

GENETIC POLYMORPHISM OF PROLACTIN RECEPTOR GENE IN MONG CAI PIG

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ABSTRACT

Prolactin receptor gene (PRLR) was investigated as a candidate gene influencing litter size in several studies. The PRLR gene was chosen because of its essential role in establishing pregnancy in pig. In this paper, a PRC-RFLP was used for testing the PRLR polymorphism in Mong Cai sow. The DNA fragment of 163 bp of PRLR was amplified by PCR using specific primers and then digested by *AluI*. The allele frequencies was 0.19 for A and 0.81 for B. Genotype AA as well as AB were found at a frequency of 0.13 and BB at 0.74. The result of t-test with using two-tailed of standard distribution showed that the difference in average number of piglet born alive of AA and BB genotype was statistically significant with probability $p=0.04$.

Keywords: Mong Cai pig, number of piglets born alive, prolactin receptor gene, PCR-RFLP.

INTRODUCTION

The number of piglets born per sow is an economically important trait therefore improvement of litter size is an important goal for pig breeders. The heritabilities for litter size and piglet survival are relatively low, but the genetic variance for these traits is large. The development of pig genome map offers the opportunity to identify individual genes controlling reproduction. A number of polymorphic genes with significant effects on litter size in pig have been identified such as estrogen receptor gene – ESR (Rothschild et al., 1996), follicle stimulating hormone – FSH (Li et al., 1998), retinol-binding protein 4 gene – RBP4 (Rothschild et al., 2000), osteopontin gene – OPN (Southwood et al., 1999), melatonin receptor 1A gene – MTNR1A (Ollivier et al., 1997), properdin gene (Buske et al., 2005), prolactin receptor gene – PRLR (Rothschild et al., 1998; Vicent et al., 1998; Putnova et al., 2002). The number of endometrial prolactin receptors increases on day 12 of pregnancy, which allows redirection of PGF₂ secretion to maintain the corpus luteum function (Pope, 1994). The porcine *PRLR* has been mapped to chromosome 16 with regional assignment to 16q1.4 (Vincent et al., 1998) and is a strong candidate gene for reproductive traits in pigs. Our study aimed to investigate the frequency of *PRLR* gene mutations and the associations between *PRLR* genotype and litter size in Mong Cai sow.

MATERIALS AND METHODS

DNA was extracted from ear tissues collected from Mong Cai pig breeding station, Thanh To farm, Hai Phong. A total of 205 litters recorded from 39 sows were included in the litter size analysis. The DNA preparation described here included three main steps: digestion by detergent and proteinase K, extraction with phenol:chloroform:isoamylalcohol and then ethanol precipitation (Ausubel et al., 1995).

The forward PRLR primers 5'-CGTGGCTCC GTTTGAAGAACC-3' and the reverse PRLR primers 5'-CTGAAAGGAGTGCATAAAGCC-3' were used to amplify a 163 bp fragment. 25 μ l PCR reaction was performed using 100 ng of DNA template, 10 pmol of each forward and reverse primers, 0.2 mmol of dNTPs, 2.0 mmol of MgCl₂ and 1 U *Taq* polymerase (Fermentas). The reactions were loaded onto Mastercycle Personal (Eppendorf) under the following conditions: 94°C for 4 min, followed by 35 cycles of 94°C for 30 sec, 58°C for 45 sec, 72°C for 30 sec, final synthesis of 72°C for 5 min and hold at 15°C.

20 μ l of PCR product was digested with 5 U of *AluI* (Fermentas) and then separated on 2.5% agarose. The result was recorded as follows: 85 bp, 59 bp, and 19 bp fragment were observed for A allele, and 104 bp and 59 bp for B allele.

Allele and genotype frequencies were calculated. The significant difference of the effect of genotypes on number of piglet born was tested using t-test method.

RESULTS AND DISCUSSION

PCR product and PCR-RFLP test for PRLR polymorphism were shown in Figure 1a and 1b.

Allele and genotype frequencies were presented in Table 1. B allele was dominant in Mong Cai sow with frequency of 0.81, and 0.19 for A allele. In comparison to these results a higher frequency of allele A was reported in Duroc, Landrace sow (Drogemüller et al. 2000), Large White pigs (Putnova et al. 2002), Jinhua pig (Xu et al., 2003) and in Polish pig (Terman 2005).

Student test method (t-test) had been used for comparing the difference of average number of piglets

born alive of AA and BB genotypes. Because of the non-significant difference between SD of number of piglet born alive, the t-test with two-tailed of standard distribution had been applied. The result showed that the difference between BB and AA genotype was significant ($p=0.04$) for average number of piglet born alive. In the current study, PRLR was investigated as potential candidate gene influencing number of piglets born alive. The result showed that B allele was a favorable allele for the number of piglet born alive. This agreed with the result of Drogemuller et al. (2001) that the B allele indicated an additive effect on the number of piglet born alive in Duroc line and with the suggestion of Isler et al. (2000) that the B allele was found to significantly influence the number of fetuses pre uterine horn, average fetal weight, and total fetal weight in Yorkshire x Large White crossbred sows. Whereas Vincent et al. (1998), Southwood et al. (1999) had shown that the A allele was significantly associated with increased litter size in three of five commercial lines. The variation among lines in that study and the different effects in the current study could be due to sampling strategies and genetic background.

IMPLICATION

This study contributed to the demonstration that the difficulties in confirming the candidate gene effects in different genetic groups and showed that mutation in PRLR gene should be tested in larger number of Mong Cai pig.

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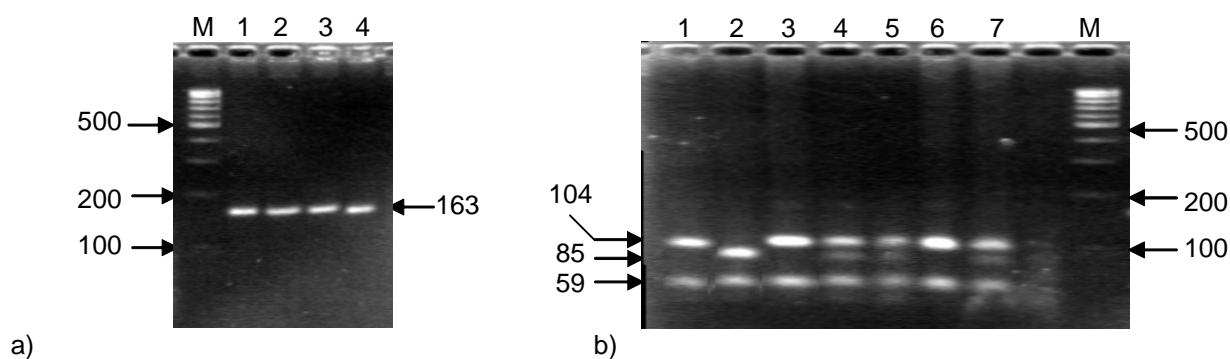


Figure 1. a) PCR product of PRLR gene
M: 100 bp DNA marker; 1-4: PCR product (163 bp)
b) The PCR-PRLR polymorphism
M: 100 bp DNA marker; 1, 3: BB genotype (104/59 bp)
2: AA genotype(85/59/19 bp) 4, 5, 7: AB genotype (104/85/59/19 bp)

Table 1. Allele and genotype distribution

		Number	Frequency (%)
Allele (n=78)	A	15	19
	B	63	81
Genotype (n=39)	AA	5	13
	AB	5	13
	BB	29	74

Table 2. Influence of PRLR on number of piglet born alive

Genotype	Mean number of piglets born alive	SD	Number of litter
AA	10.72	0.74	23
AB	11.18	0.18	29
BB	11.68	0.56	149

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