RESEARCH NOTE

Association of expression levels in skeletal muscle and a SNP in the *MYBPC1* gene with growth-related trait in Japanese Black beef cattle

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Introduction

The myosin-binding protein C, slow type (*MYBPC1*) gene is considered as a positional functional candidate gene responsible for growth performance. In this study, using real-time PCR, we first showed that the expression levels of the *MYBPC1* gene in skeletal muscle are higher in Japanese Black steers (JB) with extremely high growth performance than in the JB steers with extremely low one. Further, we indicated that the *g.70014208A > G* single nucleotide polymorphism (SNP) of the *MYBPC1* gene is significantly associated with the predicted breeding value for rib eye area in two experiments using 100 sires ($P = 0.0149$) and 745 paternal half-sib progeny steers from two sires homozygous for *A* allele at this SNP ($P = 0.0049$) in JB. These findings suggest that the possible effect of the expression levels and *g.70014208A>G* SNP of the *MYBPC1* gene on the growth-related trait in JB. The *MYBPC1* SNP may be useful for effective marker-assisted selection to increase the beef production in JB.

Growth performance as well as marbling are the main breeding objectives in JB cattle, the major beef breed in Japan. The *MYBPC1* gene is known to be one isoform of myosin-binding protein C that is one of the major myosin-binding proteins in vertebrate-striated muscles and has been previously shown to be located within genomic regions of quantitative trait loci for growth-related trait (Offer *et al.* 1973; Pepe and Drucker 1975; Sato *et al.* 2003; Takasuga *et al.* 2007). Thus, the *MYBPC1* was considered as a positional functional candidate for the gene responsible for growth performance. Our previous study showed that a *g.70014208A > G* SNP in the promoter region of the *MYBPC1* gene was associated with marbling in JB (Tong *et al.* 2014).

The objective of this study was to analyse the association of the *MYBPC1* expression levels in skeletal muscle, and the *MYBPC1 g.70014208A > G* SNP with growth-related traits in JB.

Materials and methods

Animals and traits

In the real-time PCR-based analysis, we used skeletal muscle tissues of eight JB steers with extremely high performance for each of carcass weight (CWT), rib eye area (REA) and rib thickness (RT), and eight JB steers with extremely low one, selected from 60 JB steers. The skeletal muscle tissues of these steers were collected at a slaughterhouse at Niigata prefecture (Niigata, Japan). They were chosen considering the ancestry to minimize their genetic relationship. For association study between *MYBPC1* genotype and growth-related traits, two experiments were performed. We used 100 JB sires representing a variety of sire lines in expression 1 and 745 paternal half-sib JB progeny steers produced from two sires (199 and 546 per sire) homozygous for *A* allele at the *g.70014208A > G*, with dams considered to be a random mating population in expression 2. Genomic DNA samples were obtained from the Oita Prefectural Institute of Animal

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Industry (Oita, Japan). The *g.70014208A > G* SNP was genotyped by the PCR-RFLP method as described previously (Tong *et al.* 2014)

The growth-related carcass traits, CWT, REA 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 (JMGA 1988). For real-time PCR, the phenotype data for CWT, REA and RT of the JB steers were obtained from the Niigata Prefectural Headquarters, National Federation of Agricultural Cooperative Association (Niigata, Japan). For association study, the predicted breeding values of the sires and the progeny steers for CWT, REA and RT were obtained from the Oita recording system for beef cattle reported previously by Sasaki *et al.* (2006).

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

RNA extraction, cDNA synthesis and real time PCR

Total RNA was isolated from flash-frozen skeletal muscle samples using the RNeasy Fibrous Tissue kit (Qiagen, Gmblt, Germany) according to the manufacturer's instructions. Total RNA was quantified by absorbance at 260 nm, and the integrity of total RNA was checked by agarose gel electrophoresis and ethidium bromide staining of the 28S and 18S bands. Total RNA (2 μg) was reverse-transcribed into cDNA using an iScript Advanced cDNA Synthesis kit for RT-qPCR (Biorad, Hercules, USA), according to the manufacturer's instructions.

Real-time PCR was performed using Sso Advanced SYBR Green Supermix (Biorad), the *MYBPC1* mRNA expression in skeletal muscle tissues was determined by the MiniOpticon real time PCR Detection System (Biorad), using *MYBPC1* mRNA-specific primers (F: 5′-CTCCTACTCTTCTGACC GTT-3′ and R:5′-CACATAGATCCTTGAATCCGTT-3′). *GAPDH* transcripts were amplified for normalization within each sample. The reaction was performed in 20 μL, containing 10 μL Sso Advanced SYBR Green Supermix (Biorad), 1 μL of each primer (10 μM), 2 μL cDNA (2.5 ng/μL) and 6 μL RNase/DNase-free H_2O. The thermal cycling parameters were as follows: 95°C for 30 s, followed by 40 cycles at 95°C for 3 s and 60°C for 30 s. The relative fold change was calculated using the $2^{-\Delta\Delta Ct}$ calculation (Schmittgen and Livak 2008).

Statistical analysis

Comparison of the *MYBPC1* expression level between two JB steer groups (eight JB steers with extremely high performance for each of growth-related trait and eight JB steers with extremely low one) was performed with the student's *t*-test. The effect of genotypes at the *g.70014208A > G* SNP on the predicted breeding values was analysed using the MIXED or GLM procedures of the SAS program with the model described previously (Tong *et al.* 2014).

Results and discussion

The expression levels of the *MYBPC1* gene in skeletal muscle of eight JB steers with extremely high performance for each of CWT, REA and RT and eight JB steers with extremely low one are shown in figure 1. The expression level of the *MYBPC1* gene was significantly higher in the high growth performance steer group than in low growth performance steer group, based on REA ($P = 0.0016$) and RT ($P = 0.0199$). No difference in the *MYBPC1* expression levels was detected between high and low growth performance steer groups, based on CWT ($P = 0.4365$) (figure 1).

The JB populations of the two experiments were separately analysed for association of the *MYBPC1* SNP with growth-related traits. In experiment 1, the SNP genotype had statistically significant effect on the predicted breeding values for REA ($P = 0.149$), but not for CWT and RT ($P =$ 0.1306 and 0.7596, respectively), 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 *AG* heterozygotes exhibited higher predicted breeding values for REA than the *AA* homozygotes (table 1). In experiment 2, the SNP genotype had the statistically significant effect on the predicted breeding values for REA ($P = 0.0049$) and only a marginal effect on the predicted breeding values for CWT and RT ($P = 0.765$ and 0.1172, respectively) (table 1). Genotype profiles of the predicted breeding values for REA were consistent with the results obtained in JB sires (table 1). As to CWT and RT, the *AG* heterozygotes had higher breeding values as compared with *AA* homozygotes, as well as REA.

The above result suggests the association of *MYBPC1* expression with growth-related trait. The muscle satellite

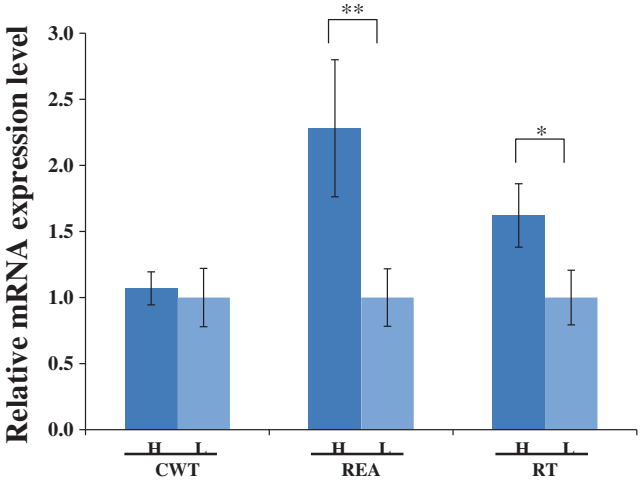

Figure 1. Expression levels of the *MYBPC1* gene in skeletal muscle of JB steers with extremely high performance for each of CWT, REA and RT and with extremely low one. CWT, carcass weight; REA, rib eye area; RT, rib thickness; H, JB steer group with high growth performance; L, JB steer group with low growth performance. Expression levels were determined by real-time PCR and normalized to *GAPDH*. Expression levels of low growth performance steer group were normalized to 1.0. Values are the mean ± SE ($n = 8$). Significantly difference: * $P < 0.05$, ** $P < 0.01$.

Table 1. Effect of the SNP genotypes of the *g.70014208A > G* on the breeding values for growth-related trait.

Breeding value[a]	Sire			Progeny steers[b]		
	AA	AG	*P* value	AA	AG	*P* value
	($n = 74$)	($n = 26$)		($n = 624$)	($n = 121$)	
Carcass weight (kg)	6.59 ± 2.97	15.47 ± 5.01	0.1306	8.16 ± 0.75	11.16 ± 1.60	0.0765
Rib eye area (cm^2)	2.46 ± 0.49	4.82 ± 0.82	0.0149	3.93 ± 0.17	4.68 ± 0.25	0.0049
Rib thickness (cm)	0.20 ± 0.42	0.45 ± 0.70	0.7596	1.42 ± 0.11	1.83 ± 0.24	0.1172

[a]The breeding values are given as estimates ± SE for sires and as least squares means ± SE for progeny steers. [b]The paternal half-sib Japanese Black progeny steers were the animals produced from two sires homozygous for *A* allele at the *g.70014208A > G* SNP.

cells are known to provide nuclei needed to support postnatal growth of muscle fiber, and to play a crucial role in determining the rate and extent of postnatal muscle growth (Kamanga-Sollo *et al.* 2011). *MYBPC1* interacts with muscle-type creatine kinase, potentially allowing it to regulate energy homeostasis during muscle contraction by coupling to the myofibril (Chen *et al*. 2011). Thus, the increase of the *MYBPC1* expression level might lead to high growth performance through enhancing muscle satellite cell proliferation. On the other hand, we could not detect difference in the *MYBPC1* expression levels between high and low growth performance steer groups, based on CWT. However, expression profile of the *MYBPC1* gene in high and low growth performance steer groups based on CWT showed similar trend to those of REA and RT. Thus, the present study might not have enough power to detect this difference.

On the basis of two experiments of association study, we showed that the *g.70014208A > G* SNP is associated with growth-related trait in JB breed, with the *G* allele resulting in high growth performance. This was especially evident in experiment 2, because the dams could be considered to represent a random sample of the JB population and thus the association is likely to be true. The effect of genotypes at the *g.70014208A > G* SNP was not statistically significant for CWT and RT in experiments 1 and 2. However, genotypic profiles of the predicted breeding value for CWT and RT in experiments 1 and 2 showed similar trend to those of REA in experiments 1 and 2. Thus, the present study might not have enough power to detect association between CWT and RT in experiments 1 and 2.

Based on the association of *g.70014208A > G* SNP with growth-related trait, together with the possible association of the *MYBPC1* expression levels with growth-related traits, we suggest that the SNP in the promoter region might have an impact on the *MYBPC1* expression, and also growth performance by affecting the *MYBPC1* promoter activity.

In conclusion, this study suggests possible effect of the *MYBPC1* gene on the growth-related trait in JB. The information on the *MYBPC1* SNP may be applied to effective marker-assisted selection to increase beef productivity in JB beef cattle.

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