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Full Length Research Paper

Association of *LXRA* gene variants with carcass and meat quality traits in beef cattle

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LXRA is an important regulator of genes involved in lipid, fatty acid and glucose metabolism in liver, and adipose tissue as well as in skeletal muscle. In this study, we discovered and evaluated the association of two SNPs (T1891C in intron 2, and A2377G in exon 3) in the bovine *LXRA* gene with carcass and meat quality traits in beef cattle. The T1891C SNP was significantly associated with carcass weight (CW), dressing percentage (DP), meat percent (MP) and loin muscle area (LMA) (P < 0.05). Animals with the genotype TT had higher CW, DP, MP and LMA than the CT genotype. No significant associations were observed between the A2377G SNP and any traits analyzed in this study (P>0.05). These results suggested that T1891C SNP of the *LXRA* gene may be useful as a genetic marker for carcass and meat quality traits in beef cattle.

Key words: Cattle, *LXRA* gene, polymorphism, carcass, meat.

INTRODUCTION

Liver X receptor alpha (LXRA) is a member of a nuclear hormone receptor superfamily that is activated by oxysterols (Lehmann et al., 1997). In humans and mice, accumulated evidence has demonstrated that LXRA act as functional regulators of several important genes involved in lipid metabolism and fatty acid biosynthesis, including sterol regulatory element binding protein 1c (SREBP-1c), acetyl CoA carboxylase (ACC) and peroxisome proliferator-activated receptor c (PPAR-c) (Repa et al., 2000; Juvet et al., 2003; Laffitte et al., 2003; Hummasti et al., 2004; Ulven et al., 2005; Gerin et al., 2005). In addition, the findings that expression of the gene encoding the insulin-sensitive glucose transporter-4 (GLUT4) in liver, adipose tissue and skeletal muscle is regulated by the LXRs have also been described (Dalen et al., 2003; Laffitte et al., 2003; Kase et al., 2005), indicating a role for LXRA in influencing glucose metabolism as well. Recent studies, using a Berkshire and Yorkshire (BY) pig resource family, have detected suggestive quantitative trait loci (QTL) for loin eye area, and marbling score, as well as total lipid measured in the *longissimus dorsi* around the regions where the *LXRA* gene were expected to be mapped (Malek et al., 2001a, b; Huff-Lonergan et al., 2002; Bosak et al., 2003 and Yu et al., 2006). No polymorphism of bovine *LXRA* gene and their association with carcass and meat quality traits had been described by now.

Therefore, based on the important physiological roles of the LXRA in lipogenesis and myogenesis as determined in human and pig, *LXRA* was considered as an attractive candidate gene for carcass composition and meat quality in bovine. The objective of this study was to detect single nucleotide polymorphism (SNP) in bovine *LXRA* gene and to explore its possible association with carcass and meat quality traits in beef cattle.

MATERIALS AND METHODS

Animals and carcass data

A total of 724 animals including Luxi (n = 273), Chinese Simmental (n = 213), Sanhe (n = 113), Angus (n = 44), Hereford (n = 30), and Simmental crossbred steers (Simmental crossed with indigenous female yellow cattle in China) (n = 51) were randomly selected from commercial populations and used to analyze the LXRA allelic fre-

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Table 1. The primer sequences and their information of bovine *LXRA* gene.

Primers	The primer sequences	Size (bp)	Time (s)	Location
LXRA-1	F: 5' GCACGGTAGCCAAGACAT 3' R: 5' CGTCCATCTCAGAGATCAGAC 3'	699	62	Exon 2 and flanking
LXRA-2	F: 5' GAGATGGACGAACTTCAGC 3' R: 5' CTCCCTGAGGATGCACTT 3'	323	61.7	Exon 3 and flanking

quencies, which were reared in the province of Shandong, Inner Mongolia, and Hebei, respectively. And a total of 211 animals including Luxi (n = 30), Chinese Simmental (n = 56), Angus (n = 44), Hereford (n = 30), and Simmental crossbred steers (n = 51) were used for the association study. Carcass and meat quality traits were measured according to the criterion GB/T 17238 - 1998 Cutting Standard of Fresh and Chilled Beef in China (China Standard Publishing House). The following traits, Live weight (LW), Carcass weight (CW), Dressing percentage (DP), Carcass length (CL), Meat percent(MP), Backfat thickness (BF), Marbing score (MS) and Loin muscle area (LMA) were measured or calculated. DNA samples were extracted from leukocytes and tissue samples according to Mullenbach et al. (1989).

SNP identification and genotyping

According to the sequence of bovine *LXRA* gene (GenBank accession No. NM_001014861.1), two pairs of primers were designed to amplify the bovine *LXRA* gene (Table 1). Polymerase chain reaction (PCR) amplifications were performed in 20 µl volume containing 50 ng DNA template, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM $MgCl_2$, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The PCR protocol was 94ºC for 5 min followed by 35 cycles of 94ºC for 30 s, annealing for 30 s, and 72ºC for 30 s and a final extension at 72ºC for 10 min.

Single-strand conformation polymorphism (SSCP) method was used to scan mutations within the amplified regions. Aliquots of 10 µl PCR products were mixed with 10 µl denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene cyanole and 0.025% bromophenol blue), heated for 10 min at 98ºC and chilled on ice for 5 min. Denatured DNA was subjected to 10% PAGE (Polyacrylamide Gel) in 1 × TBE buffer and constant voltage (200 V) for 2.5 - 3.0 h at a constant temperature of 12ºC, then gels were stained with 0.1% silver nitrate (Sun et al., 2002).

The PCR products of different homozygous genotype were separated on 1.0% agarose gels and purified with the Wizard Prep PCR purification kit (Shanghai Bioasia Biotechnology Co., Ltd. P. R. China), subcloned into the pGEM-T Easy Vector (Promega, Madison, WI, USA) and sequenced (Applied Biosystems 3730xl DNA Analyzer, Foster City, CA, USA).

By comparing the sequences of the PCR fragments amplified from 10 individuals that represented different breeds, we identified two mutations: T1891C in intron 2 and A2377G in exon 3 at the bovine *LXRA* locus. Interestingly, these SNPs could be genotyped by restriction enzymes *Higc*I and *Sch*I, respectively. An aliquot of 20 µl PCR products were digested with 15 units endonuclease (MBI, Fermentas) at 37ºC for 5 h following the supplier's directions. The restriction fragments were scored and analyzed by electrophoresis on 3% agarose gels.

Statistical analyses

The association between SNP marker genotypes of the *LXRA* gene and carcass and meat quality traits was analyzed by the least-squares method as applied in the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). According to the following statistical linear model:

$$Y_{ij} = \mu + G_i + b_{ij}SA_{ij} + \varepsilon_{ij}$$

Where Y_{ij} stands for observed value; µ: overall mean for each trait; G_i: ith genotype; b_{ij}: regression coefficient; SA_{ij}: regression variable of slaughter age; ε_{ij}: random error.

RESULTS AND DISCUSSION

SNP marker genotyping

A 323 and 699 bp fragment of the *LXRA* gene were amplified and sequenced, respectively. Two SNPs (T1891C and A2377G) were found. The T1891C mutation was detected at position 42 of the intron 2 and created a *Higc*I restriction site, another one A2377G mutation was detected at position 2 of the exon 3 and caused amino acid mutation Asp (GAT) to Glu (GAG), which created a *sch*I restriction site.

In the analyzed population, for the T1891C SNP, three size variants of restriction fragments were identified, namely: 323, 238 and 85 bp. An analysis of the localization of migration bands of the restriction fragments enabled to identify three genotypes of "mutation T>C". The genotype TT represents the occurrence of one band of 323 bp, genotype CT represents three restriction fragment bands of 323, 238 and 85 bp, and genotype CC represents two bands of 238 and 85 bp. For the A2377G SNP also, three size variants of restriction fragments were identified, namely: 699, 441 and 258 bp. The genotype AA represents the occurrence of one band of 699 bp, genotype AG represents three restriction fragment bands of 699, 441 and 258 bp, and genotype GG represents two bands of 441 and 258 bp. The electrophoresis of the PCR products was shown in Figure 1.

Allele frequencies of the two SNPs were investigated in six different beef populations (Table 2). Frequencies of LXRA-T and LXRA-A allele ranged from 0.38 to 0.85 and 0.43 to 0.82, respectively. The χ^2 test of allelic frequency of both two SNPs was performed among six breeds of cattle. There was no significant difference in the allelic frequency among breeds in both two SNPs ($P > 0.05$).

Table 2. An allelic frequency of the two SNP on the LXRA gene in different cattle breeds.

Breed	n	T1891C SNP		X^2	A2377G SNP		X^2	$X^2_{0.05(5)}$*
		T	C		A	G		
Luxi	282	0.43	0.57		0.39	0.61		
Simmental	212	0.69	0.31		0.56	0.44		
Sanhe	113	0.82	0.18		0.85	0.15		
Angus	49	0.56	0.44	0.41	0.38	0.62	0.66	11.07
Hereford	30	0.52	0.48		0.50	0.50		
Crossbreed	51	0.54	0.46		0.39	0.61		

*$X^2_{\alpha(df)}$ is the X^2 value where α is the level of significance and df degrees of freedom

Table 3. Associations of T1891C SNP genotypes with carcass and meat quality traits at bovine *LXRA* gene.

Trait	Genotypes of *Hgic*I PCR-RFLP genotyping			p-value
	TT (mean ± SE)	CT (mean ± SE)	CC (mean ± SE)	
Live weight (LW)/kg	570.12 ± 10.50	542.78 ± 8.66	551.44 ±11.39	0.1500
Carcass weight (CW)/kg	315.15^a ± 7.64	299.86^b ± 5.14	316.19^a ± 6.39	0.0216*
Dressing percentage (DP)/%	56.72^a ± 0.69	55.13^b ± 0.28	56.09^{ab} ± 0.45	0.0121*
Meat percent (MP)/%	49.10^a ± 0.59	47.74^b ± 0.25	48.74^{ab} ± 0.43	0.0238*
Marbing score (MS)/1-5	2.43 ± 0.18	2.11 ± 0.11	2.07 ± 0.16	0.6323
Loin muscle area (LMA)/cm^2	73.13^a ± 2.39	67.27^b ± 1.72	71.34^{ab} ± 2.05	0.0315*
Backfat thickness (BF)/cm	1.07 ± 0.08	1.13 ± 0.05	1.01 ± 0.06	0.3101
Meat tenderness (MT)/kg	4.16 ± 0.24	4.03 ± 0.17	4.27 ± 0.20	0.2638
Carcass depth (CD)/cm	65.52 ± 0.87	65.09 ± 0.53	63.78 ± 0.56	0.6491
Carcass length (CL)/cm	142.56 ± 0.94	140.52 ± 0.82	138.93 ± 1.02	0.3974

[a,b] Means of traits with different superscripts were significantly different.
* Effect was significant at $P < 0.05$.

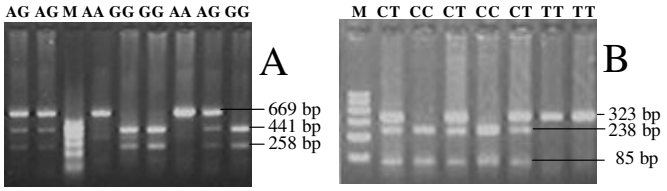

Figure 1. Agrose gel (3%) showing different genotypes of bovine LXRA gene. M: 100-600 bp. A and B represent the *Higc*I and *sch*I loci, respectively. The genotypes are given at the top of the columns

SNP marker associations

The genotypes of 211 individuals were compared with their phenotypic data for 10 traits. The results of the gene-specific SNP marker association analysis were given in Table 3 and 4. At the SNP marker of T1891C SNP in intron 2 there was a significant effect on the CW, DP, MP and LMA ($P < 0.05$) (Table 3). The T allele was associated with a significant increase in CW, DP, MP and LMA. Animals with the genotypes TT and CC had higher CW than animals with the CT genotype, and animals with the genotype TT had higher DP, MP and LMA than the CT genotype ($P < 0.05$). No significant associations were observed between T1891C SNP genotypes and other traits. The association analysis between the A2377G SNP and any traits examined in this study showed no significant genotype effects ($P > 0.05$) (Table 4).

It is interesting the bovine *LXRA* gene is mapped to several QTL regions in bovine chromosome 15, which have been indicated to affect carcass quality and yield (Sonstegard et al., 2002; Harhay et al., 2005) (http://bovineqtlv2.tamu.edu/home.php). The present study showed that the T1891C SNP was significantly associated with carcass weight, dressing percentage, meat percent and loin muscle area (Table 4). These results were similar to the results reported by Huff-Lonergan et al. (2002) and Yu et al. (2006) that a significant associated with loin muscle area in Berkshire and Yorkshire (BY) pig resource family.

In conclusion, we identified SNPs in the *LXRA* gene and investigated their association in several populations. Our results provided evidence that the *LXRA* gene might have potential effects for carcass and meat quality traits. Therefore, further work will be necessary to use these

Table 4. Associations of A2377G SNP genotypes with carcass and meat quality traits at bovine *LXRA* gene.

Trait	Genotypes of *Sch*I PCR-RFLP genotyping			p-value
	AA (mean ± SE)	AG (mean ± SE)	GG (mean ± SE)	
Live weight (LW)/kg	560.95 ± 11.25	549.16 ± 12.51	560.95 ± 7.83	0.8374
Carcass weight (CW) /kg	308.46 ± 7.94	302.36 ± 7.62	301.79 ± 4.50	0.4912
Dressing percentage (DP)/%	54.90 ± 0.73	54.97 ± 0.36	54.93 ± 0.34	0.1769
Meat percent (MP)/%	47.89 ± 0.63	47.56 ± 0.33	47.71 ± 0.31	0.2185
Marbing score (MS)/1-5	2.30 ± 0.18	2.10 ± 0.15	2.18 ± 0.12	0.4863
Loin muscle area (LMA)/cm^2	66.05 ± 1.92	70.17 ± 2.30	69.54 ± 1.21	0.5914
Backfat thickness (BF)/cm	1.16 ± 0.09	1.04 ± 0.06	1.06 ± 0.05	0.8585
Meat tenderness (MT)/kg	3.96 ± 0.22	4.31 ± 0.26	4.10 ± 0.15	0.3897
Carcass depth (CD)/cm	65.93 ± 0.79	64.59 ± 0.77	64.42 ± 0.46	0.4041
Carcass length (CL)/cm	141.47 ± 1.05	140.57 ± 1.04	140.16 ± 0.79	0.7363

SNPs for marker-assisted selection (MAS) in larger population and investigate whether the *LXRA* gene play a role in those traits or is in linkage disequilibrium with other causative mutations.

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