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Divergence of Myostatin Gene in Zebu and Normal Cattle in China

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Abstract: Using the methods of PCR amplification and sequencing, the polymorphism of myostatin gene were detected in 18 Leiqiong cattle and 18 Mongolia Cattle. The results showed that, 1128 base pairs existed in the 36 samples, 4 polymorphic site was observed, while European normal cattle, zebu and yak hold a haplotype on these sites each. The average nucleotide difference in myostatin gene between the two populations was low (0.00242±0.00031). The average nucleotide difference was 0, indicating that Leiqiong and Mongolia Cattle had a close genetic relation and their divergence was relatively late and the molecular evolution of myostatin gene in Bovinae did not relate with adaptation.

Key words: Myostatin, leiqiong cattle, mongolia cattle, nucleotide polymorphism

INTRODUCTION

The issue of the systemic origination of yellow cattle in China has long been a core of elucidating the divergence history of livestock of bovinae in Asia (Chang *et al*., 1990), the typical populations of zebu group (*Bos indicus*) and normal cattle group (*Bos taurus*) in China are Leiqiong and Mongolia Cattle, distributing in the south and north end of Chinese mainland, respectively. Solution to their divergence is an essential point to explain the phylogenic history of yellow cattle in China. Researches on morphology, ecology, Chromosome feature, structural gene, frequency distribution of microsatellite variation and historical confirmation, for a long time, have accumulated a large amount of literature (ECL, 1986).

Bovine myostatin gene (MSTN), is also called "double-muscling" gene. the loss of its function would lead to a feature of double muscle in cattle and the divergence of the features restrained by the gene is under the pressure of selection. The evolutionary situation at the molecular level would help us to acknowledge the divergence level between Leiqiong and Mongolia Cattle and reveal the systemic origination of yellow cattle in China and would provide some objective proof for exploring the relation between molecular evolution and adaptive evolution. The study was conducted based on the molecular divergence of myostatin gene between Leiqiong and Mongolia Cattle population.

MATERIALS AND METHODS

Animal population and genomic DNA extraction: Applying simple random sampling in typical colony methods in the central area of habitat, 18 blood samples were collected from Hejing county Bayibuluke district, Bayinguoleng Mongolian minority autonomy region, Xinjiang and 18 blood samples from Helu village of Nanxing and Changping village of Longmen, Leizhou, Guangdong province. The genome DNA was extracted by hydroxy benzene/chloroform method.

Polymerase chain reaction: Exon sequences and some intronic sequences were amplified from genomic DNA. GenBank sequence from *Bos taurus* (GeneBank Accession No: AB076403) was used to design primers (Shanghai Genecore company). The primers were listed below. In a typical reaction, 25uL PCR system contained 10×PCR buffer 2.5 μL, Mg^{2+} 2.5μM, dNTP 400 μM, Taq DNA enzyme 1U (Takara company, China), Forward and Reverse primer 0.4 μM each, template DNA 1 μL. The amplification program consisted of predenaturation

Table 1: Polymerase chain reaction

P1	Forward	5'- GGCTTGGCGT TACTCAAAAGC-3'
	Reverse	5'- CTCCTCCTTACGTACAAGCCAGCA-3'
P2	Forward	5'-GTTCATAGATTGATATGGAGGTGTTCG-3'
	Reverse	5'-ATAAGCACAGGAAACTGGTAGTTATT-3'
P3	Forward	5'-GAAATGTGACATAAGCAAAATGATTAG-3'
	Reverse	5'-ATACTCTAGGCTTATAGCCTGTGGT-3'

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(94°C 3 min), followed by 34 cycles of denaturation (94°C 0.5 min), primer annealing (51°C 0.5 min) and extension (72°C 0.5 min) and a final extension (72°C 3 min). The PCR products were stored at 4°C (Table 1).

Sequencing and comparison of PCR fragment: Three exons are separated by two introns in MSTN gene. PCR products were first detected using 1.2% agarose gel or 10% PAGE gel and then sequenced (SANGON company, Shanghai, China). The sequencing results were analyzed through DnaSP software and compared with the reported GenBank data, *Bos taurus* (GenBank accession number: AB076403), *Bos indicus* (GenBank accession number: AY794986) and *Bos grunniens* (GenBank accession number: AY787760), to describe the nucleotide divergences of MSTN gene between and among Leiqiong and Mongolia Cattle population.

RESULTS

PCR reaction and sequencing: The designed primers were used to amplified 36 DNA samples. The size of each PCR band was similar to literature (Meng *et al*., 1986). The sequences of PCR products for 36 DNA samples contained exon 1 (375bp), exon 2 (372bp) and exon 3 (381bp), which are identified with the reported exons in literature.

Sequence analysis: The average content for each base MSTN gene in Leiqiong Cattle was 25.4%T, 21.4%C, 31.1%A, 22.1%G and 25.27 %T, 21.37 %C, 31.21 %A, 22.16%G in Mongolia Cattle. No significant biases of base content were detected in both populations. Three nucleotide variations were observed at three sites in 18 Leiqiong Cattle: at 111bp from ATG (nt111), C took up 55.6% G took up 44.4%, while the reported normal cattle, *Bos taurus* (GenBank accession number: AB076403), zebu, *Bos indicus* (GenBank accession number: AY794986), yak, *Bos grunniens* (GenBank accession number: AY787760) and Mongolia Cattle in the study held a G haplotype; at nt1077, C took up 77.8% and A took up 22.2%, while the reported normal cattle, *Bos taurus* (GenBank accession number: AB076403), yak, *Bos grunniens* (GenBank accession number: AY787760) and Mongolia Cattle in the study held an A haplotype, but the reported zebu, *Bos indicus* (GenBank accession number: AY794986), held a C haplotype; at nt1083,T took up 77.8% and C took up 22.2%, while the reported normal cattle, *Bos taurus* (GenBank accession number: AB076403), yak, *Bos grunniens* (GenBank accession number: AY787760) and Mongolia Cattle in the study held a C haplotype, but the reported zebu, *Bos indicus* (GenBank accession number: AY794986), held a T haplotype. In 18 Mongolia Cattle, one nucleotide variation was observed at nt414, where C took up 55.6% °ÍT took up 44.4%, while the reported zebu, *Bos indicus* (GenBank accession number: AY794986), yak, *Bos grunniens* (GenBank accession number: AY787760) and Leiqiong Cattle in the study held a C haplotype, but the reported normal cattle, *Bos taurus* (GenBank accession number: AB076403) held a T haplotype (Table 2).

All the detected 4 nucleotide variations, two transitions and 2 transversions, were at the third codon and synonymous. The ratio of nonsynonymous to synonymous nucleotide substitution rates (Ka/Ks) were zero, regardless of breed and species within bovinae.

Table 2: Exon sequences of MSTN in Leiqiong and Mongolia cattle

Exon 1	1	ATGCAAAAACTGCAAATCCCTGTTTATATTTACCTATTTATGCTGATTGTTGCTGGCCCAG
	61	TGGATCTGAATGAGAACAGCGAGCAGAAGGAAAATGTGGAAAAAGAGGSGCTGTGTAAT
	121	GCATGTTTGTGGAGGGAAAACACTACATCCTCAAGACTAGAAGCCATAAAAATCCAAATC
	181	CTCAGTAAACTTCGCCTGGAAACAGCTCCTAACATCAGCAAAGATGCTATCAGACAACTT
	241	TTGCCCAAGGCTCCTCCACTCCTGGAACTGATTGATCAGTTCGATGTCCAGAGAGATGCC
	301	AGCAGTGACGGCTCCTTGGAAGACGATGACTACCACGCCAGGACGGAAACGGTCATTACC
	361	ATGCCCACGGAGTGT
Exon 2	376	GATCTTCTAACGCAAGTGGAAGGAAAACCCAAATGYTGCTTCTTTAAATTTAGCTCTAAG
	436	ATACAATACAATAAACTAGTAAAGGCCCAACTGTGGATATATCTGAGGCCTGTCAAGACT
	496	CCTGCGACAGTGTTTGTGCAAATCCTGAGACTCATCAAACCCATGAAAGACGGTACAAGG
	556	TATACTGGAATCCGATCTCTGAAACTTGACATGAACCCAGGCACTGGTATTTGGCAGAGC
	616	ATTGATGTGAAGACAGTGTTGCAGAACTGGCTCAAACAACCTGAATCCAACTTAGGCATT
	676	GAAATCAAAGCTTTAGATGAGAATGGCCATGATCTTGCTGTAACCTTCCCAGAACCAGGA
	736	GAAGATGGACTG
Exon 3	748	ACTCCTTTTTTAGAAGTCAAGGGAACAGACATCCCAAAAAGATCTAGGAGAGATTTTGGG
	808	CTTGATTGTGATGAACACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAACTGTGGAT
	868	TTTGAAGCTTTTGGATGGGATTGGATTATTGCACCTAAAAGATATAAGGCCAATTACTGC
	928	TCTGGAGAATGTGAATTTGTATTTTTGCAAAAGTATCCTCATACCCATCTTGTGCACCAA
	988	GCAAACCCCAGAGGTTCAGCCGGCCCCTGCTGTACTCCTACAAAGATGTCTCCAATTAAT
	1048	ATGCTATATTTTAATGGCGAAGGACAAATMATATAYGGGAAGATTCCAGCCATGGTAGTA
	1108	GATCGCTGTGGGTGTTCATGA

Note: Framed letters are polymorphic sites: S stands for G or C, Y for T or C, M for A or C

Table 3: Nucleotide variance of MSTN gene in Leiqiong and Menggu cattle

	111bp	414bp	1077bp	1083bp
Bt	G	T	A	C
Bi	G	C	C	T
Bg	G	C	A	C
MG	G	C/T	A	C
LQ	G/C	C	A/C	C/T

DNA divergence within and between populations: The sequences of the 36 samples were analyzed by DnaSP software. The average number of nucleotide differences (k) was, respectively 1.157 and 0.523 in Leiqiong and Mongolia cattle; the nucleotide diversity with Jukes and Cantor was, respectively 0.00103±0.00022 and 0.00046±0.00004 in Leiqiong and Mongolia cattle; the average number of nucleotide substitution per site between populations (Dxy) with Jukes and Cantor was 0.00242±0.00031. Both populations had no amino acid diversity. The above results indicated that the two populations had a low DNA diversity and a close relationship between them (Table 3).

DISCUSSION

- The Leiqiong cattle sequences contained two nucleotides, 'G '(guanine) and 'C'(cytosine), at nucleotide position 111 (the third codon)of the MSTN exon in contrast to a 'G 'in the reported normal cattle, yak, zebu and Mongolia Cattle in the study. For the frequency of the 'C' haplotype was high (0.556) at this site in Leiqiong cattle, it is reasonable to recognize that the 'G 'type at this site is an ancient nucleotide type during the evolutionary process of Bovinae and therefore, the 'C' type originates from the mutation in the ancestor populations in Leiqiong cattle.
- At nucleotide position 1077 of the MSTN exon (the third codon), Leiqiong cattle contained a transversion from 'A ' to 'C' type, originated from ancestral zebu populations and an 'A 'type, which is immanent among Bovinae populations; at nucleotide position 1083 of the MSTN exon, Leiqiong cattle contained a transition from 'C ' to 'T' type, originated from ancient zebu populations and a 'T' type, which is also immanent among Bovinae populations. The polymorphisms at the 2 sites suggested that zebu populations in South China had a closer relationship with normal cattle than other Bos populations and that the divergence between zebu and normal cattle was relatively late.
- The Mongolia cattle sequences contained two nucleotides, 'T' (thymine) and 'C'(cytosine), at nucleotide position 414 (the third codon) of the MSTN exon in contrast to a 'C' in the reported yak, zebu and Leiqiong Cattle in the study, but a 'T 'in the reported European normal cattle, suggesting that Chinese normal cattle had a closer relationship with yak and zebu than European normal cattle and that the divergence of European normal cattle was relatively late. Otherwise, the findings were different from Meng's (Meng *et al*., 2004) report that Mongolia cattle had 6 polymorphic sites in the MSTN exon.
- The myostatin protein is known to be a negative regulator of muscle mass development. Although there are multiple genes responsible for the double muscling phenotype, myostatin accounts for the primary effect seen in double-muscled cattle (Casas *et al*., 1998). It seems that the mutations in the gene of MSTN would result in a functional selection. The sequencing results in the study did not indicate any implications of DNA divergence in MSTN with positive functional selection, but demonstrated that a mutation at nucleotide position 111 originated from ancestral zebu populations in South China. For the molecular evolution of MSTN, the divergence between zebu and normal cattle was relatively late and Mongolia had a closer relationship with zebu than European normal cattle and zebu populations in South China had a closer relationship with normal cattle than other zebu populations. The little difference between bovinae species demonstrated that the molecular evolution of MSTN in Bovinae had little relation with adaptation.

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