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 variably, 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 factorβ (TGF-β) 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., 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...
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 P1: 5’-AGATTGGAAAAGGTCGCTATG-3’, Reverse primer P2: 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 MgCl2. 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% polyacryamide 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:

\[ Y_{ijk} = \mu + g_i + f_{ys} + b_k + e_{ijk} \]

Where \( Y_{ijk} \) is phenotypic value of litter size; \( \mu \) is population mean for litter size; \( g_i \) is the fixed effect of the \( i \)th genotype (\( i = 1, 2, 3 \)); \( f_{ys} \) is the fixed effect of the \( j \)th farm, year and season (\( j = 1-8 \)); \( b_k \) is the fixed effect of the \( k \)th breed (\( k = 1, 2, 3 \)); \( e_{ijk} \) is random residue. The statistical 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 P1 and P2. 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 of BMPRIB with the A→G mutation (at 746 of the coding region), and the A allele as the sequence without this mutation.

**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; F1 populations genotype BB was not
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 BMPRIB 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 wild homozygous genotype AA for litter size (p<0.05).

Least squares means (LSM) and standard error for litter size of different genotype in BMPRIB 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 BMPRIB 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 BMPRIB in three populations are different, but the difference is not significant (p>0.05).

**DISCUSSION**

The effect of the FecB\textsuperscript{B} gene is additive for ovulation rate dominant for litter size. One copy FecB\textsuperscript{B} increases ovulation rate by 1.3-1.6, litter size by 0.9-1.2; two copies FecB\textsuperscript{B} 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 BMPRIB 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 BMPRIB gene (Figures 1 and 2). The gene and genotype frequencies at the BMPRIB 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\textsubscript{1} population, which is possible due to the paternal (Poll Dorset) of the F\textsubscript{1} population containing the less B allele. Analysis of variance using GLM revealed that the BMPRIB 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, BMPRIB genotype had different effects on litter size in three populations (Table 3). Relationships of LSM for litter size of three genotypes for BMPRIB 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.

BMPRIB as a member of the transforming growth factor\(\beta\) (TGF-\(\beta\)) 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

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

<table>
<thead>
<tr>
<th>Category</th>
<th>Small Tailed Han sheep</th>
<th>Poll Dorset</th>
<th>F\textsubscript{1} populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>AA</td>
<td>12 (11.88)</td>
<td>76 (96.20)</td>
<td>17 (20.99)</td>
</tr>
<tr>
<td>AB</td>
<td>44 (43.56)</td>
<td>2 (2.53)</td>
<td>64 (79.01)</td>
</tr>
<tr>
<td>BB</td>
<td>45 (44.55)</td>
<td>1 (1.27)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Allele</td>
<td>A 68 (33.66)</td>
<td>154 (97.46)</td>
<td>98 (60.49)</td>
</tr>
<tr>
<td></td>
<td>B 134 (66.34)</td>
<td>4 (2.53)</td>
<td>64 (39.51)</td>
</tr>
</tbody>
</table>

**Table 2. Effects of sheep BMPRIB genotype on litter size traits**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No. of observation</th>
<th>Least squares means</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>106</td>
<td>1.22\textsuperscript{a}</td>
<td>0.07</td>
</tr>
<tr>
<td>AB</td>
<td>110</td>
<td>1.96\textsuperscript{b}</td>
<td>0.08</td>
</tr>
<tr>
<td>BB</td>
<td>46</td>
<td>2.26\textsuperscript{c}</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

**Table 3. Effects of BMPRIB genotype on litter size traits in three populations**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Small Tailed Han sheep</th>
<th>Poll Dorset</th>
<th>F\textsubscript{1} populations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>LSM±SE</td>
<td>No.</td>
</tr>
<tr>
<td>AA</td>
<td>12</td>
<td>1.42±0.25\textsuperscript{a}</td>
<td>76</td>
</tr>
<tr>
<td>AB</td>
<td>44</td>
<td>1.93±0.13\textsuperscript{b}</td>
<td>2</td>
</tr>
<tr>
<td>BB</td>
<td>45</td>
<td>2.27±0.13\textsuperscript{a}</td>
<td>1</td>
</tr>
</tbody>
</table>

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 BMPRIB 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 BMPRIB may be used as a marker for early selection of prolificacy in sheep.

**ACKNOWLEDGMENTS**

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**REFERENCES**


