

Distribution of Allele Frequency at PNLIP rs41648172 Between Japanese Black and Four Other Cattle Breeds with Differing Historical Selection for Marbling

¹Hideki Tanomura, ²Youji Muramatsu, ¹Takuji Yamamoto, ³Takeshi Ohta,
⁴Hiroyuki Kose and ¹Takahisa Yamada

¹Laboratory of Animal Genetics, Graduate School of Science and Technology,
Niigata University, Nishi-ku, 950-2181 Niigata, Japan

²Department of Nutritional Sciences for Well-Being, Faculty of Health Sciences for Welfare,
Kansai University of Welfare Sciences, Kashiwara, 582-0026 Osaka, Japan

³Central Pharmaceutical Research Institute, Japan Tobacco, Inc., Takatsuki, 569-1125 Osaka, Japan

⁴Department of Life Science, Division of Natural Sciences, International Christian University,
Mitaka, 181-8585 Tokyo, Japan

Abstract: Marbling defined by the amount and distribution of intramuscular fat, so-called Shimofuri is an economically important trait of beef cattle in Japan. Researchers have recently reported that a Single Nucleotide Polymorphism (SNP) referred to as rs41648172 in intron 6 region of the Pancreatic Lipase (*PNLIP*) gene which have been considered as a positional functional candidate for the gene responsible for marbling was associated with marbling in Japanese Black beef cattle with the T allele being associated with a high level of marbling. In this study, researchers investigated the allele frequency distribution of the rs41648172 SNP among the 5 cattle breeds, Japanese Black, Japanese Brown, Japanese Short Horn, Holstein and Brown Swiss breeds. The T allele at the rs41648172 SNP associated with high marbling was found at higher frequency in Japanese Black breed that has been subjected to a strong selection for high marbling than in the other breeds that have not been strongly selected for high marbling. Based on this finding, researchers hypothesized that the pressure of the strong selection for high marbling in the Japanese Black breed has increased the frequency of the T allele at the rs41648172 SNP in the PNLIP. This study, together with the previous association study, suggested that the PNLIP SNP may be useful for effective marker-assisted selection to increase the levels of marbling.

Key words: Allele frequency, cattle breed, Japanese Black breed, marbling, PNLIP, single nucleotide polymorphism

INTRODUCTION

Marbling characterized by the amount and distribution of intramuscular fat of which the increase improves the palatability and acceptability of the meat (Busboom *et al.*, 1993; Boylston *et al.*, 1995; Matsuishi *et al.*, 2001) is an economically important trait of beef cattle in Japan (JMGA, 1998). Researchers have recently reported that a Single Nucleotide Polymorphism (SNP), referred to as rs41648172 in the intron 6 region of the Pancreatic Lipase (*PNLIP*) gene which have been considered as a positional functional candidate for the gene responsible for marbling was associated with marbling in Japanese Black beef cattle with the T allele being associated with a high level of marbling (Tanomura *et al.*, 2011). The PNLIP SNP seems to be a

candidate marker for marker-assisted selection of marbling in Japanese Black beef cattle (Tanomura *et al.*, 2011). There has been a strong selection for high marbling in Japanese Black breed over the last 50 years but not in other breeds such as Japanese Brown, Japanese Short Horn, Holstein and Brown Swiss (Sasaki *et al.*, 2006). In the present study, to depict the possibility that this selection pressure has affected the allele frequency distribution of the rs41648172 SNP, researchers investigated the allele frequency distribution of the rs41648172 SNP among the 5 cattle breeds.

MATERIALS AND METHODS

Samples and data: Researchers used 101, 83, 72, 96 and 81 cows, respectively for Japanese Black, Japanese Brown,

Japanese Short Horn, Holstein and Brown Swiss breeds. There was no strong bias for a specific father or a specific maternal grandfather of the sires or cows within each breed and the animal panel for each breed likely represents a random sample of population of each breed. Furthermore, researchers used 367 paternal half-sib Japanese Black progeny steers produced from a sire homozygous for C allele at the rs41648172 SNP.

The dams of these progeny steers were considered to be a random mating population. Semen, blood or adipose tissues were collected from these animals for SNP genotyping. These materials were sampled by the Oita Prefectural Institute of Animal Industry (Oita, Japan) for Japanese Black, Holstein and Brown Swiss breeds, the Kumamoto Prefectural Agricultural Research Center Institute of Animal Industry (Kumamoto, Japan) for Japanese Brown breed and the Iwate Agricultural Research Center Animal Industry Research Institute (Iwate, Japan) for Japanese Short Horn breed. DNA samples were prepared from the materials according to standard protocols. This study conformed to the guidelines for animal experimentation of the Graduate School of Science and Technology, Niigata University (Niigata, Japan).

SNP genotyping: The rs41648172 SNP was genotyped by polymerase chain reaction-restriction fragment length polymorphism method using restriction enzyme HpyCH4IV as shown in the previous study (Tanomura *et al.*, 2011).

RESULTS

Table 1 shows the allele frequency distribution of the rs41648172 SNP among the 5 cattle breeds, Japanese Black, Japanese Brown, Japanese Short Horn, Holstein and Brown Swiss breeds. Departures from the Hardy-Weinberg equilibrium were tested for the SNP in each of sire or cow populations of the 5 cattle breeds. Statistically, significant departures at the 5% level were not observed, except for Japanese Short Horn breed in which 58 animals were homozygous for the C allele and 12 animals heterozygous for the C and T alleles and 2 animals homozygous for the T allele. In Japanese Black breed, statistical comparison between the allele frequencies in sires and the data estimated by maternal alleles in half-sib progeny steers was performed by chi-square test. No statistically significant difference was detected between the 2 allele frequencies in Japanese Black breed (Table 2). Further, statistical comparisons for the allele frequencies between breeds were performed. Statistically significant differences were detected between

Table 1: Distribution of allele frequency at the rs41648172 SNP among the 5 cattle breeds

Breeds	Frequency	
	T allele	C allele
Japanese Black-Sires	0.570	0.430
Japanese Black-Progeny Steers	0.556	0.444
Japanese Brown	0.108	0.892
Japanese Short Horn	0.111	0.889
Holstein	0.255	0.745
Brown Swiss	0.130	0.870

Table 2: Statistical significance for differences in allele frequency for the rs41648172 SNP between the 5 cattle breeds

Breeds	Breed				
	Japanese Black-Progeny Steers	Japanese Brown	Japanese Short Horn	Holstein	Brown Swiss
Japanese Black-Sires	NS	*	*	*	*
Japanese Black-Progeny Steers	-	*	*	*	*
Japanese Brown	-	-	NS	*	NS
Japanese Short Horn	-	-	-	*	NS
Holstein	-	-	-	-	*

NS: Not Significant; *: Significant

Japanese Black and the other breeds for the SNP (Table 2). Researchers have previously reported that the T allele of the SNP is associated with high marbling. The frequencies of this allele in Japanese Black breed which has been subjected to a strong selection for high marbling were 0.570 in the sires and 0.556 in the progeny steers (Table 1). As expected, as compared to these frequencies in Japanese Black breed, those in the other breeds that have not been strongly selected for high marbling were lower. The reason that the frequency of the T allele in Holstein breed was statistically higher than the frequencies of the T allele in Japanese Brown, Japanese Short Horn and Brown Swiss breeds is unknown. There were no significant differences among Japanese Brown, Japanese Short Horn and Brown Swiss breeds. We should note that all the p values reported in this study were nominal with no correction for multiple testing.

DISCUSSION

This finding leads to the hypothesis that the pressure of the strong selection for high marbling in Japanese Black breed has increased the frequency of the rs41648172 SNP T allele assuming that the allele have experienced a selective sweep. Thus, this study, together with the previous association study (Tanomura *et al.*, 2011) suggests that the SNP may be useful for effective marker-assisted selection to increase the levels of marbling. In previous study, researchers supposed that the rs41648172 SNP might affect marbling through affecting pancreatic lipase amount or activity or both (Tanomura *et al.*, 2011). The high frequency of the

rs41648172 SNP T allele in Japanese Black breed out of the 5 cattle breeds may imply that the SNP has a direct impact on marbling. On the other hand, the degree of genetic influence of European breeds on Japanese Brown breed (Sumio, 2007) and Japanese Short Horn breed (Mizuma and Sasaki, 1974; Yamamoto *et al.*, 1979) is considered to be high to some extent although, both of these and the Japanese Black breed are genetically known as Wagyu cattle. Thus, researchers cannot exclude the possibility that the difference in the allele frequency distribution of the rs41648172 SNP among the 5 cattle breeds in this study is attributable to the genetic influence of European breeds rather than the influence of selection for high marbling.

Additionally, Japanese Black breed is known to have a small effective population size. Thus, we cannot exclude the possibility that genetic drift could explain the difference in the allele frequency distribution of the SNP among breeds. Further investigation of the allele frequency distribution of the rs41648172 SNP using Asian indigenous cattle, extremely high-marbled and extremely low-marbled cattle group within each breed or historical samples of either tissue or semen from Wagyu cattle from 50 or more years ago will be needed to deny these possibilities.

CONCLUSION

In this study, researchers investigated the allele frequency distribution of the rs41648172 PNLIP SNP among the 5 cattle breeds, Japanese Black, Japanese Brown, Japanese Short Horn, Holstein and Brown Swiss breeds. The T allele at the SNP associated with high marbling was found at higher frequency in Japanese Black breed that has been subjected to a strong selection for high marbling than in the other breeds that have not been strongly selected for high marbling. This study will provide an useful information for the establishment of effective marker-assisted selection to increase the levels of marbling.

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