

Association of polymorphisms of the *DCN* gene with growth traits in cattle (Brief Report)

Assoziation des *DCN*-Gens mit Wachstumsmerkmalen beim Rind (Brief Report)

JIAJIE SUN^{1*}, CHUANWEN GU^{1*}, CHUNLEI ZHANG^{1*}, CHUZHAO LEI², XINGTANG FANG¹, QIJANG JIN¹, DANXIA CHEN¹, XIUYING SHI¹, YU DU¹ and HONG CHEN^{1,2}

¹Institute of Cellular and Molecular Biology, Xuzhou Normal University, Xuzhou, People's Republic of China,

²College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling, Shaanxi, People's Republic of China

Background

Mammalian decorin (*DCN*) consists of a protein core and a single dermatan or chondroitin sulfate glycosaminoglycan chain (CHOPRA *et al.* 1985), contributing multifunctionally to processes like matrix assembly, modulation of the activity of growth factors and cell migration and proliferation. Recently, LINDA *et al.* (2008) have reported that eight SNPs were identified in human. However, the related information in bovine is scarce. Hence, in the present experiment the exons and intron/exon boundaries of *DCN* were scanned for SNPs in the predominant cattle breeds of China.

Procedure

Based on bovine *DCN* (GenBank acc. no. NC_007303), ten pairs of primers were designed to amplify the exons and intron/exon boundaries.

D ₁ : F 5'-GGA GTA GAA GCA GGA GGT-3' R 5'-CCA AAT ACT TCG TTT CTG T-3'	D ₂ : F 5'-CAC ATA CAT TAG GCA AGG C-3' R 5'-TCA CCC AGA TCA GAA CACT-3'
D ₃ : F 5'-TTT AAT GAC TGC GTG TTG CT-3' R 5'-GCT TTA CTC CAT CAC TCC CT-3'	D ₄ : F 5'-GAT GTT GCT TCT GTT CAC TA-3' R 5'-GAT TCA ATA CCC ATT TCT CC-3'
D ₅ : F 5'-GCA GTT TCC TCA GGT TGT CC-3' R 5'-ACC CGT GGC TGA TTC AAG TC-3'	D ₆ : F 5'-ATG TAT TAT TGT AAA AGG GAT G-3' R 5'-CAG CAG AAG TTT GTG GTT-3'
D ₇ : F 5'-AAT CAC ATT AGG CAG AGG T-3' R 5'-CAC AGT AGG TAG TGG CTT T-3'	D ₈ : F 5'-GTT CAC CTG TAC GGT CTC C-3' R 5'-AAC TGC ATA TTT GGC TTT A-3'
D ₉ : F 5'-TTT CCC ACA TGA CTT ATT-3' R 5'-TTA CAT AGC CTG GAT TGA-3'	D ₁₀ : F 5'-TAA CCA TGT GCC ATT ATT-3' R 5'-ATA AGT CAT GTG GGA AAA-3'

A 15 µL reaction mixture contained 50 ng genomic DNA, 0.2 µM of each primer, 1.5 mM MgCl₂, 0.2 mM dNTPs, and 0.60 U Taq DNA polymerase (MBI). The cycling protocol was 5 min at 94°C, 32 cycles of denaturing at 94°C for 45 s, annealing at X°C for 1 min, extension at 72°C for 45 s, with a final extension at 72°C for 10 min (X was 65, 51, 64, 65, 68, 61, 60, 56, 61 and 65 for D₁, D₂, D₃, D₄, D₅, D₆, D₇, D₈, D₉ and D₁₀ primers, respectively). The SSCP were analyzed according to previous description (PAN *et al.* 2007). The PCR products from individuals which represented different PCR-SSCP patterns were purified and sequenced.

Results

SSCP polymorphisms were detected in D_2 , D_3 , D_5 and D_9 fragments of the bovine *DCN* gene in 408 individuals (without genetic relationships) belonging to three Chinese genetic types: 68 Qinchuan cattle, 240 Nanyang cattle and 100 Jiaxian cattle. In the D_2 fragment, five SSCP genotypes were identified and designated as genotype A_2A_2 , A_2B_2 , B_2B_2 , A_2C_2 and B_2C_2 , respectively. The sequencing analysis of the five genotypes revealed two SNPs: g.[4204A>G, 4264A>G] in exon2 and they formed three consistent haplotypes: $A_2(AG)$, $B_2(GA)$ and $C_2(AA)$, respectively. Genotype A_2A_2 and haplotype A_2 were more prevalent and only Nanyang cattle significantly deviated from the Hardy-Weinberg equilibrium ($P<0.01$). In the D_3 fragment, three SSCP genotypes were identified and named as genotype A_3A_3 , A_3B_3 and B_3B_3 , respectively. Only Qingchuan cattle were in Hardy-Weinberg equilibrium ($P>0.05$). The sequence analysis revealed three SNPs g.[16476A>G, 16501C>A, 16503C>T]. Through sequence comparison, haplotypes $A_3(GAT)$ and $B_3(ACC)$ were detected, respectively. In the D_5 locus, two unique SSCP banding patterns were observed and denominated as A_5A_5 and A_5B_5 genotypes. A_5 genotypes were predominant and only Nanyang cattle deviated from Hardy-Weinberg equilibrium ($P<0.01$). The sequence analysis of the two genotypes revealed three SNPs g.[24200C>A, 24259T>G, 24285G>A]. They formed two consistent haplotypes $A_5(CTG)$ and $B_5(AGA)$. Three SSCP genotypes were identified in the D_9 fragment, which were denoted as genotype A_9A_9 , A_9B_9 and B_9B_9 , respectively. A_9 genotypes were more prevalent and genotype C_9 was only detected in the Jiaxian cattle. Consistent with the D_2 and D_5 fragments, only Nanyang cattle deviated from Hardy-Weinberg equilibrium ($P<0.05$). By sequence analysis of the three genotypes a SNP g.38655G>A was identified (alleles A_9 and B_9 , respectively). The discovered sequences of alleles were deposited in GenBank (acc no. GQ249670 ~ GQ249678). The relationships between polymorphic loci and growth traits in Nanyang cattle at 12 months old were analyzed by ANOVA using the following model:

$$Y_{ijkl} = \mu + breed_i + age_j + marker_k + e_{ijk} \quad (1)$$

where Y_{ijk} is the observation of the trait, μ is the least square mean, $breed_i$ is the effect of breed, age_j is the effect of age, $marker_k$ is the effect of marker genotype and e_{ijk} is the residual effect. Significant differences between polymorphic loci and birth weight were identified and genotype A_2 , A_3 , A_5 and A_9 were obviously associated with higher values than the others ($P<0.01$) (Table 1). Hence, we firstly suggested that *DCN* gene could be regarded as molecular marker for superior birth weight.

Acknowledgements

This study was supported by the National 863 Program of China (No. 2006AA10Z197, 2008AA101010), National Natural Science Foundation of China (No.30771544, 30972080), National Key Technology R&D Program (No. 2006BAD01A10-5), Keystone Project of transgene in China (2009ZX08009-157B, 2008ZX08007-002), »13115« Sci-Tech Innovation Program of Shaanxi Province (2008ZDKG-11), MaTS-Beef Cattle System, Basic and Foreland Technology Study Program of Henan Province (No.072300430160), Natural science fund for colleges and universities in Jiangsu Province (09KJD180002).

Table 1

Least square mean (means \pm standard error of means) of growth traits for the genotypes of polymorphic loci
LSM (Mittel \pm Standardfehler) der Wachstumsmerkmale in Abhängigkeit von DCN

	G (N)	BiW, kg	BoW, kg	BH, cm	BL, cm	ChC, cm	HW, cm	ADG, kg
D ₂	A ₂ A ₂ (156)	29.2 \pm 0.2 ^A	222.6 \pm 1.8	114.1 \pm 0.3	116.8 \pm 0.6	140.8 \pm 0.6	20.6 \pm 0.1	0.54 \pm 4.8
	A ₂ B ₂ (72)	29.1 \pm 0.4 ^A	226.4 \pm 3.0	114.3 \pm 0.5	118.2 \pm 1.0	143.0 \pm 1.0	20.9 \pm 0.2	0.55 \pm 8.0
	B ₂ B ₂ (5)	25.6 \pm 0.7 ^B	228.0 \pm 7.1	115.4 \pm 0.7	120.6 \pm 3.0	142.0 \pm 3.1	20.3 \pm 1.2	0.56 \pm 21.2
	A ₂ C ₂ (2)	24.8 \pm 1.3 ^B	242.0 \pm 18	115.0 \pm 1.0	126.0 \pm 8.5	148.0 \pm 2.0	21.0 \pm 1.0	0.60 \pm 53.5
	B ₂ C ₂ (5)	25.2 \pm 0.6 ^B	223.4 \pm 7.4	111.6 \pm 1.6	118.6 \pm 2.2	146.4 \pm 4.9	20.8 \pm 0.6	0.56 \pm 20.2
	P	0.001	0.603	0.567	0.230	0.123	0.712	0.386
D ₃	A ₃ A ₃ (118)	29.1 \pm 0.3 ^A	222.8 \pm 2.1	113.9 \pm 0.4	116.6 \pm 0.7	140.7 \pm 0.7	20.6 \pm 0.1	0.54 \pm 5.6
	A ₃ B ₃ (102)	29.5 \pm 0.3 ^A	224.1 \pm 2.3	114.4 \pm 0.4	117.8 \pm 0.7	142.5 \pm 0.7	20.8 \pm 0.2	0.54 \pm 6.2
	B ₃ B ₃ (20)	25.6 \pm 0.4 ^B	230.8 \pm 5.1	114.2 \pm 0.7	120.5 \pm 1.8	143.2 \pm 2.0	20.7 \pm 0.4	0.57 \pm 14.5
	P	<0.001	0.352	0.526	0.075	0.150	0.706	0.098
D ₅	A ₅ A ₅ (170)	29.2 \pm 0.2 ^A	222.3 \pm 1.7	113.9 \pm 0.3	116.5 \pm 0.5	140.6 \pm 0.6	20.6 \pm 0.1	0.54 \pm 4.5
	A ₅ B ₅ (70)	28.4 \pm 0.4 ^B	228.3 \pm 3.0	114.6 \pm 0.5	119.7 \pm 1.0	144.3 \pm 1.0	21.0 \pm 0.2	0.56 \pm 8.1
	P	0.007	0.158	0.790	0.203	0.412	0.104	0.237
D ₉	A ₉ A ₉ (185)	29.4 \pm 0.2 ^A	222.7 \pm 1.6	114.0 \pm 0.3	116.6 \pm 0.5	140.8 \pm 0.6	20.7 \pm 0.1	0.54 \pm 4.4
	A ₉ B ₉ (55)	27.7 \pm 0.4 ^B	228.6 \pm 3.3	114.7 \pm 0.5	120.1 \pm 1.1	144.4 \pm 1.0	20.9 \pm 0.2	0.56 \pm 8.9
	P	<0.001	0.557	0.899	0.372	0.732	0.268	0.648

G (N) genotype (observed number), BiW birth weight, BoW body weight, BH body height, BL body length, ChC chest circumference, HW hucklebone width, ADG average day gain. Treatments with different capital letters are significantly different at $P < 0.01$.

References

- Chopra RK, Pearson CH, Pringle GA, Fackre DS, Scott PG (1985) Dermatan sulphate is located on serine-4 of bovine skin proteodermatan sulphate. *Biochem J* 232, 277-9
- Linda EK, Fergus JC, Shahana A, Alison MD (2008) Genetic variation in stromal proteins decorin and lumican with breast cancer: investigations in two case-control studies. *Breast Cancer Res* 10, R98
- Pan CY, Lan XY, Chen H, Hua LS, Guo YK, Zhang B, Lei CZ (2007) Five novel single nucleotide polymorphisms (SNPs) of the prophet of *PIT1* (*PROPT*) gene in bovine. *Arch Tierz* 50, 421-3

Received 30 November 2009, accepted 5 January 2010.

Corresponding author:

HONG CHEN

email: chen hong1212@263.net

Institute of Cellular and Molecular Biology, Xuzhou Normal University, Shanghai Road No. 101, Xuzhou, People's Republic of China