Expression profiles and polymorphism analysis of CDIPT gene on Qinchuan cattle

Changzhen Fu, Hong Wang, Yaokun Li, Jiali Wang, Gong Cheng, Hongbao Wang, Wucai Yang, Linsen Zan

College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, PR China
National Beef Cattle Improvement Center, Yangling, Shaanxi 712100, PR China

Abstract

Background: CDIPT (CDP-diacylglycerol–inositol 3-phosphatidyltransferase, EC 2.7.8.11) was found on the cytoplasmic side of the Golgi apparatus and the endoplasmic reticulum. It was an integral membrane protein performing the last step in the de novo biosynthesis of phosphatidylinositol (PtdIns). In recent years, PtdIns has been considered to play an essential role in energy metabolism, fatty acid metabolic pathway and intracellular signal transduction in eukaryotic cells.

Results: In this study, the results of real-time polymerase chain reaction (PCR) showed that the expression of CDIPT gene was remarkably different in diverse tissues. We also detected the polymorphism of bovine CDIPT gene and analyzed its association with body measurement and meat quality traits of Qinchuan cattle. Blood samples were obtained from 638 Qinchuan cattle aged from 18 to 24 months. DNA sequencing and PCR-restriction fragment length polymorphism (RFLP) were used to find CDIPT gene single nucleotide polymorphism (SNP). Three SNPs g.244T→C (NCBI: rs42069760), g.1496G→A and g.1514G→A were found in this study. g.244T→C located at 5′ untranslated region (5′UTR) of exon 1 showed three genotypes: TT, TC and CC. g.1496G→A and g.1514G→A detected the first time were located in intron 3 and showed the same genotypes: GG, GA and AA.

Conclusions: Analysis results showed that these three SNPs were significantly associated with body measurement traits (BMTs) and meat quality traits (MQTs). We suggested that CDIPT gene may have potential effects on BMTs and MQTs and can be used for marker-assisted selection.

1. Introduction

CDIPT (CDP-diacylglycerol–inositol 3-phosphatidyltransferase) found on the cytoplasmic side of the Golgi apparatus and the endoplasmic reticulum performs the last step in the de novo biosynthesis of phosphatidylinositol (PtdIns) by catalyzing the condensation of cytidine diphosphate-diacylglycerol and myo-inositol to produce PtdIns and cytidine monophosphate (CMP) [1,2]. As an important lipid, PtdIns participates in essential metabolic processes in all plants and animals directly or via a number of metabolites. The metabolism and biosynthesis of PtdIns are of considerable interest due to its phosphorylated derivatives in energy metabolism, fatty acid metabolic pathway and intracellular signal transduction in eukaryotic cells [3]. Breakdown products of PtdIns are ubiquitous second messengers that function downstream of many G protein-coupled receptors and tyrosine kinases regulating cell growth, protein kinase C activity and calcium metabolism [4]. Over expression of CDIPT can enhance growth and G1 progression in NIH3T3 cells [5]. In COS-7 cells, the expression of CDIPT could lead to an overproduction of protein activity, and further enhance PtdIns activity [6]. CDIPT-deficient zebra fish exhibited hepatic non-alcoholic fatty liver disease (NAFLD) pathologies, which implicated a novel link between hepatic steatosis and PtdIns [7]. These findings indicated that CDIPT is very important in fatty acid metabolism as well as in energy metabolism.

Fu et al. [8] reported a single nucleotide polymorphism (SNP) with a mutation A→G at 5′UTR of porcine CDIPT, indicating its positive effects on meat quality traits (MQTs) in swine. Studies on Qinchuan cattle suggested that SNPs in 3′UTR of CDIPT gene were also related to MQTs [9].
small intestine, rumen, reticulum, omasum and abomasum. All liver, spleen, lung, kidney, muscle, subcutaneous fat, large intestine, Center, Yangling, Shaanxi, China) were obtained, including the heart, (2 years old) (Experimental Farm of National Beef Cattle Improvement

2.1. Samples collecting

We hypothesized that CDIPT may have an influence on the body measurement traits (BMTs) and MQTs. However, there were little reports on CDIPT’s effects on cattle. In this study, we analyzed the expression level of CDIPT gene in different tissues and explore the possible association of SNP with BMTs and MQTs, aimed at selecting samples with BMTs and MQTs, aimed at selecting beef cattle with superior BMTs and MQTs.

2. Materials and methods

2.1. Samples collecting

Thirteen tissue samples from three purebred Qinchuan cattle (2 years old) (Experimental Farm of National Beef Cattle Improvement Center, Yangling, Shaanxi, China) were obtained, including the heart, liver, spleen, lung, kidney, muscle, subcutaneous fat, large intestine, small intestine, rumen, reticulum, omasum and abomasum. All samples were promptly frozen in liquid nitrogen and stored at -80°C. Total RNAs were extracted from all tissues using TRIZOL Reagent (Invitrogen, USA). To remove the contaminating genomic DNA, the RNAs extracted were treated with RNase-free DNase set (Omega, USA), and the RNA concentration of each sample was adjusted to the same level. First-strand cDNA synthesis has been obtained by reverse transcription using the treated total RNAs as template and RevertAid First Strand cDNA Synthesis kit (Takara, Dalian, China) according to the manufacturer’s instructions.

2.2. Tissue expression profile analysis

In order to enhance the understanding of the gene products’ role in different tissues of Qinchuan cattle, CDIPT gene expression profile in Qinchuan cattle was analyzed by the ABI (Applied Biosystems) 7500 real time-PCR (RT-PCR) system. RT-PCR primer (CDIPT-F: 5′-CGATGT GTCGCTGTCACA-3′ and CDIPT-R: 5′-GAGGTGTAGTAGATGCGAAGC-3′) for the Qinchuan cattle CDIPT gene was designed to amplify 184 bp products. Another pair of primers (GAPDH-F: 5′-CCAACTCTTGCTTGC-3′ and GAPDH-R: 5′-CTGCTTCAACCTTCTCTTA-3′) was designed to obtain 80 bp products of the bovine GAPDH housekeeping gene (GenBank: NM_001034034), which served as the endogenous control. The RT-PCR system in 20 μL reaction volume consisted of 50 ng cDNA, 0.4 μM of each primer, 1 × SYBR® Premix Ex Taq II, and 1 × ROX reference dye. The PCR condition was as follows: 95°C for 30 s, 40 cycles of denaturation at 95°C for 5 s, and extension at 60°C for 34 s to amplify 184 bp products, and another 15 s at 95°C with 40 cycles, 1 min at 60°C and 95°C for 15 s to obtain the melting curve. All quantitative RT-PCRs were performed in triplicate, based on a standard curve method.

2.3. DNA samples and data collection

638 individuals with complete BMT and MQT information aged from 18 to 24 months were randomly selected from Qinchuan cattle breeding farm to provide blood samples for the future analysis. The obtained blood samples were stored at -80°C after being treated with 2% heparin. DNA samples were extracted from these blood samples according to the standard procedures [10]. BMTs, including rump length (RL), body

Table 1

<table>
<thead>
<tr>
<th>Primes</th>
<th>The primer sequences</th>
<th>Product size (bp)</th>
<th>Tm-1 (°C)</th>
<th>Amplified region</th>
<th>SNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDIPT-1</td>
<td>5′-AAGGAAGGCCGCCAGATC-3′</td>
<td>431</td>
<td>56.3</td>
<td>Exon 1</td>
<td>g.244T-C</td>
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<tr>
<td>CDIPT-2</td>
<td>5′-TGGTCACTAATTGCTACC-3′</td>
<td>404</td>
<td>53.9</td>
<td>Exon 2, intron 1, partial intron 2</td>
<td>None</td>
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<tr>
<td>CDIPT-3</td>
<td>5′-CTCTGTGCTTAAAGCC-3′</td>
<td>290</td>
<td>58.3</td>
<td>Exon 3, partial intron 2, partial intron 3</td>
<td>g.1496G&gt;A, g.1514G&gt;A</td>
</tr>
<tr>
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<td>5′-CTCTCACTGTGCTTAC-3′</td>
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<td>52.0</td>
<td>Exon 4, partial intron 3, partial intron 4</td>
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<tr>
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<td>5′-GCCCTGCACTGGTACC-3′</td>
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<td>Exon 5, intron 4, partial intron 4</td>
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</table>

Table 2

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<th>SNP</th>
<th>The primer sequences</th>
<th>Product size (bp)</th>
<th>Tm-2 (°C)</th>
<th>Restriction endonuclease</th>
<th>Recognition site</th>
</tr>
</thead>
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<tr>
<td>g.244T-C</td>
<td>5′-AAGGAAGGCCGCCAGATC-3′</td>
<td>692</td>
<td>48.0</td>
<td>Sphi</td>
<td>GCATGC/C</td>
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<tr>
<td>g.1496G-A</td>
<td>5′-GACACGCTGTTAGATGAGC-3′</td>
<td>290</td>
<td>58.3</td>
<td>PstI</td>
<td>CC/CG</td>
</tr>
<tr>
<td>g.1514G-A</td>
<td>5′-TACAACCTCTAAACC-3′</td>
<td>169</td>
<td>63.0</td>
<td>MspI</td>
<td>G/CNCC</td>
</tr>
</tbody>
</table>

Fig. 1. mRNA expression profile of CDIPT gene in 13 tissues of Qinchuan cattle. RQ is relative quantity.
length (BL), hip width (HW), withers height (WH), heart girth (HG), hip height (HH), chest depth (CD) and pin bone width (PBW), were measured as previously described [11]. Ultrasound measurements were available for meat quality traits [12,13,14], including loin muscle area (ULMA), backfat thickness (UBF) and marbling score (UMAR). To minimize the systematic error, a single person was assigned to measure each of the 11 traits during the experiment.

2.4. PCR amplification and DNA sequencing

Based on the bovine CDIPT gene sequence, 5 pairs of PCR primers were designed to amplify the complete length of 5’UTR and coding sequence (CDS) region (Table 1). PCR amplified products were performed in the 20 μL reaction mixture containing 2.5 mM MgCl2, 10 pM of each primer, 20 ng DNA template, 0.20 mM dNTP and 0.5 U Taq DNA polymerase (Takara, Dalian, China). The cycling protocol was 95°C for 5 min and 95°C for 30 s with 32 cycles, annealing at Tm-1°C (Table 1) for 30 s, 72°C for 35 s, and a final extension for 10 min at 72°C. The PCR products were electrophoresed on 1.5% agarose gel stained with ethidium bromide to certify the electrophoresis pattern.

2.5. PCR-restriction fragment length polymorphism

According to the novel SNPs, new PCR primers were designed (Table 2). Aliquots (5 μL) of the PCR products from all 638 samples were digested with 0.5 U restriction endonuclease (Takara, Dalian, China) at Tm-2°C (Table 2) for 4 h following the manufacturer’s instruction. The digested PCR products were detected on 1.5% agarose gel stained with ethidium bromide to certify the electrophoresis pattern.

2.6. Statistical analysis

Genotypic frequencies, Hardy–Weinberg equilibriums, allelic frequencies, gene homozygosity (Ho), effective allele numbers (Ne), heterozygosity (He) and polymorphism information content (PIC) were statistically analyzed [15,16]. Association between the potential SNP genotypes of CDIPT and records in all these BMTs (11 traits) and MQTs (3 Traits) was analyzed by software SPSS (version 18.0) according to the following statistical linear model [Equation 1]:

\[ Y_{ijk} = \mu + G_i + S_j + A_k + F_{ik} + e_{ijk} \]

where \( Y_{ijk} \) was the observation for the BMTs and MQTs, \( \mu \) was the mean value, \( G_i \) was the genotype effect, \( S_j \) was the fixed effect of sex, \( A_k \) was the fixed effect of age, \( F_{ik} \) was the fixed effects of farm, and \( e_{ijk} \) was the random error.

3. Results

3.1. Tissue expression profile analysis

The expression of CDIPT in the selected tissues was analyzed via RT-PCR (Fig. 1). Results showed that the CDIPT gene was found in all 13 tissue samples, but the quantities were markedly different in diverse tissues, and high expression level in the kidney, rumen, spleen, small intestine, large intestine, reticulum and subcutaneous fat. The expression level was relatively low in liver, lung, omasum and heart, and especially lower in abomasum and muscle.

3.2. SNP identification and the genotype of CDIPT gene

The SNPs identified in this study were a g.244T>C (NCBI: rs42069760) mutation at the 5’UTR of exon 1 (Fig. 2), and two mutations g.1496G>A and g.1514G>C (Fig. 3 and Fig. 4) intron 3 which were detected the first time. By DNA sequencing and RFLP, the genotypes of g.244T>C were identified as follows: TT, TC and CC, the products digested with SpI showed 3 fragments (692, 406 and 286 bp) for genotype TC, 1 fragment (692 bp) for genotype CC and 2 fragments (406 and 286 bp) for genotype TT (Fig. 2); the three g.1496G>A genotypes GG, GA and AA were also identified, the products digested with PstI showed 3 fragments (290, 209 and 81 bp) for genotype GA, 1 fragment (290 bp) for genotype AA and 2 fragments (406 and 286 bp) for genotype GG (Fig. 3); the g.1514G>C polymorphism was demonstrated with 3 genotypes: GG, GA and AA, the products digested with MspI showed 3 fragments (169, 106 and 63 bp) for genotype GA, 1 fragment (169 bp) for genotype AA and 2 fragments (106 and 63 bp) for genotype GG (Fig. 4).

3.3. Genetic polymorphism of Qinchuan cattle CDIPT gene and \( \chi^2 \) test

For the mutations g.244T>C, g.1496G>A and g.1514G>A, the genotype and allele frequency were analyzed and the results were shown in Table 3. The allele frequencies for g.244T>C were 0.6654(T)/0.3346(C); for g.1496G>A, 0.7058(G)/0.2942(A); and for g.1514G>C, 0.7610(G)/0.2390(C). The \( \chi^2 \)-test indicated that the genotype distributions of the g.244T>C and g.1496G>A mutations were in Hardy–Weinberg equilibrium (\( \chi^2 < \chi^2_{0.05} \)), but the g.1514G>C mutation was not (\( \chi^2 > \chi^2_{0.05} \)). The genetic indexes of each locus including Ho, Ne, He and PIC were calculated (Table 4). The PIC values of the mutations g.244T>C, g.1496G>A and g.1514G>C were 0.3462, 0.3042 and 0.2976, respectively. According to the classification of PIC,
the QinChuan cattle population studied for the 3 mutations was all in medium polymorphism level.

3.4. Effects of the polymorphism locus on BMTs

The association of CDIPT SNPs with 8 BMTs was analyzed and the results were shown in Table 5. For g.244T–C mutation, the mean value of animals with genotype TT was significantly higher than genotype CC for BL (P < 0.05); for HW and HG, genotype TT was significantly lower than genotypes CC and TC (P < 0.05); no significant difference was found between any genotype for WH, HH, RL, CD and PBW (P > 0.05). At g.1496G–A locus, there was significant difference between genotype AA and genotype GG with BL and HW and also between genotype AA and genotype GA with HW and PBW (P < 0.05); for PBW, the mean value of individuals with genotype AA was significantly higher than individuals with genotype GG (P < 0.01); no significant difference was found between genotypes GG, AG and AA for WH, HH, RL, CD and HG (P > 0.05). For g.1514G–A mutation, there was significant difference between genotype AA and genotype GG with CD (P < 0.05); individuals with genotype AA had significantly higher expression than individuals with GG and AG genotypes (P < 0.01); no significant difference was found between any genotype for BL, WH, HH, RL, HG and PBW (P > 0.05).

3.5. Effects of the polymorphism locus on MQTs

The association between the 3 mutations and the 3 MQTs was analyzed and the results were shown in Table 6. At g.244T–C locus, the mean value of individuals with genotype CC was significantly higher than genotype TT for UMAR (P < 0.05). For g.1496G–A mutation, genotype GG was significantly better than genotype AA for UMAR (P < 0.05). At g.1514G–A locus, difference was only found between genotype GG and genotype GA for UMAR (P < 0.05). For the 3 mutations, there was no difference between any genotype for UBF (P > 0.05).

4. Discussion

The bovine CDIPT gene is located at chromosome 25, translates a 2.1-kb messenger RNA, and encodes 213 amino acids in cattle [17]. In the 13 tissues studied, the expression of CDIPT gene was not uniform. The expression was high in the rumen, spleen, small intestine, kidney, larger intestine, reticulum and subcutaneous fat, and relatively has low expression in the lung, liver and heart, lower in abomasum and muscle. However, Lykidis et al. [6] reported that mRNA expression level in different tissue samples of human CDIPT was more uniform; slightly higher level of expression was detected in liver and skeletal muscle. Perhaps, this phenomenon can be explained that the experiment was done on different species.

5′UTR, the upstream of the main Open Reading Frame (mORF), has effects on the translation initiation of mORF [18]. Zhou et al. [19] reported one SNP at 5′UTR of prolactin receptor gene which could affect fiber traits in goats, indicating that SNP of 5′UTR may have influence on animal production traits. Cenik et al. [20] reported a strong correlation between 5′UTR intron presence/absence and sequence features at the beginning of the coding region. They also claimed that genes encoding secretory and mitochondrial proteins shared a common regulatory mechanism at the level of mRNA. 5′UTR that interrupt protein-coding regions could obviously affect coding sequences and their evolution. Introns and their removal by spliceosome may also have effects on gene expression at many different levels, including polyadenylation, translational efficiency, mRNA export, transcription and the rate of mRNA decay [21].

Identifying QTL will facilitate the Chinese indigenous cattle breeding program, and molecular genetic information can also bring about significant positive developments for animals [22]. Candidate gene approach is a very effective method to analyze the association between gene's polymorphisms and valuable economical traits in farm animals [23]. Through candidate gene approach, a lot of researches have been done on animals' reproduction [24], growth [25,26,27] and meat quality traits [28,29], which will provide more valuable date for further research. And to facilitate the Chinese indigenous cattle breeding program, more molecular genetic information on quantitative trait locus should be collected [22]. So we take CDIPT as a candidate gene to explore its effects on animals' growth traits.
In this study, we revealed 3 SNPs: First (g.244T>C) in 5′UTR of exon 1; second (g.1496G>A) and third (g.1514G>A) in intron 3. Through analyzing the genetic information, the obtained He of the three SNPs was uniformly lower than Ho. This may be due to the occurrence of inadequate random sampling which could not lead to gene distribution changes. He, Ne and PIC parameters could be used to evaluate the degree of polymorphism within a given population [30]. In our study, the 3 SNP loci all showed medium polymorphism, indicating that they could be used as molecular markers. The association of CDIPT gene polymorphism with 8 BMTs (BL, RL, WH, HW, HH, HG, CD and PBW) and 3 MQTs (ULMA, UBF and UMAR) was analyzed using blood sample from 638 Qinchuan cattle population. Results showed that g.244T>C was associated with BL, HW, HG and UMAR; g.1496G>A was associated with BL, HW, PBW and UMAR; g.1514G>A was associated with HW, HG and UMAR. We found that the 3 SNPs were all significantly associated with HW and UMAR.

According to the results of this study, it is suggested that CDIPT gene may have potential effects on BMTs and MQTs in Qinchuan cattle population. Based on the results above, it can also be inferred that CDIPT has effects on Chinese native indigenous cattle. Therefore, we suggest that further research should be done in larger populations with different sizes and cattle breeds with the aim of using SNPs for marker-assisted selection.

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### Author contributions

Proposed the theoretical frame: JW; Conceived and designed the experiments: GC, HBW; Contributed reagents/materials/analysis tools: LZ; Wrote the paper: CF, WH, YKL; Performed the experiments: CF; Analyzed the data: WY.

### References


