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Impacts of single nucleotide polymorphisms and haplotypes in the bovine *Dapper1* gene on body weight

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ABSTRACT. The Dapper1 protein plays important role in multiple developmental processes via negative modulation of the Wnt signaling pathway. We detected variations in *Dapper1* in 1185 individuals from 5 Chinese cattle breeds and determined their effects on bovine body weight. Two silent mutations (g.8344C>T and g.8428C>T) in exon 6 along with two substitutions (g.10513A>G and g.10765C>G) in the 3'-untranslated region were detected with DNA pool sequencing and forced polymerase chain reaction-restriction fragment length polymorphism. Haplotype variability and the extent of linkage disequilibrium of the 4 single nucleotide polymorphisms (SNPs) were analyzed, and the results revealed 16 haplotypes and 7 combined haplotypes in the 5 cattle breeds. Statistical analyses indicated that genotypes CC and AA in the g.8344C>T and g.10513A>G loci were associated with heavier body

weight at 6 months in the Nanyang cattle population ($P < 0.05$), and the combined haplotype had consistent significant effects on body weight with a single SNP. Cattle with haplotype combinations H1H5 (CCCTAACC) displayed the heaviest body weight at 6 months compared with that of other haplotypes ($P < 0.05$). Our results provide evidence that 4 SNPs and haplotypes in *Dapper1* may be used for marker-assisted selection in beef cattle breeding programs.

Key words: Cattle; *Dapper1* gene; SNPs; Haplotype; F-PCR-RFLP; Body weight

INTRODUCTION

The Wnt signaling pathway includes canonical (Wnt/β-catenin) (Cerpa et al., 2009; Tee et al., 2009; Fukuda et al., 2010) and noncanonical (Wnt/planar cell polarity pathway and Wnt/Ca^{2+} channel) (Dale et al., 2009) pathways. The signaling pathway plays an important role in embryogenesis by regulating the expression of genes involved in cell proliferation, differentiation, and survival (Gao et al., 2008). The pathway also acts as a molecular switch that governs adipogenesis, which maintains preadipocytes in an undifferentiated state through the inhibition of adipogenic transcription factors (Kawai et al., 2007).

The Dapper family, originally identified as a Dvl-interacting protein, has been shown to inhibit both Dvl-mediated canonical and noncanonical Wnt pathways and is required for formation of the notochord and head structures in *Xenopus* embryos (Cheyette et al., 2002; Gloy et al., 2002). The Dapper family has three orthologs, *Dapper1*, *Dapper2*, and *Dapper3* (Katoh and Katoh, 2003; Waxman et al., 2004; Fisher et al., 2006; Zhang et al., 2006), which are conserved in the N-terminal leucine zipper and the C-terminal serine-rich domain. The functions of the ortholog proteins are conserved from fish to mammals (Zhang et al., 2006; Su et al., 2007).

The zebrafish *Dapper1* gene participates in distinct Wnt-dependent developmental processes (Waxman et al., 2004). The Dapper1 protein plays a regulatory role in embryonic and adult tissues by modulating Wnt signaling and transforming growth factor-beta signaling. Computer analysis has predicted that both *Dapper1* and *Dapper2* are involved in the formation of certain tumors (Katoh and Katoh, 2003). Mammalian *Dapper1* inhibits the expression of the Wnt-responsive reporters lymphoid enhancer factor-luciferase and TOPFLASH-luciferase. Considering its role in the Wnt pathway, which is important for embryogenesis, tissue homeostasis, and adipogenesis in adults (Gao et al., 2008), *Dapper1* seems to be a functional candidate gene associated with embryo development, lipid metabolism, and tumor formation, thereby affecting bovine growth performance traits such as survival rate, health status, energy intake, and body weight. However, few studies have investigated the genetic variants and function of bovine *Dapper1*.

In this study, we systematically screened the mutations in all exons, including partial introns and the 3'-untranslated region (UTR) of *Dapper1* in 5 Chinese cattle breeds with DNA pool sequencing. We then genotyped the allele frequencies using forced-polymerase chain reaction-restriction fragment length polymorphism (F-PCR-RFLP) to evaluate genetic diversity, haplotype variability, and the extent of linkage disequilibrium (LD).

In the Nanyang breed, we tested the effects of these variants on BW, which may contribute to advances in animal breeding and genetics.

MATERIAL AND METHODS

DNA samples and data collection

All procedures involving animals were approved by the Animal Care and Use Committee at the institution at which the experiment was conducted. Blood samples of 1185 unrelated female cattle were obtained from 5 breeds of Chinese bovine: Jiaxian red (JX, N = 355), Chinese Caoyuan red (CY, N = 235), Qinchuan (QC, N = 216), Nanyang (NY, N = 213), and Luxi (LX, N = 166). These breeds are important for beef production in China and are reared in the Provinces of Henan, Jilin, Shaanxi, and Shandong. The JX animals were from a breeding farm of JX cattle (Jiaxian county, Henan Province, China); the CY animals were from a breeding farm of CY red cattle (Tongyu city, Jilin Province); the QC animals were from a breeding farm and fineness breeding center of QC cattle (Fufeng county, Shaanxi Province); the NY animals were from a breeding farm of NY cattle (Nanyang city, Henan Province); and the LX animals were from a reserved farm (Juancheng county and Jining city, Shandong Province).

Blood samples were obtained via jugular venipuncture using vacuum tubes treated with 0.25% ethylenediaminetetraacetic acid. Genomic DNA were isolated from blood samples following a procedure published by Mullenbach et al. (1989) and stored at -80°C. Additionally, we quantified the body weight (BW) of 213 NY animals. These animals were weaned at an average age of 6 months and were fed a corn-corn silage diet from weaning to slaughter, and straw at 24 months of age. The traits under study were BW at birth, 6 months (BW6), 12 months (BW12), 18 months (BW18), and 24 months (BW24).

Primer design, PCR amplification, and DNA pool analysis

Seven pairs of PCR primers (P1-P7; see Table 1) were designed to amplify all exons (1-6) and the 3'-UTR of bovine *Dapper1* (GenBank accession No. NC_007308.4), including its intron-exon boundaries and the proximal flanking regions.

The 25-μL PCR volume contained 50 ng genomic DNA, 0.5 μM of each primer, 1X buffer (including 1.5 mM $MgCl_2$), 200 μM deoxyribonucleotide triphosphates (2'-deoxyadenosine triphosphate, 2'-deoxythymidine triphosphate, 2'-deoxycytidine 5'-triphosphate, and 2'-deoxyguanosine 5'-triphosphate) and 0.625 U *Taq* DNA polymerase (MBI, Vilnius, Lithuania). The cycling protocol was 5 min at 95°C, 34 cycles of 94°C for 30 s, annealing for 30 s, and 72°C for 35 s (see Table 1), with a final extension at 72°C for 10 min.

Fifty DNA samples from each of the 5 cattle breeds were selected to construct DNA pools. The selected DNA was diluted to a standard concentration, and individual DNA aliquots were transferred to a single tube to ensure that a constant amount of each sample was transferred to the pool. The pool was then mixed gently and quantitated before further dilution to a working concentration of 50 ng/μL. After amplifying the bovine genomic DNA pool (Sham et al., 2002), we sequenced PCR products using an ABI PRISM 377 DNA sequencer (Shanghai Sangon Biotech Co., Ltd., P.R. China) and then analyzed them with the BioXM version 2.6 software.

Table 1. Primers for PCR amplification and the four single nucleotide polymorphisms detected in the bovine *Dapper1* gene.

Loci	Position NC_007308.4	Sequence (5'-3')*	Ta (°C)	SAF (bp)	Restriction enzyme	Location	PCR-RFLP pattern (bp)
P1	1-177	F: ATGGTGGGCTGCAACTCGGTC R: CACCCAGTTTTTGCCTCGCTTC	62	177		Exon 1	
P2	386-580	F: CGCCGGGCAGGGCGTTATCAG R: AGGAGCGGGCGCTAGGGCTG	58	195		Exon 2	
P3	619-1065	F: GACCGGGTTGAGGGCCATGAAG R: TAGAGGCACCCACCACGGACCAC	60	447		Exon 3	
P4	3156-3446	F: CCTTTCTCTGTTTCTTCCTCATC R: TGTCTTGTTTTGCTGTAGTCTCTC	64	291		Exon 4	
P5	4136-4725	F: GGGTCAAGGAGGAAGGTCA R: TAGAAGGGTGGGATGGTGT	59	590		Exon 5	
P6	8231-8869	F: ACACCACAATCTAATTCCCTTGC R: CTTACCTTGAAGTTCGGTAGCAG	66	639		Exon 6	
P7	10266-10934	F: GGTGGTGACAGTGAGTGGA R: TTGGGCTAATGTTTAGATGG	65	669		Partial 3'-UTR	
P8 (g.8344C>T)	8213-8371	F: GTTGACTGTTCCCCCTCCACACCAC R: GAGGAACATTGGGCTCTGCACGGCC**CC**	64	159	*Msp*I C^CGG	Exon 6	TT: 159 CT: 159, 131, 28 CC: 131, 28
P9 (g.8428C>T)	8402-8612	F: GAGGAGCGGCTTGGTAACCATG**TT**AA R: CAGCGTTCACACTGGTCCTCGG	66	211	*Hin*dII GTT^AAC	Exon 6	TT: 211 CT: 211, 187, 24 CC: 187, 24
P10 (g.10513A>G)	10488-10693	F: TGTGAAGCAGATACAAGGGGCAG**CC** R: GTGGCAAAGGTTTTAGCGAATCC	63	206	*Nco*I C^CATGG	3'-UTR	GG: 206 AG: 206, 183, 23 AA: 183, 23
P11 (g.10765C>G)	10740-10981	F: TACCCAACATTGATGCCTTTTTC**GC** R: CAAGACAGGGTCAGTGGTCCAATC	65	242	*Hha*I GCG^C	3'-UTR	CC: 242 CG: 242, 219, 23 GG: 219, 23

Primers P1-P7 were used to amplify the different exons and 3'-UTR of the bovine *Dapper1* gene to detected the mutations. The last 4 pairs of primers (P8-P11) were used for detection of the 4 SNPs in the bovine *Dapper1* gene. Ta = annealing temperature; SAF = size of amplification fragment. *Nucleotides that are in bold stand for the naturally occurring nucleotides that were substituted with primers (P8-R, P9-F, P10-F, P11-F) to introduce a new recognition restriction endonuclease site. Underlined nucleotides mark recognition sites for the restriction endonuclease.

F-PCR-RFLP

F-PCR-RFLP was used to detect polymorphism sites, which introduced a point mutation into one of the primer sequences so that the PCR product contained a restriction endonuclease recognition site. Four pairs of new primers (P8-P11; see Table 1) were designed to detect four mutations through F-PCR-RFLP. Primers, selected restriction enzymes (MBI Fermentas, Vilnius, Lithuania), and fragment sizes are given in Table 1.

Aliquots of 10 µL PCR products were digested with 10 U *Msp*I, *Hin*dII, *Nco*I, and *Hha*I for 8 h at 37°C, following supplier instructions. The digested products were detected with electrophoresis on 2.5% agarose gel stained with ethidium bromide. The detection results of genotypic variation at the SNPs were based on the electrophoretic pattern of the restriction enzyme-treated PCR products.

Statistical analysis

Genotypic and allelic frequencies of polymorphism sites in bovine *Dapper1* among Chinese cattle populations were analyzed using the chi-square test performed with the SPSS version 18.0

software. Gene homozygosity (H_O), heterozygosity (H_E), effective allele numbers (N_E), and polymorphism information content (PIC) were evaluated using Nei's methods (Nei and Roychoudhury, 1974).

The LD structure determination was performed with the HAPLOVIEW version 3.32 software (Barrett et al., 2005), which measured *D*' and r^2. Some research has shown that r^2 is not as sensitive as *D*' to allele frequencies; therefore, r^2 was used as a pairwise measure of LD in our analysis (Zhao et al., 2007; Marty et al., 2010). Haplotypes were obtained for each animal using the PHASE version 2.1 computer program (Stephens et al., 2001). Association of individual SNP genotypes with BW in NY cattle were analyzed with the general linear model using SPSS version 16.0 according to the following equation:

$$Y_{ijk} = \mu + A_i + G_j + E_{ijk},$$

where Y_{ijk} is the trait measured on each 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 the j^{th} genotype; and E_{ijk} is the random error. In this model, age and marker genotypes were considered fixed effects; dam (litter), a random effect; and BW, the dependent variable.

Association analyses between the combined haplotypes of four SNPs and BW were carried out to explore the possible interaction between the SNPs. The model was similar to that of individual SNP association analysis except that the interaction between two SNPs was included as a fixed effect.

RESULTS

SNP detection in bovine *Dapper1*

Bovine *Dapper 1* was located at chromosome 10, encoding 907 amino acids (aa) that included 6 exons. In the present study, polymorphisms of all the exons, partial introns, and the 3'-UTR in bovine *Dapper1* were identified through DNA pool sequencing. Four SNPs were identified; two in exon 6 (NC_007308.4: g.8344C>T, g.8428C>T) and two in the 3'-UTR (g.10513A>G, g.10765C>G; Figures 1-4). The former two SNPs revealed two synonymous mutations: the g.8344C>T mutation GCC (Ala)>GCT (Ala) at position 340 aa and the g.8428C>T mutation AAC (Asn)>AAT (Asn) at position 368 aa. These four SNPs were novel and were deposited in the GenBank database (Table 2).

F-PCR-RFLP was successfully applied to detect the genotypic frequency of these *Dapper1* SNPs in 5 Chinese cattle breeds, and these SNPs were named according to the restriction endonuclease used in the detection: SNP1-*Msp*I locus (g.8344C>T), SNP2-*Hin*dII locus (g.8428C>T), SNP3-*Nco*I (g.10513A>G), and SNP4-*Hha*I locus (g.10765C>G).

Genetic diversity analysis

The genotype and allele frequencies, H_O, H_E, N_E, and PIC were evaluated in the 5 Chinese cattle populations (Table 3). For the SNP1-*Msp*I, SNP2-*Hin*dII, and SNP3-*Nco*I loci, the frequencies of alleles T, C, and G were higher than those of other alleles in JX, QC, NY, and LX cattle. On the contrary, in CY cattle, the frequencies of alleles C, T, and A were higher in these three loci. At the last locus (SNP4-*Hha*I), the frequency of allele C was highest in all five

breeds. The values of H_E approached 0.5 at the SNP1-*Msp*I and SNP3-*Nco*I loci; in the SNP2-*Hin*dII locus, only JX cattle displayed a lower H_E value (0.280), and in the SNP4-*Hha*I locus, CY cattle had the lowest H_E value (0.070). The values of N_E approached 2 in all five breeds, except in CY cattle in which the value at the SNP4-*Hha*I locus was 1.075.

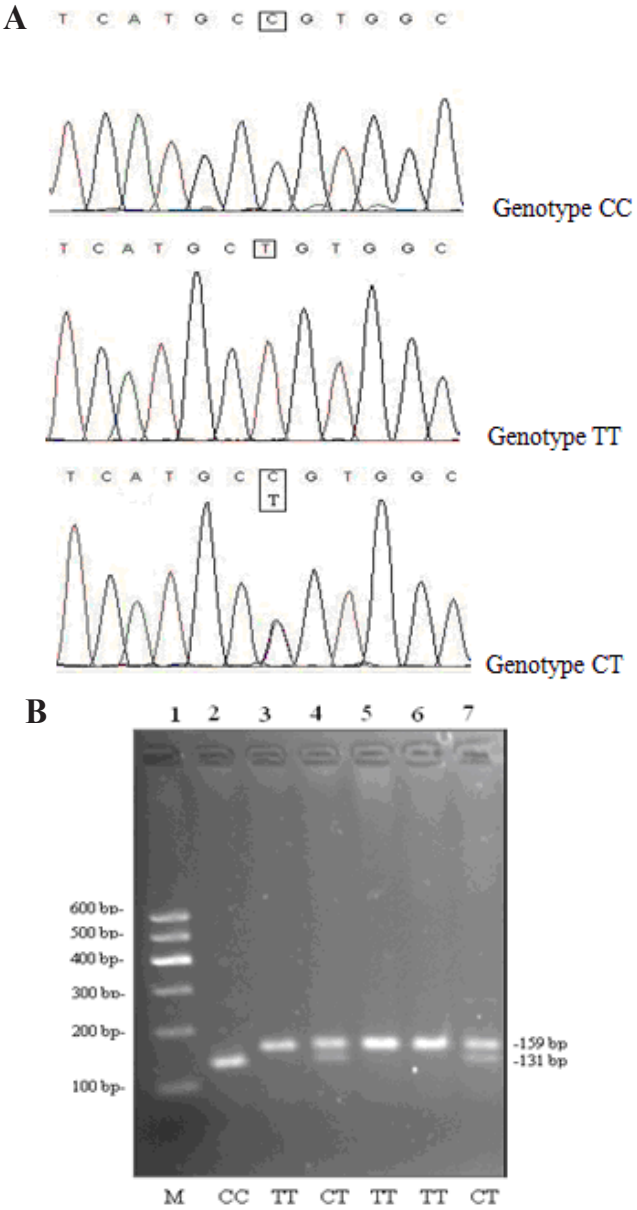

Figure 1. A. Sequencing maps at position of g.8344C>T from different genotypes in the bovine *Dapper1* gene. **B.** Electrophoresis patterns of *Msp*I F-PCR-RFLP analysis of the bovine *Dapper1* gene. TT (159 bp); CT (159+131+28 bp); CC (131+28 bp); *lane M* = marker I.

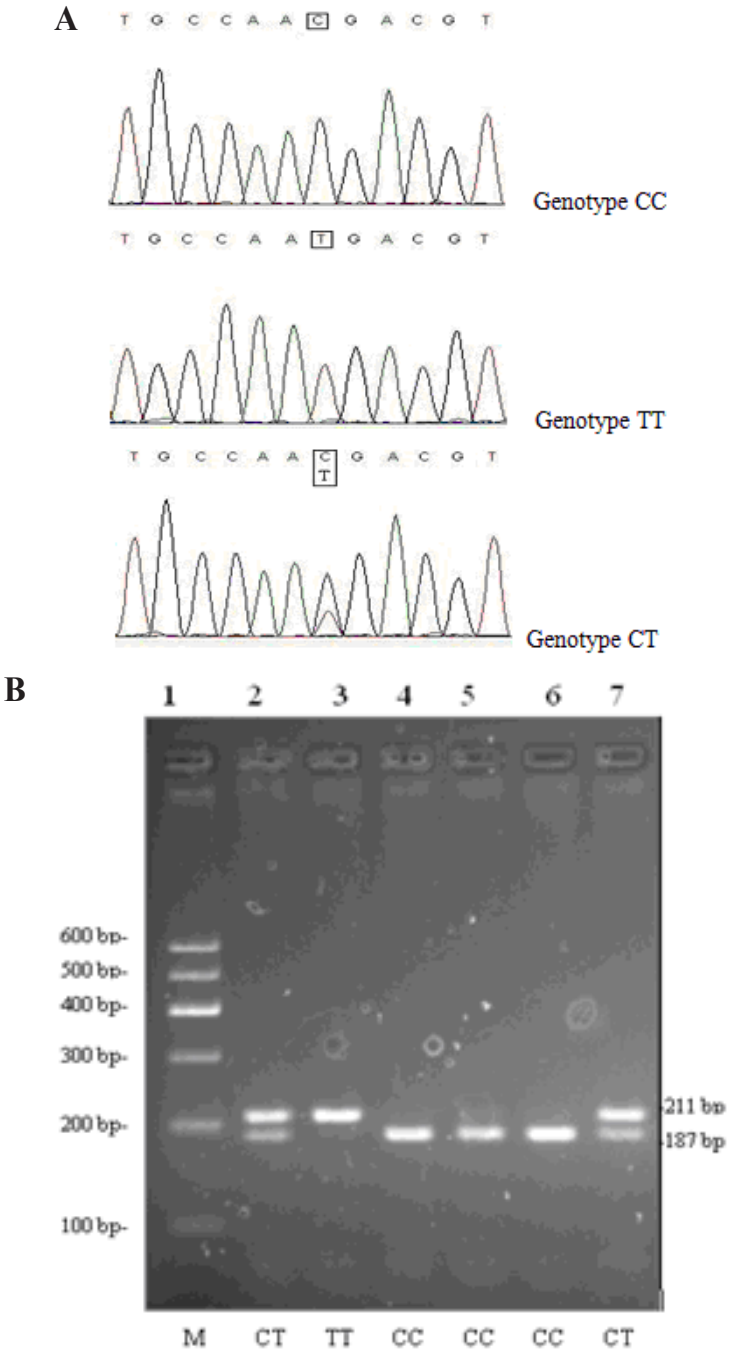

Figure 2. A. Sequencing maps at position of g.8428C>T from different genotypes in the bovine *Dapper1*gene. **B.** Electrophoresis patterns of *Hin*dII F-PCR-RFLP analysis of the bovine *Dapper1* gene. TT (211 bp); CT (211+187+24 bp); CC (187+24 bp); *lane M* = marker I.

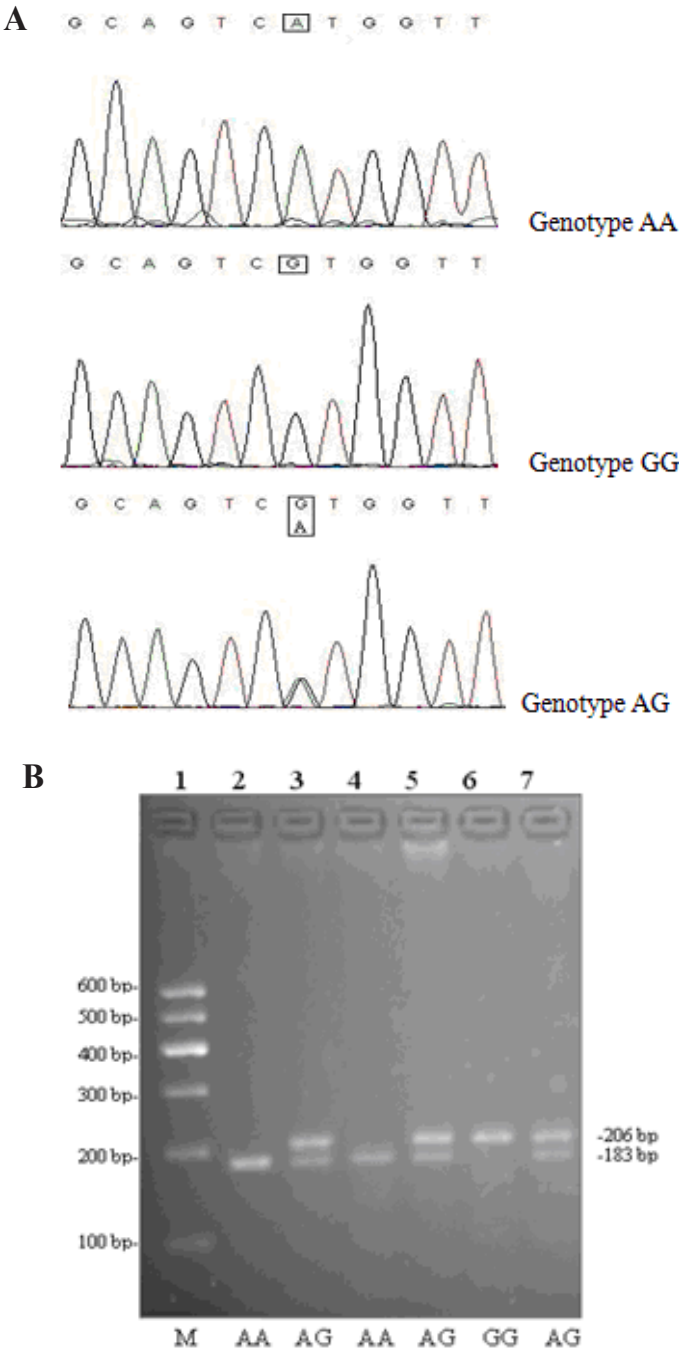

Figure 3. A. Sequencing maps at position of g.10513A>G from different genotypes in the bovine *Dapper1* gene. **B.** Electrophoresis patterns of *Nco*I F-PCR-RFLP analysis of the bovine *Dapper1* gene. GG (206 bp); AG (206+183+23 bp); AA (183+23 bp); *lane M* = marker I.

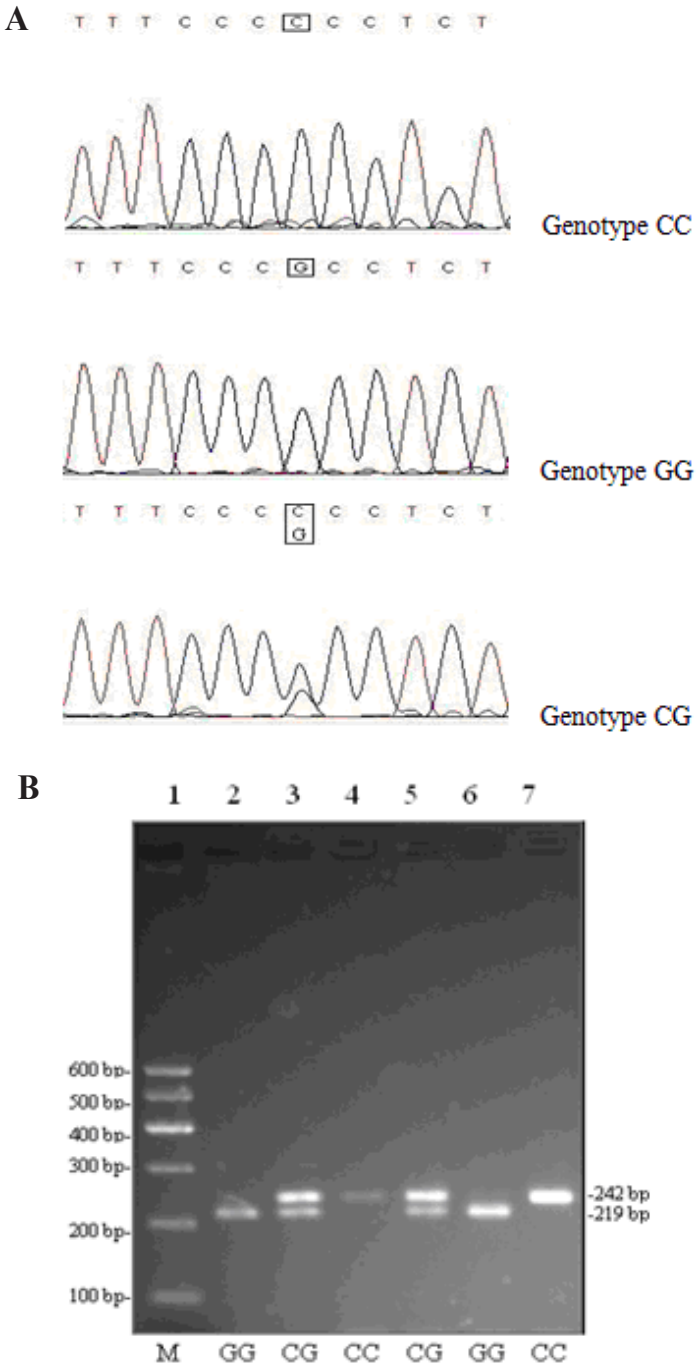

Figure 4. A. Sequencing maps at position of g.10765C>G from different genotypes in the bovine *Dapper1* gene. **B.** Electrophoresis patterns of *Hha*I F-PCR-RFLP analysis of the bovine *Dapper1* gene. CC (242 bp); CG (242+219+23 bp); GG (219+23 bp); *lane M* = marker I.

Table 2. Description of SNPs at the bovine *Dapper1* gene.

Loci	Variant type	Location	Alleles	Amino acid change	GenBank accession No.	DPS (nt)
SNP1-*Msp*I	g.8344C>T	Exon 6	GCC/GCT	Ala 340 Ala	rs410759551	0
SNP2-*Hin*dII	g.8428C>T	Exon 6	AAC/AAT	Asn 368 Asn	rs410759552	84
SNP3-*Nco*I	g.10513A>G	3'-UTR	-	-	rs410759553	2085
SNP4-*Hha*I	g.10765C>G	3'-UTR	-	-	rs410759554	252

DPS = distance from the previous sequence variant.

Table 3. Genotypic and allelic frequencies (%), value of the χ^2 test, and diversity parameters of the *Dapper1* gene in 5 cattle breeds.

Loci	Breeds	Genotype number and frequencies				Allelic frequencies			Genetic diversity parameters		
SNP1-*Msp*I locus		CC	CT	TT	Total	C	T	H_O	H_E	N_E	PIC
	JX	67	194	94	355	0.462	0.538	0.503	0.497	1.988	0.374
		(0.189)	(0.546)	(0.265)							
	CY	121	95	19	235	0.717	0.283	0.594	0.406	1.683	0.323
		(0.515)	(0.404)	(0.081)							
	QC	23	143	50	216	0.437	0.563	0.508	0.492	1.968	0.371
		(0.106)	(0.662)	(0.232)							
	NY	33	133	47	213	0.467	0.533	0.502	0.498	1.991	0.374
		(0.155)	(0.624)	(0.221)							
	LX	23	107	36	166	0.461	0.539	0.503	0.497	1.988	0.373
		(0.139)	(0.645)	(0.216)							
SNP2-*Hin*dII locus		CC	CT	TT	Total	C	T	H_O	H_E	N_E	PIC
	JX	245	102	8	355	0.834	0.166	0.720	0.280	1.389	0.241
		(0.690)	(0.287)	(0.023)							
	CY	31	139	65	235	0.428	0.572	0.510	0.490	1.959	0.370
		(0.132)	(0.591)	(0.277)							
	QC	48	136	32	216	0.537	0.463	0.503	0.497	1.989	0.374
		(0.222)	(0.630)	(0.148)							
	NY	105	101	7	213	0.730	0.270	0.606	0.394	1.651	0.316
		(0.493)	(0.474)	(0.033)							
	LX	88	69	9	166	0.738	0.262	0.613	0.387	1.631	0.312
		(0.530)	(0.416)	(0.054)							
SNP3-*Nco*I *locus*		AA	AG	GG	Total	A	G	H_O	H_E	N_E	PIC
	JX	57	202	96	355	0.445	0.555	0.506	0.494	1.976	0.372
		(0.161)	(0.569)	(0.270)							
	CY	120	106	9	235	0.736	0.264	0.612	0.388	1.635	0.313
		(0.511)	(0.451)	(0.038)							
	QC	34	130	52	216	0.458	0.542	0.503	0.497	1.986	0.373
		(0.157)	(0.602)	(0.241)							
	NY	27	135	51	213	0.444	0.556	0.506	0.494	1.975	0.372
		(0.127)	(0.634)	(0.239)							
	LX	17	99	50	166	0.401	0.599	0.520	0.480	1.924	0.365
		(0.102)	(0.596)	(0.302)							
SNP4-*Hha*I locus		CC	CG	GG	Total	C	G	H_O	H_E	N_E	PIC
	JX	106	157	92	355	0.519	0.481	0.501	0.499	1.997	0.375
		(0.298)	(0.442)	(0.260)							
	CY	218	17	0	235	0.964	0.036	0.930	0.070	1.075	0.067
		(0.928)	(0.072)	(0.000)							
	QC	99	90	27	216	0.667	0.333	0.559	0.441	1.79	0.344
		(0.458)	(0.417)	(0.125)							
	NY	65	113	35	213	0.570	0.430	0.510	0.490	1.961	0.370
		(0.305)	(0.531)	(0.164)							
	LX	56	81	29	166	0.581	0.419	0.513	0.487	1.948	0.368
		(0.337)	(0.488)	(0.175)							

JX = Jiaxian red cattle (N = 355); CY = Caoyuan red cattle (N = 235); QC = Qinchuan cattle (N = 216); NY = Nanyang cattle (N = 213); LX = Luxi cattle (N = 166); H_O = observed and H_E = expected heterozygosities; N_E = effective number of alleles; PIC = polymorphic information content.

The PIC value is effective for the assessment of genetic diversity at various loci of candidate genes. According to PIC classification, values of the four loci ranged from 0.067 to 0.375 in the 5 cattle populations. The SNP1-*Msp*I (mean PIC value = 0.363) and SNP3-*Nco*I (mean PIC value = 0.359) loci showed moderate genetic diversity. At the SNP2-*Hin*dII locus, only JX cattle showed low polymorphism (PIC value = 0.241). The PIC value at the SNP4-*Hha*I locus in CY cattle was the lowest (0.067) because of the absence of the GG genotype.

LD and haplotype analysis of bovine *Dapper1*

LD among the four SNPs of *Dapper1* in the cattle populations is shown in Table 4, which indicates that *D*' values ranged from 0.034 to 0.970; r^2 values ranged from 0.000 to 0.764. For JX cattle, SNP1, SNP3, and SNP4 were closely linked (r^2 >0.33), but SNP2 had a low level of LD with the other SNP loci. In the CY population, the level of LD in the 4 SNPs was very low. In QC cattle, only SNP1 and SNP3 were closely linked (r^2 = 0.363); SNP2 and SNP4 had the smallest r^2 value (0.000). In the NY and LX populations, SNP1 and SNP3, SNP1 and SNP4, SNP2 and SNP3, and SNP3 and SNP4 were linked closely.

Table 4. Estimated values of linkage disequilibrium analysis between four SNPs within the *Dapper1* gene of the cattle population studied.

SNPs	JX		CY		QC		NY		LX	
	r	*D*'	r^2	*D*'	r^2	*D*'	r^2	*D*'	r^2	*D*'
SNP1/2	0.176	0.867	0.025	0.219	0.208	0.701	0.249	0.768	0.322	0.880
SNP1/3	0.764	0.933	0.118	0.361	0.363	0.639	0.621	0.826	0.530	0.823
SNP1/4	0.736	0.962	0.065	0.827	0.094	0.307	0.573	0.932	0.484	0.887
SNP2/3	0.170	0.804	0.002	0.069	0.207	0.746	0.408	0.938	0.365	0.829
SNP2/4	0.134	0.848	0.015	0.545	0.000	0.034	0.260	0.967	0.235	0.959
SNP3/4	0.622	0.952	0.059	0.826	0.091	0.310	0.566	0.970	0.355	0.859
Mean	0.434	0.894	0.047	0.475	0.161	0.456	0.446	0.900	0.382	0.873

SNPs = estimated values of linkage disequilibrium (r^2 and *D*') between SNP pairs. SNP1 = SNP1-*Msp*I; SNP2 = SNP2-*Hin*dII; SNP3 = SNP3-*Nco*I; SNP4 = SNP4-*Hha*I. For abbreviations, see legend to Table 3.

Haplotypes were reconstructed in the 5 breeds by using the PHASE program. Sixteen haplotypes were identified (Table 5), of which nine (Hap1, Hap3, Hap5, Hap7, Hap9, Hap11-13, and Hap15) were shared among all 5 breeds. One haplotype (Hap2) is unique to QC. Two haplotypes were detected in only 2 breeds: Hap6 is unique to QC and NY, and Hap8 is detected only in CY and QC. Hap4 is absent in CY, and QC cattle lack Hap10. Hap16 was not detected in NY. Hap12 had the highest frequency in JX (0.454), NY (0.413), and LX (0.371). Hap5 had the highest frequency in CY (0.347) and QC (0.245).

Association between individual SNPs and growth traits in NY cattle

To investigate the effects of SNPs, we analyzed the relationship between *Dapper 1* individual SNP genotypes and BW in NY cattle (N = 213) aged 0, 6, 12, 18, and 24 months. Statistical analyses showed that in SNP1-*Msp*I and SNP3-*Nco*I loci, homozygous individuals with genotypes CC and AA had significantly higher BW6 than those of homozygous TT and GG genotype animals (P < 0.05; Table 6), and individuals with the SNP3-*Nco*I-AA genotype

showed higher BW12 than those in individuals with other genotypes ($P < 0.05$), suggesting that alleles C and T have a positive effect on growth performance in very young cattle. This result is consistent with the function of *Dapper1*, which plays a central role in embryogenesis.

Table 5. Haplotype and haplotype frequency of four SNPs in the bovine *Dapper1* gene within the population studied.

Haplotypes	SNPs				Frequency in population				
	SNP1	SNP2	SNP3	SNP4	JX (N = 355)	CY (N = 235)	QC (N = 216)	NY (N = 213)	LX (N = 166)
Hap1	C	C	A	C	0.268	0.225	0.000	0.166	0.135
Hap2	C	C	A	G			0.005		
Hap3	C	C	G	C	0.036	0.053	0.019	0.049	0.058
Hap4	C	C	G	G	0.006		0.013	0.011	0.022
Hap5	C	T	A	C	0.143	0.347	0.245	0.233	0.228
Hap6	C	T	A	G			0.117	0.003	
Hap7	C	T	G	C	0.006	0.085	0.045	0.006	0.018
Hap8	C	T	G	G		0.007	0.018		
Hap9	T	C	A	C	0.005	0.088	0.029	0.015	0.007
Hap10	T	C	A	G	0.010	0.006		0.002	0.022
Hap11	T	C	G	C	0.057	0.033	0.088	0.073	0.123
Hap12	T	C	G	G	0.454	0.023	0.145	0.413	0.371
Hap13	T	T	A	C	0.001	0.030	0.017	0.025	0.009
Hap14	T	T	A	G			0.035	0.000	
Hap15	T	T	G	C	0.005	0.103	0.018	0.004	0.004
Hap16	T	T	G	G	0.010	0.000	0.206		0.004
Number	-	-	-	-	12	12	15	13	12

Table 6. Associations of single and combined SNPs within the *Dapper1* gene with growth traits in the Nanyang (NY) cattle.

Loci	Genotypes	Growth traits (mean ± SE)				
		BW0 (kg)	BW6 (kg)	BW12 (kg)	BW18 (kg)	BW24 (kg)
SNP1-*Msp*I	CC (N = 33)	30.684 ± 0.615	166.842 ± 4.209^{a}	231.000 ± 5.411	300.00 ± 6.94	374.211 ± 8.516
locus	CT (N = 133)	30.092 ± 0.383	160.531 ± 2.621^{ab}	221.122 ± 3.369	301.55 ± 4.32	362.208 ± 5.358
	TT (N = 47)	29.596 ± 0.526	152.615 ± 3.598^{b}	220.885 ± 4.626	296.19 ± 5.93	375.115 ± 7.250
SNP2-*Hin*dII	CC (N = 105)	30.073 ± 0.415	158.317 ± 2.959	223.878 ± 3.696	295.789 ± 4.725	365.439 ± 5.865
locus	CT (N = 101)	29.840 ± 0.388	160.021 ± 2.963	220.787 ± 3.452	302.358 ± 4.467	369.848 ± 5.537
	TT (N = 7)	31.917 ± 1.086	165.333 ± 7.734	235.167 ± 9.663	307.830 ± 12.350	375.500 ± 15.330
SNP3-*Nco*I	AA (N = 27)	30.526 ± 0.619	168.737± 4.228^{a}	232.000 ± 5.364^{a}	299.421 ± 6.934	374.316 ± 8.586
locus	AG (N = 135)	29.991 ± 0.354	157.724 ± 2.420^{b}	219.414 ± 3.070^{b}	301.328 ± 3.969	364.982 ± 4.957
	GG (N = 51)	29.853 ± 0.654	155.882 ± 4.470^{b}	225.471 ± 5.670^{b}	294.765 ± 7.331	372.529 ± 9.077
SNP4-*Hha*I	CC (N = 65)	29.878 ± 0.443	161.162 ± 3.106	223.541 ± 3.933	300.297 ± 4.927	367.135 ± 6.147
locus	CG (N = 113)	30.326 ± 0.411	159.953 ± 2.881	222.326 ± 3.648	302.744 ± 4.570	365.762 ± 5.769
	GG (N = 35)	29.821 ± 0.721	154.500 ± 5.049	224.000 ± 6.394	289.143 ± 8.010	378.786 ± 9.992
	Diplotype	BW0 (kg)	BW6 (kg)	BW12 (kg)	BW18 (kg)	BW24 (kg)
SNP1-*Msp*I-	H1H5 (N = 23)	30.611 ± 0.933	171.000 ± 6.332^{a}	235.778 ± 7.853	294.898 ± 10.540	380.889 ± 12.890
SNP2-*Hin*d-	H5H5 (N = 15)	32.100 ± 1.252	165.200 ± 8.495^{ab}	238.000 ± 10.535	308.200 ± 14.141	3 82.200 ± 17.294
SNP3-*Nco*I-	H1H11 (N = 17)	30.500 ± 1.142	151.667 ± 7.755^{ab}	216.667 ± 9.618	304.333 ± 12.908	356.167 ± 15.787
SNP4-*Hha*I	H1H12 (N = 28)	30.800 ± 0.885	166.400 ± 6.007^{ab}	229.200 ± 7.450	296.100 ± 9.999	358.200 ± 12.228
	H5H11 (N = 21)	30.250 ± 0.989	162.625 ± 6.716^{ab}	219.125 ± 8.329	306.500 ± 11.179	354.750 ± 13.672
	H5H12 (N = 45)	30.441 ± 0.679	156.235 ± 4.60^{7ab}	219.765 ± 5.714	303.529 ± 7.669	376.941 ± 9.379
	H12H12 (N = 30)	29.636 ± 0.844	150.909 ± 5.728^{b}	226.000 ± 7.103	292.909 ± 9.534	385.455 ± 11.659

SE = standard error; BW0 = birth weight; BW6 = body weight of 6 months; BW12 = body weight of 12 months; BW18 = body weight of 18 months; BW24 = body weight of 24 months. LSM in a column with no common superscript letters differ significantly ($P < 0.05$).

Association between haplotype combinations and growth traits in NY cattle

To further elucidate the association between diplotypes of the four SNPs and BW in NY cattle,

we constructed the haplotypes of the SNPs. As shown in Table 5, 13 haplotypes were found in NY cattle. Because the frequenciesy of Hap4 (0.011), Hap6 (0.003), Hap7 (0.006), Hap9 (0.015), Hap10 (0.002), Hap14 (0.000), and Hap15 (0.004) were small, our association analysis for the effect of diplotype excluded their related diplotypes, and percentages of observations that were below 5% were also excluded. Therefore, 6 haplotypes were analyzed: Hap1 (0.160), Hap3 (0.049), Hap5 (0.233), Hap11 (0.073), and Hap12 (0.413), and 7 diplotypes were used in the correlation analysis (see Table 6). Taken together, the four SNPs displayed consistent significant effects on BW6 with a single SNP. Individuals with H1H5 (CCCTAACC) had higher BW6 than those with other diplotypes, particularly, H12H12 (TTCCGGGG). This difference was significant ($P < 0.05$). However, significant differences were not found in the other traits in cattle aged 0, 12, 18, and 24 months ($P > 0.05$). These results showed that the combined effects of the four SNPs were consistent with that of a single SNP.

DISCUSSION

The function of Dapper is evolutionally conserved in fish and mammals (Su et al., 2007), and studies have found that *Dapper1* is required to induce Wnt/β-catenin target genes and enhance the activity of Wnt/β-catenin in zebrafish (Waxman et al., 2004). Because the Wnt signaling pathway plays an important role in embryogenesis (Gao et al., 2008), *Dapper1* may be associated with growth in cattle.

The present study is the first to report on the polymorphism of bovine *Dapper1*. Four SNPs (NC_007308.4: g.8344C>T, g.8428C>T, g.10513A>G, and g.10765C>G) were detected in 5 Chinese cattle breeds. The first two were synonymous mutations located in exon 6, and the latter two were located in the 3'-UTR; hence, to a certain degree, this gene is conservative. These findings are consistent with the function of the evolutionally conserved Dapper protein (Su et al., 2007).

Of particular interest, no CY individual had a GG genotype in the SNP4-*Hha*I locus (see Table 3), likely because first, the number of CY cattle was limited, and therefore, we may have excluded individuals with the GG genotype from our CY cattle population; and second, different selection purposes and histories have led to the disappearance of CY individuals with the GG genotype at this locus. These results are consistent with those of our previous studies (Wang et al., 2010; Xu et al., 2011). For example, Xu et al. (2011) found that at the P7 locus of the paired box 7 gene, only two genotypes occur in CY cattle. In the absence of the GG genotype, N_E value in this locus approached 1, and the PIC value was <0.25. Consistent with PIC classification, this locus in CY cattle shows low polymorphism.

The r^2 value of SNP2 and SNP4 in QC cattle is 0.000 (see Table 4), meaning that these SNPs are not linked. Table 5 shows that QC cattle have the greatest number of haplotypes. For CY cattle, the mean r^2 value is the smallest (0.047), indicating that the four SNPs in this breed experienced the least LD, and possibly, recombination will be high and LD will be low in genovariation-dense regions.

Naturally occurring silent mutations have been found to change the function of protein - that is, protein products with the same aa sequence but different gene sequences had different structural and functional properties (Komar, 2007; Kweekel et al., 2009). For example, a silent polymorphism in multi-drug resistance gene-1 results in a substrate specificity change (Kimchi-Sarfaty et al., 2007). In our research, the g.8344C>T mutations at the SNP1-*Msp*I locus were synonymous, but the CC genotype was associated with higher BW6 than that of the TT genotype, a result consistent with other findings.

Although located in the 3'-UTR, the mutation g.10513A>G at the SNP3-*Nco*I locus may not be a causal mutation. Instead, it may regulate the expression of *Dapper1* because 3'-UTR sequences can affect the mechanism of mRNA deadenylation and degradation (Xu et al., 1997, 1998). Therefore, the 3'-UTR mutation may directly or indirectly influence the stability of *Dapper1* mRNA.

BW is one of the growth traits, which are complex, quantitative traits regulated by various genes involved in many physiological activities. For each candidate gene, growth traits were affected not only by the single SNP but also by SNP-SNP interaction; therefore, the association analysis between the combined SNPs has important biological value (Lango et al., 2008). In this study, the association analysis between the combined SNPs with growth traits was consistent with the association analysis in a single SNP. In single SNP analysis, the SNP1-*Msp*I-CC and SNP3-*Nco*I-AA genotypes were advantageous for BW6, and the combination of genotype H1H5 (CCCTAACC) showed better performance for BW6. Specifically, the genotype of the SNP1-*Msp*I locus in the H1H5 diplotype was CC and the genotype of the SNP3-*Nco*I locus in the H1H5 diplotype was AA.

To summarize, we first reported four SNPs in bovine *Dapper1*, 2 of which (g.8344C>T and g.10513A>G) had significant effects on BW6 in NY cattle. The combination of SNPs had an effect consistent with that of a single SNP; hence, it was assumed that alleles C and A were responsible for the positive effect on growth performance in very young cattle. Therefore, the CC and AA genotypes should be selected in the breeding schemes of cattle. This study may contribute to evaluations of these genotypes as genetic markers in bovine breeding. Further studies, such as functional analysis, are needed to fully elucidate how these gene mutations may affect *Dapper1* activity and whether they are appropriate as candidate markers associated with whole energy metabolism in cattle.

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