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No replicated association of the *c*.-312*A* > *G* in *EDG*1 with marbling in Niigata population of Japanese Black beef cattle

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ABSTRACT

Marbling, defined by the amount and the distribution of intramuscular fat and measured as beef marbling score (BMS), is an economically important trait of beef cattle in Japan. We recently reported that a single nucleotide polymorphism (SNP), namely, *c*.-312*A* > *G*, in the *endothelial differentiation sphingolipid G-protein-coupled receptor*, 1 (*EDG*1) gene was associated with the BMS level in the Japanese Black beef cattle population of Oita prefecture, with the *G* allele being associated with a high level of the BMS. Thus, the *c*.-312*A* > *G* SNP seems to be a candidate marker for marker-assisted selection. In this study, we investigated whether this association could be replicated in the Japanese Black beef cattle population of Niigata prefecture and analyzed the effect of the SNP genotypes on the carcass traits other than the BMS. No significant differences in the BMS level were detected among the genotypes of the *c*.-312*A* > *G* SNP in the Niigata Japanese Black beef cattle population. The SNP genotype had no significant effects on the carcass weight, rib eye area and rib thickness of the cattle population. These findings suggested that the association of the *c*.-312*A* > *G* SNP with the BMS level in the Japanese Black beef cattle population was not replicated in the Niigata population, and revealed no effects of the SNP genotype on the beef productivity in the Niigata population. Thus, we concluded that the *c*.-312*A* > *G* SNP is not useful for effective marker-assisted selection to increase meat quality and, additionally, meat productivity in Japanese Black beef cattle of Niigata prefecture.

Keywords: Association; *EDG*1; Japanese Black Breed; Marbling; Replication Study; Single Nucleotide Polymorphism

1. INTRODUCTION

Generally, marbling means the amount of intramuscular fat in *musculus longissimus* muscle [1]. In Japan, marbling is characterized as the amount and distribution of intramuscular fat in a cross section of *musculus longissimus* muscle, and called Shimofuri [1]. High levels of such marbling improve the palatability and acceptability of beef by affecting the taste and tenderness of the meat [2-4]. Because of the importance of the presence of marbling on the economics of beef production, there is great interest in gaining a better understanding of the molecular architecture of marbling and in generating new opportunities for more effective marker-assisted breeding.

The *endothelial differentiation, sphingolipid G-protein coupled receptor*, 1 (*EDG*1) gene, involved in blood vessel formation [5], has been previously shown to possess higher expression levels in a high-marbled steer group than in a low-marbled steer group in *musculus longissimus* muscle across all ages [6,7]. *EDG*1 is located within the genomic region of a quantitative trait locus for marbling on chromosome 3 [7,8], and thus has been regarded as a positional functional candidate for the gene responsible for marbling [9]. We have recently reported

that a single nucleotide polymorphism (SNP), *c*.-312*A* > *G* in the *EDG*1 was detected between the 2 steer groups, and associated with marbling in the Japanese Black beef cattle population in Oita prefecture, with the *G* allele of the SNP being associated with a high level of the marbling [9].

Thus, we have now investigated whether this association could be replicated in the Japanese Black beef cattle population of the Niigata prefecture and analyzed the effects of the SNP genotypes on the carcass traits (carcass weight (CWT), rib eye area (REA), and rib thickness (RT)) other than the beef marbling score (BMS), in order to confirm the application of the *c*.-312*A* > *G* SNP to effective marker-assisted selection in the Niigata Japanese Black cattle population.

2. MATERIALS AND METHODS

2.1. Samples and Data

Japanese Black cattle population from the Niigata prefecture were used, and we studied the association of the *c*.-312*A* > *G* SNP with BMS, CWT, REA, and RT. In this study, 130 paternal half-sib progeny steers (1 to 16 steers per sire) 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).

BMS, CWT, REA, and RT 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, CWT, REA, and RT for the steers were used as the phenotypic values in this study [10]. The breeding values were predicted using carcass records of Japanese Black steers and heifers fattened in the Niigata prefecture, and obtained from the Niigata Pre-fectural Headquarters, National Federation of Agricultural Cooperative Association (Niigata, Japan). The animals slaughtered were part of the population used in this study.

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 *c*.-312*A* > *G* SNP was genotyped by the PCR-restriction fragment length polymorphism method as described previously [9]. Using this method, 378-bp PCR fragments containing the SNP site were amplified and *Msc* I-digested into 163- and 215-bp fragments at the *A* allele, but not the *G* allele: the *GG* homozygotes, the *AA* homozygotes and the *AG* heterozygotes yielded 1 band (378 bp), 2 bands (163 and 215 bp) and 3 bands (163, 215, and 378 bp), respectively.

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 half-sib progeny steers or the frequencies estimated from maternal alleles possessed by the steers in the Niigata prefecture population and data from the Japanese Black cattle population of the Oita prefecture, as well as Japanese Brown, Japanese Short Horn, Holstein, or Brown Swiss cattle populations [9,11] were also performed by chi-square test. The effect of genotypes at the SNP on the predicted breeding values for the BMS, CWT, REA, and RT was analyzed with a model that included the SNP genotype as a fixed effect and the sire as a random effect. Statistical analysis was performed by the MIXED procedures of the SAS program version 9 (SAS Institute, Inc., Cary, NC).

3. RESULTS AND DISCUSSION

Genotyping the 130 paternal half-sib progeny steers for the *c*.-312*A* > *G* SNP revealed 46 animals homozygous for the *A* allele, 57 animals heterozygous for the *A* allele and the *G* allele and 27 animals homozygous for the *G* allele for the *c*.-312*A* > *G* SNP (**Table 1**). The observed heterozygosity value at the SNP was in agreement with the expected heterozygosity value, indicating that the Niigata prefecture population conformed to the Hardy-Weinberg equilibrium. The frequency of the *G* allele of *c*.-312*A* > *G* SNP in the Niigata prefecture population (0.427) was higher than the frequencies of this allele in Japanese Brown, Japanese Short Horn, Holstein, and Brown Swiss cattle populations that have not been strongly selected for high marbling [11], but lower than the frequency in Japanese Black cattle population of the Oita prefecture [9]. However, no statistically significant difference was detected between the allele frequencies estimated from maternal alleles possessed by the half-sib progeny steers in the Niigata prefecture population and those obtained in the Japanese Black cattle population of the Oita prefecture [9]. These frequencies were significantly higher than those of Japanese Brown, Japanese Short Horn, Holstein, and Brown Swiss cattle populations [11] (data not shown).

No statistically significant differences in BMS level were detected among the genotypes (**Table 2**). These

Table 1. Frequencies of *c*.-312*A* > *G* SNP genotypes in Japanese black cattle population of the Niigata prefecture.

Genotype	No. of animals	Frequency
AA	46	0.354
AG	57	0.438
GG	27	0.208

Table 2. Effects of *c*.-312*A* > *G* SNP genotype on BMS, CWT, REA, and RT in Japanese Black cattle population of the Niigata prefecture.

Trait[1]	*P*-value	Genotype[2]		
		AA	*AG*	*GG*
BMS	0.186	1.35 ± 0.06	1.37 ± 0.05	1.53 ± 0.08
CWT	0.157	40.60 ± 4.34	42.08 ± 3.90	53.65 ± 5.70
REA	0.546	6.45 ± 0.59	6.85 ± 0.53	7.52 ± 0.77
RT	0.133	0.53 ± 0.05	0.63 ± 0.04	0.67 ± 0.06

[1]BMS, Beef marbling score (unit); CWT, Carcass weight (kg), REA, Rib eye area (cm^2); RT, Rib thickness (cm); [2]The breeding values are given as least squares means ± SE.

results were not consistent with the data obtained in our previous study in the Oita population [9]. These results did not show replication for the association of the *c*.-312*A* > *G* SNP with BMS. Based on no replicated association, we propose that the *c*.-312*A* > *G* SNP is in linkage disequilibrium with not yet identified causative mutation for BMS level in the *EDG*1 gene in the Oita prefecture population, but not in the Niigata prefecture population. There may be differences between the history of these populations. The causative mutation may correspond to a novel SNP, *g*.1471620*G* > *T*, in 5' flanking region of the *EDG*1 gene [12]. Alternatively, a tendency towards significance for BMS was observed (P = 0.186) and genotypic profiles of the predicted breeding value for the BMS showed trends similar to data obtained in our previous study in the Oita prefecture population [9] (**Table 2**). Thus, our present study in the Niigata prefecture population might not have enough power to detect an association with the BMS.

The effect of the *c*.-312*A* > *G* SNP genotype was not statistically significant for the CWT, REA, and RT (**Table 2**). Thus, it is likely that the *c*.-312*A* > *G* SNP is not associated with the CWT, REA, and RT in the Japanese Black beef cattle population of the Niigata prefecture. The marbling quantitative trait locus corresponding to the genomic position of *EDG*1 on bovine chromosome 3 has shown a statistically significant effect on RT as well as on BMS [13]. Based on no association, the marbling quantitative trait locus was supposed to be distinct from the quantitative trait locus responsible for RT level. Otherwise, lack of linkage disequilibrium or insufficient detection power might lead to no association of the *c*.-312*A* > *G* SNP with the RT in the present study.

4. CONCLUSION

We concluded that the *c*.-312*A* > *G* SNP is not useful for effective marker-assisted selection to increase meat quality and, additionally, meat productivity in Japanese Black beef cattle of Niigata prefecture. Thus, this study will provide information on differences of populations in marker-assisted selection for 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, R.W.Jr. (1993) Effects of biological source on cooking and palatability attributes of beef produced for the Japanese market. *Meat Science*, **35**, 241-258. http://dx.doi.org/10.1016/0309-1740(93)90054-L

[3] Boylston, T.D., Morgan, S.A., Johnson, K.A., Busboom, J.R., Wright, R.W.Jr. 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. http://dx.doi.org/10.1021/jf00053a015

[4] Matsuishi, M., Fujimori, M. and Okitani, A. (2001) Wagyu beef aroma in Wagyu (Japanese Black cattle) beef preferred by the Japanese over imported beef. *Animal Science Journal*, **72**, 498-504. https://www.jstage.jst.go.jp

[5] Liu, Y., Wada, R., Yamashita, T., Mi, Y., Deng, C.X., Hobson, J.P., Rosenfeldt, H.M., Nava, V.E., Chae, S.S., Lee, M.J., Liu, C.H., Hla, T., Spiegel, S. and Proia, R.L. (2000) Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. *Journal of Clinical Investigation*, **106**, 951-961. http://dx.doi.org/10.1172/JCI10905

[6] Sasaki, Y., Nagai, K., Nagata, Y., Doronbekov, K., Nishimura, S., Yoshioka, S., Fujita, T., Shiga, K., Miyake, T., Taniguchi, Y. and Yamada, T. (2006) Exploration of genes showing intramuscular fat deposition-associated changes in musculus longissimus muscle. *Animal Genetics*, **37**, 40-46. http://dx.doi.org/10.1111/j.1365-2052.2005.01380.x

[7] Sasaki, Y., Yamada, T. and Miyake, T. (2006) Quantitative and molecular genetic approaches for the improvement of carcass traits in the Wagyu cattle. 8*th World Congress on Genetics Applied to Livestock Production*, *Session no.* 13-01. Minas Centro Convention Center, Belo Horizonte, Minas Gerais.

[8] Yamada, T., Taniguchi, Y., Nishimura, S., Yoshioka, S., Takasuga, A., Sugimoto, Y. and Sasaki, Y. (2006) Radiation hybrid mapping of genes showing intramuscular fat deposition-associated expression changes in bovine musculus longissimus muscle. *Animal Genetics*, **37**, 184-185. http://dx.doi.org/10.1111/j.1365-2052.2006.01426.x

[9] Yamada, T., Itoh, M., Nishimura, S., Taniguchi, Y., Miyake, T., Sasaki, S., Yoshioka, S., Fujita, T., Shiga, K., Morita, M. and Sasaki, Y. (2009) Association of single nucleotide polymorphisms in the endothelial differentia-

tion sphingolipid G-protein coupled receptor 1 gene with marbling in Japanese Black beef cattle. *Animal Genetics*, **40,** 209-216. http://dx.doi.org/10.1111/j.1365-2052.2008.01822.x

[10] Sasaki, Y., Miyake, T., Gaillard, C., Oguni, T., Matsumoto, M., Ito, M., Kurahara, T., Sasae, Y., Fujinaka, K., Ohtagaki, S. and Dougo, T. (2006) Comparison of genetic gains per year for carcass traits among breeding programs in the Japanese Brown and the Japanese Black cattle. *Journal of Animal Science*, **84**, 317-323. http://www.journalofanimalscience.org

[11] Watanabe, N., Yoshioka, S., Itoh, M., Satoh, Y., Furuta, M., Komatsu, S., Sumio, Y., Fujita, Y., Yamada, T. and Sasaki, Y. (2009) The G allele at the c.-312A>G SNP in the EDG1 gene associated with high marbling in Japanese Black cattle is at a low frequency in breeds not selected for marbling. *Animal Genetics*, **40**, 575-582. http://dx.doi.org/10.1111/j.1365-2052.2009.01871.x

[12] Yamada, T., Sasaki, S., Sukegawa, S., Miyake, T., Fujita, T., Kose, H., Morita, M., Takahagi, Y., Murakami, H., Morimatsu, F. and Sasaki, Y. (2009) Novel SNP in 5' flanking region of EDG1 associated with marbling in Japanese Black beef cattle. *Animal Science Journal*, **80**, 486-489. http://dx.doi.org/10.1111/j.1740-0929.2009.00665.x

[13] Takasuga, A., Watanabe, T., Mizoguchi, Y., Hirano, T., Ihara, N., Takano, A., Yokouchi, K., Fujikawa, A., Chiba, K., Kobayashi, N., Tatsuda, K., Oe, T., Furukawa-Kuroiwa, M., Nishimura-Abe, A., Fujita, T., Inoue, K., Mizoshita, K., Ogino, A. and Sugimoto, Y. (2007) Identification of bovine QTL for growth and carcass traits in Japanese Black cattle by replication and identical-by-descent mapping. *Mammalian Genome*, **18**, 125-136. http://dx.doi.org/10.1007/s00335-006-0096-5