

## Associations of T→A Mutation in the Promoter Region of Myostatin Gene with Birth Weight in Yorkshire Pigs\*\*

Y-L Jiang<sup>1,2</sup>, N Li\* , X-Z Fan<sup>2</sup>, L-R Xiao<sup>3</sup>, R-L Xiang<sup>3</sup>, X-X Hu<sup>1</sup>, L-X Du<sup>2</sup> and C-X Wu<sup>1</sup>

National Laboratories for Agrobiotechnology, China Agricultural University, Beijing 100094, P.R. China

**ABSTRACT :** A T→A mutation in the promoter region of porcine myostatin (MSTN) gene has been identified in our previous work. This study analyzed the associations of the myostatin genotypes (TT, TA) caused by this mutation with birth weight in Yorkshire pigs. Data from 211 unrelated individuals were collected three times from one breeding farm. Detections of the mutation were carried out by PCR-RFLPs approach. The effects of MSTN genotypes (TT and TA) on birth weight were compared by least square means. The results showed that for birth weight of Yorkshire pigs, individuals with TA genotype were significantly higher than those with TT genotype ( $p < 0.05$ ), and the birth weight for pigs with TA genotype were 1.37 kg in average but only 1.25 kg for pigs with TT genotype, indicating a positive effect of birth weight for *A* allele. (*Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 11 : 1543-1545*)

**Key Words :** Pig, Myostatin Gene, Mutation, Birth Weight

### INTRODUCTION

In pigs, birth weight (BW) plays an important role in breeding and production. For pigs with higher BW, the preweaning mortality decreased, while the preweaning growth and the early postweaning growth increased (Roehle, 1999). Like other quantitative traits, BW is controlled by polygene, the expression of which is affected by environmental factors. Undernutrition in utero causes low BW (Dwyer et al., 1994). Several studies have been carried out on the genetic mechanisms of BW in pigs. A QTL influencing BW was detected on porcine Chr 4 (Walling et al., 2000), Chr 1 (Wada et al., 2000; Sarker et al., 2001) or both (Malek et al., 2001). By candidate approach, studies had shown that the Msp I CC genotype in PIT1 gene were associated with heavier BW (Yu et al., 1995) and that significant differences existed between the two homozygous *myogenin* genotypes for BW (te Pas et al., 1999a). However, no associations were found of growth hormone 2 genotypes (Wang et al., 2002) or the MYF-5 gene (te Pas et al., 1999b) with BW in pigs.

Myostatin is a negative regulator of muscle mass (McPherron et al., 1997). In cattle, deletion or mutations in

exon 3 causes muscular hypertrophy (McPherron and Lee, 1997; Grobet et al., 1997; Kambadur et al., 1997; Cappuccio et al., 1998) and higher BW (Casas et al., 1999). In the previous studies, we identified three SNPs in porcine myostatin gene (MSTN) (Jiang et al., 2001) and found that the T→A mutation in promoter region of porcine MSTN was associated with average daily gain from 60 kg to 100 kg (Jiang et al., in press). This study analyzed the relationship between the mutation and the BW in Yorkshire pigs, and the positive effects of the mutation on the BW was found.

### MATERIALS AND METHODS

#### Materials

Samples of Yorkshire pig ear notch were collected from Jining Pig Breeding Farm of Shandong Province and stored in 70% ethanol at -20°C. EDTA, xylene cyanol FF, bromophenol blue, and agarose were from Sino-America Biotechnology Ltd. Ethanol, AgNO<sub>3</sub> and citrate sodium were from Beijing Chemical Reagent Company. Proteinase K were from Merck Co Ltd of Germany and dNTPs from Gibco BRL Co Ltd. *Taq* polymerase and *Dra* I were from TaKaRa Co Ltd (Dalian).

#### DNA extraction

Pig genomic DNA was extracted from ear notch by the high salt method according to Jiang, et al. (1997) and dissolved in TE solution at -20°C.

#### PCR-RFLP detection of T→A mutation

PCR-RFLP detections of T→A mutation in the promoter region of porcine myostatin (MSTN) gene were carried out according to Jiang et al. (2001).

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\* Corresponding Author: N. Li. Tel: +86-10-6289-3323, Fax: +86-10-6289-3904, E-mail: ninglbau@public3.bta.net.cn

<sup>1</sup> College of Animal Science and Technology, China Agricultural University, Beijing 100094, P.R. China

<sup>2</sup> Lab of Animal Biotechnology, Shandong Agricultural University, Taian 271018, P.R. China

<sup>3</sup> Jining Pig Breeding Farm of Shandong Province, Jining 272153, P.R. China

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### Statistical analysis

For each pig, the BW (kg), the litter size from which it comes, sex, birth-year-season and the genotype were recorded. By establishing a fixed effects model in which the effect of birth-year-season, litter size and sex were included, the relationships between the genotypes of MSTN gene and the porcine BW were analyzed. The model used was as follows:

$$Y_{ijklm} = \mu + YS_i + L_j + S_k + \alpha_l + e_{ijklm}$$

where  $Y_{ijklm}$  is the birth weight (BW) (kg) of the  $m^{\text{th}}$  individual;  $\mu$  is the least square means of the trait;  $YS_i$  is the effect of the  $i^{\text{th}}$  birth-year-season ( $i=1, 2, 3$  that the pig was born;  $L_j$  is the effect of the  $j^{\text{th}}$  litter size ( $j=1, 2, 3$  represent  $<10, 10-13$  and  $>13$  respectively) that the pig was from;  $S_k$  is the effect of the  $k^{\text{th}}$  sex ( $k=1, 2$ ) of each animal;  $\alpha_l$  is the effect of the  $l^{\text{th}}$  genotype ( $l=1, 2$  represent genotypes of TT and TA); and  $e_{ijklm}$  is the random residual effect. Difference in BW exhibited by the genotypes was compared by least square means. Statistical analysis was carried out by GLM procedure of SAS software (1998).

## RESULTS

### Genotype frequency and gene frequency

Altogether 211 animals were genotyped for the T→A mutation in the promoter region of porcine myostatin (MSTN) gene in Yorkshire pig population, of which 195 were TT and 16 were TA. No AA was found for all the Yorkshire pigs genotyped. The genotype frequencies of TT, TA and AA were 0.9242, 0.0758 and 0, and the gene frequencies of alleles *T* and *A* were 0.9621 and 0.0379 respectively. The results showed that in Yorkshire pigs both genotype AA and allele A were very rare.

### Effects of TA genotype on BW

The statistical results (Table 1) showed that in Yorkshire pigs litter size, genotype and birth-year-season were the three factors affecting Birth weight (BW). As for the mutation, the BW of individuals with TA genotype is significantly bigger than those with TT genotype ( $p<0.05$ ) (Table 1), indicating a positive effect of A allele. The least square means of BW of genotypes TA and TT were  $1.37\pm 0.06$  kg ( $n=16$ ) and  $1.25\pm 0.02$  kg ( $n=195$ ) respectively.

**Table 1.** Effects of environmental factors and MSTN genotype on BW in Yorkshire Pigs

Variable	DF	F-value	P-value
Genotype	1	4.16	0.0427*
Litter size	2	15.45	0.0001**
Sex	1	0.73	0.3930 <sup>NS</sup>
Birth-Year-season	2	3.60	0.0291*

\*  $p<0.05$ , \*\*  $p<0.01$ , NS: Not significant.

The BW of A allele carrier pigs increased by 0.12 kg in average. The result indicated that the T→A mutation in the promoter region of porcine MSTN gene produced positive effect for BW.

Litter size produced highly significant effect ( $p<0.01$ ). The BW of each pig decreased as litter size increased. The least square means of BW were  $1.41\pm 0.04$  kg ( $n=46$ ),  $1.34\pm 0.03$  kg ( $n=102$ ) and  $1.20\pm 0.04$  kg ( $n=63$ ) for litter sizes of  $<10, 10-13$  and  $>13$  respectively. Birth-year-season was the other important environmental factor significantly affecting BW ( $p<0.05$ ). The least square means of BW of samples collected on Oct 2000, Jan 2002 and March 2002 were  $1.26\pm 0.03$  kg ( $n=136$ ),  $1.33\pm 0.05$  kg ( $n=47$ ) and  $1.36\pm 0.04$  kg ( $n=28$ ) respectively. The BW of pigs born in 2000 was significantly lower than that in 2002 ( $p<0.05$ ), which was caused by differences in temperature, nutrition and management.

Of all the factors analyzed, sex did not significantly affect BW of Yorkshire pigs. The least square means were  $1.31\pm 0.04$  kg (male,  $n=114$ ) and  $1.32\pm 0.03$  kg (female,  $n=97$ ) respectively.

In conclusion, environmental factors such as litter size and birth-year-season were the causes in BW variations of Yorkshire pigs. The T→A mutation in the promoter region of porcine MSTN gene produced positive effect for BW of Yorkshire pigs.

## DISCUSSION

BW is a very important trait in pig breeding and production, the preweaning gain was positively associated with lower BW. Therefore, it is very important and feasible to increase pig production by increasing the BW of piglets. As mutations in the conserved functional region of MSTN gene cause double muscling and higher BW in cattle (McPherron and Lee, 1997; Kambadur et al., 1997; Cappuccio et al., 1998; Casas et al., 1999), and a higher expression existed for pigs with lower BW than those with higher BW (Ji et al., 1998), we speculate it may play similar role in pigs. Previous study has shown that the T→A mutation in promoter region of porcine MSTN was associated with average daily gain from 60 kg to 100 kg (Jiang et al., in press). The BW trait may be as well associated with this mutation.

Of all the mutations identified in pigs, no one caused changes of amino acid (Jiang et al., 2002, in press). The T→A mutation was located in the region 383bp upstream of translation initiation site, possibly affecting the regulation of porcine MSTN expression. The statistical results showed that piglets of A allele carrier were significantly higher than those of TT genotype ( $p<0.05$ ), indicating a positive effect caused by A allele. The explanation might be that when the

mutation occurred, the binding site or conformation of the helix may have changed, affecting the affinity of upstream binding factors.

The results also showed that the BW was affected by birth-year-season and litter size. This is reasonably true. Changes in temperature, feed nutrition and management and maybe diseases will undoubtedly affect the conditions of sow and its offspring. As for the impact caused by litter size, the effect is more obviously. The BW of each piglet is negatively associated with the size. For a certain sow, the capacity of its uterus is relatively fixed. When more piglets were born, the BW of each one would decrease.

In this study genotype AA was not detected, showing that AA is rare in Yorkshire pigs. This result is similar to our previous study (Jiang et al., 2001). In such conditions, the least square means of genotype AA can not be estimated, which also affecting the accuracy of estimating the effect of A allele. Therefore the results require further investigation. By establishing several pedigrees of TA×TA mating, three genotypes of TT, TA and AA can be obtained. The effect produced by T→A mutation on BW could be accurately estimated. In conclusion our preliminary findings suggest the BW of piglets harbouring A allele were higher than that of those piglets harbouring B allele.

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