

Full Length Research Paper

Novel polymorphism of the bovine fat mass and obesity-associated (FTO) gene are related to backfat thickness and longissimus muscle area in five Chinese native cattle breeds

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In this study, genetic variation of the fat mass and obesity-associated gene (FTO) was detected by PCR-SSCP and DNA sequencing in 618 individuals from five Chinese indigenous cattle breeds, and their genetic effects on meat quality traits were evaluated. The results showed that a novel single nucleotide polymorphism C1071T was detected in exon 5 and the allelic frequencies for the C and T alleles of the five breeds were 0.666/0.334, 0.583/0.417, 0.631/0.369, 0.653/0.347 and 0.689/0.311, respectively. Animals with CT genotype had higher mean values for backfat thickness than those with CC or TT genotypes ($P < 0.01$). Individuals with CC or CT genotypes had higher longissimus muscle area than those with TT genotype ($P < 0.05$). The FTO gene may be a candidate gene for identifying differences in meat quality traits and therefore, could be applied to marker-assisted selection of native Chinese cattle breeds.

Key words: Cattle, fat mass and obesity-associated (FTO) gene, single nucleotide polymorphism (SNP), meat quality traits.

INTRODUCTION

The fat mass and obesity-associated (FTO) gene is a member of the nonheme dioxygenase (Fe (II) - and 2-oxoglutarate-dependent dioxygenases) superfamily (Gerken et al., 2007; Sanchez-Pulido and Andrade-Navarro, 2007) and was originally cloned from a mutant mouse that had fused toes (Ft) (Peters et al., 2002). Expression studies indicate that, the FTO gene is widely

expressed in many tissues, but most abundant in the hypothalamus, the control center of energy balance (Gerken et al., 2007). The function of the gene is largely unknown, but the central distribution of FTO indicates that, the polymorphic variation in FTO may play a causal role in the regulation of energy homeostasis or in the development of fat tissue (Dina et al., 2007; Fredriksson et al., 2008). FTO mRNA levels are regulated by feeding and fasting in mice (Gerken et al., 2007; Fredriksson et al., 2008). Inactivation of the FTO gene in mice is shown to induce postnatal growth retardation and significant reduction in adipose tissue through control of energy expenditure (Fischer et al., 2009).

These findings suggest a mechanistic link between FTO and obesity and energy homeostasis. At present, human and pig genetic studies have found variation in FTO gene to be associated with obesity and related traits (Chang et al., 2008; Fan et al., 2009; Fontanesi et al., 2009, 2010; Frayling et al., 2007; Hunt et al., 2008;

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Abbreviations: PCR, Polymerase chain reaction; SSCP, single-stranded conformation polymorphism; FTO, fat mass and obesity-associated gene; EDTA, ethylenediamine tetra acetic acid; PAGE, polyacrylamide gel electrophoresis; SNP, single nucleotide polymorphism; PIC, polymorphic information content; BFT, backfat thickness; LMA, longissimus muscle area; IMF, intramuscular fat content; MAR, marbling score.

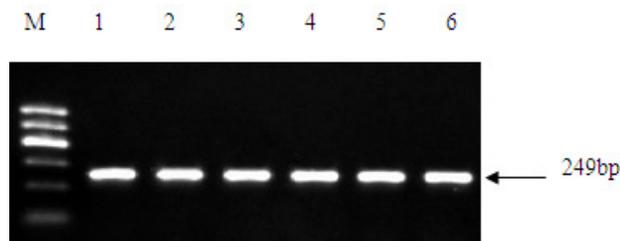


Figure 1. The PCR product of FTO gene exon 5 and its flanking region. M: Marker; Lanes 1 to 6: PCR products of the FTO gene exon5 and its flanking region.

Legrya et al., 2009; Peeters et al., 2008; Zhang et al., 2009). However, there has not been reported information so far on the polymorphism of bovine FTO gene. Therefore, the aim of this study was to detect the polymorphic variation of the bovine FTO gene and assess the effect of the variation on adipose and related traits.

MATERIALS AND METHODS

DNA samples and data collection

A total of 618 individuals of five Chinese native cattle breeds were selected and used to analyze the FTO allelic frequencies, including Qinchuan (QC, N=411, Shanxi province of China), Nan yang (NY, N=42, Henan province of China), Jiaxian red (JR, N=61, Henan province of China), Xia'n'an (XN, N=59, Henan province of China) and Simmental and Luxi crossbred steers (SL, N=45, Shandong province of China). Genomic DNA samples were extracted from blood samples of all the animals and stored at -80°C (Sambrook et al., 2002). Ultrasound measurements including backfat thickness (BFT), longissimus muscle area (LMA), intramuscular fat content (IMF) and marbling score (MAR) over the 12 to 13th longissimus muscle rib were taken in all the animals except for 250 Qinchuan cattle (Hou et al., 2010). In order to minimize systematic error, the same person was assigned to measure the same one trait.

Primers and polymerase chain reaction conditions

According to the sequence of the bovine FTO gene (GenBank accession no. NC_007316.4), one pair of polymerase chain reaction (PCR) primers (forward: 5'-GAAGGGAGGCTTGGTCAAC-3' and reverse: 5'-GCTACAAGT AGGACAGA ATCA-3') was designed to amplify a 249 bp product of FTO exon 5 and its flanking region.

PCR amplifications were performed in a 20 µl reaction mixture containing 50 ng DNA, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM MgCl₂ and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The cycling protocol was 5 min at 95°C, 32 cycles of 94°C for 30 s, annealing at 57.6°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 10 min. PCR products were electrophoresed on 1.5% agarose gels (containing 200 ng/ml ethidium bromide) using 1×TBE buffer (89 mM Tris, 89 mM boric acid and 2 mM Na₂EDTA).

Single-stranded conformation polymorphism

Polymerase chain reaction (PCR) products were analyzed for

single-strand conformation polymorphisms (SSCP). Aliquots of 5 µl of the above PCR products were mixed with 10 µl denaturing solution (95% formamide, 25 mM ethylenediamine tetra acetic acid (EDTA), 0.025% xylene-cyanole and 0.025% bromophenol blue), incubated at 98°C for 10 min and then chilled on ice. Denatured DNA was loaded onto 10% PAGE gel in 1×TBE buffer and constant voltage 250 V 0.25 h, then, 110 V for 14 h. The gel was stained with 0.1% silver nitrate and visualized with 2% NaOH solution (containing 0.1% formaldehyde) according to Yang et al. (2009).

The PCR products which represented different PCR-SSCP genotypes, including both homozygous and heterozygous genotypes were purified with the Axygen kits (MBI Fermentas, Canada) and sequenced in both directions in an ABI PRIZM 377 DNA sequencer (Perkin-Elmer, USA). The sequences were analyzed with the DNAMAN 6.0 software.

Statistical analysis

Genotypic frequencies, allelic frequencies, Hardy-Weinberg equilibrium, gene homozygosity, effective allele numbers and polymorphism information content (PIC) were statistically analyzed using the POPGENE version 1.31 software. The GLM procedure of the SPSS software (version 17.0) was used to analyze the relationship between the genotypes of the FTO gene and records of BFT, LMA, IMF and MAR. The following linear model was used in the analysis:

$$Y_{ijkl} = \mu + G_i + S_j + BF_k + Ma_l + e_{ijkl}$$

Where Y_{ijkl} is the observation for the meat quality trait, μ is the overall population mean, G_i the genotype effect (CC, TT and CT genotypes); S_j the fixed effect of sex; BF_k the fixed effect of breed and farm; Ma_l the regression variable for measure age; e_{ijkl} the random environment effect.

RESULTS

PCR-SSCP analysis of the FTO gene

A 249 bp of amplified product was obtained by PCR amplification in all the animals studied (Figure 1). Three unique SSCP banding patterns (CC homozygote, TT homozygote and CT heterozygote) were observed in the locus after PCR-SSCP analysis (Figures 2 and 3).

Genetic polymorphism of the bovine FTO gene and the χ^2 test

DNA sequencing analysis of amplified product revealed a novel single nucleotide polymorphism (SNP) (C > T mutation at 1071 bp position of mRNA, named C1071T) in exon 5 compared with the nucleotide sequence in GenBank. This SNP was a synonymous mutation, which is at position 26 in the FTO amino acid sequence (Asn 26 Asn).

Genotype frequencies are shown in Table 1. The frequency of genotype TT is low and the wild allele T had lower frequency compared with the mutant allele C in all populations studied. Allelic frequencies in QC, NY, JR, XN and SL were 0.666/0.334; 0.583/0.417; 0.631/0.369; 0.653/0.347; 0.689/0.311, respectively. The χ^2 -test showed

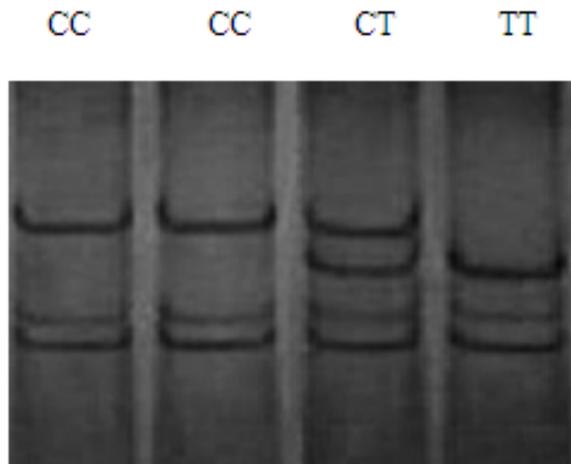


Figure 2. The Electrophoresis patterns of PCR-SSCP exon 5 and its flanking region of the bovine FTO gene, three patterns (Lanes 1 and 2 = CC genotype; lane 3 = CT genotype; lane 4 = TT genotype) were observed.

that, populations of the samples at FTO gene locus all agreed with Hardy-Weinberg equilibrium ($P > 0.05$), which might indicated that, the FTO gene locus was under homeostasis accompanied by the effect of artificial selection, migration and genetic drift and that the artificial selection had put little pressure on this gene locus.

The population genetic indices were calculated (Table 2). Gene heterozygosity varied from 0.429 (SL) to 0.486 (NY) and the effective allele numbers ranged from 1.750 (SL) to 1.946 (NY). According to the classification of PIC (low polymorphism if PIC value < 0.25 , medium polymorphism if $0.25 < \text{PIC value} < 0.5$ and high polymorphism if PIC value > 0.5), all five cattle breeds examined showed a medium polymorphic level. The minimum and maximum polymorphic information content (PIC) values were 0.337 (SL) and 0.368 (NY), respectively. The values of PIC and heterozygosity of Nan yang breed in the loci were higher than that of other populations, which implied that the polymorphism and genetic variation of Nan yang breed were higher than that of other populations.

Effect of the polymorphism locus on carcass traits of bovine

BFT, LMA, IMF and MAR were analyzed by comparison between genotypes of individuals of five populations and their phenotypic data. The results of association analysis of the gene specific marker are shown in Table 3. At the SNP marker, there were significant effects on backfat thickness ($P < 0.01$) and longissimus muscle area ($P < 0.05$). Animals with the genotype CT had higher mean values for backfat thickness than those with CC and TT genotypes ($P < 0.01$). Individuals with CC and CT

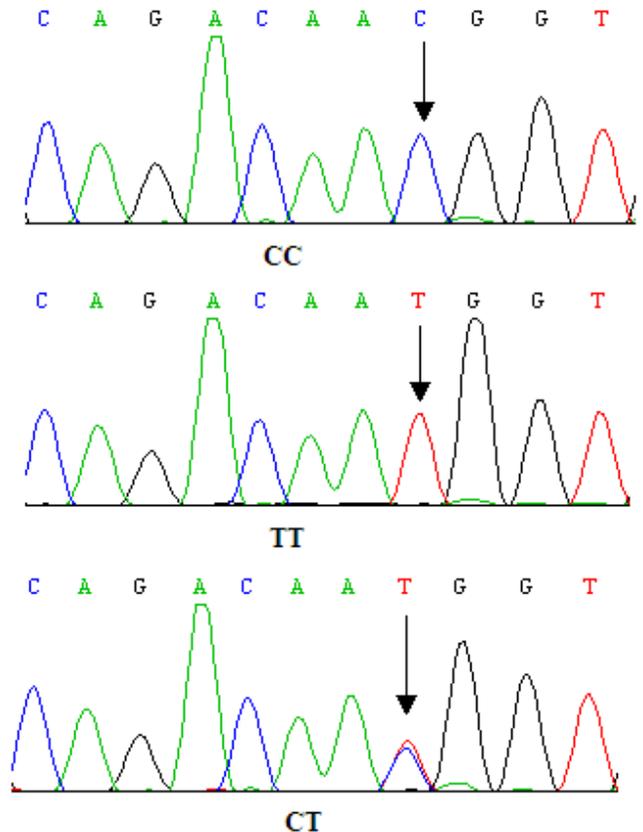


Figure 3. The sequencing map of the novel SNP in bovine FTO gene, sequencing map revealed a C1071T mutation in exon 5.

genotypes had higher longissimus muscle area (LMA) than those with TT genotype ($P < 0.05$). No significant effects of genotype on intramuscular fat content and marbling score were found ($P > 0.05$) in this study.

DISCUSSION

The present study is the first report on polymorphism of FTO gene in bovine. The FTO gene is a new candidate gene in obesity. Its important role on fatness and related traits has prompted many attempts to identify individual factors associated with its variation. At present, the gene has become one of the candidate genes for detecting polymorphism associated with the fat deposition in human and pig. Many studies have shown the strong association between fatness and related traits and polymorphism of the FTO gene. Human population genetic studies found variation in the FTO gene to be associated with fat mass, obesity, body mass index and type 2 diabetes mellitus (Chang et al., 2008; Frayling et al., 2007; Hunt et al., 2008; Legrya et al., 2009; Peeters et al., 2008). Studies on pig genetic showed the association between the polymorphism of the FTO gene and

Table 1. Genotype frequencies of the bovine FTO gene in cattle populations.

Breed	Observed genotypes (N)			Total	Allelic frequencies		χ^2 (HWE)
	CC	CT	TT		C	T	
QC	0.438(180)	0.455(187)	0.107(44)	411	0.666	0.334	0.197
NY	0.357(15)	0.452(19)	0.191(8)	42	0.583	0.417	0.202
JR	0.443(27)	0.377(23)	0.180(11)	61	0.631	0.369	2.207
XN	0.440(26)	0.424(25)	0.136(8)	59	0.653	0.347	0.254
SL	0.467(21)	0.444(20)	0.089(4)	45	0.689	0.311	0.061
Total	0.435(269)	0.443(274)	0.121(75)	618	0.657	0.343	0.165

N = Number of observations; HWE = Hardy-Weinberg equilibrium by the χ^2 test. All P values were above 0.05; QC = Qinchuan; NY = Nan yang; JR = Jiaxian red; XN = Xia'nan; SL = Simmental and Luxi crossbred steers.

Table 2. Population genetic indices at the FTO locus in cattle populations.

Breed	Homozygosity	Heterozygosity	Effective allele number	PIC
QC	0.555	0.445	1.803	0.346
NY	0.514	0.486	1.946	0.368
JR	0.534	0.466	1.871	0.357
XN	0.547	0.454	1.830	0.351
SL	0.571	0.429	1.750	0.337
Total	0.549	0.451	1.821	0.349

PIC = polymorphism information content. QC = Qinchuan; NY = Nan yang; JR = Jiaxian red; XN = Xia'nan; SL = Simmental and Luxi crossbred steers.

Table 3. Association between C1071T SNP genotypes of FTO gene and meat quality traits in *B. taurus* cattle.

Genotype	Trait (Means \pm SEM)			
	BFT (cm)	LMA (cm ²)	IMF (%)	MAR
CC	0.34 \pm 0.04 ^A	33.68 \pm 5.16 ^a	7.74 \pm 0.41	3.40 \pm 0.56
CT	0.36 \pm 0.05 ^B	33.28 \pm 5.40 ^a	7.78 \pm 0.55	3.40 \pm 0.53
TT	0.34 \pm 0.05 ^A	31.55 \pm 6.38 ^b	7.84 \pm 0.33	3.56 \pm 0.64
P value	0.001	0.013	0.210	0.071

Data are reported as means \pm SEM. Means with different superscript lower case letters are significantly different ($P < 0.05$). Means with different superscript capital letters are significantly different ($P < 0.01$). BFT= backfat thickness; LMA = longissimus muscle area; IMF=Intramuscular fat content; MAR = marbling score.

the backfat thickness, intramuscular fat content, lean meat content, total lipid percentage in muscle and average daily gain (Fontanesi et al., 2009, 2010; Fan et al., 2009; Zhang et al., 2009).

This study revealed a novel SNP (C1071T) in exon 5 within the bovine FTO gene, which is associated with BFT ($P < 0.01$) and LMA ($P < 0.05$) in five Chinese native cattle breed populations. In accordance with our results, Fan et al. (2009) discovered a synonymous mutation (c.594C > G Ala198Ala) in exon 3 within the ISU Berkshire \times Yorkshire pig resource family which was association with backfat thickness. However, we do not find any relationship between the SNP (C1071T) and IMF in this research in contrast to the findings in pig

discovered by Fontanesi et al. (2009, 2010) and Zhang et al. (2009). It may be due to specific differences or small sample size.

In conclusion, our research suggests that FTO gene may be an important candidate gene that affects fat deposition in *Bos taurus*. There after, further study will be necessary in larger populations. It is also important to determine whether the FTO gene plays a role in the development of other traits (such as body weight and average daily gain).

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