GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Novel single nucleotide polymorphisms of the bovine methyltransferase 3b gene and their association with meat quality traits in beef cattle

X. Liu[1], X.Y. Guo[1], X.Z. Xu[2], M. Wu[2], X. Zhang[1], Q. Li[1], P.P. Ma[1], Y. Zhang[1], C.Y. Wang[3], F.J. Geng[3], C.H. Qin[4], L. Liu[5], W.H. Shi[5], Y.C. Wang[1] and Y. Yu[1]

[1]Key Laboratory of Agricultural Animal and Breeding,
National Engineering Laboratory for Animal Breeding,
College of Animal Science and Technology,
China Agricultural University, Beijing, P.R. China
[2]Dalian Xuelong Industry Limited Group, Dalian, P.R. China
[3]Dingyuan SeedsStock Bulls Breeding Ltd. Company, Zhengzhou, P.R. China
[4]Ningxia Sygen BioEngineering Research Center, Yinchuan, P.R. China
[5]Beijing Dairy Cattle Centre, Beijing, P.R. China

Corresponding authors: Y.C. Wang / Y. Yu
E-mail: wangyachun@cau.edu.cn / yuying@cau.edu.cn

Genet. Mol. Res. 11 (3): 2569-2577 (2012)
Received November 3, 2011
Accepted May 10, 2012
Published June 29, 2012
DOI http://dx.doi.org/10.4238/2012.June.29.1

ABSTRACT. DNA methylation is essential for adipose deposition in mammals. We screened SNPs of the bovine DNA methyltransferase 3b (*DNMT3b*) gene in Snow Dragon beef, a commercial beef cattle population in China. Nine SNPs were found in the population and three of six novel SNPs were chosen for genotyping and analyzing a possible association with 16 meat quality traits. The frequencies of the alleles and genotypes of the three SNPs in Snow Dragon beef were similar to those in their terminal-paternal breed, Wagyu. Association analysis disclosed that SNP1 was not associated with any of the traits; SNP2 was significantly associated with lean meat color score and chuck short rib score, and SNP3

had a significant effect on dressing percentage and back-fat thickness in the beef population. The individuals with genotype GG for SNP2 had a 25.7% increase in lean meat color score and a 146% increase in chuck short rib score, compared with genotype AA. The cattle with genotype AG for SNP3 had 35.7 and 24% increases in dressing percentage and 28.8 and 29.2% increases in back-fat thickness, compared with genotypes GG and AA, respectively. Genotypic combination analysis revealed significant interactions between SNP1 and SNP2 and between SNP2 and SNP3 for the traits rib-eye area and live weight. We conclude that there is considerable evidence that *DNMT3b* is a determiner of beef quality traits.

Key words: Beef cattle; Beef quality traits; *DNMT3b*; SNPs

INTRODUCTION

DNA methylation is a key epigenetic modification that controls genes expression in the metabolic process and adipose deposition of mammals (Tidball et al., 2002; Liu et al., 2009; Wang et al., 2010). Recent studies found that the level of DNA methylation varys under environmental influences such as nutrition statues and aging (Maier and Olek, 2002; Halaschek-Wiener et al., 2009; Haggarty et al., 2009; Barres and Zierath, 2011). Twin studies depicted that DNA methylation profiles and the expression level of their related genes were more divergent in older twins than in infant twins, which proved the influences of environmental factors mediated by DNA methylation on the expression of related genes over time (Fraga et al., 2005).

Methylation of 5' position of cytocine at CpG dinucleotide is carried out by two general classes of enzymatic activities - maintenance methylation (DNMT1) and *de novo* methylation (DNMT3). DNA methyltransferase 3 (DNMT3) family mainly consists of two factors, DNMT3a and DNMT3b, which are coded by *DNMT3a* and *DNMT3b* gene, respectively. Okano et al. (1999) demonstrated that *DNMT3a* and *DNMT3b* have pivotal functions during early embryogenesis as both homozygous embryos (*DNMT3a*$^{-/-}$, *DNMT3b*$^{-/-}$) showed smaller size and abnormal morphology at E8.5 and E9.5 and died before E11.5. *DNMT3b* and *DNMT3a* were highly expressed in undifferentiated embryonic stem cells and also expressed in adult somatic tissues (Amara et al., 2010; Kurita et al., 2010). Studies have reported that mutations of *DNMT3b* were found in human ICF syndrome patients and avian Marek's disease in chickens (Okano et al., 1999; Yu et al., 2008). More recently, the report of Kamei et al. (2010) on transgenic mice showed that increased expression of *DNMT3a* in the adipose tissue may be associated with obesity-related inflammation. Our newly published study showed that the expression level of bovine *DNMT3b* in liver and muscle tissues was significantly correlated to the traits of beef quality in beef cattle (Guo et al., 2012).

Meat quality in beef cattle is an economically important trait, which is mainly related to cattle breeds and affected by fattening level and period. To investigate the genetic effects of bovine *DNMT3b* gene mutations on meat quality traits in beef cattle, single nucleotide polymorphisms (SNPs) of *DNMT3b* in the offspring of Wagyu and the crossbred of Limousin by Fuzhou yellow cattle (commercially named as Snow Dragon beef) were screened. Moreover, association studies between SNPs of bovine *DNMT3b* and meat quality traits in the beef cattle population were carried out. The potential roles of bovine *DNMT3b* mutations in the development of beef quality traits were also discussed.

MATERIAL AND METHODS

Samples and meat quality traits

Animals used in the study were the offspring of Wagyu and F_1 crossbred cows of Limousin by Fuzhou yellow cattle, which were commercially named Snow Dragon beef and reared by Xuelong Industrial Group in Dalian City (China). The fattening period of the beef population were started from around 14 to 26 months old. A total of 153 individuals were selected randomly from the fattening population and the phenotypic data of beef quality traits were collected by professional technicians according to the meat grading standards provided by Xuelong Industrial Group. The data of four carcass traits and twelve meat quality traits including live weight (LW), dressing percentage (DP), carcass weight (CW), chilled carcass weight (CCW), chilling loss (CL), rib-eye width (RW), rib-eye length (RL), rib-eye area (RA), Flank thickness (FT), chuck short rib thickness (CT), chuck short rib score (CS), backfat thickness (BFT), chuck flap weight (CF), fat color score (FCS), lean meat color score (LCS) and marbling score (MS) were used for association analysis.

Moreover, to compare the SNP frequencies among the parental breeds and Snow Dragon beef, Wagyu, Limousin, and Fuzhou cattle were also genotyped for the novel SNPs of *DNMT3b*. The samples included 11 Wagyu bulls (5 semen samples and 6 ear tissue samples were supplied by Beijing Dairy Cattle Centre and Xuelong Industrial Group, China, respectively), 23 semen samples of Limousin bulls were separately collected from Luoyang Baimasi Bull Station ($n = 4$) in Henan Province and Dingyuan Seedstock Breeding Ltd. Company ($n = 10$) in Henan Province, Beijing Dairy Cattle Centre ($n = 3$) and Ningxia Sygen BioEngineering Research Center ($n = 5$) in China, 7 semen samples of Fuzhou bulls were from the National Conservation Farm of Fuzhou yellow cattle (Dalian, China).

SNP identification

Total genomic DNA for each sample were extracted from muscle tissue using commercial kit of genomic DNA purification Wizard® (Promega Corp., Madison, WI, USA) according to manufacturer instructions. After genomic DNA isolation, the quantity and quality of DNA were measured using NanoDrop™ ND-2000c Spectrophotometer (Thermo Scientific, Inc.). A total of 23 primer pairs were designed to identify SNPs of bovine *DNMT3b*, which covered all coding regions (20 exons) and their flanking intron sequences as well as the 5' regulatory region at 2000 bp upstream from the transcription initiation site using Primer 3.0 and Oligo 6.0 softwares. A DNA pool was constructed from ten cattle (50 ng/μL per sample) randomly selected from Limousin, Wagyu, Simmental, and Angus.

Genotyping

Six novel SNPs in the gene were discovered in the beef population, of which three SNPs existing restriction enzyme sites were conducted genotyping with PCR-RFLP technique. The three SNPs were SNP1 C63029349T, SNP2 G63032883A, and SNP3 A63039420G, which are located in the 3rd, 5th and 13th intron of bovine *DNMT3b*, respectively. Features of the primers and amplicons obtained in the assays are shown in Table 1.

Table 1. Primers of three SNPs in bovine *DNMT3b*.

SNPs	Primer sequence (5'→3')	Product length (bp)	Tm (°C)
SNP1 (C63029349T)	F: AATGCTTGCAGGAAAGAAGTC R: ATTCTTGCACTTCCCACCAG	680	58
SNP2 (G63032883A)	F: ACCCTTTGAAAGTTGGAGCA R: TGAAGGTAGAGGACGGACAGA	696	56.5
SNP3 (A63039420G)	F: CTCAGCATTGGCTATGCACT R: CAAAAGCTCAAGCTTCTCTGA	580	56.5

Tm = melting temperature.

All PCRs were carried out in a final volume of 20 µL. The reactions consisted of 10 µL premix *Taq* (Takara Biotechnologh Co. Ltd., Dalian, China), which included 0.5 U *Taq* DNA polymerase, 160 µM of each deoxynucleoside triphosphate and 1.2 mM Mg^{2+}; 2 mM forward and reverse primers (Invitrogen) and 50-60 ng template DNA. PCR program was used as followed: 35 cycles of 30 s at 95°C 30 s at 56.5°~58°C and 1 min at 72°C, which included a first denaturation step at 95°C for 5 min and a final extension at 72°C or 10 min. Following PCR amplification, SNP were genotyped using restriction fragment length polymorphism (RFLP) analysis. Amplification products (15 µL) were digested overnight at 37°C, the restriction enzymes of SNP1, 2 and 3 were *Btg*I, *Msp*I and *Xba*I, respectively. Products were visualized on 2% agarose gel electrophoresis.

Statistical analysis

The frequencies of genotype and allele of the SNPs were calculated in PopGene 3.2. Association analysis between the three SNPs and beef quality traits in the population was carried out using the general linear model (GLM) procedure in SAS 8.0 as follows:

$$Y_{ij} = \mu + G + S + M + D + e_{ij} \quad \text{(Equation 1)}$$

where Y_{ij} represents the phenotype records of meat quality traits; μ is overall mean; G is the fixed effect of the genotype; S is the fixed effect of sex; M is the fixed effect of months old; D is the fixed effects of slaughtering date; e_{ij} is the random error. The data are reported as probability values and least squares means ± SE, and a P value of < 0.05 was considered to be statistically significant.

The combination effects of the three SNPs of *DNMT3b* on beef quality traits were analyzed with the following equation:

$$Y_{ij} = \mu + C + S + M + D + e_{ij} \quad \text{(Equation 2)}$$

where Y_{ij}, μ, S, M, D, and e_{ij} are the same as shown in Equation 1, and C is the effect of the combination genotypes between two SNPs or among three SNPs.

RESULTS

SNP discovery and genotypes

Nine SNPs were discovered in the bovine *DNMT3b* gene by screening in the Snow

Dragon beef population. As shown in Table 2, SNP1-SNP6 were first discovered in cattle, while the other three SNPs were annotated in GenBank with one located in intron 4 (SNP7, NCBI accession No. rs134010442) and two within intron 10 (SNP8 and SNP9, NCBI accession Nos. rs135724333 and rs132930989).

Table 2. Informations of the 9 SNPs found in the study.

SNP	Location		Mutation
SNP1	Intron 3	13chr 63029349	C→T
SNP2	Intron 5	13chr 63032883	G→A
SNP3	Intron 13	13chr 63039420	A→G
SNP4	Intron 6	13chr 63033215	T→C
SNP5	Exon 11	13chr 63037481	C→T
SNP6	Exon 20	13chr 63046526	G→C
SNP7	Intron 4	13chr rs134010442	A→G
SNP8	Intron 10	13chr rs135724333	C→T
SNP9	Intron 10	13chr rs132930989	C→T

Three of six novel SNPs, SNP1, SNP2 and SNP3, were chosen for restriction enzyme sites and further genotyped (Figure 1). Genotypic and allelic frequencies obtained in the Snow Dragon beef population were calculated and are shown in Table 3. In addition, the MassARRAY technique was used for genotyping SNP1 and SNP3 in Wagyu (N = 11), Limousin (N = 23) and Fuzhou yellow cattle (N = 7). As for SNP1, the wild-type of allele (C) and genotype (CC) were highly distributed in Wagyu and Snow Dragon beef (frequencies >0.8) as well as Fuzhou yellow cattle (>0.7), while that of Limousin was the lowest (< 0.3). For SNP3, Wagyu and Snow Dragon beef showed higher frequencies of wild-type of allele (A) and genotype of heterozygous (AG), whereas there was no polymorphism disclosed in Fuzhou yellow cattle and Limousin. Althougth SNP2 could not be included in the MassARRAY assay, its allelic and genotypic frequencies were similar between Wagyu and Snow Dragon beef population. The results indicated that the genetic background of Snow Dragon beef cattle is similar to its paternal breed Wagyu than the two parental breeds of Limousin and Fuzhou yellow cattle.

Associations and genetic effects of the SNPs

Association results between the SNPs and the beef quality traits in Snow Dragon beef population are shown in Table 4. For SNP1, there was no significant association with any beef quality trait. SNP2 was associated with lean meat color score at P value of 0.051 and with chuck short rib score at P value of less than 0.05. SNP3 was significantly associated with the traits of dressing percentage, back-fat thickness and marbling score ($P < 0.05$).

To compare the difference between two genotypes, the Bonferroni *t*-test was used for pair comparison in the current study. The results indicated that individuals with genotype GG in SNP2 showed 25.7% increases in lean meat color score and 146% increases in chuck short rib score compared with genotype AA. Individuals with genotype AG in SNP3 showed 35.7 and 24% increases in dressing percentage, 28.8 and 29.2% increases in backfat thickness compared with genotype GG and AA, respectively. Moreover, cattle with genotype AG in SNP3 showed 71.8% increased marbling score than GG individuals.

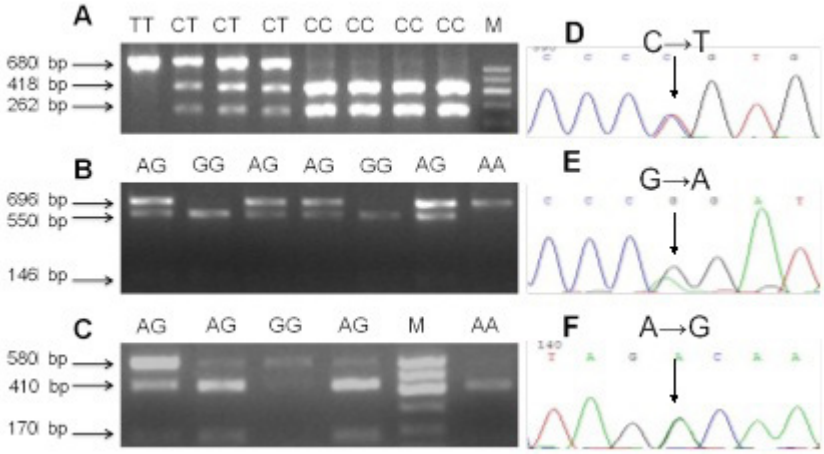

Figure 1. Detection and genotyping of the three novel SNPs in bovine *DNMT3b*. **A.** The lengths of SNP1 C>T genotypes TT, CT and CC were 680, 680/418/262 and 418/262 bp, respectively. *Lane M* = DNA marker I. **B.** The lengths of SNP2 G>A genotypes AA, AG and GG were 696, 696/550/146 and 550/146 bp, respectively. **C.** The lengths of SNP1 A>G genotypes GG, AG and AA were 580, 580/410/170 and 410/170bp, respectively. **D.** SNP1 C>T in *DNMT3b* intron6 on BTA 13. **E.** SNP2 G>A in *DNMT3b* intron5 on BTA 13. **F.** SNP3 A>G in *DNMT3b* intron13 on BTA 13.

Table 3. Frequencies of genotypes and alleles in the four cattle breeds.

	Allelic frequency		Genotypic frequency		
SNP1 (C63029349T)	C	T	CC	CT	TT
Wagyu	0.900	0.100	0.800 (8)	0.200 (2)	0 (0)
Limousin	0.281	0.719	0.1875 (3)	0.1875 (3)	0.625 (10)
Fuzhou yellow cattle	0.800	0.200	0.714 (5)	0.286 (2)	0 (0)
Snow Dragon beef	0.900	0.100	0.807 (113)	0.186 (26)	0.0072 (1)
SNP2 (G63032883A)	G	A	GG	AG	AA
Wagyu	0.500	0.500	0 (0)	1.000 (4)	0 (0)
Limousin	-*	-	-	-	-
Fuzhou yellow cattle	-	-	-	-	-
Snow Dragon beef	0.629	0.370	0.360 (32)	0.539 (48)	0.101 (9)
SNP3 (A63039420G)	A	G	AA	AG	GG
Wagyu	0.636	0.364	0.272 (3)	0.727 (8)	0 (0)
Limousin	1.000	0	1.000 (23)	0 (0)	0 (0)
Fuzhou yellow cattle	1.000	0	1.000 (7)	0 (0)	0 (0)
Snow Dragon beef	0.711	0.289	0.493 (70)	0.437 (62)	0.070 (10)

*Not genotyped by the MassARRAY technique.

The effects of combined genotypes on meat quality

To analyze the effects of combined genotypes of the three SNPs on beef quality traits, the model 2 was employed. A total of 27 combination genotypes of the three SNPs were detected in the beef population. The results indicated that combined SNP1 and SNP2 were associated with rib-eye area at P value of 0.047, back-fat thickness at P value of 0.077 and chuck short rib score at P value of 0.083, and combined genotypes of SNP2 and SNP3 were associated with live weight at P value of 0.04 and chuck short rib score at P value of 0.087. Our results showed

that the beef cattle with combination genotype CTAA (SNP1 and SNP2) have the largest rib-eye area compared with the individuals with CCAA (50.84 *vs* 35.47 cm^2); TTAG own the highest back-fat thickness, while CCAA cattle showed the lowest; moreover, individuals with combination genotype CCGG showed the highest chuck short rib score, while CTAA cattle was the lowest. For the combination genotypes of SNP2 and SNP3, individuals with GGAG showed the highest live weight and chuck short rib score (Table 5). Individuals with GG and AG presented better associations with SNP2 and SNP3, which was consistent with the results.

Table 4. Association analysis between meat quality traits and SNP effects (LSM ± SE).

Marker	Genotype (No.)	Lean meat color score	Chuck short rib score	Marbling score	Dressing percentage	Backfat thickness (cm)
SNP1	CC(113)	2.55 ± 0.22	2.74 ± 0.40	2.66 ± 0.57	0.70 ± 0.13	2.28 ± 0.31
	CT(26)	2.57 ± 0.27	2.55 ± 0.50	3.00 ± 0.71	0.81 ± 0.16	2.39 ± 0.38
	TT(1)	2.77 ± 0.82	2.49 ± 1.53	2.29 ± 2.17	0.74 ± 0.50	3.08 ± 1.16
P value		0.956^{NS}	0.913^{NS}	0.450^{NS}	0.581^{NS}	0.340^{NS}
SNP2	AA(9)	2.10 ± 0.28^{a}	1.12 ± 0.60^{A}	3.95 ± 0.50	0.53 ± 0.04	3.35 ± 0.41
	AG(48)	2.47 ± 0.20^{ab}	2.22 ± 0.42^{Bb}	4.55 ± 0.37	0.53 ± 0.03	3.02 ± 0.30
	GG(32)	2.64 ± 0.20^{b}	2.76 ± 0.41^{b}	4.76 ± 0.36	0.57 ± 0.02	3.19 ± 0.30
P value		0.051^{NS}	0.018^{*}	0.138^{NS}	0.262^{NS}	0.525^{NS}
SNP3	AA(70)	2.63 ± 0.23	2.93 ± 0.44	2.42 ± 0.61	0.59 ± 0.14^{a}	1.92 ± 0.32^{A}
	AG(62)	2.55 ± 0.22	2.74 ± 0.41	2.99 ± 0.58	0.76 ± 0.13^{b}	2.48 ± 0.30^{B}
	GG(10)	2.52 ± 0.32	2.22 ± 0.59	1.74 ± 0.83	0.56 ± 0.19^{ab}	2.00 ± 0.43^{AB}
P value		0.566^{NS}	0.573^{NS}	0.049^{*}	0.034^{*}	0.013^{*}

The Bonferroni *t*-test was used for pair comparison in the study. ^{a,b}Means within a column with no common superscript letters differ at $P < 0.05$. ^{A,B}Means within a column with no common superscript letters differ at $P < 0.01$. $^{NS}P > 0.05$.

Table 5. Association analysis between combined genotypes and meat quality traits.

Combined SNPs	Combined genotype	Rib-eye area (cm^2)	Backfat thickness (cm)	Chuck short rib score
SNP1 and SNP2	CCAA	35.47 ± 2.26^{b}	1.63 ± 0.24	2.29 ± 0.48
	CCAG	35.55 ± 0.10^{b}	1.88 ± 0.11	3.11 ± 0.21
	CCGG	35.65 ± 1.17^{b}	2.30 ± 0.13	3.77 ± 0.25
	CTAA	50.84 ± 4.23^{a}	1.85 ± 0.45	2.00 ± 0.91
	CTAG	36.80 ± 1.89^{ab}	1.92 ± 0.20	2.80 ± 0.41
	CTGG	37.96 ± 2.44^{ab}	1.90 ± 0.26	3.00 ± 0.52
	TTAG	35.70 ± 5.98^{ab}	3.00 ± 0.64	3.00 ± 1.28
P value		0.047*	0.077^{NS}	0.083^{NS}
Combined SNPs	Combined genotype	Live weight (kg)	Chuck short rib score	
SNP2 and SNP3	AAAA	617.20 ± 38.54^{ab}	2.00 ± 0.57	
	AAAG	545.00 ± 60.93^{ab}	3.00 ± 0.91	
	AAGG	583.00 ± 86.17^{ab}	2.00 ± 1.29	
	AGAA	595.18 ± 18.37^{ab}	3.45 ± 0.28	
	AGAG	606.30 ± 17.97^{ab}	2.78 ± 0.27	
	AGGG	555.50 ± 60.93^{ab}	2.50 ± 0.91	
	GGAA	525.10 ± 27.25^{a}	3.60 ± 0.40	
	GGAG	641.74 ± 17.97^{b}	3.79 ± 0.30	
	GGGG	505.67 ± 49.75^{ab}	2.67 ± 0.75	
P value		0.04^{*}	0.087^{NS}	

The Bonferroni *t* test was used for pair comparison in the study. ^{a,b}Means within a column with no common superscript letters differ at $P < 0.05$. ^{A,B}Means within a column with no common superscript letters differ at $P < 0.01$. $^{NS}P > 0.05$.

DISCUSSION

Improving beef quality is one of the most important breeding targets in beef cattle. Effec-

tive molecular markers used in the selection of beef quality traits could bring benefits for farmers and producers in beef industry (Hoey et al., 1995). In the present study, association analysis was carried out between the three novel SNPs of bovine *DNMT3b* and 16 beef quality traits in the Snow Dragon beef population in China. We found that the SNP2 (G63032883A) was associated with the traits of lean meat color score ($P = 0.051$) and chuck short rib score ($P < 0.05$), while SNP3 (A63039420G) was significantly associated with dressing percentage and back-fat thickness ($P < 0.05$). These findings suggest that the SNP2 and SNP3 of bovine *DNMT3b* may play potential roles on the improvement of meat quality traits in beef cattle.

Snow Dragon beef is a commercial beef cattle population, which is the offspring of Wagyu and the crossbred of Limousin by Fuzhou yellow cattle (50% Wagyu, 25% Limousin and 25% Fuzhou yellow cattle). Wagyu is famous for its outstanding meat quality traits especially for marbling score all over the world. As the paternal of Snow Dragon beef, the superior beef quality characteristics of Wagyu were also shown in the commercial beef cattle population. The results of the present study indicated that the frequencies of alleles and genotypes of the novel SNPs in *DNMT3b* were much similar between Snow Dragon beef and Wagyu cattle, which were consistent with their genetic background.

Recently numerous candidate genes associated with beef quality traits have been discovered. Li et al. (2009) found that SNP T2885C in bovine *PRKAG3* gene was significantly associated with meat tenderness in seven beef cattle breeds ($P < 0.05$). Moreover, bovine *PON1* (Ji et al., 2009), *PLIN* (Fan et al., 2010), *CAST* (Tidball et al., 2002) and *CAPN1* (Page et al., 2002) were also found associated with meat quality traits in beef cattle. In the present study, two novel SNPs located in the introns of bovine *DNMT3b* gene were found significantly related with beef quality traits in Snow Dragon beef cattle of China. *DNMT3b* encoded *de novo* methyltransferase 3b is one of key factors of DNA methylation. Recent studies indicated that gene expression of human *DNMT3b* was related to adipose deposition (Turek-Plewa and Jagodzinski, 2005; de Vogel et al., 2011). Our previous study showed that bovine *DNMT3a* were higher expressed in liver and muscle tissues in beef cattle than that of *DNMT3b*, and both of the genes significantly correlated to the traits of beef quality (Guo et al., 2012). Present study was the first effort to analyze the association between *DNMT3b* polymorphism and meat quality traits in beef cattle like back-fat thickness, marbling score and so on. Considering DNA methylation mediates the interaction between beef cattle, environment and nutrition, the genetic effects of more DNA methylation-related genes and their target genes in beef quality traits is imperative to study.

To sum up, the significant associations of the SNPs in bovine *DNMT3b* and the beef quality traits suggest that the *DNMT3b* gene could be a valuable gene that affects meat quality in beef cattle.

ACKNOWLEDGMENTS

The authors thank the National-Level Conservation Farms of Fuzhou Yellow Cattle (Dalian, China, Mr. Niu Zhengxin) and Luoyang Baimasi Bull Station in Henan Province for providing semen samples. Research supported by the China Agriculture Research System (#CARS-37, #CARS-38), the Key Development of New Transgenic Breeds Programs (#2009ZX08009-146B), the Fund for Basic Research from the Ministry of Education of the People's Republic of China (#2011JS006), the National Key Technologies R & D Program

(#2011BAD28B02), and the Xuelong Biological Breeding Technology Industrialization Project funded by the National Development and Reform Commission (NDRC).

REFERENCES

Amara K, Ziadi S, Hachana M, Soltani N, et al. (2010). DNA methyltransferase DNMT3b protein overexpression as a prognostic factor in patients with diffuse large B-cell lymphomas. *Cancer Sci.* 101: 1722-1730.

Barres R and Zierath JR (2011). DNA methylation in metabolic disorders. *Am. J. Clin. Nutr.* 93: 897S-900.

de Vogel S, Wouters KA, Gottschalk RW, van Schooten FJ, et al. (2011). Dietary methyl donors, methyl metabolizing enzymes, and epigenetic regulators: diet-gene interactions and promoter CpG island hypermethylation in colorectal cancer. *Cancer Causes Control* 22: 1-12.

Fan YY, Zan LS, Wang HB and Yang YJ (2010). Study on the relationship between polymorphism of *PLIN* gene and carcass and meat quality traits in Qinchuan cattle. *Chin. J. Anim. Vet. Sci.* 41: 268-273.

Fraga MF, Ballestar E, Paz MF, Ropero S, et al. (2005). Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Natl. Acad. Sci. U. S. A.* 102: 10604-10609.

Guo X, Liu X, Xu X, Wu M, et al. (2012). The expression levels of DNMT3a/3b and their relationship with meat quality in beef cattle. *Mol. Biol. Rep.* 39: 5473-5479.

Haggarty P, Hoad G, Harris SE, Starr JM, et al. (2010). Human intelligence and polymorphisms in the DNA methyltransferase genes involved in epigenetic marking. *PLoS One* 5: e11329.

Halaschek-Wiener J, Amirabbasi-Beik M, Monfared N, Pieczyk M, et al. (2009). Genetic variation in healthy oldest-old. *PLoS One* 4: e6641.

Hoey AJ, Reich MM, Davis G, Shorthose R, et al. (1995). Beta 2-adrenoceptor densities do not correlate with growth, carcass quality, or meat quality in cattle. *J. Anim. Sci.* 73: 3281-3286.

Ji AG, Zhou ZK, Zhang LP, Yang RJ, et al. (2009). PON1 gene SNPs and association with growth and carcass traits in beef cattle. *Acta Vet. Zootechnica Sin.* 40: 122-128.

Kamei Y, Suganami T, Ehara T, Kanai S, et al. (2010). Increased expression of DNA methyltransferase 3a in obese adipose tissue: studies with transgenic mice. *Obesity* 18: 314-321.

Kurita S, Higuchi H, Saito Y, Nakamoto N, et al. (2010). DNMT1 and DNMT3b silencing sensitizes human hepatoma cells to TRAIL-mediated apoptosis via up-regulation of TRAIL-R2/DR5 and caspase-8. *Cancer Sci.* 101: 1431-1439.

Li WF, Yang RJ, Gan QF, Zhang LP, et al. (2009). Polymorphism of PRKAG3 gene and Its association with carcass and meat quality traits in beef cattle. *Acta Vet. Zootechnica Sin.* 40: 1106-1111.

Liu Y, Li K, Liu WJ, Wang JF, et al. (2009). Study on the effect of down-regulation of DNMT1 on cell proliferation, metastasis ability of esophageal squamous cell carcinoma cell line EC9706 cells and its related mechanisms. *China Oncol.* 19: 826-830.

Maier S and Olek A (2002). Diabetes: a candidate disease for efficient DNA methylation profiling. *J. Nutr.* 132: 2440S-2443S.

Okano M, Bell DW, Haber DA and Li E (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 99: 247-257.

Page BT, Casas E, Heaton MP, Cullen NG, et al. (2002). Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *J. Anim. Sci.* 80: 3077-3085.

Tidball JG and Spencer MJ (2002). Expression of a calpastatin transgene slows muscle wasting and obviates changes in myosin isoform expression during murine muscle disuse. *J. Physiol.* 545: 819-828.

Turek-Plewa J and Jagodzinski PP (2005). The role of mammalian DNA methyltransferases in the regulation of gene expression. *Cell Mol. Biol. Lett.* 10: 631-647.

Wang X, Zhu H, Snieder H, Su S, et al. (2010). Obesity related methylation changes in DNA of peripheral blood leukocytes. *BMC Med.* 8: 87.

Yu Y, Zhang H, Tian F, Zhang W, et al. (2008). An integrated epigenetic and genetic analysis of DNA methyltransferase genes (DNMTs) in tumor resistant and susceptible chicken lines. *PLoS One* 3: e2672.