Polymorphism of Insulin-like Growth Factor-I Gene in 13 Pig Breeds and its Relationship with Pig Growth and Carcass Traits

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ABSTRACT : The polymorphism of insulin-like growth factor-I (IGF-I) in 13 pig breeds (total n=559) was detected by PCR-*Hha* I–RFLP, and allele A (151 bp and 28 bp) or allele B (116 bp, 35 bp and 28 bp) were observed. In these pig breeds, it was found that European pig breeds carried high frequencies of allele B, while Chinese native pig breeds carried high frequencies of allele A. Meanwhile the role of porcine IGF-I was investigated in 117 Nanchang White pigs and 360 Large Yorkshire pigs. Eight traits about growth and carcass were recorded for analyzing the associations between IGF-I gene polymorphism and performance quantitative traits. In the Nanchang White pigs, those with AA genotype generally had higher birth weight than those with AB genotype (p<0.05), but all these genotypes had no significant effect on the other traits which had been analyzed. In Large Yorkshire pigs, those with BB genotype had higher 2 months and 6 months body weight than those with AA genotype (p<0.05), and had a thicker hind-back-fat thickness and mid-back-thickness than those with AB and BB genotypes (p<0.05). And those with BB genotype were the thinnest in Large Yorkshire. Furthermore, pigs with AA genotype had a lower lean percentage than those with AB and BB genotypes (p<0.01), and the lean percentage of those with BB genotype was the highest. Based on these results, it is possible to make the IGF-Ⅰgene locus into the application of marker-assisted selection programmes. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 10 : 1391-1394)***

Key Words : Insulin-like Growth Factor-I Gene, Performance Traits, Pig, PCR-RFLP

INTRODUCTION

Growth rate and body composition are two important characteristics in livestock production. Swine production demands high growth rate and high lean percentage, together with efficient conversation of feed to meat. Pig performance traits have been successfully improved by using traditional pig selection systems; but the contribution of individual genes involving in these processes is still known little, which hinder the livestock genetics improvement.

Important economic traits are usually controlled by many genes and modified by environment factors, such as temperature, nutrition. Insulin-like growth factor-I is the candidate gene playing a role in the growth and body composition, and had been mapped on pig chromosome 5 (Winterφ et al., 1994). IGF-I is the mediator of GH (Growth Hormone), and one of its functions are to control muscle growth. The effects of IGF-I gene variants on performance traits had been described for chicken (Nagaraja, et al., 2000; Kita, et al., 2000) and mouse (Collins et al., 1993; Horvat et al., 1995). In pigs, associations between variants at the IGF-I locus and performance traits require further investigation.

In this study we investigated the polymorphism of IGF-I in 13 pig breeds and the effects of one IGF-I restriction fragment length polymorphism (RFLP) described by Fang et al. (1999) on performance traits in Nanchang White pigs and Large Yorkshire pigs.

MATERIALS AND METHODS

Animals

The ear notches of 13 pig breeds were collected, including 3 European breeds, 10 Chinese native breeds. They were Landrace (n=65, Dongxiang County Pig Farm and JAU Pig Breeding), Duroc (n=52, Dongxiang County Hongxing Pig Farm and JAU Pig Breeding), Pietrain (n=11, Jiangxi Provincial Pig Farm), Jinhua two-end Black (n=60, Jinghua county Pig Farm), Jiaxin Spotted (n=58, Jiaxing county Pig Farm), Shengxian Spotted (n=57), Leping Spotted (n=48, Leping county Pig Farm), Yushan Black (n=66, Yushan county Pig Farm), Erhualian (n=34, JAU experimental Pig Farm), Zixi Black (n=9, Zixi county Pig Farm), Xinzhi Black (n=12, Xinzhi county Pig Farm),Wild Pig (n=30, Jiaxin Pig Farm), Shangao Two-end Black (n=57, Shanggao county Pig Farm). All of the collected pigs were unrelated within 3 generations. In order to detect the effects of different IGF-I genotypes on some production performances, we also developed two pure pig breeds. Nanchang White pigs were half sibs, one male was mated fifteen females to produce 117 offsprings. For Large Yorkshire pigs, one Large Yorkshire male was mated with 10 Large Yorkshire females, and two F1 males and 36 F1

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females were subsequently used to produce 360 offsprings.

Large Yorkshire pigs were bred at Jiangxi Provincial Pig Farm and Nanchang White pigs were bred at Jiangxi Nanchang White Pig Breeding Farm. At ten days pigs were marked permanently by ear notching and this tissue was retained for DNA test. At ten weeks of age, the piglets were taken into single pens. The fattening test started at or from the 120 days old. The two pig breeds were fed with a cereal diet *at libitum* containing 19%, 16%, and 13% crude protein in the nursery, grower and finisher stages, respectively, and the weights of animals were recorded weekly. A total of eight traits were recorded at an average 210 days of age. The carcass composition traits were determined by PIGLOG105 (Denmark, 1996).

IGF-I genotypes defined by PCR-RFLP

Ear notches from offspring were frozen and DNA was extracted using a phenol/chloroform extraction method followed by ethanol precipitation (Strauss, 1991). Working dilutions of extracted DNA were prepared for each individual at a concentration of 50 ng/μl. Primers 5' AGCC CACAGGGTACGGCTC 3' and 5' CTTCTGAGCCTTG GGCATGTC 3' were used for PCR (polymerase chain reaction) amplification of insulin-like growth factor-I gene (Fang et al., 1999). The PCR mixture containing 50 ng genomic DNA, 25 pmol of each primer, 25 μM of each dNTP,1 unit of Taq DNA Polymerase and 1×reaction buffer in a 25 μl reaction volume. PCR were processed on PE9600 (PERKIN ELMER) according to the procedure: first 95°C for 300s then 35 cycles: 94°C for 45s, 59°C for 45s, 72°C for 60s, at last 72°C for 480s. The 179 bp PCR products were subsequently digested by *Hha* Ⅰ and revealed allele A (fragments of 151 bp and 28 bp) or allele B (fragments of 116 bp, 35 bp and 28 bp).The restriction digests were separated using 3.0% agarose gel in 1×TAE at a constant current of 50 mA. The gels were stained with ethidium bromide and the fragments were visualized using a UV transilluminator.

Statistics

Associations between IGF-Ⅰgenotypes and performance traits were analyzed using SAS system (1989), significance between genotypes was assessed by Student's *t*-tests.

RESULTS

The genotype frequencies and allele frequencies of IGF-Ⅰ in 13 pig breeds was listed in Table 1.

From Table 1, the AA genotype frequency in European pig breeds was low, especially in Pietrain, its frequency was 0%. While in Chinese native pig breeds, AA genotype frequency was high, and Erhualian was the highest (73.5%). Meanwhile, the B frequency in European pig breeds was high, especially in Pietrain, its frequency was 100%. And in Chinese native pig breeds the A frequency was high (above 50%), especially the A frequency of Taihu pigs was 86.8%. Also in Table 1 we found that the genotype frequency and allele frequency of Wild Pig were similar to European pig breeds.

Table 2 listed the observed genotypes and their frequencies. The genotype AA in two pig breeds was rare. AA frequency was 5.98% in Nanchang White and 5.83% in Large Yorkshire.

In Nanchang White pigs, birth weight was significantly associated with IGF-I genotypes. The pigs with AA genotype had higher birth weight than the ones with AB genotype ($p<0.05$). But no significant difference was observed among three genotypes for 2 months body weight, 4 months body weight and 6 months body weight. As far as corrected back-fat thickness and average back-fat thickness were concerned, pigs with BB genotype were the thinnest, although no significant difference was observed.

In Large Yorkshire pigs, no significant difference was observed among three genotypes for birth weight, but the birth weight of the pigs with AA genotype also was the highest, this was the same as Nanchang White pigs. For 2 months body weight, pigs with BB genotype were higher than pigs with AB genotype ($p<0.05$). For 4 months body weight, no significant difference was observed among three genotypes. For 6 months body weight, pigs with BB genotype were the highest, and had statistic significant ($p<0.05$) to the pigs with AA genotype. For hind-back-fat thickness and mid-back-fat thickness, furthermore, pigs with BB and AB genotypes were significantly different ($p<0.05$) to AA genotype. The pig with the BB genotype was the thinnest. For lean percentage, pigs with BB and AB genotypes had higher lean content than AA genotype ($p<0.01$), and the pig with BB genotype was the highest.

DISCUSSION

In Table 1, the AA genotype frequencies and A allele frequencies of European pig breeds were rare, while those of Chinese native pig breeds were much higher. As a lean-type pig breed, Wild Pig carried no AA genotype and rare A allele frequency, the reason was the long natural selection and evolution. In Table 2, the two pig breeds, AA genotypes were also rare, this was similar to the results by Fang *et al*. (1999) and Wang et al. (2000), in which western pigs carried AA genotype with a low frequency, while Chinese native pig breeds had higher frequencies.

In two different pure pig breeds, birth weight of pigs with AA genotype was higher than the other two genotypes, and pigs with BB genotype had a higher 2 months BW

Table 1. The genotype and allele frequencies of IGF-I in 13 pig breeds

Breeds	Number	Genotype frequencies (%)			Allele frequencies (%)	
		AA	AB	BB	A	B
Landrace	65	24.6	20.0	55.4	34.6	65.4
Duroc	52	0	26.9	73.1	13.5	86.5
Pietrain	11	0	0	100	0	100
Jinhua two-end black	60	61.7	16.7	21.6	70.0	30.0
Jiaxing black	58	22.8	56.1	21.1	50.9	49.1
Shengxian spotted	57	50.0	34.5	15.5	67.2	32.8
Leping spotted	48	33.3	62.5	4.2	64.6	35.4
Yushang black	66	59.1	28.8	12.1	73.5	26.5
Erhualian	34	73.5	26.5	0	86.8	13.2
Zixi black	9	55.6	44.4	0	77.8	22.2
Xinzhi black	12	33.3	41.7	25.0	54.2	45.8
Wild pig	30	0	6.7	93.3	3.3	96.7
Shanggao two-end black	57	50.0	37.9	12.1	69.0	31.0

Table 2. Effects of different genotypes on some production performances in two pure pig breeds

Breeds	Nanchang white			Large yorkshire		
Genotypes	AA	AB	BB	AA	AB	BB
Number	7	15	95	21	65	274
Birth weight (kg)	1.32±0.17[a]	1.07±0.25[b]	1.20±0.24[ab]	1.47±0.25	1.42±0.26	1.45±0.18
2 mon. BW (kg)	20.00±2.65	19.99±3.65	20.83±3.65	23.30±2.38[ab]	21.84±3.30[b]	24.28±2.27[a]
4 mon. BW (kg)	55.17±12.22	56.29±12.01	57.50±5.68	58.63±5.48	60.20±4.09	61.40±7.70
6 mon. BW (kg)	83.00±8.34	82.42±6.67	84.64±7.94	91.52±9.83[b]	97.38±9.32[ab]	101.68±12.01[a]
Hind-back-fat thickness (cm)				2.16±0.18[a]	1.93±0.21[b]	1.90±0.16[b]
Mid-back-fat thickness (cm)				2.15±0.31[a]	1.86±0.20[b]	1.77±0.21[b]
Lean percentage (%)				56.95±2.95[B]	59.26±2.44[A]	61.60±2.29[A]
Corrected back-fat depth (cm)	2.45±0.24	2.40±0.15	2.36±0.18			
Average back-fat depth (cm)	2.51±0.18	2.45±0.22	2.45±0.22			

Note: Values are M±SEM. Means with low case or capital letter within the same row in the same breed are significantly different ($p<0.05$ or $p<0.01$).

(body weight), 4 months body weight, 6 months body weight than those pigs with AB and BB genotypes. As far as lean percentage, mid-back-fat thickness, hind-back-fat thickness, corrected back-fat- thickness and average back-fat thickness were concerned, AA genotype had negative effect. From the results of two pure pig breeds we have studied, it is concluded that, if we select pigs with AB and BB genotypes in the same population, we could get more lean percentage and less back-fat thickness. Also the more BB genotype pigs are selected, the more carcass is gotten. This is similar to the results by Casas-Carrillo et al.(1997), Horvat et al.(1995) and Seo et al. (2001).

In Nanchang White pigs, there is no significant difference among three genotypes for 2 months body weight, 4 months body weight, 6 months body weight, corrected back-fat thickness and average back-fat thickness, but the body weight at these days were highest and the fat depths are the thinnest. The affective trends were similar to Large Yorkshire and the course maybe: Nanchang white is a hybrid (Large Yorkshire×Binhu Black), and only breeding for 6 generations, the pigs contents 20% blood of Binhu Black, which can effect the function of allele B. From the primary results, it is suggested to select pigs with B allele can improve the body weight and carcass lean percentage.

In two pig breeds, AA genotype had different significant effect on the birth weight. The plausible reasons are as follows: different management and nutritious level; different sample numbers. From the results of table 2, it is found that relationships between birth weight and 2 months body weight are contrary to the principle that the higher birth weight, the higher 2 months body weight, besides the above two reasons, there is a third reason that pigs with AA genotype have different biological characters such as weaning stress, suitability and genetics speciality.

Now many studies of IGF-I are only focused on its biochemical functions and nutritional modulation. But few studies were conducted to review whether polymorphism of IGF-I gene could be used as a genetic marker in pig and breeding system, thus the results could provide a theoretical and an experimental reference for pig genetic improvement.

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