

DNA polymorphism of introns 1 and 2 of Prolactin Receptor Gene and its association with litter size in goats*

Di Ran¹, Yin Jing², Chu Ming-Xing^{1}, Cao Gui-Ling¹,
Feng Tao¹, Fang Li¹, Zhou Zhong-Xiao²**

¹ Key Laboratory for Farm Animal Genetic Resources and Utilization
of the Ministry of Agriculture of China, Institute of Animal Science,
Chinese Academy of Agricultural Sciences, Beijing 100193, PR of China

² College of Animal Science and Technology, Shanxi Agricultural University,
Taigu 030801, PR of China

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The prolactin receptor (PRLR) gene was studied as a candidate gene for the high prolificacy of Jining Grey goats. Polymorphisms in intron 1 and intron 2 of PRLR gene were detected in high prolificacy (Jining Grey) and low prolificacy (Boer, Wendeng dairy, Liaoning Cashmere and Beijing) native goats using PCR-SSCP. For intron 1, five genotypes (AA, AH, AK, HH and HK) were identified in Jining Grey goats, and two (AA and AK) in the other four breeds. The Jining Grey does of genotype HH, HK, AH and AK delivered by 0.65, 0.62, 0.59 and 0.57 more kids ($P < 0.01$) than those of genotype AA, respectively. For intron 2, three genotypes (CC, CD and DD) were detected in Boer goats, and two (CC and CD) in the other four breeds. The Jining Grey does of genotype CD delivered by 0.55 ($P < 0.01$) more kids than those of genotype CC.

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**Corresponding author: mxchu@263.net

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Improvement of reproductive traits in livestock has become of increasing interest especially in goats where small increases in litter size can equal large gains in profit. Genetic improvement of reproductive traits has traditionally been restricted to the use of quantitative genetic methods but using these methods led to the limited gain only. Provided the major genes associated with reproduction are identified they can be utilized in breeding through marker-assisted selection (MAS). Reproductive traits are often suggested as prime targets for MAS for their low heritability and the fact that the trait can be measured only in one sex.

Prolactin (PRL) is an anterior pituitary peptide hormone involved in many endocrine activities, essential for reproduction and mediated by its receptor (PRLR) detected in various tissues including brain, ovary, placenta and uterus in several mammalian species [Shirota *et al.* 1990, Tzeng and Linzer 1997, Cassy *et al.* 1998]. The prolactin receptor, encoded by *PRLR* gene, is a member of the growth hormone/prolactin receptor gene family containing regions of identical sequences [Kelly *et al.* 1991]. The prolactin and growth hormone receptors are homologous to receptors for members of the cytokine superfamily [Clevenger *et al.* 1998]. Mice homozygous for null mutations in *PRLR* are sterile due to a failure of embryonic implantation, demonstrate irregular cycles, reduced fertilization rate, defective embryonic development [Ormandy *et al.* 1997, Bole-Feysot *et al.* 1998, Baran *et al.* 2002, Grosdemouge *et al.* 2003] and show impaired maternal behaviour [Lucas *et al.* 1998]. These characteristics make *PRLR* a strong candidate gene for reproductive traits. *PRLR* gene was mapped to porcine chromosome 16 [Vincent *et al.* 1997] and ovine chromosome 16 [Jenkins *et al.* 2000]. Screening of several commercial pig lines indicated existence of an association between the litter size and *AluI* polymorphism [Vincent *et al.* 1998]. Allelic additive effects found over all parities varied in different lines and ranged from 0.66 to more than two piglets per litter for numbers born alive, respectively [Rothschild *et al.* 1998, Vincent *et al.* 1998; Drogemuller *et al.* 2001, Linville *et al.* 2001, Putnova *et al.* 2002, van Rens and van der Lende 2002, van Rens *et al.* 2003].

The Jining Grey is a prolific local goat breed in China. The mean number of live born piglets for Jining Grey and Liaoning Cashmere goats as reported by Tu [1989] was 2.94 and 1.18, respectively and 1.89-2.10 for Boer goats [Malan 2000]. The objectives of the present study was to identify the polymorphism of the *PRLR* gene in goat breeds differing in prolificacy, and to investigate the association between polymorphism in *PRLR* gene and high prolificacy in goats.

Material and methods

Animals

Venous jugular blood samples (10 mL per animal) were withdrawn from 112 Jining Grey does (Jining Grey goats conservation base, Jiexiang County, Shandong

Province, PR of China), 40 Boer does (Qinshui Demonstration Farm, Zhengzhuang Town, Qinshui County, Shanxi Province, PR of China), 40 Liaoning Cashmere and 40 Beijing native goats (Qingshui Town, Mentougou District, Beijing, PR of China) and 40 Wendeng dairy goats (Wendeng City, Shandong Province, PR of China) using acid citrate dextrose as an anticoagulant. Genomic DNA was extracted from whole blood by the phenol-chloroform method, dissolved in TE buffer (10 mmol/L Tris-HCl and 1 mmol/L EDTA, pH 8.0), and kept at -20°C.

The 112 Jining Grey does kidded in 2005 and had records on litter size in parities 1, 2 and 3. The does were chosen at random and were the progeny of 5 bucks. No selection for litter size or other reproduction traits was performed in the flock over previous years. Kidding seasons consisted of 3-month groups starting with March through May as season 1 (spring), June through August as season 2 (summer), September through November as season 3 (autumn), and December through February as season 4 (winter).

Primer sequences, PCR amplification, SSCP analysis, cloning and sequencing

The primers (termed primer P1 and primer P2) were designed according to human (GenBank: AC_000048), sheep (GenBank: Y10578) and bovine (GenBank: AF042780) *PRLR* gene sequence. Primers information is shown in Table 1. The PCR, SSCP Analysis, cloning and sequencing followed the method of Chu *et al.* [2009].

Table 1. Primers of caprine *PRLR* gene designed for PCR-SSCP analysis

Primer	Size(bp)	Upstream primer (5'→3')	Downstream primer (5'→3')	Tm
P1	215	CATCTGCTGGAGGTAAGTGC	TTCATTGCCCTCTGACGCTT	66
P2	176	TGTCAGTAAGCGTCAGAGGGC	GGCTGGTGGAAGGTCACTCTT	68

Statistical

The following fixed effects model was employed for analysis of litter size in Jining Grey does and least squares mean was used for multiple comparisons of litter size among different genotypes.

$$Y_{ijklm} = \mu + S_i + KS_j + P_k + G_l + e_{ijklm}$$

where:

- Y_{ijklm} – phenotypic value of litter size;
- μ – population mean;
- S_i – the fixed effect of the i-th sire (i = 1, 2, 3, 4, 5);
- KS_j – the fixed effect of the j-th kidding season (j = 1, 2, 3, 4);
- P_k – the fixed effect of the k-th parity (k = 1, 2, 3);

G_l – the fixed effect of the l -th genotype (for primer P1, $l = 1, 2, 3, 4, 5$; for primer P2, $l = 1, 2$);

e_{ijklm} – random error effect of each observation.

Analysis was performed using the general linear model procedure of SAS (Ver 8.1) (SAS Institute Inc., Cary, NC, USA). Mean separation procedures were performed using a least significant difference test.

Results and discussion

Polymorphism in *PRLR* gene

In this study five genotypes (named AA, AH, AK, HH and HK) were found by SSCP analysis of amplicon obtained with primer P1 (Fig. 1). Three genotypes (named CC, CD and DD) were found analysing PCR products obtained with primer P2 (Fig. 2). Sequencing of amplicons synthesized with P1 primer revealed one nucleotide mutation (C119T) in intron 1 of *PRLR* gene (genotypes HH and AA – Figure 3). Two nucleotide mutations (G35A and A86G) were found in amplicons obtained with primer P2 – intron 2 of *PRLR* gene (genotypes DD and CC – Figure 4). Allele and genotype frequencies of *PRLR* gene in five goat breeds are presented in Table 2. The result indicated that H allele appeared only in Jining Grey, whereas DD genotype only in Boer goats.

Several studies have been reported on polymorphism (PCR-RFLP) of the porcine *PRLR* gene [Vincent *et al.* 1997 and 1998, Rothschild *et al.* 1998, Drogemuller *et al.* 2001, Linville *et al.* 2001, van Rens and van der Lende 2002, Putnova *et al.* 2002, van Rens *et al.* 2003].

The chicken *PRLR* gene was screened for polymorphisms by PCR-SSCP [Jiang *et al.* 2005]. One mutation A9026G in exon 3 of the chicken *PRLR* gene was detected, which led to a nucleotide transition in the 5'-untranslated region of *PRLR* cDNA. Two mutations T14771C and G14820A were detected in exon 6 of the chicken *PRLR* gene.

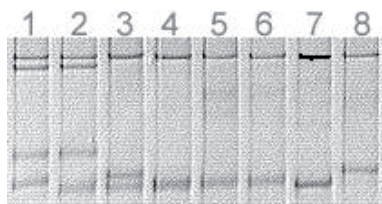


Fig. 1. SSCP of PCR products obtained using primers P1 in goats. Lane 1 – HK genotype; lane 2 – AK genotype; lane 3 – AH genotype; lanes 4, 5, 6 and 7 – AA genotype; lane 8 – HH genotype.

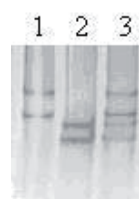


Fig. 2. SSCP of PCR products obtained using primer P2 in goats. Lane 1 – CC genotype; lane 2 – DD genotype; lane 3 – CD genotype.

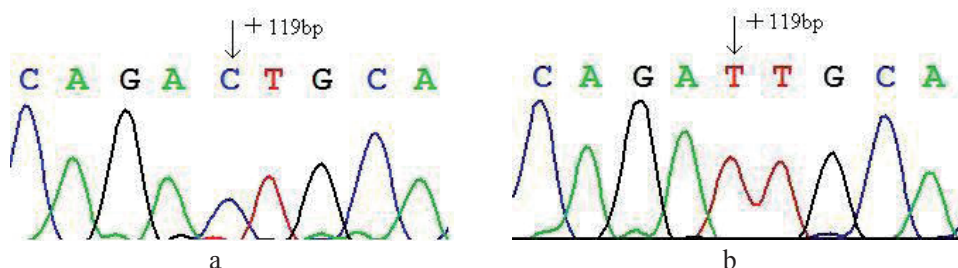


Fig. 3. Sequence comparison of AA and HH genotypes of intron 1 of *PRLR* gene in Jining Grey goats. a – AA genotype; b – HH genotype.

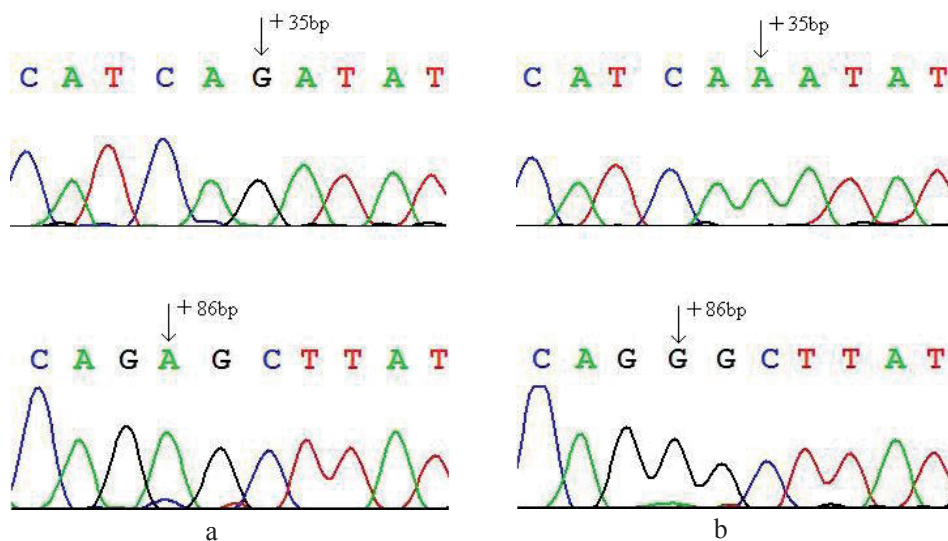


Fig. 4. Sequence comparison of CC and DD genotypes of intron 2 of *PRLR* gene in Boer goats. a – CC genotype, b – DD genotype.

The T14771C transition led to an amino acid change (Leu340Ser) in *PRLR*, whereas the G14820A transition was a synonymous mutation. These three mutations in *PRLR* gene did not affect broody traits in chickens. Polymorphisms were identified within intron 1 and exon 10 of the ovine *PRLR* gene using PCR-SSCP [Mu *et al.* 2006, Chu *et al.* 2007].

Table 2. Genotype and allele frequencies of *PRLR* gene in five goat breeds

Item	Jining Grey goat	Wendeng dairy goat	Liaoning Cashmere goat	Beijing native goat	Boer goat	
number of goats	112	40	40	40	40	
P1 genotype frequency	AA	0.732 (82)	0.975 (39)	0.900 (36)	0.975 (39)	0.975 (39)
	AH	0.107 (12)	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)
	AK	0.134 (15)	0.025 (1)	0.100 (4)	0.025 (1)	0.025 (1)
	HH	0.018 (2)	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)
	HK	0.009 (1)	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)
allele frequency	A	0.853	0.987	0.950	0.987	0.987
	H	0.076	0.000	0.000	0.000	0.000
	K	0.071	0.013	0.050	0.013	0.013
P2 genotype frequency	CC	0.875 (98)	0.950 (38)	0.950 (38)	0.975 (39)	0.625 (25)
	CD	0.125 (14)	0.050 (2)	0.050 (2)	0.025 (1)	0.325 (13)
	DD	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)	0.050 (2)
	allele frequency	C	0.937	0.975	0.975	0.987
	D	0.063	0.025	0.025	0.013	0.213

Bracketed are numbers of individuals genotyped.

Effect of *PRLR* gene polymorphism on litter size

The least squares means and standard errors for litter size of different *PRLR* genotypes in Jining Grey goats are given in Table 3. For primer P1, the Jining Grey does with genotype AH or AK had 0.59 or 0.57 ($P < 0.01$) more kids than those with genotype AA, respectively. For primer P2, the Jining Grey does with genotype CD had 0.55 ($P < 0.01$) more kids than those with genotype CC.

Table 3. Least squares means (LSM) and standard errors (\pm SE) for litter size of different genotypes of *PRLR* gene in Jining Grey goats

Primer	Genotype	No. of samples	LSM \pm SE
P1	AA	82	2.43 ^B \pm 0.18
	AH	12	3.02 ^A \pm 0.26
	AK	15	3.00 ^A \pm 0.23
P2	CC	98	2.52 ^B \pm 0.15
	CD	14	3.07 ^A \pm 0.21

^A^BMeans within primers bearing different superscripts are significantly different at $P < 0.01$.

The *PRLR* gene polymorphism was significantly associated with total number of piglets born and/or number of piglets born alive in several pig lines [Rothschild *et al.* 1998, Drogemuller *et al.* 2001, Putnova *et al.* 2002, van Rens and van der Lende

2002]. The magnitude of the effect varied by population or breed (Landrace and Large White). A significant association has also been observed between the *PRLR* genotypes and ovulation rate and a number of embryos on day 35/36 of pregnancy [van Rens *et al.* 2003].

Within intron 1 and exon 10 of the ovine *PRLR* gene, the Small Tail Han ewes with genotype BB or AB had by 0.64-0.76 or 0.44-0.54 more lambs than those with genotype AA, respectively [Chu *et al.* 2007]. The result of the present study also shows significant association between prolactin receptor *locus* and high prolificacy of Jining Grey goats. This *PRLR* gene test has the potential to be a powerful tool when used in conjunction with traditional selection methods for some goats. Without complete pedigrees of the animals, this really is a preliminary study. Before using these results for MAS, additional data are needed to confirm the significant effect seen in Jining Grey does.

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