

## Possible Association of Single Nucleotide Polymorphism in the Myosin Heavy Chain 1 Gene with Growth-Related Trait 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. The Myosin Heavy chain 1 (*MYH1*) gene is reported to encode an isoform of myosin heavy chain in type I fiber of skeletal muscle and has been previously shown to be located within genomic region of a quantitative trait locus for growth-related trait. The earlier study detected a Single Nucleotide Polymorphism (SNP), referred to as g.29850738G>A in the promoter region of the *MYH1* 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 the present study, researchers analyzed the effect of the *MYH1* g.29850738G>A SNP genotypes on the growth-related carcass trait in Japanese Black beef cattle. The SNP was significantly associated with RT and marginally associated with CWT ( $p = 0.016$  and  $0.069$ , respectively) in experiment 1 using 101 Japanese Black sires. Further, a slight trend of significance for CWT and RT was observed for the SNP in experiment 2 using 745 paternal half-sib progeny steers from 2 sires homozygous for G allele at the g.29850738G>A in Japanese Black beef cattle. These findings suggest possible effect of the g.29850738G>A SNP on the growth-related trait in Japanese Black beef cattle. The *MYH1* 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, Japanese Black breed, *MYH1*, single nucleotide polymorphism

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### 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 Myosin Heavy chain 1 (*MYH1*) gene encodes an isoform of myosin heavy chain in type I (slow-oxidative) fiber of skeletal muscle (Leinwand *et al.*, 1983) and it has been reported that a Quantitative Trait Locus (QTL) for Rib Thickness (RT) was mapped to genomic region

containing the *MYH1* gene on bovine chromosome 19 using a half-sib family of Japanese Black beef cattle (Takasuga *et al.*, 2007). Thus, the *MYH1* 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.29850738G>A was located in the promoter region of the *MYH1* gene (Tong *et al.*, 2012). In a preliminary study, researchers showed that the g.29850738G>A 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 RT and that the frequency of the A allele at the SNP was higher in animals with extremely high breeding value than with extremely low one and the G allele frequency in animals with the low one than with the high one for CWT and RT (Wu *et al.*, 2012). Thus, the MYH1 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 MYH1g.29850738G>A SNP with the growth-related carcass trait in Japanese Black beef cattle.

## MATERIALS AND METHODS

**Samples and data:** Researchers performed two experiments for the association study. Researchers used 101 Japanese Black sires in experiment 1. The sires were used either at present or previously at the Oita Prefectural Institute of Animal Industry (OPIAI, 1999) (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 lines. In experiment 2, 745 paternal half-sib Japanese Black progeny steers (199 and 546 steers per sire) produced from 2 sires homozygous for G allele at the g.29850738G>A with dams considered to represent a random sample of the female population were used. These progeny steers were fattened and shipped to a carcass market in the Oita prefecture. Semen or blood from each sires and adipose tissues of the progeny steers 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 48,045 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.29850738G>A SNP was genotyped by the PCR-Restriction Fragment Length Polymorphism Method as described previously (Tong *et al.*, 2012). Using this method, 153 bp PCR fragments containing the SNP site were amplified and Hpy 188I-digested into 74 and 79 bp fragments at the A allele but not the G allele: the GG homozygotes, the AA homozygotes and the GA heterozygotes yielded 1 band (153 bp), 2 bands (74 and 79 bp) and 3 bands (74, 79 and 153 bp), respectively.

**Statistical analyses:** The populations of the two experiments were separately analyzed. The effect of genotypes at the g.29850738G>A SNP on the predicted breeding values for CWT and RT were analyzed with the model that included the SNP genotype as the fixed effect and the sire (father of the sire) as the random effect in experiment 1. The SNP genotype effect was analyzed with the model that included the SNP genotype and the sire as the fixed effects as well as their interaction in experiment 2. Subsequently in cases where the interaction effect was not statistically significant ( $p > 0.05$ ), the interaction effect was excluded from the model to test significance of the SNP genotype effect. Statistical analysis was performed by the MIXED (experiment 1) and GLM procedures (experiment 2) of the SAS Program (SAS Institute, Inc., Cary, NC).

## RESULTS AND DISCUSSION

The populations of the two experiments were separately analyzed for association of the MYH1 SNP with CWT and RT. Genotyping 101 sires for the g.29850738G>A revealed 87 animals homozygous for G allele and 14 animals heterozygous for G allele and A allele. The SNP genotype had statistically significant

effect on the predicted breeding value for RT ( $p = 0.016$ ) and marginally significant differences among the genotypes of the SNP were detected for CWT ( $p = 0.069$ ) 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 RT was significantly higher in the GA heterozygotes than in the GG homozygotes and the CWT was marginally higher in the GA heterozygotes than in the GG homozygotes (Table 1).

To verify further the effect of the genotypes at the g.29850738G>A SNP on CWT and RT, researchers used 745 progeny steers from 2 sires homozygous for the G allele at the g.29850738G>A SNP. The steers in this experiment could be grouped only according to the alleles that they received from their dams which are considered to be a random sample of a general population in Japanese Black beef cattle. Therefore, this experiment likely allowed a linkage disequilibrium estimate of the effect of the SNP. The interaction between the SNP genotype and the sire was not statistically significant for each of CWT and RT in the model that included the SNP genotype and the sire as the fixed effects and their interaction and was excluded from the statistical model. In the model without the interaction effect, the SNP genotype had no effect on the predicted breeding value for each of CWT and RT (Table 2).

Based on the experiment 1 using the 101 sires, researchers showed that the g.29850738G>A SNP was significantly and marginally associated with RT and CWT, respectively in Japanese Black beef cattle with the A allele at the g.29850738G>A SNP resulting in high levels of RT and CWT. On the contrary, the effect of the genotypes of the SNP was not statistically significant ( $p > 0.1$ ) for each of CWT and RT in the experiment 2 using the 745 progeny steers. However, a slight trend of significance for CWT and RT was observed in the 745 progeny steers of experiment 2 and genotypic profiles of

the predicted breeding values for CWT and RT in the progeny steers of experiment 2 showed trends similar to those of the 101 sires of experiment 1 suggesting possible association of the g.29850738G>A SNP with CWT and RT. Researchers used the predicted breeding values as phenotypic values and then sire contributions to the predicted breeding values were the same for all off spring, especially in experiment 2 whereas the dams can be considered to represent a random sample of the Japanese Black population. Thus, it is likely to be desirable to use larger numbers of dams in the analyses using paternal half-sib progeny steers such as experiment 2. Researchers should note that the present study using the progeny steers of experiment 2 might not have enough power to detect an association with CWT and RT.

On the basis of the results of possible association of the g.29850738G>A SNP with RT and CWT, together with MYH1 function (Rehfeldt and Kuhn, 2006), co-localization of the RT QTL with the MYH1 (Takasuga *et al.*, 2007) and the higher frequency of the A allele at the g.29850738G>A SNP in animals with extremely high predicted breeding value for CWT and RT than with extremely low one (Wu *et al.*, 2012), researchers can hypothesize that the SNP in the promoter region might have an impact on MYH1 expression and also CWT and RT by affecting MYH1 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 MYH1 SNP is in linkage disequilibrium with an unidentified and truly relevant mutation, rather than functional and a causal mutation for CWT and RT.

The possible association with CWT and RT obtained in this study suggested possible effect of the g.29850738G>A on the growth-related trait in Japanese Black beef cattle. The information on the MYH1 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 marginally associated with growth-related trait in Japanese Black beef cattle (Yamada *et al.*, 2011). Thus, the present study seems to be the third report to show polymorphisms associated with growth-related trait using Japanese Black breed.

## CONCLUSION

In this study, researchers show that the g.29850738G>A SNP in MYH1 is possibly associated with CWT and RT in Japanese Black beef cattle. The information on the MYH1 g.29850738G>A SNP obtained

Table 1: Effect of the SNP genotypes on growth-related carcass traits in experiment 1

Genotypes	No. of animals	Breeding values <sup>1</sup>	
		Carcass weight (kg)	Rib thickness (mm)
GA	14	20.78±6.80	2.50±0.95 <sup>a</sup>
GG	87	7.30±2.72	-0.01±0.38 <sup>b</sup>

<sup>1</sup>The breeding values are given as estimates±SE; <sup>a, b</sup>Means at different genotypes without a common letter in their superscripts significantly differ ( $p < 0.05$ )

Table 2: Effect of the SNP genotypes on growth-related carcass traits in experiment 2

Genotypes	No. of animals	Breeding values <sup>1</sup>	
		Carcass weight (kg)	Rib thickness (mm)
GA	25	8.32±3.87	1.59±0.52
GG	720	3.59±0.72	1.48±0.11

<sup>1</sup>The breeding values are given as least squares means±SE

in this study may be applied to effective marker-assisted selection to increase beef productivity in Japanese Black beef cattle.

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