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Polymorphisms in Promoter Regions of *MYH1* and *IRS1* Genes 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. Previously, researchers have found that the Myosin Heavy Chain 1 (*MYH1*) gene coding for an isoform of myosin heavy chain a major contractile protein which converts chemical energy into mechanical energy in muscle which is expressed in slow-twitch oxidative fiber and involved in development of the fiber and Insulin Receptor Substrate 1 (*IRS1*) gene encoding a signaling adaptor protein which is phosphorylated by insulin receptor and associated with insulin resistance and birth weight, possess expression differences in musculus longissimus muscle between low-marbled and high-marbled steer groups. In the present study, researchers detected Single Nucleotide Polymorphisms (SNPs), g.29850738G>A and g.120947716T>C in the promoter regions of the *MYH1* and *IRS1* genes, respectively between the 2 steer groups. However, the allelic distributions of the 2 SNPs were indistinguishable between Japanese Black sires with extremely high predicted breeding value for marbling and with extremely low one. An unknown SNP in the distal region upstream of ~4 kb proximal promoter regions of the *MYH1* and *IRS1* genes might be related to changes in gene expression and/or marbling.

Key words: Allelic distribution, gene expression, IRS1, marbling, MYH1, 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). 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*., 2006a, b). Among the detected genes, myosin heavy chain 1 *(MYH1)* gene which encodes an isoform of myosin heavy chain in type I (slow-oxidative) fiber of skeletal muscle (Leinwand *et al*., 1983) 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*., 2006a, b). The Insulin Receptor Substrate 1 (*IRS1*) gene is known to encode signaling adaptor that plays a major role in the metabolic and mitogenic actions of theinsulin and Insulin-like Growth Factors (IGF). Because the IRS1 knockout mice only reach 50% of the weight of normal mice thus the signals delivered by IRS1 may regulate hepatic gene expression that coordinates glucose homeostasis and

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systemic growth (Dong *et al*., 2006). *IRS1* gene showed higher expression levels in high-marbled steer group than in low-marbled steer group across all ages of the test period (Sasaki *et al*., 2006a, b). Researchers suggested that high levels of marbling may be attributable to energy conversion manner in muscular fiber type I which may be caused by the increase of MYH1 expression and promotion of energy uptake in adipocyte by the increase of IRS1 expression, respectively.

Researchers herein explored polymorphisms in the promoterregions of the *MYH1* and *IRS1* genes and examined allelic distribution in the polymorphism between Japanese black cattle steers with extremely high predicted breeding value for marbling and with extremely low one.

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*., 2006a, b) were used for polymorphism detection in this study. The details of these steers are described earlier (Sasaki *et al*., 2006a, b). Musculus longissimus muscle tissues were obtained from these steers as described earlier (Sasaki *et al*., 2006a, b). Researchers used 2 high-marbled cloned steers to confirm the authenticity of newly discovered Single Nucleotide Polymorphisms (SNPs) in the *MYH1* and *IRS1* genes.

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 SNP genotyping in this study. The predicted breeding values were obtained from the recording system for beef cattle reported by Sasaki *et al*. (2006a, b). 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).

Polymorphism detection: Researchers screened the NCBI databases (National Center for Biotechnology Information, Bethesda, MD) and obtained bovine genomic sequences (NC_007317 and NC_007300) containing the *MHY1* and *IRS1* genes, respectively. PCR primers were designed to target ~4 kb proximal promoter regions for the 2 genes using these genomic sequences in order to screen polymorphisms in the genes 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, Madison, WI) 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 2.0% 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, Foster city, CA) 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 SNPs detected in the ~4 kb promoter regions of the MYH1 (g.29850738G>A) and IRS1 (g.120947716T>C) were genotyped by PCR-Restriction Fragment Length Polymorphism (RFLP) Method. PCR primers and restriction enzymes used for PCR-RFLP were indicated in Table 1. PCR amplifications were carried out as described in Polymorphism Detection section using a final volume of 20 μL and the annealing temperature indicated in Table 1. An aliquot of PCR-amplified products was digested at 37°C for the g.29850738G>A and g.120947716T>C SNPs for 2 h with restriction enzyme Hpy188I and SfcI, respectively and electrophoresed on a 2.0% agarose gel. Agarose gels were stained with ethidium bromide and photographed under an ultraviolet light.

Table 1: SNPs detected in the promoter regions and primers and restriction enzymes used for PCR-RFLP Method

			SNP genotype		PCR-RFLP Method	
Gene name	GenBank Accession No.[a]	SNP detected	High-marbled steer group	Low-marbled steer group	Forward primer sequence	Reverse primer sequence
MYH1	NC_007317	g.29850738G>A	GG	AG	AGACACGACTGAAGCAACTTAGGG	TGAGTTCCTACTAGACCCCAGTATCAIRS1
IRSI	NC_007300	g.120947716T>C	TT	CT	TGCGCTAAACATCAAACCTG	AACTGGGGTAGCCGGTAACT

[a]The GenBank accession number was obtained by searching against bovine genomic sequence using the NCBI databases

Statistical analysis: The allelic distributions in the detected SNPs 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 sequenced the promoter regions of the *MYH1* and *IRS1* genes from 2 low-marbled Holstein steers and 2 high-marbled cloned steers which were earlier shown to have different *MYH1* and *IRS1* genes expression patterns in ddPCR analysis (Sasaki *et al*., 2006a, b). This sequence analysis revealed only one SNP in the *MYH1* gene: a G to A transition located 2622 bp upstream from the transcription initiation site (g.29850738G>A) only one SNP in the *IRS1* gene: a T to C transition located 2910 bp upstream from the initiation site (g.120947716T>C) (Table 1). The 2 high-marbled steers were homozygous for G allele at the g.29850738G>A site, whereas the 2 low-marbled steers heterozygous for G allele and A allele. The 2 high-marbled steers were homozygous for T allele at the g.120947716T>C site whereas the 2 low-marbled steers heterozygous for C allele and T allele (Table 1).

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 each SNP site. No significant difference in the allelic distribution between 17 sires with extremely high breeding value and 17 sires with extremely low one was detected at the both SNPs (Table 2).

A number of data have shown that the type of muscle fibers is a factor that influences growth and meat quality (Rehfeldt and Kuhn, 2006). A study using histochemical staining with Sudan Black B and Oil Red O showed that all type I fibers contained neutral lipids whereas types IIA and IIB fibers only contained 26 and 1%, respectively (Karlsson *et al*., 1999). Thus, the percentage of type I fiber is positively correlated with intramuscular fat content in cattle (Calkins *et al*., 1981) and a high type I fiber content contributes more to juiciness and flavor whereas a high content of type IIB fiber tends to be associated with tougher meat. Indeed although, most of these results were obtained within limited multi-factorial designs such as relative to genetic and environmental factors there is a consensus indicating a correlation between type I fiber content and meat tenderness (Renand *et al*., 2001). Thus, the MYH1 encoding an isoform of myosin heavy chain in type I fiber was regarded as a functional candidate for the gene responsibility for marbling. In addition, many insulin responses, especially those that are associated with somatic growth and carbohydrate metabolism are mediated largely through IRS1 (Kido *et al*., 2000) moreover ablation of IRS1 results in growth retardation and mild insulin resistance (Tamemoto *et al*., 1994). This allowed the IRS1 to be regarded as a functional candidate for the causal gene. Further, it has been reported that marbling quantitative trait loci were mapped to genomic regions containing the *MYH1* and *IRS1* genes on bovine chromosomes 19 and 2, respectively using a half-sib family of Japanese Black beef cattle (Takasuga *et al*., 2007). Thus, the *MYH1* and *IRS1* genes were considered as a positional functional candidate for the gene responsibility for marbling. However, researchers obtained the results of no association between g.29850738G>A and g.120947716T>C SNPs and marbling. Taken altogether, researchers hypothesize that an unknown SNP in the distal region upstream of ~4 kb proximal promoter regions of the *MYH1* and *IRS1* genes might be related to changes in gene expression and/or marbling. The *MYH1* and *IRS1* genes have a functional relevancy to growth-related carcass traits and therefore in the further study, it is interesting to investigate the association between the SNPs, g.29850738G>A and g.120947716T>C and carcass weight, rib thickness, rib eye area subcutaneous fat thickness and daily gain in Japanese Black beef cattle.

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

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

	g.29850738G>A				g.120947716T>C			
	Frequency				Frequency			
Sires	G allele (74.79)	A allele (153)	χ^2-value	p-value	T allele (19,433)	C allele (19,101,332)	χ^2-value	p-value
With high breeding value	0.882	0.118	0.731	0.3925	0.882	0.118	0.0000	1.0000
With low breeding value	0.941	0.059	-	-	0.882	0.118	-	-

The size (bp) of the restriction fragments detected in PCR-RFLP Method is shown in parentheses. The inheritance of the restriction fragments followed Mendelian inheritance patterns

(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) the MYBPC1 g.70014208G>A SNP (Tong *et al*., 2012a) as useful molecular maker for marbling. Regrettably, researchers could not regard g.29850738G>A and g.120947716T>C SNPs as useful marbling marker.

CONCLUSION

In this study, researchers show that the g.29850738G>A SNP in *MYH1* gene and the g.120947716T>C SNP in *IRS1* gene were not associated with development of marbling in Japanese Black cattle. An unknown SNP in the distal region upstream of ~4 kb proximal promoter regions of the *MYH1* and *IRS1* genes might be related to changes in gene expression and/or 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.

Calkins, C.R., T.R. Dutson, G.C. Smith, Z.L. Carpenter and G.W. Davis, 1981. Relationship of fiber type compositon to marbling and tenderness of bovine muscle. J. Food Sci., 46: 708-710.

Dong, X.C., S. Park, X.Y. Lin, K. Copps, X.J. Yi and M.F. White, 2006. Irs1 and Irs2 signaling is essential for hepatic glucose homeostasis and systemic growth. J. Clin. Invest., 116: 101-114.

JMGA, 1988. New Beef Carcass Grading Standards. Japan Meat Grading Association, Tokyo, Japan.

Karlsson, A.H., R.E. Klont and X. Fernandez, 1999. Skeletal muscle fibres as factors for pork quality. Liv. Prod. Sci., 60: 255-269.

Kido, Y., D.J. Burks, D. Withers, J.C. Bruning, C.R. Kahn, M.F. White and D. Accili, 2000. Tissue-specific insulin resistance in mice with mutations in the insulin receptor, IRS-1 and IRS-2. J. Clin. Invest., 105: 199-205.

Leinwand, L.A., L. Saez, E. McNally and B. Nadal-Ginard, 1983. Isolation and characterization of human myosin heavy chain genes. Proc. Natl. Acad. Sci. USA., 80: 3716-3720.

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.

Rehfeldt, C. and G. Kuhn, 2006. Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. J. Anim. Sci., 84: E113-E123.

Renand, G., B. Picard, C. Touraille, P. Berge and J. Lepetit, 2001. Relationships between muscle characteristics and meat quality traits of young Charolais bulls. Meat Sci., 59: 49-60.

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., 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.

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.

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.

Takasuga, A., T. Watanabe, Y. Mizoguchi, T. Hirano and N. Ihara *et al*., 2007. Identification of bovine QTL for growth and carcass traits in Japanese Black cattle by replication and identical-by-descent mapping. Mammalian Genome, 18: 125-136.

Tamemoto, H., T. Kadowaki, K. Tobe, T. Yagi and H. Sakura *et al*., 1994. Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. Nature, 372: 182-186.

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, 2: 78-78.

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.