

Polymorphism of Growth Hormone Gene in 12 Pig Breeds and Its Relationship with Pig Growth and Carcass Traits

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ABSTRACT : The polymorphism of the growth hormone gene in 12 pig breeds (total n=475) was detected by PCR-*Apa* I -RFLP, and allele A (449 bp, 101 bp and 55 bp) or allele B (316 bp, 133 bp, 101 bp and 55 bp) were observed. In these pig breeds, we found that European pig breeds had high frequencies of allele B, while Chinese native pig breeds had high frequencies of allele A. In addition, the role of porcine GH was investigated in 117 Nanchang White pigs and 361 Large Yorkshire pigs. Eight traits about growth and carcass were recorded for analyzing associations between GH gene polymorphism and performance quantitative traits. In the Nanchang White pigs, no significant difference was observed between different genotypes and different growth and carcass traits. In Large Yorkshire pigs, those with BB genotype had more lean percentage than pigs with AA genotype ($p < 0.05$). Based on these results, we conclude that the GH locus should be further investigated in commercial breeds to determine its suitability for use in marker-assisted selection programmes. (*Asian-Aust. J. Anim. Sci.* 2003, Vol 16, No. 2 : 161-164)

Key Words : Growth Hormone 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 conversion of feed to meat. Important economic traits are usually controlled by many genes and modified by environmental factors, such as temperature and nutrition. Exogenous porcine somatotropin (GH) administration causes higher muscle mass and lower fatness in pigs. Moreover, feed conversion rate and daily gain are improved (Eherton et al., 1986; Mikel et al., 1993). The effect of porcine somatotropin depends on the genetic potential for fat deposition of the breed, e.g. animals of German Landrace and the Schwerfurter Race showed a higher response to administrated somatotropin than Pietrain (Ender and Rehfeldt, 1993). Furthermore, pigs selected for high weights at slaughter or low average back-fat thickness have higher basal concentrations of GH than unselected animals (Lund-Larsen and Bakke, 1975; Arbona et al., 1988).

Growth hormone is a peptide hormone approx. 190 amino acids in length which is produced and released by the anterior pituitary in response to the hypothalamic peptide, GH-releasing factor (Barinaga et al., 1985). Secretion of GH results in a range of metabolic actions, the end result of which is growth. Porcine growth hormone gene was

sequenced by Vize et al. (1984). It contains four introns and five exons, and the gene was assigned to porcine chromosome 12p1.2-p1.5 (Yerle et al., 1993). The effects of GH gene variations on performance traits have been described for cattle (Rocha et al., 1992; Schlee et al., 1994) and mice (Winkelman and Hodgetts, 1992). In pigs, associations between variants at the GH locus and quantitative traits were studied (Casas-Carrillo et al., 1997; Knorr et al., 1997; Wang et al., 2002), but the results obtained disagreed, thus requiring further investigation. The results of Nielsen et al. (1995) suggested differences in transcriptional activities between GH variants, which might eventually cause higher plasma GH concentrations and higher growth rates, however, their studies did not establish a direct cause and effect relationship.

In this study we investigated the polymorphism of GH (Larsen and Nielsen, 1993) in 12 pig breeds and associations of GH variants with some performance traits in Nanchang White and Large Yorkshire pigs.

MATERIALS AND METHODS

Animals

Ear notches were collected from three European breeds and nine Chinese native breeds of pigs. The breeds 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 Pig (n=60, Jinhua County Pig Farm), Leping Spotted (n=48, Leping County Pig Farm), Yujiang (n=66, Yushan County Pig Farm), Taihu Pig (n=34, JAU Experimental Pig Farm), Yanshan Black (n=12, Yanshan County Pig Farm), Xinzhi Black (n=12, Xinzhi

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County Pig Farm), Wild Boar (n=30, Jiaxin Pig Farm), Shangao Black (n=57, Shangao County Pig Farm), Wan'an Spotted (n=28, Wan'an County Pig Farm). All of the sampled pigs were unrelated within 3 generations. In order to detect the effects of different GH genotypes on some production performances, we also developed two pure pig breeds. The Nanchang White pigs were half sibs, one male was mated to 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 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 old the pigs were marked permanently by ear notching and this tissue was retained for DNA testing. At ten weeks of age, the piglets were taken into single pens. The fattening test started when the pigs were at or from the 120 days of age. The two pig breeds were fed with a cereal diet *ad libitum* containing 19%, 16%, and 13% crude protein in the nursery, grower and finisher stages, respectively, and the weights of the 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).

GH genotypes defined by PCR-RFLP

Ear notches from samples 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/ μ l. Primers designed according to Knorr et al. (1997), 5' TTA TCC ATT AGC ACA TGC CTG CCA 3' and 5' CTG GGG AGC TTA CAA CAT CCT T 3' were used for PCR (polymerase chain reaction) amplification between nucleotides -119 and +486 bp of the porcine growth hormone gene (numbering according to Vize and Wells, 1987). The PCR mixture contained 50 ng genomic DNA, 25 pmol of each primer, 25 μ M of each dNTP, 1 unit of Taq DNA Polymerase and 10 \times reaction buffer in a 25 μ l reaction volume. PCR was done using the PTC-100 (MJ Research) thermocycler according to the following procedure: first 95°C for 300s, 59°C 45s, 72°C 45s; cycles 2-30: 94°C 45s, 59°C 45s, 72°C 45s; final: 72°C for 300s. The PCR products were subsequently digested by *Apa* I and revealed allele A (fragments of 449 bp, 101 bp and 55 bp) or allele B (fragments of 316 bp, 133 bp, 101 bp and 55 bp). The restriction digests were separated using 2.0% agarose gel in 1 \times 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 GH genotypes and performance traits were analyzed for using the Statistic analysis system (SAS, 1989) and significance between genotypes was assessed by Student's *t*-test.

RESULTS

The genotypes and the allele frequencies of GH in 12 pig breeds are listed in Table 1.

From Table 1, the AA genotype frequency in European pig breeds was low, especially in Pietrain, where it was absent. However, in Chinese native pig breeds, the AA genotype frequency was high, and in Yujiang Pig it was the highest (75.8%). Meanwhile, the allele B frequency in European pig breeds was high, especially in Pietrain where its frequency was 79.1%. In Chinese native pig breeds the allele A frequency was high (above 50%), especially the allele A frequency of Yujiang which was 85.6%.

Table 2 listed the observed genotypes and their frequencies. The genotype AA was rare in two pig breeds. Its frequency was 5.10% in Nanchang White and 10.8% in Large Yorkshire.

In Nanchang White pigs, no significant difference was observed between three genotypes and the traits we analyzed. But the pigs with AA genotype had more birth weight, 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.

Table 1. Genotypes and gene frequencies of GH2 gene in 12 pig breeds

Breed	No.	Genotype frequency (%)			Allele frequency (%)	
		AA	AB	BB	A	B
Duroc	52	21.2	50.0	28.8	46.2	53.8
Landrace	65	10.8	52.3	36.9	36.9	63.1
Pietrain	11	00.0	41.8	58.2	20.9	79.1
Yujiang	66	75.8	17.9	4.5	85.6	14.4
Taihu pig	34	44.1	47.1	8.8	67.6	32.4
Jinhua pig	60	50.0	33.3	13.7	66.7	33.3
Leping spotted	48	33.3	50.0	16.7	58.3	41.7
Yanshan black	12	50.0	50.0	0.0	75.5	25.0
Xingzi black	12	50.0	41.7	8.3	70.8	29.2
Wan'an spotted	28	37.0	55.6	7.4	64.8	35.2
Shanggao black	57	29.3	53.4	17.2	56.0	44.0
Wild boar	30	26.7	50.0	23.3	56.7	43.3

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

Breed	Nanchang white			Large yorkshire		
	AA	AB	BB	AA	AB	BB
Number	6	50	61	39	176	146
Birth weight	1.23±0.25	1.20±0.21	1.18±0.27	1.47±0.24	1.47±0.18	1.45±0.21
2 month body weight (kg)	20.69±3.11	19.45±3.97	19.33±2.98	22.88±3.15	22.68±2.99	21.52±2.90
4 month body weight (kg)	58.34±10.53	56.58±8.69	55.08±10.10	65.70±8.83	60.71±7.84	59.25±4.52
6 month body weight (kg)	87.20±7.32	85.24±8.40	83.03±7.10	97.79±9.34	95.47±8.88	95.45±11.89
Average back-fat depth (cm)	2.51±0.41	2.46±0.01	2.45±0.23			
Corrected back-fat depth (cm)	2.40±0.33	2.40±0.21	2.39±0.20			
Feed to gain ratio				2.82±0.37	2.89±0.33	2.85±0.33
Lean percentage (%)				58.28±3.30 ^b	58.94±2.56 ^{ab}	59.18±2.35 ^a

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

In Large Yorkshire pigs also, no significant difference was observed among three genotypes for birth weight, 2 months body weight, 4 months body weight and 6 months body weight, but the trends shown by the genotypes were the same with the Nanchang White pigs, in that the pigs with BB genotype had more lean percentage than pigs with AA genotype ($p < 0.05$).

DISCUSSION

Table 1 shows that the AA genotype and the A allele were rare in European pig breeds, while they were more frequent in Chinese native pig breeds. As a lean-type pig breed, Wild Pig carried no AA genotype and had rare A allele frequency due to long natural selection and evolution. Table 2 shows that the two pig breeds the AA genotypes were also rare in the Nanchang White and Large Yorkshire. This is similar to the results of Larsen et al. (1993) and Knorr et al. (1997), who reported that western pigs carried AA genotype with a low frequency, while Chinese native pig breeds had higher frequencies.

In our preliminary results, in two different pig breeds, pigs with AA genotype had the highest birth weight, 2 months body weight, 4 months body weight and 6 months body weight, while pigs with BB genotype had the lowest although no significant difference was observed. After studying the offsprings of four purebred sires and two crossbred sires, Casas-Carrillo et al. (1997) found that growth hormone genotype was not associated with growth traits. But the results of Cheng et al. (2000) showed that growth performance traits in Duroc, Landrace and Tao-Yun pig breeds were highly correlated with their growth hormone genotype. Nielsen et al. (1995) using pigs from four Danish breeds found that the double-strand DNA conformation polymorphisms (DSCPs) at the promoter region of the gene was associated with change in basal-plasma GH; this suggested that the GH gene might be a QTL for GH levels. On the other hand, Larsen et al. (1995), using the same marker on a wild/Large White F2 population, found that the locus did not play a major role in defining the

genetic differences between the wild and Large White pigs for average daily gain.

In the Nanchang White pigs, the pigs with AA genotype had more average back fat thickness and corrected back fat thickness than AB and BB genotypes, but no significant difference was observed. In Large Yorkshire, pigs with BB genotype had more lean percentage than pigs with AA genotype and the difference was significant. This difference in trends in the two pig breeds maybe because Nanchang white is a hybrid (Large Yorkshire×Binhu Black) and has only bred for 6 generations. The pigs contain 20% of Binhu Black blood, which can affect the function of allele B. From our results, it is suggested that selection for pigs with B allele can improve carcass lean percentage. Knorr et al., (1997), found that in a family of Meishan×Pietrain, only traits related to fatness were associated with GH variants. The GH locus explained a significant proportion of the GH differences between F2 animals and accounted for 11.7% to 17.7% of the total phenotypic variance in the F2 population. Pierzchala et al. (1999), found that in a family of Zlotnicka Spotted boars×Polish Large White sows (ZS×PLW), the *Hae* II and *Msp* I genotypes as well as *Hae* II-*Msp* I haplotypes differed significantly for lean meat content and several traits related to carcass fatness.

From the results based on the two pure pig breeds we have studied, it suggested the relationship between different genotypes due to different PCR products and cut by different restriction enzymes and performance traits are varied. From our study, and taking into account the results of Larsen et al. (1995) and Knorr et al. (1997), it is indicated the GH locus plays a major role in defining the genetic difference between Nanchang White pigs and Large Yorkshire pigs and we regard the GH gene as a candidate gene for fatness in pigs. So it is important to build several resource families and identify the single-nucleotide polymorphism (SNP), to find out which SNP has high relationship to production performance, and to provide a basis for developing successfully mark-assisted selection programmes.

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