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Association between Single Nucleotide Polymorphisms in the Dgat2 Gene and Beef Carcass and Quality Traits in Commercial Feedlot Steers*

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ABSTRACT : Diacylglycerol acyltransferase (DGAT) is a key enzyme that catalyzes the final and rate-limiting step of triglyceride synthesis. Both DGAT1 and DGAT2 genes code proteins with DGAT activity. Studies have shown DGAT1 polymorphisms associate with intramuscular fat deposition in beef cattle, but fewer associations between DGAT2 and beef cattle economic traits have been reported. The objective of this study was to investigate single nucleotide polymorphism (SNP) in intron3 of bovine DGAT2 and evaluate the associations of that with carcass, meat quality, and fat yield traits. Test animals were 157 commercial feedlot steers belonging to 3 Chinese native breeds (22 for Luxi, 24 for Jinnan, and 23 for Qinchuan), 3 cross populations (20 for Charolais×Fuzhou, 18 for Limousin ×Luxi, and 17 for Simmental×Jinan) and 1 Taurus pure breed population (16 Angus steers). In the current study, 15 SNP were discovered in intron3 and exon4 of DGAT2 at positions 65, 128, 178, 210, 241, 255, 270, 312, 328, 334, 365, 366, 371, 415, and 437 (named as their positions in PCR amplified fragments). Only 7 of them (128, 178, 241, 270, 312, 328, and 371) were analyzed, because SNP in three groups (65-128-255, 178-210-365 and 241-334-366) were in complete linkage disequilibrium within the group, and SNP 415 was a deletion and 437 was a null mutation. Frequencies for rare alleles in the 3 native breed populations were higher than in the 3 cross populations for 178 ($p = 0.04$), 270 ($p = 0.001$), 312 ($p = 0.03$) and 371 ($p = 0.002$). A general linear model was used to evaluate the associations between either SNP genotypes or allele substitutions and the measured traits. Results showed that SNP 270 had a significant association with the fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach (KPHISY). Animals with genotype CC and CT for 270 had less (CC: -7.71±3.3 kg and CT: -5.34±2.5 kg) KPHISY than animals with genotype TT ($p = 0.02$). Allele C for 270 was associated with an increase of -4.26±1.52 kg KPHISY ($p = 0.006$) and -0.92±0.45% of retail cuts weight percentage (NMP, Retail cuts weight/slaughter body weight) ($p = 0.045$); allele G for 312 was associated with an increase of -5.45±2.41 kg KPHISY ($p = 0.026$). An initial conclusion was that associations do exist between DGAT2 gene and carcass fat traits. Because of the small sample size of this study, it is proposed that further effort is required to validate these findings in larger populations. (**Key Words :** DGAT2 Gene, SNP, Carcass Fat Traits, Feedlot Cattle)

INTRODUCTION

Triglycerides are the major energy storage molecules in eukaryotes. The final, and presumably rate-limiting, step of triglyceride synthesis is catalyzed by a diacylglycerol acyltransferase (DGAT) (Mayorek et al., 1989). DGAT1 (EC 2.3.1.20) was the first identified gene encoding a protein with DGAT activity (Cases et al., 1998). Further research indicated that DGAT1 was not the only gene coding DGAT (Smith et al., 2000). This led to DGAT2 being certified as a new gene family that also controls triglyceride synthesis (Cases et al., 2001; Lardizabal et al., 2001). Then, DGAT1 and DGAT2 were demonstrated as two unrelated proteins that exhibited DGAT activity through different pathways, but the DGAT1 gene had the highest expression level in the small intestine, whereas the DGAT2 gene had high expression levels in the liver and white adipose tissue (Cases et al., 2001; Yamazaki et al., 2005). The DGAT2 (GenBank Accession No. AJ519787) gene was mapped to bovine chromosome (BTA) 15q25-q26, and 22 single nucleotide polymorphisms (SNP) in DGAT2

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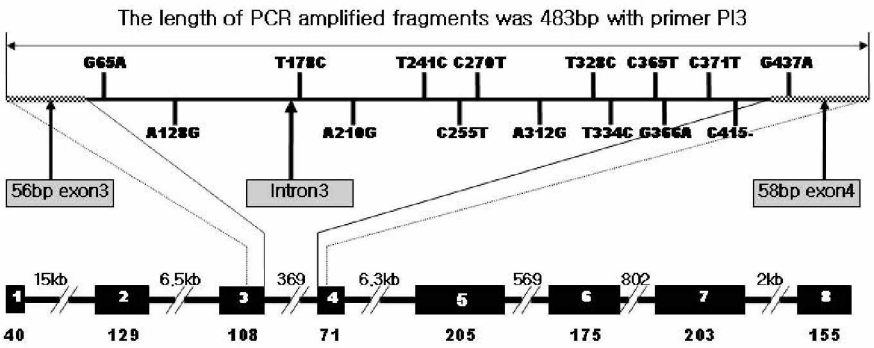

Figure 1. Exon/intron structure of bovine *DGAT2* gene and single nucleotide polymorphisms (SNP) in the last 56 bp of exon3, Intron3, and the first 58 bp of exon4 of the bovine *DGAT2* gene. Black boxes represent exons with the number (white color) in the boxes and the sizes in bp under the boxes. The sizes of the introns, in bp (or in kb if indicated), are placed above the lower horizontal line (The lower part of this figure was from Winter et al., 2003a). Vertical lines, crossing the upper horizontal line, represent single nucleotide polymorphisms (SNP) positions. The symbols at the top end (or bottom end) of the vertical lines (in the upper part) indicate the SNP names (named as their positions in the PCR amplified fragments) and the 2 corresponding nucleotides substituted for each other.

were detected (Winter et al., 2003a, 2003b). Other studies detected 15 SNP in intron3 of the bovine DGAT2 gene (Figure 1), which was found to be the most abundant polymorphisms among all 7 introns and 8 exons of DGAT2 (Xu et al., 2004). However, several studies have reported associations between DGAT1 and economic traits of cattle (Spelman et al., 2002; Thaller et al., 2003; Winter et al., 2003b). DGAT1 significantly affected intramuscular fat content (IMF) in beef cattle (Thaller et al., 2003) and milk volume, milk protein and milk fat yield in dairy cattle (Spelman et al., 2002). However, there have been fewer reports on associations between DGAT2 and beef cattle traits. The objective of this study was to investigate single nucleotide polymorphisms (SNP) in bovine DGAT2 gene intron3 and evaluate the associations between SNP and slaughter body weight, carcass and meat quality, and especially fat yield traits in commercial feedlot populations of steers.

MATERIALS AND METHODS

Animals and management

For studying associations between gene markers and beef cattle traits, comparing the performance among animals with different genetic backgrounds, and studying the effect of three diets, 196 steers with clear sources were selected in the Dachang (Hebei, China) commercial feedlot. These animals belonged to seven genetic groups (defined as different genetic backgrounds: 3 crossbreeds (Charolais× Fuzhou, Limousin×Luxi, and Simmental×Jinan); 3 Chinese native pure breeds (Luxi, Jinnan, and Qinchuan); and a pure breed (Angus). All crossed animals were F1 calves that came from native breed cows of Fuzhou, Luxi and Jinan sired respectively by bulls of Charolais, Limousin and Simmental. Charolais and Limousin bulls were progenies of pure breed animals imported from France, and Simmental bulls were progenies of pure breed Fleckvieh imported from Germany. 16 Angus steers were progenies of pure breed Angus cattle imported from Australia.

Steers within the same genetic group were arranged randomly into three subgroups corresponding to three kinds of diets (named according to their 3 different components: Rubeite, Rubeiyuan, and Palm oil powder). Because of failed data collection and genotyping, the exact number of animals that were used for allele frequencies analysis was 157 steers whose age ranged from 16 to 26 months and the average age at the start of the experiment was 20.4±3.4 months, as shown in Table 1.

During the test period, all three kinds of diets were mainly composed of 50% corn grain, 18-20% cotton seed cake, 10-11% distiller's grains, 11% wheat bran plus 4-5% vitamins and mineral supplements except for 6% Rubeite (a kind of premixed feed material containing 75% crude protein and with total energy 12.55 MJ/kg), 6% Rubeiyuan (the second kind of premixed feed material with high crude protein content and energy) and 3% Palm oil powder (the third premixed feed material with high energy); these three premixed feed materials (provided by Chinese Agric. Univ.) were added to the feed mix to form the three different diets. These 3 diets had an energy content of 0.85, 0.86, and 0.86 RND (RND = (NEm (Net energy for maintenance)+NEg (Net energy for gain))/8.08) per kilogram, respectively, and 18-20% crude protein. Straw and Silage were provided at an average of 2.5 kg and 10 kg/d per animal.

A pretest adjustment period of 20 d was allowed for the animals to adapt to the new diets and environment. Test animals were raised in a feedlot (Hebei, China) for 201 d (from Oct. 1st, 2002 to Apr. 21st, 2003) including the

Table 1. Number of Animals in test subgroup with corresponding average age in months and standard deviation

Diet	Angus			Limousin ×Luxi			Charolias ×Fuzhou			Simmental ×Jinan			Qinchuan			Luxi			Jinnan			Overall		
	No[a]	age[b]	SD[c]	No[a]	age[b]	SD[c]	No[a]	age[b]	SD[c]	No[a]	age[b]	SD[c]	No[a]	age[b]	SD[c]	No[a]	age[b]	SD[c]	No[a]	age[b]	SD[c]	No[a]	age[b]	SD[c]
Rubeite[d]	6	19	3.5	7	18	1.9	7	18	3.5	9	16	2.1	7	21	2.0	9	20	4.3	7	23	3.2	52	18	3.1
Rubeiyuan[d]	4	19	3.5	5	17	1.0	9	19	2.2	11	18	3.0	7	24	1.6	10	22	4.5	4	24	4.9	50	19	3.2
Palm oil powder[d]	6	18	3.5	9	18	2.2	8	23	4.8	6	19	3.8	10	24	3.6	9	24	2.1	7	24	3.3	55	22	3.5
Genetic group[e]	16	19	3.5	21	18	1.7	24	20	3.5	26	17	3.0	24	23	2.4	28	22	3.6	18	24	3.8	157	20	3.4

[a] The number of animals in corresponding feed subgroups. [b] Means of animal age in months in corresponding subgroups.
[c] Standard deviation of age. [d] Three feed diet subgroups according to their different components: Rubeite, Rubeiyuan and Palm oil powder.
[e] 7 genetic groups: Charolais×Fuzhou, Limousin×Luxi, and Simmental×Jinan,Luxi, Jinnan, and Qinchuan and Angus.

adjustment period of 20 d. In the feedlot, feeding stalls were housed in a shed with the long (north and south) sides closed and the 2 short (east and west) sides open to form a large pen in which animals could move freely. There were 100 feeding stalls set up along each longer side. Every feeding stall was marked with a number corresponding to the animal with the same number. A feeding regime of three times a day was followed during the experiment period. Just before the feeding time, every animal was kept in the feeding stall marked with the same number and released to the pens after feeding. Residual feed intake was daily collected, weighed and recorded.

SNP identification and genotyping

Polymerase Chain Reaction-Single Stranded Conformation Polymorphism (PCR-SSCP) was used to detect single nucleotide polymorphisms in intron3 of the bovine DGAT2 gene (GenBank accession No. AJ519787). One pair of PCR primers (named PI3), Forward: 5'-cat tgc cgt gct cta ctt cac ct-3' and Reverse: 5'-agt ctc gaa agt agc gcc aca ca-3' (http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=46850515), was designed from exon3 and exon4 sequences of the bovine DGAT2 gene.

PCR was performed using 1.0 μg of genomic DNA in a total volume of 15 μl containing the following: 1.5 μl of 10×PCR buffer (98% formamide, 20 mmol EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol), 1.5 μl of dNTPs (2 mM), 1.5 μl of each primer (0.4 μM), 0.75 μl Taq™ DNA polymerase (*TaKaRa*, Dalian, China), 8.75 μl of $2dH_2O$, and 1.0 μl of DNA Template. PCR cycling consisted of an initial 5 min denaturation at 95°C, followed by 34 cycles of 94°C for 30 s, 65°C for 25 s, and 72°C for 1 min. A final extension step at 72°C for 10 min followed the last cycle.

The mixed 1 μl of PCR reaction products and 8 μl of buffer were heated to 95°C for 10 min then placed on ice for 5 min. The sample was electrophoresed in 1×TBE buffer for 18 h at 18 V/cm. After a series of procedures of dying ($AgNO_3$), coloring (2% Na_2CO_3), and coloring stop (4% acetic acid), PCR products were isolated from agarose gel, and purified with a Qiaquick Gel Extraction Kit (BETS&TCL, China). The purified DNA fragments were linked with pGEM-T easy vector (Promega, USA) by T4 DNA ligase (Promega, USA) and transformed into Ecoli DH5α (BETS&TCL, China). Qiaprep Spin Miniprep Kit (BETS&TCL, China) was used for plasmid extraction and plasmid DNA was sequenced by Sangon Biotechnology Company (ShangHai, China).

Phenotypic information

Body weights were measured at the start, middle and end of the test, and all of the animals were harvested on the same day, Apr. 21st 2003, in Huaan commercial abattoir (Hebei, China). Slaughter body weight (SBW) was measured just before slaughter after a 24 h period of fasting. Hot carcass weight (HCW) and the fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach (KPHISY) were measured just after slaughter. Other carcass traits (retail cuts weight (RCW), marbling score (MBS), intramuscular fat percentage (IMF), rib eye area (REA), back fat thickness (BFT) and Warner-Bratzler peak shear force (WBSF)) measurements were carried out at 4 days post-mortem, and the rib area (REA, cm^2) was measured at the 12th and 13th rib interface. Meat samples for measuring WBSF (Warner-Bratzler peak shear force, kg) and intramuscular fat percentage (IMF, %) were also taken from the interface between the 12th and 13th rib. IMF (%) was determined gravimetrically via Soxhlet extraction, using petroleum ether as the solvent. Marbling score evaluation in China is a 6 point regime; according to the average amount, size, and distribution of fat particles or deposit in the rib eye muscle, the numbers 1 to 6 correspond to traces, slight, small, modest, moderate, and abundant. Net meat percentage (NMP) was calculated as the percentage of RCW over slaughter body weight (SBW), i.e., NMP = RCW/SBW×100, and the percentage of the fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach (KPHIS) was calculated as the percentage of KPHISY over hot carcass weight (HCW), i.e., KPHIS = KPHISY/HCW×100.

Record number used for various analyses

The slaughter body weight (SBW), carcass and beef quality records of 157 steers were used for summary

Table 2. Number of phenotypic records on beef cattle slaughter body weight, carcass and meat quality traits with corresponding mean, standard deviation, and coefficient of variation

Trait [a]	No. of records	Mean	SD	CV (%)
SBW (kg)	157	560.1	87.8	15.7
HCW (kg)	157	317.3	52.0	16.4
RCW (kg)	157	249.7	41.6	16.6
NMP (%)	157	44.6	2.8	6.4
MBS	157	2.4	1.2	50.0
IMF (%)	157	5.3	3.3	62.4
REA (cm^2)	157	76.6	14.4	18.8
BFT (cm)	157	1.2	0.5	42.7
WBSF (kg/cm^2)	157	4.8	1.5	30.3
KPHISY (kg)	157	47.4	11.5	24.3
KPHIS (%)	157	15.1	3.6	23.5

[a] Slaughter body weight (SBW), hot carcass weight (HCW), retail cuts weight (RCW), net meat percentage (NMP, NMP = RCW/SBW×100), marbling score (MBS), intramuscular fat percentage (IMF), rib eye area (REA), back fat thickness (BFT), Warner-Bratzler peak shear force (WBSF), fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach (KPHISY), and the percentage of KPHISY over HCW (KPHIS, KPHIS = KPHISY/HCW×100).

statistics. Table 2 presents the number of records, mean, standard deviation (SD), and the coefficients of variation (CV, %) of the analyzed traits. Most of the traits show considerable variation among the animals. These gave desirable ranges for comparing them among animals with different genotypes. The genotype data of the 157 animals was used to determine overall allelic frequencies, and the results are listed in Table 3.

If the number of animals with a given genotype was less than 4, these animals with the given genotype data were excluded from corresponding association analysis. All 16 Angus animals were excluded from any association analysis for having only 1 homozygous genotype (for dominant alleles) for any one of the 7 SNP. Genotype data for SNP 128 was also excluded from association analysis, because the number of animals with genotype AA, AG, and GG were 3, 0, and 154, respectively. Finally, the genotype data of 6 SNP (178, 241, 270, 312, 328, and 371) and the number of trait records, ranging from 139 to 141, were actually used for association analysis.

Statistical analyses

All analyses were performed using the software SAS (Version 9.1, SAS Institute, Inc., Cary, NC). Descriptive characteristics of quantitative traits were obtained using PROC MEANS. Allele frequencies were tabulated and compared by Fisher's Exact Test among genetic groups, and PROC FREQ was used to compute Monte Carlo estimates of the exact p-values. PROC ALLELE was employed to provide tests of linkage disequilibrium between each pair of markers.

Associations between genotype and BSW, HCW, RCW, NMP, MBS, IMF, BFT, REA, WBSF, KPHIS and KPHISY were evaluated using PROC GLM, fitting the following model 1 which included the Contemporary group, known

Table 3. Allele frequencies within genetic group, combined genetic group and in entire beef steer population for 7 SNP in intron3 of the DGAT2 gene

SNP[1]	Allele	Genetic group							Combined genetic group[2]			Overall
		Angus	Limousin ×Luxi	Charolias ×Fuzhou	Simmental ×Jinan	Qinchuan	Luxi	Jinnan	Angus	Crossed	Native	
128	A (%)	0^{cd}	0^{cd}	4.2^{cd}	0^{bd}	8.3^{ac}	0^{bd}	0^{cd}	0	1.4	2.9	1.9
	G (%)	100	100	95.8	100	91.7	100	100	100	98.6	97.1	98.1
178	C (%)	0	0	0	1.9	0	8.9	5.6	0^{CD}	0.7^{AC}	5.0^{BD}	2.6
	T (%)	100	100	100	98.1	100	91.1	94.4	100	99.3	95.0	97.5
241	C (%)	0^{cd}	0^{bc}	0^{bc}	3.9^{cd}	0^{bc}	5.4^{cd}	11.1^{ad}	0	1.4	5.0	2.9
	T (%)	100	100	100	96.2	100	94.6	88.9	100	98.6	95.0	97.1
270	C (%)	0^{bd}	7.1^{dgh}	4.2^{bdf}	11.5^{de}	18.8^{cefh}	39.3^{a}	22.2^{aeg}	0^{A}	7.7^{A}	27.9^{B}	15.9
	T (%)	100	92.9	95.8	88.5	81.3	60.7	77.8	100	92.3	72.1	84.1
312	A (%)	100	100	100	94.2	100	91.1	83.3	100	97.9	92.1	95.5
	G (%)	0^{bc}	0^{bc}	0^{bc}	5.8^{cd}	0^{bc}	8.9^{cd}	16.7^{ad}	0^{CD}	2.1^{AC}	7.9^{BD}	4.5
328	C (%)	0	0	0	1.9	0	7.1	5.6	0	0.7	4.3	2.2
	T (%)	100	100	100	98.1	100	92.9	94.4	100	99.3	95.7	97.8
371	C (%)	100	92.9	10	94.2	89.6	75.0	94.4	100	95.8	85.0	91.4
	T (%)	0^{bc}	7.1^{bc}	0^{bc}	5.8^{bc}	10.4^{ac}	25.0^{a}	5.6^{bc}	0^{A}	4.2^{A}	15.0^{B}	8.6
No.[3]		16	21	24	26	24	28	18	16	71	70	157

[a, b, c, d, e, f, g, h] Within a row (for genetic groups), allele frequencies without a common superscript letter differ, (Pr ($\leq P_{table}$)≤0.05, Fisher's Exact Test).

[A, B, C] Within a row (for combined genetic groups), allele frequencies without a common superscript letter differ, (Pr($\leq P_{table}$)≤0.05, Fisher's Exact Test).

[1] SNP in intron3 of DGAT2 gene according to their positions 128, 178, 241, 270, 312, 328, and 371 in PCR amplified fragments.

[2] Angus, crossed and native represent pure breed Angus, combined cross line population (Limousin×Luxi+Charolais×Fuzhou+Simmental×Jinan) and combined native pure breed population (Qinchuan+Luxi+Jinnan), respectively.

[3] Number of animals.

SNP genotypes and animal age as fixed effects.

$$Y_{ijk}= CG_i+Genotype_j+age+e_{ijk} \quad \text{(model 1)}$$

where Y_{ijk} was the trait measured in the animal *k* of the Contemporary group *i*, with DAGT2 SNP genotype j; age (animal's age in months on the experiment starting day) was a linear covariant in the model to account for its effect on the trait; and e_{ijk} was the residual random effect associated with animal k. Contemporary groups (CG) were defined as animals from the same source (3 Chinese native breed herds and 3 cross herds) with the same feed diet treatment. Therefore, a total of 18 (6×3) contemporary groups (CG) were defined. The number of animals in each contemporary group, ranging from 4 to 11, is shown in Table 1, and the range of average age of animals in each contemporary group was from 16 to 24 months.

In order to estimate average allele substitution effects, SAS PROC GLM was also employed, fitting the following model 2:

$$Y_{ij} = CG_i+Allele\_sub+age+e_{ij} \quad \text{(model 2)}$$

In model 2, Y_{ij} was the trait measured in animal j of Contemporary group i, and e_{ij} was the residual random effect associated with animal j. Contemporary group (CG) and age were the same as defined in model 1; *Allele_sub* was the number of one of the two alleles (0, 1, or 2) at the same locus and was treated as a linear covariant.

Model 3 was used to detect if feed treatments had significant effects on these measured traits, and PROC GLM was employed again. In model 3, Y_{ijkl} was the trait measured of animal l of diet i, breed j, with genotype k, and e_{ijkl} was the residual random effect associated with animal l. The fixed effect feed was 3 diets, i = 1,2,3. Breed was genetic groups, j = 1, 2, …,6,7. Genotype and age were the same as defined in model 1.

$$Y_{ijkl} = Feed_i+Breed_j+Genotype_k+age+e_{ijkl} \quad \text{(model 3)}$$

To keep reasonable probability values for Type I errors, 2 levels of tests were performed. A modified Bonferroni correction was used ($\alpha/\sqrt{n}$; Mantel, 1980) to account for the number of tests with an overall value of α = 0.05. The value of n was determined using a SNP-wise approach and equaled the number of tested genes multiplied by the number of traits within trait type. Traits were separated into 2 groups according to type as follows: yield traits (SBW, HCW, RCW, NMP, REA, BFT and KPHISY) and meat quality traits (MBS, IMF, WBSF and KPHIS). So n was equal to 42 (6×7, the number of tested genes was 6 because SNP 128 was excluded from analysis) and 24 (6×4) for yield and meat quality traits, respectively, with corresponding modified Bonferroni significant levels of 0.008 and 0.010. On the other side, to keep reasonable probability values for Type II errors, for fixed model, power was computed with the Stroup (1999) procedure which requires specification of residual variance components, probability of type I error (α) and an effect size in actual units. Here α = 0.05, residual variance component estimates and the allele substitution effect sizes were from the Proc GLM analyses of the real data.

RESULTS

Allele frequencies

PCR products were 483-bp fragments of bovine genomic DNA. One of these fragments contained the last 56 bp of exon3, intron3, and the first 58 bp of exon4 of the DGAT2 gene (Figure 1). 157 animals were genotyped and 15 new single nucleotide polymorphisms (named for their positions in PCR amplified fragments) were detected by SSCP (single-stranded conformation polymorphism). SNP positions (and corresponding nucleotide substitutions) were 65 bp (guanine-adenine, G-A), 128bp (A-G), 178 bp (thymine-cytosine, T-C), 210 bp (A-G), 241 bp (T-C), 255 bp (C-T), 270 bp (C-T), 312 bp (A-G), 328 bp (T-C), 334 bp (T-C), 365 bp (C-T), 366 bp (G-A), 371 bp (C-T), 415 bp (C-.) and 437 bp (G-A). Of them, the mutation that happened at 415 bp was a deletion and that at 437 bp occurred in a coding region (exon4). The latter mutation, being of agg→aga, was a null mutation without a change of amino acid. The remaining 13 SNP were divided into 4 groups: group1 (65, 128 and 255), group2 (178, 210, and 365), group3 (241, 334, and 366) and group4 (312, 328, 334, and 371). SNP in the first 3 groups were in complete linkage disequilibrium within their corresponding groups. So, we focused on only 7 (128, 178, 241, 270, 312, 328, and 371) of them and analyzed their allelic frequencies.

Fisher's exact test results showed significant differences for allele frequencies between pair-wise genetic groups for SNP 128, 241, 270, 312, and 371 (Table 3), but there was no significant difference among genetic groups for SNP 178 and 328 allele frequencies. Considering all genotyped cattle, alleles A of 128, C of 178, C of 241, G of 312, and C of 328 were very rare, and their frequencies were less than or equal to 5% in the overall population, whereas allele C of 270 and T of 371 had lower respective frequencies of 15.9% and 8.6%. Three native breeds (Jinnan, Luxi, and Qinchuan) and the Simmental×Jinan population tended to have higher frequencies for those rare alleles than those of the other three populations (Charolais×Fuzhou, Limousin×Luxi, and Angus). For genotyped Angus steers, neither homozygous nor heterozygous genotype individuals for those rare alleles were found for all 7 SNP genotypes.

For SNP 128, the native breed Qinchuan had a higher

frequency of the A allele than Simmental×Jinan and Luxi (8.3 vs. 0%, p = 0.05 and 8.3 vs. 0%, p = 0.04 respectively), but no significant difference was detected between Qinchuan or Simmental×Jinan or Luxi and any of the other 4 genetic groups (Angus, Charolais×Fuzhou, Limousin× Luxi and Jinnan) for A allele frequencies (0.12≤p≤1.00). For SNP 178, allele frequencies were not different among the 7 genetic groups at a significance level of 0.05, but the native breed Luxi tended to have a higher C allele frequency than that of Limousin×Luxi, Charolais×Fuzhou, and Qinchuan (8.9 vs. 0.0%, p = 0.07, 0.06, and 0.06, respectively). For SNP 241, Jinnan had a higher frequency of the C allele than Limousin×Luxi, Charolais×Fuzhou, and Qinchuan (11.1 vs. 0.0%, p = 0.04, 0.03 and 0.03, respectively), while C allele frequencies were not significantly different between Jinnan and Angus, Simmental×Jinan and Luxi (0.11≤p≤1.00). In addition, no significantly different C allele frequencies for SNP 241 were detected between any two genetic groups for Angus, Limousin×Luxi, Charolais×Fuzhou, Simmental×Jinan, Qinchuan and Luxi (0.25≤p≤1.00). For SNP 270, Luxi had a higher frequency of the C allele than Angus, Limousin×Luxi, Charolais×Fuzhou, Simmental×Jinan, and Qinchuan (39.3 vs. 0.0%, p = 0.001; 39.3 vs. 7.1%, p = 0.001; 39.3 vs. 4.2%, p = 0.001; 39.3 vs. 11.5%, p = 0.001; and 39.3 vs. 18.8%, p = 0.03, respectively), and it was not significantly different from that of Jinnan (39.3 vs. 22.2%, p = 0.11). Allele C frequencies for SNP 270 in both Jinnan and Qinchuan were higher than in Angus (22.2 vs. 0.0%, p = 0.01 and 18.8 vs. 0.0%, p = 0.01, respectively), and Allele C frequency in Jinnan was higher than that in Charolais× Fuzhou (39.3 vs. 4.2%, p = 0.02). Qinchuan tended to have a higher frequency of the C allele than Charolais×Fuzhou (18.8 vs. 4.2%, p = 0.051). Allele C frequencies among the 4 genetic groups with European breed composition (Angus, Limousin×Luxi, Charolais×Fuzhou, and Simmental×Jinan) were not significantly different (0.08≤p≤0.73), and they were also not significantly different between Jinnan and Limousin×Luxi (22.2 vs. 7.1%, p = 0.10), Jinnan and Simmental×Jinan (22.2 vs. 11.5%, p = 0.24), Jinnan and Qinchuan (22.2 vs. 18.8%, p = 0.79), Qinchuan and Limousin×Luxi (18.8 vs. 7.1%, p = 0.13), or Qinchuan and Simmental×Jinan (18.8 vs. 11.5%, p = 0.40). For SNP 312, Jinnan had a higher frequency for the G allele than Angus, Limousin×Luxi, Charolais×Fuzhou, and Qinchuan (16.7 vs. 0.0% at p = 0.03, 0.01, 0.005, and 0.005, respectively), and G allele frequency of Jinnan was not significantly different from that of Simmental×Jinan (16.7 vs. 5.8%, p = 0.15) and Luxi (16.7 vs. 8.9%, p = 0.33). G allele frequencies of SNP 312 among the 4 genetic groups Angus, Limousin×Luxi, Charolais×Fuzhou and Qinchuan were not significantly different (0.06≤p≤1.00). For SNP 328, Allele frequencies were not significantly different among all the 7 genetic groups (0.12≤p≤1.00). For SNP 371, Luxi had a higher frequency for allele T than Angus, Limousin×Luxi, Charolais×Fuzhou, and Simmental×Jinan and Jinnan (25.0 vs. 0.0%, p = 0.002; 25.0 vs. 7.1%, p = 0.03; 25.0 vs. 0.0%, p = 0.001; 25.0 vs. 5.8%, p = 0.01 and 25.0 vs. 5.6%, p = 0.02, respectively), and tended to have a higher frequency of the T allele than Qinchuan (25.0 vs. 10.4%, p = 0.07). T allele frequencies among 6 genetic groups (Angus, Limousin×Luxi, Charolais×Fuzhou, Simmental×Jinan, Qinchuan, and Jinnan) were not significantly different (0.08 ≤p≤1.00).

Allele frequencies of 7 SNP in 3 combined populations, i.e., pure breed Angus, the combined cross breed population (Limousin×Luxi+Charolais×Fuzhou+Simmental×Jinan) and the combined native pure breed (Qinchuan+Luxi+Jinnan), were also compared by Fisher's Exact Test. Allele frequencies were not significantly different between Angus and the combined cross populations for all 7 discovered SNP (0.22≤p≤1.00). For SNP 128, 241 and 328, allele frequencies were not significantly different among the 3 combined sample populations (0.07≤p≤1.00). The combined native pure breed population had higher allele frequencies of C for 178, C for 270, G for 312, and T for 371 than in the combined cross herd (5.0 vs. 0.7%, p = 0.04; 27.9 vs. 7.7%, p = 0.01; 7.9 vs. 2.1%, p = 0.03 and 15.0 vs. 4.2%, p = 0.01; respectively). Allele frequencies of C for 178 and G for 312 were not significantly different between Angus and the combined native pure breed (5.0 vs. 0.0%, p = 0.35 and 7.9 vs. 0.0%, p = 0.22; respectively), whereas allele frequencies of C for 270 and T for 371 in the combined native pure breed population were higher than in Angus (27.9 vs. 0.0%, p = 0.001; and 15.0 vs. 0.0%, p = 0.001; respectively).

The frequencies of genotypes were not in agreement with the Hardy-Weinberg equilibrium for SNP 128, 178, 241, 270, 312, and 328 (the probabilities of the x^2 tests for deviation from the equilibrium were less than or equal to 0.001, 0.004, 0.001, 0.001, 0.001, and 0.001, respectively), whereas genotype frequency for SNP 371 was in agreement with the Hardy-Weinberg equilibrium (the probabilities of the x^2 tests for deviation from the equilibrium were equal to 0.39).

Equilibrium in genotypic frequencies when considering jointly two SNP was tested by the x^2 test of expected and observed frequencies of gametic types. SNP 270 was in significant linkage disequilibrium with any single SNP of the other 6 SNP in DGAT2 Intron3 (4.5e-23≤p≤5.7e-07, data not shown). The other 4 SNP-wise tests (178 and 312, 178 and 328, 241 and 312, 312 and 328) showed significant linkage disequilibrium (p<0.0001). All other SNP-wise tests, except the above 10 pair-wise tests, showed linkage equilibrium between the two pair-wise SNP (p≥0.10).

Table 4. Association of individual single nucleotide polymorphism genotype in intron3 of DGAT2 gene with slaughter body weight, carcass and meat quality traits in commercial beef steer population

Item	SNP in intron3 of DGAT2 gene					
	178[1]	241[1]	270[1]	312[1]	328[1]	371[1]
	-	CC: 4	CC: 11	AA: 131	-	CC: 116
	CT: 6	-	CT: 28	AG: 6	CT: 5	CT: 23
	TT: 134	TT: 136	TT: 102	GG: 4	TT: 135	-
p>F[2]						
SBW[3] (kg)	0.36	0.12	0.04*	0.16	0.28	0.17
HCW[3] (kg)	0.52	0.35	0.12	0.50	0.44	0.15
RCW[3] (kg)	0.50	0.35	0.41	0.48	0.40	0.46
NMP[3] (%)	0.84	0.35	0.13	0.63	0.89	0.30
MBS[3]	0.94	0.76	0.91	0.96	0.82	0.84
IMF[3] (%)	0.97	0.98	0.18	0.99	0.26	0.21
REA[3] (cm^2)	0.61	0.82	0.34	0.86	0.97	0.17
BFT[3] (cm)	0.30	0.21	0.93	0.21	0.06[1]	0.27
WBSF[3] (kg/cm^2)	0.47	0.57	0.87	0.63	0.30	0.95
KPHISY[3] (kg)	0.31	0.07**	0.02*	0.08**	0.09**	0.30
KPHIS[3] (%)	0.63	0.19	0.13	0.33	0.24	0.74

[1] SNP in intron3 of DGAT2 named as their positions in PCR amplified fragments.

[2] Significance levels of the Wald *F*-test for genotype effect on quantitative traits.

[3] Slaughter body weight (SBW), hot carcass weight (HCW), retail cuts weight (RCW), net meat percentage (NMP, RCW/SBW×100), marbling score (MBS), intramuscular fat percentage (IMF), rib eye area (REA), back fat thickness (BFT), Warner-Bratzler peak shear force (WBSF), fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach (KPHISY), and the Percentage of KPHISY over HCW (KPHIS, KPHIS = KPHISY/HCW×100).

* Significance level, $p<0.05$. ** Significance level, $p<0.10$.

' Genotypes CC for SNP 178, CT for SNP 241, CC for SNP 328 and TT for SNP 371, having only 1, 1, 1, and 2 animals, respectively, were excluded from corresponding analysis.

Association analysis

Association analysis results showed that genotypes of SNP 270 significantly influenced slaughter body weight (SBW) and the fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach (KPHISY, Table 4). Allele substitution effect analysis (allele additive effect) showed that allele substitution of SNP 270 had significant effects on SBW ($p = 0.015$), net meat percentage (NMP, $p = 0.045$), KPHISY ($p = 0.006$) and KPHIS ($p = 0.042$), and that of SNP 312 had a significant effect on KPHISY ($p = 0.026$) (Table 5). Allele substitutions of SNP 241 and 328 tended to associate with KPHISY ($p = 0.07$ and $p = 0.09$, respectively), and that of 312 had a trend to associate with BSW ($p = 0.06$).

Genotype effect analysis : Statistics on SNP 270 indicated that animals with genotype TT had higher KPHISY (49.2±1.0 kg) than animals with genotype CC (41.5±3.2 kg) and CT (43.8±2.2 kg) ($p = 0.02$). Least square means (LSM) difference between genotype CC and TT, CT and TT were 7.7 kg and 5.4 kg, respectively, which corresponded to 0.66 and 0.46 times phenotype SD. No significant difference for KPHISY existed between genotypes CC and CT LSM. This might be due to the dominance effect of the C allele over allele T. Animals with genotype TT for SNP 270 also had higher slaughter body weight (SBW) (568.2±6.3 kg) than animals with CC (529.3 ±19.1 kg) and CT (536.5±12.8 kg) ($p = 0.04$), and there was no significant difference in SBW among animals with genotype CC and CT.

For SNP 241, 312 and 328, there was a series trend of associations between genotypes and the fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach(KPHISY, $p = 0.07$, 0.08, and 0.09, respectively). As shown in Table 4, SNP 241 genotypes (CC and TT, only 1 animal having genotype CT was excluded from analysis) least square means (LSM) for KPHISY were 37.8±5.3 kg and 47.8±0.9 kg; that of 3 SNP 312 genotypes were AA: 48.2±0.9 kg, AG: 42.5±4.76 kg and GG: 37.4±5.3 kg; and corresponding LSM of KPHISY for SNP 328 genotypes CT and TT were 39.0±5.1 kg and 47.9±0.9 kg, respectively (only 1 animal having genotype CC was excluded from analysis).

For SNP 178 and 371, individual genotype did not have a trend of association with any trait measured in the current study ($0.15 \leq p \leq 0.97$).

These genotype association analysis results indicated that animals with SNP 270 genotype CC or CT would have lower KPHISY and SBW than animals with homozygous TT; animals with genotype CT for SNP 328 would have less BFT and KPHISY than animals with TT. Compared with animals with genotype TT for SNP 241 and AA for 312, animals with genotype CC for SNP 241 and GG for SNP 312 would be expected to produce 10.0 kg and 10.8 kg less of KPHISY, respectively.

Allele substitution effect analysis : Table 5 shows results of the average allele substitution effect analysis. Allele C

Table 5. Associations between average allele substitution at given loci in intron3 of DGAT2 gene with slaughter body weight, carcass, and meat quality traits in commercial beef steer population

Item	SNP in intron3 of DGAT2 gene					
	178[1] C[2] 140[3]	241[1] C[2] 140[3]	270[1] C[2] 141[3]	312[1] G[2] 141[3]	328[1] C[2] 140[3]	371[1] A[2] 139[3]
p>F[a]						
SBW[b] (kg)	0.36	0.12	0.015*	0.06**	0.28	0.17
HCW[b] (kg)	0.52	0.35	0.07**	0.24	0.44	0.15
RCW[b] (kg)	0.50	0.35	0.22	0.23	0.40	0.46
NMP[b] (%)	0.84	0.35	0.045*	0.35	0.89	0.30
MBS[b]	0.94	0.76	0.70	0.82	0.82	0.84
IMF[b] (%)	0.97	0.98	0.67	0.89	0.26	0.21
REA[b] (cm^2)	0.61	0.82	0.29	0.67	0.97	0.17
BFT[b] (cm)	0.30	0.21	0.70	0.08**	0.06**	0.27
WBSF[b] (kg/cm^2)	0.94	0.76	0.70	0.82	0.82	0.84
KPHISY[b] (kg)	0.31	0.07†	0.006***	0.026*	0.09**	0.30
KPHIS[b] (%)	0.63	0.19	0.042*	0.14	0.24	0.74

[a] Significant level of the Wald F-test for the effect of allelic substitution on carcass and meat quality traits.

[b] Slaughter body weight (SBW), hot carcass weight (HCW), retail cuts weight (RCW), net meat percentage (NMP, RCW/SBW×100), marbling score (MBS), intramuscular fat percentage (IMF), rib eye area (REA), back fat thickness (BFT), Warner-Bratzler peak shear force (WBSF), fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach (KPHISY), and the Percentage of KPHISY over HCW (KPHIS, KPHIS = KPHISY/HCW×100).

[1] SNP positions in PCR amplified fragments of intron3 of DGAT2. [2] The number of shown allele treated as linear covariant.

[3] The number of records used for corresponding analyses. * Significance level, $p<0.05$. ** Significance level, $p<0.10$.

*** Significant effect after modified Bonferroni correction for multiple tests, $p = 0.05$.

for SNP 270 significantly associated with lower SBW, KPHISY, and KPHIS (the percentage of KPHISY over hot carcass weight) ($p = 0.015$, 0.006, and 0.042, respectively), and higher NMP ($p = 0.045$). Allele A for SNP 312 had a significant association with higher KPHISY ($p = 0.026$), and had a trend to associate with higher SBW ($p = 0.06$) and less BFT ($p = 0.08$). The average allele substitution effects, for SNP 178, 241, 328 and 371, were not significantly associated with any trait studied in this investigation ($0.06 \le p \le 0.97$).

Statistics on allele substitution effect with $p<0.10$ are listed in Table 6. Allele C for SNP 270 associated with a SBW increase of -22.65±9.19 kg ($p = 0.02$), a NMP increase of 0.8±0.40% ($p = 0.045$), a KPHISY increase of -4.26±1.52 kg ($p = 0.006$), a KPHIS increase of -0.92± 0.45% ($p = 0.042$), and tended to associate with a HCW increase of -10.32±5.71 kg ($p = 0.07$).

Allele G for SNP 312 associated with a KPHISY

Table 6. Significant ($p<0.10$) associations between single nucleotide polymorphism genotypes of Dgat2 gene intron3 (with corresponding least square means±SE) and beef cattle slaughter body weight, carcass, and meat quality traits and contrast tests of genotype effect

Trait[1]	SNP[2]	Least square mean			F	Pr>F
SBW (kg)	270	CC: 529.3±19.1[a]	CT: 536.5±12.8[a]	TT: 568.2±6.3[b]		
BFT (cm)	328	-	CT: 0.79±0.22	TT: 1.22±0.04		
KPHISY (kg)	241	CC: 37.8±5.3	-	TT: 47.8±0.9		
KPHISY (kg)	270	CC: 41.5±3.2[a]	CT: 43.8±2.1[a]	TT: 49.2±1.0[b]		
KPHISY (kg)	312	AA: 48.2±0.9	AG: 42.5±4.76	GG: 37.4±5.3		
KPHISY (kg)	328	-	CT: 39.0±5.1	TT: 47.9±0.9		
Contrast of genotype effect						
SBW (kg)	270	CC: -38.82±20.3[a]	CT: -31.62±14.9	TT: 0	3.3	0.04*
BFT (cm)	328	-	CT: -0.43±0.23	TT: 0	3.6	0.06**
KPHISY (kg)	241	CC: -9.99±5.4	-	TT: 0	3.2	0.07**
KPHISY (kg)	270	CC: -7.71±3.3	CT:-5.34±2.5	TT: 0	4.0	0.02*
KPHISY (kg)	312	AA: 10.79±5.4	AG: 5.15±6.9	GG: 0	2.4	0.08**
KPHISY (kg)	328	-	CT: -8.88±5.2	TT: 0	2.7	0.09**

[a, b] Within a row, least square means with different superscript letter differ, $p \le 0.05$.

[1] Slaughter body weight (SBW), back fat thickness (BFT), fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach (KPHISY).

[2] SNP positions in PCR amplified fragments of intron3 of DGAT2.

* Significance level, $p \le 0.05$. ** Significance level, $p \le 0.10$.

- The observation number of corresponding genotypes was less than 4 and was excluded from the analysis.

Table 7. List of significant (p<0.10) associations between the copy number of 1 allele of the 2 for the same SNP locus and beef cattle slaughter body weight, carcass and meat quality traits and corresponding regression coefficients

Trait[1]	SNP[2]	Allele[3]	Regression coefficient±SE	F	Pr>F
SBW (kg)	270	C	-22.65±9.19	6.1	0.015*
SBW (kg)	312	G	-28.03±14.60	3.7	0.057**
HCW (kg)	270	C	-10.32±5.71	3.3	0.073**
BFT (cm)	312	G	-0.19±0.11	3.1	0.082**
BFT (cm)	328	C	-0.43±0.23	3.6	0.060**
NMP (%)	270	C	0.80±0.40	4.0	0.045*
KPHISY (kg)	241	C	-5.00±2.69	3.4	0.066**
KPHISY (kg)	270	C	-4.26±1.52	7.8	0.006***
KPHISY (kg)	312	G	-5.45±2.41	4.8	0.026*
KPHISY (kg)	328	C	-8.88±5.24	2.9	0.093**
KPHIS (%)	270	C	-0.92±0.45	4.2	0.042*

[1] Slaughter body weight (SBW), Hot carcass weight (HCW), net meat percentage (NMP, Retail cuts weight/Live body weight), back fat thickness (BFT), fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach (KPHISY), and KPHIS (the percentage of KPHISY over HCW, KPHIS, KPHIS = KPHISY/HCW×100).

[2] SNP positions in PCR amplified fragments of intron3 of DGAT2 gene. [3] The number of given allele treated as covariant.

* Significance level, p≤0.05. ** Significance level, p≤0.10. *** Significant effect after modified Bonferroni correction for multiple tests, p = 0.05.

increase of -5.45±2.41 kg (p = 0.026), and had a trend to associate with a SBW increase of -28.03±14.60 kg (p = 0.06) and a BFT increase of 0.19±0.11 cm (p = 0.082).

The average allele substitutions for SNP 178, 241, 328 and 371 were not significantly associated with any trait (0.06≤p≤0.97), but there were trends of association between allele C substitution for SNP 328 and BFT increase of -0.43±0.23 cm (p = 0.06), and KPHISY increase of -8.88± 5.24 kg (p = 0.093). Substitution for allele C for SNP 241 tended to associate with a KPHISY increase of -5.00±2.69 kg (p = 0.066).

Considering the modified Bonferroni correction for multiple tests, the allele substitution effect for SNP 270 on the fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach (KPHISY) was still significant, which was not the case for the other 5 SNP in intron3 of the DGAT2 gene. This shows strong evidence for the association between 270 allele substitution effect and KPHISY.

Comparing analysis results between different models (genotype model and allele substitution model), the estimated genotype effect values were almost completely in agreement with average allele substitution effect values (Tables 6 and 7). For SNP 270, the estimated genotype values (for KPHISY) of CC and CT contrasting to TT were -7.71±3.3 kg and -5.34±2.5 kg, respectively; thus, the average C allele effect value was -4.35 kg ((-7.71 kg-5.34 kg)/3), which was almost equal to the average allele substitution effect value -4.26 kg estimated with the linear additive model (Model 2). Similar cases existed between SNP 270 and SBW, SNP 328 and BFT, SNP 241 or 312 and KPHISY.

Table 8. F test of diet effect on slaughter body weight, carcass and meat quality traits in commercial beef steer population with model 3

Item	SNP in intron3 of DGAT2 gene					
	178[1]	241[1]	270[1]	312[1]	328[1]	371[1]
p>F[2]						
SBW[3] (kg)	0.51	0.44	0.61	0.58	0.51	0.42
HCW[3] (kg)	0.49	0.41	0.58	0.52	0.49	0.43
RCW[3] (kg)	0.84	0.73	0.82	0.88	0.85	0.70
NMP[3] (%)	0.66	0.73	0.83	0.69	0.65	0.75
MBS[3]	0.41	0.42	0.39	0.41	0.41	0.40
IMF[3] (%)	0.95	0.58	0.87	0.85	0.99	0.69
REA[3] (cm^2)	0.84	0.83	0.87	0.83	0.85	0.86
BFT[3] (cm)	0.58	0.66	0.78	0.50	0.50	0.74
WBSF[3] (kg/cm^2)	0.152	0.206	0.188	0.161	0.143	0.213
KPHISY[3] (kg)	0.91	0.92	0.79	0.86	0.90	0.89
KPHIS[3] (%)	0.73	0.74	0.61	0.66	0.70	0.74

[1] SNP positions in PCR amplified fragments of intron3 of DGAT2 gene. [2] Significance levels of the Wald *F*-test for diet effect on quantitative traits.

[3] Slaughter body weight (SBW), hot carcass weight (HCW), retail cuts weight (RCW), net meat percentage (NMP, RCW/SBW×100), marbling score (MBS), intramuscular fat percentage (IMF), rib eye area (REA), back fat thickness (BFT), Warner-Bratzler peak shear force (WBSF), fat yield associated with kidney, pelvic cavity, heart, intestine, and stomach (KPHISY), and the Percentage of KPHISY over HCW (KPHIS, KPHIS = KPHISY/HCW×100).

Diet effect analysis

Table 8 shows that the type of diet had no significant effects on either yield traits or meat quality traits. All the statistical p values were more than 0.14, because the 3 diets had similar energy and protein concentrations, and these animals were fed in the same environment, although some components differed from each other.

DISCUSSION

The present study discovered 14 new bi-allelic single nucleotide substitutions (named as their positions in PCR amplified fragments) in intron3 of the bovine DGAT2 gene (according to GenBank Accession No. AY589091) and 1 single nucleotide substitution in exon4. To date, intron3 has been found to be the most abundant in single nucleotide polymorphisms among introns of the bovine DGAT2 gene. There were 1, 3, 12 and 6 single nucleotide polymorphisms in intron 4, 5, 6 and 7, respectively (Winter et al., 2003a). Our analyses revealed that intron3 SNPs in 3 groups (group1: 65, 128 and 255; group2: 178, 210 and 365; group3: 241, 334 and 366), were in complete linkage disequilibrium within SNP groups. This is similar to the case of intron6 of DGAT2 in which all 12 SNP were in complete linkage disequilibrium (Winter et al., 2003b). The x^2 tests also show that 270 is in significant linkage disequilibrium with the other 6 SNP (128, 178, 241, 312, 328, and 371) at significance levels 4.5e-23≤p≤5.7e-07 (data not shown), whereas any 2 of the other 6 SNP markers were or were not in significant linkage disequilibrium statistically with x^2 test. The degree of linkage disequilibrium for SNP in intron3 of DGAT2 was different from that for 12 SNP in intron6 of DGAT2 which were inherited in complete linkage disequilibrium (Winter et al., 2003b), but Winter's solution came from a tested population as small as 84 bulls (32 German Holstein, 32 German Simmental and 20 German Brown). These results indicated that SNP 270 in intron3 of DGAT2 can present more genetic information on the DGAT2 gene than the other SNP markers in the same intron.

An important finding of the current study is that the C allele for SNP 270 significantly associated with lower fat yield of kidney, pelvic cavity, heart, intestine, and stomach (KPHISY) and slaughter body weight (SBW). For SNP 241, 312, and 328, there were trends of association between the genotype effect and KPHISY. Genotypes for SNP 178 and 371 did not show significant association with any trait measured in this study. Allele substitution for SNP 270 had a significant effect on SBW, NMP, KPHISY, and KPHIS; and that of SNP 312 had a significant effect on KPHISY. Allele substitutions for SNP 241 and 328 tended to associate with KPHISY, and the allele substitution for SNP 312 had a trend to associate with BSW.

Both maker genotype and allele substitution of 270 showed significant association with KPHISY, at respective significance levels of p = 0.019 and p = 0.006; adjacent markers, SNP 241, 312, and 328, also had trends of associating with KPHISY (p = 0.073, 0.093, 0.097). This was in agreement with the conclusion that DGAT2 is mainly active in the liver, white adipose tissue and the mammary gland (Cases et al., 2001; Yen et al., 2005), and our results also supported that the DGAT2 gene was a high priority candidate gene for quantitative traits related to triglyceride synthesis and storage in farm animals (Winter et al., 2003; Ryo et al., 2005). According to the analysis of results in this study, selection for the favorite allele C in SNP 270 would increase RCW, decrease KPHISY, and trend to lower WBSF.

The SNP 270 allele substitution effect on NMP (the percentage of retail cuts weight over slaughter body weight) might be due to an indirect effect through itself lowering KPHISY; there was evidence that C allele substitution in SNP 270 could led to KPHIS decreasing and NMP increasing, and it did not associate with retail cuts weight (RCW) (p = 0.22) and bone yield (BY, p = 0.23; data not shown). This is similar to the case of the three SNP markers in bovine genes HEM1 and PDE1B, located at chromosome 5 (BTA5), which associated with traits relating to carcass fat; steers homozygous for the major haplotype (CGA) had 1.11±0.35% less of the predicted fat yield and 0.79±0.3% more of the predicted retail product yield than heterozygous steers (CGA/AAG) (Stone et al., 2005).

The analysis of the current work showed that intramuscular fat content (IMF) did not associate significantly with any SNP in intron3 of DGAT2 at p = 0.05 (0.18≤p≤0.99 for the individual genotype model and 0.21≤p≤0.98 for the allele substitution model). This was not in agreement with the DGAT2 gene being located in a QTL region for IMF in the porcine (Winter et al., 2003b). However, we did not find any direct report on the association between IMF and DGAT2.

Marbling score (MBS) did not associate with any SNP, either genotype or allele substitution, in intron3 of DGAT2 in the current research. This might be reasoned from DGAT2 being expressed at a high level in white adipose tissue (WAT) other than muscular (Yamazaki et al., 2005; Ryo et al., 2005). The development of adipose tissues in LM appears to disorganize the structure of the intramuscular connective tissue and contributed to tenderization of highly marbled beef in Japanese Black cattle during the late fattening period (Nishimura et al., 1999). These observations might explain why no SNP in intron3 of DGAT2 showed association with either Warner-Bratzler peak shear force (WBSF) or MBS in the current study.

For association analysis, a reliable conclusion mainly

depends on sample size, marker effect size, and the degree of linkage disequilibrium between marker and QTL. In the current study, the sample size was relatively small; but the effect of the SNP 270 was large enough to allow us to draw a robust conclusion. Based on the sample size of 140, the experimental design of this research had a power as high as 86% to detect the marker effect size of -4.26 kg of KPHISY at the condition of $\alpha = 0.05$ (Type I error) (data not shown). Therefore, the bigger effect of SNP 270 obviously raised the reliability of the conclusion of this study, even though the sample size was relatively small.

IMPLICATIONS

In summary, this work should be a basis for further study on association between DGAT2 and cattle economic traits. We obtained a stronger conclusion for the association between SNP 270 and fat yield. For rare allele frequencies in the current study, several animals with rare genotype were excluded from corresponding analysis. This means that we had no opportunity or less power to detect those effects for rare alleles. Therefore, associations need further validation work in additional populations. A new and key problem is that SNP 270 in intron3 of the DGAT2 gene showed significant association with fat yield of kidney, pelvic cavity, heart, intestine, and stomach, but did not show association with marbling score. This indicates that DGAT2 activity is not the same in different white adipose tissue, and the mechanism of effect of DGAT2 on different white adipose tissues needs further study.

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