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Preliminary Association Study of Single Nucleotide Polymorphism in *MYH1* and *TRDN* Genes for 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 the existence of Single Nucleotide Polymorphisms (SNPs), g.29850738G>A and g.3834941C>T in promoter regions of the Myosin Heavy chain 1 (*MYH1*) and the Triadin (*TRDN*) genes, respectively, in Japanese Black beef cattle population. The MYH1 encodes an isoform of myosin heavy chain in type I fiber of skeletal muscle and the TRDN is known to be involved in muscle contraction and the 2 genes have been earlier shown to be located within genomic regions of quantitative trait loci for growth-related carcass traits. Thus, the *MYH1* and *TRDN* genes were considered as positional functional candidates for the gene responsible for growth performance. In this study, we analyzed the possible effects of the 2 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.29850738G>A SNP in the MYH1, 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 allelic distribution of the g.3834941C>T SNP in the TRDN was indistinguishable between the sires with extremely high one and with extremely low one for all the traits. 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 analysis, growth-related carcass trait, Japanese Black beef cattle, MYH1, single nucleotide polymorphism, TRDN

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.

Researchers have recently reported that Single Nucleotide Polymorphisms (SNPs), referred to as g.29850738G>A and g.3834941C>T, respectively were located in the promoter regions of the Myosin Heavy Chain 1 (*MYH1*) and the Triadin (*TRDN*) genes but that the SNPs had no significant effect on marbling in Japanese Black beef cattle (Tong *et al*., 2012a, b).

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The TRDN is known to be involved in muscle contraction (Kirchhefer *et al*., 2001) and likely in pork quality traits (Kuchenmeister *et al*., 1999). Researchers have also located the *TRDN* gene within genomic region of a Quantitative Trait Locus (QTL) for Rib Eye Area (REA) which is mapped in a half-sib family of Japanese Black beef cattle to bovine chromosome 9 region (Takasuga *et al*., 2007). The *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 Rib Thickness (RT) QTL was mapped to genomic region containing the *MYH1* gene on bovine chromosome 19 (Takasuga *et al*., 2007). Thus, the *MYH1* and *TRDN* genes were regarded as positional functional candidates for the gene responsible for growth performance.

Researchers here in analyzed the allele frequency distribution in the g.29850738G>A and the g.3834941C>T SNPs between extremely high-performance and extremely low-performance Japanese Black sires for each 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), RT, REA and Subcutaneous Fat Thickness (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, 1988). The predicted breeding values of the sires for CWT, REA, RT and SFT were obtained from the Oita recording system for beef cattle, earlier 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.29850738G>A and the g.3834941C>T SNPs were genotyped by the PCR-restriction fragment length polymorphism method as described earlier (Tong *et al*., 2012a, b). For the g.29850738G>A SNP, 153 bp PCR fragments containing the SNP site were amplified and Hpy188I-digested into 74 and 79 bp fragments at the A allele but not the G allele: the GG homozygotes, AA homozygotes and the GA heterzygotes yielded 1 band (153 bp), 2 band (74 and 79 bp) and 3 bands (74, 79 and 153 bp), respectively. For the g.3834941C>T SNP, 139 bp PCR fragments containing the SNP site were amplified and AluI-digested into 39 and 100 bp fragments at the C allele but not the T allele: the CC homozygotes, TT homozygotes and the TC heterzygotes yielded 2 bands (39 and 100 bp), 1 band (139 bp) and 3 bands (39, 100 and 139 bp), respectively.

Statistical analysis: The allele frequency distributions of the 2 SNPs, g.29850738G>A and the g.3834941C>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 2 SNPs, g.29850738G>A and the g.3834941C>T.

For the g.29850738G>A SNP in the *MYH1* 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 the CWT and RT ($p = 0.020$ and 0.039, respectively). 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 both CWT and RT (Table 1). The *MYH1* gene encodes an isoform of myosin heavy chain in type I (slow-oxidative) fiber of skeletal muscle (Leinwand *et al*., 1983). A number of data have shown that the type of muscle fibers is a factor that influences growth and meat quality traits (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). Further, Calkins *et al*. (1981) reported that the percentage of type I fiber is positively correlated with intramuscular fat content in cattle. Thus, the higher type I fiber content contributes to increase of meat quality whereas the lower content of type I fiber tends to be associated with increase of meat mass. 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 quality (Renand *et al*., 2001).

Table 1: Comparison of allelic distributions in the g.29850738G>A 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
		A allele	G allele		
CWT	High	0.147	0.853	5.397	0.020
	Low	0.000	1.000		
REA	High	0.147	0.853	1.433	0.231
	Low	0.059	0.941		
RT	High	0.133	0.882	4.250	0.039
	Low	0.000	1.000		
SFT	High	0.059	0.941	0.000	1.000
	Low	0.059	0.941		

Table 2: Comparison of allelic distributions in the g.3834941C>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
		C allele	T allele		
CWT	High	0.382	0.618	2.550	0.110
	Low	0.206	0.794		
REA	High	0.441	0.559	0.060	0.806
	Low	0.412	0.588		
RT	High	0.382	0.618	1.722	0.189
	Low	0.235	0.765		
SFT	High	0.353	0.647	1.826	0.177
	Low	0.206	0.794		

Thus, researchers have also located the *MYH1* gene within genomic region of QTL for RT using a half-sib family of Japanese Black beef cattle (Takasuga *et al*., 2007).

Researchers can hypothesize that the g.29850738G>A SNP in the promoter region of the *MYH1* gene might be related to changes in gene expression and/or CWT and RT levels. In addition, although it may be true that the SNP itself is functional and directly affect the gene expression and/or CWT and RT levels, a more likely event is that the g.29850738G>A SNP is in linkage disequilibrium with an unidentified true causal mutation for growth-related carcass traits.

On the other hand, for the g.3834941C>T SNP, no significant difference in the allele frequency distribution between 17 sires with extremely high breeding value and 17 sires with extremely low one was detected for any growth-related carcass traits (Table 2). It is likely that the g.3834941C>T SNP is not in linkage disequilibrium with an unidentified true causal mutation for growth-related carcass traits.

The preliminary association of the g.29850738G>A SNP with CWT and RT obtained in this study suggested possible effect of the g.29850738G>A SNP on the growth-related carcass traits in Japanese Black beef cattle. The information on the g.29850738G>A SNP may be applied to effective marker-assisted selection to increase the beef productivity in Japanese Black beef cattle.

CONCLUSION

In this study, researchers show that the g.29850738G>A SNP in the *MYH1* gene is preliminary associated with CWT and RT in Japanese Black beef cattle. The SNP may be useful for marker-assisted selection to increase the beef productivity in Japanese Black Beef cattle.

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