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Association of Sequence Variations in DGAT 1 Gene with Economic Traits in Hanwoo (Korea Cattle)

H. S. Kong, J. D. Oh, J. H. Lee, D. H. Yoon[1], Y. H. Choi[1], B. W. Cho[2], H. K. Lee and G. J. Jeon*

Genomic Informatics Center, Hankyong National University, Korea

ABSTRACT : The effects of diacylglycerol O-acyltransferase (DGAT1) candidate gene polymorphism on the economic traits of Hanwoo were studied. Through sequencing analysis, two polymorphism sites at K232A and T11993C were established and were analyzed by PCR-RFLP. The PCR-RFLP analysis for K232A showed that the frequencies of alleles K and A were 0.75 and 0.25, respectively, and the frequencies of genotypes for K/K, K/A and A/A were estimated as 0.509, 0.491 and 0, respectively. In the PCR-RFLP analysis for T11993C, we found allele frequencies of 0.773 and 0.227 for T and A, respectively, and 0.546, 0.454 and 0 for the T/T, T/C and C/C genotype frequencies, respectively. No significant effects on economic traits in Hanwoo were found in the separate analysis of K232A and T11993C polymorphisms, but the interaction between K232A and T11993C showed a significant effect ($p<0.005$) on marbling score. The DGAT1 candidate gene was found to have a significant effect not only on milk yield and component traits but also on the metabolism of intramuscular fat. (**Key Words :** DGAT1 Gene, Single Nucleotide Polymorphism (SNP), Hanwoo)

INTRODUCTION

Mapping QTL for continuous traits of economical importance in livestock species is a frequently used application of genetic markers. Many studies in dairy cattle have shown that a quantitative trait locus (QTL) with major influence on milk production is located in the centromeric end of chromosome 14 (Coppieters et al., 1998; Heyen et al., 1999; Looft et al., 2001). This QTL had been fine-mapped to a 3-cM region (Riquet et al., 1999; Farnir et al., 2002). Diacylglycerol O-acyltransferase (DGAT1) catalyzes the last step in triglyceride synthesis (Cases et al., 1998) and abrogates milk yield when knocked out in the mouse (Smith et al., 2000). By sequencing the DGAT1 gene from individuals with known QTL genotypes, a nonconservative lysine to alanine substitution was identified at position 232, and shown to be associated with a major effect on milk yield and composition in several dairy cattle populations and breeds (Grisart et al., 2002; Spelman et al., 2002; Winter et al., 2002). Also, Homozygous lysine/lysine (DGAT1) German Holstein animals have significantly more intramuscular fat in m. semitendinosus and in m. longissimus dorsi than the heterozygous and alanine/alanine homozygous animals (Thaller et al., 2003). The DGAT1 K232A mutation was therefore considered to be the likely quantitative trait nucleotide underlying the BTA14 QTL effect. In particular, the allele encoding the lysine 232 variant proved to be more efficient with regard to milk fat synthesis. The nucleotide variation underlying the K232A substitution can be diagnosed by an RFLP assay (Sonstegard et al., 2001). The DGAT1 gene has other mutation sites and haplotypes (Spelman et al., 2002).

The objective of our study was to examine the effects of the two DGAT1 mutation regions on economic traits in the Hanwoo.

MATERIALS AND METHODS

Animal and genomic DNA extraction.

200 Hanwoo (Bos Taurus) animals were analyzed in this study. DNA samples were extracted from blood or semen by some modifications of the method used by Miller et al. (1988) and Sambrook et al. (1989).

PCR amplification

The K232A region of genomic DNA was amplified using PCR with the following primers: Forward. 5'-

* Corresponding Author: G. J. Jeon. Tel: +82-31-670-5331, Fax: +82-31-675-5331, E-mail: gjjeon@hknu.ac.kr
[1] National Livestock Research Institute, RDA, Korea.
[2] Pusan National University, Korea.
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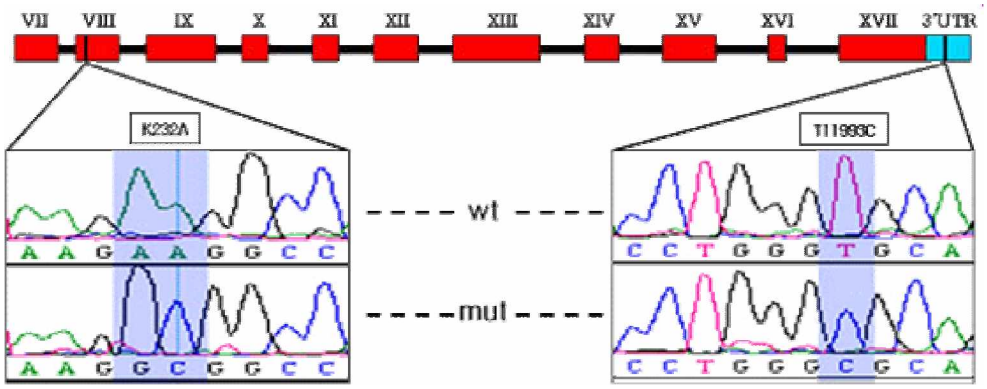

Figure 1. Genomic structure and polymorphisms found in the DGAT1 gene for Hanwoo (Korea native cattle). Coding sequence in red, trailer sequence in blue and intronic sequence in black. wt: wild type, mut: mutant type.

ttcctcaagctgttctccta-3' (196-215 nucleotides in the sequences reported by Winter et al., 2002: GenBank Accession. No. AJ318490). Reverse, 5'-cacgtacctgctggatca-3' (364-383 nucleotides). The other region (exon 17-3'UTR) was amplified using PCR with the following primers: Forward, 5'-ctcactcccgtcctgtgt-3' (196-215 nucleotides in the sequences reported by Winter et al., 2002: GenBank Accession. No. AJ318490), Reverse, 5'-gacgtctgacc acagagc-3' (364-383 nucleotides). The Polymerase Chain Reaction was conducted in 10 μl volumes, each containing 100 ng of genomic DNA, 10× PCR buffer (100 mM Tris pH 8.9, 50 mM KCl, 15 mM MgCl, 0.01% gelatin, 0.1% Triton X-100, 10 mg/ml BSA), 10 pmole of each primer, 40 μM of dNTPs and 0.5 unit TaqDNA polymerase (Promega, USA). The condition of PCR was first a denaturation step of 5 min at 94°C followed by 30 cycles, each consisting of 40 sec at 94°C, 30 sec at 56°C, 1 min at 72°C and then, a final step of 10 min at 72°C using a PTC 200 peltier thermal cycler (MJ Research, USA).

Sequence analysis

Directly sequences were generated from both stands using ET terminator Cycle Sequencing Kit on a PTC 200 peltier thermal cycler (MJ Research, USA). The extension reaction in a 10 μl volume was performed and extension products were electrophoresed on a MegaBACE DNA Analysis System (Amersham Biosciences, USA.) Searching for sequence mutation was done using the seqMAN II software (DNA STAR Inc.).

Genotyping

The PCR products of the K232A region were digested with EaeI restriction enzyme (Y^GGCC_R) and separated on a 2% agarose gel. The uncut fragment represents the lysine variant, whereas the EaeI RFLP fragments of 189 and 369 bp represent the alanine variant. The other PCR products (exon 17-3'UTR) were digested with Hpych 4 V restriction enzyme (TG^CA) and separated on a 2% agarose gel. The uncut fragment represents the cytosine variant, whereas the Hpych4V RFLP fragments of 266 and 144 bp represent the thymine variant.

Statistical analysis

The linear covariate models were used using SAS (SAS 9.1) for the two polymorphic sites. For consideration of two polymorphic sites simultaneously, the model was given as;

$$y_{ijk} = u+bSA+G1_i+G2_j+e_{ijk}$$

where y_{ijk} = a phenotypic record;
u = overall mean;
b = regression coefficient;
SA = regression variable of slaughter age;
G_i = ith genotype of the 1st polymorphic site;
G_j = jth genotype of the 2nd polymorphic site; and,
e_{ijk} = random residual.

RESULTS AND DISCUSSION

PCR amplification and investigation of SNP

To investigate the SNPs of the exon as well as intron number 8 and 17 of the DGAT1 gene, we used the PCR method to amplify the regions by using the specific primers for each region for 24 heads of Hanwoo cattle. The PCR product size of exon 8 region was 558 bp and of exon 17 region was 410 bp as expected. The PCR products were sequenced individually by the direct sequence method for nucleotide sequences of DNA. We confirmed that the regions of exon 8 and 17 in our analysis showed a homology with the sequences of the exon 8 and 17 regions of the published Bovine DGTA1 gene. After aligning the DNA sequences, we found that there were two polymorphic sites, which are 10,433 (A→G) and 10,434 (A→C) in the exon 8 region, and one polymorphic site in the 3'UTR at the site of 11,993 (T→C). The polymorphism at the exon 8

Table 1. Genotype and allele frequencies of the K232A and T11993C polymorphisms on the DGAT1 gene in Hanwoo

K232A		T11993C	
Genotype	Frequency	Genotype	Frequency
K/K	0.510	T/T	0.545
K/A	0.490	T/C	0.455
A/A	-	C/C	-
Allele frequency			
K	0.750	T	0.773
A	0.250	C	0.227

region changes the amino acid Lysine (K = AAG) to Alanine (A = GCC) at number 232 of the protein sequence denoted as K232A.

PCR RFLP analysis of the DGAT1 gene from Hanwoo

PCR-RFLP was used to analyze the SNP of the DGTA1 gene by digesting with enzyme Eae I (Y^GGCC_R) for the K232A site and with Hpych 4V (TG^CA) for the T11993C site. Through electrophoretic analysis, the PCR-RFLP products of the K232A region showed that allele K had only one fragment of 558 bp (uncut fragment) and allele A had two fragments of 189 bp and 369 bp, each For the T11993C region, it showed that the T allele had two fragments of 266 bp and 144 bp each and the C allele had only one fragment of 410 bp (uncut fragment). The genotype and allele frequencies of the K232A and T11993C regions are shown in Table 1. Also, the K232A and T11993C regions were found independent of each other.

Effect of sequence mutation on economic traits in Hanwoo

The effects of the PCR-RFLP polymorphism genotypes and the interactions between the genotypes and the economic traits are shown in Table 2. The genotypes of K/K and K/A for the K232A polymorphism and the genotypes of T/T and T/C for the T11663C polymorphism showed no significant effects on all the investigated economic traits.

DISCUSSION

Many studies in dairy cattle have shown that a quantitative trait loci (QTL) with a major influence on milk production is located in a 3 cM region of chromosome 14 (Coppieters et al., 1998; Heyen et al., 1999; Riquet et al., 1999; Looft et al., 2001; Farnir et al., 2002). The DGAT1 gene is located at the QTL region in chromosome 14 (Grisart et al., 2002; Spelman et al., 2002; Winter et al., 2002). Winter et al. (2002) reported an estimate of 0.07 for the lysine variant in a random sample of Fleckvieh bulls but a lysine variant frequency of 0.35 in German Holstein. The lower frequency of the lysine variant in Fleckvieh vs. German Holstein is somewhat unexpected since Fleckvieh has been superior in milk content traits, whereas German Holstein has a higher performance in milk yield.

In the Holstein breed, the allele frequencies of the lysine variant range from 0.30 in the New Zealand to 0.63 in the Dutch populations (Bovenhuis and Schrooten, 2002; Grisart et al., 2002). Thaller et al. (2003) reported an estimate of 0 for the K/K (lysine/lysine) genotype, 0.22 for the K/A (lysine/alanine) genotype, and 0.78 for the A/A (alanine/alanine) genotype, respectively, in a sample of the Charolais breed. In the Holstein breed, the genotype frequencies reported were 0.17 for the K/K genotype, 0.52 for the K/A genotype, and 0.28 for the A/A genotype, respectively. The K (lysine) allele had a significant effect on the intramuscular fat content of musculus longissimus dorsi and m. semitendinosus.

In our study, the PCR-RFLP analysis for K232A showed that the frequencies of alleles K and A were 0.75 and 0.25, respectively, and the frequencies of genotypes K/K, K/A and A/A were 0.509, 0.491 and 0, respectively. Similarly in the PCR-RFLP analysis for T11993C, we obtained frequencies of 0.773 and 0.227 for the T and A alleles respectively, and 0.546, 0.454 and 0 for the T/T, T/C and C/C genotypes, respectively. There were no significant effects on the economic traits for the separate analyse of the polymorphisms of K232A and T11993C. However, the interaction of K232A and T11993C showed a significant effect ($p<0.005$) on MS phenotype. Recently, several studys was reported on association with economic traits and SNPs in Hanwoo (Chung et al., 2005; Cheong et al., 2006; Kong et al., 2006; Shin et al., 2007). Our study proved that the DGAT1 candidate gene had an effect not only on milk yield but also on the metabolism of the intramuscular fat.

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Table 2. Effects of the DGAT1 interaction polymorphism on economic traits in Hanwoo

K232A	T11993C	LW	CW	DP	LMA	BF	MS
K/K	T/C	559.7±15.43	330.1±9.86	58.92±0.50	77.39±2.02	0.88±0.07	2.51±0.27[a]
K/A	T/C	543.2±16.55	319.5±10.57	58.80±0.54	75.04±2.01	0.86±0.08	1.48±0.28[b]
K/K	T/T	556.7±16.45	320.2±10.47	57.56±0.53	78.67±2.10	0.76±0.08	1.83±0.26[ab]
K/A	T/T	555.5±13.73	328.3±8.77	59.02±0.49	77.37±1.74	1.02±0.06	2.26±0.21[ab]

LW: live weight, CW: carcass weight, DP: dressing percent, LMA: longissimuscle area, BF: backfat thickness, MS: marbling score.
* Different superscripts within columns differ significantly ($p<0.05$).

Government, Republic of Korea.

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