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Effect of thyroglobulin gene polymorphisms on growth, carcass composition and meat quality traits in Chinese beef cattle

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Abstract The thyroglobulin (TG) gene has been studied as an important gene related to fat deposition, since not only does *TG* gene locate in a quantitative trait locus with an effect on fat deposition, but also it encodes the precursor of thyroid hormones which have crucial biological functions in energy metabolism. In the present study, we identified four novel SNPs at the 5′ flanking region of the bovine *TG* gene. Association analysis indicated that the G275A, G277C, G280A and C281G SNPs were significantly associated with average daily gain (ADG, $P < 0.01$ for G275A and G277C, $P < 0.05$ for G280A and C281G). Five haplotypes for the 4 SNPs were constructed and their effects on growth, carcass composition and meat quality traits were evaluated. The results showed no significant effect of haplotype on ADG. Meanwhile, no significant association was found between 4 SNPs and other growth, carcass composition and meat quality traits including intramuscular fat. Bioinformatics analysis showed that 4 SNPs may results in potential transcription factor binding site changes. Results of this study suggest that *TG* gene-specific SNPs may be a useful marker for growth traits in marker assisted selection programs in beef cattle.

Keywords Cattle · Thyroglobulin gene · Polymorphism · Growth trait · Carcass composition trait · Meat quality trait

Introduction

Thyroid hormones triiodothyronine (T3) and tyroxin (T4) are processed from thyroglobulin (TG) in thyroid follicular cells and stored in the thyroid gland [1]. Thyroid hormones have important biological functions in development and metabolism regulation and also have effects on adipocyte differentiation, growth and homeostasis of fat depots [1–4]. Because of the important function of 5′ flanking region in gene transcriptional regulation, the genetic variation in the 5′ flanking region of the *TG* gene has been used as one of the main markers to improve the beef marbling level. A SNP located in the 5′ flanking region of *TG* gene named TG5 had been proved to have a significant association with marbling score in some beef population [5–7]. However, the *TG* gene had been not only mapped to the region of the QTL for fat deposition traits, but also considered as a functional candidate gene as well as a positional candidate of USDA yield grade, post-weaning average daily gain, birth weight and weaning weight traits [8–12].

To investigate the polymorphisms in 5′ flanking region of the and their associations with carcass and meat traits in Chinese cattle population, we amplified and sequenced the 5′ flanking region of bovine *TG* gene. The result of this study provides new evidence that whether variations in *TG*

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are useful markers to be used for selection in Chinese beef cattle industry.

Materials and methods

Animals and carcass data

A total of 237 animals including Angus (n = 42), Charolais (n = 29), Luxi (n = 24), Qinchuan (n = 20), Hereford (n = 18), Limousin (n = 15), Simmental (n = 71) and Jinnan (n = 18) were randomly selected from commercial populations and used in the association analysis. The animals were reared in two farms which are in the Inner Mongolia Autonomous Region and Hebei province respectively. The animals (405 ± 50.5 kg; 30 ± 2 months of age) were housed in a concrete-floored cowshed (in a single pan for each animal) and fed for 195 days. Meat quality and carcass composition traits were measured according to the criterion GB/T 17238-1998 Cutting Standard of Fresh and Chilled Beef in China (China Standard Publishing House). Live weight (LW), carcass weight (CW), loin muscle area (LMA), marbling score (MS), backfat thickness (BF), dressing percentage (DP) and average daily gain (ADG) were measured or calculated. BF and LMA were measured between the 12 and 13th rib. MS for quality grade was evaluated on a cross section of the Loin muscle between the 12th and 13th rib, which is scored from grade 1 to 5. The ADG was calculated by subtracting the initial weight from the final weight and dividing by the number of fattening days. All experimental protocols and animal care were performed according to authorization granted by the Chinese Ministry of Agriculture.

PCR amplification and SNP genotyping

Total genome DNA were extracted from anticoagulant vein blood samples according to Mullenbach et al. [13], and diluted to 50 ng/μl for polymerase chain reaction (PCR). According to the bovine *TG* gene sequence cloned in a previous study of the authors' (unpublished data), a pair of primers (5′-GGGGGATGACTACGAG TATGAC-3′; 5′-AGCAGACCGAAGACCCATAG-3′) was designed to amplify a 785 bp fragment (checked by DNA sequencing) within 5′ flanking region of *TG* gene. PCR amplifications were performed in 20 μl volume containing 50 ng genome DNA template, 10 pmol of each primer, 0.20 mmol dNTPs, 2.5 mmol $MgCl_2$, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The PCR protocol was 95 °C for 5 min followed by 36 cycles of 94 °C for 30 s, annealing for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 10 min. The products were purified using Wizard Prep PCR purification kit (Shanghai Bioasia Biotechnology Co., Ltd. P. R. China) and sequenced in both directions (Beijing Aolaibo Biotechnology Co., Ltd. P. R. China; Applied Biosystem 3730 × 1 DNA sequencer, Foster city, CA, USA).

Statistical analysis and bioinformatic tools

The linkage disequilibrium (LD) and haplotype analysis of 4 SNPs was performed with online program SHEsis [14]. In this study, the r^2 property was used. An $r^2 > 0.33$ was considered to present strong linkage disequilibrium [15]. The relationship between single SNP genotypes and carcass composition and meat quality traits was analyzed using 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:

$$Y_{ijk} = \mu + BF_i + Month_j + G_k + e_{ijk}$$

where Y_{ijk} stands for observed value; μ: overall mean for each trait; BF_i: the effect of ith breed and farm; $Month_j$: the effect of jth month of slaughtering; G_k: kth single SNP marker genotype as a class effect; e_{ijk}: random error.

The following mixed model was used to estimate haplotype effects with SAS software.

$$Y_{ijk} = \mu + BF_i + Month_j + \sum_{k=1}^{3} \beta_k H_{km} + e_{ijkm}$$

Y_{ijkm} is the observed value; μ is the overall mean for each trait; BF_i is the effect of ith breed and farm; $Month_j$ is the effect of jth month of slaughtering; H_{km} is the number of kth haplotype for each individual; β_k is the estimated effect for the kth haplotype; e_{ijkm} is the random error.

Transcription factor binding sites (TFBS) were predicted with Tfsitescan (http://www.ifti.org).

Results

Genotype patterns of different polymorphisms

A 785 bp fragment of 5′ flanking region of the *TG* gene was amplified and sequenced successfully in 237 animals. The comparisons among these sequences revealed 4 SNPs: G275A, G277C, G280A and C281G (Fig. 1) which were located in the upstream of TG5 site (422 bp of amplified fragment).

The genotype frequencies of 4 SNPs were shown in Table 1. The AA genotype of G275A site, CC genotype of G277C site, AA genotype of G280A site and GG genotype of C281G site were not detected in the population studied.

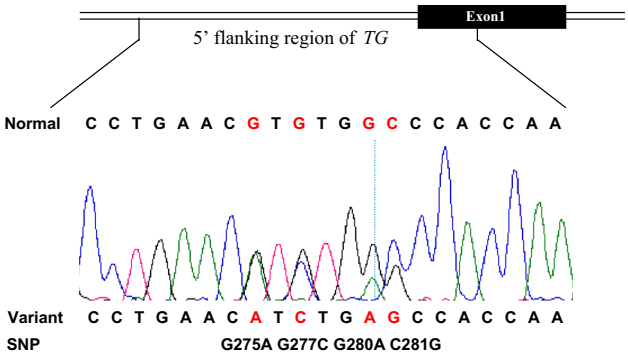

Fig. 1 Chromatograms showing sequence variation at positions 275(G275A), 277(G277C), 280(G280A) and 281(C281G) within 5′ flanking region of the *TG* gene

SNP marker associations

The LD between the 4 SNPs in the population was calculated, which indicated that G275A and G277C ($r^2 = 0.921$), G280A and C281G ($r^2 = 1$) were strongly linked. Because of the strong LD, five haplotypes were constructed. Their frequencies are in Table 2.

The relationship between genotypes of the 237 individuals and carcass composition and meat quality traits are in Table 3. The association analysis indicated that the 4 SNPs were significantly associated with ADG ($P < 0.01$ for G275A and G277C sites, $P < 0.05$ for G280A, C281G sites and diplotype). The haplotype effects on each trait are in Table 4. However, there was no significant effect of haplotype on growth, carcass composition and meat quality traits (Table 4).

Effect of haplotype on functional elements of *TG* gene promoter

The effect of the haplotype on TFBS of the *TG* gene promoter was analyzed by submitting sequences of different haplotypes to Tfsitescan website. Several different TFBS of were predicted in the region of 4 SNPs (275–281). We found that the H1 contains two potential TFBS (LF-A1_CS and Sp1-YB1) in the 275–281 reigon, while H2 contains RP58_CS, LF-A1_CS and Sp1-YB1. Interestingly, all the TFBS in the same region of H3 sequence disappeared. These 4 SNPs could lead to the disappearance of some important TFBS as well as some new TFBS emerging (Table 5).

Table 2 Frequencies of haplotypes based on the 4 SNPs in bovine *TG* gene

Haplotype	Type	Observation	Frequency
H1	G-G-G-C	416	0.878
H2	A-C-G-C	35	0.074
H3	A-C-A-G	19	0.040
H4	G-C-G-C	3	0.006*
H5	A-G-G-C	1	0.002*

*Frequency less than 0.03

Table 3 *P* values of TG gene SNP effects on production traits

Trait*	SNP/Diplotype			
	G275A	G277C	G280A	C281G
LW (kg)	0.0729	0.0421**	0.5899	0.5899
CW (kg)	0.3507	0.2694	0.6992	0.6992
DP (%)	0.315	0.2835	0.9874	0.9874
MS (1–5)	0.1416	0.3797	0.6893	0.6893
LMA (cm^2)	0.3351	0.5452	0.6014	0.6014
BF (cm)	0.6901	0.8685	0.9447	0.9447
ADG (kg)	0.0067***	0.0067***	0.0259**	0.0259**

*Live weight (LW), Carcass weight (CW), Dressing percentage (DP), Marbling score (MS), Loin muscle area (LMA), Backfat thickness (BF) and Average daily gain (ADG)

**Effect was significant at $P < 0.05$

***Effect was significant at $P < 0.01$

Table 1 Genotype frequency and MAF of 4 SNPs in *TG* gene

SNP[a]	Genotype/Allele	Total no.	Genotype no.	Genotype frequency	MAF
G275A	GG (G)	237	182	0.77	
	AG		55	0.23	0.12(A)
	AA (A)		0	0	
G277C	GG (G)	237	180	0.76	
	CG		57	0.24	0.12(C)
	CC (C)		0	0	
G280A	GG (G)	237	218	0.92	
	AG		19	0.08	0.04(A)
	AA (A)		0	0	
C281G	CC (C)	237	218	0.92	
	CG		19	0.08	0.04(G)
	GG (G)		0	0	

[a] The location of the SNP in the sequence X05380

Table 4 The estimated effects of haplotypes of *TG* gene

Trait	Estimated effect ± SE		
	H1[a]	H2[b]	H3[c]
Observation (frequency)	179 (0.768)	35 (0.150)	19 (0.082)
LW (kg)	−2.94 ± 14.81	5.37 ± 8.85	0
CW (kg)	−0.96 ± 5.83	0	0
DP (%)	0	−0.04 ± 0.17	0
MS (1-5)	0.03 ± 0.18	0	0
LMA (cm^2)	0	0	0
BF (cm)	0	0	0
ADG (kg)	−0.03 ± 0.11	0	0.01 ± 0.02

[a] H1 indicates G-G-G-C

[b] H2 indicates A-C-G-C

[c] H3 indicates A-C-A-G

Table 5 Prediction of transcription factor binding sites of the *TG* gene haplotypes

Haplotype	Site (length)	Position	Score (gaps)	Occurrence	Exp value
H1[a]	Sp1-YB1′(6)	262	6(0)	4	3.24E−01
	LF-A1_CS(6)	278	6(0)	1	7.92E−01
	Sp1-YB1(6)	281	6(0)	1	3.24E−01
	HC3(6)	282	6(0)	1	3.24E−01
	uteroglobin_HS-′(8)	283	8(0)	1	5.42E−01
H2[b]	Sp1-YB1′(6)	262	6(0)	4	3.24E−01
	RP58_CS(10)	272	10(0)	1	1.21E−02
	LF-A1_CS(6)	278	6(0)	1	7.92E−01
	Sp1-YB1(6)	281	6(0)	1	3.24E−01
	HC3(6)	282	6(0)	1	3.24E−01
	uteroglobin_HS-′(8)	283	8(0)	1	5.42E−01
H3[c]	Sp1-YB1′(6)	262	6(0)	4	3.24E−01
	HC3(6)	282	6(0)	1	3.24E− 01
	uteroglobin_HS-′(8)	283	8(0)	1	5.42E−01

[a] H1 indicates G-G-G-C

[b] H2 indicates A-C-G-C

[c] H3 indicates A-C-A-G

Discussion

A previous study has mapped a QTL with an effect on fat deposition to the *bos taurus* chromosome (BTA) 14 [16]. Because of the relationship between the *TG* gene and thyroid hormones which could affect lipid metabolism [5], *TG* gene had been proposed as a candidate gene for this QTL. The TG5 SNP has been reported to have significant association with fat deposition and could be used as a molecular marker for marbling deposition in beef cattle [5, 17]. From then on, many studies on the TG5 marker have been carried out across populations. However, the results across studies are not consistent. Some studies obtained consistent or similar results with Barendse [7, 9, 18, 19]. Nevertheless, the other studies did not detect the associations between the TG5 marker and fat deposition traits or even got an opposite result [4, 20–22].

Most researchers focused on the IMF deposition, even though *TG* gene also located in the region of the QTL for fat thickness, USDA yield grade, post-weaning average daily gain, birth weight and weaning weight traits [8–12]. Besides IMF, only a few studies reported that *TG* gene was associated with ADG, RPYD, FATYD, BONETD, FT and LMA traits [4, 23, 24]. Actually, thyroid hormones also play a crucial role in regulating animal growth, development and metabolism besides fat development [25, 26].

In this study, we identified 4 SNPs in the 5′ flanking region of *TG* gene by sequencing, constructed the haplotypes and evaluated haplotype effects on growth, carcass composition and meat quality traits in Chinese steers. There was not significant association between MS and SNPs or haplotype. However, we found that 4 SNPs had a significant association with ADG. Although haplotype

effects were not significant, H3 and H1 showed opposite trend. The inconsistent result between SNPs and haplotype might be because of the (1) low haplotype frequencies, (2) the limited sample size, (3) interference of the adjacent SNPs effects and (4) population structure such as loss of haplotype combination H2H3. Also, the potential TFBS had been predicted in our study, but whether the TFBS had effects on *TG* gene expression is needed to be verified by experiments since the false positive rate is high in TFBS prediction.

Conclusion

In conclusion, our results provide evidence that the SNPs in the 5′ flanking region of *TG* gene has potential effects on ADG. Results from association analysis provided preliminary evidence that the bovine *TG* gene could be used as a candidate gene or molecular marker for the improvement of bovine growth traits in Chinese commercial cattle. However, further work is necessary to test these SNPs in larger population and clarify the effects of *TG* gene on cattle growth traits.

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