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Associated analysis of single nucleotide polymorphisms of *IGF2* gene's exon 8 with growth traits in Wuzhishan pig

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Abstract A new SNP located in *IGF-2* gene of Wuzhishan pig was detected while a C → T silent mutation at position 17 of exon 8 was detected by Single-Strand Conformational Polymorphism (SSCP). The value of polymorphism information content (PIC) indicated that this locus had intermediate polymorphism information content ($0.25 < \text{PIC} < 0.5$). The results of the fitness of Hardy-Weinberg equilibrium was in disequilibrium ($P < 0.01$). SAS analysis together with the multiple comparisons between polymorphisms and growth traits showed that: the differences of carcass weight ($P = 0.024$), withers height ($P = 0.037$) and chest girth ($P = 0.025$) in 10-month-old generation group were very remarkable.

Keywords Wuzhishan pig · *IGF2* gene · Single nucleotide polymorphisms · Growth traits · Correlation analysis

Introduction

Wuzhishan pig, which is famous for its typical body weight and size, is becoming endangered breed in south China with high hereditary economic value. The physiology characteristics and pathogenesis of Wuzhishan pig are similar to human which has been used in many biologic experiments, for instance, cardiovascular disease, skin burn and new drug evaluation, etc. In the past decade it had been used as a valuable model animal, especially in the researches of dwarf mechanism of Wuzhishan pig.

The purport of current genetics research in livestock, as well as in plant and human, is to identify the polymorphisms which should be responsible for the variability in complex traits since most traits and disorders have a multifactorial background indicating that they are controlled by environmental factors as well as an unknown number of quantitative trait loci (QTLs).The identification of mutations underlying QTLs is a challenge, because each locus only presents a fraction of the phenotypic variation. *IGF2* (insulin-like growth factor 2) gene located in a telomeric position on pig chromosome II, which harbors a paternally expressed mutation that increases muscle growth and leanness [1–3]. In this experiment, the genetic polymorphism was detected by Single-strand Conformational Polymorphism (SSCP) and sequencing methods while analyses in combination with growth traits were associated.

Materials and methods

Sampling and DNA extraction

The ear tissue samples including 137 individuals were random selected from Wuzhishan pig groups aged from 6 months to 15 months and stored at −70°C after immersing in 75% ethanol. The sample pigs were kept in Wuzhishan pig breeding farm (CATAS, Tropical crops genetic resources institute, P. R. China) and the growth traits (carcass weight, withers height, body length, chest girth) were recorded every month.

Genomic DNA was extracted from ear tissue sample followed as the instructions in "Molecular Cloning: a

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laboratory manual" and detected by 0.8% agarose gel electrophoresis [4]. The genome DNA was diluted to 50 ng/μL after being estimated by ultraviolet spectrophotometer.

Primer design, PCR protocols

Primers (Table 1) were designed by primer premier 5.0 (Premier biosoft international, Palo Alto, CA, USA) based on the fragments of *IGF2* gene (Genbank accession No. AY044828).

The PCR reactions were carried out in a total volume of 20 μL solution containing 50 ng template DNA, 1×buffer (Tris–HCl 100 mmol/L,pH 8.3; KCl 500 mmol/L), 0.25 μmol/L primers, 2.0 mmol/L MgCl2, 0.25 mmol/L dNTPs, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The conditions for PCR amplification were a denaturing step at 95°C for 5 min, 30 cycles of 94°C for 30 s, appropriate Tm (Table 1) for 30 s, and 72°C for 30 s, and final elongation at 72°C for 8 min. PCR products were analyzed by agarose gel electrophoresis (1.5% in TAE) and visualized under UV light after ethidium bromide staining.

Genotypes of 137 individuals were ascertained by Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP). A total of 2.0 μL PCR product was mixed with 8 μL of the denaturation solution (50 mmol/L NaOH, 1 mmol/L EDTA), 1 μL of the loading buffer containing 0.25% bromophenol blue and 0.25% xylene cyannol, denatured for 10 min at 95°C, and rapidly chilled in ice block. The samples were electrophoresed in 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE). The electrophoresis was programmed as 250 V, 40 mA (preelectrophoresis) for 10 min for the first step, with the Silver Stain step of 150 V, 24 mA (Kucharczyk Techniki Elektroforetyczne) for 8 h after that. A refrigerated circulator was used to control the temperature (4°C) of gels.

Traits and statistical analysis

Genotypes and allelic frequencies were calculated by using the POPGENE software (ver. 1.31). The Hardy-Weinberg equilibrium of the mutation was exam by $\chi 2$ test.

The traits includes carcass weight (CW), withers height (WH), body length (BL), chest girth (CG). Analysis of associations between the *IGF2* genotypes and traits that reflects mastitis traits was carried out with GLM procedure by using SAS software (Statistical Analysis System 8.2,SAS Institute Inc.) according to the formula given below:

$$Y_{ij} = \mu + G_i + E_{ij}$$

Where Y_{ij} was the record of individual j with genotype i; μ was defined as population mean; G_i was defined as the genotype effect while E_{ij} was defined as the residual effect.

Results

Results of polymorphisms

The 190 bp fragment of *IGF2* gene of Wuzhishan pig which was named as exon8 was amplified by PCR (Fig. 1). The genetic polymorphism of the population was detected by SSCP in the locus of Exon8 (Fig. 2). The polymorphism

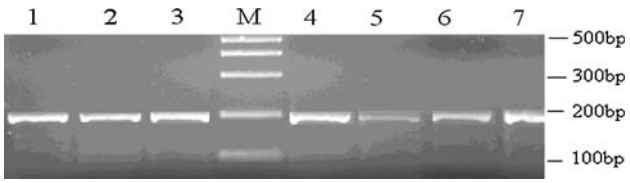

Fig. 1 PCR product products of Ex8 primer (190 bp); M: 100 bp DNA Marker

Table 1 Primer sequences of porcine insulin-like growth factor 2 gene

Primer name	Primer sequence	Size (bp)	PCR *Tm* (°C)	Amplication region
Ex5-1	Sense: 5′CGTCCTCCCCAAACAATC3′	159	56	23,241–23,401
	Anti: 5′TATCGCAAACCGAACAGC3′			
Ex5-2	Sense: 5′CCCGTCCTCCCCAAACAA3′	161	58	23,430–23,589
	Anti: 5′TATCGCAAACCGAACAGC3′			
Ex-7	Sense: CTTGTCTTTGCCCCCAGAT	198	54	26,979–27,177
	Anti: CCCCGCTGAGCTACTTACTG			
Ex-8	Sense: CCTTGGTCCTGTGGGACTT	190	58	29,046–29,235
	Anti: AGAGAGGGGCTGCCTTACC			
Ex-9	Sense: GGCAAGTTCTTCCGCTATGA	224	57	29,500–29,723
	Anti: AATTTGGCTCACTTCCGATG			

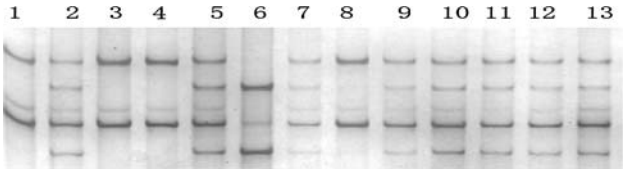

Fig. 2 Electrophoresis of PCR-SSCP for *IGF2* gene exon8; genotype AA is 1, 3, 4, 7, 8; genotype *BB* is 6; genotype *AB* is 5, 9–13

of exon8 was induced by C-T single nucleotide polymorphism (SNP) at the site of 17 bp in *IGF2* gene exon8 (Fig. 3). And the other four fragments (Ex5-1, Ex5-2, Ex7 and Ex9) of *IGF2* gene were amplified by PCR as well which included the promoter region of exon5, leader sequence exon7 and exon9. But not a single mutation site was found in those fragments.

Genotypic and allelic frequencies

The genotypic and allelic frequencies of the locus were shown as in Table 2. Among the population, allele B was the predominant one of Ex8 locus. The maximum value of *AB* genotypic frequency and minimum value of *BB* genotypic frequency were obtained in Ex8 locus as well.

Genetic characters in the population

Table 3 shows the value of heterozygosis (*H*), effective number of alleles (*Ne*), polymorphism in formation contents (*PIC*), and $\chi 2$ value. The *PIC* ranged from 0.25 to 0.50, which indicated the locus among the population was moderate polymorphism. The results of the fitness of Hardy-Weinberg equilibrium was in disequilibrium ($P < 0.01$).

The relationship between growth trait and the polymorphism of *IGF2* gene

The association analysis results (Table 4) show that the effect of *IGF2* mutation was found on CW, WH and CG in individuals aged 10 months. We speculated that the pig with the *A* allele grew more rapidly and gained weight higher than those with the *B* allele.

Table 2 Gene and genotype frequency

	Genotype				Allele	
	N_{AA} (P_{AA})	N_{BB} (P_{BB})	N_{AB} (P_A)	Number	*A*	*B*
n Sample	64	4	69	137		
Frequency	0.467	0.029	0.504	1.000	0.518	0.482

Table 3 Polymorphism genetic of exon8 of *IGF2* of Wuzhishan pig

N	H_o	H_e	PIC	N_e	χ^2
137	0.496	0.504	0.384	2.016	0.000

Discussion

As an important candidate gene effected muscle growth and lipidosis, *IGF2* attracted much attention on the polymorphism and association. A G/A transition in the 5′leader sequence was detected in an intercross population between Large White and Piétrain pig which contains 1032 F2 offspring [5]. One SNP caused by a nucleotide substitution in intron 3 of *IGF2*. The mutation occurs in an evolutionarily conserved CpG island that is hypomethylated in skeletal muscle, and affected muscle growth, fat deposition and size of the heart in pigs [1]. A causative mutation, IGF2-in3-G3072A, had substantial effect on muscle growth and backfat thickness ([6]. In this experiment, allele *A* was confirmed as the predominant one and the frequency value of *AB* genotype attained maximum in all the different age groups. The locus showed intermediate polymorphism and the result of $\chi 2$ test indicated that the locus in the population fitted with Hardy-Weinberg disequilibrium ($P < 0.01$). The Hardy-Weinberg disequilibrium might not be attributed to the fact that mutation which leading to genetic dominance of mastitis resistance but need selection through long-term.

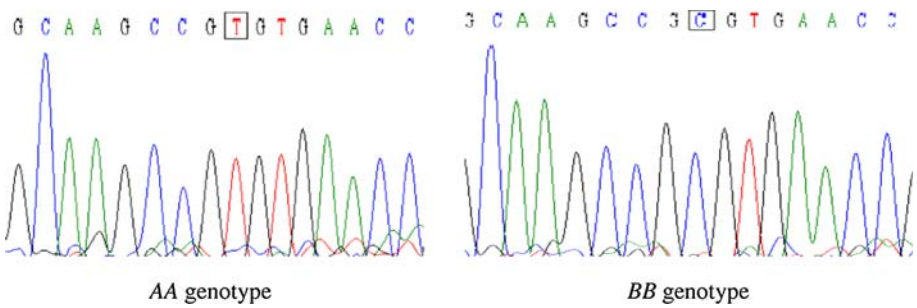

Fig. 3 Sequence comparison of *AA* and *BB* genotypes

Table 4 The correlation analysis between different genotypes and four growth characteristics of exon8 of *IGF2* gene

Months	Genotype	Trait				
		Number	BW/kg	BL/cm	WH/cm	CG/cm
6	AA	8	14.90 ± 1.74	56.12 ± 3.04	30.75 ± 1.48	55.75 ± 3.45
	AB	13	14.13 ± 1.76	54.92 ± 4.25	30.50 ± 1.52	54.46 ± 3.30
	BB	1	15.20 ± 0.00	57.00 ± 0.00	31.00 ± 0.00	57.00 ± 0.00
	P		0.583	0.364	0.557	0.429
8	AA	9	20.58 ± 2.16	60.44 ± 1.01	33.88 ± 0.78	61.77 ± 1.20
	AB	12	19.43 ± 2.06	60.00 ± 1.12	33.20 ± 0.94	61.08 ± 1.31
	BB	1	25.00 ± 0.00	61.00 ± 0.00	35.00 ± 0.00	64.00 ± 0.00
	P		0.756	0.890	0.880	0.442
10	AA	10	33.35 ± 3.45*	63.50 ± 1.08	36.20 ± 0.58*	66.60 ± 0.96*
	AB	11	31.88 ± 4.27	63.00 ± 1.34	36.00 ± 0.80	66.27 ± 1.27
	BB	1	26.10 ± 1.27*	62.00 ± 0.00	35.00 ± 0.00*	64.50 ± 0.70*
	P		0.024	0.735	0.037	0.025
12	AA	24	44.60 ± 3.63	68.00 ± 1.58	39.18 ± 1.08	72.64 ± 2.51
	AB	19	44.36 ± 3.66	67.77 ± 1.59	39.22 ± 1.16	72.77 ± 2.46
	BB	1	40.75 ± 1.06	66.50 ± 0.70	38.00 ± 0.00	70.00 ± 0.00
	P		0.836	0.794	0.757	0.386
15	AA	13	60.18 ± 8.19	76.27 ± 3.97	43.27 ± 1.94	83.45 ± 5.44
	AB	14	58.56 ± 6.99	76.93 ± 5.81	43.43 ± 1.59	83.60 ± 5.47
	P		0.558	0.711	0.828	0.543

Traits: *CW* carcass weight, *WH* withers height, *BL* body length, *CG* chest girth

* Means significantly different ($P < 0.05$)

However, we haven't found any putative SNP in exon 8 of Wuzhishan pig and its infection of growth were reported yet. The possible reason might be that Wuzhishan is so different from other pig breeds, especially in its traits of growth and dwarfism. This experiment was restricted by sample resource and number. It would be necessary for the experiments in future to verify the result by research in bigger population.

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