FSHR Gene Mutation and Its Effect on Litter Size in Pigs

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ABSTRACT : The polymorphism of the locus FSHRB in intron6-Exon7 of FSHR gene was investigated by PCR-RFLPs in Erhualian, Large White and Landrace×Large White; The association of polymorphism and litter size was analyzed by using SAS. The results showed that 1) the polymorphism of the locus FSHRB was significantly associated with litter size; 2) the total born number (TBN) and number born alive (NBA) of the sows with BB genotype were increased ($p<0.05$) with additive effects of 1.02-1.42 and 1.04-1.27 pigs per litter, respectively; 3) among the sows with genotype AA, AB or BB, there was an insignificant difference in born weight and weaning weight. This gene may be an effective potential tool used in conjunction with traditional selection methods. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 2 : 179-182)***

Key Words : FSHR Gene, Litter Size, Pig, Candidate Gene

INTRODUCTION

The follicle stimulating hormone receptor (FSHR) has been suggested as candidate gene for genetic variation in pig reproductive traits, because of the essential role of its product in the physiological mechanisms related to reproductive function, including the initiation and maintenance of qualitatively normal spermatogenesis and follicular development. A mutation in Exon7 of FSHR results in hypergonadotropic ovarian dysgenesis (ODG) in human (Aittomaki et al., 1995). A single mutation of ESR gene on chromosome1 has a large effect on litter size (Routhschild et al., 1996). The positive ESR allele increases 0.4-1.15 piglets born per litter (Routhschild et al., 1996; Short et al., 1997). The gene of PRLR is significantly associated with increasing total number born and number born alive ranged from 0.66 to greater than 1 pig per litter (Vincent et al., 1998). There is not any research that describes if the mutation in intron6-exon7 of FSHR exists in the pigs and leads to change the litter size. We want to know if the mutation locus of FSHRB are associated with the litter size in sows. In this paper we report the polymorphism of the locus FSHRB in intron6-exon7 and the relationship between the genotypes and litter size in pigs in molecular genetics.

MATERIALS AND METHODS

Pig population and traits

Blood samples of 3 populations were collected including Large White Sows from the affiliated farms of Huazhong Agricultural University; Landrace×Large White sows from Zhanghuyuan pig breeding farm, HuBei province; Erhualian sows from Xishan pig breeding farm, Jiangsu province.

Performance trains included total number born (TNB) which was calculated as live births plus stillborn animals and mummified fetuses, number born alive (NBA), body weight at birth (BW) and weaning weight of 35 days (WB35) in Large White and Landrace×Large White, body weight of 20 days (BW20) and weaning weight of 45 days (WB45) in Erhualian.

PCR and PCR-RFLP condition

Genome DNA was extracted from blood white cells and the DNA extraction procedure was described by Xiong (1999). The polymorphism at locus of the FSHRB was identified by PCR-RFLP (Zhang et al., 2000; 2001). Polymerase chain reaction (PCR) of the locus FSHRB was performed in 25 μl microcentrifuge tube containing 90 ng porcine genome DNA, 1×PCR buffer, 0.4 μM each primer, 0.2 mM dNTP, 2 mM $MgCL_2$, 1 unit Taq DNA polymerase (Amersham Pharmacia Biotech). The primer of FSHRB locus in intron6-Exon7 was as follows: Forward primer: 5'-CTTCTTTGCCATCTCTGC-3', Reverse primer: 5'-TTC TGAGCTAAATGGCTT-3'. After an initial 94°C denaturation step for 3 min, the PCR was carried out at 94°C (1 min), 58°C (1 min), 72°C (1 min) for 35 cycles, the last extension step at 72°C for 5 min. Digestion of 16 μl PCR product was performed with 4 unit of Bsm I at 37°C for 3 h. Electrophoresis was at a current of 24 mA for 3-4 h at a constant temperature of 15°C on the 8% polyacrylamide gel (29:1).

Statistical analysis

The TNB and NBA traits were analyzed with a sire

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Table 1. Genotype frequency of different population

population	Genotype and gene	Number	Frequency
Landrace×	AA	18	5.03
Large White	AB	264	73.74
	BB	76	21.23
	A		41.90
	B		58.10
Erhualian	AA	0	10.64
	AB	5	89.36
	BB	42	5.32
	A		94.68
	B		
Large White	AA	14	5.26
	AB	189	71.05
	BB	63	23.69
	A		40.79
	B		59.21

model including fixed effects of herd season, service type and parity. Sire was included as a random effect. All data were analyzed by the Duncan method of one way ANOVA procedure by using SAS software package. Chi-square test was used.

RESULTS

Allele frequency

Digestion of 78bp FSHRB locus PCR product gave the polymorphism of three genotypes. Genotype AA had the only 78 bp fragment, while BB with 52 bp and 26 bp, AB with 78 bp, 52 bp and 26 bp fragment (showed in the Figure 1). The Figure 1 showed the electrophoresis of locus FSHRB, there were only two genotypes AB and BB and the fragment of 26bp was too small to be showed on it.

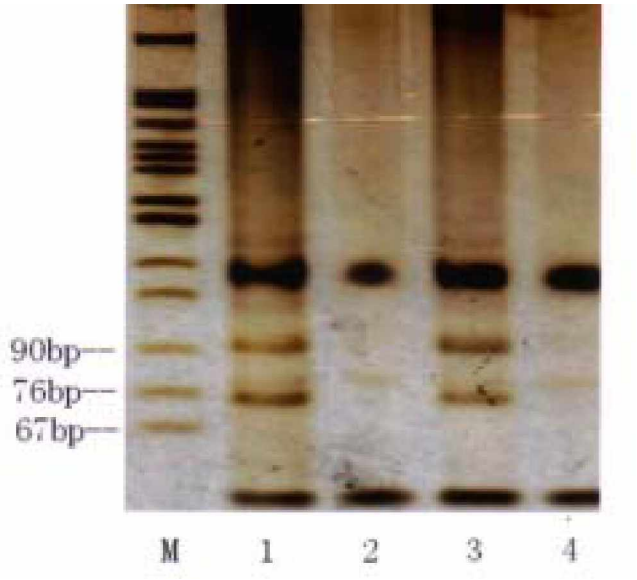

Figure 1. Electrophoresis of FSHRB. M: PBR322 DNA/MSP I markers, 2/4:BB, 1/3: AB.

Frequency of the three genotypes in different population was shown in Table 1. The frequency of genotype BB in Erhualian was significantly higher than that in Large White and Landrace×Large White by Chi-square test (Chi-square=158.253).

Effects of FSHRB polymorphism on litter size

The means and standard deviation for TNB and NBA were summarized in Table 2. In Erhualian, the sows with genotype BB of FSHRB had significantly higher NBA and TNB than that of AA ($p<0.01$) in second parity and later parities. A more than one pig per litter difference between

Table 2. Effect of polymorphism of FSHRB on litter size

Population	Genotype	First parity			Second parity			Later parities		
		N	TNB M±SD	NBA M±SD	N	TNB M±SD	NBA M±SD	N	TNB M±SD	NBA M±SD
Erhualian	AA	0			0			0		
	AB	5	11.52±1.10	11.15±1.99	5	14.54±1.98^{a}	14.11±2.54^{a}	4	14.74±2.54^{a}	14.24±1.97^{a}
	BB	42	11.68±2.43	11.53±2.12	42	15.67±2.01^{b}	15.63±2.34^{b}	40	15.87±2.11^{b}	15.42±2.30^{b}
Large	AA	14	9.25±2.09^{a}	9.28±2.35^{a}	14	9.20±1.69^{a}	9.85±1.09^{a}	14	9.43±1.88^{a}	9.64±1.52^{a}
White	AB	189	10.48±2.31^{ab}	10.37±2.46	189	10.45±2.11	10.29±2.48	186	10.52±1.11	10.35±1.28
	BB	63	11.66±1.90^{b}	11.57±1.6^{b}	63	11.24±1.90^{b}	11.87±1.54^{b}	61	12.26±2.07^{b}	11.72±2.08^{b}
	a		1.21	1.15		1.02	1.01		1.42	1.04
	d		0.03	-0.06		0.23	-0.57		-0.33	-0.32
	D		0.02	-0.05		0.23	-0.56		-0.23	-0.31
Landrace	AA	18	9.14±3.20^{a}	8.88±1.65^{a}	18	9.27±2.00^{a}	9.20±1.67^{a}	17	10.12±1.55^{a}	10.00±1.82^{a}
×Large	AB	264	9.47±1.99	9.32±1.63	264	10.45±2.22^{ab}	10.42±2.10	261	11.23±2.45	11.12±2.09^{ab}
White	BB	76	11.28±2.12^{b}	11.24±2.42^{b}	76	11.89±2.04^{b}	11.27±1.78^{b}	73	12.58±1.23^{b}	12.54±1.11^{b}
	a		1.07	1.18		1.31	1.04		1.23	1.27
	b		-0.74	-0.74		-0.16	0.19		-0.12	-0.15
	D		-0.69	-0.63		-0.10	0.18		-0.98	-0.12

M±SD in the same line with different superscripts significantly differ at $p<0.05$; Additive effect (a)=(BB-AA)/2. Dominance effect (d)=AB-(AA−BB)/2. Dominance degree (D)=d/a.

Table 3. Effect of polymorphism of FSHRB on piglet weight

Population	Trait index	First parity			Second parity			Later parities		
		AA	AB	BB	AA	AB	BB	AA	AB	BB
Erhualian	Number	0	5	42	0	5	42	0	12	125
	BW20 M±SD		3.34±0.42	3.58±0.31		3.04±0.57	3.22±0.22		3.21±0.52	3.64±0.41
	WW45 M±SD		8.52±1.82	8.47±1.45		8.60±1.85	8.54±1.42		8.59±1.36	8.96±1.47
Large White	Number	14	189	63	14	189	63	45	205	190
	BW M±SD	1.23±0.15	1.27±0.10	1.24±0.11	1.30±0.10	1.26±0.11	1.27±0.15	1.32±0.11	1.30±0.13	1.34±0.12
	WW35 M±SD	8.31±1.11	8.35±1.23	8.24±1.07	8.37±1.24	8.46±1.21	8.26±1.11	8.52±1.51	8.75±1.30	8.24±1.11
Landrace× Large White	Number	18	264	76	18	264	76	61	753	224
	BW M±SD	1.32±0.12	1.38±0.14	1.43±0.13	1.31±0.12	1.39±0.14	1.43±0.15	1.35±0.13	1.43±0.12	1.45±0.13
	WW35 M±SD	8.26±0.88	8.20±0.67	8.37±0.80	8.42±0.55	8.39±0.91	8.46±0.76	8.51±0.77	8.56±0.79	8.50±0.91

Means do not differ significantly ($p>0.05$).

BB and AA was detected for both NBA and TNB ($p<0.05$) in all parties in Large White sows, while the additive effects of TNB and NBA were 1.02-1.42 and 1.01-1.15 piglets per litter respectively. In all parity of sows of Landrace×Large White, TNB and NBA of sows with BB genotype were increased ($p<0.05$) with additive effects of 1.07-1.31 and 1.04-1.27 piglets per litter, respectively.

Effects of FSHRB polymorphism on piglet weight

Table 3 summarized the association of polymorphism with body weight at birth (BW), weaning weight 35 days (WB35) in Large White and Landrace×Large White, body weight of 20 days (BW20) and weaning weight of 45 days (WB45) in Erhualian. There was no significantly difference in above growth trains.

DISCUSSION

In this study, the allele B frequency was high in Chinese pig Erhualian that is one of the pig breeds with most litter size, while allele B was low in foreign pig breeds with middle reproduction (Large White and Landrace×Large White), and according that sows with genotype BB had highest TNB and NBA in the three pig populations, together with the study of Aittomaki (1995) that the mutation of FSHR (a C566T transition in Exon7 and Ala to Val substitution at residue 189) caused ODG (Ovarian dysgenesis) and BB genotype had positive role in human reproduction. And moreover, there was insignificant effect on body weight at birth (BW) and weaning weight (WB). We believed B allele was the favorable allele in the litter size in pigs.

The product of FSHR gene is in various tissues including brain, ovary, placenta and uterus in sows. Mutations of the FSHR could individually change the interaction of FSH with its receptor FSHR, leading to alter signal transduction and interference with ovulation/ fertilization and pregnancy, and so forth. Therefore it is possible that the FSHR gene mutation lead to change in the litter size in pigs.

IMPLICATION

Litter size is an important trait in pig reproduction, but it is difficult to improve due to its low heritability (h=0.01). Rothschild et al. (1996) found allele B of estrogen receptor gene (ESR) could control 0.5 pig per litter. In this study, the genotype BB could increase TNB and NBA by about 1 piglet per litter. In addition to positive effect of polymorphism on litter size, the significant negative effect of FSHRB on body weight at birth (BW) and weaning weight (WB) was not detected. The positive effect of FSHRB on litter size and no negative effect on piglet growth suggested that it was considerable to regard FSHRB as a marker of litter size, and with the potential economic value from marker-assisted selection. It is worth to study the causes and mechanism of FSHR effect on litter size.

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