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Genome-wide association study for fatty acid composition in Japanese Black cattle

Atsushi ISHII,1 Keita YAMAJI,1 Yoshinobu UEMOTO,2 Nanae SASAGO,2 Eiji KOBAYASHI,3 Naohiko KOBAYASHI,4 Tamako MATSUHASHI,4 Shin MARUYAMA,4 Hirokazu MATSUMOTO,1 Shinji SASAZAKI1 and Hideyuki MANNEN1

1Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, 2National Livestock Breeding Center, Fukushima, 3National Institute of Livestock and Grassland Science, Tsukuba and 4Gifu Prefectural Livestock Research Institute, Gifu, Japan

ABSTRACT

Fatty acid composition is one of the important traits in beef. The aim of this study was to identify candidate genomic regions for fatty acid composition by genome-wide association study with 50 K single nucleotide polymorphism (SNP) array in Japanese Black cattle. A total of 461 individuals and 40 657 SNPs were used in this study. We applied genome-wide rapid association using mixed model and regression (GRAMMAR) and genomic control approaches to estimate the associations between genotypes and fatty acid composition. In addition, two SNPs in fatty acid synthase (FASN) (T1952A) and stearoyl-CoA desaturase (SCD) (V293A) genes were also genotyped. Association analysis revealed that 30 significant SNPs for several fatty acids (C14:0, C14:1, C16:1 and C18:1) were located in the BTA19 FASN gene located within this region but the FASN mutation had no significant effect on any traits. We also detected one significant SNP for C18:1 on BTA23 and two SNPs for C16:0 on BTA25. The region around 17 Mb on BTA26 harbored two significant SNPs for C14:1 and SNP in SCD in this region showed the strongest association with C14:1. This study demonstrated novel candidate regions in BTA19, 23 and 25 for fatty acid composition.

Key words: fatty acid composition, GWAS, Japanese Black cattle.

INTRODUCTION

Japanese Black cattle have high meat quality characteristics, which are known for their remarkably high marbling score (Ibi et al. 2005). While beef marbling is an important factor affecting meat quality, fatty acid composition of adipose tissue in cattle has also become an important trait in the beef industry (Cameron & Enser 1991; Matsubara et al. 1998; Laborde et al. 2001). Fat tissue containing abundant monounsaturated fatty acids (MUFA)s reflects a lower melting point, which contributes positively to favorable beef flavor and tenderness (Melton et al. 1982; Smith et al. 2006). In addition, the fatty acid composition of beef products has received considerable attention for its significance in human health. Intake of saturated fatty acid (SFA), such as C14:0 and C16:0, is considered to have an adverse effect on cardiovascular diseases (Keys et al. 1974). Therefore, improvement of fat quality traits will give rise to additional meat value in the beef market.

Fatty acid composition is influenced by various environmental effects (Huerta-Leidenz et al. 1991; Chang et al. 1992; Suzuki et al. 2007) such as diet (Edwards et al. 1961; Cabezas et al. 1965), while many studies suggest that fatty acid composition could be an inherited trait (Yoshimura & Namikawa 1983; Perry et al. 1998; Oka et al. 2002). If so, fatty acid composition could be controlled by genetic factors related to lipid synthesis and fatty acid metabolism. The heritability of fatty acids was estimated to range from 0.31 to 0.73, indicating a strong genetic basis (Inoue et al. 2008).

Recently, high-density multiplex single nucleotide polymorphism (SNP) genotyping arrays have been developed with availability of numerous SNPs through whole-genome sequencing. With the advance of SNP genotyping arrays, genome-wide association study (GWAS) has been practical for exploring quantitative trait loci (QTL). GWAS enables an efficient search of
the entire genome for complex traits by assuming the linkage disequilibrium (LD) between SNP markers and QTL. Therefore, GWAS has higher power to detect QTL and to define narrower genomic regions harboring the causative genes and polymorphisms related to various traits (Barendse et al. 2007; Feugang et al. 2009; Kolbehdari et al. 2009; Settles et al. 2009).

Uemoto et al. (2011) first described QTL for the percentage of oleic acid (C18:1), which was one of the major MUFA5s in beef fat, by GWAS with the Illumina BovineSNP50 whole-genome SNP assay (Illumina, San Diego, CA, USA) in Japanese Black cattle population from Yamagata prefecture. The significant SNPs for C18:1 were detected on only BTA19 by the selective genotyping method. On the significant region, the fatty acid synthase (FASN) gene was identified as a candidate gene in their study. Fatty acid composition has been generally reported as a complex trait and could be influenced by several genes. Therefore, there should be other responsible regions affecting fatty acid composition. In fact, a few genes and polymorphisms affecting fatty acid composition have been detected in Japanese Black cattle by candidate gene analyses (Taniguchi et al. 2004; Hoashi et al. 2007). In addition, the selective genotyping method can be performed for only one trait, and it’s difficult to assess the relationship between significant SNPs for a specific trait and other related traits.

Therefore, the aim of this study was to detect novel candidate regions affecting fatty acid composition by GWAS with high-density SNP array in a Japanese Black cattle population from Gifu prefecture.

**MATERIALS AND METHODS**

**Animals and samples**

Intramuscular adipose tissue or perinephric fat tissue samples of Japanese Black cattle were collected from Gifu prefecture. A total of 468 samples were selected from 2193 samples by the criteria of at most 11 progenies in each sire and were then used for the determination of fatty acid composition. For analyzing fatty acid content, total lipid extraction was performed as described by Folch et al. (1957). The extracted fat was saponified with 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: the temperature of the inlet was 150°C, the oven was warmed from 150 to 220°C, and the temperature of the detector sensor was 228°C; and the temperature of the detector sensor was 220°C. We used helium gas as a carrier, a capillary column (TC-70, 0.25 mm internal diameter × 60 m, d.f. = 0.25 μm; GL Science, Tokyo, Japan) and a flame ionization detector for detection.

**Genotyping and quality control**

Genomic DNA was extracted from a 35-mg Trapezius muscle sample using phenol-chloroform. Sample DNA was quantified and genotyped using the Illumina BovineSNP50v2 BeadChip. The Illumina BovineSNP50v2 BeadChip assay contains 54,609 SNPs with an average probe spacing of 49.9 kb. Image data were analyzed using the Chromosome Viewer tool contained in BEADSTUDIO (Illumina) software. In this study, the autosomal chromosomes were used.

In consequence of genotyping, samples with call rate < 0.95 were excluded and 461 animals were finally used for statistical analysis. The SNP quality control was assessed using PLINK software (Purcell et al. 2007). The exclusion criteria for SNPs were minor allele frequency < 0.01, call rate < 0.95 and Hardy–Weinberg equilibrium (HWE) < 0.001.

We also genotyped two polymorphisms of candidate genes, stearoyl-CoA desaturase (SCD) (c.877T>C, V293A) and FASN (g.16024A>G, T1952A), by the PCR–RFLP (restriction fragment length polymorphism) method as described by Taniguchi et al. (2004) and Abe et al. (2009), respectively.

**Statistical analysis**

We performed genome-wide rapid association using mixed model and regression (GRAMMAR, Aulchenko et al. 2007) to detect significant SNPs. Our method is composed of three steps as follows.

In the first step, the data were analyzed by the mixed model

\[ y = X\beta + Zu + e \]

where \( y \) is the phenotype value, \( X \) and \( Z \) are the design matrices for fixed and random effects, respectively, \( \beta \) is slaughter year (3 classes), slaughter month (10 classes) and farm (37 classes) effects as a fixed effect and slaughter age effects as covariate. \( u \) is polygenic effect with \( u \sim N(0, \mathbf{A}\sigma^2_u) \), and \( e \) is residual effect with \( e \sim N(0, \sigma^2_e) \). Pedigrees of the base population animals were traced back three generations to create the numerator relationship matrix, \( \mathbf{A} \), and a total of 2289 animals were included in this study. The corrected phenotypes from this analysis are given by

\[ e = y - (X\hat{\beta} + Z\hat{u}) \]

The corrected phenotypes were estimated using ASREML software (Gilmour et al. 2006).

In the second step, these corrected phenotypes were used as the dependent trait in a simple linear regression for each SNP.

\[ e = 1, \mu + \beta_1w_i + e' \]

where \( \mu \) is the mean, \( \beta_1 \) is the allele substitution effect at \( i \)-th SNP \( w \) is the vector of the genotype at \( i \)-th SNP, and \( e' \) is the random residual at \( i \)-th SNP. We performed the Wald test based on t-distributions and regression coefficients and asymptotic P-values were obtained by using PLINK software (Purcell et al. 2007).

In the third step, we corrected the P-values of analysis by the genomic control (GC) method using an estimate of the inflation factor (\( \lambda_{\text{GC}} \)), which was obtained by comparing the observed median of all chi-square test statistics to the value predicted by theory (0.675). This approach was also carried out in the PLINK software (Purcell et al. 2007). To determine the threshold value, the Bonferroni correction was applied to account for multiple hypotheses testing in this study.

The LD coefficients (\( r^2 \)) between individual SNPs, which reached the values for 5% level of genome-wide significance.
Table 1  Statistics for fatty acid composition in Japanese Black cattle population

<table>
<thead>
<tr>
<th>Trait</th>
<th>Observed phenotype</th>
<th>Corrected phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>C14:0</td>
<td>2.75</td>
<td>0.58</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.94</td>
<td>0.24</td>
</tr>
<tr>
<td>C16:0</td>
<td>27.39</td>
<td>2.38</td>
</tr>
<tr>
<td>C16:1</td>
<td>3.79</td>
<td>0.64</td>
</tr>
<tr>
<td>C18:0</td>
<td>10.73</td>
<td>1.38</td>
</tr>
<tr>
<td>C18:1</td>
<td>51.89</td>
<td>3.32</td>
</tr>
<tr>
<td>C18:2</td>
<td>4.52</td>
<td>0.58</td>
</tr>
<tr>
<td>MUFA</td>
<td>56.62</td>
<td>3.29</td>
</tr>
<tr>
<td>SFA</td>
<td>40.87</td>
<td>3.38</td>
</tr>
</tbody>
</table>

Fatty acid compositions are calculated as percentages to total fatty acids. The monounsaturated fatty acids (MUFA) are C14:1, C16:1 and C18:1. The saturated fatty acids (SFA) are C14:0, C16:0 and C18:0. SD, standard deviation.

for single-marker associations, were also calculated in order to assess LD, and haplotype blocks were identified using the so-called four-gamete rule (Wang et al. 2002). In the four-gamete rule approach, the population frequencies of the four possible two-marker haplotypes were computed. If all four were observed with a frequency of at least 0.01, a recombination is deemed to have taken place. Blocks were then formed by consecutive markers where only three gametes were observed. These analyses were carried out in the HAPLOVIEW 4.0 software (Barrett et al. 2005).

RESULTS & DISCUSSION
Genotyping and phenotype correction
A total of 461 animals were used in this study by excluding seven animals with call rate < 0.95. We also assessed the SNP quality and excluded 11 613 SNPs with minor allele frequencies < 0.01, 558 SNPs with a call rate < 0.95 and 382 SNPs in HWE with a nominal P < 0.001. Therefore, a total of 40 657 SNPs were used in this study. The average intervals between SNPs in the whole genome were 61.5 kbp. Goddard and Hayes (2009) have reported that moderate LD in cattle populations extend up to 100 kbp. This suggests that the SNP chip in the present study may be efficient for GWAS analysis.

Fundamental statistics of the raw and corrected fatty acid composition for 461 individuals are given in Table 1. To account for the family relatedness of this population, pedigree-based mixed model was first performed to correct phenotype. The inflation factors calculated in this study were less than 1.1 in all traits, and all traits were properly corrected. Uemoto et al. (2011) showed that pedigree-based mixed model can successfully accounted for family relatedness in a Japanese Black cattle population, and our results also showed the effectiveness of this method.

Genome-wide association study in all chromosomes
A genome-wide plot of P-values corrected by the GC method is shown in Figure 1. The significant threshold of genome-wide significance at 5% and 1% accounting for multiple testing by Bonferroni correction were P = 1.23 × 10^-6 and P = 2.46 × 10^-7, respectively. The SNPs with a genome-wide significance of 5% are given in Table 2.

Figure 1  Genome-wide plots -log_{10} (P-value) for an association of loci with (a) C14:0, (b) C14:1, (c) C16:0, (d) C16:1, (e) C18:0, (f) C18:1, (g) C18:2, (h) monounsaturated fatty acids (MUFA), (i) saturated fatty acids (SFA). P-values were corrected by the genomic control method. The x-axis indicates the chromosome no., and the y-axis indicates -log_{10} (P-value). Solid line indicates the threshold of Bonferroni 1% significance level, and dashed line indicates the threshold of Bonferroni 5% significance level.

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the approximately same regions as C14:0, C14:1 and C16:1. One significant SNP for C18:1 on BTA23 was detected in 6.8 Mbp. There were no significant SNPs for stearic acid (C18:0), linoleic acid (C18:2), MUFA and SFA. Although SNPs for MUFA and SFA did not reach the significance threshold, these SNPs indicated suggestive associations on BTA19, 23 and 25 (Fig. 1h,i).

Significant SNPs for C14:0, C14:1, C16:1 and C18:1 were detected on BTA19. Although SNPs for C16:0 on BTA19 did not reach the significance threshold, these SNPs indicated suggestive association. Inoue et al. (2011) reported genetic correlations between fatty acids in Japanese Black cattle. C14:0 showed high genetic correlation with C14:1 (0.51), C16:0 (0.70), C16:1 (0.37) and C18:1 (−0.91) while a significant correlation with C18:0 and C18:2 were not observed. These results suggested that a single responsible mutation on BTA19 would be associated with multiple fatty acids (C14:0, C14:1, C16:0, C16:1 and C18:1).

GWAS in the particular chromosome

Figure 2 shows genome-wide plots of the representative candidate chromosome regions, including the significant SNPs. The detailed descriptions for each region are as follows.

**BTA19**

BTA19 harbors 30 significant SNPs for C14:0, C14:1, C16:1 and C18:1 (Table 2). Most significant SNPs were identified in the region between 49 and 52 Mbp. The FASN gene was located in the region surrounding these significant SNPs for fatty acid composition (Fig. 2a). Abe et al. (2008, 2009) identified the FASN mutations (T1952A) with an effect on the C18:1
content by linkage analysis using an F2 resource population crossed by Japanese Black sires and Limousin dams. Uemoto et al. (2011) also detected significant SNPs for C18:1 in a similar region (between 49 and 55 Mbp) by GWAS in Japanese Black cattle, and demonstrated that the mutation in *FASN* gene (T1952A) had a significant effect for C18:1.

Therefore, we performed an association analysis between the mutation and the traits using a Gifu prefecture population. However, the *FASN* mutation had no significant effect on the percentage of C18:1 ($P = 0.02$) and other fatty acids (Table 3). The result of LD analysis indicated that LD coefficient between the *FASN* mutation and the most significant SNP for C18:1 was moderate ($r^2 = 0.40$) (Fig. 3). This result suggested that the *FASN* T1952A would not be responsible for the candidate region identified in GWAS in the Gifu population. The haplotype block with high LD coefficient was observed in the region (ARS-BFGL-NGS-39328 – Hapmap39750-BTA-45775), including *FASN* T1952A mutation and the most significant SNP for C18:1 (ARS-BFGL-NGS-39328) (Fig. 3). Therefore, this is the most probable candidate region to identify the responsible mutation.

In previous studies, other *FASN* mutations affecting fatty acid composition have been identified in various breeds (Roy et al. 2006; Zhang et al. 2008; Abe et al. 2009). These *FASN* mutations might be considered as candidates in this study. Moreover, the significant SNPs spanned a wide range (between 44 and 54 Mbp), suggesting additional candidate genes, such as growth hormone (GH), which affects lipid metabolism (Ardiyanti et al. 2009) or urotensin 2 receptor (UTS2R), which plays an important role in insulin resistance and

<table>
<thead>
<tr>
<th>Trait</th>
<th>Gene</th>
<th>MAF</th>
<th>SNP effect</th>
<th>$P$-value‡</th>
<th>COD</th>
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<tr>
<td>C14:0</td>
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<tr>
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<td>0.12</td>
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<td>0.01</td>
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<td>0.01</td>
<td>0.63</td>
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<tr>
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<td>FASN</td>
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<td>0.23</td>
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<tr>
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<td>FASN</td>
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<td>0.58</td>
<td>0.18</td>
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</table>

‡The significant threshold of genome-wide significance at 5% accounting for multiple testing by Bonferroni correction were $P = 1.23 \times 10^{-6}$. COD, coefficient of determination; MAF, minor allele frequency; SE, standard error.
regulates muscle fat accumulation and fatty acid metabolism (Totsune et al. 2003) (Fig. 2a). In addition, several QTLs for fatty acid composition have been also reported in the same region on BTA19 (Morris et al. 2010; Matsuhashi et al. 2011). These candidate mutations and/or genes are expected to be responsible for this region.

BTA23 and BTA25

One significant SNP for C18:1 was detected on BTA23 and two significant SNPs for C16:0 were detected on BTA25 (Table 2). No responsible genes and QTLs for fatty acid composition have been reported on BTA23 and BTA25, while these regions harbor many genes. For example, the candidate gene in BTA23 would be retinoid X receptor, beta (RXRB), which is a transcription factor and an upstream factor of SCD gene in the signaling pathway. In BTA25, lipase maturation factor 1 (LMFI), which is initially identified in a mouse model of hypertriglyceridermia (Péterfy et al. 2007), would be one of the candidate genes (Fig. 2b,c). Therefore, further analysis in these candidate genes might lead to identifying responsible mutations.

BTA26

In this study, two significant SNPs for C14:1 were detected on BTA26 (Table 2). The SCD gene located in the region surrounding these significant SNPs (Fig. 2d) has been reported as a responsible gene for fatty acid composition. An amino acid replacement from valine (type V) to alanine (type A) in the SCD gene affected fatty acid composition (Taniguchi et al. 2004; Matsuhashi et al. 2011). Therefore, we performed an association analysis between this mutation and the traits. As a result, the SCD mutation had a higher significant effect on the percentage of C14:1 ($P = 1.56 \times 10^{-10}$, Table 3).

Matsuhashi et al. (2011) performed an association analysis between the SCD mutation and fatty acid composition in a Japanese Black population. The minor allelic frequencies (0.29) in SCD V293A in their study were similar to those in the present study (0.32). They also revealed that the SCD mutation had a significant effect for the percentage of C14:1. These results suggested that the SCD mutation would be responsible for C14:1 in GWAS performed in the present study. However, LD coefficients between the SCD mutation and significant SNPs were low ($r^2 = 0.11–0.12$) (Fig. 4). Therefore, further investigation, such as conditioned analysis, is required to confirm that the SCD mutation is responsible for fatty acid composition in the Gifu population.

In conclusion, this study detected novel candidate regions for fatty acid composition in Japanese Black
cattle by GWAS with high-density SNP array. We also confirmed previously reported regions for fatty acid composition. Further investigation on BTA19, BTA23 and BTA25 is expected to identify novel responsible genes and/or mutations associated with fatty acid composition.

ACKNOWLEDGMENTS
We thank Wagyu Registry Association for sampling and their data collection.

REFERENCES
Figure 4 Linkage disequilibrium (LD) of the significant region on BTA26. LD coefficients (r^2) between the single nucleotide polymorphisms (SNPs), which reached values of 5% genome-wide significance in single-marker associations between 17 and 21 Mbp, are indicated, and the significant region on BTA26 is illustrated. Black fields display r^2 values >0.70, white and grey fields display r^2 values <0.70 (Wang et al. 2002).
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