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Associations of polymorphisms in the promoter I of bovine *acetyl-CoA carboxylase-α* gene with beef fatty acid composition

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Summary

The objectives of this study were to identify single nucleotide polymorphisms (SNPs) in the promoter I (PI) region of the bovine *acetyl-CoA carboxylase-α* (*ACACA*) gene and to evaluate the extent to which they were associated with lipid-related traits. Eight novel SNPs were identified, which were AJ276223:g.2064T>A (SNP1), g.2155C>T (SNP2), g.2203G>T (SNP3), g.2268T>C (SNP4), g.2274G>A (SNP5), g.2340A>G (SNP6), g.2350T>C (SNP7) and g.2370A>G (SNP8). Complete linkage disequilibrium was observed among SNP1, 2, 4, 5, 6 and 8. Phenotypic data were collected from 573 cross-bred steers with six sire breeds, including Hereford, Angus, Brangus, Beefmaster, Bonsmara and Romosinuano. The genotypes of SNP1/2/4/5/6/8 were significantly associated with adjusted backfat thickness. The genotypes of SNP3 were significantly associated with triacylglycerol (TAG) content and fatty acid composition of longissimus dorsi muscle (LM) in Brangus-, Romosinuano- and Bonsmara-sired cattle. Cattle with g.2203GG genotype had greater concentrations of TAG, total lipid, total saturated fatty acid and total monounsaturated fatty acid than did cattle with g.2203GT genotype. The genotypes of SNP7 were significantly associated with fatty acid composition of LM. Cattle with genotype g.2350TC had greater amounts of several fatty acids in LM than did cattle with genotype g.2350CC. Our results suggested that the SNPs in the PI region of *ACACA* gene are associated with variations in the fatty acid contents in LM.

Keywords acetyl-CoA carboxylase, cattle, fatty acid composition, polymorphism.

Acetyl-CoA carboxylase-α (ACACA) is highly expressed in lipogenic tissues such as adipose tissues, lactating mammary gland and liver (Ponce-Castaneda *et al.* 1991; Abu-Elheiga *et al.* 1995). Transcription of bovine *acetyl-CoA carboxylase-α* (*ACACA*) can be initiated from three different promoters, promoter I (PI), II (PII) and III (PIII), which results in heterogeneous composition of the 5′ untranslated region (Mao *et al.* 2001). Promoter I-initiated transcripts are abundant in adipose tissues and mammary glands of cows, but their abundance does not increase during lactation (Mao *et al.* 2001). In contrast, PIII plays an important role in lipogenesis during lactation (Barber & Travers 1998; Mao *et al.* 2002). Unlike the other two promoters, PII is considered as a housekeeping promoter that is expressed constitutively (Luo & Kim 1990; Mao & Seyfert 2002). Because of the specific role of the PI region of *ACACA* in adipose tissue, we hypothesized that the variations in the PI region of the *ACACA* gene among individuals would be a candidate for heritable differences in lipid-related traits of beef.

In this study, we identified eight novel SNPs in *ACACA* PI in cross-bred cattle. The associations of genotype of individual SNPs with phenotypic data including lipid content, marbling score, adjusted backfat thickness and fatty acid composition then were investigated.

Steer progeny resulting from artificial insemination mating of Angus and MARC III females with Hereford, Angus, Brangus, Beefmaster, Bonsmara and Romosinuano males were used in the present study (Wheeler T.L., Cundiff L.V.,

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Shackelford S.D. & Koohmaraie M., submitted). All steers were fed the same diet and serially slaughtered in four or five groups in a commercial facility at a mean age of 426 days. The adjusted backfat thickness and marbling score were determined by the same procedures used in previous cycles of Germplasm Evaluation Program (Wheeler *et al.* 1996).

Total lipids were extracted from the longissimus dorsi muscle (LM) samples and quantified (Folch *et al.* 1957). The contents of phospholipids (PL), triacylglycerols (TAG) and fatty acids in LM were analysed as described previously (Zhang *et al.* 2008).

Genomic DNA samples were isolated by proteinase K digestion followed by phenol/chloroform extraction. The SNPs were identified by sequencing amplicons of 441 bp PCR products using an ABI 3730 DNA Analyzer (Applied Biosystems Inc.) at the Iowa State University DNA Facility. The primer sequences for the PCR reaction were as follows:

Forward primer: AGACAGTAAGGCAGGAACAGG;

Reverse primer: CTTGCATGACACCTCCAGTAT.

The PCR reactions were carried out in total volumes of 25 μl that contained 0.1 μM of each primer, 0.2 mM of dNTPs, 1.5 mM of $MgCl_2$, 1 unit of TAQ enzyme, 2 μl of DMSO and 25 ng of genomic DNA. The thermocycler program was set as initial denaturation at 94 °C for 2 min followed by 35 cycles for 15 s at 94 °C, 15 s at 56 °C and 45 s at 74 °C. All the genomic DNA samples were then genotyped by DNA sequencing.

Least squares mean (±SE) were determined using a mixed linear model (PROC MIXED; SAS Institute, Inc.) that included the fixed effects of genotype, sire line, and dam line and the random effects of assay groups and slaughter date. Tukey–Kramer adjustment was applied for multiple comparisons. The adjusted *P*-values that were less or equal to 0.05 were determined to be significant, whereas the adjusted *P*-values within the range of 0.05–0.10 were classified as statistical tendencies.

Eight novel SNPs were identified in the PI of *ACACA*. The eight SNPs are AJ276223:g.2064T>A (SNP1), g.2155C>T (SNP2), g.2203G>T (SNP3), g.2268T>C (SNP4), g.2274G>A (SNP5), g.2340A>G (SNP6), g.2350T>C (SNP7) and g.2370A>G (SNP8).

The allele frequencies of SNP1/2/4/5/6/8, SNP3 and SNP7 in cattle with sires from different breeds are shown in Table S1. Complete linkage disequilibrium was observed among SNPs 1, 2, 4, 5, 6 and 8. Two haplotypes, therefore, were defined by these six SNPs, which were TCTGAA with a frequency of 0.64 and ATCAGG with a frequency of 0.36.

Table 1 Effects of genotypes of g.2064T>A (SNP1), g.2155C>T (SNP2), g.2268T>C (SNP4), g.2274G>A (SNP5), g.2340A>G (SNP6), g.2350T>C (SNP7) and g.2370A>G (SNP8) on phenotypic traits[1].

SNP	Trait	Genotype		
SNP1/2/4/5/6/8		TT/CC/TT/GG/AA/AA (n = 214)	TA/CT/TC/GA/AG/AG (n = 290)	AA/TT/CC/AA/GG/GG (n = 68)
	PL content[2]	0.42 ± 0.01[b]	0.43 ± 0.01[a]	0.42 ± 0.01[b]
	Adjusted backfat thickness	0.44 ± 0.02[a]	0.42 ± 0.01[a,b]	0.38 ± 0.03[b]
SNP7		TT (n = 72)	TC (n = 312)	CC (n = 188)
	Total lipid content[2]	5.78 ± 0.21	5.71 ± 0.13	5.38 ± 0.15
	TAG content[2]	5.36 ± 0.21	5.28 ± 0.13	4.95 ± 0.15
	Fatty acids[3]			
	C14:0	2.14 ± 0.11[a,b]	2.14 ± 0.07[a]	1.96 ± 0.08[b]
	C14:1	0.40 ± 0.03[a,b]	0.41 ± 0.02[a]	0.36 ± 0.02[b]
	C15:0	0.26 ± 0.01	0.27 ± 0.01	0.24 ± 0.01
	C16:0	16.65 ± 0.67	16.48 ± 0.42	15.48 ± 0.48
	C16:1	2.12 ± 0.10[a,b]	2.14 ± 0.07[a]	1.94 ± 0.08[b]
	C17:0	0.74 ± 0.03	0.74 ± 0.02	0.68 ± 0.02
	C17:1	0.46 ± 0.02[a,b]	0.47 ± 0.01[a]	0.43 ± 0.01[b]
	C18:1 trans	3.09 ± 0.36[a,b]	3.02 ± 0.34[a]	2.77 ± 0.34[b]
	C18:1 n7	0.66 ± 0.04[a,b]	0.66 ± 0.03[a]	0.60 ± 0.03[b]
	SFA[4]	27.83 ± 1.09	27.35 ± 0.65	25.77 ± 0.76
	MUFA[5]	23.21 ± 0.99	22.99 ± 0.66	21.58 ± 0.74

[1]Values are expressed as least squares mean ± SE.

[2]Contents are expressed as g/100 g of beef.

[3]Contents are expressed as mg/g of beef. Only individual fatty acids that are significantly or tended to be significantly associated with SNP7 are shown in the table.

[4]Saturated fatty acids, calculated as C14:0 + C15:0 + C16:0 + C17:0 + C18:0.

[5]Monounsaturated fatty acids, calculated as C14:1 + C16:1 + C17:1 + C18:1.

SNP, single nucleotide polymorphism; PL, phospholipids; TAG, triacylglycerols; MUFA, total monounsaturated fatty acid; SFA, saturated fatty acid. Values with different superscripts letters under column of genotype differ at $P < 0.05$.

The cellular concentration of ACACA is controlled mainly by the regulation of gene expression. Promoter I of *ACACA* from cattle and rats is normally repressed by distal sequence elements (Tae *et al.* 1994; Mao *et al.* 2001). The binding of CCAAT/enhancer-binding protein (C/EBP) to PI relieves the repression of ACACA and increases the PI expression in rats (Tae *et al.* 1994). There are several proposed transcription factor binding sites in PI region of bovine *ACACA* as shown in Fig. S1 (Mao *et al.* 2001). The SNPs identified in this study are located close to several transcriptional factor binding sites. However, none of the eight SNPs disrupts any proposed transcriptional factor binding site.

A few studies detected polymorphisms in PIII or exons of sheep (Moioli *et al.* 2005; Federica *et al.* 2008) and goat (Badaoui *et al.* 2007) *ACACA* genes and investigated the associations of SNPs with milk traits. In the present study, the associations of SNPs with various phenotypic traits, including TAG, PL and total lipid content, and marbling score, adjusted backfat thickness and fatty acid composition of LM were investigated. The significant additive and dominant effects associated with SNP1/2/4/5/6/8 and SNP7 are shown in Table S2. Significant associations between the genotypes of SNP1/2/4/5/6/8 and adjusted backfat thickness and PL content were detected (Table 1).

The associations of genotypes of SNP7 with the phenotypic traits are shown in Table 1. Cattle with the g.2350TC genotype tended to have greater contents of total lipid ($P = 0.07$) and TAG ($P = 0.08$) than did cattle with the g.2350CC genotype. The contents of several SFA and MUFA in LM were significantly associated with the genotype of SNP7. Cattle with the g.2350TC genotype had greater concentrations of myristic acid (C14:0), myristoleic acid (C14:1), palmitoleic acid (C16:1) and heptadecenoic acid (C17:1) than did cattle with the genotype g.2350CC ($P < 0.05$). Moreover, cattle with g.2350TC tended to have greater contents of several SFA, including pentadecanoic acid (C15:0; $P = 0.06$), palmitic acid (C16:0; $P = 0.08$) and margaric acid (C17:0; $P = 0.06$), total saturated fatty acids (SFA; $P < 0.06$) and total monounsaturated fatty acids (MUFA; $P = 0.08$) in LM than did cattle with the g.2350CC genotype.

Because of the very low T allele frequency in the offspring of Hereford, Augus and Beefmaster bulls, the effects of SNP3 genotype were studied in the cattle with the other three sire breeds. In addition, the g.2203TT genotype was rare in Brangus- and Romosinuano-sired cattle. Therefore, only the effects of the g2203GG and g2203GT genotypes were compared. The cattle with the g.2203GG genotype had more total lipids and TAG contents in LM than did cattle with the g.2203GT genotype ($P < 0.05$; Table 2). Greater concentrations of C16:0, stearic acid (C18:0) and oleic acid (C18:1), total SFA and total MUFA were detected in the cattle with the g2203GG genotype compared with those with the g2203GT genotype ($P < 0.05$; Table 2).

Table 2 Effects of genotypes of g.2203G>T (SNP3) on phenotypic traits of Brangus-, Romosinuano- and Bonsmara-sired cattle[1].

Trait	SNP3 GG (n = 178)	SNP3 GT (n = 89)	P-value
Total lipid content[2]	5.37 ± 0.17	4.93 ± 0.20	<0.05
TAG content[2]	4.93 ± 0.17	4.50 ± 0.20	<0.05
Fatty acids[3]			
C15:0	0.25 ± 0.01	0.22 ± 0.01	<0.01
C16:0	15.39 ± 0.54	14.10 ± 0.64	<0.05
C17:0	0.69 ± 0.02	0.59 ± 0.03	<0.01
C17:1	0.45 ± 0.02	0.39 ± 0.02	<0.01
C18:0	7.15 ± 0.21	6.55 ± 0.27	<0.05
C18:1	18.63 ± 0.72	17.10 ± 0.83	<0.05
C18:1 trans	2.93 ± 0.32	2.61 ± 0.33	<0.05
SFA[4]	25.51 ± 0.84	23.34 ± 1.01	<0.05
MUFA[5]	21.46 ± 0.83	19.73 ± 0.96	<0.05

[1]Values are expressed as least squares mean ± SE.
[2]Contents are expressed as g/100 g of beef.
[3]Contents are expressed as mg/g of beef. Only individual fatty acids that are significantly associated with SNP3 are shown in the table.
[4]Saturated fatty acids, calculated as C14:0 + C15:0 + C16:0 + C17:0 + C18:0.
[5]Monounsaturated fatty acids, calculated as C14:1 + C16:1 + C17:1 + C18:1.
TAG, triacylglycerols; MUFA, total monounsaturated fatty acid; SFA, saturated fatty acid.

In the present study, the fatty acid composition was expressed as concentration of fatty acid in beef, instead of in intramuscular lipid. Cattle cannot synthesize linoleic acid (C18:2 n6) and linolenic acid (C18:3 n3), which are precursors for other polyunsaturated fatty acids (PUFA). The concentration of PUFA in LM, therefore, reflected dietary intake. Malonyl-CoA, the product of ACACA, is the intermediary substrate for fatty acid synthase (FASN). The SFA synthesized by FASN then can undergo elongation and/or desaturation in bovine adipose tissue microsomes to produce C18:0 and several MUFA such as C14:1, C16:1 and C18:1 (St John *et al.* 1991). Therefore, an increased expression of *ACACA* leads to increased production of malonyl-CoA, which could further result in increased production of even-chain SFA and MUFA. The effects of the SNP3 and SNP7 on the concentrations of even-chain SFA and MUFA in LM then could be attributed to the possible association of these SNPs with the PI activity of *ACACA* in intramuscular adipocytes. Consequently, the increased concentrations of major SFA and MUFA in LM resulted in increased contents of TAG and total lipids. An additive effect of SNP1/2/4/5/6/8 on adjusted backfat thickness was observed in this study. As the regulation of *ACACA* expression is tissue-specific (Barber *et al.* 2005), it is possible that SNP1/2/4/5/6/8 is associated with the PI activity of *ACACA* in subcutaneous adipose tissue.

In conclusion, results of the present study suggested that the eight SNPs in the PI region of *ACACA* were significantly associated with lipid-related traits.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 The positions of the SNPs in promoter I of *ACACA*. The SNPs identified in the present study are bold and underlined.

Table S1 Allele frequencies of eight SNPs located in the promoter I of the bovine *ACACA* gene.

Table S2 Estimate (±SE) of additive and dominant effects associated with g.2064T>A (SNP1), g.2155C>T (SNP2), g.2268T>C (SNP4), g.2274G>A (SNP5), g.2340A>G (SNP6), g.2350T>C (SNP7) and g.2370A>G (SNP8) on phenotypic traits[1].

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