RESEARCH NOTE

The *G* allele at the *g.70014208A>G* in the *MYBPC1* gene associated with high marbling in Japanese Black cattle is at a low frequency in breeds not selected for marbling

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Introduction

A single nucleotide polymorphism (SNP), *g.70014208A>G* in the promoter region of the *myosin binding protein C, slow type* (*MYBPC1*) gene is associated with marbling in Japanese Black beef cattle. We investigated the allele frequency distribution of the *g.70014208A>G* SNP among the five cattle breeds, Japanese Black, Japanese Brown, Japanese Short Horn, Holstein and Brown Swiss breeds. As compared to the frequency of the *g.70014208A>GG* allele associated with high marbling in Japanese Black breed that has been subjected to a strong selection for high marbling, those in the other breeds that have not been strongly selected for high marbling were null or lower. Thus, we hypothesized that the pressure of the strong selection for high marbling in Japanese Black breed has increased the frequency of the *G* allele at the *g.70014208A>G* SNP in the *MYBPC1*.

Marbling is characterized by the amount and distribution of intramuscular fat, and improves the palatability and acceptability of the meat in many parts of the world (Busboom *et al.* 1993; Boylston *et al.* 1995; Matsuishi *et al.* 2001). Marbling is an economically important trait of beef cattle in Japan (JMGA 1988).

The *MYBPC1* gene, known to encode the slow skeletal muscle isoform of the major myosin-binding proteins in vertebrate striated muscles (Offer *et al*. 1973; Pepe and Drucker 1975; Sato *et al*. 2003), plays an important role in efficient energy metabolism and homeostasis during muscle contraction (Chen *et al*. 2011), and has been regarded as a functional candidate for the gene responsible for marbling (Tong *et al*. 2012). We have recently reported that a SNP, referred to as *g.70014208A>G*, was detected in the promoter region of the *MYBPC1* between low-marbled and high-marbled steer groups, which were shown to have *MYBPC1* expression differences in musculus longissimus muscle (Sasaki *et al*. 2006a), and that the *g.70014208A>G* SNP was associated with marbling in Japanese Black beef cattle, with the *G* allele resulting in high levels of marbling (Tong *et al*. 2012).

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*. 2006b). In the present study, to depict the possibility that this selection pressure has affected the allele frequency distribution of the *g.70014208A>G* SNP, we investigated the allele frequency distribution of the *g.70014208A>G* SNP among the five cattle breeds.

Materials and methods

Samples

We used 100 sires, 87 sires, 70 sires, 190 cows and 119 cows, respectively, for Japanese Black, Japanese Brown, Japanese Short Horn, Holstein and Brown Swiss breeds. There was no

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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 the population of each breed. Further, we used 745 paternal half-sib Japanese Black progeny steers produced from two sires homozygous for the *A* allele at the *g.70014208A>G,* with dams considered to represent a random sample of the female 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 Faculty of Agriculture, Niigata University (Niigata, Japan).

SNP genotyping

The *g.70014208A>G* SNP was genotyped by polymerase chain reaction-restriction fragment length polymorphism method using restriction enzyme *Bgl*II as described previously (Tong *et al*. 2012).

Statistical analysis

Statistical comparisons among the allelic frequencies for the *g.70014208A>G* SNP in Japanese Black sires, Japanese Brown sires, Japanese Short Horn sires, Holstein cows and Brown Swiss cows, and the data estimated by maternal alleles in half-sib Japanese Black progeny steers were performed by chi-square test or Fisher's exact probability test.

Results and discussion

Table 1 summarizes the allele frequency distribution of the *g.70014208A>G* SNP among the five cattle breeds: Japanese Black, Japanese Brown, Japanese Short Horn, Holstein and Brown Swiss breeds. Departures from the Hardy–Weinberg equilibrium (HWE) were tested for the *g.70014208A>G* SNP in each of sire or cow populations of the five cattle breeds, except for Japanese Brown sires and Brown Swiss cows in which the *A* allele was fixed. Statistically significant departures at the 5% level were not observed for all the tests for the *g.70014208A>G* SNP.

Table 1. Distribution of allele frequency at *g.70014208A>G* among the five cattle breeds.

Breed	Frequency	
	G allele	*A* allele
Japanese Black-sires	0.130	0.870
Japanese Black-progeny steers	0.162	0.838
Japanese Brown	0.000	1.000
Japanese Short Horn	0.007	0.993
Holstein	0.003	0.997
Brown Swiss	0.000	1.000

No statistically significant difference was detected between the allele frequency in sires and the data estimated by maternal alleles in the half-sib progeny steers in Japanese Black breed (table 2). Further, statistical comparisons for the allele frequencies between breeds were performed. Statistically significant differences were detected between Japanese Black breed and the other breeds (table 2). Japanese Black breed has been subjected to a strong specific selection for high marbling that is likely to be selected against by natural selection (Sasaki *et al*. 2006b). The frequency of the *G* allele associated with high marbling was 0.130 in the sires and 0.162 in estimation from maternal alleles in the half-sib progeny steers in Japanese Black breed (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 zero (Japanese Brown sires and Brown Swiss cows) or lower than Japanese Black (Japanese Short Horn sires and Holstein cows) (table 1). Additionally, there were no statistically significant differences among the breeds other than Japanese Black breed (table 2).

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 *G* allele at the *g.70014208A>G*SNP in the *MYBPC1*, assuming that the *g.70014208A>GG* allele has experienced a selective sweep. We have previously supposed that the *g.70014208A>G* SNP might affect *MYBPC1* expression and also marbling through affecting *MYBPC1* promoter activity (Tong *et al*. 2012). The high frequency of the *g.70014208A>GG* allele in Japanese Black breed out of the five cattle breeds may imply that the *g.70014208A>G* SNP has an impact on marbling. On the other hand, the degree of genetic influence of European breeds on Japanese Brown breed (particularly in case of Kumamoto line) (Ito *et al*. 1988; 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, as well as the Japanese Black breed, are generically called as Wagyu cattle. Thus, we cannot exclude the possibility that the difference in the allele frequency distribution of the *g.70014208A>G* SNP between Japanese Black breed and the other 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 between Japanese Black breed and the other breeds. Further investigation of the allele

Table 2. Statistical significance for differences in allele frequency for *g.70014208A>G* between the five cattle breeds.

	Breed				
	Japanese Black-progeny steers	Japanese Brown	Japanese Short Horn	Holstein	Brown Swiss
Japanese Black-sires	n.s.	*	*	*	*
Japanese Black-progeny steers		*	*	*	*
Japanese Brown			n.s.	n.s.	n.s.
Japanese Short Horn				n.s.	n.s.
Holstein					n.s.

N.s., sinsignificant difference ($P > 0.05$); *significant difference ($P < 0.01$).

frequency distribution of the *g.70014208A>G* SNP using Asian indigenous cattle and so on or using extremely high-marbled and extremely low-marbled cattle groups within each breed will be needed to deny this possibility.

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