

## Relationship between the Polymorphisms of 5' Regulation Region of Prolactin Gene and Milk Traits in Chinese Holstein Dairy Cows

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**ABSTRACT** : Prolactin (PRL) plays an important role in promoting mammalian mammary gland development, and milk production during lactation. Therefore the PRL gene was chosen as a candidate gene for milk traits in Holstein dairy cows. PCR-SSCP and PCR-RFLP were used to analyze genetic variations in the 5' regulation region of the PRL gene. In this part of the gene, two new polymorphic sites were detected in the Chinese Holstein dairy cows. One was a *Xba*I-RFLP locus, and the other was an SSCP locus. Statistical analysis showed that the *Xba*I-RFLP locus and the SSCP locus had a significant positive effect on milk traits. (*Asian-Aust. J. Anim. Sci.* 2006. Vol 19, No. 4 : 459-462)

**Key Words** : Prolactin (PRL), PCR-RFLP, PCR-SSCP, Milk Traits

### INTRODUCTION

Prolactin (PRL), one member of growth hormone/prolactin family, is a polypeptide hormone that is synthesized in and secreted from specialized cells of the anterior pituitary gland, the lactotrophs, in vertebrate. Prolactin plays important roles in the growth and development of the mammary gland (mammogenesis), synthesis of milk (lactogenesis), and maintenance of milk secretion (galactopoiesis). These characteristics make PRL a strong candidate gene for milk traits. Till now, PRL gene has been cloned and characterized in most of the species (Kohmoto et al., 1984; Truong et al., 1984; Au et al., 2002; Cao et al., 2002). For example, in the bovine genome, a single gene, found on chromosome 20, encodes prolactin. The prolactin gene is about 10 kb in size and is composed of 5 exons and 4 introns. The bovine prolactin (bPRL) cDNA is 917 nucleotides long and contains a 699-nucleotide open reading frame encoding the prolactin prohormone. The signal peptide contains 30 amino acids, thus the mature bovine prolactin is composed of 199 amino acids (Marc et al., 2000). Cao et al. (2002) had cloned the whole sequence of bovine PRL gene cDNA (GenBank serial number: AF426315) and proved the biologic transcription activity of bovine PRL gene. To study the effect of bovine PRL gene on dairy milk traits, we chose bovine PRL gene as a candidate gene for milk traits and detected 5'-regulatory region of it, analyzed the relationship between different genotypes and milk traits. The aim was to find genetic markers highly correlated with milk traits for marker assisted selection (MAS) of dairy.

### MATERIALS AND METHODS

#### Experimental animals

Two hundred thirty six Holstein dairy cows were housed in Beijing Dairy Cattle Center in accordance with approved farm management practices. The blood samples were collected from vein, and store at -20°C with ACD anticoagulant in Sep, 2003. The milk traits recorded in the farm were 305 days correct milk yield, average milk fat yield and average milk protein yield. Genomic DNA was purified by standard procedures using proteinase K digestion followed by phenol/chloroform extraction and precipitation with isopropanol (Sambrook et al., 1989).

#### Primer design and PCR amplification

The primers were designed on the 5' region of bovine PRL gene according to the known sequence (GenBank accession number: AF426315) by Primer 5.0 software and synthesized by Shanghai BioAsia corporation.

primers P1: the forward primer 5'-AGGTTAGGAG GATAG-3' and reverse primer 5'-TTAGTCAAGTTAGATA CCG-3',

primers P2: the forward primer 5'-CCCCAGTATGA ACTCCCT-3' and reverse primer 5'-TCTGTTTTGTCCCT TCTA-3'.

The total volume of the PCR reaction was 25 µl, including 18.375 µl water, 2.5 µl 10× reaction buffer, 2 µl dNTP mixture (2.5 mmol/l), 0.5 µl primers P1 and P2 (20 µmol/l), 0.125 µl Taq DNA polymerase enzyme (TAKARA, 5 U/µl), 1 µl templet DNA (50 ng/µl). The reaction cycling included an initial denaturation at 95°C for 5 min, followed by 30 cycles 1 min at 95°C, 1min at 50.8°C, 1 min at 72°C and a final extension of 7 min at 72°C.

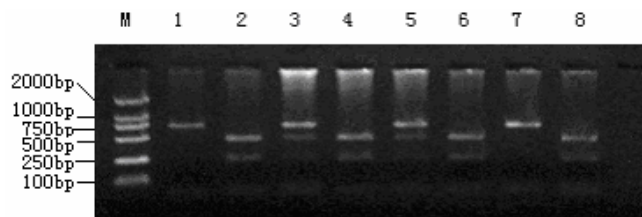
#### RFLP analysis

The 20 µl enzyme digestion mixture was composed of 1

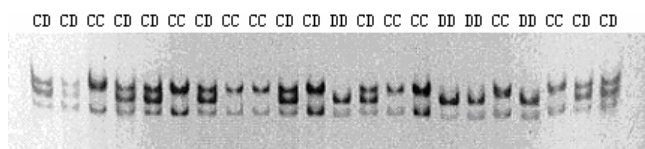
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**Figure 1.** The PCR-RFLP test for the prolactin polymorphism. Lane M: DNA marker DL 2,000; Lane 1 and 7: AA genotype; Lane 2, 4, 6, 8: BB genotype; Lane 3 and 5: AB genotype.



**Figure 2.** PCR-SSCP test for the prolactin polymorphism.

$\mu$ l enzyme, 2  $\mu$ l 10 $\times$ buffer, 2  $\mu$ l 0.1% BSA, 4  $\mu$ l PCR products, 11  $\mu$ l water. The mixture was digested at 37°C for 2-3 h and separated on 1.5% agarose gel.

#### SSCP analysis

The PCR products were separated on 8% denaturing polyacrylamide gel (arc:bis = 49:1). After being electrophoresed at 4°C overnight with electric power less than 5W, the gels were silver stained and photographed by KODAK gel screening system.

#### DNA cloning and sequencing

Different homozygote genotypes of RFLP locus and SSCP locus were recovered and constructed. Two positive clones of each genotype were sequenced by automatic PE377 sequencer.

#### Statistical analysis

The statistical analyses were examined using the Statistical Analysis System (SAS) general linear models (GLM) procedure. First, the polymorphisms were analyzed separately using model I. Then the combined genotypes were analyzed using model II. Statistical models included the fixed effects of parity, period of farrowing and genotypes of PRL gene. The models were as follows:

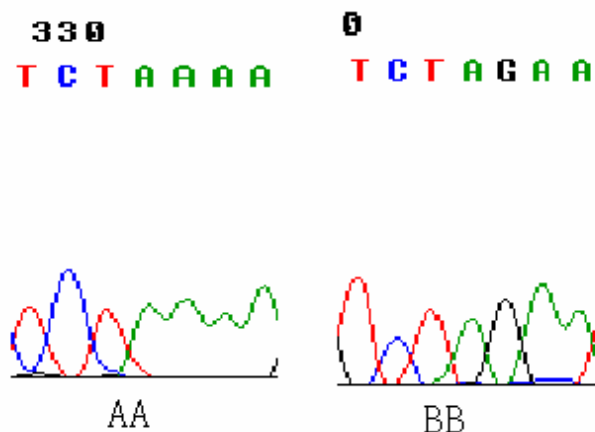
Statistical model I:

$$Y_{ijk} = \mu + G_i + T_j + F_k + e_{ijk}$$

Statistical model II:

$$Y_{ijkl} = \mu + G_i + G_j + G_i \cdot G_j + T_k + F_l + e_{ijkl}$$

Where, Y = trait value;  $\mu$  = general mean; G = effect of



**Figure 3.** The partial sequence of AA and BB genotype of *XbaI*-RFLP locus.

genotype; T = effect of parity; F = effect of period of farrowing; e = random residual.

## RESULTS

#### RFLP analysis

A new polymorphism in the PRL gene was detected with *XbaI* restriction endonuclease, which cut the amplicon to several fragments. Allele A in the *XbaI* locus, in which polymorphic restriction site was absent, was characterized by the presence of the largest fragment of 678 bp, while for allele B, which possessed the polymorphic restriction site, this fragment was cut to yield fragment of 447 bp and 231 bp (Figure 1).

#### SSCP analysis

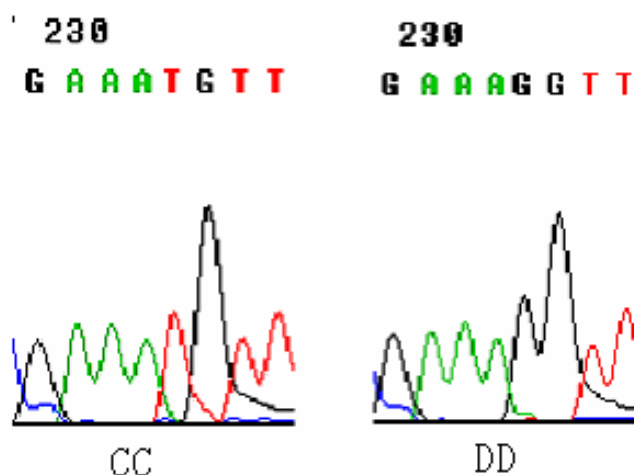
The primer P2 yielded 304 bp fragment. SSCP analysis of the fragment displayed polymorphisms in the Chinese Holstein dairy cows. There were three genotypes with two homozygotes, defined as CC and DD, and one heterozygote, defined as CD (Figure 2).

#### Cloning and sequencing of polymorphic fragments

Sequencing of the homozygotes of *XbaI*-RFLP locus and SSCP locus indicated that polymorphisms of *XbaI*-RFLP locus were caused by A  $\rightarrow$  G mutation at 446 position (Figure 3) and polymorphisms of SSCP locus were caused by T  $\rightarrow$  G mutation at 175 position (Figure 4).

#### Association analysis of PRL gene and milk traits

The effect of genotypes of *XbaI*-RFLP and SSCP loci of the PRL gene on milk traits were given in Table 1. Because only two AA genotypes were detected in the 263 Chinese Holstein dairy cows, no significant effects of the *XbaI*-RFLP locus were found on milk yield, milk protein yield and milk fat yield from parity 2 to parity 5. But the *XbaI*-



**Figure 4.** The partial sequence of CC and DD genotype of SSCP locus.

RFLP locus significantly affected milk protein and fat yield on parity 1 ( $p < 0.05$ ). The BB cows yielded more milk protein and fat than AB cows ( $p < 0.05$ ). The SSCP locus significantly affected the milk fat yield on parity 1 and milk protein yield on parity 4 ( $p < 0.05$ ). The estimated increase was 43.96 kg milk fat yield per copy of allele C. While for the milk protein yield on the parity 5, 19.55 kg milk protein per copy of allele C was estimated.

Statistical analysis of the combined genotype of *Xba*I-RFLP and SSCP loci found no significant association between the combined genotypes and milk yield ( $p > 0.05$ ) (Table 2). But there was a tendency that the BBCC cows

**Table 2.** Least squares means and standard errors for milk yield of the combined genotypes in the PRL gene

Genotype		Number	Milk yield (kg)
<i>Xba</i> I-RFLP locus	SSCP locus		
BB	CC	61	8,467.21±145.36
BB	CD	53	8,402.35±138.42
AB	CC	48	8,387.54±143.68
AB	CD	45	8,324.84±189.57

had highest milk yield.

## DISCUSSION

PRL initiate JAK2/STAT5 signaling pathway through binding to PRL receptor distributed at the surface of target cell membrane, and activate STAT5 to affect target sequences in the promoter region of lactoprotein gene and enhance the expression of target gene. Therefore, prolactin plays an important role in the growth and mammatogenesis, lactogenesis and galactopoiesis. Till now, many polymorphisms have been found in the bPRL gene and the associations between polymorphisms and production traits have been analyzed. For example, Cowan et al. (1990) analyzed bovine PRL gene with RFLP analysis and estimated breeding values of 700 granddaughter of breeder bull. The results indicated that for milk yield, cheese ratio and milk protein ratio traits the differences between AA genotype and BB genotype were 282.9 kg, 48.58% and 53.67%. Udina et al. (2001) analyzed the samples of Russian Ayrshire and Gorbato Red cattle breeds and found one polymorphic *Rsa*I site in exon 3 and one variation of

**Table 1.** Least squares means and standard errors for milk traits of the polymorphisms in the PRL gene

Genotype	AA	AB	BB	CC	CD	DD
Number	2	107	127	130	90	15
Parity 1						
Milk yield (kg)	-	7,815.53±206.93	8,103.27±178.62	7,988.39±195.72	7,831.37±236.25	6,917.00±806.96
Milk protein (kg)	-	257.55±6.97 <sup>b</sup>	283.59±6.01 <sup>a</sup>	262.06±5.98	258.06±7.22	217.67±24.65
Milk fat (kg)	-	333.58±10.14 <sup>b</sup>	360.31±8.75 <sup>a</sup>	353.59±9.32 <sup>a</sup>	334.91±11.25 <sup>a</sup>	265.67±38.42 <sup>b</sup>
Parity 2						
Milk yield (kg)	-	8,344.90±308.62	8,519.12±282.85	8,600.76±286.20	8,098.62±286.20	8,133.50±655.76
Milk protein (kg)	-	275.71±8.99	276.12±8.24	278.48±9.08	273.90±9.08	273.25±20.80
Milk fat (kg)	-	365.33±17.28	360.52±15.83	371.62±17.37	357.33±17.37	344.25±39.80
Parity 3						
Milk yield (kg)	8,550±605.94	8,445.12±207.84	8,558.56±201.98	8,704.05±240.72	8,480.45±316.36	8,665.57±396.58
Milk protein (kg)	306.50±25.11	279.18±8.61	295.56±8.37	286.47±7.61	279.27±10.00	280.57±12.54
Milk fat (kg)	405.00±50.55	355.00±17.34	355.78±16.85	349.68±15.77	344.00±20.73	374.43±25.98
Parity 4						
Milk yield (kg)	8,103.00±1186.97	8,529.33±395.66	8,735.11±395.66	8,763.55±338.30	8,318.43±404.34	8,618.00±676.59
Milk protein (kg)	274.00±36.90	280.28±12.30	289.67±12.30	317.30±9.32 <sup>a</sup>	274.57±11.14 <sup>b</sup>	278.20±18.65 <sup>ab</sup>
Milk fat (kg)	352.00±60.78	379.78±20.26	380.89±20.26	376.75±17.03	362.50±20.35	350.60±30.05
Parity 5						
Milk yield (kg)	-	8,514.00±362.48	8,805.43±433.25	8,806.10±359.06	8,388.14±429.16	-
Milk protein (kg)	-	266.60±8.24	288.43±9.85	282.80±8.51	265.28±10.18	-
Milk fat (kg)	-	390.20±17.37	419.86±20.76	402.30±18.05	402.57±21.58	-

<sup>a, b</sup> Means within a column without a common superscript differ ( $p \leq 0.05$ ).

the microsatellite dinucleotide repeat in the regulatory region of the PRL gene.

In our study, 5' regulation region of bovine prolactin (bPRL) gene was screened by PCR-RFLP and PCR-SSCP techniques and two mutation sites were discovered for the first time. Whether these mutations could affect the expression of PRL gene remains further study. Analysis of the association between the polymorphisms of PRL gene and milk traits showed that the *Xba*I-RFLP locus significantly affected milk protein and milk fat in first fetus ( $p < 0.05$ ), individual of BB genotype yield more milk protein and fat than those of AB genotype. The SSCP locus significantly affected the milk fat yield on parity 1 and milk protein yield on parity 4 ( $p < 0.05$ ). Because of the small population, no significant effect was found for the combined genotypes on the milk yield. It is necessary to increase the number of animals and records for each population.

Our finding together with aforementioned literature data indicated that neither the *Xba*I-RFLP nor the SSCP locus of the PRL gene were causative mutations. It is hypothesized that the effect is caused by linkage disequilibrium between marker loci and a causative mutation, which is either within the PRL gene or at its near proximity.

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