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Chinese Yellow Cattle *PPARA* Gene: Analyses of Expression, Polymorphism and Trait Association

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ABSTRACT

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Peroxisome proliferator-activated receptor alpha (PPARA) is a member of the nuclear receptor superfamily that regulates key proteins involved in fatty acid oxidation and extracellular lipid metabolism. Given the importance of PPARA in modulating energy metabolism, *PPARA* may be a suitable candidate gene for assessing economic traits in cattle. In the present study, the genetic diversity of the *PPARA* gene in Chinese cattle breeds and its effects on growth traits in the Nanyang and Jiaxian breeds were investigated. First, the transcript profiles for *PPARA* were determined in eight adult cattle tissue types. Next, polymorphisms were identified in the coding and predicted promoter regions of the bovine *PPARA* gene in 424 animals. Finally, an association study was carried out to evaluate the relationship between *PPARA* and the development of cattle. Based on quantitative real-time polymerase chain reaction, *PPARA* was mainly expressed in the kidney and liver. Nine single nucleotide polymorphisms (SNPs) were identified in Chinese domestic cattle, including one novel SNP. Haplotype frequencies and linkage disequilibrium were also investigated. Four SNPs (g.17148558A>T, g.117195348A>G, g.117228160T>C, and g.117233248A>G) showed significant associations with growth traits in NY and JX cattle, including body weight, average daily gain, and hipbone width. These results confirmed the importance of *PPARA* as a candidate gene for marker-assisted selection for growth traits in cattle.

Keywords: molecular markers; sequence variation; cattle growth; *PPARA*; body weight

Peroxisome proliferator-activated receptor alpha (PPARA), a transcription factor in the nuclear receptor superfamily, regulates fatty acid oxidation. When activated by a ligand, PPARA heterodimerises with the retinoic X receptor (RXR), binds to DNA, and modulates gene transcription (Desvergne and Wahli 1999). Key proteins involved in lipid metabolism are under the control of PPARA at the transcriptional level. Previous studies have demonstrated that a deficiency in PPARA diminishes insulin secretion in response to elevated glucose levels (Lalloyer et al. 2006).

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These results suggest that PPARA plays an important role in the regulation of genes related to lipid and glucose metabolism.

In humans, *PPARA* is most commonly expressed in organs and tissues where fatty acid oxidation is active, such as the liver, muscle, kidney, heart, vascular endothelium, and smooth muscle cells (Lefebvre et al. 2006). In recent years, numerous efforts have been made to predict susceptibility to diseases based on sequence variation in the *PPARA* gene (Sparso et al. 2007; Chen et al. 2008; Dongiovanni et al. 2010). Several variants near *PPARA* are associated with metabolic disorders in humans. For example, rs1800206 at the *PPARA* locus is associated with increased serum fasting cholesterol and triglyceride levels (Sparso et al. 2007) and high insulin resistance (Dongiovanni et al. 2010). The *PPARA* L162V polymorphism is associated with reduced lipid levels and a lower body mass index (BMI) in patients with diabetes mellitus (Evans et al. 2001). These studies have clearly demonstrated a genetic link between *PPARA* and the regulation of metabolic disorders, suggesting that genetic variations in *PPARA* affect human body weight.

Common variants of the *PPARA* gene have been associated with economic traits in domestic animals. The porcine *PPARA* gene was mapped to chromosome SSC5p15 in the region harbouring quantitative trait loci (QTL) for performance and carcass traits (Szczerbal et al. 2007). Polymorphisms in the 3' untranslated region (UTR) of the *PPARA* gene are associated with fatness traits in some pig breeds, and one of these influences transcript levels and is associated with adipose tissue accumulation (Stachowiak et al. 2014). In chickens, an association has been described between a polymorphism in the *PPARA* gene and fatness traits (Meng et al. 2002). These findings make the *PPARA* gene a candidate gene for growth and meat quality traits. The bovine *PPARA* gene is located on chromosome 5 and encodes a 470 amino acid protein (GenBank accession number NM_001034036.1). Few studies have examined the bovine *PPARA* gene in cattle. In a commercial Angus-cross population, a single nucleotide polymorphism (SNP) in the *PPARA* gene has shown to be associated with carcass and meat quality traits, such as sirloin fat depth (Gill et al. 2010). Three intronic SNPs in Chinese Holstein cattle are associated with heat tolerance (Fang et al. 2014). However, the relationships between genetic variants of the bovine *PPARA* gene and growth traits are unclear. Thus, further research is needed to evaluate the bovine *PPARA* gene as a genetic determinant of growth traits in cattle. The aims of this study were to determine the spatial expression of *PPARA* in Chinese native yellow cattle (Zaobei), investigate the genetic diversity of the *PPARA* gene in Chinese cattle breeds, and evaluate the relationship between detected SNPs and growth traits in local cattle in Henan Province. We expect to discover candidate markers related to growth traits in Chinese native cattle.

MATERIAL AND METHODS

Animals and samples. Zaobei cattle are a native Chinese cattle breed, mainly reared in Hubei Province. Eight samples (heart, liver, spleen, lung, kidney, muscle, back fat, and small intestine) were collected from three adults from the breeding farm of Zaobei cattle (Suizhou, Hubei Province, P.R. China). Samples were collected within 30 min postmortem, immediately frozen in liquid nitrogen, and stored at −80°C for the expression analysis.

Sequence variation in the bovine *PPARA* gene was detected in 424 animals representing four prominent Chinese cattle breeds: Jiaxian (JX, n = 141), Luxi (LX, n = 114), Nanyang (NY, n = 139), and Qinchuan (QC, n = 30) cattle. Animals were weaned at an average of 6 months of age, and were switched to a corn-corn silage diet, according to the nutrient requirements of growing heifers (NRC 2000; Ma et al. 2014). The NY animals were randomly chosen from the breeding centre for Nanyang cattle (Nanyang City, Henan Province, P.R. China); the JX animals were randomly chosen from the breeding farm of Jiaxian cattle (Jiaxian county, Henan Province, P.R. China); the QC animals were randomly chosen from the breeding farm of Qinchuan cattle (Fufeng county, Shaanxi Province, P.R. China); the LX animals were randomly chosen from the breeding farm of LX cattle (Juancheng City, Shandong Province, P.R. China).

In China, the farms where NY and JX cattle have been bred are two of the largest farms with complete production records. NY and JX cattle are local draught and meat cattle breeds that perform well and exist mainly in Henan. For the association study, a total of 173 NY (n = 100) and JX (n = 73) cows were measured for growth traits, including body weight at birth, 6 (weaning), 12, 18, and

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24 months, body length, heart girth, hip height, hipbone width, and average daily weight gain over the periods of 6, 12, 18, and 24 months. These traits were measured following previously described methods (Gilbert et al. 1993). All experimental procedures were performed as authorised by the Chinese Ministry of Agriculture. All international, national, and institutional guidelines for the care and use of animals were followed.

RNA extraction, cDNA synthesis, and quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was extracted from eight tissue samples using the RNAiso™ Plus reagent (TaKaRa, Japan). Total RNA concentration and purity were measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop, Denmark). The integrity and quality of the RNA was confirmed using the 2100 Bioanalyzer (Agilent Technologies, USA), based on the RNA integrity number. The first-strand cDNA was synthesised from 1 µg of total RNA using the PrimeScriptRT Reagent Kit with gDNA Eraser (TaKaRa). The following protocol for reverse transcription was used: 37°C for 15 min, 85°C for 5 s, and cooling at 4°C.

The expression levels of genes were quantified by the SYBR Green I assay and qRT-PCR reactions were performed as three-step reactions using an ABI 7300 (Applied Biosystems, USA) as previously reported (Ma et al. 2014). Primers for *PPARA* (GenBank accession number NM_001034036.1) were designed using Primer Premier 5.0. To ensure unbiased normalisation of relative expression data, two reference genes, *tubulin, alpha 1* (*TUBA1A*, NM_001166505.1) and *actin beta* (*ACTB*, NM_173979.3) were used (Supplementary Table S1 in Supplementary Online Material (SOM)) (Vandesompele et al. 2002). Each sample was run in triplicate along with the reference genes using a standard cycling PCR protocol (95°C for 30 s, 54°C for 15 s, and 72°C for 30 s for 40 cycles). Expression results were calculated using the delta-delta cycle threshold ($2^{-\Delta\Delta Ct}$) method. Expression levels were considered undetectable when the Ct value of the target gene exceeded 35 for the sample tissues. Expression data were analysed using Sequence Detector software (Applied Biosystems) and results are expressed as means ± standard deviation (SD) for each type of tissue (Chen et al. 2015).

SNP detection and genotyping. To detect the sequence variation in the bovine *PPARA* gene, nine pairs of primers (PPARA-P1 to PPARA-P9) (Supplementary Table S2 in SOM) were designed based on the sequence of the bovine *PPARA* gene (NCBI Gene ID: 281992). Primers were designed (Primer Premier 5.0) and synthesised by Nanjing Genscript Biological Engineering Technology Company (P.R. China). Samples from 40 animals were selected to prepare the DNA pool, with 10 DNA samples randomly selected from each breed. PCR was performed in a 25-µl reaction volume containing 50 ng of genomic DNA, 10 pmol/l of each primer, 1× buffer (including 1.5 mmol/l $MgCl_2$), 200 µmol/l dNTPs, and 1.5 U of *Taq* DNA polymerase. SNP discovery was accomplished by direct sequencing. The PCR products were sequenced using the ABI PRISM 3730 DNA analyzer (Applied Biosystems), and variations were analysed using BioXM software (Version 2.6) (Ma et al. 2015). The genotyping of nine SNPs was conducted using PCR-restriction fragment length polymorphisms (PCR-RFLP) and artificially created restriction site-PCR (ACRS-PCR) from the same selected animals (Chen et al. 2017). Primers, restriction enzymes (MBI, Lithuania), and fragment sizes are given in Supplementary Table S2 in SOM. Briefly, 6 µl of PCR products were digested with 0.5 U of restriction enzymes for 3 h at 37°C and 3 µl of the digested products were easily separated by 12% polyacrylamide gel electrophoresis (PAGE) in 1 × Tris-borate-EDTA (TBE) buffer under a constant voltage (130 V) for 2 h at room temperature. The gels were stained with 0.1% silver nitrate and the different electrophoretic patterns represent different genotypes (Ma et al. 2014).

Statistical analysis. Gene frequencies were determined by direct counting. Population genetic indices, such as heterozygosity (H_e), effective allele numbers (N_e), and polymorphism information content (PIC) were calculated according to Nei's methods. Hardy–Weinberg equilibrium (HWE) was also analysed (Emigh 1980). The linkage disequilibrium (LD) structure and haplotypes were determined using SHEsis (Shi and He 2005).

Statistical analysis was performed using records of six growth traits for NY and JX cattle at five different ages. The reduced model was used in the final analysis (Hickford et al. 2010). Observed relationships between genotypes and growth traits in the cattle were analysed as previously described using SPSS (IBM, USA) (Ma et al. 2014). The following linear model was used:

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$Y_{ijkl} = \mu + B_i + G_j + A_k + e_{ijkl}$

where:

Y_{ijkl} = phenotype of the animal

μ = population mean

B_i = effect of breed

G_j = effect of genotype

A_k = effect of age

e_{ijkl} = random error

Differences in means between each group were considered significant at $P < 0.05$. The Least Squares Means estimates with standard errors for different genotypes and growth traits were used. Age, breeds, and genotypes were considered fixed effects and growth traits were dependent variables.

RESULTS

Gene expression profile of bovine PPARA. The mRNA levels of the bovine *PPARA* gene were analysed in eight tissue types by qRT-PCR with normalisation against *TUBA1A* and *ACTB*. The specificity of the intended product was inferred by a melting curve analysis of the amplicons. *PPARA* was predominantly expressed in the kidney and liver, with low levels of expression observed in the heart and adipose tissues (Figure 1).

Identification of genetic variation in PPARA. The bovine *PPARA* gene contains seven exons and six introns (NC_007303.6). In this study, totally nine SNPs were identified (Table 1), of which eight were previously reported and one was novel. Among these variants, g.117148558 A>T was located in a potential promoter region, and g.117195033 A>G and g.117195348 A>G were located in introns 2 and 3, respectively. Four SNPs (g.117204210 G>A, g.117204336 T>C, g.117228031 T>C, and g.117228160 T>C) were silent mutations in exons 5, 5, 7, and 7, respectively. Two SNPs (g. 117232845 T>C and g.117233248 T>C) were detected in the 3'UTR.

Genetic characterisation of the nine SNPs detected in the bovine PPARA gene. To evaluate genetic diversity among four Chinese native cattle populations, genetic indices (H_e, N_e, and PIC) and genotypic and allelic frequencies were calculated for each of the nine SNPs. As shown in Supplementary Table S3 in SOM, there were differences in genotype frequency between the tested breeds. Interestingly, only two genotypes were found at the g.117228031 T>C and g.117233248 A>G loci in all breeds. For the g.117148558 A>T locus, the *AA* genotype was absent in the JX, QC, and BH populations, suggesting a specific selection history and/or a smaller sample size. Moreover, the χ^2 test indicated that only two mutations, g.117204210 G>A and g.117204336 T>C, in all breeds were in agreement with HWE, indicating that there was dynamic equilibrium, despite artificial selection, migration, and genetic drift. The ranges of minor allele frequencies, H_e, N_e, and PIC of nine SNPs (from SNP g.117148558 A>T to g.117233248 A>G) were 0.027–0.500, 0.053–0.500, 1.056–2.000, and 0.062–0.375 among all populations, respectively. For nine SNPs, only the loci g.117228031 T>C and g.117232845 T>C had a low genetic diversity (PIC < 0.25), while at the other seven loci, all of the analysed populations had moderate genetic diversity (0.25 < PIC < 0.5).

LD and haplotype structure analysis. LD between SNP pairs and a haplotype structure analysis of the bovine *PPARA* gene in four cattle populations are summarised in Table 2 and Table 3. LD between each pair of the nine SNPs was estimated. The D' values ranged from 0.044 to 1.000. The

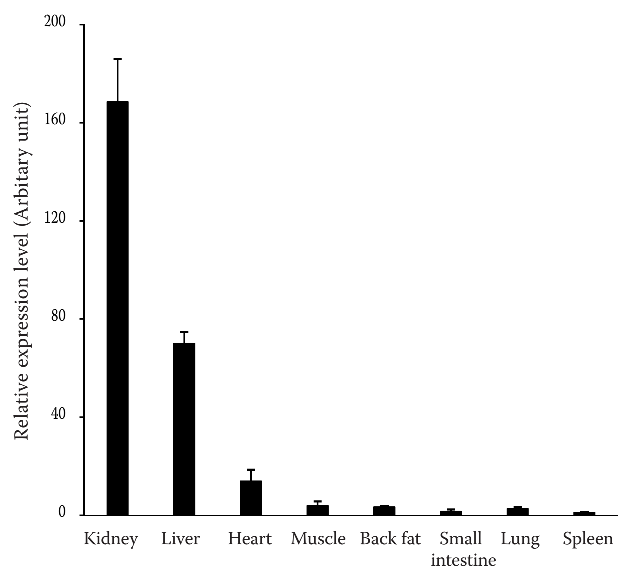

Figure 1. Tissue distribution of bovine *PPARA* mRNA assessed by qRT-PCR

values shown in the figure are the averages of three independent experiments; error bars represent the standard deviation (SD) ($n = 3$) of relative mRNA levels of bovine *PPARA;* the expression data were normalised using the geometric mean of the mRNA levels of two reference genes (*TUBA1A* and *ACTB*)

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Table 1. Identification of polymorphisms in *PPARA* gene among Chinese cattle according to the reference sequence (GenBank accession No. NC_007303.6)

No.	SNPs	Position (cDNA)[1]	Chromosome	rsID[2]	Allele	Amino acid[3]	Type of SNPs
1	g.117148558A>T	promoter (–1910)	5:117148558	rs471506343	*A/T*		upstream variant
2	g.117195033A>G	intron 2	5:117195033	rs134580633	*A/G*		intron variant
3	g.117195348A>G	intron 3	5:117195348	rs135735531	*A/G*		intron variant
4	g.117204210G>A	exon 5 (574)	5:117204210	rs477982176	*G/A*	129 Arg (agG)/ Arg (agA)	synonymous
5	g.117204336T>C	exon 5 (700)	5:117204336	rs137668765	*T/C*	171 Asn (aaT)/ Asn (aaC)	splice region/ synonymous variant
6	g.117228031T>C	exon 7 (1048)	5:117228031	rs110745628	*T/C*	287 Thr (acC)/ Thr (acT)	synonymous
7	g.117228160T>C	exon 7 (1177)	5:117228160	rs446377435	*T/C*	330 Asp (gaT)/ Asp (gaC)	synonymous
8	g.117232845T>C	3'UTR	5:117232845	novel	*T/C*		3' UTR variant
9	g.117233248A>G	3'UTR	5:117233248	rs432147085	*A/G*		3' UTR variant

SNPs = single nucleotide polymorphisms
[1]comparison of sequences of *PPARA* mRNA (GenBank accession No. NM_001034036.1), [2]% rsID in database of dbSNP in NCBI, [3]comparison of sequences of PPARA mature protein sequence with reference sequence (GenBank accession No. NP_001029208)
(http://www.ensembl.org/Bos_taurus/Transcript/Sequence_cDNA?db=core;g=ENSBTAG00000008063;r=5:117151549-117233112;t=ENSBTAT00000010606)

r^2 values were between 0.001 and 0.680. Applying a threshold pair-wise r^2 of 0.33, a strong linkage was found between g.117195033A>G and g.117195348A>G within the studied population (D' = 0.965 and r^2 = 0.680). However, the values for other loci indicated that the variants were not in LD in all populations.

There are, theoretically, 512 (2^9) haplotypes for the nine SNPs in bovine *PPARA*; however, only 92 haplotypes were observed. Among them, ten common haplotypes (frequency > 0.02) were constructed. Haplotype 5 (Hap 5: TAGGTCTTA) had the highest observed frequency (21.0%) in the population (Table 3).

Table 2. Linkage disequilibrium tests among nine loci[1] within the *PPARA* gene in Chinese native cattle (n = 424)

Loci[1]	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9
SNP1		0.433	0.192	0.596	0.154	0.996	0.442	0.341	0.749
SNP2	0.059		**0.965**	0.374	0.747	0.053	0.645	0.044	0.464
SNP3	0.012	**0.680**		0.377	0.462	0.415	0.457	0.793	0.403
SNP4	0.209	0.026	0.036		0.826	0.997	0.684	0.892	0.612
SNP5	0.005	0.264	0.138	0.084		0.606	0.182	0.002	0.371
SNP6	0.012	0.000	0.006	0.007	0.006		1.000	0.971	0.971
SNP7	0.151	0.100	0.068	0.379	0.005	0.009		0.873	0.294
SNP8	0.044	0.000	0.080	0.028	0.000	0.004	0.034		0.532
SNP9	0.314	0.120	0.098	0.122	0.054	0.020	0.037	0.060	

SNP = single nucleotide polymorphism, D' = linkage disequilibrium coefficient, r^2 = correlation coefficient
[1]SNP1: g.117148558A>T, SNP2: g.117195033A>G, SNP3: g.117195348A>G, SNP4: g.117204210G>A, SNP5: g.117204336T>C, SNP6: g.117228031T>C, SNP7: g.117228160T>C, SNP8: g.117232845T>C, SNP9: g.117233248T>C
D' and r^2 values for pair-wise linkage disequilibrium analysis are shown above diagonal and below diagonal of the table, respectively
bolded = strong linkage

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Table 3. Haplotype frequencies in the bovine *PPARA* gene in Chinese cattle

Haplotype	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	Frequency[1]
Hap1	*A*	*A*	*G*	*A*	*T*	*C*	*T*	*T*	*G*	0.027
Hap2	*A*	*A*	*G*	*G*	*T*	*C*	*T*	*C*	*G*	0.021
Hap3	*A*	*G*	*A*	*A*	*T*	*C*	*C*	*T*	*G*	0.089
Hap4	*T*	*A*	*G*	*G*	*T*	*C*	*T*	*C*	*G*	0.021
Hap5	*T*	*A*	*G*	*G*	*T*	*C*	*T*	*T*	*A*	0.207
Hap6	*T*	*A*	*G*	*G*	*T*	*C*	*T*	*T*	*G*	0.066
Hap7	*T*	*G*	*A*	*G*	*C*	*C*	*C*	*T*	*A*	0.026
Hap8	*T*	*G*	*A*	*G*	*C*	*C*	*T*	*T*	*A*	0.177
Hap9	*T*	*G*	*A*	*G*	*C*	*C*	*T*	*T*	*G*	0.025
Hap10	*T*	*G*	*A*	*G*	*T*	*C*	*T*	*T*	*A*	0.048
Others										0.293

nine single nucleotide polymorphisms (SNPs) were SNP1: g.117148558A>T, SNP2: g.117195033A>G, SNP3: g.117195348A>G, SNP4: g.117204210G>A, SNP5: g.117204336T>C, SNP6: g.117228031T>C, SNP7: g.117228160T>C, SNP8: g.117232845T>C, SNP9: g.117233248T>C; haplotypes with frequency > 0.02 are presented, those with frequency < 0.02 are ignored in the analysis
[1]number of animals investigated $n = 424$

Relationships between PPARA SNPs and growth traits. We examined correlations between the nine SNPs and growth traits in the NY and JX populations, and the results are shown in Table 4. Four mutations showed significant effects on growth trait(s). At g.117148558A>T, animals with either the *TT* or *TA* genotype showed a better performance body weight and average daily gain at

Table 4. Association analysis between four single nucleotide polymorphisms (SNP) and growth traits in Nanyang ($n = 100$) and Jiaxian ($n = 73$) cattle at different ages

SNP	Ages (months)	Growth traits	Genotypes (Least Squares Means ± standard error)			*P*-value
			AA (12)	*AT* (71)	*TT* (90)	
g.117148558A>T	6	body weight (kg)	$147.042^{b} \pm 6.914$	$164.021^{a} \pm 2.842$	$165.139^{a} \pm 2.524$	0.024
		average daily gain (kg)	$0.646^{b} \pm 0.038$	$0.741^{a} \pm 0.016$	$0.749^{a} \pm 0.014$	0.023
			AA (34)	*AG* (87)	*GG* (52)	
g.117195348A>G	6	hipbone width (cm)	$14.676^{b} \pm 0.753$	$16.638^{a} \pm 0.471$	$15.577^{ab} \pm 0.609$	0.028
	24	body weight (kg)	$351.412^{b} \pm 7.334$	$370.236^{a} \pm 4.585$	$365.808^{ab} \pm 5.930$	0.049
			TT (134)	*TC* (30)	*CC* (9)	
g.117228160T>C	6	hipbone width (cm)	$15.554^{b} \pm 0.401$	$17.017^{b} \pm 0.805$	$18.944^{a} \pm 1.469$	0.026
	12	hipbone width (cm)	$18.260^{b} \pm 0.387$	$18.917^{b} \pm 0.777$	$21.389^{a} \pm 1.419$	0.033
	18	hipbone width (cm)	$21.343^{b} \pm 0.364$	$21.950^{b} \pm 0.730$	$24.222^{a} \pm 1.333$	0.036
	24	body weight (kg)	$360.764^{b} \pm 3.974$	$371.700^{b} \pm 7.981$	$390.778^{a} \pm 14.572$	0.046
		hipbone width (cm)	$24.065^{B} \pm 0.374$	$24.310^{b} \pm 0.744$	$27.938^{A} \pm 1.416$	0.008
			AA (26)	*AG* (147)		
g.117233248A>G	birth	body weight (kg)	$32.308^{A} \pm 0.717$	$29.522^{B} \pm 0.302$	–	0.000
	18	hipbone width (cm)	$24.558^{A} \pm 0.739$	$21.099^{B} \pm 0.311$	–	0.000
		average daily gain (kg)	$0.440^{A} \pm 0.028$	$0.336^{B} \pm 0.012$	–	0.005
	24	body weight (kg)	$384.250^{A} \pm 8.330$	$361.837^{B} \pm 3.503$	–	0.009
		hipbone width (cm)	$26.712^{A} \pm 0.752$	$23.728^{B} \pm 0.316$	–	0.001

[a,b]means with no common superscripts in the same row differ at $P < 0.05$, [A,B]means with no common superscripts in the same row differ at $P < 0.01$; only significant associations are presented

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6 months compared with those with the *AA* genotype ($P < 0.05$). At locus g.117195348 A>G, significant differences in hipbone width at 6 months and body weight at 24 months were observed between *AA* and *AG* individuals ($P < 0.05$), indicating that the *G* allele could be associated with an increased pelvic width and body weight at 6 and 24 months of age, respectively. At locus g.117228160 T>C, NY and JX animals with the *CC* genotype had a significantly greater hipbone width at four different ages compared to those of individuals with the *TT* genotype ($P < 0.05$), demonstrating that the allele C might be associated with increased hipbone width at all ages. Animals with the *CC* genotype also exhibited significantly greater body weights at 24 months than those with the *TT* and *TC* genotypes. For g.117233248 A>G, NY and JX animals with the *AA* genotype had a better greater hipbone width at 18 and 24 months in relation to animals with the *AG* genotype ($P < 0.01$). This indicated that the *A* allele is a possible factor leading to an increased hipbone width. Animals with the *AA* genotype also weighed significantly more at birth and 24 months than animals with the *AG* genotype ($P < 0.01$). Similarly, cattle with the *AA* genotype also had significantly greater average daily gains at 18 months compared with the *AG* genotyped animals ($P < 0.01$). Other mutations were not significantly associated with growth traits ($P > 0.05$, data not shown).

DISCUSSION

A broad spectrum of physiological functions are regulated by *PPAR* family genes, including lipid metabolism, glucose metabolism, and insulin sensitivity (Lee et al. 2002). Evidence from *PPARA* knockout mice has indicated that PPARA is involved in the development of obesity, and *PPARA* null mice fed a diet high in fat exhibit a dramatic increase in body weight (Monsalve et al. 2013; Akanno et al. 2015). Conversely, the activation of PPARA reduces weight gain in rodents, whereas the inactivation of PPARA results in the late onset of obesity (Tetens et al. 2013). Considering the important role of PPARAs as master controllers of energy metabolism and likely development, we hypothesised that *PPARA* was important for the growth of cattle. In this study, the expression profile and variation in *PPARA* gene were evaluated, and effects of genetic variation on growth performance were examined in native Chinese cattle.

qRT-PCR analysis revealed that *PPARA* was highly expressed in the kidney and liver of adult cattle (Figure 1), similar to the distribution observed in humans and mice (Braissant et al. 1996). *PPARA* plays a key role in the regulation of genes involved in lipid metabolism and immune responses (Bionaz et al. 2012). PPARA is a key determinant of metabolic adaptation to increases in fatty acid concentrations (Monsalve et al. 2013). The expression of genes in the PPARα signaling pathway is also associated with the fatty acid content in yak and cattle *longissimus dorsi* muscles (Qin et al. 2015). Changes in the fat composition within muscle tissues affect meat quality. Taken together, the relatively high expression level of the *PPARA* gene in cattle livers confirms that the gene and its product play an important role in lipid metabolism.

It has been well established that the genetic variability among native Chinese cattle breeds is high (Chen et al. 2018). Therefore, it is necessary to evaluate variation within different breeds, and to determine the effects of variation in *PPARA* on growth in cattle. The bovine *PPARA* gene contains seven exons and six introns. In this study, we preliminarily identified nine SNPs within the bovine *PPARA* gene (Table 1), showing a remarkable allelic diversity. These variants were located in a potential promoter region, introns, exons, and the 3'UTR. No amino acid changes were detected in cattle in this study, indicating high conservation at the amino acid level. According to population genetic indices and PIC, g.117228031 T>C exhibited low polymorphism, which may explain why two genotypes were detected, while other SNPs exhibited abundant polymorphism. The JX and LX cows were not in HWE at three loci (g.117148558 A>T, g.117228160 T>C, and g.117233248 A>G). This could be attributed to artificial selection of JX and LX cattle over the past few decades. Furthermore, genetic drift and migration might have contributed to the observed disequilibrium.

Many genes have been mapped to QTL, some of which may have strong effects on a specific trait. Bovine *PPARA* is located on chromosome 5 (from 117, 151, 549 to 117, 233, 112 bp). Alignment of bovine chromosome 5 on the radiation hybrid map of the USDA-MARC cattle database indicated QTLs associated with growth traits in this region (Table 5), such as average daily gain (Saatchi et al. 2014a), body weight (Snelling et al. 2010; Saatchi et al. 2014b; Akanno et al. 2015), and body energy

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Table 5. Quantitative trait loci (QTL) of economic traits around *PPARA* gene

Trait name	Trait type	QTL symbol	Detected type	QTL centre location (cM)	QTL span (Mbp)	Reference
Average daily gain	growth	ADG	QTL	n/a	106.3–107.0	(Saatchi et al. 2014a)
Body weight (birth)	growth	BW	association	n/a	119.9–119.9	(Akanno et al. 2015)
Body weight (mature)	growth	MWT	association	122.69	106.2–107.0	(Saatchi et al. 2014b)
Body weight (weaning)	growth	WWT	association	122.69	106.2–107.0	(Saatchi et al. 2014b)
Body weight (yearling)	growth	W365	association	130.05	107.2–107.2	(Snelling et al. 2010)
Body weight gain	growth	BWG	association	130.05	107.2–107.2	(Snelling et al. 2010)
Body energy content	production	BENERC	association	32.30	103.6–103.6	(Tetens et al. 2013)

n/a = not available

content (Tetens et al. 2013). These results suggest that *PPARA* is a candidate gene for growth and meat quality. The statistical analysis in this study indicated that animals with the *TT* and *TA* genotypes of the g.117148558A>T locus, *GA* genotype of the g.117195348A>G locus, *CC* genotype of the g.117228160T>C locus, and *AA* genotype of the g.117233248A>G locus tend to have greater body weights and hipbone widths (Table 4). These results suggest that cattle with these genotypes could be selectively bred to produce beef cattle with greater body weights and hipbone widths. In recent years, numerous efforts have been made to predict the effects of sequence variations within the *PPARA* gene on body weight. Ahmetov et al. (2013) demonstrated that male humans with the *C* allele at rs4253778 are taller, and have larger body weights and BMIs than those of their counterparts expressing the *G* allele. A L162V polymorphism at the *PPARA* locus has been associated with a lower BMI in patients with metabolic disorders (Evans et al. 2001). Studies of both humans and mice have demonstrated the importance of PPARA in lipid homeostasis and protection against obesity (Tai et al. 2002; Yamakawa-Kobayashi et al. 2002). Taken together, based on our current understanding of its biological functions and the observation that bovine *PPARA* maps closely to many growth trait QTLs, *PPARA* is a promising candidate for the optimisation of growth traits. The SNPs described in this study may be used as genetic markers for the prediction of commercially desirable growth traits.

CONCLUSION

In conclusion, we report the tissue expression profile of the bovine *PPAPA* gene in Zaobei cattle. Our results provide insight into the role of the *PPAPA* gene and genetic variation among populations. The results of association study suggested that four SNPs (g.17148558A>T, g.117195348A>G, g.117228160T>C, and g.117233248A>G) in bovine *PPARA* are potential genetic markers for improving growth traits in cattle.

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