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Polymorphism of Insulin-like Growth Factor Binding Protein-4 Gene in 17 Pig Breeds and Its Relationship with Growth Traits

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ABSTRACT : Insulin-like growth factor binding protein-4 (IGFBP-4) is a member of the IGF super family, and regulates the action of IGFs. The polymorphism of porcine IGFBP-4 gene in 17 pig breeds (total n = 570) was detected by PCR-SSCP, and alleles A and B were detected. In these pig breeds, it was found that exotic pig breeds carried high frequencies of allele A, while Chinese native pig breeds carried high frequencies of allele B. The role of porcine IGFBP-4 was investigated in 172 F2 offspring of a Lantang×Lantang population. Forty eight growth traits were recorded for analyzing the association between IGFBP-4 gene polymorphism and quantitative performance traits. In this resource family, pigs with AA genotype had higher fore-body weight, bone weight of mid-body, bone weight of rear-body, fore-leg weight and rear-leg weight than those pigs with BB genotype ($p<0.05$), while pigs which carried BB genotype had higher back-fat thickness at C point and lard weight than those pigs with AA genotype ($p<0.05$); pigs with AA genotype had higher body weight than those with BB genotype; for meat quality traits, pigs with AA genotype had higher meat color than those of BB genotype ($p<0.01$), and pigs with BB genotype had higher marbling than those of AA and AB genotypes ($p<0.01$ and $p<0.05$, respectively).Based on these results, it is necessary to do more studies on IGFBP-4 before using the IGFBP-4 locus for the application of marker-assisted selection programs. (**Key Words** : IGFBP-4 Gene, Performance Traits, Swine, PCR-SSCP)

INTRODUCTION

The insulin-like growth factor (IGF) system includes two ligands, IGF1 and IGF2; a membrane-spanning, tyrosine kinase receptor that transduces both ligands known as the IGF1 receptor (Le Roith et al., 2001); and a family of high-affinity IGF binding proteins (IGFBPs 1-6). IGFBPs are produced by a variety of biological tissues and found in various biological fluids (Rajaram et al., 1997). Although all six known IGFBPs belong to the same gene family, several features distinguish these IGFBPs from each other. As one of the members of the IGFs superfamily, IGFBP-4, a nonglycosylated protein of 25 kDa and 32-36 kDa, was first isolated from medium conditioned by human osteosarcoma TE-89 cells (Mohan et al., 1989) and from adult rat serum (Shimonaka et al., 1989). Subsequently this nonglycosylated protein was isolated from a variety of cell types from different animal species and from human adult serum (Shimasaki and Ling, 1991; Rechler, 1993).

IGFs are essential for promoting cell growth, survival, migration and differentiation *in vitro*, and have insulin-like activity (Jones and Clemmons, 1995). Its actions are determined by the availability of free IGFs to interact with the IGF receptors. The level of free IGF in a system is modulated by rates of IGF production and clearance and the degree of binding to IGFBPs. Commonly, unlike IGFBP1-3, IGFBP-4 inhibits the actions of IGFs, and it can not bind cell surface. IGFBP-4's function is unique in that it inhibits IGF actions in all cell systems studied *in vitro* thus far (Lawrence et al., 1999). This has been shown to occur via high-affinity binding to both IGF-I and IGF-II, thus preventing interaction of either IGF with its receptor (Kelly et al., 1996). Recently, experiments developed transgenic mice that overexpressed IGFBP-4 selectively in smooth muscle. IGFBP-4 overexpression was associated with smooth muscle hypoplasia (Wang et al., 1998).

Human IGFBP-4 gene is mapped on chromosome 17, it contains four exons and spans 15.3 kb on chromosome (Allander et al., 1993), recently, we have mapped the porcine IGFBP-4 gene on SSC12 by FISH and RH panel (Wang et al., 2005). Also a fragment of porcine IGFBP-4 gene was cloned and submitted to NCBI (GenBank accession No. DQ 537340). In this study, we firstly

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Table 1. Statistic results of the F2 traits in Lantang×Landrace resource family

Traits	Means	SE	Traits	Means	SE
Fore-body weight	12.06	1.29	Head weight	7.11	0.95
Mid-body weight	11.80	1.53	Fore-leg weight	0.79	0.15
Rare-body weight	9.64	1.09	Rare-leg weight	1.22	0.23
Lean weight of fore-body	6.10	0.85	Left carcass weight	34.99	2.89
Lean weight of mid-body	4.61	0.74	Right carcass weight	34.91	3.18
Lean weight of rare-body	5.25	0.69	Lard weight	1.22	0.25
Skin fat weight of fore-body	4.07	0.73	Kidney weight	0.16	0.03
Skin fat weight of mid-body	5.98	1.17	Liver weight	1.33	0.25
Skin weight of rare-body	3.20	0.66	Stomach weight	0.66	0.18
Bone weight of fore-body	1.81	0.42	Heart weight	0.28	0.05
Bone weight of mid-body	1.17	0.25	Lung weight	0.95	0.20
Bone weight of rare-body	1.12	0.23	Length of small intestine	1,690.52	207.76
Lean percentage	48.00	4.21	Length of large intestine	438.91	97.25
The thickest back fat thickness	4.33	0.84	No. of rib	14.35	0.53
Back-fat thickness at A point	3.41	0.54	Body straight length	90.52	6.25
Back-fat thickness at B point	2.59	0.52	Body slanting length	76.38	5.56
Back-fat thickness at C point	2.69	0.56	Body weight	96.19	7.24
Loin muscle	28.40	4.75	Body length	116.35	7.68
Meat color	3.59	0.30	Body height	60.55	4.72
SFK meat color	86.82	16.77	Chest width	31.11	2.36
Marbling	3.11	0.38	Hump girth	73.65	15.95
pH value	6.32	0.36	Circumference of cannon	106.76	5.32
Water capacity	87.10	6.00	Back-fat thickness at point A of living body	2.87	0.59
			Back-fat thickness at point B of living body	2.48	0.45
			Back-fat thickness at point C of living body	2.52	0.47

investigate the effects of one IGFBP-4 single nucleotide polymorphism (SNP) on performance traits in population family.

MATERIALS AND METHODS

Animals

The ear notches of 17 pig breeds were collected, including 3 European breeds and 14 Chinese native breeds. They were Landrace (n = 36, Dongxiang County Pig Farm and Jiangxi Seed Pig Farm), Duroc (n = 30, Dongxiang County Pig Farm and JAU Seed Pig Farm), Large White (n = 60, Jiangxi Seed Pig Farm), Kele (n = 30, Kele County Seed Pig Farm, Guizhou Province), Zang (n = 30, Haiyang County Seed Pig Farm, Guizhou Province), Qinping (n = 30, Qinping County Seed Pig Farm, Hubei Province), Tongcheng (n = 30, Tongcheng County Seed Pig Farm, Huibai Province), GuangDong Large White (n = 30, Dongguang City Pig Farm, Guangdong Province), Ming (n = 30, Lanxi County Seed Pig Farm, Helongjiang Province), Janjiang Black (n = 32, Heihe Seed Pig Farm of Mian County, Shan'anxi Province), Bamei (n = 32, Bamei County Seed Pig Farm, Qinghai Province), Yujiang (n = 32, Yushan County Seed Pig Farm, Jiangxi Privince), Erhualian (n = 48, Chanshu Pig Farm of Jiangsu Province), Jinhua Pig (n = 30, Jinhua County Pig Farm, Zhejiang Perovince), Taoyuan Pig (n = 45, Taoyuan County pig Farm), Xiang (n = 30, Guizhou Provincial Seed Pig Farm) and Wild boar (n = 30, Jinhua County Pig Farm, Zhejiang Province). All of the collected pigs were unrelated within 3 generations. In order to detect the effects of different IGFBP-1 genotypes on production performance traits, we also investigated one population family of Lantang×Landrace (n = 172, gifted by Professor Chen Yaosheng, SCAU, China). A total of 48 traits were recorded according to the management experimental design (Li, 2001) (Table 1).

IGFBP-4 genotypes detected by PCR-SSCP

Genomic DNA was extracted from ear notches, using a phenol/chloroform extraction method followed by ethanol precipitation. Working dilutions of extracted DNA were prepared for each individual at a concentration of 50 ng/μg. Primers 5'-TGCCTCTCTTCCTTCTGCTGA-3' and 5'-ACAGAGACCCA CCTGCTTGG-3' (from 691 bp-844 bp, GenBank accession no. DQ537340) were used for polymerase chain reaction (PCR) amplification of IGFBP-4 gene. The PCR mixture containing 50 ng genomic DNA, 25 pmol of each primer, 25 μM of each dNTP, 1 unit of *Taq* DNA Polymerase (TAKARA) 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 300 s then 35 cycles: 94°C for 45 s, 60°C for 45 s, 72°C for 45 s, at last 72°C for 480 s.

The PCR products were separated by 12% non-denaturalization PAGE gel, after electrophoresis for 16 h at

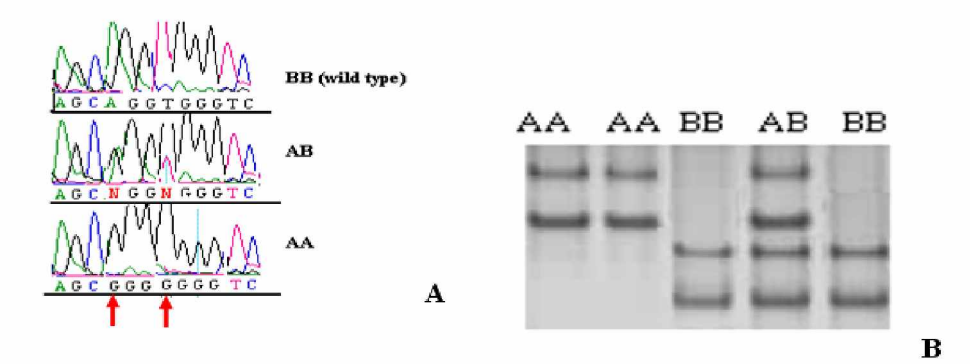

Figure 1. Photos for PCR-SSCP and sequencing of *IGFBP-4* gene. (A) Sequence photo for different genotypes, arrows indicate the mutant sites (A868G and T871A); (B) photo of the PCR-SSCP electrophoresis of different genotypes.

Table 2. Genotypic and allelic frequencies distribution of *IGFBP-4* gene in different pig breeds and *Chi*-square test for Hardy-Weinberg equilibrium

Breeds	Number	Genotypic frequencies (%)			Allelic frequencies (%)		H-W
		AA	AB	BB	A	B	χ^2
Kele	30	40.00 (12)[1]	20.00 (6)	40.00 (12)	50.00	50.00	4.05
Zang	30	33.33 (7)	16.67(5)	60.00 (18)	31.67	68.33	7.02*
Qinping	30	13.33 (4)	46.67 (14)	40.00 (12)	36.67	63.33	1.12
Tongcheng	30	33.33 (10)	13.33 (4)	53.33 (16)	40.00	60.00	6.65*
Guangdong Large White	30	10.00 (3)	26.67 (8)	63.33 (19)	23.33	76.67	5.62
Ming	30	13.33 (4)	33.33 (10)	53.33 (16)	30.00	70.00	3.65
Hanjiang Black	32	12.50 (4)	25.00 (8)	62.50 (20)	25.00	75.00	7.00*
Bamei	32	25.00 (8)	31.25 (10)	43.75 (14)	40.62	59.38	2.25
Yujiang Black	32	15.63 (5)	50.00 (16)	34.37 (11)	40.62	59.38	0.56
Jinhua	48	18.75 (9)	31.25 (15)	50.00 (24)	34.38	65.62	4.88
Erhualian	30	3.33 (1)	63.33 (19)	33.33 (10)	35.00	65.00	2.15
Taoyuan	30	23.33 (7)	53.33 (16)	23.33 (7)	50.00	50.00	0.05
Xiang	30	16.67 (5)	26.67 (8)	56.67 (17)	30.00	70.00	4.85
Wild boar	30	6.67 (2)	83.33 (25)	10.00 (3)	48.33	51.67	5.02
Large Yorkshire	60	46.67 (28)	33.33 (20)	20.00 (12)	63.33	36.67	4.63
Landrace	36	91.67 (33)	8.33 (3)	0.00 (0)	95.83	4.17	22.5**
Duroc	30	100.00 (30)	0.00 (0)	0.00 (0)	100.00	0.00	26.25**

[1] Digits in the blanket is the number of pigs; * $p<0.05$. ** $p<0.01$ ($\chi^2_{0.05} = 5.99$, $\chi^2_{0.01} = 9.21$).

constant 120 V. the gels were stained with silver-staining method as described by our lab. And different bands were named as AA, AB and BB genotypes. PCR products of different genotypes were recovered and sequenced on ABI 377 (ABI, Foster City, CA, USA).

Statistics

The χ^2-test was used for estimation of Hardy-Weinberg equilibrium within the breeds.

For data analysis, a fix model was established as follows:

$$Y_{ijm} = \mu + S_i + G_j + e_{ijm}$$

Where Y_{ijm} is the individual observation; μ is the population mean; S_i and G_j are the estimated effect for sex and genotype, respectively; and e_{ijm} is the random residue effect. Least square means (LSM) method was used for statistical analysis for data collected. Associations between IGFBP-4 genotypes and performance traits were analyzed using SAS system (1989) and significance between genotypes was estimated by Duncan's multiple range Test.

RESULTS

Using PCR-SSCP, we found one SNP. After sequence analysis, two mutation sites were detected. One was at A868G and the other was T871A (Figure 1). The A868G mutation was in exon 2, which induced Glu changed to Arg, and the T871A was in intron 2.

The genotype frequencies and allele frequencies of IGFBP-4 in 17 pig breeds were listed in Table 1.

From Table 2, the AA genotype frequency in European pig breeds was high, all were over 50%, and Duroc is the highest, its AA frequency was 10.00%. Meanwhile, the allele A frequency in European pig breeds was high.

Table 3. Relationship of different genotypes of *IGFBP-4* gene with the partial production performances in Lantang×Landrace population family

Genotype	No.	Fore-body weight	Skin fat weight of fore body	Bone weight of mid-body	Bone weight of rear-body	Back-fat thickness at C point	Fore-leg weight
AA	47	12.48±0.33^{a}	3.93±0.12^{b}	1.23±0.04^{a}	1.20±0.03^{a}	2.49±0.10^{b}	0.81±0.02^{a}
AB	58	11.99±0.19	4.00±0.11	1.13±0.04	1.08±0.04^{b}	2.72±0.16	0.74±0.03^{b}
BB	67	11.81±0.14^{b}	4.34±0.14^{a}	1.12±0.04^{b}	1.07±0.04^{b}	2.87±0.13^{a}	0.74±0.02^{b}
Genotype	No.	Rear- leg weight	Lard weight	Heart weight	Body length	Meat color	Marbling
AA	47	1.27±0.04^{a}	1.08±0.08^{b}	0.26±0.01^{B}	118.97±1.23^{A}	3.68±0.05^{A}	2.98±0.05^{Bb}
AB	58	1.20±0.03	1.28±0.09	0.28±0.01^{b}	115.59±1.45	3.58±0.05	3.16±0.06^{a}
BB	67	1.17±0.04^{b}	1.35±0.11^{a}	0.31±0.01^{Aa}	114.08±1.18^{B}	3.46±0.05^{B}	3.25±0.08^{A}

Values are LSM±SE.

Means with different character on superscript within the same row in the same breed are significantly different ($p<0.05$ or $p<0.01$).

especially in Duroc, its frequency was 100.00%. In Chinese native pig breeds, the B frequency was high (above 50%), especially the B frequency in Guangdong Large White was 76.67%. Chi-square test showed that among all the pig breeds we investigated, only Zang, Tongcheng, Hanjiang Black, Landrace and Duroc were not in Hardy-Weinberg equilibrium.

Table 3 listed the observed genotypes and the effects of different genotypes on production performances in Lantang×Landrace population family. In this population family, the genotypic frequencies of AA, AB and BB were 20.01%, 35.04% and 37.95%, respectively, and genotypic distribution was at Hardy-Weinberg equilibrium (χ^2 = $5.42<\chi^2_{0.05}=5.99$).

Among all the 48 traits we detected, 12 traits associated with different genotypes greatly. Pigs with AA genotype had higher fore-body weight, bone weight of mid-body, bone weight of rear-body , fore-leg weight and rear-leg weight than those pigs with BB genotype ($p<0.05$); while pigs carried BB genotype had higher back-fat thickness at C point and lard weight than those pigs with AA genotype ($p<0.05$); pigs with AA genotype had higher body weight than those with BB genotype; for meat quality traits, pigs with AA genotype had higher meat color than those of BB genotype ($p<0.01$), and pigs with BB genotype had higher marbling than those of AA and AB genotypes ($p<0.01$ and $p<0.05$).

DISCUSSION

As a member of IGFBPs, IGFBP-4 mostly inhibits the activity of IGF-1. In human, IGFBP-4 was found abundantly in bone. Zazzi et al. (1998) studied the structure and transcriptional regulation of human IGFBP-4 gene. While the study of porcine IGFBP-4 gene is a blank, only we previously mapped the gene on SSC12 q21-24 (Wenjun et al., 2005).

Using PCR-SSCP, we found a SNP of the known sequence, this SNP is the combination of a mutation in exon and a mutation in intron. The distribution of genotypic and allelic frequencies in Chinese and European pig breeds showed that Chinese carried higher frequencies of BB genotype and allele B, while European pig breeds carried higher frequencies of AA genotype and allele A. In our study we found that preponderant genotype of Chinese native pig breed had lower growth traits and had higher fat traits than those of the preponderant genotype of European genotype, these were consisted with the hypothesis that Chinese pigs had higher fat and lower growth rate than European pigs (Müller et al., 2000). Lantang is a Chinese native pig breed, it grows slower than that of the European pig breed, Landrace. Even though we had detected only 12 traits were associated with different genotypes, but other 36 traits (Table 1) like mid- and rare-body weight, lean percentage, loin muscle, length of small and large intestine in the pigs with AA genotype were higher than those pigs with BB genotype ($p>0.05$), while traits like back-fat thickness at B and C points, pH value in the pigs with AA genotype were lower than those pigs with BB genotype ($p>0.05$), these further verify the hypothesis that Chinese pigs had higher fat and lower growth rate than European pigs (Müller et al., 2000).

In porcine fetal liver and muscle, Gerrard et al. (1999) found that with the pregnancy went on, IGFBP-4 expression level in skeletal muscle decreased and no change was found in liver, which indicates IGFBP-4 expression is time- and tissue-dependent in fetal liver and muscle. Harrell et al. (1999) found that among 10 d to 129 d of age after birth, circulating concentration of IGFBP-4 was unchanged. Hathaway et al. (2003) also found that serum levels of nonglycosylated IGFBP-4 were not affected by either weaning or Aureozol supplementation. Clapper et al. (2000) found that at 70 d of age, boars and barrows had greater relative amounts of 24-kDa IGFBP-4 than gilts, but from 84 d of age through 140 d of age, its concentration did not change among boar, barrow and gilt, which indicated 24-kDa IGFBP-4 may regulate the porcine growth at early stage, and another form 28-kDa IGFBP-4 were greater in boars than in barrows or gilts from 84 to 140 d of age. Perhaps the increase in relative amounts of the 28-kDa form

of IGFBP-4 in boars acts to protect cells from overstimulation by binding with IGF-I and preventing it from interacting with its receptor (Cohick et al., 1993). In mammals, 24-kDa IGFBP-4 is the major form of this IGFBP-4, relative stable of this protein in the body helps animal to grow and develop normally.

On SSC12 many QTLs were found. IGFBP-4 was mapped on SSC12 q21-24, and linked to S0147. In three different population families, Yue et al. (2003) found many QTLs affected fat contents and meat quality, and in wild boar×Meishan family, a QTL affected 10th-rib back fat thickness was located near S0147, and in Meishan×Pietrain family, a QTL affected CK20-value was found, in our study, we found that IGFBP-4 affected lard weight, marbling and meat color. Malek et al. (2001a, b) found a QTL (81 cM) affected the back fat thickness at the last rib and a QTL (73 cM) affected meat color, in our study, we found IGFBP-4 affected back-fat thickness at C point and meat color, but these two QTL were far from S0147 (61.9 cM), so they may not affect the gene or less. Paszek et al. (1999) found a QTL (SW37-SW467, 58-75 cM) affected the average daily gain between 35-65 kg, in our study, we did not find IGFBP-4 associated with growth rate at different growth stages, but we found that IGFBP-4 associated with body length and other traits such as fore-body weight, bone weight and legs weight, and these traits can reflect the growth status. Meanwhile, GH and IGF1R genes were mapped on SSC12, and many studies found that these two genes affected porcine growth and development (Wang et al., 2003; Wang et al., 2006), so it is important to study the effect of SSC12 on porcine growth and development.

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