Study on pig growth hormone gene polymorphisms in western meat-type breeds and Chinese local breeds*

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Abstract: Chinese Meishan and Jiangquhai pigs are two of the most prolific pigs in the world, but their growth rate is lower than that of Duroc, Landrace and Pietrain pigs. It is suggested that growth rate is regulated by growth hormone. The objective of the current study was to analyze the porcine growth hormone (p*GH*) gene polymorphisms based on the polymerase chain reaction restriction fragment-length polymorphism method (PCR-RFLP) for three western meat-type breeds (Duroc, Landrace and Pietrain) and two local Chinese pigs (Meishan and Jiangquhai). Five polymorphic restriction sites were detected with the *Apa*I, *Msp*I, *Bsp*I and *Hha*I restriction enzymes in two amplified fragments (605 bp, −119 to +486; 506 bp, +206 to +711). Breed difference was found only in the 506 bp fragment. There was no difference in allelic frequencies of *Bsp*I and *Hha*I restriction sites among the five breeds ($P>0.05$). Landrace and Meishan pigs lacked allele *G*3 of *Msp*I site. The allele *G*3 frequency of restriction *Msp*I site of the 506 bp fragment in Pietrain pigs was higher than that in Duroc and Jianquhai pigs ($P<0.001$). For *Apa*I site, the Meishan pigs lacked allele *G*1; no difference was found in allelic frequencies among Pietrain, Duroc, Landrace and Jiangquhai pigs ($P>0.05$). This new and rapid PCR-RFLP typing method is an attractive tool for analysis of porcine growth hormone gene restriction sites. The differences in *Msp*I and *Apa*I restriction sites may explain the growth difference between the foreign meat-type breeds above mentioned and local Chinese pigs.

Key words: Pigs, Growth hormone gene, Polymorphisms
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INTRODUCTION

Growth hormone (*GH*) is a peptide hormone which regulates growth and various metabolic activities (Sterle, 1995; Yuan, 1996). Injections of *GH* into growing pigs increased growth rate of the animals and the percentage of muscle, and while fat accretion was decreased (Bonneau, 1991; Fabry, 1991; Mikel *et al.*, 1993). *GH* gene is thus a major candidate for controlling growth and fat deposit in pigs. Associations between *GH* polymorphisms and variation in growth and fatness traits have been established in pigs (Knorr *et al.*, 1997; Krenkova *et al.*, 1999; Pierzchala *et al.*, 1999; Cheng *et al.*, 2000; Song *et al.*, 2001). Compared to Duroc, Landrace and Pietrain pigs, Chinese local pigs appear to lower growth rate and percentage of muscle. The merit of the above local Chinese breeds is their high prolificacy; studies focused on their reproductive characteristics have been conducted in many countries (Rohrer *et al.*, 1999; Rothschild, 1994). In contrast, less research has been carried out to investigate their growth performance and regulation. Therefore, the present study was carried out to analyze the differences in polymorphisms of porcine growth hormone (p*GH*) gene among two local Chinese breeds and three western meat-type breeds.

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MATERIALS AND METHODS

Animals

Female adult pigs (21 Landrance, 28 Pietrain, 35 Duroc, 33 Chinese Meishan and 73 Jiangquhai pigs) were used for this experiment. Landrance, Chinese Meishan and Jiangquhai pigs were from Jiansu Province. Pietrain and Duroc pigs were purchased from Shanghai and Guangdong Province.

Chemicals

Restriction enzymes (MBI), proteinase K, Taq polymerase (Sangon) and primers were obtained from the Shanghai Sangon Biological Technology, Ltd., China. $MgCl_2$, ethidium bromide and 4dNTPs were obtained from Promega (USA). Other reagents were commercial preparations of the highest purity available.

DNA samples

DNA extraction was performed as previously described (Song *et al.*, 2001). Briefly, one half gram of hair sample was cut off from 20 roots of freshly plucked hairs, placed in 0.4 ml buffer with Rnase A (100 μg/ml) for 1 h at 37 ℃; then incubated with proteinase K(100 μg/ml) at 56 ℃ for 3 h, after which, DNA was purified twice with a mixture of phenol, chloroform and isoamyl alcohol (25:24:1, v/v/v). Then, DNA samples were washed with 75% ethanol and dissolved with TE (pH 8.0, 10 mmol/L Tris, 1mmol/L EDTA).

Polymerase Chain Reaction (PCR)

Primers were constructed as previously described by Larsen and Nielsen (1993) and Kirkpatrick (1992). Briefly, the primer pairs of PCR1 and PCR2 were designed from 5′ upstream to the second intron, from first intron to third exon respectively (refer to Table 1). The PCR amplification was carried out for each primer pair on a Hybaid Omnigene themocycler (Hybaid, Middlesex, UK). The amplification of PCR1 was carried out in a total volume of 25 μl reaction buffer (Sangon, Shanghai, China) containing 10 pmol of primer; 1 U Taq polymerase; 0.4 mmol/L each of dATP, dCTP, dGTP, dTTP; and 100 ng DNA sample. DNA was denatured for 4 min at 95 ℃. Polymerase chain reaction 1 was run for 30 cycles at 95 ℃ for 45 s, 59 ℃ for 60 s, 76 ℃ for 1.5 min, then extended for 10 min at 76 ℃. The amplification of PCR2 was carried out in a total volume of 10 μl reaction buffer containing 10 pmol primer; 1 U Taq polymerase; 0.2 mmol/L of each of dATP, dCTP, dGTP, dTTP; and 100 ng DNA. DNA was denatured for 5 min at 94 ℃. Polymerase chain reaction 2 was run for 5 cycles at 94 ℃ for 60 s, 60 ℃ for 30 s, 74 ℃ for 30 s, then 30 cycles at 94 ℃ for 30 s, 60 ℃ for 40 s, 74 ℃ for 30 s, and final extension for 10 min at 72 ℃. The amplified fragments were identified by molecular weight marker (*pGEM3Zf*(+)/*Hae* III).

Table 1 Primer sequence, restriction site and corresponding PCR Product site for *GH* gene

	Primer sequence	Site	Size
PCR1	Forward: 5′ TTATCCATTAGCACATGCCTGCCAG 3′ Reverse: 3′ CTGGGGAGCTTACAAACTCCTT 5′	−119 to +486	605 bp
PCR2	Forward: 5′ GCCAAGTTTTAAATGTCCCTG 3′ Reverse: 3′ CTGTCCCTCCGGGATGTAG 3′	+206 to +711	506 bp

Restriction Fragment Length Polymorphism (RFLP)

The amplified fragment was digested at 37 ℃ for 3 hours with restriction enzyme (Table 2) in a total volume of 20 μl reaction buffer containing 10 μl PCR products and 10 units of enzyme. The digests were electrophoresed through 8% acryl amide gel (composed of 8% acryl amide (19:1, acryl amide: N N′-bismethylene-acrylamide, w/w), 5%(v/v) glycerol and 1 × TBE buffer), and stained with 0.5 μg/ml ethidium bromide. Gels were visualized on an ultraviolet transilluminator and photographed. The images were analyzed with Biotechs Gel290 software (1.0 version). The allele frequency was calculated by the formula: $F = A/2T$, where A is the allele number, T is the total number of samples from each breed.

Table 2 Restriction enzymes used in the current study, digestion sites of growth hormone gene and identifying sequences of each restriction enzymes

	Restriction Enzyme	Site	Sequence
PCR1	*Hha*I	+ 330 and + 379	5′ G * CGC 3′
	*Bsp*I	+ 193	5′ G * GGCC 3′
PCR2	*Apa*I	+ 300	5′ GGGCC * C 3′
	*Msp*I	+ 566	5′ C * CGG 3′

Statistics

Chi-square test was used to detect significant differences in allelic frequencies among different breeds by SPSS software (SPSS, 10.0 version).

RESULTS

Polymorphism

One hundred ninety adult femal pigs representing five breeds were examined for growth hormone gene polymorphisms in the current experiment. Five polymorphic restriction sites were detected. Two polymorphic restriction sites were identified in the 506 bp fragments with *Apa*I (at the base 300) and *Msp*I (at the base 566), respectively. Two polymorphic restriction sites were identified with *Hha*I in the 605 fragment (at the bases 330 and 379). One was identified with *Bsp*I in the same fragment at the base of 193. The amplified fragments of PCR1 digested with *Hha*I and *Bsp*I restriction enzymes yielded allele *A*1, *A*2, *C*1, *C*2 and *C*3 (refer to Figs. 1 and 2). The amplified fragments of PCR2 digested with *Apa*I and *Msp*I restriction enzymes yielded allele *G*1, *G*2, *G*3, and *G*4 (refer to Figs.3 and 4).

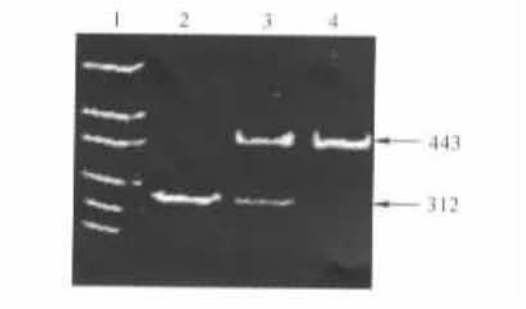

Fig.1 Representative gel of *Bsp*I digested 605 bp PCR product. Lane 1 is a molecular weight marker (*pGEM7Zf*(+)/*Hae*III), Lanes 2 – 4 are *A2A2*, *A1A2* and *A1A1*, respectively

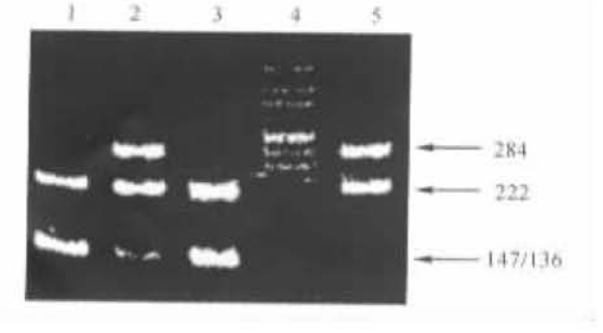

Fig.2 Representative gel of *Hha*I digested 605 bp PCR product. Lane 1 is a molecular weight marker (*pGEM7Zf*(+)/*Hae*III), Lanes 2 – 7 are *C1C2*, *C1C2*, *C3C3*, *C2C3*, *C2C2* and *C1C3*, respectively

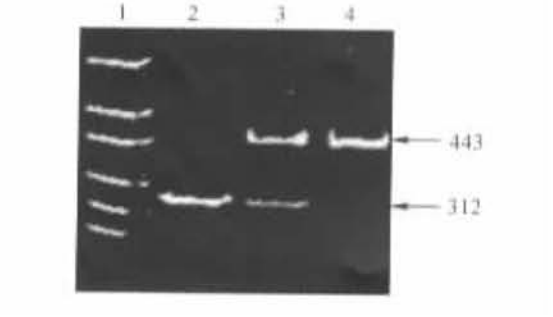

Fig.3 Sample gel of *Msp*I degested 506 bp PCR procuct. Lane 4 is a molecular weight Marker (*pGEM7Zf*(+)/*Hae*III), Lanes 1 – 3 and 5 are *G4G4*, *G3G4*, *G4G4* and *G3G3*, respectively

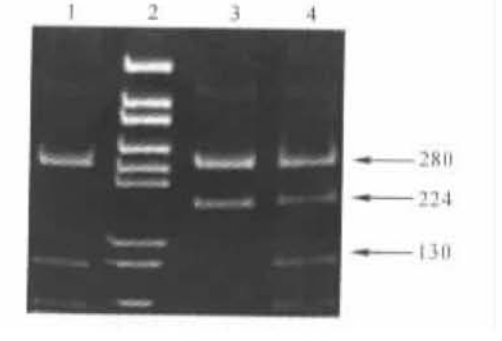

Fig.4 Sample result of *Apa*I degested 605 bp PCR procuct. Lane 2 is a molecular weight Marker (*pGEM7Zf*(+)/*Hae*III), Lanes 1, 3 and 4 are *G2G2*, *G1G1* and *G1G2*, respectively

Allelic frequencies

The allelic frequencies of the 506 bp and 605 bp fragments in the five pig breeds are summarized in Tables 3 and 4. Among the five breeds, there was significant difference ($P < 0.001$) in the allelic frequency of the polymorphic site of *Msp*I 506 bp fragment. The Landrace and Meishan pigs lacked allele *G*3. The allele *G*3 frequency of restriction *Msp*I site of the 506 bp fragment in Pietrain pigs was higher than that in Duroc, and Jianquhai pigs ($P < 0.001$), while there was no difference between Duroc and Jianquhai pigs ($P > 0.05$). The allelic frequency of the *Apa*I 506 bp *GH* fragment was different among the five breeds too ($P < 0.001$). Meishan pigs lacked allele *G*1. No difference existed in the allelic frequency of the *Apa*I site of the 506 bp fragment among Pietrain, Duroc, Landrace and Jiangquhai pigs ($P > 0.05$, see Table 3). No differences were detected in the allelic frequency of the *Hha*I and *Bsp*I restriction site of the 605 bp fragment among the five pig breeds ($P > 0.05$). In general, there was no difference in allelic frequency in the 605 bp fragment restriction sites among the five pig breeds ($P > 0.05$, see Table 4).

Table 3 The PCR-RFLPs allelic frequencies of *GH* gene (506 bp fragment) in five porcine breeds[a]

Restriction Enzyme	Allele	Pietrain	Duroc	Landrace	Jianquha	Meishan	
N^{b}		28	35	21	73	33	
*Apa*I	*G*1	0.300[b]	0.255[b]	0.385[b]	0.310[b]	0.000[a]	$X^2 = 29.14$
	*G*2	0.700[b]	0.745[b]	0.615[b]	0.690[b]	1.000[a]	$P < 0.001$
*Msp*I	*G*3	0.550[c]	0.345[b]	0.000[a]	0.250[b]	0.000[a]	$X^2 = 68.49$
	*G*4	0.450[c]	0.655[b]	1.000[a]	0.750[b]	1.000[a]	$P < 0.001$

[a] Frequencies with same superscript in the same row mean no difference ($P > 0.05$) and with different superscript in the same row mean significant difference ($P < 0.001$)

[b] *N*: The number of pigs per breed

Table 4 The PCR-RFLPs allelic frequencies of *GH* gene (605 bp fragment) in five porcine breeds

Restriction Enzyme	Allele	Pietrain	Duroc	Landrace	Jianquha	Meishan	
N^{a}		28	35	21	73	33	
*Bsp*I	*A*1	0.286	0.300	0.3095	0.2875	0.303	$X^2 = 0.13$
	*A*2	0.714	0.700	0.6905	0.7125	0.697	$P > 0.05$
*Hha*I	*C*1	0.3755	0.400	0.381	0.349	0.3785	$X^2 = 1.59$
	*C*2	0.3030	0.3285	0.310	0.3625	0.303	$P > 0.05$
	*C*3	0.3215	0.2715	0.309	0.2885	0.3185	

[a] *N*: The number of pigs per breed

DISCUSSION

The growth hormone plays an important role in the regulation of growth, development and metabolism in mammals (Kirkwood *et al.*, 1989; Leger *et al.*, 1998; Lough *et al.*, 1989; Shimoda *et al.*, 1997; Skarda, 1998). Growth hormone is an essential mediator of normal postnatal growth and its expression is regulated by other hormones and nutritional and developmental factors (Lauterio and Scanes, 1988; Nelson *et al.*, 1988; Pfeuffer *et al.*, 1988; Pisanty *et al.*, 1997; Tannenbaum *et al.*, 1998). Therefore, growth hormone gene may be potential candidate marker for marker assisted selection programs.

The sequence of porcine *GH* was identified by Vice and Wells (1987), and the total length of porcine *GH* gene is 2231 bp, containing four introns and five exons. Besides, several growth hormone gene polymorphsic sites had been reported and the effects of some sites on growth performance were investigated (Handler *et al.*, 1996; Knorr *et al.*, 1997; Krikpatrick and Huff, 1990; Krikpatrick, 1992; Korwin-Kossakowska *et al.*, 1999; Larsen and Nielsen,

1993; Nielsen and Larson, 1991; Pierzchala *et al*., 1999; Schellander *et al*., 1994). The exception Knorr *et al*. (1997), they did not find any differences in growth performance between the pigs differing in *GH* genotypes. Knorr *et al*. (1997) reported that in Meishan × Pietrain crosses, eight traits related to fatness were associated with *GH* genotypes. The *GH* locus explained 11.7% to 17.7% of the total phenotypic variance in the F2 population. Also in our previous study, some significant differences in early growth rate were found among the pigs with different genotypes of *Apa*I locus (Song *et al*., 2001). In the present study, five polymorphic sites were identified. Among them, two sites were in the first intron (193/*Bsp*I, 300/*Apa*I), one site in the second intron (577/*Msp*I), and two sites in the second exon (330, 379/*Hha*I). Those sites were assumed to be functionally related to growth (Jiang *et al*., 1996; Song *et al*., 2001).

In this study we found Landrace and Meishan pigs lacked allele *G*3 of *Msp*I site, Meishan pig lacked allele *G*1 of *Apa*I site. Others also reported similar results, in which local Chinese pigs (Jinhua, Taihu and Wanzhehua) did not have some of these allele sites, but those sites did exist in the two western pigs (Large White and Landrace) (Jiang *et al*., 1996). Previous study revealed that among Chinese Tao-yuan, Duroc and Landrace pigs, the significant difference of allelic frequencies in *Taq*I and *Dra*I of *GH* locus were identified (Cheng *et al*., 2000). We also found that in the polymorphic site of *Msp*I and *Apa*I, the difference of allelic frequency was significant among the five breeds ($P < 0.001$). The growth rate of local Chinese pigs is lower, but their reproductive performance is better compared to the Landrace, Duroc and Pietrain pigs. Furthermore, several binding sites of units regulating *GH* gene expression in the 5′ flank and intron of *GH* gene were identified (Schaufele *et al*., 1990; Tansey *et al*., 1993). These indicated the possibility that these sequences may be involved in the regulation of *GH* production. Therefore the mutations in intron may provide markers on growth performance for future studies.

In contrast to previous observation by Jiang *et al*. (1996), we did not find any differences in the allelic frequencies in *Hha*I site. Some studies have identified that the two mutations result in amino-acid substitution (330 bp site, alanine to valine, 379 bp site, glycine to glutamiacid) (Vice and Wells, 1987). But the biological effect of that substitution was not clear. In fact, the sequence of the first and partial second exon encodes signal peptide of *GH* production, the sequence of partial second's, third, fourth and fifth exons encode mature peptide of *GH* production (Vice and Wells 1987). It suggested that the signal peptide is not involved in regulating gene expression. The two amino-acid substitutions were in signal peptide, which did not change the regulatory function of *GH*. Therefore, the two *Hha*I polymorphsic sites of the second exon were suggested to be not essential for growth-promoting activity.

In conclusion, the polymorphic sites of *Msp*I and *Apa*I were important; they might functionally relate to growth. The amino-acid substitutions in the signal peptide might have no biological effect. Further study should focus on the mutations of mature *GH* peptide.

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