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Useful g.70014208G>A SNP Marker in *MYBPC1* Gene Showing Marbling-Associated Expression Changes

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Abstract: Marbling, defined by the amount and distribution of intramuscular fat is an economically important trait of beef cattle in Japan. Researchers have earlier showed that the Myosin Binding Protein C, slow type (*MYBPC1*) gene, known to interact with muscle protein including myosin, actin and titin to regulate muscle contraction and to express in slow-twitch oxidative fiber that is observed in high-marbled muscle and involved in development and maintenance of the fiber, 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 (DIK4787) close to the MYBPC1 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 3 Single Nucleotide Polymorphisms (SNPs) in the promoter region of the *MYBPC1* gene between low-marbled Holstein and high-marbled Japanese Black steer groups. A SNP in the upstream of the promoter of the MYBPC1, referred to as g.70014208G>A, exhibited significantly different allelic distribution between animals with extremely high predicted breeding value for marbling and with extremely low one ($p = 0.046$). The other 2 SNPs seemed not to segregate in Japanese Black beef cattle. The findings suggest that the g.70014208G>A SNP may be related to changes in *MYBPC1* gene expression and/or marbling. The g.70014208G>A SNP marker may be useful for effective marker-assisted selection to increase the levels of marbling.

Key words: Allelic distribution, close marker, marbling, MYBPC1, 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, 1998). 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 earlier 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 Myosin Binding Protein C, slow type (*MYBPC1*) gene which is known to be one isoform (in slow skeletal muscle) of myosin binding protein C that is one of the major myosin-binding

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proteins in vertebrate striated muscles (Offer *et al*., 1973; Pepe and Drucker, 1975; Sato *et al*., 2003), exhibited higher expression levels in high-marbled Japanese Black steer group than in low-marbled Holstein steer group in the early and middle stages of the test period (Sasaki *et al*., 2006b). It has been reported that MYBPC1 is physically and functionally associated with Muscle-type Creatine Kinase (MM-CK) and MYBPC1 acts as an adaptor to connect the ATP consumer (myosin) and the regenerator (MM-CK) for efficient energy metabolism and homoeostasis during muscle contraction (Chen *et al*., 2011). The MYBPC1 is expressed in slow-twitch oxidative fiber that is observed in high-marbled muscle and involved in development and maintenance of the fiber. Researchers suggested that the high level of MYBPC1 expression in muscle may increase the efficiency of energy metabolism and homoeostasis to play a role in the development of marbling. Thus, the MYBPC1 was considered as a functional candidate for the gene responsible for marbling.

To examine whether polymorphism in the MYBPC1 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 MYBPC1 in high-marbled and low-marbled cattle. Researchers further explored polymorphism in the promoter region of the *MYBPC1* 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 earlier 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 earlier (Sasaki *et al*., 2006b). Researchers used 2 high-marbled cloned steers to confirm the authenticity of newly discovered Single Nucleotide Polymorphism (SNP) in the *MYBPC1* 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 DIK4787 microsatellite marker as the close marker to the *MYBPC1* gene. PCR amplification of the DIK4787 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_007303) containing the *MYBPC1* gene. PCR primers were designed to target ~7 kb proximal promoter region of the *MYBPC1* 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). Primer sequences will be available on request.

SNP genotyping: The 3 SNPs, g.70014208G>A, g.70015100T>G and g.70015741A>G SNPs, detected in the promoter region of the *MYBPC1* gene were genotyped by PCR-Restriction Fragment Length Polymorphism (RFLP) Method. For g.70014208G>A SNP, PCR primers used for PCR-RFLP were 5'-GATCCCATGGACTACAGCC TACC-3' and 5'-ACGGTAAAGCGACTGCCTACA-3'. For g.70015100T>G SNP, PCR primers used for PCR-RFLP were 5'-AAGACCCTGATGCTACGAAAG-3' and 5'-AAGAATACGGGAGTCGGTTG-3'. For g.70015741A>G SNP, PCR primers used for PCR-RFLP were 5'-AAAAT AAGAGAGGAAGACTCACCC-3' and 5'-CAAAGTA TTGTACCTGGTTACCGA-3'. PCR amplifications were carried out as described in polymorphism detection section using a final volume of 20 μL and the annealing temperature of 65°C (g.70014208G>A), 58°C (g.70015100T>G) and 58°C (g.70015741A>G). An aliquot of PCR-amplified products was digested at 37°C for 2 h with restriction enzyme BglII (g.70014208G>A), HpaII (g.70015100T>G) and AluI (g.70015741A>G) 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 DIK4787 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 earlier shown to have different *MYBPC1* gene expression patterns in ddPCR analysis (Sasaki *et al*., 2006b) for the DIK4787 microsatellite marker close to the *MYBPC1* gene. This genotyping analysis revealed polymorphism of the DIK4787 between the high-marbled and low-marbled steer groups. The high-marbled steers were homozygous for 222 bp allele at the DIK4787 whereas the low-marbled steers homozygous for 226 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 DIK4787. 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 DIK4787 (Table 1). The frequency of the 222 bp allele at the DIK4787 was higher in animals with the high breeding value than with the low one, on the contrary, the frequency of 226 bp allele was lower in animals with the high one than with the low one (Table 1).

Researchers second sequenced the ~7 kb proximal promoter region of the *MYBPC1* gene from 2 low-marbled Holstein steers and 2 high-marbled Japanese Black steers. This sequence analysis revealed 3 SNPs in the promoter region from the *MYBPC1* gene: g.70014208G>A is a G to A substitution located 4927 bp upstream from the transcription initiation site; g.70015100T>G is a T to G substitution located 4035 bp upstream of the transcription initiation site; g.70015741A>G is an A to G substitution located 3394 bp upstream from the transcription initiation site. The low-marbled steers were homozygous for A allele at the g.70014208G>A whereas the high-marbled steers heterozygous for A and G alleles at the SNP. The low-marbled steers were heterozygous for G and T alleles at the g.70015100T>G whereas the high-marbled steers homozygous for T allele at the SNP. The low-marbled steers were heterozygous for G and A alleles at the g.70015741A>G whereas the high-marbled steers homozygous for A allele at the SNP.

Researchers third established genotyping method based on PCR-RFLP. For the g.70014208G>A SNP, the AA homozygotes, the AG heterozygotes and GG homozygotes resulted in 2 bands (217 and 61 bp), 3 bands (278, 217 and 61 bp) and 1 band (278 bp), respectively. For the g.70015100T>G SNP, the TT homozygotes, the TG heterozygotes and GG homozygotes resulted in 3 bands (330, 60 and 40 bp), 4 bands (390, 330, 60 and 40 bp) and 2 bands (390 and 40 bp), respectively. For the g.70015741A>G SNP, the AA homozygotes, the AG heterozygotes and GG homozygotes resulted in 1 band (334 bp), 3 bands (334, 245 and 89 bp) and 2 bands (245 and 89 bp), respectively.

Table 1: Comparison of allelic distribution in DIK4787 between 17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one

	Frequency		
Sires	222 bp allele	226 bp allele	p-value
With high breeding value	0.447	0.553	<0.005
With low breeding value	0.125	0.875	-

Researchers fourth 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 using this PCR-RFLP Method. The g.70014208G>A SNP exhibited significant difference in the allele frequency distribution between 17 sires with extremely high breeding value and 17 sires with extremely low one (Table 2). The frequency of the G allele at the g.70014208G>A SNP was higher in animals with extremely high breeding value than with extremely low one and the A allele frequency in animals with the low one than with high low (Table 2). Genotyping 34 sires for the g.70015100T>G and g.70015741A>G SNPs showed no detection of the G allele in each SNP, suggesting that the g.70015100T>G and g.70015741A>G SNPs seem to fix at the T allele and A allele, respectively and not to segregate in Japanese Black beef cattle.

Thus, researchers showed the higher frequency of the DIK4787 222 bp allele and the MYBPC1 g.70014208G>A G allele in high-marbled cattle as compared to low-marbled cattle. This study suggests that the g.70014208G>A SNP in promoter region of the *MYBPC1* gene might be related to changes in gene expression and/or marbling. On the other hand, although, it may be true that the SNP itself is functional and directly affect the gene expression and/of marbling, a more likely event is that an unidentified true causal mutation which is in linkage disequilibrium with the DIK4787 microsatellite and g.70014208G>A SNP, directly affects changes in *MYBPC1* gene expression and/or marbling. The MYBPC1 g.70014208G>A SNP may be useful for effective marker-assisted selection to increase the levels of marbling. Researchers have identified the EDG1 g.1471620G>T SNP (Yamada *et al*., 2009a), the TTN g.231054C>T SNP (Yamada *et al*., 2009b), the RPL27A g.3109537C>T SNP (Yamada *et al*., 2009c), the AKIRIN2 c.*188G>A SNP (Sasaki *et al*., 2009), BM6437 microsatellite close to the CDC10 (Tong *et al*., 2012a), BMS817 microsatellite close to the TRDN (Tong *et al*., 2012b), IDVGA-49 microsatellite close to the MFN2 (Tong *et al*., 2012c) as useful molecular maker for marbling. This study is the eighth report on useful marbling marker.

Table 2: Comparison of allelic distribution in the g.70014208G>A SNP between 17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one

	Frequency		
Sires	G allele	A allele	p-value
With high breeding value	0.176	0.824	0.046
With low breeding value	0.029	0.971	-

CONCLUSION

In this study, researchers show that the frequencies of the 222 bp allele and the G allele at the DIK4787 microsatellite close to the *MYBPC1* gene and the *MYBPC1* gene, respectively are higher in high-marbled cattle than in low-marbled cattle. This study suggests that the g.70014208G>A SNP itself or an unidentified true causal mutation which is in linkage disequilibrium with the DIK4787 marker and the g.70014208G>A SNP may be related to changes in *MYBPC1* gene expression and/or marbling. The g.70014208G>A SNP may be useful for effective marker-assisted breeding to increase the levels of marbling.

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REFERENCES

Boylston, T.D., S.A. Morgan, K.A. Johnson, J.R. Busboom, R.W. Wright Jr. and J.J. Reeves, 1995. Lipid content and composition of Wagyu and domestic breeds of beef. J. Agric. Food Chem., 43: 1202-1207.

Busboom, J.R., L.E. Jeremiah, L.L. Gibson, K.A. Johnson, C.T. Gaskins, J.J. Reeves and R.W. Wright, 1993. Effects of biological source on cooking and palatability attributes of beef produced for the Japanese market. Meat Sci., 35: 241-258.

Chen, Z., T.J. Zhao, J. Li, Y.S. Gao, F.G. Meng, Y.B. Yan and H.M. Zhou, 2011. Slow skeletal muscle myosin-binding protein-C (MyBPC1) mediates recruitment of muscle-type Creatine Kinase (CK) to myosin. Biochem. J., 436: 437-445.

JMGA, 1998. New Beef Carcass Grading Standards. Japan Meat Grading Association, Tokyo, Japan.

Matsuishi, M., M. Fujimori and A. Okitani, 2001. Wagyu beef Aroma in Wagyu (Japanese black cattle) beef preferred by the Japanese over imported beef. Anim. Sci. J., 72: 498-504.

OPIAI, 1999. Sire summary. Oita Prefectural Institure of Animal Industry, Oita, Japan.

Offer, G., C. Moos and R. Starr, 1973. A new protein of the thick filaments of vertebrate skeletal myofibrils: Extraction, purification and characterization. J. Mol. Biol., 74: 653-676.

Pepe, F.A. and B. Drucker, 1975. The myosin filament. III. C-protein. J. Mol. Biol., 99: 609-617.

Sasaki, S., T. Yamada, S. Sukegawa, T. Miyake and T. Fujita *et al*., 2009. Association of a single nucleotide polymorphism in *akirin* 2 gene with marbling in Japanese Black beef cattle. BMC Res. Notes, Vol. 2. 10.1186/1756-0500-2-131.

Sasaki, Y., T. Miyake, C. Gaillard, T. Oguni and M. Matsumoto *et al*., 2006a. Comparison of genetic gains per year for carcass traits among breeding programs in the Japanese Brown and the Japanese Black cattle. J. Anim. Sci., 84: 317-323.

Sasaki, Y., K. Nagai, Y. Nagata, K. Doronbekov and S. Nishimura *et al*., 2006b. Exploration of genes showing intramuscular fat deposition-associated expression changes in musculus longissimus muscle. Anim. Genet., 37: 40-46.

Sato, N., T. Kawakami, A. Nakayama, H. Suzuki, H. Kasahara and T. Obinata, 2003. A novel variant of cardiac myosin-binding protein-C that is unable to assemble into sarcomeres is expressed in the aged mouse atrium. Mol. Biol. Cell, 4: 3180-3191.

Shiga, K., T. Fujita, K. Hirose, Y. Sasae and T. Nagai, 1999. Production of calves by transfer of nuclei from cultured somatic cells obtained from Japanese black bulls. Theriogenology, 52: 527-535.

Tong, B., Y. Muramatsu, T. Yamamoto, H. Tanomura and T. Ohta *et al*., 2012a. Polymorphism in promoter region of *CDC10* gene showing marbling-associated expression changes. J. Anim. Vet. Adv., 11: 1930-1933.

Tong, B., Y. Muramatsu, T. Yamamoto, H. Tanomura and T. Ohta *et al*., 2012b. Useful marker around TRDN gene showing marbling-associated expression changes. J. Anim. Vet. Adv., 15: 2792-2795.

Tong, B., Y. Muramatsu, T. Yamamoto, H. Tanomura and T. Ohta *et al*., 2012c. Preliminary association study of SNPs in *MFN2* gene showing marbling-associated expression changes. J. Anim. Vet. Adv., 15: 2796-2800.

Yamada, T., S. Sasaki, S. Sukegawa, T. Miyake and T. Fujita *et al*., 2009a. Novel SNP in 5' flanking region of EDG1 associated with marbling in Japanese Black beef cattle. Anim. Sci. J., 80: 486-489.

Yamada, T., S. Sasaki, S. Sukegawa, S. Yoshioka and Y. Takahagi *et al*., 2009b. Association of a single nucleotide polymorphism in titin gene with marbling in Japanese Black beef cattle. BMC Res. Notes, Vol. 2. 10.1186/1756-0500-2-78.

Yamada, T., S. Sasaki, S. Sukegawa, T. Miyake and T. Fujita *et al*., 2009c. Association of a single nucleotide polymorphism in ribosomal protein L27a gene with marbling in Japanese Black beef cattle. Anim. Sci. J., 80: 631-635.