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MSTN Gene Polymorphism and its Relationships with Growth Performance in Ma Shen Porcine

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Abstract: Myostatin gene (*MSTN*) is a negative regulator of myogenesis which is related to total lean meat yield. A total of 339 individuals of three breeds were used to analyzed polymorphisms by the Polymerase Chain Reacton Single-Strand Conformation Polymorphism (PCR-SSCP) method in the 5'-promoter region of MSTN. This study analyzed the relationship of mutation with the growth traits including Weight at Birth (WB) and Weaning Weight (WW) in Ma Shen swine breeds. Two alleles (A and B) and three genotypes (AA, AB and BB) were identified in MSTN promoter region of Ma Shen swine. The result showed that WB of individuals with genotype BB was significant higher than that with genotype AA and AB ($p<0.05$). No statistically significant differences were observed in WW ($p>0.05$) among MSTN variation.

Key words: Ma Shen swine, 5'-regulator region of the Myostatin gene, mutation, growth traits, genotype

INTRODUCTION

Myostatin (also known as growth and differentiation factor 8, GDF8) is a secreted TGF-β protein super-family member which is encoded by Myostatin gene (*MSTN*) (Hickford *et al*., 2010). Alexandra McPherron and Se-Jin Lee who discovered the gene encoding myostatin also produced a strain of mutant mice that lack this gene. These mice with MSTN knockout had approximately twice as much muscle as normal mice (McPherron *et al*., 1997). Researches suggested that loss of function mutations in the MSTN are related to increased skeletal muscle yield in sheep (Clop *et al*., 2006; Masri *et al*., 2011), cattle (Marchitelli *et al*., 2003) and human (Schuelke *et al*., 2004). The MSTN is highly polymorphic. The high level of polymorphism already described for MSTN in cattle (Miranda *et al*., 2002), chicken (Ye *et al*., 2007) and human.

Belgian Blue cattle is known to have an increase in muscle mass compared with conventional cattle. Grobet reported that an 11 nucleotide deletion existed in third exon of MSTN resulting more lean meat yield (Grobet *et al*., 1997). Shaoquan described the expression of MSTN at birth was higher in longissimus muscle of low-birth-weight than normal in swine, irrespective of gender (Ji *et al*., 1998). A polymorphism concerning two nucleotide changes existed in the 5' regulatory region of MSTN in porcine. Allele B with frequency of 0.475 has the positive effect on body weight on day 21st in Yorshire pigs (Yu *et al*., 2007). These results illustrated that MSTN may be a gene related to lean meat yield in swine.

In this study, we used Polymerase Chain Reaction Single-Stranded Conformational Polymorphism (PCR-SSCP) analysis to investigate allelic variation of *MSTN* gene in 5'-regulatory region and estimated the effects of the mutations on the performance of WB and BB of three swine breeds.

MATERIALS AND METHODS

Animals: Ear tissue samples of 245 Ma Shen swines, 49 SD-III of Shanxi Lean swines and 45 Duroc were collected randomly from Changzhi farm and Datong pig farm in Shanxi province.

PCR-SSCP: Genomic DNA was extracted from ear tissue samples by the phenol-chloroform alcohol extraction protocol. Using oligo 6.0 program, pair of primers was designed according to swine myostatin gene sequence for forward: 5'-AAGTGCAGTTT-ATATTATTG-3', reverse: 5'-AACAGTA-TTTTG-TTACAGT-3'. PCR amplification was performed in a reaction volume of 25 μL including 2 μL of genomic DNA, 0.8 pm of each primer, 0.2 mM d NTP, 2.5 μL 10×buffer, 0.4 μL Taq DNA polymerase (5 U L^{-1}) and 14.8 μL dd H_2O. The PCR condition was 95°C for 2.5 min,

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followed by 30 cycles of 95°C for 40 sec, annealing for 40 sec and 72°C for 1 min with a final extension at 72°C for 7 min. SSCP method was used to scan mutations within the amplified regions.

About 5 μL of PCR product were mixed with 5 μL denaturing buffer (95% formamide, deionized, 25 mM EDTA, 0.025% xylene-cyanole and bromophenol blue) and then denatured at 98°C for 10 min, chilled in ice immediately for 10 min. The denatured PCR products were electrophoresed on 10% PAGE (Polyacrylamid Gel Electrophoresis) in 1×TE buffer and constant voltage for 18 h at 4°C and then gels were stained by 0.2% $AgNO_3$ for 20 min and then 3% Na_2CO_3 for about 5 min (Qu *et al*., 2005). After polymorphism was detected, genotypes were recorded according to band patterns.

Association analysis: Associations between genotypes and early growth traits including weight at birth and weaning weight were performed with GLM procedure of SPSS 10.0 Software. The significant of differences among genotype effects of each SNP on Estimated Breeding Values (EBV) for WB and WW were calculated by Duncan's multiple-range test. The model used was as follows:

$$Y_{ij} = \mu + B_i + \alpha_j + e_{ij}$$

Where:

Y_{ij} = EBV for each early growth traits (WB and WW)
μ = Overall means
B_i = Sire effect
α_j = Genotype effect
e_{ij} = Random residual effect

RESULTS AND DISCUSSION

Identification of SNP within MSTN: On Single Nucleotide Polymorphism (SNP) was identified (Fig. 1) in which three patterns (named as genotypes AA, BB and AB, respectively) were observed representing 2 MSTN alleles (designed A and B). As shown in Table 1, allele and genotype frequencies were identified. Gene *A* was superior at swine *MSTN* gene of three swine breeds. The frequency for allele A (0.656) of Ma Shen swine was lower than that of Duroc and SD-III of Shanxi Lean swine (0.944 and 0.847). Three genotypes (AA, AB, BB) could found in all three breeds. The frequency of AA was higher than that of AB and BB of three swine breeds. The frequencies of AA were 0.933, 0.816 and 0.552 in Duroc, SD-III of Shanxi Lean swine and Ma Shen swine, respectively. The genotype AA occurred at a frequency 0.552 and genotype BB and AB at almost same (0.241 and 0.207) frequency of Ma Shen swine.

Association between MSTN and early growth traits: Associations were tested of MSTN for two early growth traits, Weight at Birth (WB) and Weaning Weight (WW) on 245 Ma Shen swines. Means of WB and WW are shown in Table 2. Very significant difference was observed between BB and AA ($p = 0.005<0.01$) and significant difference between BB and AB ($p = 0.046<0.05$). Means: AA: 1.34050.2107 (N = 178), BB: 0.4389±0.2184 (N = 46); AB: 1.3286±0.1471 (N = 21). Individuals with genotype BB have the significant higher WB than that with genotype AA and AB. No significant associations with WW were observed for this SNP ($p>0.05$). Means: AA: 8.1503±3.0664 (N = 178); BB: 8.1043±1.9094 (N = 46; AB: 7.3238±1.3030 (N = 21). AA>BB>AB ($p = 0.433>0.05$).

Associations of MSTN with weight at birth, weaning and 1 year of age were reported by Casa. The Piedmontese cattle with mutation at MSTN had heavier birth weight (Casas *et al*., 1999). McPherron reported that MSTN knockout mice had a widespread increase in skeletal muscle mass (McPherron *et al*., 1997). Particular breed of pigs also shows a heavily-muscled phenotype. This similarity of phenotypes between doubled-muscled cattle and pigs revealed that MSTN was a candidate gene for muscular hypertrophy in swine (Stinckens *et al*., 2006). The data indicated that MSTN greater expression was associated with low weight at birth in swine

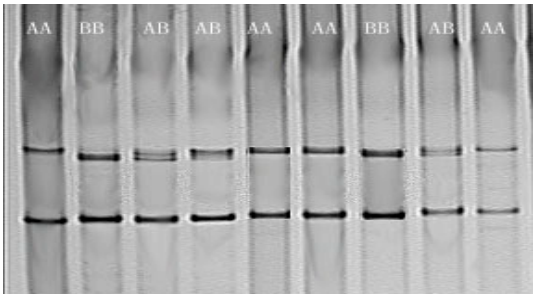

Fig. 1: PCR-SSCP result of MSTN. Lanes 1, 5, 6, 9: genotype AA; Lanes 2, 7: genotype BB and Lanes 3, 4, 8: genotype AB

Table 1: Genotype and allele frequencies of MSTN in three different pig breeds

		Genotype			Allele		
Breeds	Number	AA	BB	AB	A	B	Hardy-weinberg
Duroc	45	0.933 (42)	0.044 (2)	0.022 (1)	0.944	0.055	15.412
SD-III of Shanxi Lean swine	49	0.816 (40)	0.122 (6)	0.061 (3)	0.847	0.153	23.849
Ma Shen swine	245	0.552 (136)	0.241 (59)	0.207 (50)	0.656	0.344	15.257

Table 2: Least square meaning value for WB and WW

	Genotypes			
Traits	AA (n = 178)	AB (n = 21)	BB (n = 46)	Sig.
WB	1.3405±0.2107	1.3286±0.1471	1.4389±0.2184	0.015
WW	8.1503±3.0664	7.3238±1.3030	8.1043±1.9094	0.433

WB: Weight at Birth, WW: Weaning Weight

(Ji *et al*., 1998). Study of Jiang *et al*. (2002) has shown that mutation in promoter region of porcine MSTN was associated with average daily gain. All these results showed that mutation in promoter region of swine MSTN had association with growth traits. As the described polymorphisms in the promoter region for the MSTN regulated gene expression in porcine (Stinckens *et al*., 2005). Despite the importance of MSTN in skeletal muscle development, little is known about its role in the myogenic growth and growth traits in swine.

In eukaryotic cells, transcription occurs with the regulation of two types of regulatory regions promoters and enhancers which was name transcription signal. Promoters with TATA-box, CAAT-box and core promoter are regions of DNA that promote transcription. Expression of MSTN was regulated by Myogenic Regulator Factors (MRFs) and Myocyte Enhancer Factor 2 (MEF2). These studies revealed that 5'-promotor region was important regulatory region to MSTN expression of swine.

The test showed significant ($p<0.01$) deviation from Hardy-Weinberg equilibrium for both loci of Ma Shen and SD-III of Shanxi Lean swine. It may be the result of random genetic drift, selection and assortative mating. Genotype frequency in Duroc also deviated from Hardy-Weinberg equilibrium which was caused by acclimatization and artificial selection. This result was similar to the previous study (Jiang *et al*., 2002).

In addition, studies were performed based on effect on growth traits, WB and WW of MSTN. Despite the genetic effect, MSTN had the direct effect on WB. The result demonstrated that significant association was found between the MSTN genotype and weight at birth ($p = 0.015<0.05$). Very significant differences were found between genotype AA and genotype BB ($p = 0.005<0.01$). Significant differences were detected between genotype AB and genotype BB ($p = 0.046<0.05$). No significant association between the SNP and WW in Ma Shen swine ($p>0.05$). WB is a very important trait in pig breeding and production. The preweaning gain was positively related to lower WB (Jiang *et al*., 2002). Significant association was observed between MSTN variation and WB, BB>AA>AB which was similar to the result of Ji *et al*. (1998).

CONCLUSION

Results from this study suggest that there is one SNP in 5'-promoter region of MSTN and its effect on early growth trait of Ma Shen swine which could promote the breeding of Ma Shen swine in Shanxi province. These studies will also be of practical importance for the improvement of Chinese native swine and its breeding in China.

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