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Effect of the *PLAG1* gene polymorphism on oleic acid percentage in Japanese Black cattle populations

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ABSTRACT. We investigated the effect of the *PLAG1* gene (on bovine chromosome 14) polymorphism on oleic acid percentage (C18:1) in four Japanese Black cattle populations (JB1: n = 900, JB2: n = 560, JB3: n = 456, JB4: n = 450). We genotyped the *PLAG1* polymorphism (rs109231213) for four populations and then analyzed the effects on carcass weight (CW) and C18:1. This polymorphism was significantly associated with CW in these four populations ($P<0.001$), in agreement with previous reports. In addition, it also had a significant effect on C18:1 in JB1, JB2 and JB3 ($P<0.05$). However, considering that the effect of *PLAG1* differed among populations, it was suggested that the *PLAG1* polymorphism would not have a direct effect on C18:1 and would be in linkage disequilibrium (LD) with a causative mutation. Turkey-Kramer's honestly significant difference test revealed that the qq genotype had a higher percentage of C18:1 than the QQ genotype in JB1 and JB2 (1.62 and 2.23, respectively), while the qq genotype showed lower CW in all four populations. These results suggest that the *PLAG1* gene polymorphism would be useful as a DNA marker for C18:1 in Japanese Black cattle populations; this

information could contribute to the identification of a causative mutation on BTA14 for fatty acid composition.

Key words: Japanese Black cattle; C18:1; *PLAG1*

INTRODUCTION

The pleomorphic adenoma gene 1 (*PLAG1*) on bovine chromosome 14 expresses during late somitogenesis and beyond (Pendeville et al., 2006). It is associated with cattle growth by regulating the expression of genes that effect cell multiplication and cell division (Voz et al., 2000). Some studies have reported that *PLAG1* is a causative gene for certain economically important traits in cattle growth. The study by Karim et al. (2011) identified a polymorphism associated with body length and height in cattle; this polymorphism is also regarded as a causative mutation for carcass weight (CW) in Japanese black, a native Japanese cattle breed (Nishimura et al., 2012; Hoshiba et al., 2013; Takasuga 2016).

On the other hand, quantitative trait loci (QTL) for fatty acid composition have been reported in the region near the *PLAG1* gene on BTA14. In the Japanese Black cattle population, Kawaguchi et al. (2018) detected a QTL for C18:1 on 19 Mb and Kelly et al. (2014) also reported a QTL for C16:0 and C18:0 on 16-31 Mb in a population consisting of seven breeds. Furthermore, several studies have reported an association between the *PLAG1* polymorphism and fatty acid composition. Sasago et al. (2016) reported that the *PLAG1* polymorphism had an association with C16:1 in Japanese Black cattle and Kim et al. (2017) also reported an association of *PLAG1* polymorphism with C20:1 in Hanwoo cattle. Although an association between *PLAG1* polymorphism and fatty acid composition has been observed in some populations, considering the function of *PLAG1*, it could not have a direct effect on fatty acid composition. Sasago et al. (2016) suggested that fatty acid composition might be affected by other genes in linkage disequilibrium (LD) with *PLAG1*. However, considering the reported QTL for fatty acid composition and the association between the *PLAG1* polymorphism and fatty acid composition, there is a causative mutation near *PLAG1* on BTA14.

The objective of this study was to evaluate the effectiveness of the *PLAG1* polymorphism as a DNA marker for improving C18:1 by investigating the association between *PLAG1* and fatty acid composition in various Japanese Black cattle populations.

MATERIAL AND METHODS

Animals

In this study, we used four Japanese Black cattle populations: 900 animals in the Hyogo prefecture (JB1); 560 animals in the Miyazaki prefecture (JB2); 456 animals in the Gifu prefecture (JB3); and 450 animals in different prefectures throughout Japan (JB4). The average age (± standard deviation) at slaughter was 31.83 ± 1.37 months (JB1), 29.10 ± 1.62 months (JB2), 28.32 ± 1.09 months (JB3), and 28.58 ± 1.35 months (JB4). Carcass traits were systematically measured by a certified graded and included CW, rib eye area (REA), rib thickness (RT), subcutaneous fat thickness (SFT), yield estimate (YE), and BMS. Table 1

presents summary statistics of carcass traits and fatty acid composition in these four Japanese Black cattle populations. Genomic DNA was extracted from each 50 mg longissimus cervicis muscle sample using a standard phenol-chloroform method. Intramuscular fat samples were collected from the longissimus thoracis muscle in JB1, JB3, and JB4, and perirenal fat tissue was collected in JB2 to analyze the fatty acid composition.

Table 1. Summary statistics of carcass traits and fatty acid composition in four Japanese Black cattle populations

Trait	JB1		JB2		JB3		JB4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Carcass weight (kg)	388.34	39.63	432.06	46.87	446.18	41.92	450.99	55.97
Rib eye area (cm^2)	52.50	7.06	55.44	7.31	56.07	7.44	56.27	8.58
Rib thickness (cm)	6.65	0.66	7.46	0.80	7.66	0.85	7.71	0.87
Subcutaneous fat thickness (cm)	2.36	0.67	2.38	0.70	2.38	0.69	2.87	0.79
Yield estimate (%)	73.70	1.19	74.08	1.26	74.10	1.25	73.66	1.46
BMS	5.83	0.93	6.07	1.98	6.43	2.20	6.44	2.24
Fatty acid composition (%)								
C14:0	2.16	0.47	2.64	0.65	2.75	0.58	2.67	0.59
C14:1	0.98	0.24	0.89	0.35	0.93	0.24	0.88	0.30
C16:0	23.61	3.07	24.35	3.27	27.38	2.36	26.94	2.06
C16:1	4.42	0.69	2.75	0.71	3.79	0.64	3.91	0.78
C18:0	10.55	1.64	19.43	3.45	10.73	1.39	11.50	1.66
C18:1	53.32	3.50	48.00	5.20	51.90	3.31	51.71	2.93
C18:2	2.07	0.44	1.95	0.47	2.52	0.58	2.31	0.78
MUFA	60.03	3.87	51.63	5.40	56.62	3.29	56.50	3.10
SFA	37.81	3.96	46.42	5.47	40.86	3.37	41.11	3.15

Fatty acid composition

To analyze the fatty acid composition, a total lipid extraction was performed as described by Folch et al. (1957). The extracted fat was saponified with a potassium hydrate-ethanol solution and then methyl-esterified with a boron trifluoride-methanol complex. The processed fat was analyzed by gas chromatography (6890A; Agilent Technologies, Santa Clara, CA, USA) under the following conditions: inlet temperature of 150°C, oven was warmed from 150 to 220°C, and detector sensor temperature was 220°C. We used helium gas as a carrier, a capillary column (TC-70, 0.25 mm I.D. × 60 m, df = 0.25 μm; GL Science, Tokyo, Japan), and a flame ionization detector for detection. Each fatty acid percentage (C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, and C18:2) was expressed as a percentage of the total fatty acid content. The monounsaturated fatty acid percentage (MUFA) and saturated fatty acid percentage (SFA) were then calculated from the fatty acid composition.

Genotyping

Stature quantitative trait nucleotides (QTNs) in the *PLAG1* gene were difficult to amplify by regular PCR. Therefore, we genotyped FJX_250879 (rs109231213) SNP according to the studies by Nishimura et al. (2012) and Hoshiba et al. (2013). They reported that FJX_250879, which is located downstream of the *PLAG1* gene, was in nearly complete LD (r^2 = 0.998) with stature QTNs in 1156 Japanese black steers. The genotyping was performed by the TaqMAN assay, according to the study by Karim et al. (2011).

Statistical analysis

The effect of the *PLAG1* polymorphism on fatty acid composition was statistically tested using analysis of variance (ANOVA) and Tukey's honestly significant difference

(HSD) test. The analytical model included the effect of shipment year, shipment month, linear and quadratic covariates for age at slaughter, sire, sex, and genotype without interactions in JB1, the effect of linear and quadratic covariates for age at slaughter, sire, sex, and genotype in JB2, the effect of linear and quadratic covariates for age at slaughter and genotype in JB3, and the effect of linear and quadratic covariates for age at slaughter, sex, year, and genotype in JB4.

RESULTS

We genotyped the *PLAG1* polymorphism (109231213) in four Japanese Black cattle populations (Table 2). The minor allele q frequencies were 0.462, 0.329, 0.224, and 0.236 in JB1, JB2, JB3, and JB4, respectively.

Table 2. Genotype and allele frequency of *PLAG1* gene polymorphism in four Japanese Black cattle populations

Population	N	Genotype frequency (n)			Allele frequency	
		QQ	Qq	qq	Q	q
JB1	900	0.247 (222)	0.582 (524)	0.171 (154)	0.538	0.462
JB2	560	0.471 (264)	0.400 (224)	0.129 (72)	0.671	0.329
JB3	456	0.614 (280)	0.325 (148)	0.061 (28)	0.766	0.224
JB4	450	0.587 (264)	0.356 (160)	0.058 (26)	0.764	0.236

We investigated the associations between the *PLAG1* polymorphism and carcass traits and fatty acid composition using ANOVA (Table 3). Significant associations were observed between CW, REA, BMS, C14:0, C16:0, C18:0, C18:1, MUFA, and SFA in JB1; with CW, REA, SFT, YE, C16:0, C18:0, C18:1, MUFA, and SFA in JB2; with CW, REA, C18:0, C18:1, and MUFA in JB3; and with CW, RT, BMS, C14:0, and C18:0 in JB4 ($p < 0.05$).

Table 3. ANOVA for carcass traits and fatty acid composition in four Japanese Black cattle populations

Trait	JB1	JB2	JB3	JB4
Carcass weight (kg)	<0.0001	<0.0001	<0.0001	<0.0001
Rib eye area (cm^2)	<0.0001	<0.0001	0.0341	0.0029
Rib thickness (cm)	ns	ns	ns	0.0061
Subcutaneous fat thickness (cm)	ns	0.0027	ns	ns
Yield estimate (%)	ns	0.0405	ns	ns
BMS	0.0074	ns	ns	0.0081
Fatty acid composition (%)				
C14:0	0.0319	ns	ns	0.0380
C14:1	ns	ns	ns	ns
C16:0	<0.0001	0.0227	ns	ns
C16:1	<0.0001	ns	ns	ns
C18:0	<0.0001	0.0422	ns	0.0003
C18:1	<0.0001	0.0028	0.0429	ns
C18:2	ns	ns	0.0364	ns
MUFA	<0.0001	0.0013	0.0243	ns
SFA	<0.0001	0.0024	ns	ns

ns, not significant

A Tukey–Kramer HSD test was conducted to investigate the detailed effect of the *PLAG1* polymorphism on CW and C18:1. Table 4 presents the least square means for CW and C18:1 and significant differences between genotypes. The QQ genotype showed a significantly higher CW than the qq genotype in JB1, JB2, JB3, and JB4 (30.76, 38.43, 39.35, and 66.48, respectively). On the other hand, the qq genotype showed a significantly higher C18:1 than that of the QQ genotype in JB1 and JB2 (1.62 and 2.23, respectively). In JB3,

although the difference was not significant in C18:1, a similar tendency between genotypes was observed as in the JB1 and JB2 populations.

Table 4. Effect of *PLAG1* gene polymorphism on carcass weight and C18:1 in four Japanese Black cattle populations

	Carcass weight			C18:1		
	QQ	Qq	qq	QQ	Qq	qq
JB1	$392.57^{a} \pm 5.16$	$383.36^{b} \pm 4.91$	$361.81^{c} \pm 5.51$	$52.62^{a} \pm 0.44$	$53.53^{b} \pm 0.42$	$54.24^{c} \pm 0.47$
JB2	$437.67^{a} \pm 3.84$	$426.11^{b} \pm 6.35$	$399.22^{c} \pm 4.28$	$48.11^{a} \pm 0.41$	$48.88^{ab} \pm 0.46$	$50.34^{b} \pm 0.68$
JB3	$453.13^{a} \pm 2.42$	$439.15^{b} \pm 3.33$	$413.78^{c} \pm 7.76$	51.59 ± 0.20	52.32 ± 0.27	52.69 ± 0.63
JB4	$455.55^{a} \pm 8.84$	$429.26^{b} \pm 3.65$	$389.07^{c} \pm 2.90$	51.70 ± 0.18	52.24 ± 0.22	51.84 ± 0.54

Means with different superscript (a,b,c) are significantly different between genotypes.

DISCUSSION

In this study, we focused on the *PLAG1* polymorphism associated with CW and investigated this association with C18:1 for four Japanese Black cattle populations. We evaluated the effectiveness of the *PLAG1* polymorphism as a DNA marker for the improvement of C18:1.

Biases in allele frequency among populations were not observed in genotyping results, suggesting that these Japanese Black cattle populations are not affected by the selection of the *PLAG1* polymorphism. This result reflected the genetic background as Japanese Black has not been strongly selected for CW compared with beef quality such as marbling.

Based on the results of an association analysis, *PLAG1* polymorphism showed a highly significant association with CW in all four populations ($P<0.0001$), which agrees with the results of Nishimura et al. (2012) and Hoshiba et al. (2013). In addition, it also had a significant association with C18:1 in all local populations (JB1, JB2, and JB3; $P<0.05$). This suggests that this marker would also be useful for other local populations in Japan. On the other hand, no significant association was observed in JB4, which could be explained by the fact that JB4 consisted of several different local populations that have different genetic backgrounds.

Sasago et al. (2016) reported on the association between the *PLAG1* polymorphism and C16:0, and they suggested that this association would be attributed to another gene which is in LD with *PLAG1*. Based on our results in which the effect of PLAG1 on C18:1 differed among populations, we suggest that the *PLAG1* polymorphism would not have a direct effect on C18:1 and would be in LD with a causative mutation. The different P-values observed in the JB1, JB2, and JB3 populations could be explained by differences in the degree of LD in each population.

Based on the HSD test, the QQ genotype showed a significantly higher CW than did the qq genotype in all four populations, which agrees with the results of the studies by Nishimura et al. (2012) and Hoshiba et al. (2013). On the other hand, the qq genotype showed a significantly higher C18:1 than did the QQ genotype in JB1 and JB2. In JB3, although the difference was not significant in C18:1, a similar tendency between genotypes was observed as in JB1 and JB2. The q allele frequency was relatively low (0.224 - 0.462) in all four populations, suggesting that the *PLAG1* polymorphism would be effective for the improvement of C18:1 as a DNA marker. However, we should also pay attention to the negative effect of the q allele on CW when we use this polymorphism to improve C18:1.

In conclusion, the *PLAG1* polymorphism has a significant association with C18:1 in three Japanese Black cattle populations, which suggests that this polymorphism could also be effective in other local populations. These results are expected to contribute to the improvement of beef quality in Japanese Black cattle and also to identification of a causative mutation for fatty acid composition on BTA14.

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