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MEF2A Gene Polymorphisms are Associated with Growth Traits in Chinese Indigenous Cattle Breeds

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Abstract: Myocyte enhancer factor 2A (MEF2A) gene encodes a member of the myocyte enhancer factor 2 (MEF2) protein family, which involves in vertebrate skeletal muscle development and differentiation. The important function of MEF2A implies this gene is a potential candidate gene for considering association analysis between polymorphism and growth trait. Sequencing and forced-PCR-RFLP were used to analyze the polymorphisms of the MEF2A gene in 1009 cattle from three Chinese indigenous breeds. Three SNPs (C1598T, G1641A and C1734T) were detected in the cattle MEF2A gene, leading to a missense (P420L) and two silent mutations (E434 E, P465P). The frequencies of 1598CC genotype and the frequencies of allele 1598C varied from 0.884-1.000 in the analyzed population (NY, QC and JX). The genotypic and allelic frequencies of G1641A and C1734T in three populations were 0.034-1.000. The C1598T was associated with body length at 6 and 12 months of cattle. The G1641A was associated with average daily gain at 12 months of cattle. And the C1734T affected the average daily gain and body weight at 6 months of cattle. Those three mutations affected the early growth and development of cattle, which could be potential molecular makers in assistant selection of cattle breeding.

Key words: MEF2A gene, cattle, Single Nucleotide Polymorphisms (SNPs), growth traits, association, mutation

INTRODUCTION

As a family of transcription factor, Myocyte Enhancer Factor-2 (MEF2) proteins are important regulators of cellular differentiation and consequently play a critical role in embryonic development (Potthoff and Olson, 2007). The MEF2 gene family is widely expressed in all branches of eukaryotes from yeast to human. There are four subtypes of the MEF2 gene in vertebrates (human versions are denoted as MEF2A, MEF2B, MEF2C and MEF2D) rather than just one copy in Drosophila. They are all expressed in distinct but overlapping patterns during embryogenesis till adulthood (McKinsey *et al*., 2002). All of the mammalian MEF2 gene family share two domains, MADS-box (56 amino acids) and MEF2 domain (29 amino acids). Their sequences were highly conserved in their N-terminal and diverge in C-terminal (Black and Olson, 1998).

MEF2 family are essential for vertebrate skeletal muscle development and differentiation, which are selectively expressed in differentiated myocytes and activates nearly all skeletal and cardiac muscle genes by binding a conserved A/T-rich DNA sequence (Gossett *et al*., 1989; Davis *et al*., 1990; Morisaki and Holmes, 1993; Feo *et al*., 1995; Rao *et al*., 1996; Wang *et al*., 2001). During myogenesis, MEF2A and bHLH proteins cooperatively activate skeletal muscle genes and physically interact through the MADS domain of MEF2A and three myogenic amino acids of the muscle bHLH proteins (Kaushal *et al*., 1994). Moreover, MEF2 controls skeletal muscle formation after terminal differentiation in nascent fibers and drives expression of genes encoding thick filament proteins (Hinits and Hughes, 2007).

Kaushal *et al*. (1994) proved that MEF2A induced myogenic development, when ectopically expressed in clones of nonmuscle cells of human clones. The MEF2A gene previously had been linked to patients with coronary artery disease and myocardial infarction. In particular, a 21-bp deletion and missense mutations were

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demonstrated either to reduce MEF2A transcriptional activity or to impair its nuclear translocation (Wang *et al*., 2003; Guella *et al*., 2009; Lieb *et al*., 2008; Elhawari *et al*., 2009). From 90-270 days, MEF2A expressions level were significant varied among cardiac muscle, dorsal muscle and leg muscle and the highest was leg muscle, the second was dorsal muscle, the lowest was cardiac muscle (Gao *et al*., 2009). Recently it was reported that the polymorphism of chicken MEF2A gene associated with body weight (Zhou *et al*., 2010).

The DNA sequencing and Forced-PCR-RFLP were used to identify the polymorphisms of the MEF2A gene in three Chinese indigenous cattle breeds. And the associations between the Single Nucleotide Polymorphisms (SNPs) within the bovine MEF2A gene and the growth traits of cattle were investigated, in order to identify the potential molecular markers in assistant cattle breeding.

MATERIALS AND METHODS

Animals: Genomic DNA samples were obtained from 1009 individuals belonged to three cattle breeds: Qinchuan (QC, n = 287), Nanyang (NY, n = 272), Jiaxian (JX, n = 450). Growth traits of Nanyang and Jiaxian population were collected every six monthss from birth to twenty-four monthss (birth, 6, 12, 18, 24 months), including birth weight, Body Weight (BW), Hucklebone Width (HBW), Withers Height (WH), Heart Girth (HG), Body Length (BL) and Heart Girth Index (HGI). Growth traits of Qinchuan cattle were collected after 3 years old including WH, BL, HBW, HG, BLI, HGI, Height at Hip Cross (HHC), Rump Length (RL) and Hip Width (HW).

PCR conditions: According to the whole genome shotgun sequence of Bos taurus MEF2A (GenBank accession No. NC_007319.3), nine pairs of primers were designed to investigate the potential SNPs in the MEF2A gene, The 15 μL PCR solution contained 30 ng DNA templates, 1 μmolL^{-1} of each primer, 2×Reaction Mix 7.5 μL (500 μM dNTP each, 200 mmolL^{-1} Tris-HCl, 100 mmol L^{-1} KCl, 3 mmol L^{-1} $MgCl_2$) and 0.25 U Taq DNA polymerase (Tiangen, Beijing, China). DNA pool was mixed with 100 DNA samples with equal concentration (50 ng μL^{-1}) for each group. And then the PCR products of DNA pool were used to sequence.

The PCR was performed using the following program: an initial step at 94°C for 4 min, followed by 35 cycles (denaturation at 94°C for 40 sec, annealing temperatures for 40 sec and extension at 72°C for 40 sec) and there is a final extension at 72°C for 10 min.

Forced PCR restriction fragment length polymorphism (F-PCR-RFLP): Three mutations were detected using the ABI 377 sequencer from both directions (Applied Biosystems, USA) of pooling DNA samples after PCR amplification (NM_001083638: 1734 C>T (465aa), 1641 A>G (434aa), 1598 T>C (420aa)) in MEF2A gene. In order to exactly detect these mutations, the forced PCR-RFLP method was used. The primer sequences were in the following:

F1: 5'-AGAGTTTGGGGGCCGGCCGAGCACACC-3'
R1: 5'-AGGCCCCACAGCCGCAGCCCCGGCTGCA-3'
R2: 5'-TCCAGCAGCAGCAGCAGCCACAGGCGC-3'

The underlined bases show the incorporated mismatch creating restriction sites. The length of PCR product was 191 bp, which contains three enzyme restriction sites to detect the three mutations of the MEF2A gene. In which 1734C>T, 1641A>G and 1598T>C could be detected by EcoR II, Pst I and BspT107 I, respectively (Fig. 1).

PCR products were obtained from all individuals in this study and aliquots of 5 μL PCR products were digested with 4U EcoR II, Pst I and BspT107 I (Takala,

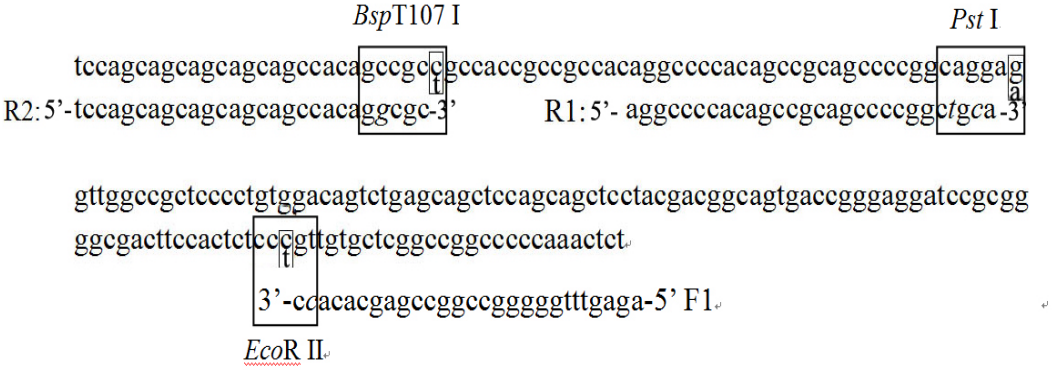

Fig. 1: The primer sequences for forced PCR-RFLP amplification. In the new forward, the italicized letters were in order to create a restriction site and the lettets in were polymorphic loci

Dalian, China) for 10 h at 37°C, respectively. The digested products were detected by electrophoresis in 3.0% agarose gel stained with ethidium bromide.

Statistical analysis: Genotypic and allelic frequencies of the 1598T>C, 1641A>G and 1734C>T were directly calculated. All sequences determined in this study were edited using the DNA star 5.0 package.

The associations between SNP marker genotypes and growth traits in cattle were analyzed by the least-squares method as applied in the General Liner Models (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, USA) according to the following linear model:

$$Y_{ijklmn} = \mu + B_i + F_j + M_k + G_l + S_m + e_{ijklmn}$$

Where:

Y_{ijklm} = Observed value
μ = Overall mean for each trait
B_i = Fixed effect of ith breed
F_j = Fixed effect of jth farm
M_k = Fixed effect of kth months of surveying
G_l = Fixed effect of lth single SNP marker genotype
S_m = Fixed effect of sex
e_{ijklmn} = Random error

RESULTS AND DISCUSSION

Nine exons of MEF2A gene were scanned in three Chinese indigenous cattle breeds and three mutations in exon11 were identified. The locus C1598T (Fig. 2, GenBank accession No. NM_001083638, same below) was identified as a missense mutation leading to a proline (CCG) to leucine (CTG) exchange. The loci G1641A (Fig. 3) and C1734T (Fig. 4) were all silent mutation.

The allele frequency and genotype frequency of the MEF2A gene were presented in Table 1. The C1598T and G1641A loci showed low Polymorphism (PIC = 0.13) and intermediate Polymorphism (PIC = 0.35) in NY but no polymorphism was found in QC and JX. In locus C1734T, the CC was the dominant genotype in NY and QC cattle but no polymorphism was detected in JX.

The relationship between SNPs and the cattle growth traits were analyzed (Table 2). The individuals with genotype 1598CT and CC had larger BL and HG at 12 months than the individuals with genotype 1598TT ($p<0.05$). This missense mutation maybe affects protein localization and transcription activity and then affects the cattle growth and development. The individuals with genotype 1641AA had better Average Daily Gain (ADG)

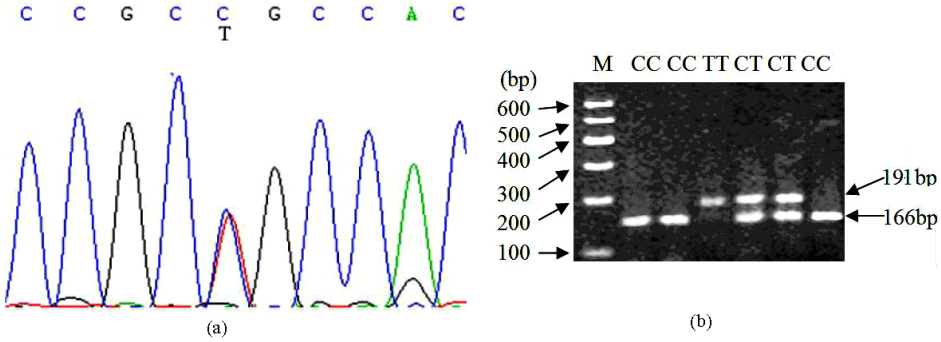

Fig. 2: (a) DNA sequencing map (b) BspT107 II restriction fragment length polymorphisms pattern

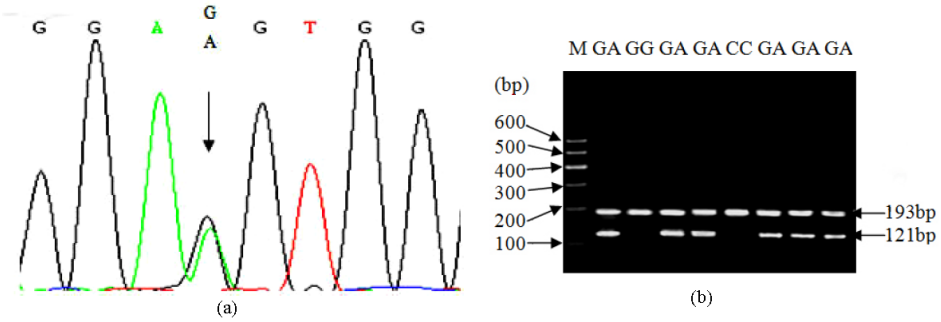

Fig. 3: (a) DNA sequencing map (b) Pst I restriction frangment length polymorphisms

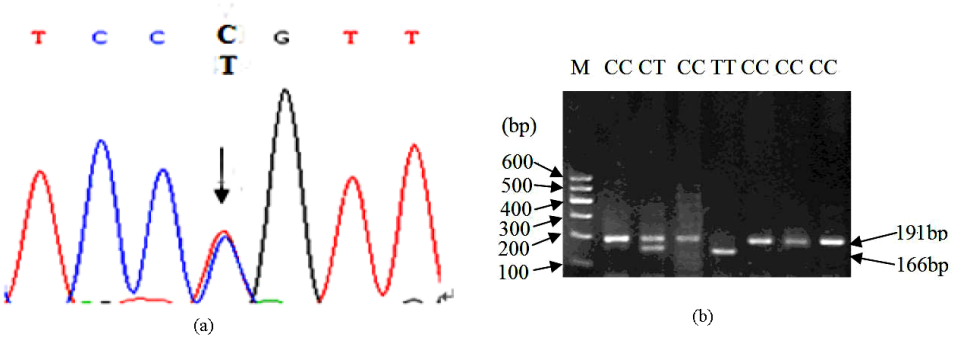

Fig. 4: (a) DNA sequencing map (b) EcoR II restriction fragment length polymorphisms

Table 1: Primers information of of the cattle MEF2A gene

Loci	Primers	Primer position	Product length (bp)	Annealing (°C)	Note
P1	1F: 5'-AAACACCATTCTATTCTACACC-3'	5690283-5690304	162	56	Exon 1 and its flanking region
	1R: 5'-ATTTGGACTACAAGGAAGGA-3'	5690420-5690444			
P2	2F: 5'-ATAAATGGGTCATCACATAGTA-3'	5670919-5670941	426	55	Exon 2 and its flanking region
	2R: 5'-GAACTCATCTTCAGGTAGCC-3'	5671325-5671344			
P3	3F: 5'-ATCAAAGCAAGAACATTATGAGTAG-3'	5647385-5647409	381	59	Exon 4 and its flanking region
	3R: 5'-AAGTAGATGAAAATGAAACTAACAG-3'	5647742-5647765			
P4	4F:5'- ATTATGTAGTAGTGACTCTTGCTC-3'	5647361-5647384	478	55	Exon 5 and its flanking region
	4R: 5'- TACCTCCATCTTCTGCTTTT-3'	5647815-5647834			
P5	5F: 5'- AGATAAGACAGATGCCAACA-3'	5646636-5646655	264	55	Exon 7 and its flanking region
	5R: 5'- ACATCATAATGCCTAGTTCAG-3'	5646778-5646798			
P6	6F: 5'- ATACTCATTAGGAAAAGGTCT-3'	5632635-5632654	500	54	Exon 8 and its flanking region
	6R: 5'- GTAAGGCTGGAAACAAGTGG-3'	5633111-5632130			
P7	7F: 5'- GGGAATCTTTCTATAATCAATG-3'	5614802-5614923	456	59	Exon 9 and its flanking region
	7R: 5'- TCACATAATCACTTGCTTCAGG-3'	5615239-5614260			
P8	8F: 5'- TGGGAAGCGTAGGTAGAGGT-3'	5611018-5611037	323	60	Exon 10 and its flanking region
	8R: 5'- TGAGCGACTAAGCACAAAGG-3'	5611322-5611341			
P9	9F: 5'- CAAAGTACAAACACCTGCCT-3'	5609435-5609454	462	60	Exon 11 and its flanking region
	9R: 5'- ATCTGTAGTATCCCATTCGTG-3'	5609875-5609895			

Reference to NC 007319.3

Table 2: Genotypic and allelic frequencies of different polymorphisms with in E11 of MEF2A gene

		Genotype frequencies			Allele frequencies			
SNPs	Breeds	1598CC	1598CT	1598TT	1598C	1598T	Mutations	PIC
C1598T	NY	0.884	0.079	0.037	0.926	0.076	C1598T	0.131
	QC	1.000	0	0	W[b] 1.000	0	-	-
	JX	1.000	0	0	W[b] 1.000	0	-	-
		1641GG	1641AG	1641AA	1641G	1641A		
G1641A	NY	0.304	0.696	0	0.652	0.348	G1641A	0.351
	QC	1.000	0	0	1.000	0	-	-
	JX	1.000	0	0	1.000	0	-	-
		1734CC	1734CT	1734TT	1734C	1734T		
C1734T	NY	0.500	0.284	0.216	0.642	0.357	C1734T	0.354
	QC	0.740	0.130	0.130	0.805	0.195	C1734T	0.265
	JX	1.000	0	0	1.000	0	-	-

at 12 months than the individuals with genotype 1641AT ($p<0.05$). The genotype 1734TT was better than the genotype 1734CT in BW and ADG at 6 months in cattle ($p<0.05$) (Table 3). Although, the two silence mutations didn't cause amino acid change, which could affect the growth traits cattle through codon usage biases or mRNA structure changing, further experiments were needed to investigate the mechanisms. If $r^2>0.33$, then the linkage disequilibrium was considered strong (Li *et al*., 2009). The linkage disequilibrium between the three SNPs were estimated, which indicated that the three SNPs were not linked strongly in the analyzed populations (r^2 = 0.001, 0.023 and 0.006, respectively).

The sequence conservation and function domain of MEF2A gene were shown in Table 4. The MEF2A gene has highly conserved N-terminal MADS-box and MEF2 domains, while diverge C-terminal transactivation domain. No polymorphism was detected except exon11 in the nine exons of MEF2A gene in this study showed the consistent conservation mode.

Table 3: The association between the body measurement of cattle and SNPs of MEF2A gene

	Genotype				Genotype				Genotype		
Traits	1598CC	1598CT	1598TT	Trait	1641GG	1641AG	1641 AA	Traits	1734GG	1734AG	1734AA
BL of 12 M	129.5±0.737[a]	130.3±2.330[a]	122.0±2.331[b]	ADG of 12 M	0.421±0.031[a]	0.331±0.017[b]	-	BW of 6 M	160.0±2.930	156.2±3.875[b]	168.1±4.746[a]
HG of 12 M	156.3±0.989[a]	156.0±3.127	147.8±3.126[b]	-	-	-	-	ADG of 6 M	0.7243±0.0960	0.6988±0.1126[b]	0.7624±0.1106[a]

Mean±SE; Values with different shoulder letter differ ($p<0.05$); M: Months

Table 4: The domain organization and sequence comparison of MEF2 CDS and proteins from some species

Genes	MADS	MEF2	MEFA2	TAD
g.Human	100	100	100	NM_005587
g.Bos	99	96	90	NM_00108363
g.Mus	98	96	89	NM_00109969
p.Human	100	100	100	(Potthoff
p.Yeast	61	11	7	and Olson,
p.Drosophila	90	68	14	2007)
p.C.elegans	95	84	7	

The nucleotide sequence numbering shown is of the human MEF2A sequence and the percent sequence identities are all relative to Human MEF2A. The domain organization and sequence comparison of MEF2 proteins from representative species (Potthoff and Olson, 2007). The amino acid numbering shown is of the human MEF2A sequence and the percent sequence identities are all relative to hMEF2A

The MEF2 proteins were involved in regulation of many muscle specific genes and played active roles in myogenesis, proliferation and differentiation (Olson *et al*., 1995; Kaushal *et al*., 1994). The functional importance of the MEF2A gene and the phenotype influence of the mutation implied that the three SNPs detected in this study could be potential markers in molecular marker assisted selection of cattle breeding. But only SNP association analysis is not enough, further research need to be done to investigate the mechanisms of these mutations and more research need to be done to investigate the MEF2 family's function in cattle's growth and development.

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