nt216-235)
P2-R2: 5′ GGC AGA ACG CTT TCC AGA TAA GCT 3′ (*Hind*III, A^AGCTT; nt517-540).

The 205 bp fragment of the *NPM1* gene was amplified by P1-F2 and P1-R1 primers. The naturally-occurring ›T‹ nucleotide at nt232 was substituted by an ›A‹ in the forward primer (underlined) in order to introduce a new recognition site (GAT^ATC) for the *EcoR*V (MBI, Vilnius, Lithuania) restriction endonuclease (size: 205 bp; primers: P1-F2/P1-R1). Also, the new reverse P2-R2 primer was designed to produce a new *Hind*III (MBI) restriction site (A^AGCTT) in PCR products (size: 149bp; primers: P2-F1/P2-R2) by the same method. Thus, the amplification contains two recognition sites for the detection of SNPs. Then, aliquots of 10 μL PCR products were digested with 10 U *EcoR*V and *Hind*III for 6 h at 37°C, respectively. The digested products were detected by electrophoresis in 3.0% agarose gel stained with ethidium bromide, respectively.

Results

SNPs were detected in the exon in 1 032 unrelated cattle from four cattle breeds in China (Nanyang – 262, Qinchuan – 235, Jiaxian – 441, Chinese Holstein - 94). Comparison between the nucleotide sequences of the bovine *NPM1* gene and the above sequences revealed six mutations (GenBank acc no. NC_007307: g.236C>G, 489G>A, 516G>A, 624T>C, 630T>C, 632A>C). Moreover, the SNPs at nt489, nt516, nt624, nt630 and nt632 are in complete linkage disequilibrium: with GGT TA always together and AAC CC always together. The discovered sequence of the first missense mutation (236C>G) was deposited in GenBank (acc no. GQ144334; size: 593 bp; primers: P1-F1/P2-R1), the complete linkage of five SNPs were deposited in GenBank (acc no. FJ794270; size: 972 bp; primers: P1-F1/P1-R2). The variation at two SNP loci caused amino acid mutation 236C>G: Ser to Cys; 632A>C: Glu to Asp, respectively. While variation at the other four SNPs loci were synonymous mutations. Two SNPs (236C>G and 516G>A) were genotyped among four cattle populations by forced PCR-RFLP methods. The genotypic frequencies of two SNP loci in the Chinese Holstein population agreed with Hardy-Weinberg equilibrium

(P>0.05) (Table 1). The association of six mutations with body weight and average daily gain of Nanyang and Jiaxian were analyzed together. The statistical model:

$$Y_{ijkl} = \mu + A_i + G_j + e_{ijk} \quad (1)$$

where Y_{ijk} is the trait measured on each of the *ijk*-th animal, μ is the overall population mean, A_i is the fixed effect due to the *i*-th age, G_j is the fixed effect associated with *j*-th genotype and e_{ijk} is the random error. The least square means estimates (LSM) with standard errors for three genotypes and growth traits were used. The result indicated that the first missense mutation (236C>G) genotype was significantly associated with body weight and average daily gain. The individuals with GG genotype had higher body weight and average daily gain than the individuals with CG and CC genotypes (P<0.05) (Table 2). However, the five linked mutations (489G>A, 516G>A, 624T>C, 630T>C, 632A>C) were not statistically significantly associated with growth traits (P>0.05).

Table 1
Genotype distribution and allelic frequencies at *NPM1* gene in cattle
Genotypen-Verteilung und relative Allelehäufigkeit des NPM1-Gens beim Rind

SNP/Amino acid change	Breeds	Observed Genotypes				Allelic frequencies		χ^2
		CC	CG	GG	Total	C	G	(HWE)
236C>G/Ser43Cys	NY	170	63	29	262	0.769	0.231	P<0.05
	QC	184	36	15	235	0.86	0.14	P<0.05
	JX	257	123	61	441	0.722	0.278	P<0.05
	CH	81	11	2	94	0.92	0.08	P>0.05
		WW	WM	MM		W	M	
516G>A/Leu136Leu	NY	128	84	50	262	0.649	0.351	P<0.05
	QC	119	86	30	235	0.689	0.311	P>0.05
	JX	264	107	70	441	0.72	0.28	P<0.05
	CH	88	6	0	94	0.968	0.032	P>0.05

χ2 (HWE) Hardy-Weinberg equilibrium χ2 value, P>0.05 showed that the SNP locus in the population was at Hardy-Weinberg equilibrium. NY Nanyang breed, QC QinChuan breed, JX Jiaxian breed, CH Chinese Holstein breed

Table 2
Associations of different genotypes within the NPM1 gene with growth traits in cattle
Genotypen-Verteilung und relative Allelehäufigkeit des NPM1 beim Rind

Breeds	Ages	Traits	Genotypes at *NPM1* gene (236C>G)			*P*-value
			CC (Mean ± SE)	CG (Mean ± SE)	GG (Mean ± SE)	
JX	6 months	BW, kg	161.90 ± 8.82^{a}	167.59 ± 5.12^{a}	182.63 ± 4.21^{b}	0.027
		ADG, g	747.00 ± 48.00^{a}	775.00 ± 28.00^{a}	857.00 ± 23.00^{b}	0.029
NY	18 months	BW, kg	284.07 ± 7.66^{a}	293.80 ± 4.84^{ab}	305.80 ± 4.05^{b}	0.024

JX Jiaxian breed, NY Nanyang breed, BW body weight, ADG average daily gain, mean least square means, SE standard error of the means, Values with different superscripts within the same line differ significantly at ^{a,b} P<0.05.

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Corresponding author:

HONG CHEN

email: chenhong1212@263.net

College of Animal Science and Technology, Northwest A&F University, Yangling, No. 22 Xinong Road, Shaanxi 712100, People's Republic of China