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

SNPs at 3'-UTR of the bovine *CDIPT* gene associated with Qinchuan cattle meat quality traits

C.Z. Fu[1], H. Wang[1], C.G. Mei[1], J.L. Wang[1], B.J. Jiang[1], X.H Ma[1], H.B. Wang[1], G. Cheng[1] and L.S. Zan[1,2]

[1]Animal Science and Technology College, Northwest A&F University, Yangling, Shaanxi, China
[2]National Beef Cattle Improvement Center of China, Yangling, Shaanxi, China

Corresponding author: L.S. Zan
E-mail: zanls@yahoo.com.cn

Genet. Mol. Res. 12 (1): 775-782 (2013)
Received June 18, 2012
Accepted November 24, 2012
Published March 13, 2013
DOI http://dx.doi.org/10.4238/2013.March.13.6

ABSTRACT. The CDIPT is crucial to the fatty acid metabolic pathway, intracellular signal transduction and energy metabolism in eukaryotic cells. We detected three SNPs at 3'-untranslated regions (UTR), named 3'-UTR_108 A > G, 3'-UTR_448 G > A and 3'-UTR_477 C > G, of the *CDIPT* gene in 618 Qinchuan cattle using PCR-RFLP and DNA sequencing methods. At each of the three SNPs, we found three genotypes named as follows: AA, AB, BB (3'-UTR_108 A > G), CC, CD, DD (3'-UTR_448 G > A) and EE, EF, FF (3'-UTR_477 C > G.). Based on association analysis of these SNPs with ultrasound measurement traits, individuals of genotype BB had a significantly larger loin muscle area than genotype AA. Individuals of genotype CC had significantly thicker back fat than individuals of genotype DD. Individuals of genotype EE also had significantly thicker back fat than did individuals of genotype FF. We conclude that these SNPs of the *CDIPT* gene could be used as molecular markers for selecting and breeding beef cattle with superior body traits, depending on breeding goals.

Key words: Cattle; *CDIPT* gene; Ultrasound measurement traits; SNP; PCR-RFLP

INTRODUCTION

Two enzymes, cytidine diphosphate (CDP)-diacylglycerol synthetase and phosphatidylinositol (PtdIns) synthase (CDIPT, National Center for Biotechnology Information gene ID: 515135), are involved in the PtdIns biosynthetic pathway. CDIPT acts in the last step of the *de novo* biosynthesis of PtdIns by catalyzing the condensation of cytidine diphosphate-diacylglycerol and myo-inositol (Deguchi et al., 2002). The biosynthesis and metabolism of PtdIns and its phosphorylation derivatives play essential roles during intracellular signal transduction (White et al., 1973). However, Lykidis et al. (1997) have reported that overexpression of CDP-diacylglycerol synthetase or CDIPT alone or in combination does not enhance the rate of PtdIns biosynthesis, possibly because the exchange activity of PtdIns is regulated by the reverse reaction of CDIPT and it depends on cytidine monophosphate, which is tightly bound to CDIPT. The expression of the *CDIPT* gene is uniform among tissues, but slightly higher expression levels are detected in the human liver and skeletal muscles (Lykidis et al., 1997). Moreover, Deguchi et al. (2002) have reported that overexpression of the *CDIPT* gene enhances growth and G1 progression in NIH3T3 cells. CDIPT has an optimum pH of 9.0, requires Mn^{2+} or Mg^{2+}, and is inhibited by Ca^{2+} and Zn^{2+}, suggesting that chemical elements of signal pathways could regulate the enzyme activity of CDIPT. Therefore, CDIPT may also be involved in some signal pathways (Antonsson, 1994).

In 2009, Fu et al. obtained the sequence of the porcine *CDIPT* gene 5'-UTR from 3 swine breeds, namely, Large White, Landrace, and Meishan. They detected a significant SNP in the analyzed population. According to the association analysis between SNP and the meat quality traits of pigs, they concluded that the detected SNP could be used as candidate molecular marker in porcine selection and breeding.

Taken together, these findings suggest that the *CDIPT* gene plays an important role during fatty acid and energy metabolism. No research to date has reported on *CDIPT* gene polymorphisms in cattle. Therefore, the objective of this study was to detect potential SNPs in the Qinchuan cattle *CDIPT* gene and explore possible associations between SNPs and ultrasound measurement traits (UMTs) in cattle with the aim of selecting beef cattle with better UMTs.

MATERIAL AND METHODS

DNA samples and data collection

Blood samples were collected from 618 individuals (18-24 months old) randomly selected from a Qinchuan cattle breeding population to analyze *CDIPT* gene genotypic and allelic frequencies. The UMTs, including ultrasound back fat (UBF), ultrasound loin muscle area (ULMA), and ultrasound marbling score, were measured as described elsewhere (Brethour, 1994; Hamlin et al., 1995). Blood samples obtained from the 618 animals were stored at -80°C after being treated with 2% heparin. DNA samples were extracted from the blood samples according to standard procedures (Chen and Leibenguth, 1995).

Polymerase chain reaction (PCR) and DNA sequencing

Based on the bovine *CDIPT* gene sequence, 3 pairs of PCR primers were designed to

amplify the complete sequence of the 3'-UTR (Table 1). PCR was performed in 20-μL reaction mixtures containing 50 ng DNA template, 10 pM of each primer, 0.20 mM deoxyribonucleoside triphosphate, 2.5 mM $MgCl_2$, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The cycling protocol was 5 min at 95°C, 32 cycles at 95°C for 30 s, annealing at Tm-PCR °C (see Table 1) for 30 s, 72°C for 35 s, and a final extension at 72°C for 5 min. The PCR products were electrophoresed on 1.5% agarose gel (containing 200 ng/mL ethidium bromide) using 1X TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2ethylenediaminetetraacetic acid).

Table 1. Sequences of three PCR amplification primer pairs.

Fragment names	Primer pairs	Tm-PCR (°C)	Size (bp)
Fragment-1	F: 5'-TCACAGTTGGCTCGGTGGGTC-3' R: 5'-TTACGGGGGTGGGAGAACTGG-3'	58.2	357
Fragment-2	F: 5'-TCACCCTGTGTGCTGGAAATG-3' R: 5'-CTGCTCCAAGAGTATCTCCAAG-3'	59.5	914
Fragment-3	F: 5'-TGGGATAGGGGCCAGGGTTC-3' R: 5'-CATCATTTTTCCCCCAACCTCT-3'	60.0	244

Tm = melting temperature.

PCR-restriction fragment length polymorphism (RFLP)

Aliquots (5 μL) of the PCR products from all 618 samples were digested with 0.5 U restriction endonuclease (MBI Fermentas, Lietuva) at Tm-RFLP °C (Table 2) for 4 h following manufacturer instructions. The digested products were detected with electrophoresis on 1.5% agarose gel stained with ethidium bromide. The PCR products of the electrophoresis patterns were sequenced in both directions in an ABI PRIZM 377 DNA sequencer (Perkin-Elmer Corporation, USA). The sequences were analyzed using the DNAStar package.

Statistical analyses

Genotypic frequency, allele frequency, Hardy-Weinberg equilibrium, gene homozygosity, gene heterozygosity (H_E), effective allele numbers (N_E), and polymorphism information content (PIC) were statistically analyzed according to the approaches of Nei and Roychoudhury (1974) and Nei and Li (1979). Associations between SNP marker genotypes of the *CDIPT* gene and records of UMTs were analyzed with SPSS (version 18.0) according to the following statistical linear model:

$$Y_{ijk} = \mu + S_i + E_j + P_k + \varepsilon_{ijk} \quad \text{(Equation 1)}$$

where Y_{ijk} is the observation for the UMTs; μ is the mean for each trait; S_i is the fixed effect of age; E_j is the genotype effect; P_k represents the fixed effects of the farm, and ε_{ijk} is the random error.

RESULTS

The National Center for Biotechnology Information Nucleotide Basic Local Alignment Search Tool result for the *CDIPT* gene revealed that Qinchuan cattle had 89, 87, 83, and 81% nucleotide sequence similarities with *Sus scrofa* (GU144289), *Mus musculus* (NM_138754.3),

Homo sapiens (NM_006319.3), and *Rattus norvegicus* (NM_138899.2), respectively. We amplified 3 DNA sequence fragments - Fragment-1, Fragment-2, and Fragment-3 - to obtain the complete 3'-UTR sequence of Qinchuan cattle and detected 3 novel mutations located at 108, 448, and 477 bp using PCR-RFLP and DNA sequencing methods. The first mutation (A > G) at 108 bp was named 3'-UTR_108 and is a *Tas*I restriction enzyme site in Fragment-1. The second mutation (G > A) at 448 bp was named 3'-UTR_448 and is an *Nco*I restriction enzyme site in Fragment-2. The third mutation (C > G) at 477 bp was named 3'-UTR_477 and is an *Mbo*I restriction enzyme site in Fragment-3 (see Table 2).

Table 2. Genotypic frequencies at *CDIPT* gene 3'-UTR for the SNPs in Qinchuan cattle and restriction endonuclease information.

Location	Restriction endonuclease	Tm-RFLP (°C)	Genotypic frequency (number)		Total	Allele frequency		χ^2 (HW)	P (HW)
3'-UTR_108	*Tas*I	65	AA (399)	0.646	618	A	0.803	0.054	0.973
			AB (194)	0.314					
			BB (25)	0.040		B	0.197		
3'-UTR_448	*Nco*I	37	CC (411)	0.665	618	C	0.815	0.044	0.978
			CD (185)	0.299					
			DD (22)	0.036		D	0.185		
3'-UTR_477	*Mbo*I	37	EE (418)	0.676	618	E	0.819	1.007	0.604
			EF (176)	0.285					
			FF (24)	0.039		F	0.181		

UTR = untranslated regions; RFLP = restriction fragment length polymorphism; HW = Hardy-Weinberg equilibrium, $\chi 0.05^2 = 5.991$, $\chi 0.01^2 = 9.21$.

Mutation 3'-UTR_108 had 3 genotypes, AA, AB, and BB (Figure 1), with genotypic frequencies of 0.646, 0.314, and 0.040, respectively. The frequencies of alleles A and B were 0.803 and 0.197, respectively. The chi-square test revealed that the analyzed cattle population was in Hardy-Weinberg equilibrium (P > 0.05; see Table 2). H_E, N_E, and PIC values were 0.317, 1.464, and 0.267, respectively (Table 3). The SPSS analysis indicated that individuals with genotype BB had a ULMA larger than that of individuals with genotype AA (52.341 *vs* 46.103 cm^2; Table 4).

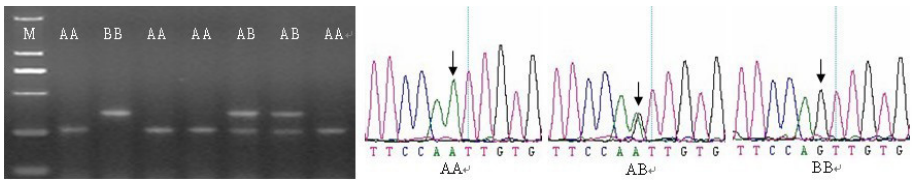

Figure 1. Agarose gel electrophoresis of *Tas*I PCR-RFLP. The AA genotype shows 1 fragment (258 bp), the AB genotype shows 2 fragments (357 and 258 bp) and the BB genotype shows 1 fragment (357 bp). *Lane M* = DNA marker, DL2000 (TaKaRa, Dalian, China).

Table 3. Population genetic indices at the *CDIPT* gene locus in Qinchuan cattle.

Location	H_O	H_E	N_E	PIC
3'-UTR _108	0.683	0.317	1.464	0.267
3'-UTR _448	0.698	0.302	1.433	0.256
3'-UTR _477	0.703	0.297	1.422	0.253

H_O = gene homozygosity; H_E = gene heterozygosity; N_E = effective allele numbers; PIC = polymorphism information content.

Table 4. Association between the 3'-UTR_108 A > G SNP genotype of the *CDIPT* gene and UMTs in Qinchuan cattle.

Traits (mean ± SE)	Genotypes (number)		
	AA (399)	AB (194)	BB (25)
UBF (cm)	0.873 ± 0.015	0.891 ± 0.022	0.917 ± 0.060
ULMA (cm^2)	46.103 ± 0.732[b]	47.124 ± 1.050	52.341 ± 2.926[a]
UMAR	4.298 ± 0.030	4.340 ± 0.043	4.480 ± 0.121

[a,b]Means with different superscript letters are significantly different (P < 0.05); UTR = untranslated regions; UMT = ultrasound measurement trait; UBF = ultrasound back fat thickness; ULMA = ultrasound loin muscle area; UMAR = ultrasound marbling score.

Mutation 3'-UTR_447 also had 3 genotypes, CC, CD, and DD (Figure 2), with genotypic frequencies of 0.665, 0.299, and 0.036. The frequencies of alleles C and D were 0.815 and 0.185, respectively. The analyzed population was in Hardy-Weinberg equilibrium (P > 0.05) according to the chi-square test (see Table 2). H_E, N_E, and PIC values were 0.302, 1.433, and 0.256, respectively (see Table 3). Individuals of genotype CC had a UBF thicker than that of individuals of genotype DD (0.955 *vs* 0.803 cm; Table 5).

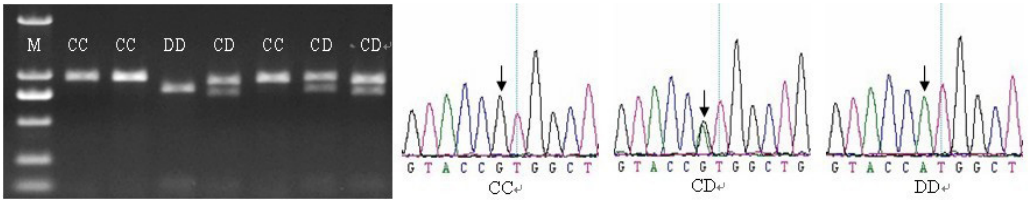

Figure 2. Agarose gel electrophoresis of *Nco*I PCR-RFLP. The CC genotype shows 1 fragment (914 bp), the CD genotype shows 2 fragments (914 and 783 bp) and the DD genotype shows 1 fragment (783 bp). *Lane M* = DNA marker, DL2000 (TaKaRa, Dalian, China).

Table 5. Association between the 3'-UTR_448 G> A SNP genotype of the *CDIPT* gene and UMTs in Qinchuan cattle.

Traits (mean ± SE)	Genotypes (number)		
	CC (411)	CD (185)	DD (22)
UBF (cm)	0.955 ± 0.017[a]	0.897 ± 0.025	0.803 ± 0.073[b]
ULMA (cm^2)	48.797 ± 0.837	48.613 ± 1.247	46.777 ± 3.617
UMAR	4.290 ± 0.035	4.330 ± 0.053	4.364 ± 0.153

[a,b]Means with different superscript letters are significantly different (P < 0.05); UTR = untranslated regions; UMT = ultrasound measurement trait; UBF = ultrasound back fat thickness; ULMA = ultrasound loin muscle area; UMAR = ultrasound marbling score.

Mutation 3'-UTR_477 had 3 genotypes, EE, EF, and FF (Figure 3), with genotypic frequencies of 0.676, 0.285, and 0.039, respectively. The frequencies of alleles E and F were 0.819 and 0.181, respectively. The analyzed population was in Hardy-Weinberg equilibrium (P > 0.05; see Table 2). H_E, N_E, and PIC values were 0.297, 1.422, and 0.253, respectively (see Table 3). Individuals with genotype EE had a UBF thicker than that of individuals of genotype FF (0.912 *vs* 0.785 cm; Table 6).

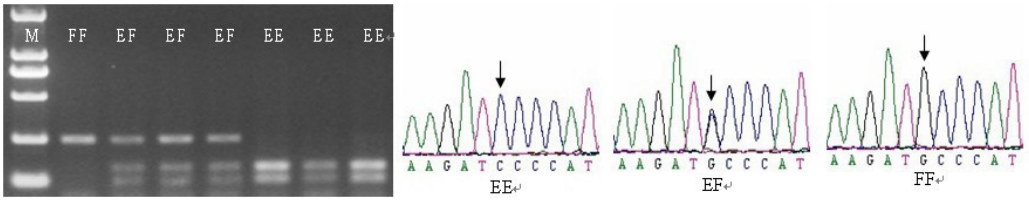

Figure 3. Agarose gel electrophoresis of *Mbo*I PCR-RFLP. The EE genotype shows 2 fragments (143 and 101 bp), the EF genotype shows 3 fragments (244, 143 and 101 bp) and the FF genotype shows 1 fragment (244 bp). *Lane M* = DNA marker, DL2000 (TaKaRa, Dalian, China).

Table 6. Association between the 3'-UTR_477 C> G SNP genotype of the *CDIPT* gene and UMTs in Qinchuan cattle.

Traits (mean ± SE)	Genotypes (number)		
	EE (418)	EF (176)	FF (24)
UBF (cm)	0.912 ± 0.015[a]	0.866 ± 0.023	0.785 ± 0.062[b]
ULMA (cm^2)	48.014 ± 0.809	46.866 ± 1.246	46.871 ± 3.374
UMAR	4.234 ± 0.038	4.290 ± 0.058	4.375 ± 0.157

[a,b]Means with different superscript letters are significantly different ($P < 0.05$); UTR = untranslated regions; UMT = ultrasound measurement trait; UBF = ultrasound back fat thickness; ULMA = ultrasound loin muscle area; UMAR = ultrasound marbling score.

DISCUSSION

The bovine *CDIPT* gene is located at chromosome 25, translates a 2.1-kb messenger RNA, and encodes 213 amino acids in cattle (Zimin et al., 2009). Our study found that the bovine *CDIPT* gene had comparatively high nucleotide sequence similarities (>80%) with other animals, indicating good sequence conservation during evolution.

The 3'-UTR is a section of messenger RNA that starts with the nucleotide immediately following the stop codon of the coding region. The processing of the 3'-UTR is important for transcription termination downstream of cleavage sites. The polyadenylation signal of the 3'-UTR has been demonstrated to impact gene expression in many mammals (Zhao et al., 1999; Neilson and Sandberg, 2010). In addition, Zhou et al. (2011) have reported one SNP at the UTR of the prolactin receptor gene that could affect fiber traits in goats, indicating that the SNP of the UTR may influence animal production traits. In this study, we analyzed the entire 3'-UTR of the bovine *CDIPT* gene and found one CCAAT binding site from 106 to 110 bp, which indicated that the *CDIPT* gene may regulate cattle body metabolism via a response to some CCAAT box-binding proteins. Such a crucial role played by the 3'-UTR stimulated us to search for and analyze its composing structure and potential SNP positions.

H_E, N_E, and PIC were used to measure the genetic information of each site, and the H_E value obtained in the 3 groups was uniformly lower than the value of gene homozygosity. This difference may be due to gene random drift owing to the low frequency of alleles B, D, and F. In addition, such observation may also be the result of inadequate random sampling that could not detect gene distribution changes. H_E, N_E, and PIC parameters can be used to evaluate the degree of polymorphism within a given population (Chakraborty et al., 1994). Generally, PIC can be classified into 3 types of polymorphism: low (PIC value <0.25), medium (0.25 < PIC

value < 0.50), and high (PIC value >0.50). In our study, all SNP loci showed medium polymorphism (see Table 3), indicating they could be used as molecular markers.

Three genotypes were observed at the 3'-UTR_108 mutation: AA, AB, and BB. Individuals of genotype BB performed better than those of genotype AA in ULMA (52.341 *vs* 46.103 cm^2), suggesting that producers could select individuals of genotype BB if their aim is to have a better ULMA. The 3'-UTR_448 mutation also had 3 genotypes (CC, CD, and DD). Individuals of genotype CC had UBF greater than that of individuals of genotype DD (0.955 *vs* 0.803 cm). Moreover, mutation 3'-UTR_477 in Fragment-3 showed genotypes EE, EF, and FF. Individuals of genotype EE had UBF thicker than that of individuals of genotype FF (0.912 *vs* 0.785 cm). All of the analyses in this study suggest that individuals of genotype CC and EE could be selected if the goal is to breed cattle with greater UBF.

In summary, our study is the first to report 3 SNPs of the *CDIPT* gene 3'-UTR in Qinchuan cattle. We also demonstrated an association between the *CDIPT* gene and UMT. The results of this research could be used to select and breed high-quality beef cattle.

ACKNOWLEDGMENTS

Research supported by the National Twelfth "Five Year" Science and Technology Support Project (#2011BAD28B04-03), the China National 863 Program (#2011AA100307), the GMO New Varieties Major Project (#2011ZX08007-002), the National Beef and Yak Industrial Technology System (#CARS-38), the Program for Changjiang Scholars and Innovative Research Team (#IRT0940), and the "13115" Scientific and Technological Innovation Program of Shaanxi Province (#S2010ZDGC109).

REFERENCES

Antonsson BE (1994). Purification and characterization of phosphatidylinositol synthase from human placenta. *Biochem. J.* 297 (Pt 3): 517-522.

Brethour JR (1994). Estimating marbling score in live cattle from ultrasound images using pattern recognition and neural network procedures. *J. Anim. Sci.* 72: 1425-1432.

Chakraborty R, Zhong Y, de AM, Clemens PR, et al. (1994). Linkage disequilibria among (CA)n polymorphisms in the human dystrophin gene and their implications in carrier detection and prenatal diagnosis in Duchenne and Becker muscular dystrophies. *Genomics* 21: 567-570.

Chen H and Leibenguth F (1995). Studies on multilocus fingerprints, RAPD markers, and mitochondrial DNA of a gynogenetic fish (*Carassius auratus gibelio*). *Biochem. Genet.* 33: 297-306.

Deguchi A, Segawa K, Hosaka K, Weinstein IB, et al. (2002). Overexpression of phosphatidylinositol synthase enhances growth and G1 progression in NIH3T3 cells. *Jpn. J. Cancer Res.* 93: 157-166.

Fu YY, Xiong YZ, Pan G, Cheng L, et al. (2009). Association of the polymorphism of porcine *CDIPT* gene with carcass and meat quality traits. *Chin. J. Anim. Vet. Sci.* 40: 787-791.

Hamlin KE, Green RD, Perkins TL, Cundiff LV, et al. (1995). Real-time ultrasonic measurement of fat thickness and longissimus muscle area: I. Description of age and weight effects. *J. Anim. Sci.* 73: 1713-1724.

Lykidis A, Jackson PD, Rock CO and Jackowski S (1997). The role of CDP-diacylglycerol synthetase and phosphatidylinositol synthase activity levels in the regulation of cellular phosphatidylinositol content. *J. Biol. Chem.* 272: 33402-33409.

Nei M and Roychoudhury AK (1974). Sampling variances of heterozygosity and genetic distance. *Genetics* 76: 379-390.

Nei M and Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. U. S. A.* 76: 5269-5273.

Neilson JR and Sandberg R (2010). Heterogeneity in mammalian RNA 3' end formation. *Exp. Cell Res.* 316: 1357-1364.

White DA (1973). The Phospholipid Composition of Mammalian Tissues. In: Form and Function of Phospholipids (Ansell GB, Hawthorne JN and Dawson RMC, eds.). Elsevier, Amsterdam, 441-482.

Zhao J, Hyman L and Moore C (1999). Formation of mRNA 3' ends in eukaryotes: mechanism, regulation, and interrelationships with other steps in mRNA synthesis. *Microbiol. Mol. Biol. Rev.* 63: 405-445.

Zhou JP, Zhu XP, Zhang W, Qin F, et al. (2011). A novel single-nucleotide polymorphism in the 5' upstream region of the prolactin receptor gene is associated with fiber traits in Liaoning cashmere goats. *Genet. Mol. Res.* 10: 2511-2516.

Zimin AV, Delcher AL, Florea L, Kelley DR, et al. (2009). A whole-genome assembly of the domestic cow, *Bos taurus*. *Genome Biol.* 10: R42.