Polymorphisms of coding region of BMPR-IB gene and their relationship with litter size in sheep

Mingxing Chu · Lihua Jia · Yingjie Zhang · Mei Jin · Hongquan Chen · Li Fang · Ran Di · Guiling Cao · Tao Feng · Qianqian Tang · Yuehui Ma · Kui Li

Abstract The bone morphogenetic protein receptor IB (BMPR-IB) was studied as a candidate gene for the prolificacy of sheep. Nine pairs of primers (P1–P9) were designed to detect single nucleotide polymorphisms (SNPs) of exons 1–4 and 6–10 of the BMPR-IB gene in both high (Small Tail Han and Hu sheep) and low prolificacy breeds (Texel and Chinese Merino sheep) by polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP). Only the products amplified by primers P2, P5, P6, P7, P8 and P9 displayed polymorphisms. The present study identified 22 SNPs in partial coding regions of ovine BMPR-IB, in which 20 SNPs were reported for the first time. In total of the 22 mutations, 18 DNA variations were originated from the Hu breed, three were found in the Small Tail Han breed (two of them were found in other sheep breeds), three in the Chinese Merino breed, and none in the Texel breed. These results preliminarily demonstrated that BMPR-IB is a major gene affecting the hyperprolificacy in Small Tail Han and Hu sheep, and could be used as a molecular genetic marker for early auxiliary selection for hyperprolificacy in sheep.

Keywords Bone morphogenetic protein receptor IB gene · PCR-SSCP · Prolificacy · Sheep

Introduction

Ewes from the Booroola strain of Australian Merino sheep are characterized by high ovulation rate and litter size. The Booroola gene (FecB) is an autosomal mutation identified on the basis of segregation studies on litter size [1] and ovulation rate [2]. FecB was the first major gene for prolificacy identified in sheep. The FecB locus is situated in the region of ovine chromosome 6 corresponding to the human chromosome 4q22–23 that contains the bone morphogenetic protein receptor IB (BMPR-IB) gene, which encodes a member of the transforming growth factor β (TGFβ) receptor family [3, 4]. One point mutation at base 746 of the coding region (746A→G) in the highly conserved intracellular kinase signaling domain of the BMPR-IB caused an amino acid change (249Q→R) was associated fully with the hyperprolific phenotype of Booroola ewes [3–5].

The Small Tail Han and Hu sheep are excellent local breeds in China for their significant characteristics of hyperprolificacy and year-round estrus. The mean litter sizes alive of Small Tail Han, Hu, Chinese Merino and Texel sheep have been reported to be 2.61 [6], 2.29 [6], 1.23 [6] and 1.41 [7], respectively. Based on the crucial role of BMPR-IB gene in the regulation of terminal folliculogenesis and the control of ovulation rate, BMPR-IB was considered as a possible candidate gene for prolificacy of
sheep. The objectives of the present study were firstly to
detect single nucleotide polymorphisms (SNPs) of exons
1–4 and 6–10 of BMPR-IB gene in high (Small Tail Han
and Hu sheep) and low prolificacy breeds (Texel and
Chinese Merino sheep) by PCR-SSCP, and secondly to
investigate the association between BMPR-IB gene and
prolificacy in Small Tail Han sheep in which the poly-
morphisms are segregating.

Materials and methods

Animals

All procedures involving animals were approved by the
animal care and use committee at the respective institution
where the experiment was conducted. All procedures
involving animals were approved and authorized by the
Chinese Ministry of Agriculture.

Venous jugular blood samples (10 ml per ewe) were
collected from 140 Small Tail Han ewes lambed in 2006,
along with data on litter size in the first, second, or third
parity (Jiaxiang Sheep Breeding Farm, Shandong Province,
China), 40 Chinese Merino ewes (Ziniqun Breeding
Sheep Farm, Shihezi City, Xinjiang Uygur Autonomous
Region, China), 40 Texel ewes (HITEK Ranch [Beijing]
Ltd. Co., Beijing, China) and 38 Hu ewes (Yuhang Hu
Sheep Breeding Farm, Hangzhou, China) using acid citrate
dextrose as an anticoagulant. Genomic DNA was extracted
by phenol–chloroform method, and dissolved in TE buffer
(10 mmol/l Tris–HCl [pH 8.0], 1 mmol/l EDTA [pH 8.0])
and kept at −20°C.

The 140 Small Tail Han ewes were selected at random
and they were the progeny of five rams. Because the five
rams had been sold, their blood was not collected for
genotyping. No selection on litter size or other fertility
traits was performed in the flock over previous years.
Lambing seasons consisted of 3 month groups starting with
March through to May as season 1 (spring), June through to
August as 2 (summer), September through to November as
3 (autumn) and December through to February as 4
(winter).

Primers and PCR amplification

Nine pairs of primers were designed according to mRNA
sequence (GenBank accession AF357007) of ovine BMPR-
IB. Exons 1–4 and 6–10 of BMPR-IB were amplified.
 Primer sequence, amplified region, product size and
annealing temperature were listed in Table 1.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence (5’→3’)</th>
<th>Product size (bp)</th>
<th>Amplified region</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
</table>
| P1     | F: AAGCAAAACTTCCCTTGATAACAT  
R: CTGCAAAATTTGGTGAGCCA | 163  
Exon 1 (137–299) | 54.6 |
| P2     | F: GCAGCAGATGGATAATGTTT  
R: CGACACTGAAAATCTGAGCTT | 106  
Exon 2 (296–401) | 58.4 |
| P3     | F: GGACACTCCCATCCTCATC  
R: CTCTGTTTTTCAGTGAGGAGGAAG | 104  
Exon 3 (402–505) | 56.8 |
| P4     | F: GAGATTTTTGTGGACGGACCTA  
R: TACCTGAAGTAAACAGAAATAAATGA | 103  
Exon 4 (503–605) | 57.5 |
| P5     | F: GGTCCAGGGGACACATAGCAA  
R: GCCCAAGATGTGTTTCAATGC | 196  
Exon 6 (741–936) | 60.2 |
| P6     | F: GCTTCCATTGCTGAGATAT  
R: CCTAATAAATTTACAGGCCCA | 299  
Exon 7 (935–1233) | 59.5 |
| P7     | F: TATAGTGACCGAATGAAGT  
R: CTATACCTCCCTGACACACAT | 188  
Exon 8 (1227–1414) | 61.1 |
| P8     | F: GTCAGGAGGTATAGTGGGAAATATC  
R: CGTCACTGCTCGCCGAGT | 137  
Exon 9 (1401–1537) | 55.8 |
| P9     | F: GACGAGTGTCTCAGGCGAGATG  
R: CTCAGAGGCTAATGTGCCTGGGA | 133  
Exon 10 (1534–1666) | 59.5 |

F stands for forward primer, R stands for reverse primer
Single strand conformation polymorphism detection and cloning and sequencing

These steps followed the method of Yan et al. [8].

Statistical analysis

The following fixed effects model was employed for analysis of litter size in Small Tail Han ewes and least squares mean was used for multiple comparisons in litter size among different genotypes.

\[ y_{ijklm} = \mu + S_i + L_{ij} + P_k + G_l + e_{ijklm}, \]

where \( y_{ijklm} \) is the phenotypic value of litter size; \( \mu \) is the population mean; \( S_i \) is the fixed effect of the \( i \)th sire (\( i = 1, 2, 3, 4, 5 \)); \( L_{ij} \) is the fixed effect of the \( j \)th lambing season (\( j = 1, 2, 3, 4 \)); \( P_k \) is the fixed effect of the \( k \)th parity (\( k = 1, 2, 3 \)); \( G_l \) is the fixed effect of the \( l \)th genotype (\( l = 1, 2, 3 \)) and \( e_{ijklm} \) is the random residual 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

Only the PCR products amplified by primers P2, P5, P6, P7, P8 and P9 displayed polymorphisms. Four genotypes (AA, AC, CC and DD) were detected for primer P2, three (\( ++, B+ \) and BB) for primer P5, five (GG, GH, HH, KK and LL) for primer P6, three (MM, MN and PP) for primer P7, two (QQ and RR) for primer P8, and two (UU and ZZ) for primer P9.

Polymorphic sequence variations in \( BMPR-IB \) in four sheep breeds were showed in Table 2. The 22 SNPs were identified in partial coding regions of ovine \( BMPR-IB \) gene in the present study, in which 20 SNPs were reported for the first time. In total of the 22 mutations, 18 base variations were originated from the Hu breed, 3 were found in the Small Tail Han breed (two of them were found in other sheep breeds), 3 in the Chinese Merino breed, and none in the Texel breed.

Table 2 Polymorphic sequence variations in \( BMPR-IB \) gene in four sheep breeds

<table>
<thead>
<tr>
<th>Primer</th>
<th>Genotype</th>
<th>Variant</th>
<th>Amino acid change</th>
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<tbody>
<tr>
<td>P1</td>
<td>AA→CC</td>
<td>A1</td>
<td>G192A</td>
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<tr>
<td></td>
<td>AA→DD</td>
<td>B1</td>
<td>T195C</td>
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<tr>
<td>P5</td>
<td>++→BB</td>
<td>C1</td>
<td>A746G</td>
</tr>
<tr>
<td>P6</td>
<td>GG→KK</td>
<td>D1</td>
<td>T864C</td>
</tr>
<tr>
<td></td>
<td>GG→LL</td>
<td>F1</td>
<td>T852C</td>
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<tr>
<td></td>
<td>MM→MN</td>
<td>G1</td>
<td>C1113A</td>
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<tr>
<td>P7</td>
<td>MM→PP</td>
<td>H1</td>
<td>C1113A</td>
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<tr>
<td>P8</td>
<td>QQ→RR</td>
<td>J1</td>
<td>C1311T</td>
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<tr>
<td>P9</td>
<td>UU→ZZ</td>
<td>K1</td>
<td>A1467G</td>
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</tbody>
</table>

Except for genotypes MM, MN and PP, amino acid sequences of genotypes AA, ++, GG, QQ and UU were the same as that of ovine \( BMPR-IB \) gene published in GenBank (AF357007).

The frequencies of 19 genotypes and 16 alleles in four sheep breeds were presented in Table 3. As for primers P2, P5, P6, P7, P8 and P9, the genotypes and alleles were 4, 3, 5, 3, 2, 2 and 3, 2, 4, 3, 2, 2, respectively. These results indicated that the polymorphisms in coding region of \( BMPR-IB \) gene were rather abundant among these sheep breeds.

The test result of difference for \( BMPR-IB \) genotype distribution of primer P5 in four sheep breeds was summarized in Table 4. There was highly significant difference (\( P < 0.001 \)) in the \( BMPR-IB \) genotype distribution between high (Small Tail Han and Hu sheep) and low prolificacy breeds (Texel and Chinese Merino sheep). And in either group, no significant difference (\( P > 0.05 \)) was monitored.

Litter size was significantly influenced by sire, lambing season and parity (\( P < 0.05 \), \( P < 0.05 \), and \( P < 0.05 \), respectively). The least squares means (LSM) and standard
errors for litter size of different genotypes of *BMPR-IB* in Small Tail Han sheep were given in Table 5. For primer P2, the differences of the LSM for litter size between AA, AC and CC were not significant (*P* > 0.05). For primer P5, the ewes with genotype BB or B+ had 1.51 (*P* < 0.001) or 1.02 (*P* < 0.001) lambs more than those with genotype ++; the ewes with genotype BB had 0.49 (*P* < 0.01) lambs more than those with genotype B+.

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<th>CC</th>
<th>DD</th>
<th>Allele frequency</th>
<th>A</th>
<th>C</th>
<th>D</th>
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<td>GH</td>
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</tbody>
</table>

The numbers in the parentheses are the individuals that belong to the respective genotypes.
The present study also showed that the FecB G922T, T1043C, G192A) led to the change of amino acids in sheep breeds. The C1113A mutation was only in Hu sheep, not in other three Han and Hu sheep, not in Texel or Chinese Merino sheep. Six–10 of ovine BMPR-IB[5]. The present study also identified these two point mutations. A746G mutation was in both Small Tail Han and Hu sheep (China). Therefore, these six ovine breeds may share a common ancestor.

Small Tail Han sheep (China), South African Mutton Merino, Chinese Merino and Corriedale sheep, 17 of 21 SNPs in Inhibin βA gene were from the Hu sheep breed[21]. In the current study, 18 of 22 SNPs were from the Hu sheep breed. The Hu sheep breed is a special local breed in China. Chinese Hu sheep are used mainly for lambskin production and may be found throughout the Taihu Lake area that covers Jiangsu province, Zhejiang province and the vicinity of Shanghai. This sheep breed is famous for its beautiful lambskin, sexual precocity, year-round estrus, and hyperprolificacy[6, 22]. This is why so many variations in the Hu sheep breed deserve further study. For primers P2, P6, P8 and P9, no heterozygotes were reported in the Hu sheep breed. Possible reasons include: (1) Heterozygotes exist in the Hu sheep breed, but infertile heterozygotes were probably rapidly removed from the population, or heterozygotes suffered from distinct developmental and reproductive defects, they suffered from the embryonic death, or they were sifted out before arriving at reproductive age. (2) Heterozygotes do not exist in the Hu sheep breed. To verify this hypothesis, a more comprehensive sample should be reported in the Hu sheep.

**Table 4** Test of difference for BMPR-IB genotype distribution of primer P5 in four sheep breeds

<table>
<thead>
<tr>
<th>Primer</th>
<th>Breed</th>
<th>Hu sheep</th>
<th>Texel sheep</th>
<th>Chinese Merino sheep</th>
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<tbody>
<tr>
<td>P5</td>
<td>Small Tail Han</td>
<td>4.583</td>
<td>110.924***</td>
<td>113.949***</td>
</tr>
<tr>
<td></td>
<td>sheep</td>
<td></td>
<td>71.000***</td>
<td>73.000***</td>
</tr>
<tr>
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<td>Hu sheep</td>
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<tr>
<td></td>
<td>Texel sheep</td>
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</tbody>
</table>

The effect of BMPR-IB mutation on sheep litter size

The effect of FecB gene is additive for ovulation rate (the number of ova shed at each ovulatory cycle) and partially dominant for litter size. One copy increases ovulation rate by 1.3–1.6 and two copies by 2.7–3.0; litter size is increased by 0.9–1.2 in ewes carrying a single copy and 1.1–1.7 in ewes with two copies [1, 2]. In the BB or B+ ewes, Q249R substitution would impair the inhibitory effect of BMPR-IB on granulosa cell steroidogenesis, leading to their advanced differentiation and an advanced maturation of follicles [3]. This A746G mutation could cause the phenotype characteristic of the
Booroola animals which is the precocious development of a large number of small antral follicles resulting in increased ovulation rate [5]. The results in the present study showed that the mean litter size of BB, B+ and ++ genotypes was 2.65, 2.16 and 1.14 in Small Tail Han ewes, respectively. The fact that the FecB mutation is present in prolific Small Tail Han and Hu sheep will promote us to develop breeding strategies to maximize the benefits of increased prolificacy in these breeds and their crosses.

Acknowledgments
This work was supported by National Key Technology R and D Program of China (nos. 2006BADB2B01, 2006BAD01A11 and 2006BAD13B08), by the earmarked fund for Modern Agro-industry Technology Research System of China (no. 2006BAD01A11 and 2006BAD13B08), by National Key Basic Research and Development Program of China (nos. Y0705003041131 and BJNY2006-03), by Beijing Natural Science Foundation of China (no. 5042017).

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