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Useful Marker Around *TRDN* Gene Showing Marbling-Associated Expression Changes

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Abstract: Researchers have previously showed that the *triadin* (*TRDN*) gene involved in muscle contraction possesses expression differences in musculus longissimus muscle between low-marbled Holstein and high-marbled Japanese Black steer groups. In the present study, researchers found that a marker (BMS817) close to the TRDN was polymorphic between low-marbled Holstein and high-marbled Japanese Black steer groups and exhibited significantly different allelic distribution between Japanese Black sires with extremely high predicted breeding value for marbling and with extremely low one. Further, researchers detected Single Nucleotide Polymorphism (SNP) in the *TRDN* gene between low-marbled Holstein and high-marbled Japanese Black steer groups. The allelic distribution of the SNP referred to as g.3834941C>T in the TRDN was indistinguishable between Japanese Black sires with extremely high predicted breeding value for marbling and with extremely low one. The findings suggest that an unidentified true causal mutation which is in linkage disequilibrium with the BMS817 marker but not the g.3834941C>T SNP may be related to changes in *TRDN* gene expression and/or marbling. The BMS817 marker may be useful for effective marker-assisted selection to increase the levels of marbling.

Key words: Allelic distribution, TRDN, close marker, marbling, single nucleotide polymorphism, Japan

INTRODUCTION

Marbling characterized by the amount and distribution of intramuscular fat in a cross section of musculus longissimus muscle is one of the economically important traits of beef cattle (JMGA, 1988). High levels of marbling improve the palatability and acceptability of beef by affecting the taste and tenderness of the meat (Busboom *et al*., 1993; Boylston *et al*., 1995; Matsuishi *et al*., 2001). Because of the importance of marbling on the economics of beef production, there is great interest in gaining a better understanding of the molecular architecture of marbling and in generating new opportunities for more effective marker-assisted breeding. Researchers have previously undertaken differential-display PCR (ddPCR) in low-marbled and high-marbled steer groups at 8, 10, 12 and 14 months of age, encompassing the time that marbling starts to appear to explore genes showing marbling-associated expression changes in musculus longissimus muscle (Sasaki *et al*., 2006a). Among the detected genes, the *triadin* (*TRDN*) gene which is known to be involved in muscle contraction (Kirchhefer *et al*., 2001) exhibited higher expression levels in low-marbled Holstein steer group than in high-marbled Japanese Black steer group in the late stage of the test period (Sasaki *et al*., 2006b). TRDN proteins are also reported to interact with RYR1 (Zhang *et al*., 1997) which is likely involved in pig meat quality traits (Kuchenmeister *et al*., 1999).

Researchers suggested that the decrease in TRDN expression was thought to promote proliferation, differentiation or maturation of adipocyte-lineage cells by weakening the structural integrity of the sarcomere, thereby resulting in intramuscular fat deposition in high-marbled steer (Sasaki *et al*., 2006a, b). *TRDN* gene regarded as intriguing gene of which expression change may cause intramuscular fat deposition (Sasaki *et al*., 2006a).

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It has been reported that a marbling quantitative trait locus was mapped to genomic region containing the *TRDN* gene on bovine chromosome 9, using a half-sib family of Japanese Black beef cattle (Takasuga *et al*., 2007). Thus, the TRDN was considered as a positional functional candidate for the gene responsible for marbling.

To examine whether polymorphism around the TRDN is useful for effective marker-assisted selection to increase the levels of marbling, researchers herein analyzed the allele frequency distribution of a marker close to the TRDN in high-marbled and low-marbled cattle. Researchers further explored polymorphism in the *TRDN* gene and examined allelic distribution in the polymorphism between high-marbled and low-marbled cattle.

MATERIALS AND METHODS

Samples: Two Holstein steers and 2 somatic nuclear-derived cloned steers (Shiga *et al*., 1999) from a Japanese Black Itofuku sire with a very high estimated breeding value for marbling (OPIAI, 1999) which were assigned for low-marbled and high-marbled steer groups, respectively in the previous ddPCR analysis (Sasaki *et al*., 2006b) were used for microsatellite marker genotyping and polymorphism detection in this study. The details of these steers are described before (Sasaki *et al*., 2006a, b). Musculus longissimus muscle tissues were obtained from these steers as described previously (Sasaki *et al*., 2006a). Researchers used 2 high-marbled cloned steers to confirm the authenticity of newly discovered Single Nucleotide Polymorphism (SNP) in the *TRDN* gene. Further, researchers used 34 Japanese Black unrelated sires (17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one) selected from 101 unrelated sires, a panel of that represent almost all of the lines within a Japanese black beef cattle population, for microsatellite marker genotyping and SNP genotyping in this study. The predicted breeding values were obtained from the recording system for beef cattle reported by Sasaki *et al*. (2006a). The accuracy of the predicted breeding values in the 101 sires was 0.935±0.008 ranging from 0.770-0.990. Semen or blood were collected and DNA samples were prepared from the materials according to standard protocols. This study conformed to the guidelines for animal experimentation of the Faculty of Agriculture, Niigata University (Niigata, Japan).

Microsatellite marker genotyping: Researchers screened the cattle genome maps (NAGRP Cattle Genome Coordination Program) and obtained BMS817 microsatellite marker as the close marker to the *TRDN* gene. PCR amplification of the BMS817 was performed in a 10 μL volume containing 25 ng of template DNA, 0.2 mM of each dNTP, 0.1 μM of each primer, 0.5 U of Go Taq polymerase (Promega, Madison, WI) and 1 X Go Taq buffer (Promega). The amplification condition was as follows: 94°C for 2 min, 35 cycles of 94°C for 30 sec, 57°C for 30 sec and 72°C for 1 min followed by 72°C for 5 min. Forward primer was labeled with FAM fluorescent dye. Primer sequences were obtained from NAGRP Cattle Genome Coordination Program (http://www.animal genome.org/cattle/). PCR products were electrophoresed in an ABI3730 sequencer (ABI, Foster city, CA). Allelic sizes were scored using software GeneMapper 4.0 (ABI).

Polymorphism detection: Researchers screened bovine genome sequence (National Center for Biotechnology Information, Bethesda, MD) by BLAST search with human TRDN sequence (NM_006073) and obtained bovine genomic sequence (NW_001495548) containing the *TRDN* gene. PCR primers were designed to target ~4 kb proximal promoter region, exon region and intron region adjacent to exon sequences for the *TRDN* gene using this genomic sequence in order to screen polymorphisms in the gene between 2 low-marbled Holstein steers and 2 high-marbled cloned steers. PCR amplifications were performed using 25 ng of the prepared DNA as template in a final volume of 100 μL containing 1 μM of each primer, 0.25 mM of each dNTP, 2.5 U of Go Taq polymerase (Promega) and 1 X Go Taq buffer (Promega). The PCR conditions were carried out as follows: 94°C for 2 min 35 cycles of 94°C for 30 sec the appropriate annealing temperature for 30 sec and 72°C for 1 min followed by a further 5 min extension at 72°C. PCR products were examined by electrophoresis through a 1.0% agarose gel to determine the quality and quantity for DNA sequencing. DNA sequencing of PCR-amplified products was performed by the direct sequencing with an ABI3730 sequencer (ABI) following standard Big Dye protocols (ABI). Primers used for PCR amplifications and obtained from primer walking were used as sequencing primers. Nucleotide polymorphisms were identified by comparison of sequence traces among the 4 DNA samples and designated according to nomenclature for the description of sequence variations in the HGVS (Human Genome Variation Society, Fitzroy, VIC, Australia) (http://www. genomic.unimelb.edu.au/mdi/ mutnomen/). Primer sequences will be available on request.

SNP genotyping: The SNP, g.3834941C>T detected in the intron 39 region of the *TRDN* gene was genotyped by PCR-Restriction Fragment Length Polymorphism (RFLP)

Method. PCR primers used for PCR-RFLP were 5'-GAAGATGTACCAACTTCAAAGAATGCTAAAGGT AAGATAG-3' and 5'-TCTAAACTTTGCTTAGGTAAAG TTGAGAACCAGAATAG-3'. PCR amplifications were carried out as described in Polymorphism detection section, using a final volume of 20 μL and the annealing temperature of 60°C. An aliquot of PCR-amplified products was digested at 37°C for 2 h with restriction enzyme AluI and electrophoresed on a 3.0% agarose gel. Agarose gels were stained with ethidium bromide and photographed under an ultraviolet light.

Statistical analysis: The allelic distributions of the BMS817 and the detected SNP were compared between 17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one by Chi-square (χ^2) test. Statistical analysis was performed by the FREQ procedure of SAS program (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Researchers first genotyped 2 low-marbled Holstein steers and 2 high-marbled Japanese black steers which were previously shown to have different *TRDN* gene expression patterns in ddPCR analysis (Sasaki *et al*., 2006a) for the BMS817 microsatellite marker close to the *TRDN* gene. This genotyping analysis revealed polymorphism of the BMS817 between the high- and low-marbled steer groups. The low-marbled steers were homozygous for 123 bp allele at the BMS817 whereas the high-marbled steers heterozygous for 121 and 123 bp allele. Researchers further genotyped 34 Japanese black unrelated sires consisting of 17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one for the BMS817. Statistically significant difference in the allelic distribution between 17 sires with extremely high breeding value and 17 sires with extremely low one was detected for the BMS817 (Table 1). The frequency of the 121 bp allele at the BMS817 was higher in animals with the high breeding value than with the low one and the 123 bp allele frequency in animals with the low one than with the high one (Table 1).

Researchers second sequenced the proximal promoter region, exon region and intron region adjacent to exon sequences of the *TRDN* gene from 2 low-marbled Holstein steers and 2 high-marbled Japanese black steers. This sequence analysis revealed only one SNP (g.3834941C>T) in the intron 39 region of the *TRDN* gene: a C to T substitution located 10 bp downstream of the exon 39. The low-marbled steers were homozygous for C allele at the g.3834941C>T site whereas the high-marbled steers heterozygous for C allele and T allele. Using PCR-RFLP Method, researchers genotyped 34 Japanese black unrelated sires consisting of 17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one for the g.3834941C>T SNP. The CC homozygotes, the TT homozygotes and the CT heterozygotes resulted in two bands (39 and 100 bp), one band (139 bp) and three bands (39, 100 and 139 bp), respectively. No significant difference in the allele frequency distribution between 17 sires with extremely high breeding value and 17 sires with extremely low one was detected for the g.3834941C>T SNP (Table 2).

Table 1: Comparison of allelic distributions in BMS817 between 17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one

	Frequency		
Sires	121 bp allele	123 bp allele	p-value
With high breeding value	0.450	0.550	<0.05
With low breeding value	0.225	0.775	-

Table 2: Comparison of allelic distributions in the detected SNP between 17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one

	Frequency		
Sires	C allele	T allele	p-value
With high breeding value	0.412	0.588	0.451
With low breeding value	0.324	0.676	-

Thus, researchers showed the higher frequency of the BMS817 121 bp allele in high-marbled cattle as compared to low-marbled cattle although, the allelic distribution of the g.3834941C>T SNP detected in intron 39 region of the *TRDN* gene was indistinguishable between high-marbled and low-marbled cattle. This study together with the mapping of a marbling quantitative trait locus within genomic region containing TRDN on bovine chromosome 9 (Takasuga *et al*., 2007) suggests that an unidentified true causal mutation which is in linkage disequilibrium with the BMS817 marker but not the g.3834941C>T SNP, directly affects changes in *TRDN* gene expression and/or marbling. The BMS817 microsatellite marker may be useful for effective marker-assisted selection to increase the levels of marbling.

CONCLUSION

In this study, researchers show that the frequency of the 121 bp allele at the BMS817 microsatellite marker close to the *TRDN* gene is higher in high-marbled cattle than in low-marbled cattle, although the allelic distribution of the g.3834941C>T SNP detected in intron 39 region of the *TRDN* gene is indistinguishable between high-marbled and low-marbled cattle. This study

suggests that an unidentified true causal mutation which is in linkage disequilibrium with the BMS817 marker but not the g.3834941C>T SNP may be related to changes in *TRDN* gene expression and/or marbling. The BMS817 marker may be useful for effective marker-assisted breeding to increase the levels of marbling.

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