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Association of Single Nucleotide Polymorphism in *IRS1* Gene with Growth-Related Trait in Japanese Black Beef Cattle

[1, 7]Yao Wu, [2]Bin Tong, [3]Kaifeng Wu, [2]Seiki Sasaki, [4]Youji Muramatsu,
[5]Takeshi Ohta, [6]Hiroyuki Kose, [2]Takahisa Yamada, [3]Dong Zhang and [1]Guoxin Ni
[1]Department of Orthopeadics and Traumatology,
Southern Medical University, 510515 Guangzhou, China
[2]Department of Agrobiology, Faculty of Agriculture, Niigata University,
Nishi-ku, 950-2181 Niigata, Japan
[3]College of Life Science, Inner Mongolia Key Lab of Bio-Manufacture,
Inner Mongolia Agricultural University, 010018 Huhhot, China
[4]Department of Nutritional Sciences for Well-Being, Faculty of Health Sciences for Welfare,
Kansai University of Welfare Sciences, Kashiwara, 582-0026 Osaka, Japan
[5]Central Pharmaceutical Research Institute, Japan Tobacco, Inc., Takatsuki, 569-1125 Osaka, Japan
[6]Department of Life Science, Division of Natural Sciences,
International Christian University, Mitaka, 181-8585 Tokyo, Japan
[7]Department of Joint and Traumatology, The First Hospital of Hohhot, 010050 Hohhot, China

Abstract: Growth performance as well as marbling is the main breeding objectives in Japanese Black cattle, the major beef breed in Japan. The Insulin Receptor Substrate 1 (*IRS1*) gene is reported to be associated with insulin resistance and birth weight in human and has been previously shown to be located within genomic region of a quantitative trait locus for growth-related trait. The previous study detected a Single Nucleotide Polymorphism (SNP), referred to as g.120947716T>C in the promoter region of the *IRS1* gene which exhibited significantly different allelic distribution between Japanese Black sires with extremely high predicted breeding value and the sires with extremely low one for Carcass Weight (CWT) and Rib Thickness (RT) in a preliminary study. In this study, researchers analyzed the effect of the IRS1 g.120947716T>CSNP genotypes on the growth-related carcass trait in Japanese Black beef cattle. The SNP was associated with CWT and RT ($p = 0.030$ and 0.005, respectively) in experiment using 100 Japanese Black sires. This finding suggests possible effect of the g.120947716T>C on the growth-related trait in Japanese Black beef cattle. The IRS1 SNP may be useful for effective marker-assisted selection to increase the beef productivity in Japanese Black beef cattle.

Key words: Association, beef cattle, growth-related trait, IRS1, Japanese Black breed, single nucleotide polymorphism

INTRODUCTION

Growth performance has an important effect on the economics of beef production (JMGA, 1988). 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.

The Insulin Receptor Substrate 1 (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 a Quantitative Trait Locus (QTL) for growth-related carcass trait using a half-sib family of Japanese Black beef cattle (Takasuga *et al*., 2007). Thus, the *IRS1* gene was regarded as a positional functional candidate for the gene responsible for growth performance.

Researchers have recently reported that a Single Nucleotide Polymorphism (SNP), referred to as g.120947716T>C was located in the promoter region of the

Corresponding Author: Guoxin Ni, Department of Orthopeadics and Traumatology, Nanfang Hospital,
Southern Medical University, 1838 Guangzhou Avenue (N), 510515 Guangzhou, China

IRS1 gene (Tong *et al*., 2012a). In a preliminary study, researchers showed that the g.120947716T>C SNP exhibited significantly different allelic distribution between Japanese Black sires with extremely high predicted breeding value and the sires with extremely low one for Carcass Weight (CWT) and Rib Thickness (RT) and that the frequency of the C allele at the SNP was higher in animals with extremely high breeding value than with extremely low one and the T allele frequency in animals with the low one than with the high one for CWT and RT (Tong *et al*., 2012b). Thus, the IRS1 SNP seems to be a candidate marker for marker-assisted selection of growth performance in Japanese Black beef cattle.

Researchers herein analyzed association of the IRS1 g.120947716T>C SNP with the growth-related carcass trait in Japanese Black beef cattle.

MATERIALS AND METHODS

Samples and data: Researchers used 100 Japanese Black sires for the association study. The sires were used either at present or previously at the Oita Prefectural Institute of Animal Industry (Oita, Japan). There was no strong bias for a specific father or a specific maternal grand father of the sires and the sire panel likely represents a variety of the sire lines. Semen or blood from each sires were collected for SNP genotyping. DNA samples were prepared from the materials according to standard protocols.

The growth-related carcass traits, CWT and RT 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, 1988). The predicted breeding values of the sires and the progeny steers for CWT and RT 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, 45 and there were 89 market-year subclasses, 332 farms and 228 sires.

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.120947716T>C SNP was genotyped by the PCR-restriction Fragment Length Polymorphism Method as described previously (Tong *et al*., 2012a). Using this method, 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, the CC homozygotes and the TC heterozygotes yielded 2 bands (19 and 433 bp), 3 bands (19, 101 and 332 bp) and 4 bands (19, 101, 332 and 433 bp), respectively.

Statistical analyses: The effect of genotypes at the g.120947716T>C SNP on the predicted breeding values for CWT and RT was analyzed with the model that included the SNP genotype as the fixed effect and the sire (father of the sire) as the random effect. Statistical analysis was performed by the MIXED procedure of the SAS Program (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Genotyping 100 sires for the g.120947716T>C revealed 83 animals homozygous for the T allele and 17 animals heterozygous for the C and the T allele. Statistically significant differences among the genotypes of the SNP were detected for the predicted breeding values for CWT ($p = 0.030$) and RT ($p = 0.005$) by the analysis with the model that included the SNP genotype as the fixed effect and the sire (father of the sire) as the random effect (Table 1). The predicted breeding values for CWT and RT were significantly higher in the TC heterozygotes than in the TT homozygotes (Table 1). This result showed that the g.120947716T>C SNP is associated with CWT and RT in Japanese Black beef cattle with the C allele at the g.120947716T>C resulting in high levels of CWT and RT consistent with the previous data of the higher frequency of the C allele at the SNP in animals with

Table 1: Effect of the SNP genotypes on CWT and RT in 100 Japanese Black sires

		Breeding values[1]	
Genotypes	No. of animals	CWT (kg)	RT (cm)
TC	17	21.05±6.10^{a}	2.55±0.86^{a}
TT	83	6.31±2.76^{b}	-0.14±0.39^{b}

[1]The breeding values are given as estimates±SE; $^{a,\,b}$Estimates at different genotypes without a common letter in their superscripts significantly differ ($p<0.05$)

extremely high predicted breeding values for CWT and RT than with extremely low one (Tong *et al*., 2012b).

On the basis of the results of association of the g.120947716T>C SNP with CWT and RT, together with the IRS1 function (Tamemoto *et al*., 1994; Kido *et al*., 2000; Dong *et al*., 2006) and the co-localization of the QTL for growth-related carcass trait with the IRS1 (Takasuga *et al*., 2007) and the difference in allele frequency distribution of the g.120947716T>C SNP between sires with extremely high predicted breeding values for CWT and RT and with extremely low one (Tong *et al*., 2012b), researchers can hypothesize that the SNP in the promoter region might have an impact on IRS1 expression and also CWT and RT by affecting IRS1 promoter activity. However, the SNP is not located within any of as yet identified canonical sequences involved in gene transcription. Thus, a more likely event is that the IRS1 SNP is in linkage disequilibrium with an unidentified and truly relevant mutation, rather than functional and a causal mutation for CWT and RT.

The association with CWT and RT obtained in this study suggested possible effect of the g.120947716T>C on the growth-related trait in Japanese Black beef cattle. The information on the IRS1 SNP may be applied to effective marker-assisted selection to increase beef productivity in Japanese Black beef cattle. Setoguchi *et al*. (2009) have recently reported that a SNP in the chromosome condensation protein *G* gene was associated with growth-related trait in Japanese Black breed. Further, researchers have recently reported that a SNP in promoter region of the titin gene was associated with growth-related trait in Japanese black beef cattle (Yamada *et al*., 2011). Thus, the present study seems to be an additional report to show polymorphisms associated with growth-related trait using Japanese Black breed.

CONCLUSION

In this study, researchers show that the g.120947716T>C SNP in IRS1 is associated with CWT and RT in Japanese Black beef cattle. This study will provide a useful information for the establishment of effective marker-assisted selection to increase beef productivity in Japanese Black beef cattle.

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