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Polymorphism in Promoter Region of *CDC10* Gene Showing Marbling-Associated Expression Changes

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Abstract: Researchers have previously showed that the septin 7 (*CDC10*) gene involved in cellular proliferation 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 (BM6437) close to the CDC10 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 promoter region of the *CDC10* gene between low-marbled Holstein and high-marbled Japanese Black steer groups. The allelic distribution of the SNP, referred to as g.63629097G>C in the CDC10 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 BM6437 marker but not the g.63629097G>C SNP may be related to changes in *CDC10* gene expression and/or marbling.

Key words: Allelic distribution, CDC10, close marker, marbling, single nucleotide polymorphism

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).

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*., 2006b). Among the detected genes, the septin 7 (*CDC10*) gene which is known to be involved in cellular proliferation (Nagata *et al*., 2004), exhibited higher expression levels in high-marbled Japanese Black steer group than in low-marbled Holstein steer group across all ages of the test period (Sasaki *et al*., 2006b). Researchers suggested that high levels of marbling may be attributable to enhancement of preadipocyte proliferation which may be caused by the increase of CDC10 expression. The change of expression predicted from gene function, compared with the result obtained by ddPCR, made the *CDC10* gene strong candidate for involvement in marbling.

It has been reported that a marbling quantitative trait locus was mapped to genomic region containing the *CDC10* gene on bovine chromosome 4, using a half-sib family of Japanese Black beef cattle (Takasuga *et al*., 2007). Thus, the CDC10 was considered as a positional

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functional candidate for the gene responsible for marbling. Researchers herein analyzed the allele frequency distribution of a marker close to the CDC10 in high-marbled and low-marbled cattle. Researchers further explored polymorphism in the promoter region of the *CDC10* 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 previously (Sasaki *et al*., 2006b). Musculus longissimus muscle tissues were obtained from these steers as described previously (Sasaki *et al*., 2006b). Researchers used 2 high-marbled cloned steers to confirm the authenticity of newly discovered Single Nucleotide Polymorphism (SNP) in the *CDC10* 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 (Nigata, Japan).

Microsatellite marker genotyping: Researchers screened the cattle genome maps (NAGRP Cattle Genome Coordination Program) and obtained BM6437 microsatellite marker as the close marker to the *CDC10* gene. PCR amplification of the BM6437 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.animalgenome.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 the NCBI databases (National Center for Biotechnology Information, Bethesda, MD) and obtained bovine genomic sequence (NC_007302) containing the *CDC10* gene. PCR primers were designed to target ~4 kb proximal promoter region for the *CDC10* 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 ABI 3730 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.63629097G>C detected in the promoter region of the *CDC10* gene was genotyped by PCR-restriction Fragment Length Polymorphism (RFLP) Method. PCR primers used for PCR-RFLP were 5'-TTAACCTTAGCGGCGGTGTT-3' and 5'-CTCGCGAGA TTACTTCATTGCTG-3'. PCR amplifications were carried out as described in polymorphism detection section using a final volume of 20 μL and the annealing temperature of 58°C. An aliquot of PCR-amplified products was digested at 37°C for 2 h with restriction enzyme HpaII and electrophoresed on a 2.0% agarose gel. Agarose gels were stained with ethidium bromide and photographed under an ultraviolet light.

Statistical analysis: The allelic distributions of the BM6437 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 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 *CDC10* gene expression patterns in ddPCR analysis (Sasaki *et al*., 2006b) for the BM6437 microsatellite marker close to the *CDC10* gene. This genotyping analysis revealed polymorphism of the BM6437 between the high-marbled and low-marbled steer groups. The low-marbled steers were homozygous for 267 bp allele at the BM6437 whereas the high-marbled steers heterozygous for 239 bp allele and 267 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 BM6437. 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 BM6437 (Table 1). The frequency of the 239 bp allele at the BM6437 was higher in animals with the high breeding value than with the low one and the 267 bp allele frequency in animals with the low one than with the high one (Table 1).

Researchers second sequenced the promoter region of the *CDC10* gene from 2 low-marbled Holstein steers and 2 high-marbled Japanese Black steers. This sequence analysis revealed only one SNP (g.63629097G>C) in the *CDC10* gene: a G to C substitution located 323 bp upstream of the transcription initiation site. The low-marbled steers were homozygous for G allele at the g.63629097G>C site whereas the high-marbled steers homozygous for C 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.63629097G>C SNP. The GG homozygotes, the CC homozygotes and the CG heterozygotes resulted in two bands (57 and 129 bp), one band (186 bp) and three bands (57, 129 and 186 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.63629097G>C SNP (Table 2).

Thus, researchers showed the higher frequency of the BM6437 239 bp allele in high-marbled cattle as compared to low-marbled cattle although, the allelic distribution of the g.63629097G>C SNP detected in promoter region of the *CDC10* 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 CDC10 on bovine chromosome 4 (Takasuga *et al*., 2007) suggests that the BM6437 microsatellite marker is associated with marbling and may be useful for effective marker-assisted selection to increase the levels of marbling. Researchers can hypothesize that an unidentified true causal mutation which is in linkage disequilibrium with the BM6437 marker but not the g.63629097G>C SNP directly affects changes in *CDC10* gene expression and/or marbling.

Table 1: Comparison of allelic distributions in BM6437 between 17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one

	Frequency		
Sires	239 bp allele	267 bp allele	p-value
With high breeding value	0.250	0.750	<0.005
With low breeding value	0.000	1.000	-

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	G allele	p-value
With high breeding value	0.559	0.441	0.209
With low breeding value	0.706	0.294	-

CONCLUSION

In this study, researchers show that the frequency of the 239 bp allele at the BM6437 microsatellite marker close to the *CDC10* gene is higher in high-marbled cattle than in low-marbled cattle although, the allelic distribution of the g.63629097G>C SNP detected in promoter region of the *CDC10* 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 BM6437 marker but not the g.63629097G>C SNP may be related to changes in *CDC10* gene expression and/or marbling.

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