Asian-Aust. J. Anim. Sci.
Vol. 20, No. 2 : 172 - 177
February 2007
www.ajas.info

Association of SNP Marker in the Thyroglobulin Gene with Carcass and Meat Quality Traits in Korean Cattle

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ABSTRACT : Thyroid hormones play an important role in regulating metabolism and can affect homeostasis of fat depots. The gene encoding thyroglobulin (TG), producing the precursor for thyroid hormones, has been proposed as a positional and functional candidate gene for a QTL with an effect on fat deposition. The SNP occurs in the 5′ promoter region of the TG gene and is widely used in marker assisted selection (MAS) programs to improve the predictability of marbling level and eating quality in beef cattle. In this study, we identified three SNPs at the 5′ promoter region of the TG gene in Korean cattle. Of the three SNPs identified in TG gene, the C257T and A335G were previously unreported new SNPs. The sequence data were submitted to GenBank (GenBank accession number: AY615525). The previously reported C422T SNP showed three genotypes, CC, CT and TT, by digestion with the restriction enzyme *Mfl*I using the PCR-RFLP method. A new allelic variant corresponding to the C→T and A→G mutations at positions 257 and 335, respectively, could be detected by the SSCP analysis. The gene–specific SNP marker association analysis indicated that the C422T SNP marker was significantly associated ($p<0.05$) with marbling score. Animals with the CC and CT genotypes had higher marbling score than those with the TT genotype. Results from this study suggest that TG gene-specific SNP may be a useful marker for meat quality traits in future MAS programs in Korean cattle. (**Key Words :** TG Gene, SNP Marker, Marbling Score, Korean Cattle)

INTRODUCTION

Carcass and meat quality traits, which are under the control of multiple genes, are economically important traits in beef cattle industry. Selection of animals with better carcass composition and higher meat quality is a great significance to breeders and consumers. The major objective of the application of genomic research to animal genetic improvement at present is to identify, map and analyze QTL (quantitative trait loci) affecting production traits and to use genetic markers for marker-assisted selection (MAS) to increase frequency of favorable QTL alleles in target populations (Stone et al., 2005). The MAS can increase the accuracy and efficiency of selection by providing more information on breeding values compared with traditional selection methods (Meuwissen and Goddard, 1996). The use of DNA- based markers could be particularly beneficial for genetic evaluation of economic traits for which phenotypic measurements are difficult or expensive to obtain. The MAS will first require identification of candidate gene or anonymous genetic markers associated with the traits of interest. The candidate gene may be selected on the basis of a known relationship between physiological or biochemical processes and production traits, and can be tested as QTL. Although a number of potential candidate genes have been recognized, few genetic markers have been identified for carcass and meat quality traits in beef cattle (Chung and Kim, 2005).

Thyroglobulin (TG) is the molecular store for the thyroid hormones T3 and T4. These hormones affect fat cell growth and differentiation. The TG has been mapped to the region of the QTL, and as its product is the precursor of hormone that affect lipid metabolism, TG was considered as a functional candidate gene as well as a positional candidate (Thaller et al., 2003). The genetic variation occurs in the 5′ promoter region of the TG gene and is widely used in marker assisted selection (MAS) programs to improve the predictability of marbling level and eating quality in beef cattle. An allele of the TG gene was identified as having a significant association with marbling score (Barendse, 2001; Burrell et al., 2004). Mears et al. (2001) also proved that the both T3 and T4 have been associated with marbling

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Received April 7, 2006; Accepted August 22, 2006

deposition in Japanese Black cattle. GeneSTAR Marbling is a DNA diagnostic test for the TG gene and commercially available marker for carcass quality traits. The DNA test has been used by breeders in the United States, Japan, Canada, Argentina and Australia. The objective of this study was to evaluate the association of reported single nucleotide polymorphism (SNP) in the TG gene with carcass composition and meat quality traits in Korean cattle. We also described identification of a new allelic variant in the TG gene of Korean cattle.

MATERIALS AND METHODS

Animals and carcass data

Three hundred-nine korean native steers with pedigree information and carcass data through national progeny testing program from the Hanwoo Experiment Station of the National Livestock Research Institute (NLRI) were used in this study. Meat samples were collected from 13th thoracic rib to the first lumbar vertebrae of the steers within 24 h of slaughter and evaluated by mechanical and physical methods. The carcass data included were carcass weight (CW), carcass percentage (CP), M. *longissimus dorsi* area (LDA), backfat thickness (BF) and marbling score (MS). BF and LDA were measured at the 12th- and 13th- rib interface. MS for quality grade was evaluated on a cross section of the longissimus muscle at the 12^{th}-to 13^{th}-rib interface. MS is scored on a scale from 1 to 7 with 7 being associated with the most marbling.

SNP identification and marker genotyping

Genomic DNA was extracted from white blood cells using standard techniques. Genotyping of the C/T polymorphism at position 422 of the TG gene (GenBank accession number X05380) was carried out using PCR-restriction fragment length polymorphism (RFLP). The PCR amplification was performed using primers TG5U2 (5′ -GGGGATGACTACGAGTATGACTG-3′) and TG5D1 (5′-GTGAAAATCTTGTGGAGGCTGTA-3′)(Barendse, 1999). The 20 μl reaction mixture contained 50 ng of genomic DNA, 10 pmol of each primer, 1× PCR buffer, 1.5 mM $MgCl_2$, 250 μM of each dNTP and 1.0 unit *Taq* polymerase. Amplification conditions were 94°C for 5min followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 7 min in a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT). For the PCR-RFLP analysis, amplified fragments were digested with restriction enzyme *Mfl*I at 37°C for at least 2 h. The digested DNA fragments were separated on 3% agarose gels by electrophoresis with 1×TBE buffer. In addition, to search for new mutations in the amplified fragments of the TG gene, the PCR- SSCP analysis was carried out. The PCR reaction for the SSCP analysis was the same conditions as described above for the PCR-RFLP analysis. After PCR amplification, 2 μl of PCR product was mixed with 8μl of gel loading solution containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol. The mixture was then denaturated at 96°C for 5 min, cooled on ice for 5 min and loaded on a nondenaturing 12%

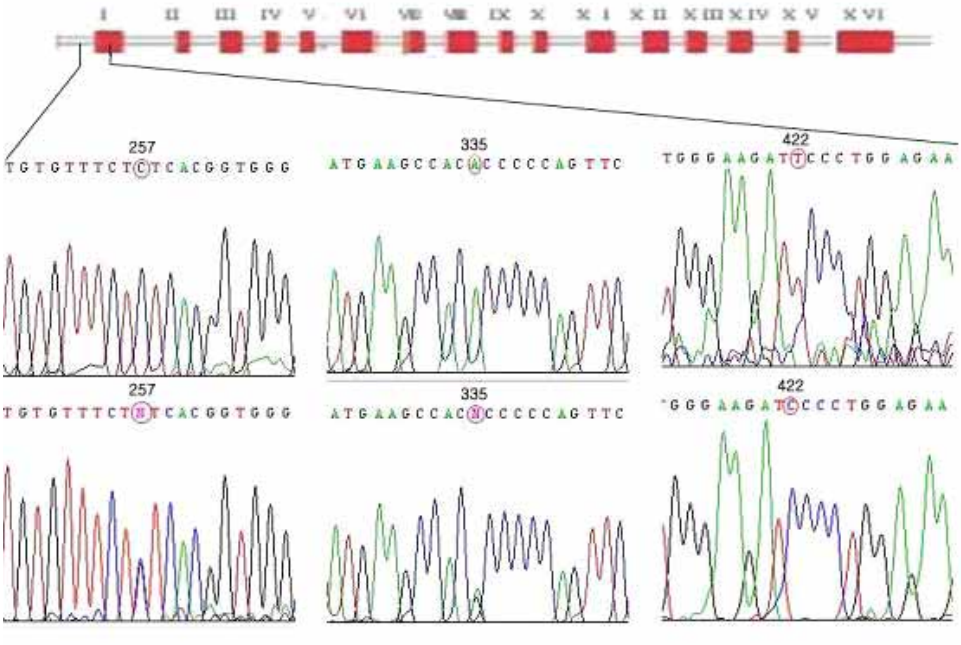

Figure 1. Chromatograms showing sequence variation at positions 257 (C257T), 335 (A335G) and 422 (C422T) within 5′ promoter region of the TG gene in Korean cattle. The two new SNPs, C257T and A335G, were discovered in the new allele of TG gene.

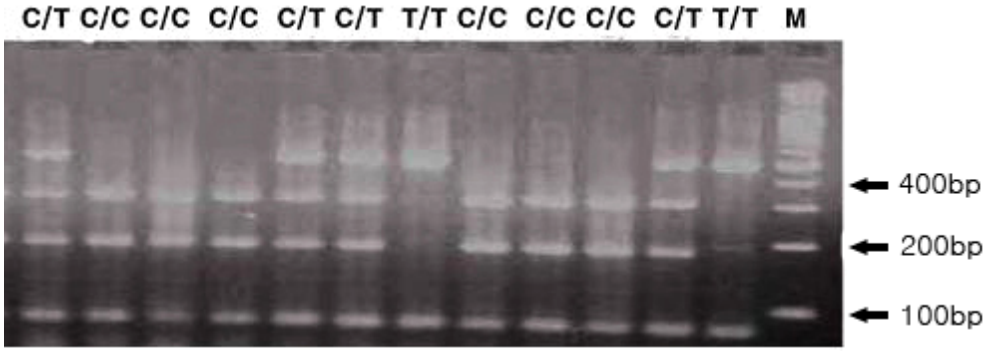

Figure 2. PCR-RFLP genotyping of C422T SNP in the TG gene with restriction enzyme *Mfl*I in Korean cattle. Three SNP genotypes, C/C, C/T and T/T, were detected. M: 100 bp DNA ladder.

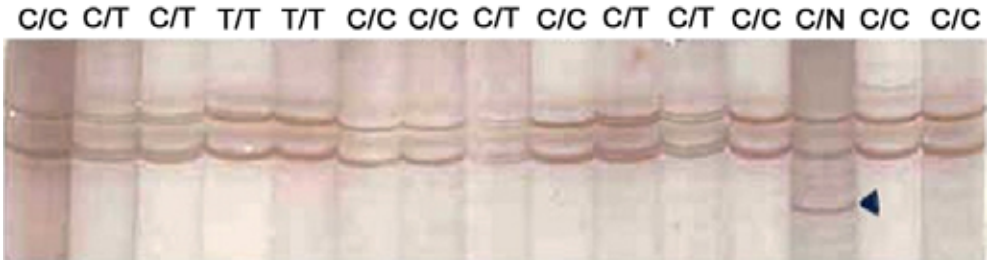

Figure 3. PCR-SSCP genotyping of C422T SNP in the TG gene in Korean cattle. Three SNP genotypes, C/C, C/T and T/T, were detected. Arrowhead indicates the new allelic variant, named as N, in the TG gene.

polyacrylamide gels (49:1 acrylamide to bis-acrylamide). Electrophoresis was performed in 1×TBE buffer at 250 V for 4 h at room temperature. After electrophoresis, the DNA fragments in the gel were detected by silver staining. The direct sequencing of the PCR products was performed using ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City CA).

Statistical analysis

The association between SNP marker genotypes of the TG gene and carcass and meat quality traits measured were analyzed by the least-squares method as applied in the GLM procedure of SAS (SAS, Inst. Inc., Cary NC) according to the following statistical linear model:

$$Y_{ijkl} = \mu + YS_i + P_j + G_k + e_{ijkl}$$

Where Y_{ijkl} is the observation of the carcass traits, μ is the overall mean for each trait, YS_i is the effect of i_{th} year and season of calving, P_j is the effect of j_{th} parity, G_k is the fixed effect of K_{th} SNP genotype and e_{ijkl} is the random residual effect. Additive effects were estimated by the difference between solutions for the two homozygous genotypes. Dominance effects were estimated by the differences between the solution for the heterozygous genotype and the average of the solutions for the two homozygous genotypes.

RESULTS

SNP identification and genotyping

We have amplified a fragment (545 bp) at the 5′ promoter region of the TG gene. Three SNPs closely linked in the fragment were identified by DNA sequence analysis of the PCR products: C/T at position 257, A/G at position 335 and C/T at position 422 (Figure 1). Of the three SNPs identified in TG gene, the C257T and A335G were previously unreported new SNPs. The sequence data were submitted to GenBank (GenBank accession number: AY615525). The previously reported C422T SNP showed three genotypes, CC, CT and TT, by digestion with the restriction enzyme *Mfl*I using the PCR-RFLP method. The C allele was cleaved into three fragments of 72, 178 and 295 bp, while T allele showed two fragments of 72 and 473 bp (Figure 2). For the C422T SNP, the frequencies of alleles C and T were 0.641 and 0.359, respectively, and genotype frequencies for CC, CT and TT were 41.1, 45.9 and 13.0%, respectively, in the population. A new allelic variant corresponding to the C→T and A→G mutations at positions 257 and 335, respectively, could be detected by the SSCP analysis (Figure 3), and was tentatively designated as N. This new SSCP allele with two point mutations (C257T and A335G) was found in 12 animals, only in combination allele C. The genotypes determined using the PCR-SSCP method were consistent with the RFLP typing results except for the new SSCP allele.

Table 1. Least squares means and standard errors for carcass traits and meat quality of different TG genotype in Korean cattle population (n = 309)

Traits	SNP Genotype			p-value	Additive effect	Dominance effect
	CC (127)[1]	CT (142)	TT (40)			
LW/kg	543.255±4.539	534.788±4.326	533.947±8.363	0.347	9.308±9.515	7.625±12.861
CW/kg	309.534±2.870	305.936±2.736	304.736±5.289	0.580	4.798±6.018	2.398±8.134
DP/%	56.926±0.141	57.174±0.134	57.055±0.260	0.445	-0.128±0.296	-0.367±0.400
BF/cm	0.696±0.025	0.680±0.024	0.697±0.046	0.879	-0.0004±0.053	0.033±0.072
LMA/cm^2	75.813±0.722	74.971±0.688	76.131±1.331	0.608	-0.317±1.515	2.001±2.047
MS/1-7	2.255±0.118^a	2.288±0.112^a	1.815±0.218^b	0.044*	0.440±0.248*	-0.505±0.335

LW: Live weight; CW: Carcass weight; DP: Dressing percentage; BF: Backfat thickness; LMA: M. *Longissimus dori* area; MS: Marbling score.
* Effect was significant at $p<0.05$.
[a, b] Within a row, means without a common superscript letter differ ($p<0.05$).
[1] Parentheses indicate number of animals.

Gene-specific SNP marker association analysis

The gene-specific SNP marker association analysis indicated that the C422T SNP marker was significantly associated ($p<0.05$) with marbling score. Animals with the CC and CT genotypes had higher marbling score than those with the TT genotype. In contrasts testing for additive and dominant gene action, a significant additive effect ($p<0.05$) on the MS was detected as shown in Table 1. Animals with the genotypes CC and CT gained 0.44 and 0.47 score more than animals with the genotype TT for MS, respectively. No significant association, however, was detected between any of the marker genotypes and other traits measured in this study.

DISCUSSION

Thyroglobulin is a glycoprotein hormone that is synthesized from the thyroid follicular cell. The TG is the carrier for both triiodothyronine (T3) and thyroxin (T4) and is stored in the thyroid gland (Ailhaud et al., 1992) Thyroid hormones play an important role in regulating metabolism and can affect adipocyte growth, differentiation and homeostasis of fat depots (Ailhaud et al., 1992; Casas et al., 2005). Likewise, T3 and T4 hormones play a role in fat cell development (Darimont et al., 1993; Smas and Sul, 1995). Barendse (1999) identified a C/T SNP in a repetitive element upstream from the promoter of the TG gene (position 422 of accession X05380). The C/T alleles identified by Barendse (1999) can be detected by using PCR-RFLP method. In this study, we detected a new allelic variant in the 5′ promoter region of the TG gene by PCR-SSCP method. Sequence analysis showed that this new allele was caused by both C to T and A to G transitions at positions 257 and 335, respectively. However, the new allele was very rare with a frequency of 3.9%. With respect to C422T SNP, previous studies have reported TT genotype frequencies of this SNP marker at 6.7 to 18.9% in *Bos Taurus* (Moore et al., 2003; Thaller et al., 2003; Rincker et al., 2006), whereas its frequency was very low (1.5%) in *Bos indicus* (Casas et al., 2005). In this study, the TT genotype frequency (13%) was similar to previously reported frequencies in *Bos Taurus*.

Recent work has mapped a QTL with an effect on fat deposition to the centromeric region of bovine chromosome (BTA) 14 in multiple populations (Casas et al., 2000; Moore et al., 2003), with the gene encoding TG being proposed as a positional and functional candidate gene for this QTL because its product is the precursor of hormones that affect lipid metabolism (Barendse, 1999). The C422T SNP of the TG gene, producing the precursor for thyroid hormones, has been associated with an improvement in overall fattening and could be used as a gene marker for marbling deposition in beef cattle (Barendse, 1999; Grisart et al., 2001). Animals with genotype TT had significantly higher marbling scores than those genotypes CC and CT (Barendse, 1999). Recently, Burrell et al. (2004) also reported that the TT genotype was associated with higher level of marbling relative to the CC genotype with the CT heterozygote being intermediate in beef cattle. Similarly, Thaller et al. (2003) found a significant effect on the intramuscular fat content only in musculus longissimus dorsi of German Holstein cattle, but the authors were unable to detect this effect in Charolais. It should be noted that the sample size in the German study was extremely small (28 Genrman Holstein and 27 Charolais animals), that the data indicated a recessive mode of effect with the T allele homozygotes having higher marbling. In this study, we have also found a significant association between the C422T SNP in the TG gene and MS. In contrast to the previous studies, however, the CC and CT genotypes were associated with higher MS compared with TT genotypes in Korean cattle population. Casas et al. (2005) reported that the TG marker was associated with fat thickness and LMA ($p<0.05$), but not with MS in *Bos indicus* cattle. Although, the association between the SNP marker in TG gene and MS did not reach a significance level, there was a trend ($p<0.078$) for allele C with higher marbling; the average

marbling score for genotypes CC, CT and TT was 324±5, 301±13 and 295±20, respectively. On the other hand, the association analysis between the TG gene-specific SNP and backfat EBV indicated no significant genotype effects in a commercial line of *Bos taurus* (Moore et al., 2003), but the results showed a trend for allele T to decrease the backfat EBV; the average backfat EBV for genotype CC, CT and TT was 0.02321, -0.0459 and -0.0701, respectively. De et al.(2004) observed that in the TG gene, the genotype differences between homozygotes CC and TT were -0.074±0.093 for marbling score in Wagyu×Limousin F2 crosses ($p>0.05$). Recently, using the commercially available GeneStar marbling marker (TG gene marker), Rincker et al. (2006) showed that Simmental steers with different allele types had no effect on marbling score, chemically determined intramuscular fat (IMF) percentage, quality grade or percent low choice and better. These data suggest that the GeneSTAR marbling marker was not an efficacious predictor of IMF deposition in early weaned Simmental steers fed a high-energy diet. Such the inconsistent results may have arisen from differences in the number of animals analyzed and statistical models used, or more importantly, the genetic background of the breed populations studied. Quantitative traits such as carcass composition and meat quality are regulated by many genes and affected by interactions among them, and thus, a candidate gene associated with a trait in one population may have a different effect, or show no effect at all, in another population due to negative effects of other genes and epistatic interactions of the candidate gene with other genes in the population (Ge et al., 2003). This theory is supported by many association studies, in which a SNP was significantly associated with meat quality traits in one population or breed, but not in another population or breed (Barendse, 1999; Burrell et al., 2004; Casas et al., 2005; Rincker et al., 2006).

The significant association between the SNP marker of TG gene and the MS indicates that the SNP of the TG gene may be one of the causative genes that control intramuscular fat deposition or that the gene is very close to the causative mutations that affects MS in the population of Korean cattle examined in this study, indicating that TG gene-specific SNP may be a useful marker for meat quality traits in future MAS programs in Korean cattle. However, the associated effects of the SNP marker need to be verified in other cattle populations.

ACKNOWLEDGMENTS

This study was supported by a grant (Code# 20050301-034-442-016-01-00) from BioGreen 21 program, Rural Development Administration, Republic of Korea.

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