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Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Polymorphism in the *PRLR/Alu*I gene and its effect on litter size in Large White sows

Marek Kmieć, Arkadiusz Terman

Department of Genetics and Animal Breeding, Agricultural University of Szczecin, Doktora Judyma 6, 71-466 Szczecin, Poland

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The prolactin receptor (PRLR) has been detected in various tissues including brain, ovary, placenta and uterus of several mammalian species. In the pig, the *PRLR* gene has been mapped to chromosome 16. This study was aimed to detect the DNA mutations in gene *PRLR* and to find propable relations between the *PRLR* genotype and some reproductive traits in sows. The polymorphism in *PRLR* gene *locus* was detected using PCR-RFLP method, with specific primers. Digestion of PCR product was performed with 5 I.U. of appropriate restriction endonuclease *Alu*I (MBI Fermentas) for three hours at 37°C. Two *PRLR* alleles were identified: $PRLR^A$ (0.3106) and $PRLR^B$ (0.6894), controlling three genotypes: $PRLR^APRLR^A$ (0.1030), $PRLR^APRLR^B$ (0.4153) and $PRLR^BPRLR^B$ (0.4817). The results suggest a possibility of using the existing polymorphism in the *PRLR/Alu*I gene to improve certain reproductive performance traits of sows.

KEY WORDS: gene polymorphism / litter size / prolactin / sows

Prolactin (PRL) is a peptide hormone primarily secreted by the anterior pituitary in response to factors such as oestrogen. PRL acts in many ways on mammalian tissue and is essential for reproductive seccess [Vincent *et al*. 1998]. In sows PRL is one of the factors involved in controlling luteal and follicular steroidogenesis. The PRL receptor (PRLR) exists as a short form of 310 amino acids (first cloned from the rat liver [Boutin *et al*. 1988] and a long form of 610 amino acids (subsequently cloned from the rat ovary [Zhang *et al*. 1990]. The *PRLR* has been detected in various tissues of several mammalian species [Kelly *et al*. 1991] and was mapped by linkage analysis and by means of somatic cell hybrid panel to porcine chromosome 16. An *Alu*I PCR-RFLP polymorphism was identified in porcine 157 bp-long fragment of the gene [Vincent *et*

al. 1997] and was found associated with total number of piglets born and/or number born alive in three genetic lines tested – Rothschild *et al*. [1998], Vincent *et al*. [1998]. The mechanism through which *PRLR* affects litter size remains unknown [Van Rens and Van Der Lende 2002].

The present study was conducted to determine the effect of *PRLR* gene polymorphism on reproduction traits in Large White sows.

Material and metods

Large White sows (n=301) were raised and mated on the same farm. Genomic DNA was extracted from blood using Master Pure kit of EPICENTRE TECHNOLOGIES. Genotypes of the *PRLR* were determined by the PCR-RFLP method. The *PRLR* fragment was amplified from genomic template using the PCR with primers of sequences proposed by Drogemüller *et al*. [2000]. Information on primers' sequences, restriction enzyme and allele size are presented in Table 1. PCR reactions were performed in total volume 25 µl using 100 ng porcine genomic DNA, 15 pmol of each primer, 100 µM each dNTP, 1.5 mM $MgCl_2$ and 0,6 units Taq DNA polymerase (MBI FERMENTAS) in standard PCR buffer. Thermal conditions were as follows: 94°C for 4 min, followed by 35 cycles of 30 s at 94°C, 60 s at 55°C, 30 s at 72°C and the last extension 5 min at 72°C. Digestion of PCR product was performed with 5 I.U. of appropriate restriction endonuclease *Alu*I (MBI FERMENTAS) at 37°C overnight. The restriction fragment of DNA was separated by electrophoresis in 3% agarose gel stained with ethidium bromide. After the electrophoresis the gels were analysed in UV rays.

Tabl. Primer sequences and allele sizes of *PRLR* gene

Candidate Gene	Primer sequence	PCR product size (bp)	Endonuclease	Allele size (bp)	Source
PRLR	F-CGTGGCTCCGTTTGAAGAACC R-CTGAAAGGAGTGCATAAACGCC	163	*Alu*I	A – 85, 59, 19 B – 104, 59	Drogemüller *et al* [2001]

F – forward primer.
R – reverse primer

Sow's performance data were collected from farm records and contained: total number of piglets born (TNB), number born alive (NBA), number weaned (NW) and sow age (SA) at the day of farrowing. The relations between *PRLR* genotype and reproductive traits studied were analysed with one-way analysis of variance and the significance of differences was verified using Duncan test with computer program STATISTICA'99.

Results and discussion

The polymorphism in the *PRLR* gene was detected with *Alu*I restriction enzyme, which cuts the amplimer into several fragments. In the sows' group studied two *PRLR* alleles were identified: $PRLR^A$ and $PRLR^B$ controlling three genotypes: $PRLR^APRLR^A$, $PRLR^APRLR^B$ and $PRLR^BPRLR^B$. The length of restriction fragments detected were: 85, 59, and 19 bp for $PRLR^A$ and 104 and 59 bp for $PRLR^B$ (Photo 1).

Studied was the relation between *Alu*I polymorphism detected and sows' reproduc-

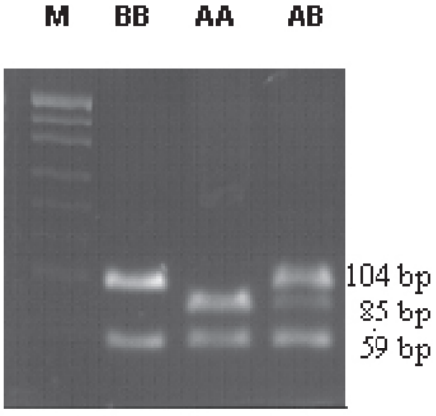

Photo 1. PCR-RFLP products of porcine *PRLR* gene. Shown are three different genotypes: $PRLR^APRLR^A$, $PRLR^APRLR^B$ and $PRLR^BPRLR^B$. M – DNA marker pUC 19/*Msp*I.

Table 2. The frequency of *PRLR/AluI* genotypes and alleles of sows under study

Breed		*PRLR/AluI* genotype			*PRLR* allele	
		$PRLR^APRLR^A$	$PRLR^APRLR^B$	$PRLR^BPRLR^B$	$PRLR^A$	$PRLR^B$
Large White	number	31	125	145	0.3106	0.6894
	frequency	0.1030	0.4153	0.4817		

tion traits. Allele and genotypes frequency estimated at *Alu*I *loci* of the *PRLR* gene are given in Table 2. A lower frequency of $PRLR^A$ was observed by Kmieć *et al*. [2001], Drogemüller *et al*. [2001] and Linville *et al*. [2001] who studied the Landrace sows. A similar, also lower frequency of $PRLR^A$ was observed in Large White pigs by Putnova *et al*. [2002]. On the other hand, in Duroc pigs a higher frequency of $PRLR^A$ was reported [Drogemüller *et al*. 2001].

Effect of genotypes at *Alu*I *loci* of the *PRLR* gene on reproduction traits (TNB, NBA, NW and SA) are given in Table 3. For all the traits only small and not significant differences were found between sows of different *PRLR* genotypes. However, the $PRLR^APRLR^A$ genotype sows produced by 0.2 piglet more than those of $PRLR^BPRLR^B$

Table 3. Means and standard deviations of total number born (TNB), number born alive (NBA), number weaned (NW) and sow age (SA) at the day of farrowing

PRLR genotype	Parity	Number of sows	TNB		NBA		NW		SA	
			mean	SD	mean	SD	mean	SD	mean	SD
$PRLR^A PRLR^A$	I	31	8.16	1.88	8.03	1.94	7.87	1.89	407.6	37.8
$PRLR^A PRLR^B$		123	7.77	2.32	7.72	2.33	7.48	2.37	399.8	44.9
$PRLR^B PRLR^B$		138	7.94	2.07	7.75	2.19	7.49	2.24	397.2	51.6
Total		292	7.89	2.16	7.77	2.22	7.53	2.26	399.4	49.5
$PRLR^A PRLR^A$	II	22	9.00	1.88	9.00	1.88	8.81	1.87	392.5	68.5
$PRLR^A PRLR^B$		93	8.40	2.56	8.19	2.74	7.92	2.63	364.6	62.4
$PRLR^B PRLR^B$		101	8.44	2.41	8.29	2.36	8.01	2.26	367.5	67.6
Total		216	8.48	5.43	8.32	2.49	8.06	2.40	368.8	65.7
$PRLR^A PRLR^A$	III	16	10.38	1.63	10.19	1.60	9.56	1.79	722.1	90.2
$PRLR^A PRLR^B$		70	9.46	2.36	9.32	2.35	8.76	2.12	716.4	75.1
$PRLR^B PRLR^B$		74	9.28	2.37	9.22	2.40	8.61	2.27	720.6	84.0
Total		160	9.47	2.31	9.36	2.32	8.77	2.17	718.9	80.4

genotype. Rothschild *et al*. obtained similar results [Drogemüller *et al.* [2001]; the effect of *PRLR* genotype was 0.25 piglet per litter, but in successive litters that effect was greater.

The frequencies of $PRLR^A$ and $PRLR^B$ allele observed in this study were 0,3106 and 0,6894, respectively. This preliminary report showed that sows of the $PRLR^A PRLR^A$ genotype produced (not significantly) more piglets than those of the $PRLR^A PRLR^B$ and $PRLR^B PRLR^B$ genotypes. The analysis of the total number of piglets born, number born alive and number weaned as well as of sow age at farrowing showed small and not significant differences between sows of different *PRLR* genotypes. The results obtained, however, should be verified by further investigation on greater number of animals.

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Marek Kmieć, Arkadiusz Terman

Polimorfizm genu *PRLR*/*Alu*I i jego wpływ na wielkość miotu świń rasy wielkiej białej polskiej

S t r e s z c z e n i e

Gen *PRLR* wykryto w mózgu, jajniku, łożysku i macicy poszczególnych gatunków ssaków. U świń gen ten zmapowano na chromosomie 16. Celem przedstawionych badań było wykrycie ewentualnych mutacji DNA w tym genie i znalezienie zależności między genotypami *PRLR* a niektórymi cechami reprodukcyjnymi świń wbp. Polimorfizm genu *PRLR* badano metodą PCR-RFLP z wykorzystaniem specyficznych starterów. Trawienie produktu PCR przeprowadzono 5 jednostkami odpowiedniego enzymu restrykcyjnego *Alu*I przez 3 godziny w temperaturze 37°C. Podczas badan zidentyfikowano dwa allele *PRLR*: $PRLR^{A}$ (0.3106) i $PRLR^{B}$ (0.6894), które kontrolowały występowanie trzech genotypów – $PRLR^{A}PRLR^{A}$ (0.1030), $PRLR^{A}PRLR^{B}$ (0.4153) oraz $PRLR^{B}PRLR^{B}$ (0.4817). Przedstawione badania sugerują możliwość wykorzystania polimorfizmu genu *PRLR/Alu*I w doskonaleniu niektórych cech reprodukcyjnych loch.