

Review

Genes Associated with Fatty Acid Composition of Beef

Hideyuki MANNEN*

Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, 1-1 Rokkoudai, Nada, Kobe 657-8501, Japan

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Fatty acid composition has become recognized as an important trait in beef because of its relationship with beef quality, including beef flavor and tenderness. Our laboratory attempted to identify genes responsible for the fatty acid composition of cattle. We found several such genes and developed genetic markers of fatty acid composition. The findings were as follows. 1) Genetic polymorphisms of the *stearoyl-CoA desaturase (SCD)* gene are associated with fatty acid composition. The effects of *SCD* type A gene substitution on the percentage of monounsaturated fatty acid (MUFA) and the melting point of intramuscular fat were on average approximately +1.0% and -1.0°C , respectively. 2) Intron polymorphisms of sterol regulatory element binding protein-1 (SREBP-1) also affected MUFA. 3) No effect of *SCD* or SREBP-1 genotypes on any representative carcass traits of Japanese Black cattle was observed in the field population. 4) Additional genetic markers, *adipocytes fatty acid binding protein 4 (FABP4)* and *liver X receptor α* , also affected the fatty acid composition. 5) *SCD* and *FABP4* significantly affected fatty acid composition of Holstein steers. These findings bring new insights into the fat-related carcass traits of beef cattle. In addition, the developed DNA makers will contribute to improved meat quality of beef.

Keywords: adipose tissue, beef quality, fatty acid composition, *stearoyl-CoA desaturase*, sterol regulatory element binding protein

Introduction

Beef of Japanese Black cattle in Japan is valued for its highly marbled meat. Moreover, the melting point of its fat is relatively low compared to that of other breeds. The lower melting point is caused by the higher percentage of unsaturated fatty acids in the fat (Yang *et al.*, 1999a). Recently, the fatty acid composition of adipose tissue in beef cattle has become recognized as an important trait in the beef industry. In cattle, higher concentrations of monounsaturated fatty acids (MUFA) in the adipocytes and a lower fat-melting point are considered to contribute to the softness of beef fat, favorable beef flavor, and may decrease the circulating concentration of LDL cholesterol (Melton *et al.*, 1982; Rudel *et al.*, 1995; Smith *et al.*, 1994).

The fatty acid composition of cattle, unlike that in non-ruminants, is much less dependent on diet. Microorganisms within the rumen hydrogenate a majority of the dietary

unsaturated fatty acids and most dietary fatty acids are absorbed as saturated fatty acids (Jenkins, 1993). However, it is reported that diet also influences bovine fatty acid composition (Edwards *et al.*, 1961; Cabezas *et al.*, 1965). Zembayashi *et al.* (1995) demonstrated that adipose tissue of Japanese Black cattle contains a higher proportion of MUFA than that of Holstein, Japanese Brown or Charolais cattle. Oka *et al.* (2002) demonstrated that sire groups had a significant effect on the fatty acid composition of Japanese Black cattle. In addition to various environmental effects (Huerta-Leidenz *et al.*, 1991; Chang *et al.*, 1992; Suzuki *et al.*, 2007), these results suggest that fatty acid composition could be controlled by genetic factors such as lipid synthesis and fatty acid metabolism-related genes.

Recently, genes responsible for the fatty acid composition of beef have been identified, and the number of reports on this topic is increasing. Here, I would like to review the current research on genes responsible for the fatty acid composition of cattle.

*To whom correspondence should be addressed.

E-mail: mannen@kobe-u.ac.jp

Stearoyl-CoA desaturase

Stearoyl-CoA desaturase (SCD) is an enzyme responsible for the conversion of saturated fatty acids into MUFA in mammalian adipocytes. The fatty acid composition reflects the preceding actions of SCD on substrates such as stearic acid or palmitic acid (Kim and Ntambi, 1999). Nutrition has been shown to contribute to the fatty acid profile, while genetic factors are still being elucidated. SCD is a candidate for genetic variation in fatty acid composition. Yang *et al.* (1999b) presented interesting correlations between SCD enzyme activity and fatty acid composition in a survey of bovine adipose tissues.

We sequenced full-length bovine *SCD* cDNA and the 5'-upstream region (Taniguchi *et al.*, 2004a). The full length of the *SCD* cDNA was 5,331 bp and the protein-coding sequence (CDS) contained 1,080 nucleotides coding for 359 amino acids. A comparison of the *SCD* sequence between 20 Japanese Black animals showed eight nucleotide substitutions: three substitutions were in CDS and five were in the 3' UTR (Fig. 1). Nucleotide substitution at 878 bp in CDS causes replacement of the amino acid valine (V type) with alanine (A type) in the SCD peptide.

SCD polymorphism and fatty acid composition

Genotyping of 1,003 Japanese Black steers for the non-synonymous polymorphism was carried out to investigate the relationship between *SCD* genotypes and fatty acid composition or fat-melting point of fattened Japanese Black steers (Taniguchi *et al.*, 2004a). The means of the proportion of MUFA and the melting point of intramuscular fat for three *SCD* genotypes are shown in Table 1. The differences were significant in the percentage of MUFA (high in type AA, medium in type AV, low in type VV). The melting point of intramuscular fat tissue was also significantly different between *SCD* genotypes (high in type VV, medium in type AV, low in type AA). The effect of *SCD* type A gene substitution on the

percentage of MUFA and the melting point of intramuscular fat was +0.805% and -1.03°C , respectively (Taniguchi *et al.*, 2004a). The genotype accounted for 4% of the total variation in MUFA and 3% of that in the melting point of intramuscular fat.

Sterol regulatory element binding protein-1

As described above, polymorphism of the *SCD* gene is associated with fatty acid composition. Since *SCD* gene expression is possibly related to this trait, the effect of gene expression was investigated. The results showed that the level of *SCD* mRNA expression in Japanese Black cattle (132.1 ± 34.1) was significantly higher ($P < 0.05$) than that in Holstein cattle (39.5 ± 12.9) (Taniguchi *et al.*, 2004b). Japanese Black cattle had a consistently higher ($P < 0.05$) percentage of mono-unsaturated fatty acids than did Holstein cattle. These results suggest that differences in *SCD* gene expression may contribute to the fatty acid compositional differences seen between Japanese Black and Holstein cattle.

The above studies revealed that *SCD* mRNA expression level is related to the MUFA percentage differences between cattle breeds. Sterol regulatory element binding proteins (SREBPs) are transcription factors that play a central role in energy homeostasis by promoting glycolysis, lipogenesis, and adipogenesis. SREBPs belong to the original basic helix-loop-helix-leucine zipper family of transcription factor (Eb-

Table 1. Comparison of MUFA content and melting point of fat tissue between two *SCD* genotypes.

Genotype	n	MUFA (%)	Melting point ($^{\circ}\text{C}$)
AA	278	58.8 ± 0.1^a	25.4 ± 0.2^a
AV	635	58.2 ± 0.1^b	26.1 ± 0.1^b
VV	90	57.1 ± 0.3^c	27.6 ± 0.3^c

Adipose from *M. trapezius* was used in this study.
Modified from Taniguchi *et al.* (2004a).

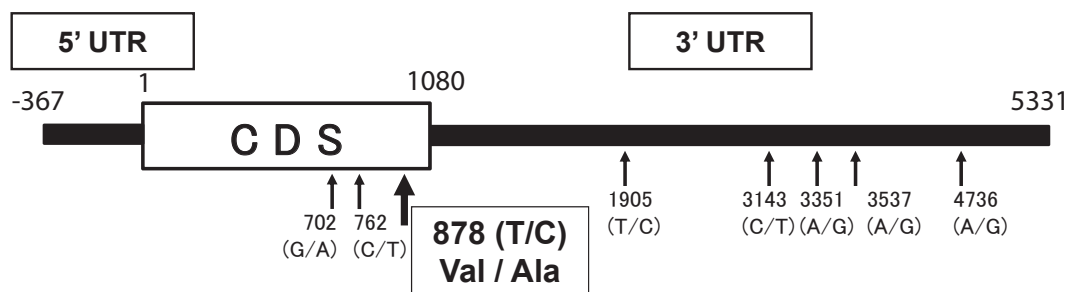


Fig. 1. Full-length bovine stearoyl-CoA desaturase cDNA. The full-length bovine SCD is 5,331 bp long. The protein-coding sequence (CDS) contains 1,080 nucleotides coding for 359 amino acids, and the 3' un-translated region (UTR) contains 3,884 nucleotides. Arrows show the positions of eight nucleotide substitutions, and the box indicates nucleotide mutation sites resulting in amino acid replacement.

erle *et al.*, 2004). In humans and mice, the *SREBP-1* gene produces two proteins by alternative splicing: SREBP-1a and 1c (Eberle *et al.*, 2004). It is known that *SREBPs* regulate gene transcription activation by binding to sterol regulatory element sequences contained in the promoter of their downstream genes, including the *SCD* gene (Shimano, 2001).

Subsequently, we determined the full-length sequence of bovine *SREBP-1* cDNA and then surveyed polymorphisms in whole exons and introns in the bovine genome (Hoashi *et al.*, 2007). Although there were no notable mutations (e.g., nonsynonymous polymorphisms) in exon regions, a large 84-bp insertion (long type: L) and a deletion (short type: S) were found in intron 5 of bovine *SREBP-1* in Japanese Black cattle. The associations between the *SREBP-1* genotypes and fatty acid composition were analyzed in 606 Japanese Black cattle. The SS type of cattle exhibited a 1.3% higher proportion of MUFA and a 1.6°C lower melting point in intramuscular fat than the LL type of cattle (Table 2). Bovine *SREBP-1* genotype is considered to reflect a genetic variation associated with the physiological characteristics of fat tissue in Japanese Black cattle.

No effect of *SCD* or *SREBP-1* genotypes on any representative carcass traits of Japanese Black cattle in the field population

Our studies showed that the *SCD* and *SREBP* polymorphisms affect fatty acid composition of bovine adipose tissue. However, the effects on field populations had not been confirmed, and the effects of these DNA markers on carcass traits had not been evaluated. Therefore, we investigated the effects of *SCD* and *SREBP-1* on beef carcass traits (carcass weight, rib eye area, rib thickness, subcutaneous fat thickness, yield estimate, beef marbling score, beef color standard) using field cattle populations (Ohsaki *et al.*, 2009).

The results showed no effect of *SCD* and *SREBP-1* genotypes on any representative carcass traits of Japanese Black cattle in the field population. This implies that there are no adverse effects on other representative carcass traits when marker-assisted selection is performed using these genetic markers in Japanese Black cattle.

Other genes associated with fatty acid composition

Fatty acid synthase (*FASN*) is a multifunctional enzyme that regulates de novo biosynthesis of long chain fatty acids in mammals (Smith, 1994). Abe *et al.* (2009) revealed that the *FASN* genotype had a significant effect on the fatty acid composition of an F₂ population from Japanese Black and Limousin cattle. Two mutations led to amino acid substitutions of threonine (T) with alanine (A) and of tryptophan (W) with arginine (R). The TW haplotype was associated with

Table 2. Comparison of MUFA content and melting point of intramuscular fat among *SREBP* genotypes.

Genotype	n	MUFA (%)	Melting point (°C)
LL	98	57.7 ± 0.3 ^a	26.7 ± 0.4 ^a
LS	437	58.1 ± 0.2 ^a	26.3 ± 0.2 ^a
SS	71	59.0 ± 0.3 ^b	25.1 ± 0.4 ^b

Mean values with different superscripts in the same column differ significantly ($P < 0.05$).

Modified from Hoashi *et al.* (2007).

increased C18:0 and C18:1 content and in the ratio of mono-unsaturated to saturated fatty acids, along with decreased C14:0, C14:1, C16:0, and C16:1 content.

We also surveyed additional genetic markers associated with fatty acid composition (Hoashi *et al.* 2008). The full-length CDS for six genes, *adipocytes fatty acid binding protein (FABP4)*, *liver X receptor α (LXR α)*, *cytochrome b_5* , *long-chain acyl-CoA synthetase (ACSL) 1*, *ACSL4*, and *diacylglycerol acyltransferase 2*, were sequenced to identify polymorphisms in Japanese Black and Holstein cattle. For *FABP4*, two nucleotide substitutions were predicted to cause amino acid substitutions, from isoleucine to valine at 220 bp A/G (I74V) and from valine to methionine at 328 bp G/A (V110M). For *LXR α* , two nucleotide substitutions were predicted to cause amino acid substitutions, from glycine to glutamic acid at 152 bp G/A (G51E) and from valine to isoleucine at 397 bp G/A (V133I).

The *FABP4* is a member of the cytoplasmic protein family involved in intracellular free fatty acid transport and metabolism, and binds long-chain fatty acids with high affinities. Fatty acid trafficking during lipolysis is mediated by *FABP4*, and the complex with hormone-sensitive lipase is the first step in an organized lipid-transfer process (Shen *et al.*, 1999). *LXR α* is a transcription factor that regulates genes involved in cholesterol and lipid metabolism. *LXR* directly activates *SREBP-1* gene transcription, and the *SREBP-1* subsequently activates lipogenic genes such as *FASN* and *SCD-1* (Schultz *et al.*, 2000; DeBose-zboyd *et al.*, 2001).

The effects of four nonsynonymous substitutions in *FABP4* and *LXR α* were investigated using a Japanese Black cattle population (Hoashi *et al.*, 2008). The *FABP4* I74V had a highly significant effect on the percentage of C16:1. *LXR α* V133I had a significant effect on the percentage of C18:2, while no significant effect was found with the polymorphisms *FABP4* V110M and *LXR α* G51E. Table 3 presents the means of the significant differences in fatty acid and index proportion among genotypes. In *FABP4* I74V, I/I homozygotes exhibited a significantly higher percentage of C16:1 than V/V homozygotes, reflecting a high proportion

Table 3. Effect of FABP4 I74V and LXR α V133I on fatty acid composition of Japanese Black cattle.

	I74V of <i>FABP4</i>			V133I of <i>LXRα</i>		
	I/I n = 82	I/V n = 113	V/V n = 39	V/V n = 174	V/I n = 54	I/I n = 6
Fatty acid composition (%)						
C16:1	3.76 ^a	3.56 ^{ab}	3.25 ^b	3.65	3.67	3.25
C18:2	1.95	2.1	2.14	2.08 ^b	2.30 ^a	1.79 ^{ab}
MUFA	56.45	56.73	56.21	56.2	56.83	56.36
C16:1/(C16:0 + C16:1)	12.23 ^a	11.88 ^{ab}	10.95 ^b	11.94	12.25	10.87

Mean values with different superscripts in the same column differ significantly ($P < 0.05$).

Modified from Hoashi *et al.* (2008).

of C16:1 / C16:0 + C16:1. V/I heterozygotes in LXR α V133I exhibited a significantly higher percentage of C18:2 than V/V homozygotes.

Additional analyses using different field populations are needed in the near future to confirm the effects of these three genes.

The gene effect in other breeds

While Holstein is most famous for milk production, they are also an important source of domestic beef in Japan. Therefore, we investigated the effects of genetic polymorphisms in *SCD*, *SREBP-1*, *FASN*, *LXR α* and *FABP4* on the fatty acid composition of Holstein steers (Narukami *et al.*, in press).

The major allele frequencies were 0.705 in *SCD*, 1.000 in *SREBP-1*, 0.518 in *FABP4*, 0.888 in *FASN*, and 0.984 in *LXR α* . The *SCD* V293A had significant effects on the percentages of C14:0, C14:1, C18:0, C18:1, MUFA and SFA. The *FABP4* I74V had a significant effect on the percentage of C16:0, while no significant effect was observed with *FASN* T1952A. Analysis of the effects on *LXR α* and *SREBP-1* genotypes were not possible due to the extremely biased genotype frequencies.

Zhang *et al.* (2008) reported that amino acid substitution in the TE domain of the *FASN* gene was associated with beef fatty acid composition of Angus bulls. Michal *et al.* (2006) demonstrated that the *FABP4* genotype significantly affects the marbling score using F₂ animals in a Wagyu \times Limousin cross.

Conclusions

Recently, fatty acid composition has become recognized as an important beef trait. In this review paper, I summarized recent studies on the identification of genes associated with the fatty acid composition of cattle. Economic traits, such as

the fatty acid composition, have been considered as a polygenic trait. Quantitative trait locus analysis has indicated that the fatty acid composition of livestock species is regulated by many potential candidate genes with small effects (Taylor *et al.*, 1998; Sanchez *et al.*, 2007; Abe *et al.*, 2008). Additionally, differential expression of adipogenic factors is known to play a key role in regulating multiple responsive pathways involved in fat development and lipid metabolism in cattle adipocytes (Taniguchi *et al.*, 2008). Although the adipogenic mechanism is extremely complicated, some genes have been elucidated and confirmed to be associated with the fatty acid composition of cattle. Further analysis will bring new insights into fat-related carcass traits of beef cattle, while contributing to the beef industry and to human food health throughout the world.

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