Frequencies, Inheritance of Porcine FSH-β Retroposon and its Association with Reproductive Traits**

Li Feng'e*, Xiong Yuanzhu, Deng Changyan, Jiang Siwen and Zheng Rong
College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070. P. R. China

ABSTRACT : The fragment in intron I of FSH-β gene was amplified by PCR. According to the polymorphism, we analyzed the distribution of FSH-β retroposon in different pig breeds; its inheritance pattern in Large White×Meishan reference family; and the association of FSH-β retroposon with litter size, female reproductive organs measurement, ultrasonic backfat and other traits. The results showed that almost each Chinese indigenous pig had the retroposon, while foreign pig breeds rarely had; the frequencies of porcine FSH-β retroposon were strongly associated with breeds ($p<0.01$); the pattern of inheritance was consistent with Mendelian fashion; total number born (TNB) and number born alive (NBA) were increased per FSH-β retroposon ($p<0.01$) with additive effects of 1.2-1.8 and 1.4-1.8 pigs/litter, respectively; between the FSH-β retroposon carriers and non-carriers, there was an insignificant difference in the measurement of female reproductive organs, body weight at birth, backfat thickness, loin meat height, lean meat percentage, teat number, days to 100 kg, and average daily gain. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 2 : 179-183)***

Key Words : Pigs, FSH-β Gene, Retroposon, Litter Size

INTRODUCTION

Retroposons are a class of genes that were created by making a reverse transcriptase copy of a processed mRNA and inserting the DNA copy into genomic DNA (Kenneth et al., 1999). Zhao et al. (1999) found a retroposon in intron I of porcine FSH-β gene. a DNA fragment which came from the reverse transcription of a RNA fragment transcipted by RNA polymerase III and was transposed into porcine FSH-β gene. As it inserts into intervening sequence. the sequence of the expression product of FSH-β gene doesn't change. however. the retroposon probably regulate gene expression.

Mammalian follicular stimulating hormone (FSH) is one of glycoproteins secreted by the anterior pituitary gland. FSH interacts with its receptor on granular cell. stimulates the maturation and differentiation of ovarian follicle in females and it is known that FSH signaling is considered essential for pubertal initiation of spermatogenesis and maintenance of normal sperm production in males. FSH belongs to a family of α/β heterodimeric glycoproteins including luteinizing hormone (LH). thyroid stimulating hormone (TSH) and chorionic gonadotropin(CG). α is a common subunit of these hormones while β is unique. It's the unique β subunit that gives each hormone in this family its physiological specialty (Jameson et al.. 1988; Kato, 1988; Esch et al., 1986).

To better understand the mechanisms of retransposition and the prolificacy in Chinese indigenous pigs. we chose FSH-β gene as a candidate gene of the major genes influencing litter size and studied the distribution of FSH-β retroposon in Chinese and foreign pig populations and several synthetic lines: the association of FSH-β retroposon with litter size, reproductive organs measurement, ultrasonic backfat and other traits: and its inheritance pattern in Large White×Meishan (LW×M) reference family.

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* Corresponding Author: Li Feng'e, Tel: +86-27-87287139, Fax: +86-27-87394184, E-mail: hzauswin@public.wh.hb.cn
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MATERIALS AND METHODS

Animals

Blood samples of 11 pig populations were collected including Erhualian pigs from Xishan pig breeding farm. Jiangsu Province; Meishan pigs from Jiading pig breeding farm. Shanghai city; Lingao from Hainan Province: Bamei pigs from Qinghai Province: Landrance from Daqiao pig breeding farm, Hubei Province: Qingping and Tongcheng pigs from Hubei Province: Large White pigs. Duroc. LW×M reference family F1 and F2 offspring. and DIV2 line from the farms owned by Huazhong Agricultural University.

Traits

Several performance traits were recorded. Litter trait included total number born (TNB). which was calculated as live births plus stillborn animals and mummified fetuses, and number born alive (NBA). Body weight at birth (BWB) and total number of teats (TN) were recorded at birth. Growth performance records including ultrasonically measured backfat thickness (BFT). ultrasonically measured loin meat height (LMH). estimated lean meat percentage (LMP), days to 100 kg, average daily gain (ADG) over the whole period were recorded when the pigs were slaughtered. F2 offspring were slaughtered at about 100 kg. The measurement of female reproductive organs including length of uterine horn (LUH), length of uterine cervix (LUC), length of uterine body (LUB), uterine weight (UW), weight of two ovaries (WO) and volume of uterine lumen (VUL) were collected. LUH, LUC and LUB were measured according to the method of Lin (1992); VUL was the maximum volume of filled water.

DNA Preparation

Blood was collected in 50 mM EDTA at pH 8.0 to prevent coagulation, and genomic DNA was extracted from blood white cells. DNA extraction procedure was described as Xiong (1999). PCR was used to amplify DNA in micro-centrifuge tube. Reaction included 2.5 μl 10×buffer, 1.5 μl 25 mM $MgCl_2$, 1.5 μl 2.0 mM dNTP, 50-100 ng DNA, 1.5 μl 5 mM primers, 1 IU *Taq* DNA polymerase. FSH-β genotypes were analyzed by PCR, using primers as reported (Zhao et al., 1999). Primers were as follows: 5'-AGT TCT GAA ATG ATT TTT CGG G-3'; 5'-ACT GGT CTA TTC ATC CTC TC-3'. The amplified fragment is located in the intron I. The fragment of the retroposon allele is about 1,100 bp, and the fragment without the retroposon is about 800 bp. The reaction was loaded onto a PE-9600 thermal cycler under the following conditions: 1 cycle at 94°C for 4 min; 30 cycles of 94°C for 45sec, 55°C for 45 sec, 72°C for 45 sec; 1 cycle at 72°C for 10 min; 4°C for 2 h. PCR products were separated with 2% agarose and 1×TAE, and visualized under UV light after staining with ethidium bromide (EB).

Statistical analysis

The data obtained were analyzed by the Duncan method of one way ANOVA procedure of SAS package (1987). Chi-square test was also used.

RESULTS

Allele frequency

Frequencies of porcine FSH-β retroposon in different pig populations are showed in table 1. The results demonstrated that almost each Chinese indigenous pig had retroposon while foreign pig breeds rarely had, and the synthetic line (DIV2 line) and cross line (LW×M) had intermediate frequencies of porcine FSH-β retroposon. By Chi-square test (Chi-square=155.138), the frequencies of porcine FSH-β retroposon were strongly associated with breeds ($p<0.01$).

Inheritance pattern

Retroposon insertion consistent with autosomal Mendelian fashion was observed in a 3-generation LW×M reference family. Mendelian segregation of the retroposon allele among F2 offspring whose F1 parents had genotypes BB and AB was observed. In Lane 1 to 8, upper and lower bands represent alleles A (retroposon alleles) and B, respectively (figure 1).

Table 1. Frequencies of porcine FSH-β retroposon in different pig populations (%)

populations	Number of observed	Frequency (%)
Erhualian	47	100.0
Lingao	46	100.0
Meishan	55	80.2
Duroc	54	15.8
Large White	60	6.7
Landrace	48	9.1
DIV2 line	47	34.0
LW×M	31	33.9
Tongcheng	70	100.0
Qingping	68	85.0
Bamei	39	73.1

Porcine FSH-β retroposon is associated with increased litter size

As shown in table 2, TNB and NBA were increased per FSH-β retroposon ($p<0.01$) with additive effects of 1.2-1.8 and 1.4-1.8 pigs/litter, respectively.

Effect of porcine FSH-β retroposon on female reproductive organ measurement

Analyses of the measurement of female reproductive organs of F2 offspring of LW×M reference family are in table 3 and 4. Retroposon-carriers sows had larger but insignificant length of uterine horn, uterine weight and volume of uterine lumen than non-carriers. On non-rutting condition the ovaries of carriers were heavier than those of non-carriers, while on rutting condition the ovaries of

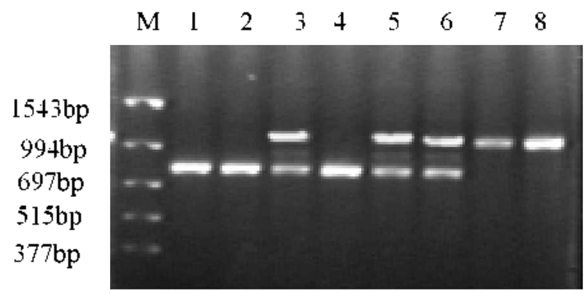

Figure 1. Amplified length polymorphisms of FSH-β locus and segregation pattern of the retroposon allele among F2 offspring. M: DNA size markers; Lane 1, 2, 3, 6: F2 offspring; Lane 4, 5: parental pigs

Table 2. Effect of porcine FSH-β retroposon on litter size

Genotype	First parity			Second parity			Later parities		
	n	TNB	NBA	n	TNB	NBA	n	TNB	NBA
AA	43	11.674 ±0.320[a]	11.023 ±0.358[a]	39	13.974 ±0.386[a]	13.410 ±0.376[a]	89	15.596 ±0.290[a]	14.381 ±0.305[a]
AB	24	10.803 ±0.390[ab]	9.000 ±0.710[b]	22	10.227 ±0.366[b]	9.227 ±0.455[b]	49	14.000 ±0.393[b]	12.816 ±0.451[b]
BB	37	9.270 ±0.476[b]	8.088 ±0.560[b]	38	11.342 ±0.391[b]	10.050 ±0.460[b]	67	11.940 ±0.359[c]	10.838 ±0.331[c]
A		1.202	1.468		1.316	1.680		1.828	1.773
D (d)		0.331	-0.556		-2.431	-2.503		0.232	0.208
D		0.275	-0.379		-1.847	-1.490		0.127	0.117

Means±SE in the same column with different superscripts significantly differ at $p<0.01$; Additive effect(a)=(AA-BB)/2, Dominance effect(d)=AB-(AA+BB)/2, Dominance degree(D)=d/a.

Table 3. Effect of porcine FSH-β retroposon on non-rutting female reproductive organ measurement

Genotype	n	LUH (cm)	LUC (cm)	LUB (cm)	UW (g)	WO (g)	VUL (ml)
AA	2	40.000 ±3.000	12.250 ±2.250	8.100 ±0.600	158.050 ±1.650	8.500 ±0.500	112.250 ±9.550
AB	12	37.600 ±1.910	12.950 ±1.223	6.777 ±0.434	138.050 ±10.556	8.380 ±0.746	108.420 ±13.740
BB	31	34.784 ±1.592	13.081 ±0.610	6.742 ±0.308	133.624 ±7.981	8.195 ±0.360	81.569 ±9.109

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

Table 4. Effect of porcine FSH-β Retroposon on rutting female reproductive organ measurement

Genotype	n	LUH (cm)	LUC (cm)	LUB (cm)	UW (g)	WO (g)	VUL (ml)
AA	2	92.500 ±2.500	16.250 ±1.750	8.000 ±1.000	572.200 ±1.400	14.600 ±1.200	983.600 ±120.500
AB	14	88.179 ±8.732	17.821 ±1.167	8.023 ±0.409	542.279 ±35.143	15.682 ±1.517	936.164 ±77.840
BB	31	79.450 ±4.326	19.933 ±0.984	8.083 ±0.324	517.557 ±30.518	16.863 ±1.950	862.897 ±85.220

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

carriers were lighter. Lengths of uterine cervix of carriers were shorter than those of non-carriers.

Effects of porcine FSH-β retroposon on performance measures

Table 5 summarized the association of porcine FSH-β retroposon with body weight at birth, backfat thickness, loin meat height, lean meat percentage, teat number, days to 100 kg, and ADG. The results showed that porcine FSH-β retroposon allele was not significantly associated with above performance measures ($p>0.10$).

DISCUSSION

Most retroposons that arose by reverse transcription of cellular mRNA and by reintegration into genome are nonfunctional, and they exist in the present genomes as processed pseudo-genes (Zdenek et al., 1999).

Few studies about the inheritance pattern of retroposons have been reported. In the present study, the results showed that segregation of allele A, B in a 3-generation Large White×Meishan reference family was observed, and the inheritance pattern of porcine FSH-β retroposon was consistent with Mendelian fashion.

In this study, the results demonstrated that allele B was abundant in foreign pig breeds, while allele A was rich in Chinese indigenous pigs. However, litter size is so deviant

Table 5. Effects of porcine FSH-β retroposon on performance measures in F2 offspring

Genotype	n	BWB (kg)	BFT (mm)	LMH (mm)	LMP	TN (pair)	Days to 100 kg	ADG (kg/d)
AA	6	1.139 ±0.097	19.033 ±0.841	43.750 ±1.931	51.375 ±0.818	7.167 ±0.167	223.852 ±9.251	0.405 ±0.020
AB	25	1.413 ±0.058	15.709 ±0.922	41.792 ±1.067	53.708 ±0.933	7.440 ±0.083	244.597 ±4.845	0.377 ±0.008
BB	61	1.449 ±0.027	16.073 ±0.472	40.881 ±0.782	53.422 ±0.495	7.385 ±0.069	242.256 ±3.064	0.390 ±0.058

Means do not differ significantly (p>0.10).

(>13 pigs/litter in Erhualian and Tongcheng pigs, while only about 7 pigs/litter in Lingao) that it is difficult to determine which allele is favorable. According to its relationship with litter size and female reproductive organs measurement, together with the experience of Rothschild et al. (1996), we believe A allele as the favorable allele. Meanwhile, we detected the effect of FSH-β retroposon in F1 of Large White×Meishan reference family which rarely has genotype AA, and the findings showed that litter size of AB was more than that of BB by 0.61 piglets/litter. The results were different from the previous studies of Zhao et al. (1999) who reported that B allele was the favorable allele, probably due to different pig populations.

Porcine FSH-β retroposon was associated with increased litter size. Each copy of FSH-β retroposon could control 1.2-1.8 pigs/litter TNB and 1.4-1.8 pigs/litter NBA (p<0.01), respectively. The effect didn't decrease with increase of parity, which was also not similar to the results of Zhao et al. (1999). We suppose that in the first and second parity litter size is liable to be influenced by environment, while in the later parities litter size is comparatively constant.

The measurements of female reproductive organs of FSH-β retroposon carriers were insignificantly larger than those of non-carriers, which provides a abundant material for prolificacy. On non-rutting condition the ovaries of carriers were heavier than those of non-carriers, whereas on rutting condition the result was just the opposite, maybe because rutting carriers had more but smaller follicles than rutting non-carriers (just like the follicles of Chinese pigs have). In this study, FSH-β retroposon was not significantly related to the measurement of female reproductive organs, which could support that the hypotheses of the mechanism of the prolificacy in Chinese indigenous pigs are high ovulation rate and good uterine environment including the concentration of oestradiol and other hormone levels (Yang et al., 1993) and that the prolificacy is not related to the size and weight of uterus and ovary.

From a commercial standpoint, it is very important that beneficial alleles for a given trait have no antagonistic relationship with other growth performance traits. The beneficial retroposons have no significantly negative pleiotropic effects on body weight at birth, backfat thickness, loin meat height, lean meat percentage, teat number, days to 100 kg, and average daily gain. Therefore, porcine FSH-β retroposon is a promising marker to be exploited in pig breeding and selection.

IMPLICATION

Litter size is one of the most economically important traits in pig production, and because of its low heritability and sex-limited nature, the improvement is very slow. Rothschild et al. (1996) chose estrogen receptor (ESR) gene as a candidate gene of the major genes influencing litter size, studied the linkage of allele B with litter size, and found allele B could control 0.5 pigs/litter. In this study, we concluded each copy porcine FSH-β retroposon could increase TNB and NBA by more than 1.2 pigs/litter. The positive effect of porcine FSH-β retroposon on litter size suggests that potential economic value from marker-assisted selection using this retroposon is considerable. In addition to the positive effect for litter size, significant negative effects were not detected in backfat thickness, weight at birth, teat number and other traits. But why porcine FSH-β retroposon has so large an effect on litter size and how does it work? These problems still need to be studied further. Moreover, FSH-β retroposon has been seen primarily in Chinese indigenous pigs, maybe it is one of the fundamental reasons to account for the prolificacy of Chinese pigs.

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