

## Association of Single Nucleotide Polymorphisms in Exon 6 Region of BMPRIB Gene with Litter Size Traits in Sheep

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**ABSTRACT** : The objective of this study was to investigate polymorphisms of BMPRIB (bone morphogenetic protein type IB receptor) gene and its effect on litter size traits in sheep. Three populations including 101 Small Tailed Han sheep, 79 Poll Dorset and 81 hybrids (Poll Dorset×Small Tailed Han sheep) were used to detect the polymorphisms in exon 6 region of sheep BMPRIB gene. A fragment of approximately 190bp was amplified by one pair of primers, the polymorphism was revealed from the analysis of three populations by the technique of PCR -SSCP, and a mutation from A to G at 746 of the coding region was confirmed by sequencing in several individual. Statistical results indicated the distribution of allele B (with a A→G mutation) and A (without mutation) or genotype AA, AB and BB frequencies differed in three populations. BB genotype (44.55%) and B allele (66.34%) frequencies of Small Tailed Han sheep were higher than those of the others. Analysis of variance showed that the polymorphism of BMPRIB gene was associated with positive effect on litter size traits. The means of genotype BB and AB were about 1.04 and 0.74 more than genotype AA for litter size ( $p < 0.05$ ). Analysis of BMPRIB genotype effects on litter size in three populations indicates the existence of genotype BB or B allele increases the litter size. It suggested that the polymorphism in exon 6 (at 746 in the coding region) of sheep BMPRIB gene may be used as a marker for early selection of prolificacy in sheep. (*Asian-Aust. J. Anim. Sci.* 2005. Vol 18, No. 10 : 1375-1378)

**Key Words** : Sheep, Litter Size, BMPRIB, PCR-SSCP

### INTRODUCTION

Multiple ovulation in mammals is a complex trait influenced by genetic and environmental factors. Primates and many ruminants typically release a single oocyte at each cycle whereas species such as mice and pigs, capable of rearing many offspring, have consistently high ovulation rate. The ovulation rate in different sheep breeds ranges variedly, providing a convenient model to study the molecular genetics of this phenomenon (Montgomery et al., 2001). The identification and use of specific major genes for production traits in livestock enable an increased rate of genetic improvement (Hoque et al., 2002; Zhu et al., 2004).

BMPRIB gene is a member of the transforming growth factor $\beta$  (TGF- $\beta$ ) receptor superfamily. It has a coding sequence of 1,509 bp, which is composed of 10 exons and mainly expressed in granulose cell. BMPRIB gene is located in sheep chromosome 6 containing the FecB locus (Mulsant et al., 2001). FecB gene is considered as a major gene inducing the high prolificacy of Booroola sheep. On average, the gene increases litter size by one to two extra lambs with one copy of the FecB mutation (Montgomery et al., 1992). Only recently have researchers discovered that a mutation in the BMPRIB is associated fully with the FecB

Booroola mutation. (Mulsant et al., 2001; Souza et al., 2001). These discoveries make it possible to select prolific strains of sheep earlier by determine mutation, without the need for the pedigree information in sheep (Montgomery et al., 1993).

Small Tailed Han sheep that has significant characteristics of high prolificacy and non-seasonal ovulation activity is an excellent local sheep breed in P. R. China (Chu et al., 2003a). Some studies about the high prolificacy of Small Tailed Han sheep are being done. It has been reported that three microsatellite loci (OarAE101, BM1329 and BMS2508) (Chu et al., 2003a), Rsa I site in the exon 2 of melatonin receptor 1a gene (Chu et al., 2003b) and the deletion in the exon 1 of BMP15 (bone morphogenetic protein 15) (Guo et al., 2004) were related to the high prolificacy of Small Tailed Han sheep. In the present study, prolific Small Tailed Han sheep, low fecundity Poll Dorset sheep and hybrids (Poll Dorset ×Small Tailed Han sheep) were firstly used to identify polymorphisms in the BMPRIB gene by PCR single strand conformation polymorphism (SSCP) and secondly to evaluate the association of these polymorphisms with litter size traits.

### MATERIALS AND METHODS

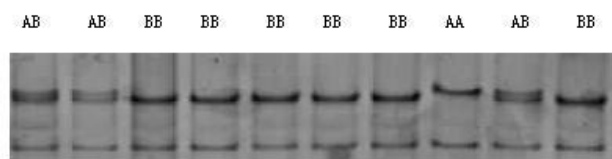
#### Animals and DNA samples

The blood samples of three populations with 261 ewes including 101 Small Tailed Han sheep, 79 Poll Dorset and 81 hybrids with the records of litter size were collected

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**Figure 1.** Genotypes of SSCP analysis of the exon 6 in the sheep BMPRIB. Individuals with one slow band, one fast band and two bands were designed as genotype AA, BB and AB, respectively, besides one mutual band.

from two breeding sheep bases of China Agricultural University of Beijing in P. R. China. Genomic DNA from blood cells was extracted by phenol-chloroform method.

### PCR condition and SSCP analysis

The PCR was performed to amplify sequence fragments of exon 6 regions of BMPRIB gene. The primer sequences were indexed from Mulsant et al. (2001). The primer of BMPRIB locus in exon6-intron6 was as follows: Forward primer P<sub>1</sub>: 5'-AGATTGAAAAGGTCGCTATG-3', Reverse primer P<sub>2</sub>: 5'-ACCCTGAACATCGCTAATACA-3'. Each 25 µl of PCR reaction contained 50 ng ovine genomic DNA, 0.2 µM of each primer, 200 µM dNTP, 1 U of Taq polymerase (Dingguo Biotechnology Company, Beijing, China) and reaction buffer with 1.5 mM MgCl<sub>2</sub>. PCRs were performed at 94°C for 5min, followed by 35 cycles at 94°C for 30 s, 56.8°C for 30 s, 72°C for 1 min and final extension at 72°C for 7 min. The PCR products were then directly genotyped by SSCP. The PCR products were mixed (1:5) with loading buffer (95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol FF), denatured at 95°C for 5 min, followed by a rapid chilled on ice and loaded on 12% polyacrylamide gels. Gels were run at 8 V/cm at room temperature for 16 h. The DNA bands were visualized with silver staining technique.

### Direct sequencing of PCR products to confirm mutation

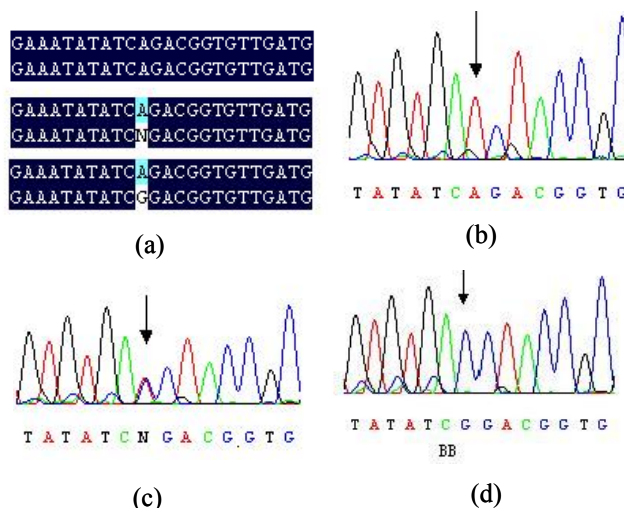
The PCR products from the sheep with different bands were sequenced respectively after purified PCR products using the quick PCR Purification kit Protocol (Dingguo Biotechnology Company, Beijing, China).

### Statistical analysis

Association of BMPRIB genotypes with litter size trait was analyzed using the following model:

$$Y_{ijk} = \mu + g_i + f_{ys_j} + b_k + e_{ijk}$$

Where  $Y_{ijk}$  is phenotypic value of litter size;  $\mu$  is population mean for litter size;  $g_i$  is the fixed effect of the  $i^{\text{th}}$  genotype ( $i = 1, 2, 3$ );  $f_{ys_j}$  is the fixed effect of the  $j^{\text{th}}$  farm, year and season ( $j = 1-8$ );  $b_k$  is the fixed effect of the  $k^{\text{th}}$  breed ( $k = 1, 2, 3$ );  $e_{ijk}$  is random residue. The statistical



**Figure 2.** Nucleotide sequence comparison of the PCR products amplified by P<sub>1</sub>/P<sub>2</sub>; (a) BLAST results of the nt sequence of genotype AB and BB in this study with previously published nt sequence in GenBank; (b) nt sequence of genotype AA (arrow's position is consistent with nt sequence in GenBank); (c) and (d) nt sequence of genotype AB and BB (the arrow pointed to the mutation site).

program used was by the GLM procedure of SAS 8.2 (version).

## RESULTS

### The analysis of polymorphism by SSCP

A fragment of approximately 190 bp was amplified by primer P<sub>1</sub> and P<sub>2</sub>. The polymorphisms of 261 sheep were analyzed by PCR-SSCP, and three distinct band patterns were observed (Figure 1). The B allele was defined as the nucleotide sequence in exon 6 of BMPRIB with the A→G mutation (at 746 of the coding region), and the A allele as the sequence without this mutation.

### Direct sequencing of PCR products

The individual with different bands were direct sequenced of PCR products. Comparative sequencing of BMPRIB amplified fragments obtained from individuals of three populations revealed a 'A→G' transition in exon 6 (Figure 2).

### Association of the BMPRIB gene SNP with litter size traits

Two hundred and sixty-one sheep from three populations were genotyped for the SNP (Single Nucleotide Polymorphisms). The gene and genotype frequencies at the BMPRIB locus in three populations are different. Table 1 indicated that in Small Tailed Han sheep genotype frequencies are BB>AB>AA and allele frequencies is B>A; in Poll Dorset genotype AA were dominant and A allele frequencies is higher; F<sub>1</sub> populations genotype BB was not

**Table 1.** Distribution of genotypes and allele frequencies in three populations

Category		Small Tailed Han sheep		Poll Dorset		F <sub>1</sub> populations	
		No.	%	No.	%	No.	%
Genotypes	AA	12	11.88	76	96.20	17	20.99
	AB	44	43.56	2	2.53	64	79.01
	BB	45	44.55	1	1.27	0	0
Allele	A	68	33.66	154	97.46	98	60.49
	B	134	66.34	4	2.53	64	39.51

**Table 2.** Effects of sheep BMPRI<sup>B</sup> genotype on litter size traits

Genotypes	No. of observation	Least squares means	Standard error
AA	106	1.22 <sup>a</sup>	0.07
AB	110	1.96 <sup>b</sup>	0.08
BB	46	2.26 <sup>c</sup>	0.10

Least-squares means for genotypes in the same column with different superscripts are significantly different (p<0.05).

discovered in this study. BB genotype and B allele frequencies of Small Tailed Han sheep were higher than those of others among the populations.

Analysis of variance showed that the BMPRI<sup>B</sup> genotypes have significant effect on litter size in sheep. Table 2 showed the least square means litter size is the highest in BB genotype, followed by AB and AA and is significantly different among them (p<0.05). The ewes with genotype BB and AB had about 1.04 and 0.74 more than genotype AA for litter size (p<0.05).

Least squares means (LSM) and standard error for litter size of different genotype in BMPRI<sup>B</sup> gene in three populations are showed in Table 3. It can be seen from Table 3, relationships of LSM for litter size of two genotypes for BMPRI<sup>B</sup> in Small Tailed Han sheep and Poll Dorset are BB>AA and the different is significant (p<0.05). The LSM for litter size of two genotypes (AA and AB) for BMPRI<sup>B</sup> in three populations are different, but the difference is not significant (p>0.05).

### DISCUSSION

The effect of the FecB<sup>B</sup> gene is additive for ovulation rate dominant for litter size. One copy FecB<sup>B</sup> increases ovulation rate by 1.3-1.6, litter size by 0.9-1.2; two copies FecB<sup>B</sup> increase ovulation rate and litter size by 2.7-3.0 and 1.1-1.7, respectively, in ewe (Montgomery et al., 1992). However, the Booroola (FecB) phenotype was associated with a A→G mutation at 746 of the coding region in BMPRI<sup>B</sup> gene (Mulsant et al., 2001; Souza et al., 2001).

The current study showed a mutation from A to G was identified by SSCP at 746 in the coding region of the BMPRI<sup>B</sup> gene (Figures 1 and 2). The gene and genotype frequencies at the BMPRI<sup>B</sup> locus in three populations are different (Table 1). BB genotype and B allele frequencies of Small Tailed Han sheep were higher than those of others among the populations. The genotype of BB is not discovered in F<sub>1</sub> population, which is possible due to the paternal (Poll Dorset) of the F<sub>1</sub> population containing the less B allele. Analysis of variance using GLM revealed that the BMPRI<sup>B</sup> genotypes have significant effect on litter size (Table 2). The ewes with homozygous mutant BB and heterozygous mutant AB had about 1.04 and 0.74 more than wild homozygous genotype AA for litter size (p<0.05) in sheep. However, BMPRI<sup>B</sup> genotype had different effects on litter size in three populations (Table 3). Relationships of LSM for litter size of three genotypes for BMPRI<sup>B</sup> in Poll Dorset are BB>AA>AB, which is possibly contributed to the fewer samples, but the others are BB>AA (p<0.05) and AB>AA (p = 0.07 for two populations). So the existence genotype BB or B allele is benefit to the high litter size.

BMPRI<sup>B</sup> as a member of the transforming growth factorβ (TGF-β) receptor superfamily played the important roles in signal transduction. The current model of induction of signalling responses is at the cell surface, the ligand binds a complex of transmembrane receptor serine/threonine kinases (types I and II) and induces transphosphorylation of the Gly-Ser (GS) segments in the type I receptor by the type II receptor kinases. The consequently activated type I receptors phosphorylate selected Smads at C-terminal serines, and these receptor-activated Smads (R-Smads) then form a complex with a common Smad4. Activated Smad complexes translocate into the nucleus, where they regulate transcription of target genes, through physical interaction and functional cooperation with DNA-binding transcription factors (X) and CBP or p300 coactivators (Derynck et al., 2003). Signal

**Table 3.** Effects of BMPRI<sup>B</sup> genotype on litter size traits in three populations

Genotypes	Small Tailed Han sheep		Poll Dorset		F <sub>1</sub> populations	
	No.	LSM±SE	No.	LSM±SE	No.	LSM±SE
AA	12	1.42±0.25 <sup>b</sup>	76	1.14±0.04 <sup>b</sup>	17	1.53±0.17 <sup>a</sup>
AB	44	1.93±0.13 <sup>ab</sup>	2	1.00±0.25 <sup>b</sup>	64	1.88±0.09 <sup>a</sup>
BB	45	2.27±0.13 <sup>a</sup>	1	2.00±0.35 <sup>a</sup>	0	/

Least-squares means for genotypes in the same column with different superscripts are significantly different (p<0.05).

specificity is determined both by the specific ligand and the different smad proteins used in the signal transduction (Wilson et al., 2001). It is noteworthy for the study on what mechanisms BMPRII gene uses leading to differences in ovulation rate including the type of the gland and target gene.

The result of this study suggested that the polymorphism at 746 in the coding region of sheep BMPRII may be used as a marker for early selection of prolificacy in sheep.

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