Development of DNA Markers Associated with Beef Quality

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ABSTRACT

The fatty acid composition of adipose tissue in beef has been recognized as an important trait because of its relationship with beef quality, including favorable beef flavor and tenderness. Our laboratory has tried to identify the genes responsible for the fatty acid composition in cattle. We have found the following. 1) Genetic polymorphism of stearoyl-CoA desaturase (SCD) is one of the responsible genes associated with fatty acid composition. The average effects of gene substitution of the SCD type A gene on the monounsaturated fatty acid (MUFA) percentage and the melting point of intramuscular fat were approximately +1.0 % and -1.0°C, respectively. 2) Intron polymorphism 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 in the field population was observed. 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 in Holstein steers. 6) Genomic wide association study using high density SNP array (54K SNP) is useful to find new candidate chromosomal regions for fatty acid composition. These findings and will bring new insight into the fat-related carcass traits of beef cattle. In addition, the developed DNA makers will contribute to improve meat quality of beef.

Key words: Adipose tissue, Beef quality, Fatty acid composition, Genome wide association study, 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 the fat is relatively lower than those 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 an important trait in the beef industry. In cattle, higher concentrations of monounsaturated fatty acid (MUFA) in the adipocytes and a lower fat-melting point are considered to contribute to the softness of bovine fat, favorable beef flavor and may decrease the circulating concentration of LDL cholesterol (Melton et al., 1982; Rudel et al., 1995; Smith, 1994).

The fatty acid composition in cattle, unlike that for non-ruminants, is much less dependent on the 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 influence 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 cattle or Charolais. Oka et al. (2002) demonstrated that sire groups had a significant effect on the fatty acid composition in Japanese Black. In addition to various environmental effects (Huerta-Leidenz et al., 1991; Chang et al., 1992; Suzuki et al., 2007), these results suggested that fatty acid composition could be controlled by genetic factors such as lipid synthesis and fatty acid metabolism related genes.

Recently, identification of the genes responsible for the fatty acid composition of beef has been made, and the number of related reports is increasing. Here, I would like to review the research of the genes responsible for the fatty acid composition in cattle.

Stearoyl-CoA desaturase

Stearoyl-CoA desaturase (SCD) is an enzyme responsible for conversion of saturated fatty acids into MUFA in mammalian adipocytes. The fatty acids composition reflects the earlier action of SCD on substrates such as stearic acid or palmitic acid (Kim and Ntambi, 1999). Nutrition contributes to the fatty acid profile, while the genetic factors are still defined. SCD was 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.

Therefore, 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 long and the protein coding region (CDS) had 1,080 nucleotides, coding 359 amino acids. A comparison of the *SCD* sequences among 20 Japanese Black animals detected eight nucleotide substitutions; three substitutions were in CDS and five in 3' UTR. The nucleotide substitutions at 878bp in CDS would cause replacement of the amino acid valine (V type) to alanine (A type) in the SCD peptide.

***SCD* Polymorphism and fatty acid composition**

The genotyping of 1,003 Japanese Black steers for the nonsynonymous polymorphism was carried out to investigate the relationship between the *SCD* genotypes and the fatty acid compositions or fat melting point of fattened Japanese Black steers (Taniguchi et al., 2004a). The means of the MUFA proportion and the melting point of intramuscular fat for the three *SCD* genotypes were indicated in Table 1. The differences were significant in MUFA percentage (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 average effect of gene substitution of the *SCD* type A gene on the MUFA percentage and melting point of intramuscular fat was +0.805 and -1.03, respectively. The genotypes explained 4% of the total variation in MUFA and 3% of the melting point of intramuscular fat.

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

Genotype	n	MUFA (%)	Melting point (°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
Mean values with different superscripts in the same column differ significantly ($P < 0.05$)
Modified from Taniguchi et al. (2004a)

Sterol regulatory element binding protein-1

As described above, the polymorphism of the *SCD* gene is associated with fatty acid composition. Since *SCD* gene expression was also possibly related to this trait, the effect of the gene expression was investigated. As the results, the levels of *SCD* mRNA in Japanese Black cattle (132.1 ± 34.1) revealed significant higher expression ($P < 0.05$) than that of Holstein cattle (39.5 ± 12.9) (Taniguchi et al., 2004b). Japanese Black had consistently higher ($P < 0.05$) mono-unsaturated fatty acids percentage than did Holstein. 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 the *SCD* mRNA expression level was related to different MUFA percentages 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 (Eberle 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 down-stream genes, including the *SCD* gene (Shimano, 2001).

Subsequently, we determined the full-length sequence of bovine *SREBP-1c* DNA and then surveyed polymorphisms in whole exons and introns in the bovine genome (Hoashi et al., 2007). Although there was no notable mutation (ex. Nonsynonymous polymorphism) 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 for 606 Japanese Black cattle. The SS type of cattle exhibited a 1.3% higher MUFA proportion and a 1.6°C lower melting point in intramuscular fat than the LL type of cattle (Table 2). Genotyping of bovine *SREBP-1* is considered to reflect a genetic variation associated with the physiological characteristics of fat tissue in Japanese Black cattle.

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).

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

Our studies showed that the *SCD* and *SREBP* polymorphisms would affect on fatty acid composition in bovine adipose tissue. However, the effects in the field populations were never confirmed. Moreover, the effects of these DNA markers on carcass traits were not 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).

As the results, this investigation showed the absence of any effect of *SCD* and *SREBP-1* genotypes on any representative carcass traits of Japanese Black in the field population. This implies that there will be no adverse effects on the 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 in an F_2 population from Japanese Black and Limousin cattle. Two mutations led to amino acid substitutions of threonine (T) to alanine (A) and tryptophan (W) to arginine (R). The TW haplotype was associated with increases C18:0 and C18:1 content and in the ratio of monounsaturated to saturated fatty acids, along with decreases C14:0, C14:1, C16:0, and C16:1 content.

We have surveyed additional genetic markers associated with fatty acid composition (Hoashi et al., 2008). The full length of 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 detect the polymorphisms in Japanese Black and Holstein cattle. In *FABP4*, two nucleotide substitutions were predicted to cause amino acid substitutions, isoleucine to valine at 220bpA/G (I74V) and valine to methionine at 328bpG/A (V110M).

In *LXRα*, two were predicted to cause amino acid substitutions, glycine to glutamic acid at 152bpG/A (G51E) and valine to isoleucine at 397bpG/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). LXRα is a transcription factor regulating 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-Boyd et al., 2001).

The effects of four nonsynonymous substitutions in *FABP4* and *LXRα* were investigated using Japanese Black cattle population (Hoashi et al., 2008). The FABP4 I74V had a highly significant effect on the percentage of C16:1. LXRα V133I had a significant effect on the percentage of C18:2, while no significant effect was found with the polymorphisms FABP4 V110M and LXRα G51E. Table 3 presents the means of the significant fatty acid and index proportion among genotypes. In FABP4 I74V, II homozygote exhibited a significantly higher percentage of C16:1 than VV homozygote, reflecting a high proportion of C16:1 / C16:0+C16:1. VI heterozygote in LXRα V133I exhibited a significantly higher percentage of C18:2 than VV homozygote.

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

Table 3. Effect of FABP4 I74V and LXRα V133I on fatty acid composition in Japanese Black.

	I74V of *FABP4*			V133I of *LXRα*		
	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)

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 have investigated the effects of genetic polymorphisms of *SCD*, *SREBP-1*, *FASN*, *LXRα* and *FABP4* on fatty acid composition in Holstein steers (Narukami et al., 2011).

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 *LXRα*. 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 in FASN T1952A. Effects of *LXRa* and *SREBP-1* genotypes were unable to analyze because of 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 in Angus bulls. Michal et al. (2006) demonstrated that the *FABP4* genotype significantly affected the marbling score, using F_2 animals in a Wagyu x Limousin.

Genome wide association study for bovine fatty acid composition

High-density multiplex single nucleotide polymorphism (SNP) genotyping arrays have been developed with the recent availability of numerous SNPs through whole-genome sequencing. These tools have widely been used in studies of genes associated with human diseases. In cattle, similar tools have also been developed, and used in linkage disequilibrium (LD) analyses (Khatkar et al., 2007), estimating population structure and divergence (Gautier et al., 2007), past effective population size (Sargolzaei et al., 2008), and detection of selection signatures in cattle population (Prasad et al., 2008). In addition to these population genetic studies, high-density SNP panels have also been widely used in genome-wide association studies intended to identify the causative genes and polymorphisms related to various traits (Barendse et al., 2007; Feugang et al., 2009).

In order to find novel responsible genes associated with fatty acid composition, we applied the Illumina BovineSNP50 BeadChip whole genome single nucleotide polymorphism (SNP) assay. In this experiment, we applied a mixed model and Genomic Control approach using selective genotyping to perform a genome-wide association study. A total of 468 animals, selected from 2,193 animals from a Japanese Black population. In this study, a total of 40,657 SNPs were used, and the estimated inflation factors on any traits were less than 1.10.

As a result, we have found novel candidate chromosomal regions for traits of several fatty acid compositions (detail data not shown since before publication). Genome wide association study is powerful tool to identify candidate genes on economic traits in livestock.

CONCLUSION

Recently, fatty acid composition has become an important beef trait. In this review paper, I summarized recent studies in terms of identification of genes associated with fatty acid composition in 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. 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. Although the adipogenic mechanism is extremely complicated, some genes have been elucidated and confirmed to be associated with fatty acid composition in cattle. Further analysis, especially hunting of novel responsible gene using genome wide association study, would bring new insight into fat-related carcass traits in beef cattle, while contributing to the beef industry and to human food health throughout the world.

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