No replicated association of the c.-312A > G in EDG1 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.-312A > G, in the endothelial differentiation sphingolipid G-protein-coupled receptor, 1 (EDG1) 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.-312A > 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.-312A > 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.-312A > 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.-312A > 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; EDG1; 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 (EDG1) 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]. EDG1 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.-312A > G in the EDG1 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.-312A > 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.-312A > 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.-312A > 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.-312A > 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.-312A > 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.-312A > 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
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.-312A > G SNP with BMS. Based on the replicated association, we propose that the c.-312A > G SNP is in linkage disequilibrium with yet-identified causative mutation for BMS level in the EDG1 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.1471620G > T, in 5' flanking region of the EDG1 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.-312A > G SNP genotype was not statistically significant for the CWT, REA, and RT (Table 2). Thus, it is likely that the c.-312A > 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 EDG1 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.-312A > G SNP with the RT in the present study.

4. CONCLUSION

We concluded that the c.-312A > 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.

5. ACKNOWLEDGEMENTS

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REFERENCES


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

<table>
<thead>
<tr>
<th>Trait</th>
<th>P-value</th>
<th>Genotype2</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMS</td>
<td>0.186</td>
<td></td>
<td>1.35 ± 0.06</td>
<td>1.37 ± 0.05</td>
<td>1.53 ± 0.08</td>
</tr>
<tr>
<td>CWT</td>
<td>0.157</td>
<td></td>
<td>40.60 ± 4.34</td>
<td>42.08 ± 3.90</td>
<td>53.65 ± 5.70</td>
</tr>
<tr>
<td>REA</td>
<td>0.546</td>
<td></td>
<td>6.45 ± 0.59</td>
<td>6.85 ± 0.53</td>
<td>7.52 ± 0.77</td>
</tr>
<tr>
<td>RT</td>
<td>0.133</td>
<td></td>
<td>0.53 ± 0.05</td>
<td>0.63 ± 0.04</td>
<td>0.67 ± 0.06</td>
</tr>
</tbody>
</table>

1BMS, Beef marbling score (unit); CWT, Carcass weight (kg), REA, Rib eye area (cm²); RT, Rib thickness (cm); 2The breeding values are given as least squares means ± SE.
http://dx.doi.org/10.1111/j.1365-2052.2008.01822.x

http://www.journalofanimalscience.org

http://dx.doi.org/10.1111/j.1365-2052.2009.01871.x

http://dx.doi.org/10.1111/j.1740-0929.2009.00665.x

http://dx.doi.org/10.1007/s00335-006-0096-5