

Asian-Aust. J. Anim. Sci. Vol. 20, No. 12 : 1798 - 1804 December 2007

www.ajas.info

Association between Polymorphisms of MSTN and MYF5 Genes and Growth Traits in Three Chinese Cattle Breeds

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ABSTRACT: The objective of this study was to assess the association of polymorphisms in MSTN and MTF5 genes with growth traits in three Chinese cattle breeds. Only one homozygous animal with BB genotype at MSTN locus was observed in Jiaxian population which was at Hardy-Wemberg disequilibrium (p<0.05). The frequencies of allele A at MSTN locus and allele B at MTF5 locus in the three Chinese breeds were 0.9550/0.9730/0.9720 and 0.8275/0.7581/0.7523, respectively. Allele A at MSTN locus and allele B at ATF5 locus were dominant in these three populations. No statistically significant differences in growth traits were observed between the genotypes of the Jiaxian breed at MSTN and MTF5 loci and the Nanyang breed at MTF5 locus. However, there were statistically significant differences between the genotypes at MSTN locus of the Nanyang breed for WH, HG, HGI and HGBLR (p<0.05), and of the Qinchuan breed for BLI (p<0.05). The SNP in MTF5 had significant effects on WH and HHC of Qinchuan animals (p<0.05). These results suggest that MSTN and MTF5 are strong candidate genes that influence growth traits in cattle. Other SNPs of MSTN and MTF5 or other linked genes should also be studied, which could lead to the development of selection plans to improve the performance of Chinese cattle and also promote the breeding of genuine beef cattle in China. (**Key Words**: Cattle, MSTN, MTF5, Growth Traits)

INTRODUCTION

Nanyang. Qinchuan, and Jiaxian cattle are three of the eight best cattle breeds in inland China. Due to their uniqueness, these breeds have been designated as nationally protected resources. They have been the representative of Chinese cattle breeds for their good performance traits and especially fleshy characteristics. For thousands of years, Nanyang, Qinchuan and Jiaxian breeds, famous for their large body size and excellent farming ability, have been the main labor force in agriculture and an important beef source. However, with the development of science and technology and the ever-increasing mechanization of farming, these good farming abilities have become obsolete. Compared

with imported commercial beef cattle breeds, these three breeds have several drawbacks, such as an underdeveloped hind hip, slower growth rate and poor milk production. In an effort to adapt to the development of a commercial economy, it has become necessary for these three breeds to improve meat performance while maintaining their other excellent inherent characteristics.

Several candidate genes affecting muscle mass in farm animals may be selected on the basis of their participation in the processes of muscle development. Myostatin (MSTN), or growth and differentiation factor 8 (GDF8), is a member of the transforming growth factor β (TGF- β) superfamily, which includes proteins that mediate key events in cell growth and development through signal transduction. Myostatin acts as a negative regulator of myogenesis (Lee and McPherron, 2001) and inhibits myoblast proliferation during the cell cycle and myogenic differentiation (Thomas et al., 2000; Taylor et al., 2001; Rios et al., 2002; Langley et al., 2002; Lu et al., 2007). In the absence of myostatin, the skeletal musculature of mice is two to three times greater in mass than that of wild-type nuce (McPherron et al., 1997). Several cattle breeds are characterized by double muscling phenotype (an increase in the number of normal-sized

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Table 1. The primer sequences, position, PCR fragment sizes and the annealing temperatures of two genes

| Genes | Primer sequence | Position* | Size (bp)** | Annealing temperature |
|-------|-------------------------------|-----------|-------------|-----------------------|
| MSTN | 5'-CCCTACAGAGGCCACTTCAA-3' | 9,142 | 1,346 | 63°C |
| | 5'-CTCGCTGTTCTCATTCAGATC-3' | 11,486 | | |
| MYF5 | 5'-GATAGCTGGCTGTGAATGAT-3' | 966 | 1,190 | 60°C |
| | 5' -CTGGCAACTGGGGAGAGAGAAG-3' | 2,155 | | |

^{*} The position of the primers was established based on Genbank sequence AF348479 and M95684.

muscle fibers that result in enlarged muscles with deep creases) caused by six different loss-of-function mutations at the MSTN locus (Grobet et al., 1997; Kambadur et al., 1997; Karim et al., 2001). Homogeneous and heterogeneous individuals with the mutations showed enlarged musculature, increased birth weight and greater growth rate (Casa et al., 1999; 2004). Although a large number of alleles of the gene have been identified in cattle, most are silent or neutral in their resultant effect. In some breeds double muscling is not associated with any disruptive mutation in the gene. Muscle fiber formation takes place during embryonic development and is regulated by the MyoD gene family, which consists of four genes, MyoD1 (MTF3), myogenin (MTOG or MTF4), MTF5 and MTF6 (MRF4). MYF5, in combination with MYOD1, determines the muscular lineage and is the first factor of this family to be expressed in the embryo (Braun et al., 1989, 1990). Expression of MYF5 in adult skeletal muscle is restricted to the satellite cell population (Beauchamp et al., 2000) and to the muscle spindles (Zammit et al., 2004). Knockout mouse experiments showed that MTF5 and MTOD1 do affect muscle development, although they are redundant to a certain extent. Mice lacking both MYF5 and MYOD1 myogenic regulator factors were born alive but they died soon after birth (Rudnicki et al., 1993). MYF5 gene was mapped at bovine chromosome 5 region (0 to 30 cM) which was identified as having significant associations with the growth traits (Crosse et al., 1999; Li et al., 2002a, b; 2004). Because of its functions and support from the results of QTL studies. MTF5 is considered as a candidate gene for growth related traits in meat producing animal species.

The objective of this study was to analyze the genetic variations of MSTN and MYF5 genes in Nanyang, Qinchuan and Jiaxian cattle breeds and to investigate their corresponding effects on the growth traits of these three Chinese cattle breeds.

MATERIALS AND METHODS

Animal and data source

Four hundred and eleven female animals (Nanyang, 210; Qinchuan, 93; Jiaxian, 108) were used in this study. The animals originated from three farms. The Nanyang animals were from the breeding center of Nanyang cattle (Nanyang city, Henan Province, China), the Jiaxian animals

were from the breeding farm of Jiaxian cattle (Jiaxian county. Henan Province. China) and the Qinchuan animals were from the reserved farm (Weinan city, Shaanxi Province, China). The Nanyang and Jiaxian animals were housed individually in the farms under strictly standardized conditions. The Qinchuan animals were born in 2006. The Nanyang animals were all of the same age and growth traits were measured every six months from six to twenty-four months old according to methods of Chen (1998). Growth traits measured in the Nanyang population were: birth weight, body weight (BW), hucklebone width (HBW), withers height (WH), heart girth (HG), body length (BL), body length index (BLI), heart girth index (HGI) and ratio of heart girth and body length (RHGBL). Only phenotypic data of Qinchuan animals aged four months and Jiaxian animals aged twenty-four months were available. Growth traits of the Qinchuan and Jiaxian animals measured were: WH, BL, HBW, HG, BLI, HGI, RHGBL, height at hip cross (HHC), rump length (RL), and hip width (HW).

DNA preparation and PCR amplification

Genomic DNA of 411 animals was isolated from 2% heparin-treated blood samples and stored at -80°C. following standard procedures (Sambrook et al., 2002). Using available sequence information from bovine *MSTN* (Genbank accession number AF348479) and bovine *MSTN* (Genbank accession number M95684). PCR primers were designed. PCR amplification was performed in 25 μl reactions containing 100 ng of genomic DNA. 1.5 mM MgCl₂. 200 μM each of the four dNTP, 5 pmol of each primer and 1 U of Taq DNA polymerase (TaKaRa, China) under the following conditions: one cycle 94°C 3 min; 39 three-step cycles 94°C 30 s, the appropriate temperature 40 s. and 72°C 1 min followed by a last extension for 10 min at 72°C. The primer sequences, position, PCR fragment sizes and the annealing temperatures are listed in Table 1.

PCR-RFLPs analysis

Enzyme digestion was carried out in a total volume of 20 µl reaction mixture using DraI, HaeIII, HinfI, HinfII, HinfII, HpaI, and TaqI according to product manuals. The products were analysed on ethidium bromide-stained 3% agarose gel and allele frequencies were estimated. Significant departure from Hardy-Weinberg equilibrium was tested by a x^2 test.

^{**} Expected size of the fragments after amplification according to the same sequences.

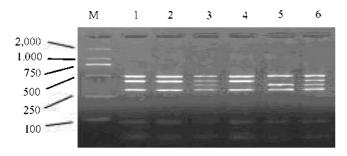


Figure 1. Agarose gel electrophoresis (3%) of PCR fragment of *MSTN* gene digested with *Dra*I. Genotype AA is in lanes 1, 2, 4, genotype AB is in lanes 3, 6, genotype BB is in lane 5. M is the D2000 marker.

Statistical analysis

Data of the breeds were analysed by ANOVA (SAS software GLM procedure) using the following model:

$$Y_{ijklmn} = \mu + Breed_i + Age_j + Marker_k + (Breed \times Marker)_1$$

$$+ (Breed \times Age)_m + (Marker \times Age)_n + e_{ijklmn}$$

to estimate the effects of markers on growth traits; where Y_{ijklmn} is the observation of the trait. μ is the least square mean. Breed, is the effect of breed. Age_j is the effect of age. Marker, is the effect of marker genotype, $(Breed \times Age)_m$ is the interaction of breed and marker, $(Breed \times Age)_m$ is the interaction of breed and age, $(Marker \times Age)_n$ is the interaction of marker and age and ϵ_{ijklm} is the residual effect.

RESULTS

Polymorphism/alleles frequencies

Two polymorphisms, MSTN-DraI and MYF5-TaqI were observed after products were digested with enzymes. The polymorphism of MSTN-DraI is caused by T/A transversion at position -371 (relative to ATG start codon) that introduces a site for DraI restriction enzyme (Crisa et al., 2003). Digestion of the PCR fragment of MSTN promoter with DraI resulted in fragment lengths of 505, 427, 321, and 93 bp for phenotype A4, 505, 365, 321, 93, and 62 bp for phenotype BB and 505, 427, 365, 321, 93 and 62 bp for phenotype AB (Figure 1). The frequency of allele A was dominant in all three Chinese cattle breeds. Only

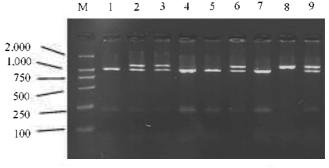


Figure 2. Agarose gel electrophoresis (3%) of PCR fragment of *MYF5* gene digested with *Taq*I. Genotype AA is in lane 8, genotype BB is in lanes 1, 4, 5, 7, genotype AB is in lanes 2, 3, 6, 9. M is the D2000 marker.

homozygous BB individuals were present in the Jiaxian population (Table 2). The genotype frequencies of *MSTN* in the Nanyang and Qinchuan populations agreed with Hardy-Weinberg equilibrium. whereas those in the Jiaxian population were at Hardy-Weinberg disequilibrium (p<0.05).

The MYF5-TaqI is a SNP (A/G) at the 1.948 bp position of intron 2 (Genbank accession number M95684) (Li et al., 2004). PCR-RFLP testing by digestion of a 1.190 bp amplicon with TaqI produced fragments of 1.190 bp in homozygous AA animals, 983 and 207 bp in homozygous BB animals, and 1.190, 983, 207 bp in heterozygous AB animals (Figure 2). The frequencies of the two alleles in the three breeds are given in Table 2. The frequency of allele B was dominant in the three breeds and individuals with AA genotype were less frequent than individuals with other genotypes. The genotype frequencies of MYF5 in the three populations all agreed with Hardy-Weinberg equilibrium.

Association of polymorphism with growth traits

No statistically significant differences at MSTN locus were observed between the AA and AB genotypes of the Jiaxian breed concerning growth traits (Table 4). However, there were statistically significant differences between the genotypes of the Nanyang breed for the following growth traits (p<0.05) (Table 3): WH at eighteen-months, AA was significantly higher than AB; HG, HGI and HGBLR at sixmonths. AA was significantly lower than AB. In the Qinchuan breed, the individuals with genotype AB at MSTN

Table 2. Allele and genotype frequencies at MSTN and MTF5 genes in three Chinese cattle breeds

| Breeds | Individuals | Genes - | Genotype frequencies | | | Allele frequencies | | Locus equilibrium x^2 test |
|----------|-------------|---------|----------------------|--------|--------|--------------------|--------|------------------------------|
| Diccus | | | .4.4 | .AB | BB | Ή. | В | Locus equinorium a test |
| Nanyang | 210 | MSTN | 0.9100 | 0.0900 | 0.0000 | 0.9550 | 0.0450 | Equilibrium (p>0.05) |
| | | MYF5 | 0.0400 | 0.2650 | 0.6950 | 0.1725 | 0.8275 | Equilibrium (p>0.05) |
| Qinchuan | 93 | MSTN | 0.9460 | 0.0540 | 0.0000 | 0.9730 | 0.0270 | Equilibrium (p>0.05) |
| | | MYF5 | 0.0430 | 0.3978 | 0.5592 | 0.2419 | 0.7581 | Equilibrium (p>0.05) |
| Jiaxian | 108 | MSTN | 0.9351 | 0.0556 | 0.0093 | 0.9629 | 0.0371 | Disequilibrium (p<0.05) |
| | | MYF5 | 0.0360 | 0.4234 | 0.5406 | 0.2477 | 0.7523 | Equilibrium (p>0.05) |

Table 3. Least squares means, and standard errors for the effect of MSTN and MIF5 genes on growth traits in Nanyang cattle

| Ασο | Growth traits | Genotypes at | t MSTN gene | Genotypes at MYF5 gene | | |
|--------------------------|------------------|-----------------|-----------------------------|------------------------|---------------|--|
| Ages | Growth traits | .4.4 | .4B | AВ | BB | |
| Birth | Body weight (kg) | 31.333±0.867 | 29.743±0.256 | 29.962±0.541 | 29.949±0.335 | |
| Six months | BW (kg) | 159.680±1.878 | 169.333±6.355 | 160.115±6.124 | 159.941±3.713 | |
| | HBW (cm) | 18.379±0.215 | 18.333±0.726 | 18.308±0.426 | 18.235±0.263 | |
| | WH (cm) | 106.117±0.499 | 107.444±1.687 | 106.577±0.900 | 105.897±0.556 | |
| | HG (cm) | 128.748°±0.838 | 134.444 ^b ±2.821 | 128.500±1.748 | 129.235±1.060 | |
| | BL (cm) | 105.544±0.740 | 108.556±2.504 | 105.654±1.540 | 105.618±0.934 | |
| | BLI | 0.995±0.005 | 1.011±0.017 | 0.991±0.010 | 0.997±0.006 | |
| | HGI | 1.214 °±0.006 | 1.252 b±0.019 | 1.206±0.011 | 1.221±0.007 | |
| | HGBLR | 1.221 °±0.005 | 1.238 ^b ±0.018 | 1.217±0.010 | 1.224±0.006 | |
| Twelve months | BW (kg) | 222.794±2.289 | 221.000±7.707 | 223.280±6.124 | 223.779±3.713 | |
| | HBW (cm) | 20.729±0.215 | 20.667±0.726 | 20.620±0.434 | 20.624±0.263 | |
| | WH (cm) | 114.088±0.501 | 114.333±1.687 | 114.600±0.917 | 113.941±0.556 | |
| | HG (cm) | 141.088±0.838 | 145.667±2.821 | 141.200±1.748 | 141.500±1.060 | |
| | BL (cm) | 116.892±0.740 | 119.556±2.504 | 116.640±1.540 | 117.485±0.934 | |
| | BLI | 1.024±0.005 | 1.045±0.017 | 1.017±0.010 | 1.031±0.006 | |
| | HGI | 1.237 °±0.006 | 1.275 b±0.019 | 1.232±0.012 | 1.242±0.007 | |
| | HGBLR | 1.209±0.005 | 1.221±0.018 | 1.211 ± 0.011 | 1.207±0.006 | |
| Eighteen months | BW (kg) | 297.373±3.129 | 297.000±10.532 | 292,760±6.124 | 301.706±3.713 | |
| | HBW (cm) | 23.240±0.215 | 22.500±0.726 | 22.900±0.434 | 23.184±0.263 | |
| | WH (cm) | 121.284°±0.501 | 120.778 ^b ±1.687 | 120.800±0.917 | 121.059±0.556 | |
| | HG (em) | 156.029±0.838 | 156.444±2.821 | 154.880±1.748 | 156.588±1.060 | |
| | BL (cm) | 129.461±0.740 | 129.444±2.504 | 129.000±1.540 | 129.735±0.934 | |
| | BLI | 1.069±0.005 | 1.072±0.017 | 1.068±0.010 | 1.071±0.006 | |
| | HGI | 1.288±0.006 | 1.295±0.019 | 1.282±0.012 | 1.294±0.007 | |
| | HGBLR | 1.206±0.005 | 1.210±0.018 | 1.201±0.011 | 1.208±0.006 | |
| Twenty-four months | BW (kg) | 366.951±4.034 | 365.333±13.581 | 360.240±6.124 | 373.044±3.713 | |
| | HBW (cm) | 25.407±0.215 | 24.778±0.726 | 25.020±0.434 | 25.353±0.263 | |
| | WH (cm) | 126.225±0.501 | 126.667±1.687 | 126.080±0.917 | 126.603±0.556 | |
| | HG (cm) | 168.642±0.838 | 172.000±2.821 | 167.520±1.748 | 169.390±1.060 | |
| | BL (cm) | 138.127±0.740 | 137.778±2.504 | 137.320±1.540 | 138.485±0.934 | |
| | BLI | 1.094±0.005 | 1.088±0.017 | 1.089±0.010 | 1.094±0.006 | |
| | HGI | 1.336 ± 0.006 | 1.358 ± 0.019 | 1.329±0.012 | 1.338±0.007 | |
| | HGBLR | 1.221 ± 0.005 | 1.250 ± 0.018 | 1.221±0.011 | 1.224±0.006 | |
| Average daily gains (kg) | | 0.462±0.007 | 0.458±0.024 | 0.452±0.011 | 0.470±0.007 | |

LSM in a column with no common superscripts differ significantly, low-case character represents significance at p<0.05, capital character represents significance at p<0.01.

locus had higher BLI than those with genotype AA (p<0.05). The animals with genotype AA at MYF5 locus were excluded when association analysis was carried out because the sample size for AA genotypes was too small.

No statistically significant differences in growth traits at MIF5 locus were observed between the AB and BB genotypes of the Nanyang and Jiaxian breeds. However, in the Qinchuan breed animals with BB genotype showed lower WH and HHC when compared with AB genotypes (p<0.05).

DISCUSSION

Myostatin acts as a negative regulator of skeletal muscle growth and keeps the skeletal musculature within

appropriate proportions (Lee and McPherron. 1999). Piedmontese crossbreds with an inactive MSTN allele have higher birth weights and yearling weights (Casas et al.. 1999). Investigations of myostatin developmental expression in bovine skeletal muscles (Shibata et al.. 2003) and its function in myogenesis and adipogenesis showed myostatin expression is related to animal growth (Lin et al.. 2002; Joulia et al., 2003; Rebbapragada et al.. 2003; Wagner et al.. 2005). Mutations in MSTN promoter could lead to changes of the gene expression and thereby influence cattle growth and development. A T/A transversion at -371 (relative to ATG start codon) has been identified in the promoter region of porcine MSTN (Strail and Kopecny, 1999). A study with pigs analyzed the relationship between this mutation and growth traits, showing that individuals

Table 4. Least squares means, and standard errors for the effect of MSTN and MTF5 genes on growth traits in Qinchuan and Jiaxian breeds

| Breeds | Traits | Genotypes a | t <i>MSTN</i> gene | Genotypes at MYF5 gene | | |
|-----------------------|----------|---------------|---------------------------|------------------------|----------------------------|--|
| Dieeus | | .A.H | AB | .4B | BB | |
| Qinchuan | WH (cm) | 93.113±0.633 | 89.000±2.922 | 94.200°±0.900 | 92.553 b±0.744 | |
| (Four months) | HHC (em) | 97.266±0.648 | 95.833±2.995 | 99.096°±0.994 | 96.211 ^b ±0.882 | |
| | BL (cm) | 93.571±0.939 | 95.333±4.336 | 94.346±1.511 | 93.395±1.249 | |
| | HBW (cm) | 9.439±0.272 | 8.333±1.257 | 9.704±0.426 | 9.268±0.352 | |
| | RL (cm) | 31.774±0.331 | 33.000±1.529 | 32.404±0.516 | 31.514±0.426 | |
| | HG (cm) | 112.042±1.058 | 112.833±4.885 | 113.046±1.714 | 111.566±1.418 | |
| | HW (cm) | 25.206±0.409 | 24.667±1.887 | 25.785±0.640 | 24.929±0.530 | |
| | BLI | 1.005°±0.006 | 1.071 ^b ±0.029 | 1.001±0.010 | 1.009±0.008 | |
| | HGI | 1.203±0.007 | 1.268±0.034 | 1.200±0.011 | 1.206±0.009 | |
| | HGBLR | 1.200±0.007 | 1.185±0.031 | 0.836±0.010 | 0.837±0.009 | |
| Jiaxian | WH (cm) | 125,463±0.501 | 124.200±2.263 | 124.740±0.662 | 125.813±0.630 | |
| (Twenty- four months) | HHC (em) | 124.132±0.514 | 122.400±2.320 | 123.969±0.732 | 124.019±0.696 | |
| | BL (cm) | 154.100±0.744 | 151.200±3.359 | 152.740±1.112 | 155.011±1.058 | |
| | HBW (cm) | 23.196±0.216 | 22.600±0.974 | 22.896±0.313 | 22.887±0.298 | |
| | RL (cm) | 47.574±0.262 | 47.200±1.185 | 47.354±0.379 | 47.736±0.361 | |
| | HG (cm) | 178.424±0.838 | 174.800±3.784 | 177.375±1.262 | 178.792±1.201 | |
| | HW (cm) | 44.191±0.324 | 43.400±1.462 | 43.906±0.471 | 44.170±0.448 | |
| | BLI | 1.229±0.005 | 1.219±0.022 | 1.225±0.007 | 1.233±0.007 | |
| | HGI | 1.422±0.006 | 1.409±0.026 | 1.422±0.008 | 1.421±0.008 | |
| | HGBLR | 1.161±0.005 | 1.157±0.024 | 1.163±0.008 | 1.157±0.007 | |

LSM in a column with no common superscripts differ significantly, low-case character represents significance at p<0.05.

with AB genotype had a higher average daily gain than those with A4 genotype (Jiang et al., 2002). Crisa et al. (2003) found no statistically significant differences with the mutation among genotypes of nine cattle breeds, but a significant difference between individuals bearing the combination mh/+ at the third exon and A4 at the DraI site, versus \pm/\pm at exon 3 and AB or BB at the DraI site, with an index about 30% higher in the former group. In the present study, the mutation had significant effects on WH, HG HGI and HGBLR (p<0.05) and the traits affected significantly were different at different growth stages, which indicated that the selected traits and the growth stages should be comprehensively considered in the breeding practice for Nanyang cattle. The mutation is located within two transcriptional factor-binding motifs and could affect the binding of these factors. The allele B (nucleotide A at the position) at the DraI site generates a Pit1 and a WAP-US6 binding motif at -372 and at -374 respectively (Crisas et al., 2003). These results indicate a regulatory role for the promoter region in myostatin expression. In the published bovine sequence (Genbank accession number AF 348479), a C base is found at position -360 (relative to start codon). whereas Crisas et al. (2003) detected an A base at that position. There may be a polymorphism in bovine MSTN promoter which can be detected by PCR-RFLP with TaqL This study confirmed the presence of nucleotide A at -360(relative to start codon) after digestion of the MSTN promoter fragment with TaqI. Therefore no polymorphism was present in the PCR fragment after digestion with TaqI

in this study and we suspect an occurrence of sequencing error in the published bovine sequence (Genbank accession number AF 348479). The result is contrary to the study by Min (2005) on goats and may contribute to the difference between species.

Knowledge of the possible mutations at the promoter region of MSTN gene helps elucidate the regulatory mechanisms of the gene in mammalian species. The observations have economic implications because the finding could lead to the development of selection plans that can be useful both when the double muscling trait is considered advantageous (breeding in controlled conditions), and when the trait should be avoided (range breeding). Since myostatin plays a role in breeds not carrying double muscling, the mutations occurring at the promoter region can be useful also to fine tune selection schemes aimed to obtain a desired grade of muscularity.

The MYF5 gene has been considered to play an important role in growth and development of mammals. Li et al. (2002a, b) fine-mapped QTL for birth weight, preweaning average daily gain and average daily gain on feed on bovine chromosome 5. Three chromosomal regions (0 to 30 cM, 55 to 70 cM and 70 to 80 cM) of chromosome 5 were identified as having significant associations with growth traits. MYF5 gene was considered to be a positional candidate gene underlying one of the chromosomal regions (0 to 30 cM) as it was mapped at the chromosomal location of 19.0 cM (Grosse et al., 1999). Extensive investigations led to the detection of several polymorphisms in MYF5

gene in swine and cattle. However, association analyses mostly revealed contradictory results in different lines and breeds or failed to show any relationship to production traits (Strail and Cepica, 1999; Te Pas et al., 1999; Drogemuller and Kempers, 2000; Cieslak et al., 2002; Li et al., 2002; Urbanski and Kuryl, 2004; Klosowska et al., 2004; Fausto et al., 2005). In this study, the association between the SNP of MIF5 and birth weight of Nanyang cattle did not reach a level of significance, which is consistent with the results of Li et al. (2004). The association between the SNP of MYF5 and the average daily gain of Nanyang also did not reach a significant level, whereas Li et al. (2004) and Chung et al. (2005) showed that the SNP had a significant effect on the average daily gain of cattle. In the Qinchuan population, only association between the SNP of MYF5 and WH and HHC reached a level of significance (p<0.05). In short, results of extensive investigations suggest that MYF5 gene may be one of the causative genes that control growth traits in beef cattle or that the gene is very close to the causative genes. Being located in one of the intron regions, the SNP studied here may not be a causal mutation. Thus, one may suggest that it is linked to another mutation in the coding or regulatory regions of the gene which is a causal mutation for the growth traits. However, introns have been shown to affect transcriptional efficiency of numerous genes in a variety of organisms (Greenwood and Kelsoe, 2003; LeHir et al., 2003). An association study using more SNPs of MIF5, including SNPs in the promoter and extronic regions. and further functional tests of candidate genes may lead to the identification of causative mutations in the genes that control growth traits in beef cattle. These studies will be of practical importance for the improvement of Chinese native cattle and the breeding of genuine beef cattle in China.

ACKNOWLEDGMENTS

This study was supported by grants from the National 863 Program of China (No.2006AA10Z197), National Natural Science Foundation of China (No.30471238), National Key Technology R&D Program (No. 2006BAD01A10-5). Innovative Foundation of Outstanding Talent from Henan Government (No.0521001900). Sustaining Program for Topnotch Persons of Northwest A&F University (No. 01140101) and and Natural Science Foundation of Xuzhou Normal University (XY200234).

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