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SNP Identification and Analysis in Part of Exon 3 of Beef Cattle *MSTN* Gene and Variation among Breeds

Liu Guifen, Wan Fachun, Song Enliang, Liu Xiaomu, Tan Xiuwen and Cheng Haijian
Shandong Key Lab of Animal Disease Control and Breeding,
Shandong Academy of Agricultural Sciences,
Institute of Animal Science and Veterinary Medicine, 250100 Jinan, China

Abstract: This study investigated the effects of four SNP in exon 3 of myostatin gene on growth, carcass and beef quality traits in China. A total four SNPS (A→C, C→T, G→A, C→A) were detected among the 117 sequenced individuals. The experiment used four crossed breeds include the Limousin cattle x Luxi yellow cattle (LL), Simmental cattle x Luxi yellow cattle (SL), Charolais cattle x Simmental cattle x Luxi yellow cattle (CSL), Limousin cattle x Simmental cattle x Luxi yellow cattle (LSL). In addition, research group also included Qinchuan cattle (Chinese local breed) and one unidentified breeds. The results showed that there are no differences in all detected traits wherever between breed or within breed of different genotype in four mutative sites. However, there are dominant differences between breeds under without considering genotype circumstance. The results provided strong evidence that three mutative site in exon 3 of MSTN might be some invalid mutation to the researched groups. However, the body measurement, growth and carcass traits have dominant difference in all breeds.

Key words: SNP, MSTN, meat quality, cattle, exon

INTRODUCTION

The Myostatin (*MSTN*) gene has been generally acknowledged as an important candidate gene for growth and development of domestic animal due to its key role in muscle growth and polymorphism of *MSTN* gene can have important economic consequences in animal husbandry (Miranda *et al*., 2002; Li *et al*., 2006). *MSTN* gene was first discovered in mice and acts as a negative regulator of skeletal muscle mass. In cattle, mutation in this gene are responsible for double muscling, a trait by an increase in skeletal muscle amount (McPherron and Lee, 1997; Grobet *et al*., 1997). This study aimed to determine whether or not four mutations in the exon 3 of *MSTN* gene were influencing in some cross cattle groups and to study the effects of the mutation on various qualities of body measurement, growth and carcass.

MATERIALS AND METHODS

Animal: All animals used in this study from slaughterhouse and were reared and slaughtered in compliance with national regulations for human care and use of animals in research. Blood samples of 117 beef cattle were collected in centrifuge tubes (1.5 mL) with 70% ethanol and stored at 4°C until DNA extraction.

Simultaneously, traits of body measurement (body depth, body length, chest circumference, circumference of cannon bone, rump length), growth and carcass (body weight, carcass weight, dressing percentage, backfat, high-grade meat proportion, pure meat percentage) have been collected for statistical analysis.

Genomic DNA was extracted from blood by phenol and chloroform extraction and stored at -20°C. For genotyping the polymorphism of *MSTN* gene mutative locus, primers for MSTN were designed from cattle *MSTN* gene sequence (NCBI nucleotide database accession AB076403). For this research, researchers used a gene specific primer to amplify a 376 bp region located at the exon 3 (upper primer 5'-ATGCTGTCGTTACCCTCTAA-3' and lower primer 5'-TAGCCTGTGGTACATAATTT CA-3'). The polymerase chain reaction amplification was performed using 100-500 ng of genomic DNA, 2.5 μL of 10×PCR buffer (containing 100 mM Tris-HCl (pH8.0), 500 mM KCl, 10 mM of $MgCl_2$ and 0.1% glutin), 200 μM of each dNTP, 10 pM of each primers and 2 U of Taq DNA polymerase in a 25 μL final volume. The amplifying conditions of PCR for *MSTN* gene were 94°C for 5 min followed by 35 cycles of 95°C for 30 sec, 60°C for 1 min, 72°C for 40 sec then 72°C for 10 min. The PCR products were resolved by electrophoresis in 1.5% agarose gel, stained with EB and visualized by UV irradiation.

Corresponding Author: Wan Fachun, Shandong Academy of Agricultural Sciences,
Institute of Animal Science and Veterinary Medicine, 250100 Jinan, China

Statistical analysis: Allele and genotype frequencies of MSTN were calculated from the number of animals in each group of cattle breed under study, respectively. One linear model was established to analyze the genotype effects of MSTN:

$$Y_{ij} = \mu + P_i + G_j + e_{ij}$$

Where:

Y = The detected traits
μ = The overall mean
P_i = The effect of breed (i = 1-6)
G_j = The effect of genotype (j = 1, 2)
e = The random residual

When there are no differences of different genotype in all breeds, one-way ANOVA was used to analyze the data.

RESULTS AND DISCUSSION

The four mutations in exon 3 of *MSTN* gene are shown in Fig. 1. The allele, genotype frequencies and genetic polymorphism parameters of *MSTN* gene are shown in Table 1 and 2. Mutative sites not found in Simmental cattle x Luxi yellow cattle, Charolais cattle x Simmental cattle x Luxi yellow cattle, Limousin Cattle x Simmental cattle x Luxi yellow cattle and unidentified breeds. However, these mutations have been found in L×L and Qinchuan cattle. AA genotype is higher than BB in all groups and sites. The homozygosity of four sites are high in all groups, however the polymorphism information content and effective number of alleles is low, the reason may be that artificial selection pressure of cattle is bigger.

The different genotype in all research sites of MSTN has no effect to the body measurement, growth and carcass traits. From Table 3, researchers can conclude that there are differences of above traits among different breeds. The S×L cross cattle higher than the other five groups in body weight, carcass weight, rump length and body length and the difference is dominant. L×L and

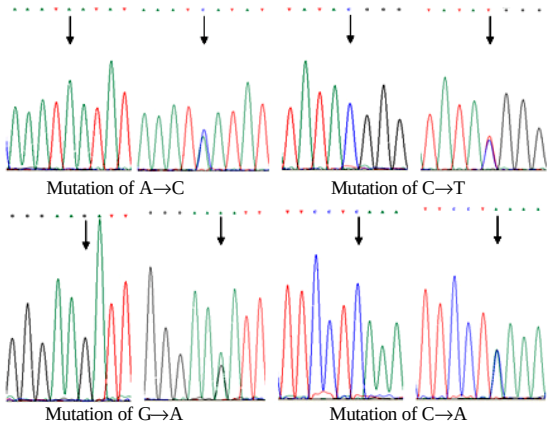

Fig. 1: The mutation sites in exon 3 of *MSTN* gene

Table 1: Allele and genotype frequencies of MSTN in different beef cattle

Breeds	No. of samples	Genotype frequency of site 1		Allele frequency of site 1		Genotype frequency of site 2		Allele frequency of site 2	
		AA	BB	A	B	AA	BB	A	B
LL	49	0.94 (46)	0.06 (3)	0.94	0.06	0.92 (45)	0.08 (4)	0.92	0.08
Qinchuan cattle	17	0.88 (15)	0.12 (2)	0.88	0.12	0.76 (13)	0.24 (4)	0.76	0.24
SL	16	1 (16)	0 (0)	1.00	0.00	1 (16)	0 (0)	1.00	0.00
CSL	12	1 (12)	0 (0)	1.00	0.00	1 (12)	0 (0)	1.00	0.00
LSL	13	1 (13)	0 (0)	1.00	0.00	1 (13)	0 (0)	1.00	0.00
Others	10	1 (10)	0 (0)	1.00	0.00	0.9 (9)	0.1 (1)	0.90	0.10
Breeds	No. of samples	Genotype frequency of site 3		Allele frequency of site 3		Genotype frequency of site 4		Allele frequency of site 4	
		AA	BB	A	B	AA	BB	A	B
LL	49	1 (49)	0 (0)	1.00	0.00	0.78 (38)	0.22 (11)	0.78	0.22
Qinchuan cattle	17	0.88 (15)	0.12 (2)	0.88	0.12	0.59 (10)	0.41 (70)	0.59	0.41
SL	16	1 (16)	0 (0)	1.00	0.00	1 (16)	0 (0)	1.00	0.00
CSL	12	1 (12)	0 (0)	1.00	0.00	1 (12)	0 (0)	1.00	0.00
LSL	13	1 (13)	0 (0)	1.00	0.00	1 (13)	0 (0)	1.00	0.00
Others	10	1 (10)	0 (0)	1.00	0.00	0.9 (9)	0.1 (1)	0.90	0.10

Table 2: Genetic polymorphism parameters of MSTN in different beef cattle

Genetic polymorphism parameters	Site 1				Site 2				Site 3				Site 4			
	Ho	He	Ne	PIC	Ho	He	Ne	PIC	Ho	He	Ne	PIC	Ho	He	Ne	PIC
LL	0.8872	0.1128	1.1271	0.1064	0.8528	0.1472	1.1726	0.1364	1	0	1	0	0.6568	0.3432	1.5225	0.2843
Qinchuan cattle	0.7888	0.2112	1.2678	0.1889	0.6352	0.3648	1.5743	0.2983	0.7888	0.2112	1.2678	0.1889	0.5162	0.4838	1.9372	0.3668
SL	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
CSL	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
LSL	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
Others	1	0	1	0	0.82	0.18	1.2195	0.1719	1	0	1	0	0.82	0.18	1.2195	0.1719

Table 3: Effect of different breeds on the body measurement, growth and carcass traits (LS means±SE)

Traits	Breed					
	Limousin x LYC	Qinchuan cattle	Simmental x Simmental LYC	Charolais x x LYC	Limousin x Simmental x LYC	Other breed
Traits						
Body depth	132.66±0.75^{a}	136.26±1.69^{b}	135.31±1.020^{abd}	131.75±2.500^{acd}	130.15±1.510^{ace}	133.00±1.280^{abd}
Body length	139.02±1.19^{a}	140.24±1.95^{a}	147.62±2.080^{b}	142.58±3.550^{a}	141.69±2.840^{a}	139.20±4.170^{a}
Chest circumference	204.33±1.67^{a}	206.29±2.31^{a}	207.06±1.910^{a}	202.92±3.960^{a}	192.85±5.130^{b}	196.80±4.140^{ab}
Circumference of cannon bone	19.97±0.24^{a}	19.62±0.18^{a}	22.28±0.300^{b}	21.29±0.250^{b}	21.58±0.420^{b}	21.20±0.380^{b}
Rump length	40.38±0.53^{a}	40.88±1.04^{a}	44.44±0.810^{b}	40.25±0.960^{a}	40.38±0.770^{a}	39.10±1.070^{a}
Body weight	561.90±9.27^{a}	517.06±8.21^{b}	680.31±22.29^{bc}	577.08±23.08^{acd}	552.30±19.38^{abd}	560.50±24.28^{abd}
Carcass weight	295.61±6.48^{a}	282.76±5.50^{a}	353.75±12.23^{b}	314.33±16.31^{a}	295.69±11.81^{a}	286.50±15.61^{a}
Dressing percentage (%)	52.41±0.64^{a}	54.74±0.89^{a}	50.18±2.180^{ab}	54.34±1.330^{ac}	53.51±0.930^{a}	53.10±0.940^{a}
Bone weight	17.23±1.22	19.81±0.57	21.22±2.640	18.95±2.880	19.70±1.950	18.55±2.710
Backfat	1.10±0.15^{a}	1.20±0.10^{a}	0.57±0.050^{b}	0.41±0.070^{b}	0.40±0.040^{b}	0.60±0.150^{b}
High-grade meat proportion (%)	9.90±0.17^{a}	10.08±0.30^{ab}	9.02±0.200^{a}	8.64±0.220^{b}	9.20±0.140^{a}	9.03±0.250^{a}
Pure meat percentage (%)	46.38±0.85^{a}	47.05±0.82^{a}	45.70±1.030^{a}	50.06±2.070^{b}	46.76±1.330^{a}	46.68±1.240^{a}

Values with different superscripts show significant levels ($p \leq 0.05$)

Chinese local breed Qinchuan cattle higher than the other four groups in Backfat. Qinchuan cattle are highest than the other breeds in high-grade meat proportion and the difference is dominant. The C×S×L has highest than others in pure meat percentage, however, the high-grade meat proportion is lowest. The bone of six groups is no difference.

The S×L cross have high number in all growth traits than other groups. Qinchuan cattle and Luxi yellow cattle all are one of Chinese local yellow breeds. Although, their growth and development are lower they have tall physical, plump muscle, excellent meat quality. So, these local breeds have been crossed with exotic breeds animals to improve growth rate. In this study, Qinchuan cattle are highest than the other breeds in high-grade meat proportion.

In cattle, MSTN is considered to be one of the most important marker gene responsible for meat quality traits (Sadkowski *et al*., 2008; De la Rosa-Reyna *et al*., 2006). Casas *et al*. (1998) has reported that breed source of the MSTN was not significant for birth weight and carcass composition traits in Piedmontese and Belgian blue.

Several polymorphisms have been found in *MSTN* gene and Grobet *et al*. (1998) and Dunner *et al*. (2003) reported that the *MSTN* gene is highly variable within beef cattle. Moreover, in the study, four mutative sites of *MSTN* gene have been found in six groups, however there were no difference among groups. Hamrick *et al*. (2000) demonstrated that despite the impressive musculature of the MSTN mull "Might mouse", its femora were not altered in either shape or size. In this study there was no effect on bone weight.

CONCLUSION

The data on different breeds of *MSTN* gene polymorphism provide evidence for the exon 3 polymorphism of *MSTN* gene has no effect on this researched cattle's groups. However, there are dominant differences in detected traits among these groups except the gene effect.

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