Assessing the association of single nucleotide polymorphisms at the thyroglobulin gene with carcass traits in beef cattle[1,2]

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ABSTRACT: The objective of this study was to assess the association of SNP in the thyroglobulin gene, including a previously reported marker in current industry use, with marbling score in beef cattle. Three populations, designated GPE6, GPE7, and GPE8, were studied. The GPE6 population sampled breeds that could be used as alternative germplasm sources in beef cattle production, including Wagyu, Swedish Red and White, Friesian, and Norwegian Red. The GPE7 population sampled 7 popular beef cattle breeds used in temperate climates of the United States: Angus, Charolais, Gelbvieh, Hereford, Limousin, Red Angus, and Simmental. The GPE8 population sampled *Bos indicus*-influenced breeds used in subtropical regions of the country and subtropical and tropical regions of the world, including Beefmaster, Bonsmara, Brangus, and Romosinuano. Evaluation of 6 SNP in the thyroglobulin gene, including 5 newly described variations, showed no association ($P > 0.10$) with marbling score in these populations, except a tendency ($P < 0.10$) for an association with the previously described marker in GPE6. Closer examination of the GPE6 data revealed that the source of the tendency was an association ($P < 0.02$) with marbling in animals of Wagyu inheritance. Animals having Wagyu background and inheriting the TT genotype had a greater marbling score (599 ± 20) than those inheriting the CC (540 ± 10) or the CT (541 ± 11) genotype. No association was detected with any other carcass trait for this marker in the 3 populations. Furthermore, none of the 5 newly described markers in the gene displayed an association with marbling score. The data indicate that markers at the thyroglobulin gene may be a useful predictor of marbling performance for producers raising Wagyu-based cattle. Although associations with marbling score in the remaining populations were not large or significant, the TT genotype had the numerically greatest marbling score in each population.

Key words: beef cattle, carcass trait, marbling, thyroglobulin

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INTRODUCTION

An important factor in determining carcass value is the amount of marbling, with high-premium carcasses exhibiting abundant intramuscular fat and little intermuscular and subcutaneous fat (USDA, 1997). Carcasses with Grade A maturity and at least a Slightly Abundant marbling score are classified as Prime, whereas those with much less marbling fat that have a Slight marbling score are classified as Select (USDA, 1997). Marbling is one of the most important factors in determining the value of beef carcasses, yet genetic selection programs to modify marbling in beef cattle have been limited, largely because of the time and expense necessary for progeny testing potential sires (Barendse et al., 2004). Several breed associations publish ultrasound-based marbling EPD. Deoxyribonucleic acid tests with predictive merit for marbling propensity would provide a useful tool to facilitate genetic progress in increased marbling.

Marbling is a quantitative trait affected by multiple genes and can display marked variation among individuals and breeds. A candidate gene proposed to affect marbling produces thyroglobulin (gene symbol *TG*), the precursor to thyroid hormones with known endocrine

[1]Mention of trade names, proprietary products, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable.

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roles in fat metabolism (Barendse, 1999). An SNP upstream from the promoter of *TG* (marker TG5) has a reported association with marbling and is the source of a commercially available DNA test (Barendse, 1999; Barendse et al., 2004). However, studies of its effect on marbling in beef cattle have produced conflicting results (Barendse et al., 2004; Casas et al., 2005; Rincker et al., 2006).

Thus, the objective of this study was to assess the association of the TG5 and additional SNP in TG with marbling score in several populations by sampling a wide variety of beef cattle breeds.

MATERIALS AND METHODS

All experimental procedures were reviewed and accepted by the ARS Animal Care and Use Committee and were in accordance with the Federation of Animal Science Society's Guide to care and use of agricultural animals in agricultural research and teaching.

Populations

Three populations were studied, representing different cycles of the US Meat Animal Research Center Germplasm Evaluation Project (Wheeler et al., 2006). Hereford and Angus sires were included in all 3 cycles to provide a link for statistical comparison; however, no purebred Hereford or Angus matings were made to avoid confounding sire breed effects with heterosis effects. These 2 breeds were treated as 1 breed group (British breeds) for statistical analysis. Cycle 6 of this project (**GPE6** population) sampled sire breeds that could be used as alternative germplasm sources in beef cattle production (Wheeler et al., 2004; Casas and Cundiff, 2006). In the first year of the cycle, semen from Norwegian Red, Swedish Red and White, Wagyu, or Friesian sires was used on Hereford, Angus, and MARC III (one-fourth Angus, one-fourth Hereford, one-fourth Red Poll, one-fourth Pinzgauer) cows to produce a mixed F_1 population. Heifers in the F_1 generation were mated in 4 separate breeding pastures for 2 consecutive years, to 54 Charolais sires via multisire natural service (without record of individual sire-progeny relationships). This produced 653 crossbred steers and heifers. Details of the population structure, animal management, and feeding regimen have been described previously (Casas and Cundiff, 2006).

The second population was cycle 7 of the Germplasm Evaluation Project (**GPE7**), evaluating popular sire breeds used in the temperate areas of the United States. Details of the population structure, animal management, and feeding regimen have been reported previously (Wheeler et al., 2005). The GPE7 population was produced by using semen from Red Angus, Simmental, Gelbvieh, Limousin, and Charolais (as well as Angus and Hereford sires), with approximately equal numbers of calves produced from each sire breed (149 total sires, ranging from 18 to 23 sires per breed) produced from a similar population of Hereford, Angus, and MARCIII cows as GPE6. The population included 554 F_1 steers.

The third population of animals came from cycle 8 of the Germplasm Evaluation Project (**GPE8**), and sampled sires from tropically adapted *Bos indicus*-influenced breeds, which are used in subtropical regions of the country and subtropical and tropical regions of the world (Wheeler et al., 2006). The GPE8 population was produced by using semen from Beefmaster, Brangus, Bonsmara, and Romosinuano bulls (as well as Angus and Hereford sires) on Angus and MARCIII cows. A total of 127 purebred sires were sampled, producing the 578 crossbred steers (approximately equal numbers of calves per sire breed) that were used in this study (Wheeler et al., 2006). Management of these animals and collection of phenotypes were similar to GPE7 (Wheeler et al., 2006).

Traits Evaluated

Marbling score was evaluated on a cross-section of the LM at the 12th- to 13th-rib interface as follows: Practically Devoid = 200 to 299; Traces = 300 to 399; Slight = 400 to 499; Small = 500 to 599; Modest = 600 to 699; Moderate = 700 to 799; Slightly Abundant = 800 to 899; Moderately Abundant = 900 to 999; and Abundant = 1,000 to 1,099 (USDA, 1997; Wheeler et al., 2005). In addition to marbling score, traits recorded for the animals were live BW (kg), postweaning ADG (kg/d), dressing percentage, yield grade, fat thickness (cm), LM area (**LMA**, cm^2), HCW (kg), estimated KPH (%), retail product yield (%), fat yield (%), and bone yield (%). Retail, fat, and bone yields were estimated by using prediction equations that included carcass yield grade traits (LMA, adjusted fat thickness, and estimated KPH) and marbling score (Shackelford et al., 1995).

Markers Used and Genotyping Procedure

The previously reported SNP TG5 is a C/T in a repetitive element upstream from the promoter of the *TG* gene, located at position 422 of accession no. X05380 (Barendse, 1999). This polymorphism was genotyped as described by Casas et al. (2005). Additional SNP were detected by amplification of portions of the gene and sequencing of the PCR products from a panel of 32 animals, including representatives from each of the sire breeds in the GPE7 and GPE8 populations. The SNP were detected and annotated as described by Stone et al. (2002). The data for the markers has been deposited in the dbSNP database at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov; last accessed 7 September 2007) with accession numbers given in Table 1. The SNP were genotyped by PCR of each locus, followed by primer extension and product detection via mass spectrometry by using a Sequenom MassARRAY genotyping system, as recommended by the

Table 1. Amplification and probe primers for genotyping newly identified polymorphisms in the bovine *TG* gene

Marker	dbSNP accession no.[1]	Forward PCR primer[1]	Reverse PCR primer[1]	Probe primer	SNP alleles
551	BV718460	CATGGCTTTCTGCATCCTTC	AGGACCAGACAGAGGGATGA	CCACTGTCCTAGCTTAAGTC	C/T
668	BV718458	TCATCAGAAGAGGGTCATAG	TTGGACAATGTCCTGGTGTG	TCAGAAGAGGGTCATAGTAATGA	G/T
776	BV718459	TGTAGGCACTCCTGGAAATG	CCACACAGGAGACACTTAAC	AAAGTGCTGGGGAAACC	A/G
957	BV718459	ATGAGGGTAGTTTAAGGGCG	CGCCCCCTTGGCTGTATTTG	TTTTTCCTCCTCCATCT	C/T
993	BV718457	TCCACTCTTGCATCAGTACC	TGGGAGGGATGTCTATCTAC	AGCTTCCCAGGGAAAGTCAT	A/G

[1]Amplification primers are shown without the standard mass tag for matrix-assisted laser desorption ionization time of flight assays.

manufacturer (Sequenom, La Jolla, CA). The primer sequences used for amplification and detection are given in Table 1.

A saturated salt procedure (Miller et al., 1988) was used to obtain DNA from white blood cells. Blood samples were collected in 60-mL syringes with 4% EDTA. Blood samples were collected from the jugular vein via needle puncture. Blood was spun at 2,500 rpm (1,300 $\times g$) for 25 min and buffy coats were aspirated, cleaned, and frozen until DNA was extracted. A genotype for each animal was collected on the MassARRAY system, and the automated calls were checked by visualization of the spectrographs to minimize errors. Limited availability of buffy coats and problems with the degradation of existing DNA samples hampered the collection of a complete data set of all animals for all markers. When necessary, genotype assays were performed a second time to increase the number of successful genotypes, but genotype assays were not attempted a third time.

Statistical Methods

Models were evaluated by using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model for GPE6 included fixed effects of maternal grandsire breed, maternal granddam breed, interaction of maternal grandsire breed and maternal granddam breed, sex class, year of birth, slaughter group within year, and TG5 genotype. The random effect of maternal grandsire within maternal grandsire breed and a linear covariate based on age at weaning also were included in the model. Further analysis of this population was pursued by evaluating animals with Wagyu inheritance and animals without Wagyu inheritance. When evaluating the population with Wagyu inheritance, a similar model was used, but the effects of maternal grandsire and the interaction of maternal grandsire breed and maternal granddam breed were excluded. When evaluating animals without Wagyu inheritance, the original statistical model was used. The model used for GPE7 and GPE8 included sire breed, dam breed, the interaction between sire breed and dam breed, year of birth, slaughter group within year, and TG5 genotype as fixed effects (White et al., 2005). Weaning age was included as a linear covariate. Sire was included as a random effect nested within sire breed. Probability values shown are nominal and not corrected for multiple testing.

RESULTS

The first goal was to determine the predictive merit of the previously reported TG5 marker, a C/T SNP upstream of the promoter now in commercial use, in the 3 crossbred populations. Genotyping of the GPE6, GPE7, and GPE8 animals indicated that T was the less frequent allele, present at 25, 24, and 21% frequency, respectively (Table 2). The frequency of the TT genotype was 7.7, 5.6, and 5.2% in GPE6, GPE7, and GPE8, respectively, providing sufficient numbers to estimate the effect of genotype for all 3 genotypic classes in each population. The models used for estimation of putative effects varied slightly among the populations because of their different structures; specifically, the GPE6 animals were of both sexes and had uncertain sires because of the natural service multisire pasture matings, whereas the GPE7 and GPE8 animals had known parentage, but were all steers. Classification of animals in the 3 populations by SNP genotype did not reveal any statistically significant association with marbling score (Table 3); however, in GPE6 there was a tendency ($P < 0.10$) for an association between marbling score and TG5.

The original study reporting the TG5 marker (Barendse, 1999) was performed in a crossbred population by using the greatly marbled Wagyu breed, which suggested further examination of the marker in the GPE6

Table 2. Number of individuals inheriting the CC, CT, and TT genotypes at the TG5 marker in the GPE6,[1] GPE7, and GPE8 populations

	TG5 genotype			
Population[1]	CC	CT	TT	Total
GPE6	373	230	50	653
GPE7	314	209	31	554
GPE8	369	179	30	578
Total	1,056	618	111	1,785

[1]GPE6 = Germplasm Evaluation Program, cycle 6, includes animals with Hereford, Angus, Norwegian Red, Swedish Red and White, Friesian, and Wagyu inheritance. GPE7 = Germplasm Evaluation Program, cycle 7, includes animals with Hereford, Angus, Red Angus, Simmental, Gelbvieh, Limousin, and Charolais inheritance. GPE8 = Germplasm Evaluation Program, cycle 8, includes animals with Hereford, Angus, Beefmaster, Brangus, Bonsmara, and Romosinuano inheritance.

Table 3. Level of significance, least squares means, and SE for the effect of the TG5 marker on various traits[1] of the GPE6, GPE7, and GPE8 populations

	GPE6				GPE7				GPE8			
Trait[2]	*P*-value	CC	CT	TT	*P*-value	CC	CT	TT	*P*-value	CC	CT	TT
MAR[3]	0.09	537 ± 5	535 ± 6	560 ± 11	0.30	538 ± 4	530 ± 5	542 ± 11	0.13	497 ± 4	490 ± 5	513 ± 12
LWT, kg	0.61	563 ± 3	567 ± 3	564 ± 6	0.62	604 ± 3	604 ± 3	597 ± 8	0.80	558 ± 3	556 ± 3	561 ± 9
ADG, kg/d	0.14	1.32 ± 0.01	1.33 ± 0.01	1.35 ± 0.02	0.47	1.50 ± 0.01	1.49 ± 0.01	1.46 ± 0.02	0.22	1.34 ± 0.01	1.32 ± 0.01	1.36 ± 0.02
HCW, kg	0.78	346 ± 2	348 ± 2	348 ± 4	0.71	369 ± 2	369 ± 2	365 ± 5	0.77	341 ± 2	339 ± 2	342 ± 5
DRESS, %	0.68	61.5 ± 0.1	61.4 ± 0.1	61.6 ± 0.3	0.81	61.1 ± 0.1	61.0 ± 0.1	61.2 ± 0.2	0.69	61.2 ± 0.1	61.1 ± 0.1	61.0 ± 0.3
FAT, cm	0.86	1.05 ± 0.02	1.04 ± 0.03	1.04 ± 0.06	0.49	2.35 ± 0.04	2.28 ± 0.05	2.34 ± 0.12	0.95	1.00 ± 0.03	1.02 ± 0.04	1.00 ± 0.08
KPH, %	0.59	1.96 ± 0.01	1.95 ± 0.01	1.95 ± 0.02	0.73	2.32 ± 0.03	2.31 ± 0.04	2.24 ± 0.1	0.69	2.17 ± 0.04	2.13 ± 0.05	2.24 ± 0.12
LMA, cm^2	0.82	80.1 ± 0.4	81.1 ± 0.4	80.4 ± 1.0	0.57	84.9 ± 0.5	84.3 ± 0.6	85.5 ± 1.4	0.65	82.4 ± 0.5	81.9 ± 0.6	82.6 ± 1.3
YG	0.92	2.81 ± 0.03	2.80 ± 0.04	2.83 ± 0.08	0.31	2.94 ± 0.05	2.99 ± 0.05	2.82 ± 0.12	0.94	2.69 ± 0.04	2.72 ± 0.06	2.70 ± 0.12
RPYD, %	0.49	63.9 ± 0.2	64.1 ± 0.2	63.5 ± 0.5	0.98	61.8 ± 0.2	61.8 ± 0.2	61.9 ± 0.5	0.27	62.5 ± 0.2	62.8 ± 0.2	62.0 ± 0.5
FATYD, %	0.56	21.4 ± 0.2	21.2 ± 0.3	21.9 ± 0.6	0.98	24.9 ± 0.2	25.0 ± 0.3	24.8 ± 0.6	0.50	24.2 ± 0.2	23.9 ± 0.3	24.4 ± 0.7
BONEYD, %	0.82	14.9 ± 0.04	14.9 ± 0.05	14.9 ± 0.1	0.92	14.1 ± 0.06	14.2 ± 0.07	14.1 ± 0.2	0.55	14.4 ± 0.06	14.5 ± 0.08	14.5 ± 0.2

[1]GPE6 = Germplasm Evaluation Program, cycle 6, includes animals with Hereford, Angus, Norwegian Red, Swedish Red and White, Friesian, and Wagyu inheritance. GPE7 = Germplasm Evaluation Program, cycle 7, includes animals with Hereford, Angus, Red Angus, Simmental, Gelbvieh, Limousin, and Charolais inheritance. GPE8 = Germplasm Evaluation Program, cycle 8, includes animals with Hereford, Angus, Beefmaster, Brangus, Bonsmara, and Romosinuano inheritance.

[2]LWT = live BW; ADG = postweaning ADG. Marbling score (MAR), percentage of carcasses classified as Choice, dressing percentage (DRESS), yield grade (YG), fat thickness (FAT), LM area (LMA), HCW, estimated KPH, retail product yield (RPYD), fat yield (FATYD), and bone yield (BONEYD).

[3]Marbling score: 400 = Slight^{00} and 500 = Small^{00}.

population that also incorporated Wagyu germplasm. As a consequence of the population structure in GPE6, the Wagyu alleles were transmitted to the phenotyped generation from the maternal grandsire. Table 4 shows a breakdown of GPE6 genotypes by maternal grandsire breed. All breeds had T as the allele with the lowest frequency (20 to 31% frequency). The Wagyu subpopulation had the greatest T allele frequency of any of the alternative maternal grandsire breeds (31%). The number of animals in the Wagyu subpopulation having the TT genotype was low (n = 17), potentially limiting the power to detect an association if the mode of inheritance is recessive. Despite this limitation, a significant ($P < 0.02$) association of the TG5 genotypes was detected with an apparently recessive mode of action, with the TT genotype having greater marbling than the CT or CC genotype class (Table 5). The remainder of the GPE6 animals, when analyzed separately from Wagyu as a group, showed no sign of a tendency toward association (Table 5).

Because the TG5 marker is in current application, it was of interest to determine whether it had detectable effects on other production-related traits despite a lack of detectable effect on marbling. However, no association was detected for any of the other 11 traits analyzed in these populations, indicating that selection would be neither beneficial nor detrimental to these phenotypes (Table 3).

The data suggest the possibility that variation in *TG* might influence marbling, but that the reported TG5 marker is not in linkage disequilibrium with other functional loci, except in the Wagyu breed. Therefore, we developed additional markers in the gene to determine whether a marker in disequilibrium with a common variant affecting marbling in the most common US beef cattle breeds could be identified. Bovine *TG* is a relatively large gene including 48 exons spanning more than 200 kilobases of the genome. Fifteen genomic fragments of approximately 1 kilobase each, spread from intron 5 through exon 45, were sequenced in a discovery panel of 32 bovine genomic DNA samples (White et al., 2005) representing the sire breeds from GPE7 and GPE8 (data not shown). Five SNP were chosen for investigation based on the allele with the lowest frequency in the panel. Table 6 reports the genomic positions in the gene, as well as genotype frequencies of these markers in GPE7. The markers span a range of alleles, with the lowest frequencies from 3% (marker 668) to 46% (marker 957), and were not part of gene-wide haplotypes (i.e., the genotype at one marker was not predictive of the genotype at another; data not shown). Analysis of these 5 markers in GPE7 did not detect an association with marbling score ($P > 0.1$). Nominally significant associations ($P < 0.05$) of marker 551 with fat yield and bone yield, and of marker 668 with ADG, retail product yield, and fat yield were observed (Tables 7 and 8). However, these associations must be interpreted with caution.

Table 4. Number of individuals inheriting the CC, CT, and TT genotypes, and allelic frequencies, at the TG5 marker by sire breed of the dam in the GPE6[1] population

Maternal grandsire breed	Genotype count				Allelic frequency	
	CC	CT	TT	Total	C	T
British	119	72	20	211	0.74	0.26
Norwegian	47	22	3	72	0.80	0.20
Swedish	39	22	7	68	0.73	0.26
Friesian	94	52	3	149	0.80	0.20
Wagyu	74	62	17	153	0.69	0.31
Total	373	230	50	653		

[1]GPE6 = Germplasm Evaluation Program, cycle 6, includes animals with Hereford, Angus, Norwegian Red, Swedish Red and White, Friesian, and Wagyu inheritance.

DISCUSSION

The objective of this study was to determine whether variation in the thyroglobulin gene was associated with marbling score and other carcass traits in 3 cattle populations. Conflicting reports have been published about the association of the TG5 SNP with marbling score in beef cattle (Thaller et al., 2003; Barendse et al., 2004; Casas et al., 2005; Rincker et al., 2006). This could be a result of the variable populations and production systems used in the analyses. Barendse et al. (2004) used purebred Angus and Shorthorn cattle of undetermined parentage fed for less than 250 d in Australia, and observed an association of the TG5 marker with marbling; Casas et al. (2005) examined Brahman cattle raised in Florida and fed approximately 140 d and failed to detect an association; Rincker et al. (2006) used Simmental cattle fed for approximately 250 d in Montana and failed to detect an association; Thaller et al. (2003) found an association between the TG5 marker and marbling score in a small German Holstein population but failed to find such an association in a Charolais population.

It is possible that the TG5 marker is in linkage disequilibrium with other functional loci in some genetic backgrounds but not others, or that the effect of the allele is dependent on the production system. The likelihood of the former is related to the average distance of linkage disequilibrium in this area of the bovine genome, the distance between the marker and functional variation responsible for the observed effect, and the natural history of cattle carrying the functional variant. The likelihood of environmental differences is even more difficult to determine, but could be critical for determining circumstances in which the marker can be successfully applied.

The populations used for our study represent a test for the association of marker genotype with phenotype (Page et al., 2004). The animals included a wide variety of breeds, individuals, and sire lines. Analyses of the 3 populations did not detect a significant association of the TG5 genotypes with marbling score. However, it is important to note that, whereas differences were not significant in any population, the TT genotype class had the greatest marbling score in all 3 populations (Table 3). The probability of this occurring at random, if the genotype has no effect on marbling, is less than 0.04 ($0.33 \times 0.33 \times 0.33$), suggesting that in these cattle populations the genotypic effect was obscured by some unaccounted for effect. Two of these populations (GPE7 and GPE8) have been used to identify significant effects of markers associated with meat tenderness (Page et al., 2004; White et al., 2005) and growth (White et al., 2007), demonstrating that they can be successfully used for this purpose. To increase the power of the study, it would be necessary to increase the frequency of the T allele in the populations to establish statistical differences, if they exist in outbred cattle populations, because no population with greater minor allele frequency and marbling phenotypes is currently available.

Table 5. Number of individuals, level of significance, least squares means, and SE for the effect of TG5 marker on marbling score[1] in offspring derived from Wagyu maternal grandsires and non-Wagyu maternal grandsires in the GPE6[2] population

Subpopulation	Individuals, n	TG5			
		P-value	CC	CT	TT
Wagyu	152	0.019	540 ± 10[a]	541 ± 11[a]	599 ± 20[b]
Non-Wagyu	497	0.897	536 ± 5	533 ± 7	539 ± 13

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).
[1]Marbling score: 400 = Slight[00]; 500 = Small[00].
[2]GPE6 = Germplasm Evaluation Program, cycle 6, includes animals with Hereford, Angus, Norwegian Red, Swedish Red and White, Friesian, and Wagyu inheritance.

Table 6. Position in the gene and number of individuals per genotype in GPE7[1] for markers 993, 776, 551, 957, and 668

	Genotype							
SNP position	AA	CC	GG	TT	AG	CT	GT	Total
993 intron 43	4		470		80			554
776 intron 41	158		133		262			553
551 intron 38		22		333		186		541
957 intron 41		156		118		259		533
668 intron 24			0	509			38	547

[1]GPE7 = Germplasm Evaluation Program, cycle 7, includes animals with Hereford, Angus, Red Angus, Simmental, Gelbvieh, Limousin, and Charolais inheritance.

The current study detected an association of the TG5 marker with marbling in one subpopulation: the segment of GPE6 with Wagyu inheritance. In this subpopulation, animals with the TT genotype had significantly greater marbling scores than those with the other 2 genotypes. The fact that the effect was detected in a pedigree-defined subsample supports the hypothesis that the failure to observe an association was not due to environmental effects. One obvious source of this complication could be that the marker has a different phase with respect to causative variation in different genetic backgrounds. This phenomenon has previously been observed in studies of markers in the bovine μ-calpain (*CAPN1*) gene, in which the allele encoding Ile at AA 530 was found to be associated with decreased tenderness in the GPE7 population, but was found to increase tenderness in certain commercial populations used for validation studies (Page et al., 2004; R. Quaas, personal communication). It is likely that the TG5 marker will produce unreliable results in US beef cattle herds if used in a selection program to increase marbling.

Two hypotheses for the conflicting results with the TG5 marker are that it is relatively distant (in genetic units) from the causative variation, such that recombination has changed the phase of the marker allele in some cattle genomes, or that the T allele of the marker is associated with a mixture of multiple variations with differing effects on marbling, whose composition may differ among populations. The latter is more likely, because the mean for all populations was consistently greater for the TT genotype. Previous studies of *CAPN1* locus markers and meat tenderness revealed a series of markers having associations in various phases in different populations, as mentioned above. By testing a number of markers in multiple populations, it was possible to develop marker systems with consistent predictive merit for this trait (White et al., 2005; Casas et al., 2006). These results suggested the possibility that, if functional variation in the *TG* gene commonly affects marbling in the GPE populations, different markers might produce more reliable results. To address these issues, additional SNP in the gene were identified and tested in an attempt to ascertain their utility in selection for increased marbling. However, none of the SNP developed displayed an association with marbling score in GPE7. These new markers were not evaluated in the other populations, because the goal was to identify markers with robust predictive merit for use in US beef cattle selection and management. A likely explanation for the data is that the causative variation being detected in populations showing an association lies relatively distant from the marker and is not an allele of the *TG* gene itself.

Quantitative trait loci for subcutaneous fat thickness and marbling score have been detected on chromosome 14, containing the *TG* gene, in a population of F_2 cattle obtained from Wagyu and Limousin (Michal et al., 2006). However, the marbling score QTL was positioned telomeric from the TG gene, and the study reported an association of an SNP in the bovine fatty acid binding protein 4 (FABP4) gene with both marbling score and subcutaneous fat. The FABP4 gene is also positioned under QTL for fat thickness, as identified by Casas et al. (2000), Casas et al. (2003), and Moore et al. (2003), suggesting that it may be a reasonable candidate gene

Table 7. Level of significance, least squares means, and SE for the effect of marker 551 of the thyroglobulin gene on fat yield (FATYD) and bone yield (BONEYD) in the GPE7[1] population

Trait	*P*-value	CC	CT	TT
FATYD, %	0.030	26.8 ± 0.8[a]	25.1 ± 0.3[b]	24.8 ± 0.2[b]
BONEYD, %	0.015	13.6 ± 0.2[a]	14.1 ± 0.1[b]	14.2 ± 0.1[b]

[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]GPE7 = Germplasm Evaluation Program, cycle 7, includes animals with Hereford, Angus, Red Angus, Simmental, Gelbvieh, Limousin, and Charolais inheritance.

Table 8. Level of significance, least squares means, and SE for the effect of marker 668 of the thyroglobulin gene on postweaning ADG, retail product yield (RPYD), and fat yield (FATYD) in the GPE7[1] population

Trait	*P*-value	GT	TT
ADG, kg/d	0.048	1.45 ± 0.02	1.49 ± 0.01
RPYD, %	0.041	60.8 ± 0.5	61.9 ± 0.2
FATYD, %	0.035	26.1 ± 0.6	24.9 ± 0.2

[1]GPE7 = Germplasm Evaluation Program, cycle 7, includes animals with Hereford, Angus, Red Angus, Simmental, Gelbvieh, Limousin, and Charolais inheritance.

for harboring the putative causative variation for the marbling effect of TG5 in some populations. Two studies using Wagyu purebred families identified QTL for BW, carcass weight, and growth rate on chromosome 14, but did not detect an effect of the TG5 polymorphism or QTL for marbling (Mizoshita et al., 2004; Mizoguchi et al., 2006). This result suggests that the functional variation may be fixed in Wagyu, and previous studies with Wagyu crosses have detected the difference between this fixed allele in Wagyu and alleles present in other beef cattle breeds.

Additional markers developed in the thyroglobulin gene have shown an inconsistent association with carcass traits. Barendse et al. (2004) indicated that further discovery of SNP in the thyroglobulin gene should allow identification of the causal mutation. Markers developed at the gene in the current study indicate that the causal mutation is yet to be identified. Additional markers developed in the gene were not associated with marbling score in the GPE7 population; therefore, results of their association with other traits should be interpreted with extreme caution.

Further research will be needed to clarify the role of markers at the thyroglobulin gene in marbling or to develop alternative marker systems to track what appears to be likely variation in beef cattle on chromosome 14. Although associations of the TG5 marker have been observed (Barendse, 1999; Thaller et al., 2003; Barendse et al., 2004), there are also studies in which no association has been detected. The TG5 marker in the thyroglobulin gene promoter region does not appear to be a consistent, effective predictor of marbling score performance in common production environments in the United States.

The commercially available SNP reported in the thyroglobulin gene was associated with marbling score in cattle with Wagyu inheritance. The marker may explain a portion of the variation observed for marbling score in beef cattle. Further studies are needed to ascertain the effect of this marker on marbling score. Five additional markers developed in this gene were not associated with marbling score and were inconsistently associated with variation in other carcass traits of beef cattle.

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