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Possible Effects of Single Nucleotide Polymorphism in *CDC10*, *IRS1* and *MFN2* Genes on Growth-Related Traits in Japanese Black Beef Cattle

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Abstract: Growth performance as well as marbling is the main breeding objectives in Japanese Black cattle, the major beef breed in Japan. Researchers have recently reported that existence of Single Nucleotide Polymorphisms (SNPs), g.63629097G>C, g.120947716T>C and g.38437771C>T in the promoter regions of the septin 7 (CDC10), the insulin receptor substrate 1 (*IRS1*) and the mitofusin 2 (*MFN2*) genes, respectively in Japanese Black beef cattle population. The CDC10 is known to be involved in cellular proliferation and the IRS1 is reported to be associated with insulin resistance and birth weight. The 2 genes have been earlier shown to be located within genomic regions of quantitative trait loci for growth-related traits. Thus, the *CDC10* and *IRS1* genes were considered as positional functional candidates for the gene responsible for growth performance. In addition, the MFN2 is known to play a role in energy balance through mitochondrial fusion, so the *MFN2* gene was considered as functional candidate. In this study, researchers analyzed the possible effects of the 3 SNPs on the growth-related carcass traits: Carcass Weight (CWT), Rib Thickness (RT), Rib Eye Area (REA) and Subcutaneous Fat Thickness (SFT) in Japanese Black beef cattle. The g.63629097G>C and g.120947716T>C SNPs in the CDC10 and IRS1, respectively, exhibited significantly different allelic distribution between Japanese Black sires with extremely high predicted breeding value and the sires with extremely low one for CWT and RT but not for the others. The g.38437771C>T SNP in the MFN2 had significantly different allelic distribution for RT. These findings suggest possible effects of the g.63629097G>C, g.120947716T>C and g.38437771C>T SNP on the growth-related trait in Japanese Black beef cattle. The SNPs in the *CDC10*, *IRS1* and *MFN2* genes may be useful for effective marker-assisted selection to increase the beef productivity in Japanese Black beef cattle.

Key words: Association analysis, CDC10, growth-related carcass trait, IRS1, Japanese Black beef cattle, MFN2, single nucleotide polymorphism

INTRODUCTION

Growth performance has an important effect on the economics of beef production (JMGA, 1998). Thus, it is greatly interesting to obtain better knowledge on the molecular architecture of growth characteristics and to generate new opportunities for more effective marker-assisted selection.

Researchers have recently reported that Single Nucleotide Polymorphisms (SNPs) referred to as g.63629097G>C, g.120947716T>C and g.38437771C>T SNPs, respectively were located in the promoter regions of the septin 7 (CDC10), the Insulin Receptor Substrate 1 (*IRS1*) and the Mitofusin 2 (*MFN2*) genes but the SNPs had no significant effect on marbling in Japanese Black beef cattle (Tong *et al*., 2012a-c).

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The CDC10 is known to be involved in cellular proliferation (Nagata *et al*., 2004). Researchers have also located the *CDC10* gene within genomic region of Quantitative Trait Loci (QTL) for Rib Eye Area (REA) and Subcutaneous Fat Thickness (SFT) which are mapped in a half-sib family of Japanese Black beef cattle to bovine chromosome 4 region (Takasuga *et al*., 2007). The IRS1 is reported to be signaling adaptor that plays a major role in the metabolic and mitogenic actions of the insulin and insulin-like growth factors. 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 systemic growth (Dong *et al*., 2006). Furthermore, the *IRS1* gene was located in bovine chromosome 2 region containing QTL for REA and SFT (Takasuga *et al*., 2007). Thus, the *CDC10* and *IRS1* genes were regarded as positional functional candidates for the gene responsible for growth performance.

In addition, the Mitofusin 2 (*MFN2*) gene is known to be mitochondrial membrane protein that participates in mitochondrial fusion in mammalian cells and is crucial to the maintenance and operation of the mitochondrial network and to mitochondrial metabolism in muscle cells (Bach *et al*., 2003). So, researchers hypothesized that the *MFN2* gene may be a functional candidate gene for growth-related carcass trait.

Researchers herein analyzed the allele frequency distribution in the g.63629097G>C, the g.120947716T>C and the g.38437771C>T SNPs between extremely high-performance and extremely low-performance Japanese Black sires for growth-related carcass trait.

MATERIALS AND METHODS

Samples: Researchers used 34 Japanese Black unrelated sires (17 sires with extremely high predicted breeding value for each growth-related carcass trait 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 sires were used either at present or earlier at the Oita Prefectural Institute of Animal Industry (Oita, Japan). There was no strong bias for a specific father or a specific maternal grandfather of the sires and the sire panel likely represents a variety of the sire line. Semen or blood were collected and DNA samples were prepared from the materials according to standard protocols.

The growth-related carcass traits, Carcass Weight (CWT), Rib Thickness (RT), REA and SFT were measured on carcasses dissected at the sixth and seventh rib section, according to the Japanese meat grading system by certified graders from the Japan Meat Grading Association (Tokyo, Japan) (JMGA, 1998). The predicted breeding values of the sires for CWT, REA, RT and SFT were obtained from the Oita recording system for beef cattle previously reported by Sasaki *et al*. (2006). In the recording system, the breeding values were predicted from carcass records of Japanese Black steers and heifers, fattened in the Oita prefecture. The fattened animals were shipped to various carcass markets from 1988 to 2003 where they were slaughtered and their carcasses evaluated. The data were edited to connect across subclasses such that each market-year subclass had 50 or more animals and each farm had 10 or more animals. The final number of animals was 48,045 and there were 89 market-year subclasses, 332 farms and 228 sires. The accuracy of the predicted breeding values in the 101 sires was 0.935±0.008, ranging from 0.770-0.990.

Data were analyzed by the REML Method using the MTDFREML programs (Boldman *et al*., 1995) and genetic and environmental variances were estimated. The BLUP option in the programs using the estimated variance components was chosen to predict the breeding values of animals with a single trait model. Sex, market-year and farm were considered fixed effects. Fattening period and slaughter age were also considered as up to quadratic covariates. The fattening period denotes the period from the start of fattening to shipping to market for each animal. These fixed effects were all significant ($p<0.001$). Random effects included the additive genetic effect of the individuals that is the animal model was adopted to predict the breeding values.

This study conformed to the guidelines for animal experimentation of the Graduate School of Science and Technology, Niigata University (Niigata, Japan).

SNP genotyping: The g.63629097G>C, the g.120947716T>C and the g.38437771C>T SNPs were genotyped by the PCR-restriction fragment length polymorphism method as described earlier (Tong *et al*., 2012a-c). For the g.63629097G>C SNP, 186-bp PCR fragments containing the SNP site were amplified and *Hpa*II-digested into 57 and 129 bp fragments at the G allele but not the C allele: the GG homozygotes, the CC homozygotes and the GC heterozygotes yielded 2 bands (57 and 129 bp), 1 band (186 bp) and 3 bands (57, 129 and 186 bp), respectively. For the g.120947716T>C SNP, 452 bp PCR fragments containing the SNP site were amplified and SfcI-digested into 19, 101 and 332 bp fragments at the C allele but into 19 and 433 bp fragments at the T allele: the TT homozygotes, CC homozygotes the TC heterzygotes yielded 2 bands (19 and 433 bp), 3 bands

(19, 101 and 332 bp) and 4 bands (19, 101, 332 and 433 bp), respectively. For the g.38437771C>T SNP, 292 bp PCR fragments containing the SNP site were amplified and Hpy 99I-digested into 57 and 235 bp fragments at the C allele but not the T allele: the CC homozygotes, the TT homozygotes and the CT heterozygotes resulted in 2 bands (57 and 235 bp), 1 band (292 bp) and 3 bands (57, 235 and 292 bp), respectively.

Statistical analysis: The allele frequency distributions of the 3 SNPs, g.63629097G>C, the g.120947716T>C and the g.38437771C>T were compared between 17 sires with extremely high predicted breeding value for each growth-related carcass trait 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 selected 34 Japanese Black unrelated sires consisting of 17 sires with extremely high predicted breeding value and 17 sires with extremely low one for each growth-related carcass trait of CWT, REA, RT and SFT from the 101 sires and genotyped the selected sires for the 3 SNPs, g.63629097G>C, the g.120947716T>C and the g.38437771C>T.

For the g.63629097G>C SNP in the *CDC10* gene, 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 CWT and RT (p = 0.009 and 0.011, respectively). The frequency of the G allele was higher in animals with extremely high breeding value than with extremely low one and the C allele frequency in animals with the low one than with the high one for both CWT and RT (Table 1). The CDC10 is known to be involved in cellular proliferation (Nagata *et al*., 2004) and the *CDC10* gene was located within genomic region of QTL for REA and SFT using a half-sib family of Japanese Black beef cattle (Takasuga *et al*., 2007; Yokouchi *et al*., 2009). These findings suggest that the g.63629097G>C SNP in CDC10 has possible effect on the growth-related trait in Japanese Black beef cattle.

For the g.120947716T>C SNP in the *IRS1* gene, the frequency of the T allele was significantly higher in animals with extremely high breeding value than with extremely low one and the C allele frequency in animals with the low one than with the high one for CWT and RT (p = 0.005 and 0.005, respectively) (Table 2). The IRS1 is known to be signaling adaptor that plays a major role in the metabolic and mitogenic actions of the insulin and insulin-like growth factors. Many insulin responses, especially those that are associated with somatic growth and carbohydrate metabolism are mediated largely through the *IRS1* gene (Kido *et al*., 2000). Moreover, ablation of the *IRS1* gene results in growth retardation and mild insulin resistance (Tamemoto *et al*., 1994). The *IRS1* gene was located in the bovine chromosome 2 region of QTL for REA and SFT (Takasuga *et al*., 2007). Taken together, researchers considered that the *IRS1* gene was regarded as functional positional candidate for the causal gene. This study suggest that the g.120947716T>C SNP in the *IRS1* gene shows possible effect on the growth-related trait in Japanese Black beef cattle.

For the g.38437771C>T SNP in the *MFN2* gene, 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 RT (p = 0.014). The frequency of the T allele was higher in animals with extremely high breeding value than with

Table 1: Comparison of allelic distributions in the g.63629097G>C SNP between 17 sires with extremely high predicted breeding value and 17 sires with extremely low one for each growth-related carcass trait

Growth-related carcass traits	Sires	Frequency		χ^2-value	p-value
		G allele	C allele		
CWT	High	0.438	0.563	6.783	0.009
	Low	0.147	0.853		
REA	High	0.294	0.706	0.706	0.401
	Low	0.206	0.794		
RT	High	0.500	0.500	6.439	0.011
	Low	0.206	0.794		
SFT	High	0.206	0.794	0.706	0.401
	Low	0.294	0.706		

Table 2: Comparison of allelic distributions in the g.120947716T>C SNP between 17 sires with extremely high predicted breeding value and 17 sires with extremely low one for each growth-related carcass trait

Growth-related carcass traits	Sires	Frequency		χ^2-value	p-value
		T allele	C allele		
CWT	High	0.794	0.206	7.803	0.005
	Low	1.000	0.000		
REA	High	0.853	0.147	0.146	0.703
	Low	0.882	0.118		
RT	High	0.794	0.206	7.803	0.005
	Low	1.000	0.000		
SFT	High	0.941	0.059	0.349	0.555
	Low	0.971	0.029		

Table 3: Comparison of allelic distributions in the g.38437771C>T SNP between 17 sires with extremely high predicted breeding value and 17 sires with extremely low one for each growth-related carcass trait

Growth-related carcass traits	Sires	Frequency		χ^2-value	p-value
		T allele	C allele		
CWT	High	0.294	0.706	0.706	0.401
	Low	0.206	0.794		
REA	High	0.206	0.794	0.327	0.567
	Low	0.265	0.735		
RT	High	0.412	0.588	6.028	0.014
	Low	0.176	0.824		
SFT	High	0.265	0.735	0.078	0.779
	Low	0.235	0.765		

extremely low one and the C allele frequency in animals with the low one than with the high one for RT (Table 3). The MFN2 is known to be mitochondrial membrane protein that participates in mitochondrial fusion in mammalian cells and is crucial to the maintenance and operation of the mitochondrial network and to mitochondrial metabolism in muscle cells. Skeletal muscle contains two subpopulations of mitochondria, Subsarcolemmal (SS) and Intermyofibrillar (IMF) mitochondria (Hood, 2001; Cogswell *et al*., 1993). The maintenance, operation and metabolism in these mitochondria are likely concerned with growth traits. In this study, possible effect of the g.38437771C>T SNP in the *MFN2* gene on growth-related trait is suggested.

CONCLUSION

In this study, researchers showed that the g.63629097G>C and the g.120947716T>C SNPs in the *CDC10* and *IRS1* genes have possible effect on CWT and RT and the g.38437771C>T SNP in the *MFN2* gene on RT in Japanese Black beef cattle. Although, it may be true that the each SNP itself is functional and directly affects the gene expression and/or CWT and RT or RT levels, a more likely event is that the each SNP is in linkage disequilibrium with an unidentified true causal mutation for growth-related carcass traits. Each SNP may be useful for marker-assisted selection to increase the beef productivity in Japanese Black beef cattle.

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