

Replicated association of the single nucleotide polymorphism in *PNLIP* with marbling in Niigata population of Japanese Black beef cattle

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ABSTRACT

Marbling is regarded as an economically important trait of beef cattle in Japan, and measured as a beef marbling score (BMS). Our previous study reported an association between a single nucleotide polymorphism (SNP), *rs41648172*, in the pancreatic lipase (*PNLIP*) gene and the BMS level, using the Japanese Black beef cattle population of Oita prefecture. Further, we showed that the *T* allele at the *rs41648172* SNP is associated with a high level of the BMS. Thus, we suggested that the *rs41648172* SNP seems to be a candidate marker for marker-assisted selection. Our present study was designed to investigate whether this association could be replicated in other independent Japanese Black cattle population and analyze the effect of the SNP genotypes on the carcass traits other than the BMS. We detected the marginally significant effect of the genotypes of the *rs41648172* SNP on the BMS level by using the Japanese Black beef cattle population of Niigata prefecture ($P = 0.0919$), and obtained the result of the *T* allele associated with an increase in the BMS level, consistent with our previous data. In addition, we showed no significant association of the SNP with the subcutaneous fat thickness, carcass weight, rib eye area, rib thickness and yield estimate in the Japanese Black beef cattle population of Niigata prefecture. Thus, we concluded that the *rs41648172* SNP was useful for effective marker-assisted selection to increase the BMS

level in Japanese Black beef cattle, based on the replicated association of the *rs41648172* SNP with the BMS level in the other independent Japanese Black beef cattle population and no effect of the SNP genotypes on the carcass traits other than BMS.

Keywords: Association; *PNLIP*; Japanese Black Breed; Marbling; Replication Study; Single Nucleotide Polymorphism

1. INTRODUCTION

Intramuscular fat deposition (marbling) measured as a beef marbling score (BMS) is one of the economically important traits of beef cattle [1]. A high level of the BMS enriches the taste and tenderness of beef, improving the palatability [2-4], therefore, the BMS affects the evaluation of beef quality [1]. The BMS is regarded as the most important trait especially in Japan. Thus, it would be desirable to construct a more effective marker-assisted breeding scheme for increasing the BMS level in Japanese Black beef cattle.

We recently showed that a single nucleotide polymorphism (SNP), namely, *rs41648172*, in the pancreatic lipase (*PNLIP*) gene was associated with the BMS level in a Japanese Black beef cattle population in Oita prefecture, with the *T* allele of the SNP being associated with a highest level of the BMS [5].

Thus, it was necessary to investigate whether this association could be replicated in other independent populations of Japanese Black beef cattle and to analyze the

effects of the SNP genotypes on the carcass traits other than the BMS, in order to confirm the application of the *rs41648172* SNP to effective marker-assisted selection.

2. MATERIALS AND METHODS

2.1. Samples and Data

We used independent Japanese Black cattle population of Niigata prefecture, and studied the association of the *rs41648172* SNP with the BMS, subcutaneous fat thickness (SFT), carcass weight (CWT), rib eye area (REA), rib thickness (RT), and yield estimate (YE). In this study, 134 paternal half-sib progeny steers (1 to 16 steers per sire) produced from 39 sires were used. Hair root specimens of the progeny steers were collected for genotyping the SNP. DNA samples were prepared from the materials using DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany).

The BMS, SFT, CWT, REA, RT and YE were measured according to the Japanese meat grading system by certified graders from the Japan Meat Grading Association (Tokyo, Japan) [1]. The predicted breeding values of the BMS, SFT, CWT, REA, RT and YE for the progeny steers were used as the phenotypic values in this study. The breeding values were predicted using carcass records of Japanese Black steers and heifers fattened in the Niigata prefecture, and obtained from the Niigata Prefectural Headquarters, National Federation of Agricultural Cooperative Association (Niigata, Japan).

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

2.2. SNP Genotyping

The *rs41648172* SNP was genotyped by the PCR-restriction fragment length polymorphism method as described previously [6]. Using this method, 546-bp PCR fragments containing the SNP site were amplified and *Hpy*CH4IV-digested into 131- and 415-bp fragments at the *C* allele, but not the *T* allele: the *TT* homozygotes, the *CC* homozygotes and the *CT* heterozygotes yielded 1 band (546-bp), 2 bands (131- and 415-bp) and 3 bands (131-, 415-, and 546-bp), respectively. PCR amplifications were performed using 25 ng of the prepared DNA as template in a final volume of 25 μ l containing 0.5 μ M of each primer, 0.2 mM of each dNTP, 0.625 U of Ex Taq polymerase (Takara, Shiga, Japan), and 1 \times Ex Taq buffer (Takara). The PCR conditions were as follows: 94°C for 3 min, 35 cycles of 94°C for 50 s, 66°C for 50 s, and 72°C for 50 s, followed by a further 5-min extension at 72°C. An aliquot of PCR-amplified product was digested at 37°C for 1 h with restriction enzyme *Hpy*CH4IV and electrophoresed on a 3.0% agarose gel. Agarose gels were stained with ethidium bromide and photographed under

an ultraviolet light.

2.3. Statistical Analyses

Departures from the Hardy-Weinberg equilibrium were tested for the SNP by chi-square test. Statistical comparisons between the allele frequencies at the SNP in the progeny steers or estimated by maternal alleles in half-sib progeny steers in the Niigata prefecture population and the previous date obtained in Japanese Black cattle population of the Oita prefecture, Japanese Brown, Japanese Short Horn, Holstein, or Brown Swiss cattle populations [7] were also performed by chi-square test. The effect of genotypes at the SNP on the predicted breeding values for the BMS, SFT, CWT, REA, RT and YE was analyzed with the model that included the SNP genotype as the fixed effect and the sire as the random effect. Statistical analysis was performed by the MIXED procedures of the SAS program (SAS Institute, Inc., Cary, NC).

3. RESULTS AND DISCUSSION

Genotyping the 134 progeny steers for the *rs41648172* SNP revealed 46 animals homozygous for the *C* allele, 56 animals heterozygous for the *C* allele and the *T* allele and 32 animals homozygous for the *T* allele for the *rs41648172* (**Table 1**). The observed frequencies of the SNP genotypes in the Niigata population are shown in **Table 1**. The observed and expected heterozygosity values at the SNP conformed to the Hardy-Weinberg equilibrium in the Niigata population. The frequency of the *T* allele of *rs41648172* SNP in the Niigata prefecture population was higher than the frequencies of this allele of Japanese Brown, Japanese Short Horn, Holstein, or Brown Swiss cattle populations that have not been strongly selected for high marbling [7], but lower than the frequency of this allele in Japanese Black cattle population of the Oita prefecture determined in our previous study [7] (data not shown). However, no statistically significant difference was detected between the allele frequencies estimated by maternal alleles in half-sib progeny steers in the Niigata population and obtained in Japanese Black cattle population of the Oita prefecture [7], and those frequencies were higher than those of Japanese Brown, Japanese Short Horn, Holstein,

Table 1. Frequencies of the *rs41648172* SNP genotypes in Japanese Black cattle population of the Niigata prefecture.

Genotype	No. of animals	Frequency
<i>CC</i>	46	0.343
<i>CT</i>	56	0.418
<i>TT</i>	32	0.239

Table 2. Effects of the *rs41648172* SNP genotypes on BMS, SFT, CWT, REA, RT and YE in Japanese Black cattle population of the Niigata prefecture.

Trait ¹	P-value	Genotype ²		
		CC	CT	TT
BMS	0.0919	1.32a ± 0.06	1.38ab ± 0.05	1.51b ± 0.07
SFT	0.1901	-0.20 ± 0.05	-0.12 ± 0.05	-0.07 ± 0.06
CWT	0.4059	42.28 ± 4.36	47.35 ± 4.11	51.40 ± 5.34
REA	0.8176	6.62 ± 0.57	7.10 ± 0.53	6.76 ± 0.69
RT	0.4410	0.57 ± 0.04	0.64 ± 0.04	0.64 ± 0.05
YE	0.5116	1.02 ± 0.10	1.02 ± 0.09	0.86 ± 0.12

¹BMS, beef marbling score (unit); SFT, subcutaneous fat thickness (cm); CWT, carcass weight (kg); REA, rib eye area (cm²); RT, rib thickness (cm); YE, yield estimate (%). ²the breeding values are given as least squares means ± SE. mean values at different genotypes without a common superscript letter significantly differ ($P < 0.10$).

or Brown Swiss cattle populations [7] (data not shown).

Marginally significant differences in the BMS level were detected among the genotypes of the *rs41648172* SNP in the Niigata prefecture population, by analysis using a model that included the SNP genotype as the fixed effect and the sire as the random effect (Table 2). The BMS level in the *TT* homozygotes was marginally higher than that in the *CC* homozygotes, and the values in the heterozygotes were intermediate between those in the 2 homozygotes (Table 2). These results were consistent with the data obtained in our previous study in the Oita prefecture population [5]. These results suggest that the association of the *rs41648172* SNP with the BMS level was replicated in the general Japanese Black beef cattle populations.

In previous study, we showed that the *rs41648172* SNP in *PNLIP* is associated with growth-related traits of the CWT, REA, RT and daily gain in the Oita prefecture population [8]. In this study, the *rs41648172* SNP genotype had no significant effects on the CWT, REA and RT, as well as YE and SFT, in the Niigata prefecture population. Many quantitative trait loci (QTL) for the growth-related trait are thought to be fixed in the Oita prefecture population, but not in the Niigata prefecture population. Our present study might not have sufficient power to detect the association of the *rs41648172* SNP with the growth-related trait in the Niigata prefecture population, because of the larger number of segregating QTL for the growth-related trait in the Niigata prefecture population as compared with that in the Oita prefecture population. Further study using a larger number of samples will be needed for the Niigata prefecture population.

In this study, we demonstrated replication of the association of the *rs41648172* SNP with the BMS level, and revealed a favorable effect of the *T* allele on the beef quality in general Japanese Black beef cattle populations. Together with the results of our previous study [5], these

findings suggest that the *rs41648172* SNP in the *PNLIP* is a useful marker for effective marker-assisted selection to increase the level of marbling in Japanese Black beef cattle.

4. CONCLUSION

In this study, we showed that the *rs41648172* SNP in the *PNLIP* replicated association with marbling in Japanese Black beef cattle population of Niigata prefecture, and that the *T* allele at the SNP had a favorable effect on the beef quality. This study will provide useful information for the establishment of effective marker-assisted selection to increase the levels of marbling in Japanese Black beef cattle.

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REFERENCES

- [1] JMGA (1988) New beef carcass grading standards. Japan Meat Grading Association, Tokyo.
- [2] Busboom, J.R., Jeremiah, L.E., Gibson, L.L., Johnson, K.A., Gaskins, C.T., Reeves, J.J. and Wright Jr., R.W. (1993) Effects of biological source on cooking and palatability attributes of beef produced for the Japanese market. *Meat Science*, **35**, 241-258. [doi:10.1016/0309-1740\(93\)90054-L](https://doi.org/10.1016/0309-1740(93)90054-L)
- [3] Boylston, T.D., Morgan, S.A., Johnson, K.A., Busboom, J.R., Wright Jr., R.W. and Reeves, J.J. (1995) Lipid content and composition of Wagyu and domestic breeds of beef. *Journal of Agricultural and Food Chemistry*, **43**, 1202-1207. [doi:10.1021/jf00053a015](https://doi.org/10.1021/jf00053a015)
- [4] Matsuishi, M., Fujimori, M. and Okitani, A. (2001) Wag-

- yu beef aroma in Wagyu (Japanese Black cattle) beef preferred by the Japanese over imported beef. *Animal Science Journal*, **72**, 498-504.
- [5] Tanomura, H., Yamamoto, T., Muramatsu, Y., Ohta, T., Kose, Y. and Yamada, T. (2011) The pancreatic lipase gene is associated with marbling in Japanese Black beef cattle. *Journal of Animal and Veterinary Advances*, **10**, 1719-1723. [doi:10.3923/javaa.2011.1719.1723](https://doi.org/10.3923/javaa.2011.1719.1723)
- [6] Tanomura, H., Yamamoto, T., Muramatsu, Y., Ohta, T., Kose, Y. and Yamada, T. (2011) Difference in *PNLIP* allele frequency distribution between high-marbled and low-marbled cattle. *Journal of Animal and Veterinary Advances*, **10**, 1714-1718. [doi:10.3923/javaa.2011.1714.1718](https://doi.org/10.3923/javaa.2011.1714.1718)
- [7] Tanomura, H., Yamamoto, T., Muramatsu, Y., Ohta, T., Kose, Y. and Yamada, T. (2012) Distribution of allele frequency at *PNLIP* rs41648172 between Japanese black and four other cattle breeds with differing historical selection for marbling. *Journal of Animal and Veterinary Advances*, **11**, 1350-1352. [doi:10.3923/javaa.2012.1350.1352](https://doi.org/10.3923/javaa.2012.1350.1352)
- [8] Tanomura, H., Yamamoto, T., Muramatsu, Y., Ohta, T., Kose, Y. and Yamada, T. (2012) Association of single nucleotide polymorphism in pancreatic lipase gene with growth-related traits in Japanese Black beef cattle. *Journal of Animal and Veterinary Advances*, **11**, 1346-1349. [doi:10.3923/javaa.2012.1346.1349](https://doi.org/10.3923/javaa.2012.1346.1349)