Association of a microsatellite flanking *FSHB* gene with reproductive traits and reproductive tract components in pigs

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ABSTRACT: Microsatellite FSHBMS polymorphism at the 5' flanking region of *FSHB* gene was genotyped and associations with reproductive traits in several pig populations and with reproductive tract components in the Large White × Meishan F_2 offspring were studied. The results showed that FSHBMS allele 98 carriers had a non-significantly higher total number born and number born alive in multiple parities; 118/118 animals had a significantly higher number of piglets at weaning than 98/98 and 98/118 animals ($P < 0.05$) and significantly higher litter weight at weaning and individual weight at weaning than 98/98 animals ($P < 0.05$). The 98/118 animals had the shortest gestation length ($P < 0.05$); the length of uterine horns and the length of oviducts of 98/98 individuals were shorter and longer, respectively, than those with 98/118 genotype ($P < 0.05$).

Keywords: pig; *FSHB* gene; microsatellite FSHBMS; reproductive trait; reproductive tract components

Mammalian follicle stimulating hormone (FSH) is one of the glycoproteins secreted by the anterior pituitary gland. FSH interacts with its receptor in a granular cell, stimulates the maturation and differentiation of ovarian follicle in females (Li et al., 2002). FSH belongs to the family of α/β heterodimeric glycoproteins, and β subunit gives this hormone its physiological specificity (Esch et al., 1986; Jameson et al., 1988; Kato, 1988). Zhao et al. (1999) found a retroposon in intron 1 of porcine *FSHB* gene, and after statistical analysis they found that *BB* (non-retroposon carrier allele) homozygote females produced on average 2.53 piglets more than did *AA* sows for total number born (TNB) of the first parity and 2.12 for number born alive in Landrace and Yorkshire pigs. Ellegren (1993) reported a microsatellite 4kb 5' upstream of *FSHB* gene, but no reports have been published about the association of this microsatellite locus with reproductive traits. Porcine *FSHB* gene was located to 2p12-16 by physical mapping (Mellink et al., 1995) and to SSC2 by linkage mapping (Rohrer et al., 1994). To

Supported by the National High Technology Development (Project No. 2007AA10Z162), the open funds of the Hubei Key Laboratory of Animal Embryo Engineering and Molecular Breeding of China (102ZD) and the National Natural Science Foundation of China (Project No. 30700571).

further study the genetic effects of *FSHB* gene, we chose a microsatellite flanking *FSHB* as a candidate locus for association studies with litter size and other reproductive traits.

MATERIAL AND METHODS

Animals

Three Large White boars were crossed with seven Chinese Meishan sows. From the F_1 offspring 5 boars were mated to 23 sows producing 287 offspring in 41 families. The pigs were slaughtered in three contemporary groups between 1998 and 2000 following a common protocol (Xiong and Deng, 1999). F_2 offspring were slaughtered at about 100 kg. Reproductive tract characteristics, including the length of uterine horn (LUH), length of uterine cervix (LUC), length of uterine body (LUB), uterine weight (UW), weight of two ovaries (OW), volume of uterine lumen (VUL), length of oviduct (LO) and ovulation rate (OR) were recorded. Ovulation rates were estimated by counting the corpora lutea on the surface of the ovaries. LUH, LUC and LUB were measured according to Lin (1992), VUL was defined as the maximum volume of filled water (Li et al., 2002).

Blood samples were collected from 44 Erhualian pigs from Xishan pig breeding farm, Jiangsu Province; 42 Large White pigs, 17 Duroc and 45 Line DIV_2 and 24 Large White × Meishan (LW × M) F1 pigs from the farms owned by Huazhong Agricultural University. Piglets were weaned at 35–45 days of age. Total number born (TNB), number born alive (NBA), number at weaning (Nw), litter weight at weaning (LWT), average individual weight at weaning (WT), teat number of offspring (TNO), litter weight at birth (LWB), average body weight at birth (ABWT), gestation length (GL), litters per year (LPY), survival rate from birth to weaning ($SR_{B\text{-}W}$), weaning to oestrus interval (WOI), weaning to conception interval (WCI) were recorded according to Xiong and Deng (1999).

Individual weight at weaning (WT) was corrected according to weaning age, and the correction formula was described as follows. Corrected:

$$WT = aX + b$$

where:

a = regression coefficient of the weaning age
b = intercept
X = weaning age

Genotyping

The microsatellite *FSHBMS* primers were as follows: 5'-GACCCCACCTGAGAA-TTTCCATAT- 3', 5'-ATGGTTTCTAATCC-ACAGTAGGC-3', and the procedure of genotyping was as described by Ellegren et al. (1993).

Statistical analysis

Association analysis was performed using the General Linear Model (GLM) procedure of SAS package (Windows V8). Pairwise *t*-test was used to test differences between the genotypes of microsatellite *FSHBMS*. These statistical models used were:

Model 1 for reproduction traits:

$$Y_{ijk} = \mu + P_i + F_j + G_k + e_{ijk}$$

where:

Y_{ijk} = observation of the trait
μ = least squares mean
P_i = effect of i^{th} parity (i = 2, 3, 4 (parity ≥ 4))
F_j = effect of j^{th} farm and population (j = 1 for Erhualian, 2 for Large White, 3 for Duroc, 4 for Line DIV_2 and 5 for LW × MF_1 pigs)
G_k = effect of k^{th} genotype (k = *1, 2, 3, 4, 5, 6*)
e_{ijk} = random residual

Model 2 for reproduction component traits:

$$Y_{ij} = \mu + E_i + G_j + e_{ij}$$

where:

Y_{ij} = observation of the trait
μ = least squares mean
E_i = effect of j^{th} oestrous cycle (i =1 for oestrus, 2 for dioestrum)
G_j = effect of k^{th} genotype (k = *1, 2, 3, 4, 5*)
e_{ij} = random residual

RESULTS

The microsatellite was amplified by PCR and analyzed by polyacrylamide gel electrophoresis (PAGE). Three alleles (98bp, 108bp and 118bp) were identified in pig populations studied for the *FSHBMS* microsatellite, and the alleles were designated according to the number of base pairs detected in the fragment after PCR amplification when compared with a molecular weight marker (pBR322DNA-*Msp*I digest).

Table 1. Effect of *FSHBMS* microsatellite locus on the reproductive traits over multiple parities

Traits	Least squares means ± Standard error (LSM ± SE)					
	98/98	*98/108*	*98/118*	*108/108*	*108/118*	*118/118*
No. litters	20	36	105	102	86	144
TNB	12.814 ± 0.656	12.978 ± 0.480	13.097 ± 0.284	12.481 ± 0.299	12.535 ± 0.314	12.664 ± 0.271
NBA	11.758 ± 0.678	11.747 ± 0.496	12.111 ± 0.293	11.548 ± 0.310	11.437 ± 0.323	11.497 ± 0.280
N_w	9.198 ± 0.502^{a}	9.831 ± 0.382^{a}	9.868 ± 0.251^{a}	10.198 ± 0.259^{a,b}	10.004 ± 0.300^{a,b}	10.622 ± 0.258^{b}
LWT/kg	93.631 ± 6.343^{a}	100.548 ± 5.121^{a,b}	104.895 ± 3.338^{a,b}	104.983 ± 3.442^{a,b}	102.384 ± 3.911^{a,b}	107.083 ± 3.522^{b}
WT/kg	10.531 ± 1.546^{a}	10.609 ± 1.248^{a,b}	10.756 ± 0.807^{a}	10.482 ± 0.839^{a}	10.578 ± 0.958^{a,b}	11.433 ± 0.852^{b}
TNO	13.480 ± 0.285	13.468 ± 0.118	13.424 ± 0.074	13.393 ± 0.092	13.449 ± 0.074	13.459 ± 0.057
LWB/kg	14.101 ± 2.041	15.621 ± 0.846	16.296 ± 0.532	14.896 ± 0.649	15.497 ± 0.527	15.553 ± 0.410
ABWT/kg	1.335 ± 0.169	1.412 ± 0.061	1.394 ± 0.038	1.441 ± 0.047	1.453 ± 0.038	1.447 ± 0.030
GL/d	116.724 ± 1.422^{a}	115.833 ± 0.456^{a}	114.863 ± 0.286^{b}	16.104 ± 0.338^{a}	116.167 ± 0.268^{a}	115.598 ± 0.218^{a}
LPY/litter/year	2.141 ± 0.077^{a}	2.028 ± 0.042^{a,b}	1.995 ± 0.028^{b}	2.036 ± 0.028^{a,b}	2.016 ± 0.025^{a,b}	2.051 ± 0.020^{a,b}
SR_{B-W}	0.755 ± 0.061^{a}	0.839 ± 0.046^{a,b}	0.827 ± 0.030^{a,b}	0.863 ± 0.031^{a,b}	0.882 ± 0.036^{a,b}	0.905 ± 0.031^{b}
WOI/day	4.370 ± 4.032^{a}	3.038 ± 1.041^{a}	4.978 ± 0.622^{a}	5.609 ± 0.678^{c}	5.159 ± 0.542^{a,c}	6.505 ± 0.485^{b,c}
WCI/day		15.312 ± 3.083	13.581 ± 1.841	12.030 ± 2.006	14.561 ± 1.606	13.096 ± 1.437

values in the same row with different superscripts are significantly different at the level $P < 0.05$

total number born (TNB); number born alive (NBA); number at weaning (Nw); litter weight at weaning (LWT); average individual weight at weaning (WT); teat number of offspring (TNO); litter weight at birth (LWB); average body weight at birth (ABWT); gestation length (GL); litters per year (LPY); survival rate from birth to weaning (SRB-W); weaning to oestrus interval (WOI); weaning to conception interval (WCI)

Microsatellite *FSHBMS* was tested in 172 sows with litter size records encompassing five populations, in which 3 alleles and 6 different genotypes were detected. Table 1 shows that *118/118* animals had significantly more piglets at weaning (N_W) than *98/98* and *98/118* animals ($P < 0.05$), significantly higher litter weight at weaning (LWT) and average individual weight at weaning (WT) and a significantly lower survival rate from birth to weaning (SR_{B-W}) than *98/98* animals ($P < 0.05$). So we presumed in multiple parities that allele *118* was favourable for nursery performance. Animals with genotype *98/118* had the shortest gestation length, but the lowest number of litters per year ($P < 0.05$). Table 2 shows that the LUH of *98/98* individuals was significantly shorter, while its OR was higher when compared to *98/118* genotype ($P < 0.05$).

DISCUSSION

Genetic improvement of litter size has been limited by traditional selection because of the low heritability and sex-limited expression of reproductive traits. The identification of a specific gene or an anonymous genetic marker for litter size traits in pigs could have a major impact on the improvement of reproductive performance by increasing the accuracy of selection (Rothschild et al., 1996). Rohrer et al. (1994) reported a PCR-RFLP marker located within the first intron of *FSHB* gene, and assigned this gene to SSC2 by linkage mapping. Li et al. (1998) and Zhao et al. (1999) reported an association of *FSHB* gene locus in intron 1 and litter size in pigs. Li et al. (2000) identified an *FSHB* allele in Chinese breeds of pigs that had an 11bp deletion (*D* allele) in the 3' UTR, and *D* allele was expressed at a level similar to WT allele (no 11bp deletion) in the anterior pituitary glands of heterozygous males. The aim of research reported here was to check *FSHB* gene association with litter size by analyzing polymorphism at another locus.

Table 1 shows that allele *98* carriers had larger (but not significantly) litter size and number born alive in multiple parities. But *118/118* animals had a significantly higher number at weaning, litter weight at weaning and individual weight at weaning than *98/98* animals ($P < 0.05$). So probably allele *118* was favourable for nursery performance for multiparous sows.

Table 1 also shows that *98/118* animals had the shortest gestation length, but it was not logical that *98/118* animals had the lowest number of litters per year ($P < 0.05$). Litters per year were determined by the length of gestation, weaning age and non-pregnant days, and so we think the reason for *98/118* lowest LPY is a relatively older weaning age.

In the present study, even though OR of *98/98* genotype was higher than that of *98/118* genotype ($P < 0.05$), litter sizes of *98/98* animals were non-significantly smaller than those of *98/118* animals. The major reason was that *98/98* genotype had

Table 2. Effect of *FSHBMS* microsatellite locus on the female reproductive tract components in LW × MF_2 offspring

Traits	Least squares means ± Standard error (LSM ± SE)				
	98/98 (n = 10)	*98/118* (n = 35)	*108/108* (n = 4)	*108/118* (n = 9)	*118/118* (n = 42)
VUL/cm^3	428.520 ± 116.331	492.106 ±62.053	330.547 ± 74.346	385.813 ± 51.458	526.901 ± 55.533
LUH/cm	46.563 ± 6.794[a]	64.145 ± 3.766[b]	53.444 ± 10.692[a,b]	53.314 ± 7.081[a,b]	60.298 ± 3.407[a,b]
LUC/cm	6.429 ± 1.290	7.654 ± 0.723	6.988 ± 2.031	7.563 ± 1.345	8.238 ± 0.653
LUB/cm	14.7371 ± 1.435	16.283 ± 0.796	18.213 ± 2.258	17.436 ± 1.496	15.082 ± 0.720
UW/g	321.849 ± 41.951	337.999 ± 23.257	329.336 ± 66.016	308.529 ± 43.721	326.324 ± 21.035
OW/g	15.541 ± 3.046	13.022 ± 1.689	12.366 ± 4.793	9.897 ± 3.174	12.514 ± 1.527
OR	14.722 ± 1.112[a]	11.963 ± 0.775[b]	14.322 ± 2.447[a,b]	12.176 ± 1.106[a,b]	13.472 ± 0.610[a,b]
OL/cm	20.804 ± 4.038	23.554 ± 2.025		22.471 ± 2.336	22.304 ± 1.046

values in the same row with different superscripts are significantly different at the level $P < 0.05$

length of uterine horn (LUH); length of uterine cervix (LUC); length of uterine body (LUB); uterine weight (UW); weight of two ovaries (OW); volume of uterine lumen (VUL); length of oviduct (LO); ovulation rate (OR)

smaller LUH and lower embryo survival rate than *98/118* animals.

Except for a QTL for the number of nipples, no other reproduction QTLs linked to *FSHB* gene have been described so far (http://www.animalgenome.org/QTLdb/pig) while QTLs affecting reproduction have been verified on porcine chromosomes 4, 6–8 (Rothschild, 1998). The entire genome scan for regions that affected plasma FSH in boars from a Meishan-White Composite resource population showed that 3 genomic regions located on chromosomes 3, 10, and *X* apparently possessed genes that significantly affect the FSH level, but no evidence has been found that any regions located on SSC2 possessed genes affecting the FSH level (Rohrer et al., 2001). This may have resulted from small sample sizes or from the fact that the *FSHB* allele was involved in both parental populations in the QTL scan and it was not specifically genotyped in those experiments.

Rathje et al. (1997) reported a sizable QTL for ovulation rate (+3.07 ova) on chromosome 8. This QTL was later confirmed by Milan et al. (1998). The large ovulation rate/litter size QTL on chromosome 8 is of interest as it maps to the region syntenic with the Booroola fecundity gene in sheep. Interestingly, Short et al. (1997) reported the association between microsatellite within *OPN* gene located in the same region of chromosome 8 and litter size in Landrace and Meishan populations (US patent No. 6410227).

Acknowledgments

The authors also gratefully acknowledge the teachers and graduate students of the Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture and the workers of all the pig farms mentioned above for their cooperation.

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Received: 2007–08–01
Accepted after corrections: 2007–12–05

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