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Polymorphisms of *BMPR-IB* gene and their relationship with litter size in goats

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Abstract The bone morphogenetic protein receptor IB (*BMPR-IB*) gene was studied as a candidate gene for the prolificacy of goats. According to mRNA sequence of ovine *BMPR-IB* gene, ten pairs of primers were designed to detect single nucleotide polymorphisms (SNPs) of exon 1, exon 2, exon 6 to exon 10 and 3′ untranslated region (UTR) of the *BMPR-IB* gene in both high prolificacy breed (Jining Grey goat) and low prolificacy breeds (Wendeng Dairy and Inner Mongolia Cashmere goats) by polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) method. Only the products amplified by primers P8 and P10 of the 3′UTR displayed polymorphisms. For primer P8, three genotypes (AA, AB and BB) were detected in Jining Grey and Wendeng Dairy goats, two genotypes (AA and AB) were in Inner Mongolia Cashmere goats. Sequencing revealed one mutation (71C→T) of the *BMPR-IB* gene in genotype BB compared with AA. The differences of least squares mean (LSM) for litter size between genotypes AA, AB and BB were non-significant ($P > 0.05$) in Jining Grey goats. For primer P10, three genotypes (CC, CD and DD) were detected in Jining Grey and Wendeng Dairy goats and one genotype (CC) in Inner Mongolia Cashmere goats. Sequencing revealed one mutation (130T→C) of the *BMPR-IB* gene in genotype DD compared with CC. The differences of LSM for litter size between genotypes CC, CD and DD were non-significant ($P > 0.05$) in Jining Grey goats. These results preliminarily showed that the detected loci of the *BMPR-IB* gene had no significant effect on prolificacy of Jining Grey goats.

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

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The bone morphogenetic proteins (BMPs) are members of the transforming growth factor β (TGFβ) superfamily. They are multifunctional proteins that regulate growth and differentiation in many cell types and play essential roles during embryogenesis and the fertility in mammals [1–5]. BMPs cannot exert their physiological functions without bone morphogenetic protein receptors [6, 7]. 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 [8] and ovulation rate [9]. The *FecB* was the first major gene for prolificacy identified in sheep and located 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 TGFβ receptor family [7, 10]. Mulsant et al. [7], Wilson et al. [10] and Souza et al. [11] indicated that Booroola sheep have one point mutation at base 746 of the encoding region (A in the

$FecB^{+}/FecB^{+}$ to a G in $FecB^{B}/FecB^{B}$ animals) in the highly conserved intracellular kinase signaling domain of the BMPR-IB which results in a change from a glutamine (Q) in the wild type to an arginine (R) in the $FecB^{B}/FecB^{B}$ animals. This nonconservative substitution (Q249R) in the *BMPR-IB* encoding sequence was associated fully with the hyperprolific phenotype of Booroola ewes [7, 10, 11].

The Jining Grey goat breed that has significant characteristics of hyperprolificacy and year-round estrus is an excellent local breed in P.R. China. The mean litter sizes alive of Jining Grey, Wendeng Dairy and Inner Mongolia Cashmere goats have been reported to be 2.94 [12], 1.83 [13] and 1.04 [12] respectively. Based on the crucial role of *BMPR-IB* gene in the regulation of terminal folliculogenesis and the control of ovulation rate, *BMPR-IB* gene was considered as a possible candidate gene for prolificacy of goats. The objectives of the present study were firstly to detect single nucleotide polymorphisms (SNPs) of exon 1, exon 2, exon 6 to exon 10 and 3′untranslated region (UTR) of the *BMPR-IB* gene in both high prolificacy breed (Jining Grey goat) and low prolificacy breeds (Wendeng Dairy and Inner Mongolia Cashmere goats) by PCR-SSCP method, and secondly to investigate the association between SNPs of *BMPR-IB* gene and prolificacy in Jining Grey goats in which the polymorphisms 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 goat doe) were collected from 140 Jining Grey goat does kidded in 2007, along with data on litter size in the first, second, or third parity (Jining Grey goats conservation base, Jiaxiang County, Shandong Province, P.R. China), 40 Wendeng Dairy goat does (Wendeng City, Shandong Province, P.R. China), and 38 Inner Mongolia Cashmere goat does (Inner Mongolia White Cashmere Goat Breeding Farm, Etuokeqi, Ordos City, the Inner Mongolia Autonomous Region, P.R. China) using acid citrate dextrose as an anticoagulant. Genomic DNA was extracted from whole blood by phenol-chloroform method as described by Sambrook and Russell [14], and then 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 Jining Grey goat does were selected at random and were the progeny of five goat bucks. Because the five goat bucks were sold, their blood was not collected and they were not genotyped. No selection on litter size or other fertility traits was performed in the flock over previous years. Kidding seasons consisted of 3-month groups starting with March through to May as season 1 (spring), June through to August as season 2 (summer), September through to November as season 3 (autumn) and December through to February as season 4 (winter).

Primers and PCR amplification

Ten pairs of primers were designed according to mRNA (GenBank accession number AF357007) of ovine *BMPR-IB* gene. Exon 1, exon 2, exon 6 to exon 10 and 3′UTR of the *BMPR-IB* gene were amplified. These primers were synthesized by Shanghai Invitrogen Biotechnology Limited Corporation (Shanghai, P.R. China). Primer sequence, product size, amplified region and annealing temperature were listed in Table 1.

Polymerase chain reaction was carried out in 25 μl volume containing approximately 2.5 μl of 10 × PCR buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.0), 0.1% Triton X-100), 1.5–2.0 μl of 25 mmol/l Mg^{2+}, 2.5 μl of 2.5 mmol/l each dNTP, 1.0 μl of 10 μmol/l each primer, 3.0 μl of 50 ng/μl genomic DNA, 1.0 μl of 2.5 U/μl *Taq* DNA polymerase (Promega, Madison, WI, USA), and the rest is ddH_2O. Amplification conditions were as follows: initial denaturation at 94°C for 8 min; followed by 32 cycles of denaturation at 94°C for 30 s, annealing for 30 s (annealing temperature in Table 1), extension at 72°C for 30 s; with a final extension at 72°C for 10 min on Mastercycler® 5333 (Eppendorf AG, Hamburg, Germany).

SSCP detection

A volume of 2 μl PCR product was transferred in an Eppendorf tube, mixed with 7 μl gel loading solution containing 98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol, 20 mmol/l EDTA (pH 8.0) and 10% glycerol. The mixture was centrifugalized and denatured at 98°C for 10 min, then chilled on ice for 5 min and loaded on 12–14% neutral polyacrylamide gels (acrylamide:bisacrylamide = 29–49:1). Electrophoresis was performed in 1 × Tris borate (pH 8.3)-EDTA buffer at 9–15 V/cm at 4°C overnight. After electrophoresis, the DNA fragments in the gels were visualized by silver staining, photographed and analyzed by an AlphaImager™ 2200 and 1220 Documentation and Analysis Systems (Alpha Innotech Corporation, San Leandro, CA, USA).

Cloning and sequencing

After SSCP analysis, PCR products of different genotypes were separated on 1% agarose gels and recovered using

Table 1 Primer sequence, product size, amplified region and annealing temperature of goat *BMPR-IB* gene

Primer	Primer sequence (5′→3′)	Product size (bp)	Amplified region	Annealing temperature (°C)
P1	F: AAGCAAACTTCCTTGATAACAT	163	Exon 1 (137–299)	56.8
	R: CTGCAAATATTGTTGACCGA			
P2	F: GCAGCACAGATGGATATTGTTT	106	Exon 2 (296–401)	57.1
	R: CGACACTGAAAATCTGAGCCT			
P3	F: GGTCCAGAGGACAATAGCAA	196	Exon 6 (741–936)	59.8
	R: GCCCAAGATGTTTTCATGC			
P4	F: GCTTCATTGCTGCAGATAT	299	Exon 7 (935–1233)	58.4
	R: CCTAATAAACTTAACAGCCAA			
P5	F: TATTAGTGACACGAATGAAGT	188	Exon 8 (1227–1414)	58.4
	R: CTATACCTCCTGACACACAT			
P6	F: TCAGGAGGTATAGTGGAAGAATATC	137	Exon 9 (1401–1537)	61.1
	R: CGTCACTGCTCCACCGGTT			
P7	F: GACGAGTGTCTCAGGCAGATG	133	Exon 10 (1534–1666)	61.1
	R: CTCAGAGCTTAATGTCCTGGGA			
P8	F: CCTGTTTGTGGGCAGAGCAAA	234	3′UTR	59.8
	R: CAATCCCAAAATACCGGGCTT			
P9	F: AATTTTGCCAAAATAAAACAA	162	3′UTR	54.0
	R: TGTCTTTTGGTTGGTAGAGTG			
P10	F: CATTAAACACAAACAAAGCTTTT	157	3′UTR	58.4
	R: CTTACAGCTCAAGAGCAAATTA			

F stands for forward primer, R stands for reverse primer

Geneclean II kit (Promega). The ligation reaction was conducted as per the instructions of the manufacturer (Promega). Each DNA fragment was then transformed into *Escherichia coli* DH5α competence cell. Positive clones of transformed cells were identified by restriction enzyme digestion. Two clones of each genotype were selected and sequenced. Each clone was sequenced for twice. The target DNA fragments in recombinant plasmids were sequenced from both directions using an ABI3730 automatic sequencer (Perkin Elmer Applied Biosystems, Foster City, CA, USA) by Shanghai Invitrogen Biotechnology Ltd. Co., (Shanghai, P.R. China).

Statistical analysis

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

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

where y_{ijklm} is the phenotypic value of litter size; μ is the population mean; S_i is the fixed effect of the ith sire ($i = 1, 2, 3, 4, 5$); KS_j is the fixed effect of the jth kidding season ($j = 1, 2, 3, 4$); P_k is the fixed effect of the kth parity ($k = 1, 2, 3$); G_l is the fixed effect of the lth 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

PCR amplification

Genomic DNA of three goat breeds was amplified using ten pairs of primers for *BMPR-IB* gene. PCR products were detected by running a 2% agarose gel electrophoresis (Fig. 1). The amplified products were consistent with the target fragments and had a good specificity, which could be directly analyzed by SSCP.

SSCP analysis

Only the PCR products amplified by primers P8 and P10 displayed polymorphisms. Three genotypes (AA, AB and BB) were detected by primer P8, and three genotypes (CC, CD and DD) were detected by primer P10 (Fig. 2).

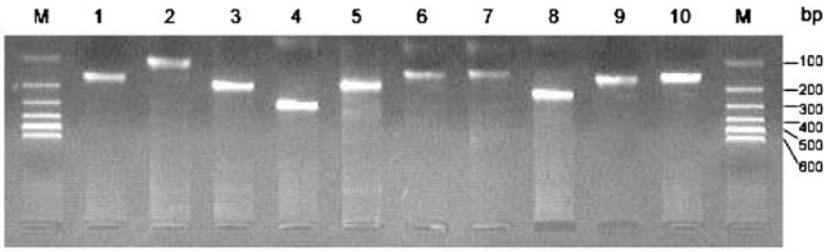

Fig. 1 PCR products of ten pairs of primers. M: SD002 marker; 1–10: PCR products of primers P1 to P10

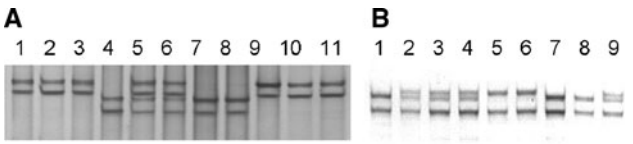

Fig. 2 SSCP analysis of PCR amplification using primer P8(A) and P10(B) in different goat breeds. A:1–3,9–11: AA genotype; 4,7,8: BB genotype; 5,6: AB genotype. B: 5,6: CC genotype; 1,7,8: DD genotype; 2,3,4,9: CD genotype

Sequencing of different genotypes and nucleotide mutations

For primer P8, sequencing revealed one nucleotide mutation (71C→T) (Fig. 3) of *BMPR-IB* gene between genotype BB and genotype AA. For primer P10, sequencing revealed one nucleotide mutation (130T→C) (Fig. 3) of *BMPR-IB* gene between genotype DD and genotype CC. All the two mutations located in the 3′UTR of goat *BMPR-IB* gene.

Allele and genotype frequencies of *BMPR-IB* gene in three goat breeds

Allele and genotype frequencies of *BMPR-IB* gene in three goat breeds were presented in Table 2.

For primer P8, three genotypes (AA, AB and BB) were detected in Jining Grey and Wendeng Dairy goats, two genotypes (AA and AB) were in Inner Mongolia Cashmere goats. For primer P10, three genotypes (CC, CD and DD) were detected in Jining Grey and Wendeng Dairy goats, one genotype (CC) was in Inner Mongolia Cashmere goats.

Influence of fixed effects on litter size in Jining Grey goats

Litter size was significantly influenced by sire, kidding season and parity ($P < 0.05$, $P < 0.05$, and $P < 0.05$ respectively). For primers P8 and P10, the *BMPR-IB* genotype had no significant influence on litter size in Jining Grey goats ($P > 0.05$). The least squares mean and standard error for litter size of different genotypes of *BMPR-IB* gene in Jining Grey goats were given in Table 3.

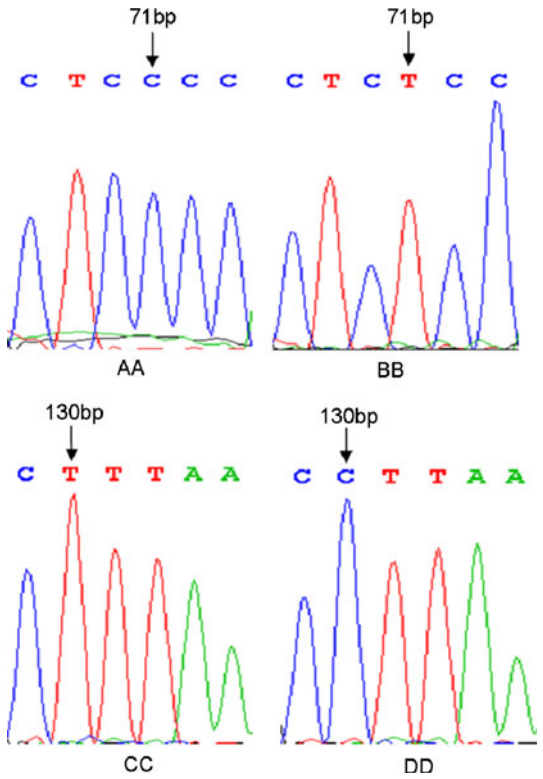

Fig. 3 Sequence comparison of different genotypes of the *BMPR-IB* gene in goats

Discussion

Polymorphisms of sheep and goat *BMPR-IB* gene

The *FecB* mutation (A746G or Q249R) is present in Booroola Merino (Australia) [7, 10, 11], Garole (India) [15], Javanese (Indonesia) [15], Small Tail Han (China) [16–22], and Hu (China) sheep [17, 19, 20, 23–25]. Therefore, these five ovine breeds may share a common ancestor.

Hyperprolificacy in sheep carrying the Booroola gene (*FecB*) is the result of a mutation in the *BMPR-IB* gene, which had provided a direct marker for the DNA mutation test for *FecB*. Chinese Merino, Tan, Dorset, Suffolk, Thoka, Coopworths, Gotland, Perindale, Romney, Texel, Merinos d'Arles, Woodlands, Olkuska, Lacaune, Belclare,

Table 2 Allele and genotype frequencies of *BMPR-IB* gene in three goat breeds

Breed	No.	Primer 8					No.	Primer 10				
		Allele frequency		Genotype frequency				Allele frequency		Genotype frequency		
		A	*B*	*AA*	*AB*	*BB*		*C*	*D*	*CC*	*CD*	*DD*
Jining Grey goat	140	0.88	0.12	0.78 (109)	0.21 (29)	0.01 (2)	135	0.97	0.03	0.96 (129)	0.03 (4)	0.01 (2)
Inner Mongolia Cashmere goat	38	0.91	0.09	0.82 (31)	0.18 (7)	0.00 (0)	38	1.00	0.00	1.00 (38)	0.00 (0)	0.00 (0)
Wendeng Dairy goat	40	0.48	0.52	0.25 (10)	0.45 (18)	0.30 (12)	40	0.91	0.09	0.88 (35)	0.07 (3)	0.05 (2)

The numbers in the brackets are the individuals that belong to the respective genotypes

Table 3 Least squares mean and standard error for litter size of different genotypes of *BMPR-IB* gene in Jining Grey goats

Primer	Genotype	No. of does	Litter size
Primer 8	*AA*	109	2.26 ± 0.15^{a}
	AB	29	2.35 ± 0.20^{a}
	BB	2	2.62 ± 0.35^{a}
Primer 10	*CC*	129	2.28 ± 0.13^{a}
	CD	4	2.39 ± 0.25^{a}
	DD	2	2.58 ± 0.32^{a}

Least squares means with the same superscript for the same pair of primer have nonsignificant difference ($P > 0.05$)

Cambridge, Romanov (2 strains), Finn (2 strains), East Friesian, Teeswater, Blueface Leicester, D'Man, Chios, Mountain Sheep (three breeds), German Whiteheaded Mutton, Lleyn, Loa, Galician, Barbados Blackbelly (pure and crossbred), St. Croix sheep had no Q249R mutation [7, 10, 15–20, 23, 24]. Souza et al. [11] found two point mutations by screening 20 Scottish Blackface Merino cross ewes of Booroola genotypes in the kinase domain of BMPR-IB, one at base 746 of the encoding region (A in the $FecB^{+}/FecB^{+}$ to a G in $FecB^{B}/FecB^{B}$ animals), which resulted in a change from a glutamine in the wild type to an arginine in the Booroola animals. Another point mutation was identified at position 1113 (C to A), but this mutation did not change the encoding amino acid. Five Chinese native goat breeds (Jining Grey, Anhui White, Wendeng Dairy, Liaoning Cashmere and Beijing native goats) had no Q249R mutation [26].

Jia et al. [27] identified two mutations (121T→C and 195T→C) in 3′UTR of sheep *BMPR-IB* gene which were present in prolific Hu sheep, not in prolific Small Tail Han sheep or low fecundity breeds (Texel and Chinese Merino sheep). The present study identified two mutations (71C→T and 130T→C) in 3′UTR of goat *BMPR-IB* gene. Mutation 71C→T was present in Jining Grey, Wendeng Dairy and Inner Mongolia Cashmere goats, and mutation 130T→C was only present in Jining Grey and Wendeng Dairy goats.

Effect of *BMPR-IB* gene Q249R mutation on sheep and goat litter size

The *FecB* was the first major gene for prolificacy identified in sheep [8, 9]. 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 [8, 9, 28, 29]. The mean ovulation rate was 4.65 for Booroola ewes ($n = 280$) and 1.62 for the control Merino ewes ($n = 69$); the mean litter size was 2.29 (range 1–7) for Booroola ewes ($n = 522$) and 1.22 (range 1–2) for the control Merino ewes ($n = 835$) [8, 9].

In the $FecB^{B}$ carrier 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 [7]. This 746 A to G mutation in the subdomain 3 of the kinase domain could result in an alteration in the expression and/or phosphorylation of SMADs (SMADs are evolutionarily conserved proteins identified as mediators of transcriptional activation by members of the TGFβ superfamily of cytokines, including TGFβ, activins and BMP. Upon activation these proteins directly translocate to the nucleus where they may activate transcription.), resulting in 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 [11]. The *FecB* mutation (A746G or Q249R) had highly significant influence on ovulation rate and/or litter size in Booroola Merino (Australia) [7, 10, 11], Garole (India) [15], Javanese (Indonesia) [15], Small Tail Han (China) [16–22], and Hu [China] sheep [17, 19, 20, 23–25]. Knowledge that the *FecB* mutation is present in prolific Small Tail Han and Hu sheep will allow breeding strategies to be developed that maximize the benefits of increased prolificacy in these breeds and their crosses.

The *FecB* mutation (A746G or Q249R) had no significant influence on litter size in Jining Grey, Boer, Anhui

White, Wendeng Dairy, Liaoning Cashmere or Beijing native goats [26]. These results of the present study preliminarily showed that the detected loci of the *BMPR-IB* gene had no significant effect on prolificacy of Jining Grey goats.

In the present study, the samples of each goat breed were obtained from a single farm; therefore, it is likely that the degree of relatedness between goats is very high. This in turn would affect the effective population size of the goats genotyped and would undoubtedly impact the significance of the detected association. Moreover, the size of the breed groups between Jining Grey goats and other breeds genotyped is markedly different. Surely this, in conjunction with relatedness issues, would also impact the reported association between PCR-SSCP of *BMPR-IB* gene and prolificacy; therefore, the results in the present study were preliminary.

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References

1. Galloway SM, McNatty KP, Cambridge LM, Laitinen MPE, Juengel JL, Jokiranta TS, McLaren RJ, Luiro K, Dodds KG, Montgomery GW, Beattie AE, Davis GH, Ritvos O (2000) Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat Genet 25(3):279–283
2. Lan ZJ, Gu P, Xu X, Jackson KJ, DeMayo FJ, O'Malley BW, Cooney AJ (2003) GCNF-dependent repression of BMP-15 and GDF-9 mediates gamete regulation of female fertility. EMBO J 22(16):4070–4081
3. Fang X, Xu H, Zhang C, Chen H, Hu X, Gao X, Gu C, Yue W (2009) Polymorphism in BMP4 gene and its association with growth traits in goats. Mol Biol Rep 36(6):1339–1344
4. Monget P, Fabre S, Mulsant P, Lecerf F, Elsen JM, Mazerbourg S, Pisselet C, Monniaux D (2002) Regulation of ovarian folliculogenesis by IGF and BMP system in domestic animals. Domest Anim Endocrinol 23(1–2):139–154
5. Murakami M, Matsuzaki F, Funaba M (2009) Regulation of melanin synthesis by the TGF-beta family in B16 melanoma cells. Mol Biol Rep 36(6):1247–1250
6. Yi SE, LaPolt PS, Yoon BS, Chen JYC, Lu JKH, Lyons KM (2001) The type I BMP receptor BmprIB is essential for female reproductive function. Proceedings of the National Academy of Sciences of the USA 98(14):7994–7999
7. Mulsant P, Lecerf F, Fabre S, Schibler L, Monget P, Lanneluc I, Pisselet C, Riquet J, Monniaux D, Callebaut I, Cribiu E, Thimonier J, Teyssier J, Bodin L, Cognie Y, Chitour N, Elsen JM (2001) Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. Proceedings of the National Academy of Sciences of the USA 98(9):5104–5109
8. Piper LR, Bindon BM (1982) The Booroola Merino and the performance of medium non-Peppin crosses at Armidale. In: Piper LR, Bindon BM, Nethery RD (eds) The Booroola Merino. CSIRO, Melbourne, Australia, pp 9–20
9. Davis GH, Montgomery GW, Allison AJ, Kelly RW, Bray R (1982) Segregation of a major gene influencing fecundity in progeny of Booroola sheep. N Z J Agric Res 25:525–529
10. Wilson T, Wu XY, Juengel JL, Ross IK, Lumsden JM, Lord EA, Dodds KG, Walling GA, McEwan JC, O'Connell AR, McNatty KP, Montgomery GW (2001) Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biol Reprod 64(4):1225–1235
11. Souza CJH, MacDougall C, Campbell BK, McNeilly AS, Baird DT (2001) The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (BMPRIB) gene. J Endocrinol 169(2):R1–R6
12. Tu YR (1989) Sheep and goat breeds in China. In: Zheng PL (ed) Shanghai Science and Technology Press, Shanghai, pp 88–90, 98–101
13. Wu ZH, Chu MX, Li XW, Fang L, Ye SC, Liu ZH, Chen GH (2006) PCR-SSCP analysis on exon 2 of growth differentiation factor 9 gene in goats. Scientia Agricultura Sinica 39(4):802–808
14. Sambrook J, Russell DW (2001) Molecular cloning—a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA
15. Davis GH, Galloway SM, Ross IK, Gregan SM, Ward J, Nimbkar BV, Ghalsasi PM, Nimbkar C, Gray GD, Subandriyo InounuI, Tiesnamurti B, Martyniuk E, Eythorsdottir E, Mulsant P, Lecerf F, Hanrahan JP, Bradford GE, Wilson T (2002) DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biol Reprod 66(6):1869–1874
16. Liu SF, Jiang YL, Du LX (2003) Studies of BMPR-IB and BMP15 as candidate genes for fecundity in Little Tailed Han sheep. Acta Genetica Sinica 30(8):755–760
17. Wang GL, Mao XZ, Davis GH, Zhao ZS, Zhang LJ, Zeng YQ (2003) DNA tests in Hu sheep and Han sheep (small tail) showed the existence of Booroola (FecB) mutation. J Nanjing Agric Univ 26(1):104–106
18. Jia CL, Li N, Zhao XB, Zhu XP, Jia ZH (2005) Association of single nucleotide polymorphisms in exon 6 region of BMPRIB gene with litter size traits in sheep. Asian-Australasian J Anim Sci 18(10):1375–1378
19. Yan YD, Chu MX, Zeng YQ, Fang L, Ye SC, Wang LM, Guo QK, Han DQ, Zhang ZX, Wang XJ, Zhang XZ (2005) Study on bone morphogenetic protein receptor IB as a candidate gene for prolificacy in Small Tailed Han sheep and Hu sheep. J Agric Biotechnol 13(1):66–71
20. Davis GH, Balakrishnan L, Ross IK, Wilson T, Galloway SM, Lumsden BM, Hanrahan JP, Mullen M, Mao XZ, Wang GL, Zhao ZS, Zeng YQ, Robinson JJ, Mavrogenis AP, Papachristoforou C, Peter C, Baumung R, Cardyn P, Boujenane I, Cockett NE, Eythorsdottir E, Arranz JJ, Notter DR (2006) Investigation of the Booroola (FecB) and Inverdale ($FecX^I$) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Animal Reproduction Science 92(1–2):87–96
21. Yin ZH, Jiang YL, Fan XZ, Wang Y, Tang H, Yue YS (2006) Polymorphism, effect and linkage analysis of BMPR-IB gene in Small Tailed Han sheep. Acta Veterinaria et Zootechnica Sinica 37(5):510–513
22. Chu MX, Liu ZH, Jiao CL, He YQ, Fang L, Ye SC, Chen GH, Wang JY (2007) Mutations in BMPR-IB and BMP-15 genes are associated with litter size in Small Tailed Han sheep (*Ovis aries*). J Anim Sci 85(3):598–603

23. Wang QG, Zhong FG, Li H, Wang XH, Liu SR, Chen XJ (2003) The polymorphism of BMPR-IB gene associated with litter size in sheep. Grass-feeding Livestock 2003(2):20–23
24. Wang QG, Zhong FG, Li H, Wang XH, Liu SR, Chen XJ, Gan SQ (2005) Detection on major gene on litter size in sheep. Hereditas (Beijing) 27(1):80–84
25. Guan F, Liu SR, Shi GQ, Ai JT, Mao DG, Yang LG (2006) Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. Acta Genetica Sinica 33(2):117–124
26. Chu MX, Fang L, Ye SC (2006) RFLP analysis on bone morphogenetic protein receptor IB as a candidate gene for prolificacy in goats. J Agric Biotechnol 14(1):139–140
27. Jia LH, Chu MX, Chen HQ, Fang L, Ma YH, Li K (2008) Polymorphism analysis of partial 3′UTR of the bone morphogenetic protein receptor IB (*BMPR-IB*) gene in sheep. J Agric Biotechnol 16(1):47–50
28. Bindon BM (1984) Biology of the Booroola Merino sheep. Austral J Biol Sci 37(3):163–189
29. Piper LR, Bindon BM, Davis GH (1985) The single gene inheritance of the high litter size of the Booroola Merino. In: Land RB, Robinson DW (eds) Genetics of reproduction in Sheep. Butterworths, London, pp 115–125