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A novel polymorphism of exon2 in *IGF2* gene and its influence on growth and backfat traits of pig breeds

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Insulin-like growth factor 2 (IGF2) is a potent cell growth and differentiation factor of many tissues, particularly muscles and fat (Duan *et al.* 2010*,* Burgos *et al.* 2012), and is considered a candidate molecular marker of meat production in domestic animals. In the porcine IGF2 gene, a number of polymorphic loci were identified and found to be associated with important traits, such as the number of born piglets, muscle development and fat deposit, lean meat ratio, backfat thickness and IGF2 gene expression, etc. (Nezer *et al.* 1999*,* Liu *et al.* 2003*,* Vykoukalová *et al.* 2006*,* Xue and Xu 2008).

Several SNPs in exon 2 of IGF2 gene were reported in different species, and they influenced various traits. For example, A C292T transition in exon 2 in the bovine IGF2 gene affected LM (*musculus longissimus dorsi*) area, backfat thickness, average daily gain, diary and meat production and the carcass fat, etc. (Goodall and Schmutz 2007*,* Bagnicka *et al.* 2010). In chicken, a C71G mutation in the exon 2 was associated with growth and carcass traits (Wang *et al.* 2005). However, the association of polymorphism in the exon 2 of IGF2 gene with growth, meat and fat traits in pigs has not been shown. Using PCR-SSCP method, here we identified a novel single nucleotide polymorphism in exon 2 of the porcine IGF2 gene, and analyzed the association of this SNP with growth and fat deposition traits.

Landrace (87), Large White (91), Laiwu pigs (119), Large Polin pigs (78), Yimeng Black pigs (107) and Licha pigs (81) from 2006 to 2008 were randomly selected as the tested animals. Birth litter size and birth parity number were recorded for all samples. The 10 ml blood were exsanguinated from the anterior vena cava of the tested animals, added to anticoagulant citrate dextrose solution (6:1), and stored at -70°C for later using. Backfat thickness was defined as the mean of the depth of fat (mm) measured by ultrasound (A-mode) at the level of the last rib approximately 8 cm on both sides of mid line.

Genomic DNA was extracted as described (Chacon-Cortes *et al.* 2012). Primer sequence for exon 2 of IGF2 was designed according to the IGF2 DNA sequence (AY044828) of porcine published in the GenBank (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) was used to design. The primer sequences were 5'-GACCCATTATGATGCACACG-3' and 5'-CTGCACCCAAACACTCAATG-3', and were synthesized.

The PCR amplification reactions system was: 10 × PCR buffer, 2.5 µl, 1.5 µl 25 mmol / L $MgCl_2$, 1.0 µl 0.01 mmol / L of primers, 2.5 µl 2.0 mmol / L dNTPs, 0.2 µl 5U/µl Tag enzyme, 2 µl 50 mg / L DNA. The PCR program was: 94°C 5 min; 95°C 45 sec, 58°C 45 s, 72°C extension of 45 sec, 30 cycles; 72°C extension of 8 min, and 4°C preservation. PCR products were electrophoresed on 2% agarose gel, and the amplification results were analyzed by gel imaging system.

Genotyping of the amplified region was carried out using the SSCP method as described (Zeng *et al.* 2013) with modifications. In brief, PCR product (1µl) and 5 µl of loading (98% formamide, 0.025% bromophenol blue, 0.025% xylene Green, 10 mmol / L EDTA (pH8.0), 10% glycerol) were mixed, and kept at 98°C for 10 min to denature. The mixture was then put in the ice bath for 5 min to keep the products at denatured state. The denatured PCR products were analyzed by non-denaturing polyacrylamide gel electrophoresis (Acr: Bis = 29:1) for 10 ~ 11 h, and silver stained. The homozygous were sequenced on the PRISM 377-96 automatic DNA Sequencer, using the reagent of BigDye terminator v2.0 and the method of Sanger dideoxy chain termination.

Values of the genotype frequencies were calculated for the examined pig breeds and were analyzed by the significance test. Based on the fixed effects model, the effects on birth weight, weaning weight, 6-month weight and backfat thickness at 6 month of age, species and IGF2 genotype were analyzed using the GLM (General Linear Model) process of SPSS software package. The statistical analysis model is:

$$Y_{ijkl}=\mu+B_i+m_k+e_{ijkl}$$

where, Y_{ijkl} is the observed value of *l*th individual from the breed *i*, of genotype *k*, in the *j*th farm-year-season; μ is the least square means of the observed values; B_i is the

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effective value of the breed *i*; m_k is the effective value of the genotype *k*; and e_{ijkl} is the random residual effect corresponding to the observed value.

The size of the PCR product amplified by the primers designed from the genomic DNA template was consistent with the expected size, and no non-specific bands existed. Polymorphic site was detected by the SSCP analysis, and defined as AA, AB, BB genotypes respectively. Homozygous were sequenced, and a polymorphic site A4G was found. Birth litter size and birth parity number were evaluated using multifactorial statistical models. No statistical significance was found in these factors among the tested breeds.

Differences of genotype frequencies exist among various pig breeds (Table 1). Allele A was the prevalent gene in Landrace and Large White pig breeds, with the allele frequencies of 0.59 and 0.71 respectively; allele B was the prevalent gene in Laiwu, big Polin, Yimeng Black and Licha Black pig breeds, with the allele frequencies of 0.73, 0.74, 0.69 and 0.72 respectively. Multiple comparison results showed that significant difference (P <0.01) exist when compare the genotype distribution of Landrace and Large White pig breeds with that of Laiwu, big Polin, Yimeng Black and Licha Black pig breeds. No significant difference (P> 0.05) on genotype distribution was detected among other pig breeds (Table 2).

Least-squares analysis showed that among different pig breeds, there exist highly significant differences (P <0.01) on birth weight, weaning weight, and 6-month weight, and significant difference (P <0.05) on 6-month backfat thickness; while no significant difference (P > 0.05) of the examined traits were detected among every farm – year – season status. Among different genotypes, there were significant differences (P < 0.05) on birth weight and backfat thickness, but not (P > 0.05) on weaning weight and 6-month weight (Table 3). The birth weight and backfat thickness of individuals with genotype of AA are significantly different from those of individuals with genotypes of AB and BB. The order of the three genotypes in the birth weight and the back fat thickness is AA> AB>

Table 1. The distribution of genotype and gene frequencies in different pig breeds

Genotype and Gene frequenciesm	Landrace	Large White	Laiwu	Dapuliang	Yimeng black	Licha Black
AA	0.44 (38)	0.64 (58)	0.09 (11)	0.08 (6)	0.17 (18)	0.17 (14)
AB	0.31 (27)	0.15 (14)	0.36 (43)	0.36 (28)	0.29 (31)	0.22 (18)
BB	0.25 (22)	0.21 (19)	0.55 (65)	0.56 (44)	0.54 (58)	0.60 (49)
A	0.59	0.71	0.27	0.26	0.31	0.28
B	0.41	0.29	0.73	0.74	0.69	0.72

Number of body was shown in bracket. For the data statistics of all traits and alleles, see supplementary table.

Table 2. The χ^2 test of genotypes in different pig breeds

	Landrace	Large White	Laiwu	Dapuliang	Yimeng black
Large White	4.6407				
Laiwu	27.1966**	24.1674**			
Dapuliang	29.7293**	17.4311**	1.4251		
Yimeng Black	19.9431**	19.3160**	2.9780	6.0174	
Licha Black	14.5868**	25.7384**	4.8753	3.9582	2.7950

Value with ** differ at P < 0.01.

Table 3. Effect of the source of variation on the measured traits

Source	df[2]	F value			
		Birth Weight	Weaning Weight	Weight at the six-month	Backfat thickness
Breed	5	17.421**	21.335**	25.692**	11.661*
Fys[1]	5	4.055	8.829	6.905	5.016
Genotype	2	8.322*	3.740	2.734	7.635*

[1] Farm-year-season; [2] degree of freedom. Value with * differ at P < 0.05; Value with ** differ at P < 0.01 the abomasal mucosa after the administration of larvae.

Table 4. Least square means and standard errors for the measured traits of different genotypes

Genotype	Birth Weight	Weaning Weight	Weight at the six-month	Backfat thickness
AA	1.821± 0.154[a]	7.441± 0.054	110.433± 12.115	7.388± 1.061[b]
AB	1.499± 0.212[b]	7.099± 1.118	112.328± 10.074	8.827± 1.175[a]
BB	1.502± 0.146[b]	7.574± 0.327	113.502± 9.107	8.764± 1.377[a]

Means with the different superscripts within the same column differ significantly (P < 0.05).

Supplementary table. Average backfat thickness for different breeds

Breed	Genotype	N	Backfat thickness
Landrace	AA	38	10.50±2.36
	AB	27	10.44±2.44
	BB	22	10.45±2.61
Large White	AA	58	10.59±2.37
	AB	14	11.14±2.44
	BB	19	10.63±2.50
Laiwu	AA	11	7.64±1.57
	AB	43	7.42±1.38
	BB	65	7.60±1.40
Dapuliang	AA	6	6.50±1.05
	AB	28	7.57±1.40
	BB	44	7.68±1.41
Yimeng black	AA	18	7.78±1.35
	AB	31	7.42±1.41
	BB	58	7.66±1.38
Licha Black	AA	14	7.71±1.44
	AB	18	7.06±1.35
	BB	49	7.49±1.42

Data are presented as Mean ±SD. Backfat thickness was recorded as 6 month of age.

BB and AA <AB <BB, respectively (Table 4).

In pigs, a G/A transition was identified at the 41st nucleotide of IGF2 exon 2, but its effects on meat production and other traits have not been investigated (Li *et al.* 2008). Here we identified a novel SNP of G4A transition in exon2 of the porcine IGF2 gene, which significantly impacted on birth weight and backfat thickness, but not on weaning weight and 6-month weight. These findings of ours and others highlight the importance roles of exon 2 in fetal growth, backfat and other traits.

We showed that allele A was the dominant gene in the introduction pig breeds such as Landrace and Large White pig breeds, while allele B was the dominant gene in the Shandong local pig breeds including Laiwu, big Polin, Yimeng Black and Licha Black pig breeds. Our results are consistent with that of Nezer *et al.* (1999), suggesting long-term natural selection of IGF2 genotypes can produce unfavorable impacts on growth rate and fat deposition traits among different pig breeds. Therefore, protection and usage of the local pig breeds should be strengthened.

The polymorphism of the exon 2 should not alter the amino acids, but may impact on imprinting to affect fetal growth. As shown in a previous study, a G42A transition in exon 2 did significantly altered paternal imprinting of the porcine IGF2 gene, and the imprinting effect was lost in one week old pigs (Li *et al.* 2008). Consistently, the G4A polymorphism in our study affected birth weight but not weaning weight and 6-month weight, suggesting this polymorphism impacted fetal growth via influencing imprinting. In contrast, the impact on 6-month backfat thickness may involve mechanisms other than imprinting. A candidate mechanism is altered expression of IGF2 gene in adipose or muscle tissues. It was found that the G3072A transition in intron 3 of the porcine IGF2 gene resulted in a 3-fold higher expression of IGF2 mRNA in postnatal skeletal muscle, which is believed to be associated with increased lean growth (Gardan *et al.* 2008). The growth of lean tissue, in turn, may suppress fat tissue development through intercellular signaling between adipose and muscle tissues (Kokta *et al.* 2004). The expression profile of IGF2 gene in relevance to the exon 2 polymorphism is a primary goal of our future study.

The A4G polymorphic loci found in our study could be used as genetic marker for muscle growth and fat deposition in the porcine marker-assisted selection. Such selection is expected to raise the birth weight and backfat development of the offspring in the breeding process. One limitation of our study is that we have not examined the lean meat content and intramuscular fat deposit that are important traits for the meat quality. These traits should be evaluated in the future studies.

SUMMARY

Using the PCR-SSCP method, we identified a novel polymorphism (A4G) in exon 2 of the porcine IGF2 gene, producing three genotypes (*AA*, *AB*, *BB*). Significant differences of genotype distribution were found comparing the Landrace and the Large White with the Laiwu, the Dapuliang, the Yimeng and the Licha Black pig breeds. Least square analysis shows that individuals of the *AA* genotype have significantly higher birth weight and significantly lower backfat thickness than those of *AB* and *BB* genotypes, with the orders of AA > AB > BB and BB > AB > AA for birth weight and 6-month backfat thickness, respectively. These data provide references for the conservation and breeding processes of different pig breeds.

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REFERENCES

Bagnicka E, Siadkowska E, Strzalkowska N, Zelazowska B, Flisikowski K, Krzyzewski J and Zwierzchowski L. 2010. Association of polymorphisms in exons 2 and 10 of the insulin-like growth factor 2 (IGF2) gene with milk production traits in Polish Holstein-Friesian cattle. *The Journal of Dairy Research* **77**: 37–42.

Burgos C, Galve A, Moreno C, Altarriba J, Reina R, Garcia C and Lopez-Buesa P. 2012. The effects of two alleles of IGF2 on fat content in pig carcasses and pork. *Meat Science* **90**: 309–13.

Chacon–Cortes D, Haupt L M, Lea R A and Griffiths L R. 2012. Comparison of genomic DNA extraction techniques from whole blood samples: a time, cost and quality evaluation study. *Molecular Biology Reports* **39**: 5961-66.

Duan C, Ren H and Gao S. 2010. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation. *Gen Comp Endocrinol* **167**: 344–51.

Gardan D, Gondret F, Van den Maagdenberg K, Buys N, De Smet S and Louveau I. 2008. Lipid metabolism and cellular features of skeletal muscle and subcutaneous adipose tissue in pigs differing in IGF-II genotype. *Domestic animal endocrinology* **34**: 45–53.

Goodall J J and Schmutz S M. 2007. IGF2 gene characterization and association with rib eye area in beef cattle. *Animal Genetics* **38**: 154–61.

Kokta T A, Dodson M V, Gertler A and Hill R A. 2004. Intercellular signaling between adipose tissue and muscle tissue. *Domestic Animal Endocrinology* **27**: 303–31.

Li C, Bin Y, Curchoe C, Yang L, Feng D, Jiang Q, O'Neill M, Tian X C and Zhang S. 2008. Genetic imprinting of H19 and IGF2 in domestic pigs *(Sus scrofa)*. *Animal Biotechnology* **19**: 22–27.

Liu G L, Jiang S W, Xiong Y Z, Zheng R and Qu Y C. 2003. [Association of PCR-RFLP polymorphisms of IGF2 gene with fat deposit related traits in pig resource family]. *Yi chuan xue bao = Acta genetica Sinica* **30**: 1107–12.

Nezer C, Moreau L, Brouwers B, Coppieters W, Detilleux J, Hanset R, Karim L, Kvasz A, Leroy P and Georges M. 1999. An imprinted QTL with major effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. *Nature Genetics* **21**: 155–56.

Vykoukalová Z, Knoll A, Dvoøák J and Èepica S. 2006. New SNPs in the IGF2 gene and association between this gene and backfat thickness and lean meat content in Large White pigs. *Journal of Animal Breeding and Genetics* **123**: 204–07.

Wang G, Yan B, Deng X, Li C, Hu X and Li N. 2005. Insulin-like growth factor 2 as a candidate gene influencing growth and carcass traits and its bialleleic expression in chicken. *Science China Ser. C.-Life Sci.* **48**: 187–94.

Xue H L and Xu L X. 2008. [Genetic polymorphisms and genetic effects of IGF2 gene in pigs]. *Yi chuan = Hereditas / Zhongguo yi chuan xue hui bian ji* **30**: 179–84.

Zeng Y F, Ding X Z, Cheng S R and Yu S J. 2013. Genetic polymorphisms of the IGF-II gene intron 8 coding region and its association with growth and carcass traits in yak. *Genet Mol Res* **12**: 6602–10.