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A novel SNP of the C/EBPα gene associated with superior meat quality in indigenous Chinese cattle

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ABSTRACT. CCAAT/enhancer-binding protein alpha (C/EBPα) is an essential transcriptional factor regulating the differentiation of adipocytes. We report a novel single nucleotide polymorphism (C271A) of the C/EBPα gene in six indigenous Chinese cattle breeds using PCR-SSCP and DNA sequencing methods. Allele frequencies were investigated and evaluated by the χ^2 test in 817 individuals; all populations were found to be in Hardy-Weinberg equilibrium. Gene heterozygosity, effective allele numbers and polymorphism information content of the C/EBPα locus varied from 0.50 to 0.54, 1.84 to 1.99 and 0.35 to 0.37, respectively. We also evaluated a potential association of the C/EBPα SNP with ultrasound traits in 555 individuals; individuals of the AA genotype had greater ultrasound backfat thickness than did genotype CC (0.36 versus 0.34 cm, $P < 0.01$); genotypes AA and CA had higher ultrasound marbling scores than did genotype CC (3.53, 3.52 versus 3.37, $P < 0.05$). Analysis based on meat quality data in another 204 Qinchuan cattle showed that animals with genotype AA had bigger loin eye areas than did genotype CA (87.10 versus 79.08 cm^2, $P < 0.05$).

These results indicate that the C271A SNP of the C/EBPα gene could be used as a molecular marker for selecting beef cattle with superior carcass traits.

Key words: Bovine C/EBPα gene; Genetic polymorphism; PCR-SSCP; Meat quality traits

INTRODUCTION

The differentiation of preadipocytes into mature adipocytes is regulated by a series of transcription factors (MacDougald and Lane, 1995). CCAAT/enhancer-binding proteins (C/EBPs), which have highly conserved basic leucine zipper domains (bZIP) and a variable N-terminal region, were described as positive transcriptional regulators of adipocytes, and to date, six members of C/EBPs have been found: C/EBPα, -β, -δ, -ε, -γ, and -ζ (Graves et al., 1986; Simon et al., 1991; Lin and Lane, 1992; Lekstrom-Himes et al., 1998). C/EBPα is composed of 353 amino acids and is expressed just prior to the transcription of most adipocyte-specific genes that possess C/EBPα binding sites. Therefore, C/EBPα could mediate reporter gene expression (MacDougald and Lane, 1995; Constance et al., 1996). Placing C/EBPα gene expression under the control of the Lac repressor or using a retroviral vector demonstrated that the expression of C/EBPα was sufficient to induce the differentiation of 3T3-L1 preadipocytes into adipocytes without using external hormonal inducers (Lin et al., 1993; Freytag et al., 1994). Wang et al. (1995) reported that C/EBPα knockout mice failed to accumulate interscapular fat within the first 32 h after birth. C/EBPα also could regulate energy and nutrition metabolism by activating some specific genes such as phosphoenolpyruvate carboxykinase and insulin receptor (Park et al., 1990). Taken together, these findings indicate that C/EBPα is an important regulator of adipocyte differentiation.

We are not aware of reports of C/EBPα gene polymorphisms in animals. Based on the important role that C/EBPα plays in regulating adipose differentiation, the C/EBPα gene is an attractive candidate gene for selecting beef cattle with specific carcass traits, such as marbling. Therefore, the objective of this study was to detect single nucleotide polymorphisms (SNPs) of the bovine C/EBPα gene and to explore possible associations between C/EBPα SNPs and meat quality traits in Chinese indigenous cattle.

MATERIAL AND METHODS

DNA samples and data collections

DNA was extracted from blood samples with a routine phenol-chloroform extraction method (Mullenbach et al., 1989) from 817 individuals representing six Chinese native cattle breeds: Nanyang (NY, N = 57, Henan Province), Luxi (LX, N = 67, Shandong Province), Xianan (XN, N = 97, Henan Province), Simmental x Luxi (SL, N = 79, Shandong Province), JiaxianRed (JXR, N = 143, Henan Province), and Qinchuan (QC, N = 374, Shanxi Province). Ultrasound measurements can be used to predict carcass merit at slaughter (Hamlin et al., 1995; Brethour, 2000; Wall et al., 2004), so we measured ultrasound backfat thickness (UBF),

ultrasound loin muscle areas (ULMA) and ultrasound marbling scores (UMAR) in 555 cattle of the initial 817 animals used for DNA extraction. Marbling areas were 14, 11, 8, 6, 4, 2, and 0.5% of total area for marbling grades 5, 4, 3, 2.5, 2, 1.5, and 1, respectively) (Tang et al., 2006; Zhou et al., 2010).

Meanwhile, records from 204 QC cattle from 817 samples used for DNA extraction were selected to analyze five carcass traits at slaughter: marbling scores (MAR), loin eye areas (LEA), meat tenderness (TD), backfat thickness (BFT), and water holding capacity (WHC).

PCR amplification and sequencing

According to the NCBI sequence of the bovine C/EBPα gene (GenBank: NM_176784.2), a pair of polymerase chain reaction (PCR) primers named P1 (P1f: 5' AGCAGCGCCGCTTTC GGCTT 3' and P1r: 5' TCAAAGTCGTTGCCGCCTCC 3') was designed to amplify a 236-bp product. The 15-μL PCR mixture contained 50 ng genomic DNA, 10 pM of each primer, 1X buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs, and 0.5 U Taq DNA polymerase (MBI). PCR conditions were as follows: initial denaturation step at 94°C for 5 min, 32 cycles of denaturation at 94°C for 30 s, annealing at 62.5°C for 30 s, extension at 72°C for 35 s, and a final extension of 10 min at 72°C.

Aliquots of 6 μL of the PCR products were mixed with 10 μL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole, and 0.025% bromophenol blue), and heated for 10 min at 98°C followed by chilling on ice. The 13-μL mixture was then applied to a 12% polyacrylamide gel (acrylamide:bis, 29:1), 14% (v/v) glycerol and 10X TBE buffer. Electrophoresis was carried out at 250 V for 25 min and then 110 V for 16 h at room temperature in 1X TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH 8.3). The gel was stained with 0.1% silver nitrate (Lan et al., 2007) and visualized by 2% NaOH solution (containing 0.1% formaldehyde) (Zhang et al., 2007). After the polymorphism was detected, the PCR products were sequenced in an ABI PRIZM 377 DNA sequencer. The sequences were analyzed by the DNASTAR 5.0 package.

Statistical analysis

Genotypic frequencies and allelic frequencies, Hardy-Weinberg equilibrium and the population genetic indices H_E (gene heterozygosity), H_O (gene homozygosity), N_e (effective allele numbers), and PIC (polymorphism information content) were calculated using the POPGENE Version 1.31 software (Nei and Roychoudhury, 1974; Nei and Li, 1979; Liu et al., 2009). The GLM procedure (SPSS 17.0, SPSS Inc.) was used to analyze the associations between the genotypes and ultrasound traits based on UBF, ULMA and UMAR records according to the following statistical linear model:

$$Yijlm = \mu + Gi + Sj + Al + BFm + \varepsilon ijlm \quad \text{(Equation 1)}$$

Meat quality traits (BFT, LEA, MAR, WHC, and TD) were evaluated according to the statistical linear model:

$$Yijl = \mu + Gi + Sj + Al + \varepsilon ijl \quad \text{(Equation 2)}$$

where *Yijl (m)* is the observation for the meat quality traits, μ is the overall mean for each trait, *Gi* is the genotype (CC, CA and AA genotypes) effect, *Sj* is the fixed effect of sex, *BFm* is the fixed effect of breed and farm, *Al* is the measure of age, and $\varepsilon ijl\ (m)$ is the random environment effect.

RESULTS

PCR-SSCP analysis of the *Bos taurus* C/EBPα gene

The 236-bp products were obtained by PCR amplification in all six breeds, and three unique single-strand conformational polymorphism (SSCP) banding patterns (CC, CA and AA) were observed (Figures 1 and 2).

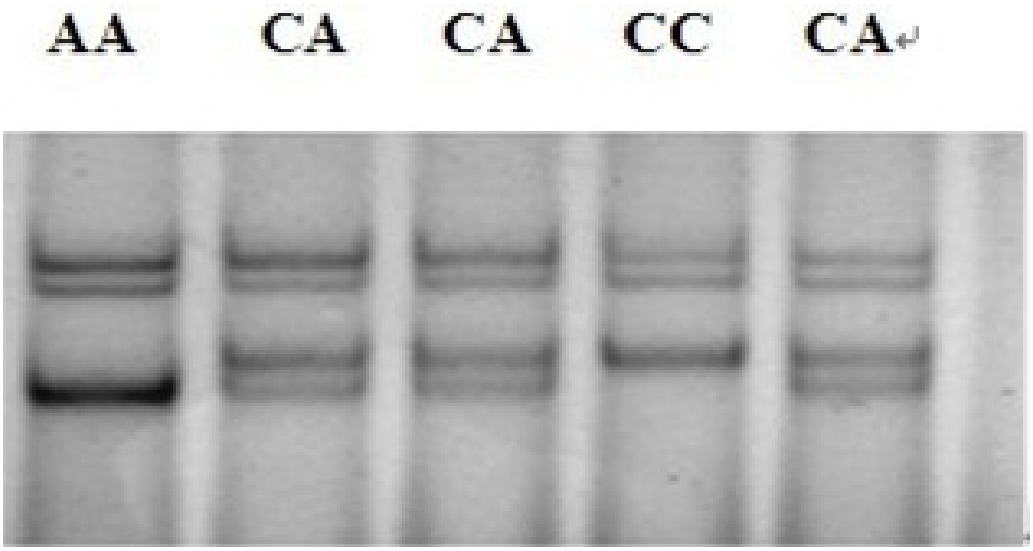

Figure 1. The PCR-SSCP patterns of the C/EBPα gene; three patterns (CC, CA, AA) were observed in the Chinese indigenous cattle, with CC, CA and AA being three different genotypes.

Genetic polymorphism of the C/EBPα gene and χ^2 test

Sequence analysis of the C and A alleles revealed a novel SNP (C271A) of the amplified products. The SNP caused a synonymous mutation: Arg > Arg for the C/EBPα protein. The mutant allele A had higher frequency than the wild-type allele C in all the experimental populations (Table 1).

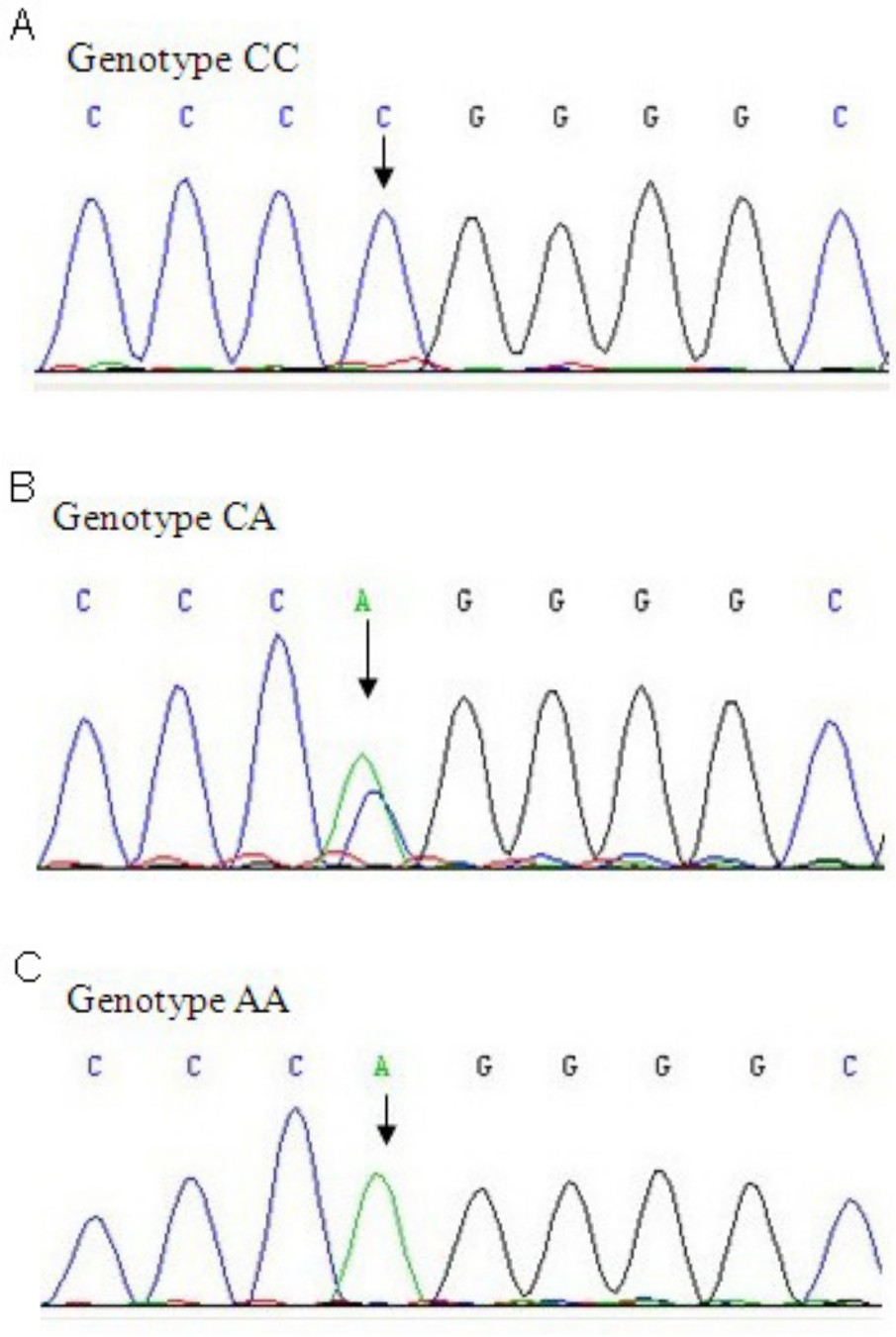

Figure 2. The sequencing trace of the SNP in the bovine C/EBPα gene, revealing a C271A mutation; **A.** CC, **B.** CA and **C.** AA are the three different genotypes.

Table 1. Genotype frequencies at the C/EBPα gene for the SNP in bovine populations.

Breed	Observed genotypes (number)			Total	Allelic frequencies		χ^2 test (HW)	P value (HW)
	CC	CA	AA		C	A		
Nanyang	0.26 (15)	0.39 (22)	0.35 (20)	57	0.46	0.54	2.81	0.28
Luxi	0.28 (19)	0.36 (24)	0.36 (24)	67	0.46	0.54	5.24	0.06
Xianan	0.22 (21)	0.41 (40)	0.37 (36)	97	0.42	0.58	2.33	0.29
Simmental x Luxi	0.19 (15)	0.37 (29)	0.44 (35)	79	0.37	0.63	3.67	0.16
JiaxianRed	0.15 (21)	0.41 (59)	0.44 (63)	143	0.35	0.65	1.34	0.55
Qinchuan	0.12 (45)	0.48 (180)	0.40 (149)	374	0.36	0.64	0.70	0.70
Total	0.17 (136)	0.43 (354)	0.40 (327)	817	0.38	0.62	5.67	0.06

HW = Hardy-Weinberg equilibrium; Simmental x Luxi = Simmental and Luxi crossbred steers.

There was a medium polymorphism for the SNP (Table 2) (low polymorphism PIC value <0.25, medium polymorphism 0.25 < PIC value < 0.5, and high polymorphism PIC value >0.5). Minimum and maximum PIC values were 0.35 (JXR, QC) and 0.37 (LX, NY, XN), respectively. H_E varied from 0.50 (LX, NY) to 0.54 (JXR, QC) and N_e ranged from 1.84 (JXR) to 1.99 (LX).

Table 2. Population genetic indices at the C/EBPα locus in six bovine populations.

Breed	Gene homozygosity	Gene heterozygosity	Effective allele number	Polymorphism information content
Nanyang	0.50	0.50	1.98	0.37
Luxi	0.50	0.50	1.99	0.37
Xianan	0.49	0.51	1.95	0.37
Simmental x Luxi	0.47	0.53	1.88	0.36
JiaxianRed	0.46	0.54	1.84	0.35
Qinchuan	0.46	0.54	1.86	0.35
Total	0.47	0.53	1.90	0.36

Simmental x Luxi = Simmental and Luxi crossbred steers.

Effect of the polymorphism locus on bovine carcass traits

Individuals with genotype AA had greater UBF than those with genotype CC ($P < 0.01$); animals with genotypes AA and CA had better UMAR than animals with genotype CC ($P < 0.05$) (Table 3). No significant associations of different genotypes with other traits were detected ($P > 0.05$).

Table 3. Association between the C271A SNP genotype of the C/EBPα gene and ultrasound traits in *Bos taurus*.

Genotype	Traits		
	UBF (cm)	ULMA (cm^2)	UMAR
CC	0.34 ± 0.007^{A}	66.20 ± 1.174	3.37 ± 0.054^{a}
CA	0.35 ± 0.004^{AB}	66.75 ± 0.741	3.53 ± 0.034^{b}
AA	0.36 ± 0.004^{B}	67.32 ± 0.729	3.52 ± 0.034^{b}

Data are reported as means ± standard error. ^{ab}Means with different superscript letters were significantly different ($P < 0.05$). ^{AB}Means with different superscript letters were significantly different ($P < 0.01$). UBF = backfat thickness; ULMA = ultrasound loin muscle areas; UMAR = ultrasound marbling scores.

We also analyzed the association between genotype and meat quality traits, including BFT, LEA, MAR, WHC, and TD in another 204 QC individuals (Table 4). There were significant differences in LEA ($P < 0.05$) between the three genotypes; animals with genotype AA had larger LEA than animals with genotype CA.

Table 4. Association between C271A SNP genotypes of the C/EBPα gene and meat quality traits in Qinchuan cattle.

Genotype	Traits				
	BFT (mm)	LEA (cm^2)	MAR	WHC	TD
CC	0.94 ± 0.08	85.54 ± 4.14^{ab}	2.04 ± 0.17	0.24 ± 0.01	2.15 ± 0.10
CA	0.90 ± 0.05	79.08 ± 2.52^{a}	2.27 ± 0.10	0.24 ± 0.01	2.03 ± 0.06
AA	0.86 ± 0.04	87.10 ± 2.11^{b}	2.18 ± 0.08	0.25 ± 0.01	2.13 ± 0.05

Data are reported as means ± standard error. ^{ab}Means with different superscript letters were significantly different ($P < 0.05$). BFT = backfat thickness; LEA = loin-eye areas; MAR = marbling scores; WHC = water holding capacity; TD = meat tenderness.

DISCUSSION

C/EBPα has been detected in adipose tissue, placenta, liver, and a variety of other organs, such as reproductive tissues and cells of the inflammatory system (McKnight et al., 1989; Chumakov et al., 1997), and several studies showed that C/EBPα in combination with other adipocyte transcriptional regulators controls the transcriptional pathway of adipogenesis (Wu et al., 1999). Therefore, we proposed that the C271A SNP would be related to carcass adiposity traits in Chinese native cattle (Komar, 2007). The ultrasound results for the 555 cattle confirmed our hypothesis.

All six native Chinese cattle populations were in Hardy-Weinberg equilibrium, suggesting that the gene frequencies and genotype frequencies remain stable during the process of generational change and can, therefore, be used to detect associations with carcass traits. Our present study revealed that the mutant homozygotes (AA) were at higher frequency than wild-type homozygotes (CC); accordingly, allele A had higher frequency than allele C in all the experimental populations.

H_O and H_E are used to measure specific sites in the homozygous alleles, and the greater H_E is, the more accurate the genetic variation is. H_E varied from 0.50 (LX, NY) to 0.54 (JXR, QC). N_e, as the reciprocal of H_E, which ranged from 1.84 (JXR) to 1.99 (LX), can objectively reflect the genetic variation. Comparisons of carcass traits among genetic groups showed that in animals with genotypes CA and AA, allele A may be a beneficial allele for some economic traits of Chinese indigenous cattle.

The C271A SNP of the C/EBPα gene resulted in a synonymous mutation in C/EBPα, which may lead to a protein with the same amino acid sequence but different structural and functional properties (Komar, 2007). The degeneracy of the genetic code enables the same amino acid sequences to be encoded and translated in different ways (Kurland, 1991). The C271A SNP of the bovine C/EBPα gene influences some meat quality traits such as UBF, UMAR, and LEA. Others have described associations between polymorphisms and carcass traits. Yang et al. (2009) confirmed the association between genetic variation of the calsarcin-1 gene and carcass traits in three Chinese indigenous cattle using the PCR-SCCP method.

It is not clear why the C/EBPα SNP was associated with LEA in the Qinchuan cattle, as this gene does not regulate muscle growth. There may be a reciprocal relationship between LEA and MAR in Qinchuan cattle, but this has not been documented. The relationship between the C/EBPα SNP and measures of carcass adiposity (BFT and MAR) was not significant, indicating that the C/EBPα SNP had no functional significance in this population of Qinchuan cattle.

In summary, we report a novel SNP of the bovine C/EBPα gene for the first time and demonstrate associations of the bovine C/EBPα gene with some carcass traits. Based on these data, individuals homozygous or heterozygous for the A allele could be selected for breeding in the future. However, further analysis on other herds and larger sample size should be performed in order to validate this association.

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