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Possible Association of Single Nucleotide Polymorphism in *Titin* 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 researchers have recently reported that a Single Nucleotide Polymorphism (SNP), referred to as g.231054C>T in promoter region of the *Titin* (*TTN*) gene was associated with marbling in Japanese Black beef cattle with the T allele being associated with a high level of marbling. The TTN is known to be involved in myofibrillogenesis and has been previously shown to be located within genomic region of a quantitative trait locus for rib eye area. Thus, the TTN was considered as a positional functional candidate for the gene responsible for growth performance. In this study, the researchers analyzed the effect of the TTN g.231054C>T SNP genotypes on the growth-related carcass traits in Japanese Black beef cattle. The SNP was marginally associated with rib eye area in two experiments using 101 sires ($p = 0.067$) and 820 paternal half-sib progeny steers from 3 sires homozygous for C allele at the g.231054C>T ($p = 0.090$) in Japanese Black beef cattle. These findings suggest possible effect of the g.231054C>T on the growth-related trait in Japanese Black beef cattle. The TTN SNP polymorphism 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, single nucleotide polymorphism, TTN

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.

The researchers have recently reported that a Single Nucleotide Polymorphism (SNP), referred to as g.231054C>T in promoter region of the *Titin* (*TTN*) gene was associated with marbling in Japanese Black beef cattle with the T allele being associated with a high level of marbling (Yamada *et al*., 2009). The TTN SNP seems to be a candidate marker for marker-assisted selection of marbling in Japanese Black beef cattle (Yamada *et al*., 2009). The TTN is known to be involved in myofibrillogenesis (Itoh-Satoh *et al*., 2002). The researchers have also located the *TTN* gene within genomic region of a quantitative trait locus for rib eye area (Takasuga *et al*., 2007) which is mapped in a half-sib family of Japanese Black cattle to bovine chromosome two region (Yamada *et al*., 2006). Thus, the *TTN* gene was regarded as a positional functional candidate for the gene responsible for growth performance. We herein analyzed association of the TTN SNP, g.231054C>T with the growth-related carcass traits in Japanese Black beef cattle.

MATERIALS AND METHODS

Samples and data: The researchers performed two experiments for the association study. The researchers

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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 (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, 820 paternal half-sib Japanese Black progeny steers (88-535 steers per sire) produced from 3 sires homozygous for C allele at the g.231054C>T 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, carcass weight, rib eye area and rib thickness were measured on carcasses dissected at the 6th and 7th 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 and the progeny steers for carcass weight, rib eye area and rib thickness 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 1988-2003 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.231054C>T was genotyped using PCR-Restriction Fragment Length Polymorphism (RFLP) method (Yamada *et al.*, 2009). PCR primers used for PCR-RFLP were 5'-TCATCTCCTAACTACTTCCCA-3' and 5'-ACAAAATCTGAACCTGGCTT-3' (Nucleotide positions relative to the putative transcription initiation site of the *TTN* gene were -842 to 822 and -616 to 635, respectively). PCR amplifications were performed using 25 ng of the prepared DNA as template in a final volume of 25 μL containing 0.5 μM of each primer, 0.2 mM of each dNTP, 0.625 U of Ex Taq polymerase (Takara, Shiga, Japan) and 1 X Ex Taq buffer (Takara). The PCR conditions were as follows: 94°C for 2 min, 35 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 60 sec, followed by a further 5 min extension at 72°C. An aliquot of PCR-amplified products was digested at 37°C for 1 h with restriction enzyme HpyCH4III and electrophoresed on a 3.0% agarose gel. Agarose gels were stained with ethidium bromide and photographed under an ultraviolet light. Using this method, 227 bp PCR fragments containing the SNP site were amplified and HpyCH4III-digested into 36 and 191 bp fragments at the C allele but not at the T allele: the CC homozygotes, the TT homozygotes and the CT heterozygotes resulted in 2 bands (36 and 191 bp), 1 band (227 bp) and 3 bands (36, 191 and 227 bp).

Statistical analyses: The populations of the two experiments were separately analyzed. The effect of genotypes at the g.231054C>T SNP on the predicted breeding values for carcass weight, rib eye area and rib thickness 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 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 TTN SNP with carcass weight, rib eye area and rib thickness.

Experiment 1: Genotyping 101 sires for the g.231054C>T revealed 50 animals homozygous for C allele, 47 animals

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

		Breeding value[1]		
Genotype	No. of animals	Carcass weight (kg)	Rib eye area (cm^2)	Rib thickness (mm)
CT	47	9.89±3.54	3.75±0.58[a]	0.59±0.53
CC	50	6.38±3.46	2.17±0.57[b]	0.41±0.55

[1]The breeding values are given as estimates±SE; [a,b]Estimates at different genotypes without a common letter in their superscripts reach marginally significant difference ($p<0.1$)

heterozygous for C allele and T allele and 4 animals homozygous for T allele. The 4 TT homozygotes were too small sample size to give reliable estimates and then excluded from the statistical analysis. Marginally significant differences among the genotypes of the SNP were detected for rib eye area ($p = 0.067$) 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 SNP genotype had no significant effect on carcass weight and rib thickness ($p>0.1$) (Table 1). The rib eye area was marginally higher in the CT heterozygotes than in the CC homozygotes (Table 1).

Experiment 2: To verify further the effect of genotype at the g.231054C>T SNP on carcass weight, rib eye area and rib thickness, we used 820 progeny steers from 3 sires homozygous for the C allele at the g.231054C>T 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 all of the traits 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 effect reached marginal significance ($p = 0.090$) for rib eye area but not for carcass weight and rib thickness ($p>0.1$) (Table 2). The rib eye area exhibited a tendency to be higher in the CT heterozygotes than in the CC homozygotes (Table 2).

On the basis of two experiments using the 101 sires and the 820 progeny steers from 3 sires homozygous for the C allele at the g.231054C>T SNP, the researchers showed that the g.231054C>T SNP is marginally associated with rib eye area in Japanese Black beef cattle with the T allele at the g.231054C>T SNP resulting in high levels of rib eye area. Especially in experiment 2 because the dams can be considered to represent a random sample of the Japanese Black population, the association is likely to be true. The researchers used the predicted breeding values as phenotypic values and then sire contributions

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

		Breeding value[1]		
Genotype	No. of animals	Carcass weight (kg)	Rib eye area (cm^2)	Rib thickness (mm)
CT	270	5.36±1.14	4.27±0.18[a]	1.22±0.18
CC	550	4.02±0.84	3.95±0.13[b]	1.01±0.13

[1]The breeding values are given as least squares means±SE; [a,b]Mean values at different genotypes without a common superscript letter reach marginally significant difference ($p<0.1$)

to the predicted breeding values were the same for all offspring. 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. The researchers should note that the experiments in this study were the case.

Based on the marginal association of the g.231054C>T with rib eye area, together with TTN function (Itoh-Satoh *et al*., 2002) and co-localization of the rib eye area quantitative trait locus with the TTN (Yamada *et al*., 2006), the researchers can hypothesize that the SNP in the promoter region might have an impact on TTN expression and also rib eye area by affecting TTN 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 TTN SNP is in linkage disequilibrium with an unidentified and truly relevant mutation, rather than functional and a causal mutation for rib eye area.

The effect of genotypes of the SNP was not statistically significant ($p>0.1$) for carcass weight and rib thickness. Further, the rib eye area quantitative trait locus corresponding to chromosomal position of the TTN did not have statistically significant effect on carcass weight and rib thickness (Takasuga *et al*., 2007). However, a slight trend of significance for carcass weight and rib thickness was observed and genotypic profiles of the predicted breeding value for carcass weight and rib thickness showed trends similar to those of rib eye area in all two experiments. Thus, the present study might not have enough power to detect an association with carcass weight and rib thickness.

The marginal association with rib eye area obtained in this study suggested possible effect of the g.231054C>T on the growth-related trait in Japanese Black beef cattle whereas the effect of the SNP genotypes on carcass weight and rib thickness was not significant in this study. The effect of the g.231054C>T on the growth related trait in Japanese Black beef cattle was corroborated by an independent study using ~100 paternal half-sib Japanese Black families with a total of ~1000 progeny steers. The information on the TTN 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. Thus, the present study seems to be the second report to show polymorphisms associated with growth related trait using Japanese Black breed.

CONCLUSION

In this study, we show that the g.231054C>T SNP in TTN is marginally associated with rib eye area in Japanese Black beef cattle. This research will provide an useful information for the establishment of effective marker-assisted selection to increase beef productivity in Japanese Black beef cattle.

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REFERENCES

Boldman, K.G., L.A. Kriese, L.D. van Vleck, C.P. van Tassell and S.D. Kachman, 1995. A Manual for Use of MTDFREML, A Set of Programs to Obtain Estimates of Variances and Covariances. ARS and USDA, Washington, DC. USA.

Itoh-Satoh, M., T. Hayashi, H. Nishi, Y. Koga and T. Arimura *et al*., 2002. Titin mutations as the molecular basis for dilated cardiomyopathy. Biochem. Biophys. Res. Commun., 291: 385-393.

JMGA, 1998. New Beef Carcass Grading Standards. Japan Meat Grading Association, Tokyo, Japan.

Sasaki, Y., T. Miyake, C. Gaillard, T. Oguni and M. Matsumoto *et al*., 2006. 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.

Setoguchi, K., M. Furuta, T. Hirano, T. Nagao, T. Watanabe, Y. Sugimoto and A. Takasuga, 2009. Cross-breed comparisons identified a critical 591-kb region for bovine carcass weight QTL (CW-2) on chromosome 6 and the Ile-442-Met substitution in NCAPG as a positional candidate. BMC Genet., 10: 43-43.

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.

Yamada, T., S. Sasaki, S. Sukegawa, S. Yoshioka and Y. Takahagi *et al*., 2009. Association of a single nucleotide polymorphism in titin gene with marbling in Japanese Black beef cattle. BMC Res. Notes, 2: 78-78.

Yamada, T., Y. Taniguchi, S. Nishimura, S. Yoshioka, A. Takasuga, Y. Sugimoto and Y. Sasaki, 2006. Radiation hybrid mapping of genes showing intramuscular fat deposition-associated expression changes in bovine musculus longissimus muscle. Anim. Genet., 37: 184-185.