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A missense mutant of the *PPAR-γ* gene associated with carcass and meat quality traits in Chinese cattle breeds

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ABSTRACT. The peroxisome proliferator-activated receptor gamma (*PPAR-γ*) is a key molecule in adipocyte differentitation; it transactivates multiple target genes in lipid metabolic pathways. Using PCR-SSCP and DNA sequencing, we evaluated a potential association of an SNP (72472 G>T in exon7) of the bovine *PPAR-γ* gene with carcass and meat quality traits in 660 individuals from five Chinese indigenous cattle breeds, Qinchuan (QC), Luxi (LX), Nanyang (NY), Jiaxian (JX), and Xianan (XN). This 72472 G>T mutation identified a missense mutation, Q448H. Two alleles were named C and D. Allele frequencies of *PPAR-γ*-C/D in the five breeds were 0.78/0.22, 0.90/0.10, 0.74/0.26, 0.71/0.29, and 0.83/0.17 for QC, NY, JX, LX, and XN, respectively. Except for the XN breed, all breeds were in Hardy-Weinberg equilibrium at this locus. The polymorphism information content was low for NY and XN (0.16 and 0.24, respectively), while it was moderately high for QC, JX, and LX (0.28, 0.31 and 0.33, respectively). Correlation analysis showed significant association of this missense mutation with carcass length, backfat thickness and water holding capacity in the QC breed. Animals with the genotype CD had significantly

greater carcass length than those with genotypes CC and DD, while animals with genotype CC had significantly greater backfat thickness than those with genotypes CD and DD. Animals with genotype CC had lower water holding capacity than those with the genotypes CD and DD. In conclusion, this locus is a candidate for a major quantitative trait locus affecting production traits and could be used for beef breeding selection.

Key words: *PPAR-γ* gene; SNPs; Meat traits; Molecular marker

INTRODUCTION

Meat quality is determined on the basis of marbling, meat color, fat color, texture, and overall maturity. The deposition of intramuscular adipose tissue is positively related to beef flavor and palatability (Judge et al., 1989). Ideal marbling of muscle is the result of increased quantity and volume hypertrophy of intramuscular fat cells (Albrecht et al., 2006). Qinchuan (QC) cattle is one of the most famous beef breeds in inland China, it is a representative of good Chinese cattle breeds known for its good performance traits, especially fleshy characteristics. Meat traits are controlled by multiple genes with pleiotropic effects (Choudhary et al., 2007), Therefore, analysis of the associations of single nucleotide polymorphisms (SNPs) of important functional regions of candidate genes linked with economically important traits may be very useful (Wang et al., 2005). Some studies have reported improvements in carcass quality, especially high-quality beef yield and tenderness and marbling scores, in the QC breed through marker-assisted selection (Liu et al., 2010; Zhong et al., 2010).

Peroxisome proliferator-activated receptor gamma (*PPAR-γ*) is a ligand-activated nuclear hormone receptor that forms a heterodimer with the retinoid X receptor (Issemann and Green, 1990). It is abundantly expressed in adipose tissue, controls adipocyte differentiation and lipid storage (Tontonoz et al., 1994; Kliewer et al., 1995; Forman et al., 1997), affects the action of insulin, and regulates glucose homeostasis (Spiegelman, 1998; Berger and Moller, 2002). It is believed to regulate target genes in adipocyte differentiation (Spiegelman and Flier, 1996; Kliewer et al., 1999) and insulin sensitization (Fajas et al., 2001; Meirhaeghe et al., 2003) by directly or indirectly enhancing the transcription of genes encoding proteins such as lipoprotein lipase, fatty acid binding protein, and liver X receptor α (Escher and Wahli, 2000; Evans et al., 2004; Rosenson, 2007). *PPAR-γ* signaling pathways control lipid uptake, transport, storage, and disposal (Walczak and Tontonoz, 2002). *PPAR-γ* induces fibroblasts or preadipocytes to differentiate into adipocytes (Schoonjans et al., 1996; Matsusue et al., 2004).

Therefore, the present study was performed to investigate SNPs in bovine *PPAR-γ* as a candidate gene in Chinese indigenous cattle breeds and to explore their possible association with meat traits, with a view to develop more efficient selection programs for the improvement of genetic characters for Chinese indigenous cattle.

MATERIAL AND METHODS

Animals and DNA samples

Five breeds of Chinese cattle [QC, N = 405; Nanyang (NY), N = 40; Jiaxian (JX), N =

86; Luxi (LX), N = 78; Xianan (XN), N = 51) were used in this study. The QC animals were selected from the Kingbull Livestock Development Co., Ltd., Shaanxi, China; NY animals, from the breeding center of Nanyang cattle (Nanyang City, Henan Province, China); the JX animals, from the breeding farm of Jiaxian cattle (Jiaxian County, Henan Province, China); LX animals, from the reserved farm (Hezhe City, Shandong Province, China), and XN animals, from the breeding farm of Xianan cattle (Biyang City, Henan Province, China). They were fed according to the National Institute for Agricultural Research, France (INRA) standards. A total of 660 animals were used to analyze the *PPAR-γ* allelic frequencies, and 108 of QC steers, which were 1.5-2 years old, were selected in random and slaughtered to collect data on the carcass traits (slaughter weight, carcass weight, carcass length, backfat thickness, loin-eye area, water holding capacity [WHC], beef marbling score, and tenderness) for statistical analysis.

Genomic DNA of 660 animals was isolated from acid-citrate dextrose (ACD) blood (ACD:blood is 1:6) samples and stored at -80°C according to standard procedures (Sambrook and Russell, 2002).

Polymerase chain reaction (PCR) amplification

Each exon of the *PPAR-γ* gene (NC_007320) was amplified. The specific primers shown in Table 1 were designed using the Primer 5.0 software.

Table 1. Primer sequences and information of the *PPAR-γ* gene in cattle.

Name	Sequences	Length (bp)	Tm (°C)	Region
Primer 7	5'-AATGTGAGTCATTGGCTG-3' 5'-GGTGTCAGATTTTTCCCT-3'	365	57.8	7th exon

Tm = melting temperature.

The 25-μL PCR mixture contained 50 ng genomic DNA, 0.5 μM of each primer, 1X buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs, and 0.625 U Taq DNA polymerase. The reaction conditions were 95°C for 3 min, 32 cycles of 94°C for 30 s, 57.8°C annealing for 30 s, 72°C for 40 s, with a final extension at 72°C for 10 min.

Single-stranded conformation polymorphism (SSCP) and DNA sequencing

PCR products (3 μL) were mixed with 8 μL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene cyanol, and 0.025% bromophenol blue), heated for 10 min at 98°C, and chilled on ice. Then, the samples were loaded onto 12% polyacrylamide gels (200 x 175 x 1 mm) in 1X TBE buffer (Tris-EDTA, pH = 8.3) for 0.5 h at 4°C under a constant voltage (220 V) and maintained for 12 h at 4°C under a constant voltage (90 V). Then, the gel was stained with 0.1% silver nitrate and visualized with 2% NaOH solution (supplied with 0.1% formaldehyde) (Sun et al., 2002).

After the polymorphism was detected, the PCR products of different electrophoresis patterns were subjected to bidirectional sequencing in the ABI PRIZM 377 DNA sequencer (Perkin-Elmer). Before sequencing, the PCR products were purified with Axygen kits (MBI Fermentas), and the sequences were analyzed with the BioXM software (version 2.6).

Statistical analysis

The following population genetic indices were evaluated for: genotype, allele frequencies, the Hardy-Weinberg equilibrium, and population genetic indexes: H_E (gene heterozygosity), H_O (gene homozygosity), N_E (effective allele numbers), and polymorphism information content (PIC) was calculated using the POPGENE Version 1.31 software.

The analysis of the associations between genotypes of *PPAR-γ* and carcass and growth performance traits of the 1.5-2-year-old animals was conducted by the general linear model (GLM) procedure (SPSS 13.0, SPSS Inc.). The following Equation was used:

$$Y_{ijm} = \mu + \sigma + Age_i + marker_j + e_{ijm,} \quad \text{(Equation 1)}$$

where Y_{ijm} was the result for the carcass measurement traits; μ, the overall population mean; σ, the mean of genotype; Age_i, the effect of jth month of slaughtering; $marker_j$, the effect of genotype, and e_{ijm}, the random error.

RESULTS

We selected 660 individuals belonging to 5 breeds and performed PCR-SSCP (Figure 1) analysis for the genotypes CC, CD, and DD. SNP loci were identified using the sequencing method in the 7th exon of *PPAR-γ*, which was 72472 G>T (shown in Figure 2), and the 72472 G>T mutant resulted in an amino acid change of glutamine→histidine at the 448th site (shown in Figure 3).

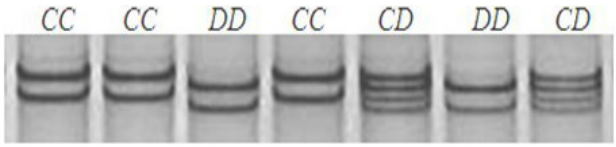

Figure 1. Polymorphic patterns demonstrated by PCR-SSCP of the *PPAR-γ* gene 72472 locus.

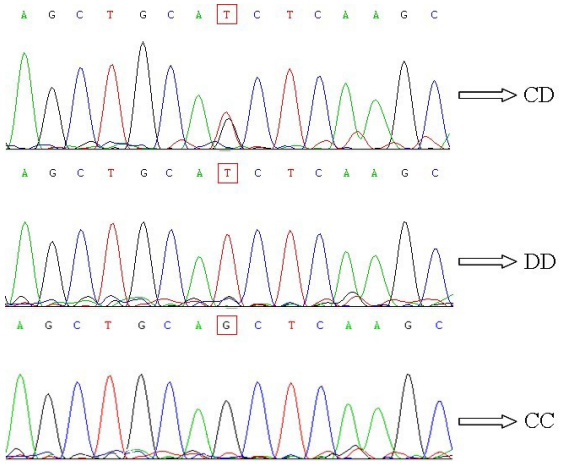

Figure 2. Sequencing map of wild and mutational nucleotide sequences in the exon 7 of the *PPAR-γ* gene. Sequencing map revealed a 72472 G>T in exon 7 of the *PPAR-γ* gene.

Figure 3. Result of the comparison of amino acid at the 448th site in the *PPAR-γ* gene by the DNAman software.

The relationship of the 72472 G>T missense mutant with some meat traits of 108 QC breed individuals, which were aged 1.5-2 years and maintained under identical feeding conditions, was analyzed by the GLM. Genotype distribution and allelic frequencies are shown in Table 2. Genetic diversity is summarized in Table 3.

Table 2. Genotype distribution and allelic frequencies of the *PPAR-γ* gene 72472 locus in Qinchuan (QC) breed.

Breeds	Observed genotypes			Total	Allelic frequencies		HWE*
	CC	CD	DD		C	D	
QC	253	127	25	405	0.78	0.22	P > 0.05
NY	32	8	0	40	0.90	0.10	P > 0.05
JX	47	34	5	86	0.74	0.26	P > 0.05
LX	38	34	6	78	0.71	0.29	P > 0.05
XN	38	9	4	51	0.83	0.17	P < 0.05

HWE = Hardy-Weinberg equilibrium; NY = Nanyang; JX = Jiaxian; LX = Luxi; XN = Xianan.

Table 3. Genetic diversity of the *PPAR-γ* gene 72472 locus in Qinchuan (QC) breed.

Breeds	H_O	H_E	N_E	PIC	χ^2
QC	0.66	0.34	1.52	0.28	2.71
NY	0.82	0.18	1.22	0.16	0.49
JX	0.62	0.38	1.61	0.31	0.13
LX	0.58	0.42	1.71	0.33	0.18
XN	0.72	0.28	1.38	0.24	6.78**

H_O = gene homozygosity; H_E = gene heterozygosity; N_E = effective allele numbers; PIC = polymorphism information content. For other abbreviations, see legend to Table 2. *P < 0.01 for significant differences.

The homozygous 72472-CC genotype was the most frequent in all the 5 breeds. All breeds, except for XN (P < 0.05), were in Hardy-Weinberg equilibrium at the locus (P > 0.05). The frequencies of the C/D alleles in the populations analyzed were 0.78/0.22, 0.90/0.10, 0.74/0.26, 0.71/0.29, and 0.83/0.17 for the QC, NY, JX, LX, and XN populations, respectively. H_O varied from 0.58 (LX) to 0.82 (NY); H_E varied from 0.18 (NY) to 0.42 (LX); N_E ranged from 1.2195 (NY) to 1.7119 (LX), and PIC was 0.2832, 0.1638, 0.3083, 0.3294, and 0.2392 for QC, NY, JX, LX, and XN, respectively. Hence, allele C was the preponderant allele. According to the classification on the basis of PIC (low polymorphism, if PIC value was <0.25; moderate polymorphism, if PIC value was >0.25 and <0.5, and high polymorphism, if PIC value was >0.5), NY and XN possessed low genetic diversity (PIC value <0.25) and QC, JX, and LX had moderate polymorphisms (0.25 < PIC value < 0.5).

Correlation analysis showed that the genotypes were related to carcass length, backfat thickness, and WHC (shown in Table 4). Multi-comparison showed that the frequency of the genotype CC was significantly higher than those of genotypes CD and DD in backfat thick-

ness and WHC ($P < 0.05$); the difference between the frequencies of the CC and CD genotypes was very significant for backfat thickness ($P < 0.01$). The frequency of the genotype CD was significantly higher than that of the other genotype in carcass length ($P < 0.05$). However, no other significant association was noted between genotypes and traits ($P > 0.05$).

Table 4. Relationships between genotypes and meat traits in the Qinchuan breed.

Traits	Genotype (mean ± SE)		
	CC (N = 69)	DC (N = 31)	DD (N = 8)
Slaughter weight (kg)	414.22 ±17.28	420.58 ± 13.35	383.78 ± 24.23
Carcass weight (kg)	210.10 ± 7.21	215.86 ± 8.81	189.87 ± 14.29
Carcass length (cm)	139.56 ± 1.97^{b}	145.45 ± 1.84^{a}	137.83 ± 2.51^{b}
Back fat thickness (cm)	1.04 ± 0.08A^{a}	0.71 ± 0.06^{B}	0.70 ± 0.08^{b}
Loin-eye area (cm^2)	85.58 ± 4.50	77.02 ± 5.42	83.66 ± 6.21
Water holding capacity (%)	0.21 ± 0.06^{a}	0.23 ± 0.01^{b}	0.24 ± 0.09^{b}
Beef marbling score (No.)	3.26 ± 0.19	2.89 ± 0.90	2.89 ± 0.60
Tenderness	4.91 ± 0.09	4.95 ± 0.61	5.14 ± 0.19

Beef marbling score: scored from 1 (abundant) to 5 (poor). ^{a,b}Different lower case superscript letters were significantly different by the least square means in a column ($P < 0.05$); ^{A,B}mean very significant ($P < 0.01$).

DISCUSSION

The nuclear receptor, *PPAR-γ*, is a crucial regulator of adipocyte differentiation. Some mutations of the gene *PPAR-γ* have been proposed to play an essential role in glucose, lipid, and blood pressure homeostasis. Studies have shown that bovine *PPAR-γ* is located on chromosome 22, contains 7 exons, and encodes 507 amino acids. In a previous study, the polymorphism of human *PPAR-γ* mainly focuses on the mutants of Pro12Ala and C1431T (Yen et al., 1997). Few studies related to the polymorphism of bovine *PPAR-γ* have been reported. In the present study, we tested cattle breeds for mutations of the 7th exon of the *PPAR-γ* gene and found a missense mutation, namely, G-to-T in the 7th exon, which was caused by the change from glutamine to histidine at the 448th site amino acid. Analysis of the genetic diversity at the 72472 G>T locus showed that the frequencies of the C allele varied from 0.74 (JX) to 0.90 (NY) in the 5 breeds and that C is the preponderant allele. Among the 5 breeds, XN was in disagreement with the Hardy-Weinberg equilibrium ($P < 0.05$). This may be attributed to the background of breeding: the XN cattle breed had a wider breeding space and the QC, NY, JX, and LX cattle breeds had a higher level of artificial selection and breeding space. The PIC values indicated that there was more genetic diversity within QC, JX, and LX than NY and XN, but the level of PIC was not high ($0.16 < PIC < 0.33$) in the populations analyzed, suggesting that the animals may have been subjected to a high level of pressure from artificial selection during breeding.

The association between the 72472 G>T polymorphism and meat traits was analyzed in the QC population. The results showed that there was no significant relationship between the genotypes and carcass traits (including slaughter weight, carcass weight, loin-eye area, beef marbling score, and tenderness) ($P > 0.05$). The genotype CC was determined to have a significant association with backfat thickness and WHC ($P < 0.05$), and genotype CD was found to be significantly associated with carcass length ($P < 0.05$), which indicates that the allele C might be a beneficial allele for some growth traits in the QC population. As a consequence, the individuals with the DD genotype are limited. Hence, artificial selection must be strengthened during the breeding of the QC population. Several extensive studies have been conducted to improve the quality of QC cattle during the last few decades, and the role of QC

cattle has changed from work cattle to beef cattle. However, it is still necessary to augment the character of the hindquarter and increase meat production for breeding improved QC cattle.

We conclude that the 72472 G>T polymorphism in exon 7 of the gene *PPAR-γ* is associated with the carcass and meat quality traits in QC cattle. Our findings can be practically applied for the improvement of Chinese native cattle and the breeding of genuine beef cattle in China. The study should be further extended to include other breeds, emphasizing on the interaction of 72472 G>T (in exon 7), 200 A>G (in exon 1), and 42895 C>T (in exon 5).

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