



## Polymorphism of Exon 2 of BMP15 Gene and Its Relationship with Litter Size of Two Chinese Goats

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**ABSTRACT :** Polymorphisms of BMP15 gene exon 2 and its relationship with prolificacy of goats were detected by PCR-SSCP and DNA sequencing methods in Chinese two local goat breeds. The results showed that the product amplified by the primers displayed polymorphisms. Three genotypes (AA, BB and AB) were detected in Funiu white goats, and their frequency was 0.071, 0.715, 0.214, respectively. Two genotypes (AB and BB) were detected in Taihang black goats, and their frequency was 0.342 and 0.658, respectively. Sequencing revealed that four mutations (456T→G, 466C→G, 510C→T, 511T→C) occurred in genotype BB of Funiu white goat, which resulted in amino acid substitution of V155G and S171P. No mutation was detected in Taihang black goat. The Funiu white goat with genotype BB had 0.91 or 0.82 kids, more than those with AB or AA, respectively. The difference of the least squares means for litter size between BB and AB was not significant ( $p>0.05$ ) in Taihang black goat. It is concluded that the BMP15 gene may be a major gene which affects the prolificacy in Funiu white goats. This study could provide basic molecular data on the reproductive characteristics of local breeds of Henan province in China, and a scientific basis for the conservation and utilization of those two goat breeds. (**Key Words :** Bone Morphogenetic Protein 15 (BMP15), Goat, Prolificacy)

### INTRODUCTION

In 1998 two groups independently described the bone morphogenetic protein 15 (BMP15), also known as growth and differentiation factor 9B (GDF9B), in the mouse ovary. It is a member of the transforming growth factor beta superfamily (TGF $\beta$ ) which in humans, rodents and sheep is expressed exclusively in the oocyte (Vitt and Hsueh, 2001). In mammals BMP-15 is the product of an X-linked gene expressed in oocytes (Dube et al., 1998; Laitinen et al., 1998; Grapes and Rothschild, 2002; Silva et al., 2005). BMP-15 acts as a paracrine/autocrine factor to stimulate follicle development and plays a very important role in regulating ovulation rate and oocyte quality. The relative importance of BMP-15 in early follicle development is species-specific and appears to be related to differences between mono- and polyovulatory species (Moore and Shimasaki, 2005). Mutations in BMP-15 gene in ewes and women have been shown to cause defects in folliculogenesis. Natural occurring mutations in sheep

BMP-15, such as FecX<sup>G,B,I,H,L</sup> (Chu et al., 2005; McNatty et al., 2005; Bodin et al., 2007) lead to infertility in homozygous ewes due to defects in early folliculogenesis, whereas heterozygous ewes have increased ovulation rate and litter size. Heterozygous ewes carrying the naturally occurring BMP-15 point mutations have increased ovulation rates. Extensive research has been carried out on different prolific sheep breeds to identify the genes involved in controlling ovulation rate and prolificacy (Chu et al., 2005; Guang et al., 2005; Vacca et al., 2010). Based on results indicating a major gene effecting sheep prolificacy, the possibility of a major gene also effecting the prolificacy capability of some goat breeds was widely investigated using biology information and molecular biotechnology. Recent studies on prolific goat breeds such as Boer, Haimen, Huanghuai, Nubi, Matou and Jining Grey goat, suggested that higher prolificacy in goats is not like that of sheep (Chu et al., 2007; Hua et al., 2008). It is reported that the point mutations of BMP15 (Fec X<sup>H</sup>, Fec X<sup>I</sup>, Fec X<sup>G</sup> and Fec X<sup>B</sup>) genes in the prolific goat breeds are monomorphic. Henan has a goat population of 20.38 million, which accounts for 7.26% of the national total goat flocks in 2008. Funiu white goat and Tainhang black goat are two local Chinese goat

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breeds distributed in the Yuxi Taihang Mountain and Funiu Mountain regions. Funiu white goat is a prolific and major meat-skin producing animal which kids twice a year or more commonly thrice in 2 years with 7.37% single births, 68.42% twins, 24.74% triplets. The multiple birth rate is higher in Neixiang county which distributes to the south of the Funiu Mountains. This breed is also found in other Yuxi mountain regions such as Xichuang, Xixia, Nanzhao and Zhenping County and the region northwards of the Funiu Mountains. At present, there are about 700,000 Funiu white goats in China.

Taihang black goat is also called the Xiuwu goat, because it originates in the Xiuwu region where it is the predominant breed. This breed is also found in adjacent counties such as Xinxiang, Anyang and Qinyang. The Taihang Black goat population has been decreasing over the past 30 years due to strengthened environmental protection, enforced restricted grazing in pastoral areas, semi-agricultural and semi-pastoral areas, and a conflict between forest and animal husbandry. In the 1980's, the data genetic resources investigations showed that a minimum of 227,000 Taihang black Goats were in existence. By the end of the 20<sup>th</sup> century, Taihang black goat was endangered with extinction. However, in recent years, protective measures taken by local government has ensured their survival. Today, less than 400,000 Taihang black goats exist and they have an average litter size of 1-2 lambs.

These two goat breeds have a better adaptability to extensive management in mountain areas; better immunity to infection and better meat-skin quality, and are a natural gene reservoir for improving crossbreed predominance. Rearing these two goat breeds has brought local farmers considerable economic benefits. However, since of no scientific selective breeding system has been conducted over the past 10 years many recessive traits were not adequately protected. Although, improvement of productive traits in goats is an important issue from the viewpoint of producers, prolific traits were not studied and selected, which has restricted the development of those two goat breeds. Protection and use of these indigenous goat breed resources is the most urgent research problem in local goat genetics and breeding. Consequently, it is essential to study the genetics and reproduction of these goat breeds using modern genetic methods. Until now, the association of BMP15 exon 2 genetic variations with litter size has been reported in some goat breeds but not in the Funiu white goat or the Taihang black goat. Since the BMP15 gene is associated with the folliculogenesis of sheep, it may be a potential candidate gene for litter size of local goats. Therefore, a preliminary test of association between BMP15 exon 2 polymorphisms and litter size in those two goat breeds was conducted.

## MATERIALS AND METHODS

### Experimental animals and DNA isolation

Genomic DNA samples were obtained from 205 female goats belonging to two goat breeds: Funiu white goats (105) and Taihang black goats (100). Goats were obtained from different villages of Funiu mountain region of Henan province, China. Approximately 10 ml of blood was collected from the jugular vein and the collected samples were transported to the laboratory at 4°C before DNA isolation. The DNA was isolated according to the procedure described by a routine protocol. Records of kids for different parities of the two goat breeds were collected for statistical analysis. For each doe, the number of kids born, the date of kidding, the flock number, the season of kidding, and the prolific performance of the first three parities were recorded. Does with incomplete performance records, does lacking birth information and records with other obvious errors were removed.

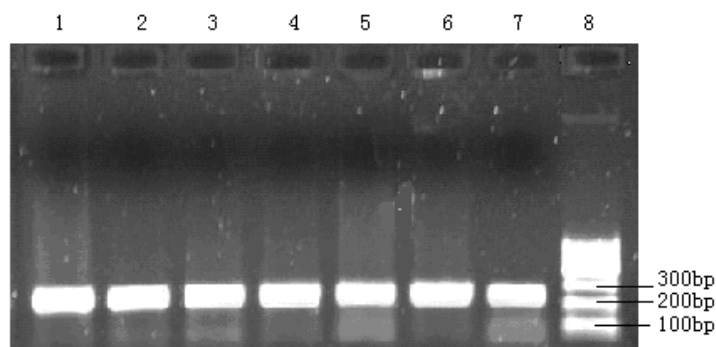
### PCR amplification

Based on the *Ovis aries* genbank database (GenBank Accession No.: AF236079.1), one pair of PCR primers for exon 2 of BMP15 (forward 5'-TA CAGACCCTGGACTT TCCTCT-3' and reverse 5'-GCCCAACATGTTCCAT GATATCC-3') was designed by Oligo 6.0 software (Biolytic lab Performance Inc) and was composed by Shanghai Sangon Biotech Co., Ltd.

The PCR reaction was performed in a 25 µl reaction volume containing about 50-100 ng genomic DNA, 0.5 µl of each primer (10 mM), 10× buffer (including 1.5 mM MgCl<sub>2</sub>), 200 µM dNTPs and 0.5 units of *Taq* DNA polymerase. Initial denaturation at 95°C for 4 min; 32 cycles of denaturation at 94°C for 30 s; annealing at 59.7°C for 30 s, extension at 72°C for 15s, then holding at 72°C for 10 min. The PCR products were separated by horizontal submarine agarose gel (2%, free from DNase and RNase) electrophoresis in 1×TAE buffer at 80 V. The gel was stained with ethidium bromide solution (0.5 µg/ml) and maintained for 10 min in darkness and photographed using a molecular imager (Kodak 120, USA).

### Single-stranded conformation polymorphism and DNA sequencing

Aliquots of 5 µl PCR products were mixed with 5 µl denaturing solution (98% formamide, 0.025% xylene-cyanole and 0.025% bromophenol blue, 10 mM EDTA), incubated at 98°C for 10 min and then chilled on ice. Denatured DNA was loaded on 10% PAGE gel (80 mm×73 mm×0.75 mm) in 1×TBE buffer and constant voltage 200V for 2.5 h. The gel was stained with 0.1% silver nitrate



**Figure 1.** PCR products of BMP15 gene exon 2 amplified by the primer. Lanes 1-7: PCR products from two goat breeds; lane 8: Marker.

solution. PCR products of BMP15, Homozygous and heterozygous genotypes from different SSCP patterns in different breeds were sequenced in both directions by Shanghai Sangon Biotech Co., Ltd, China.

### Statistical analysis

Least square analysis of the variance for litter size of different genotypes was carried out by the follow model:

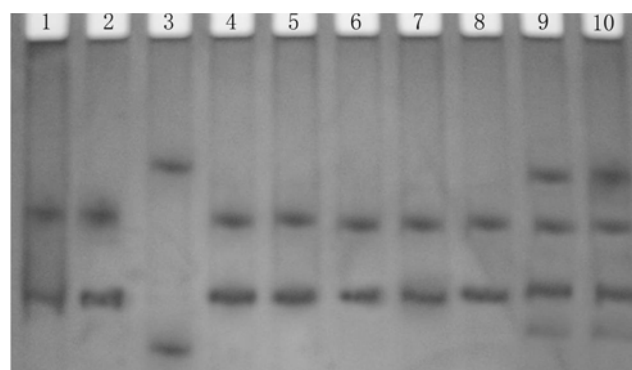
$$y_{ijklm} = \mu + S_i + KS_j + P_k + G_l + e_{ijklm}$$

Where  $y_{ijklm}$  was phenotypic value of litter size;  $\mu$  was the population mean;  $S_i$  was fixed effect of  $i$ th buck,  $i = 1, 2, 3$ ;  $KS_j$  was fixed effect of season;  $P_k$  was fixed effect of the  $k$ th partary,  $k = 1, 2, 3$ ;  $G_l$  was fixed effect of the  $l$ th genotype;  $e_{ijklm}$  was random residual error.

GLM (General Linear Model) of SAS (V8.12) is used for multiple testing.

## RESULTS

A 235 bp generated DNA fragment was subjected to 2% agarose gel electrophoresis. The results showed that amplification fragment sizes were consistent with the expected size as determined from their gene sequence information (Figure 1).



**Figure 2.** SSCP analysis of PCR products of primer. Lanes 1-2, 4-8, BB genotype; lane3, AA genotype; lane 9-10, AB genotype.

### Polymorphisms of the BMP15 gene exon 2 in the two goat breeds

The PCR products of BMP15 gene exon 2 were polymorphic in the two goat breeds with BB and AB genotypes found in both breeds while AA genotype was detected only in Funiu white goats (Figure 2). The haplotypes were named A and B, respectively. Frequencies of A allele were 0.179 and 0.171, and frequencies of B allele were 0.821 and 0.829, and the PIC was 0.250 and 0.243, and the Ne was 1.416 and 1.396, and h was 0.294 and 0.283, respectively in Funiu white goats and Taihang black goats (Table 1).

**Table 1.** Genetic structure of the exon 2 of BMP15 gene for two goat breeds

|                    |                      | Genotype and Allele | Frequencies | PIC   | Ne    | H     |
|--------------------|----------------------|---------------------|-------------|-------|-------|-------|
| Funiu white goat   | Genotype frequencies | AA                  | 0.071       | 0.250 | 1.416 | 0.294 |
|                    |                      | AB                  | 0.214       |       |       |       |
|                    |                      | BB                  | 0.715       |       |       |       |
|                    | Allele frequencies   | A                   | 0.179       |       |       |       |
|                    |                      | B                   | 0.821       |       |       |       |
| Taihang black goat | Genotype frequencies | AA                  | 0.000       | 0.243 | 1.396 | 0.283 |
|                    |                      | BB                  | 0.658       |       |       |       |
|                    |                      | AB                  | 0.342       |       |       |       |
|                    | Allele frequencies   | A                   | 0.171       |       |       |       |
|                    |                      | B                   | 0.829       |       |       |       |

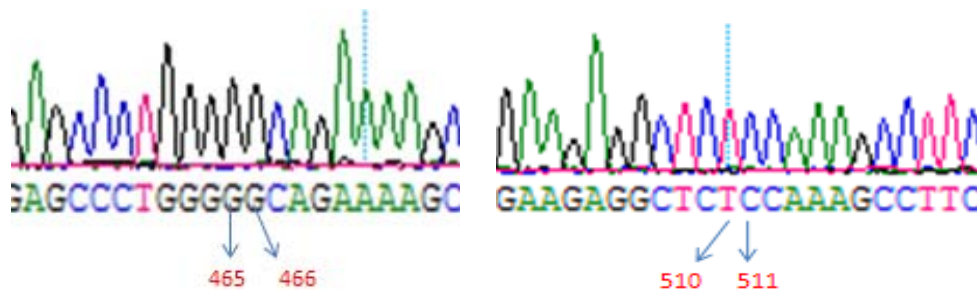


Figure 3. Partial sequence comparison of altered bases in goats with genotype BB.

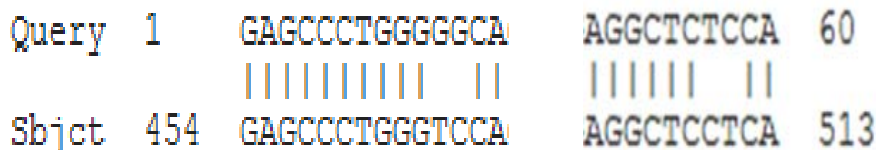


Figure 4. Basic sequence mutational site by exon2 by blast.

**BMP15 gene sequence and amino acid sequence mutations**

The different SSCP patterns AA (only for Funiu white goat), BB and AB of the BMP15 gene exon 2 for two goat breeds amplified by primer locus were sequenced in both directions and by using the software DNASTAR, Bio Edit and information from BLAST, <http://www.ncbi.nlm.nih.gov/>. Comparisons between the protein sequences of genotype AA and BB, show that four mutations (465T→G, 466C→G, 510C→T, 511T→C) in AB for Funiu White Goat, which resulted in amino acid substitution of V155G and S171P. No mutation was detected in Taihang black goat (Figures 3-5).

**Association of polymorphisms with litter size in the goat breeds**

In this study, sample size of Funiu white goat with AA, BB and AB genotypes was 7, 70 and 21 does respectively, does with BB and AB genotype of Taihang black goat was 58 and 30, respectively. Kids from the first three parities were calculated for two goat breeds. The total litter size of Funiu white does with AA genotype was 34, AB genotype was 101, and BB genotype was 479. In Taihang black goat, the total litter size of does with BB and AB genotypes was

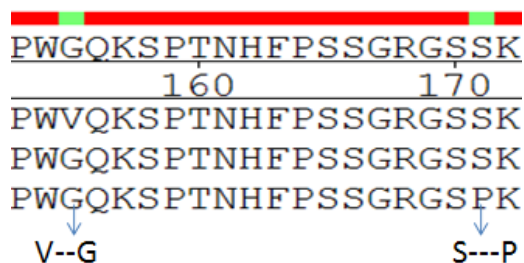


Figure 5. Encoded sequence compare of amino acids by exon2 by blast.

253 and 141, respectively. Does with BB genotype had greater litter size than those with AA genotype ( $p < 0.05$ ) and AB ( $p < 0.05$ ) from the first three parities of Funiu white goats. Does with AB genotype had greater litter size than those with BB genotype ( $p > 0.05$ ) from the first three parities of Taihang black goats. No difference in litter size between the two genotypes was found in Taihang black goats (Table 2). The results indicate that B allele is dominant allele in these two goat breeds. The three genotypes in Funiu white goats deviated from Hardy-Weinberg equilibrium ( $p < 0.01$ ). The Taihang black goat breed was also in a state of Hardy-Weinberg disequilibrium ( $p < 0.05$ ). It is possible that the two goat breeds have been

Table 2. Association of BMP15 genotypes with litter size (mean±SE) in two locals

| Breeds             | Genotypes | First parity litter size | Second parity litter size | Third parity litter size | Average litter size    |
|--------------------|-----------|--------------------------|---------------------------|--------------------------|------------------------|
| Funiu white goat   | AA        | 1.29±0.22 <sup>b</sup>   | 1.71±0.52 <sup>b</sup>    | 1.86±0.55 <sup>b</sup>   | 1.62±0.32 <sup>b</sup> |
|                    | AB        | 1.38±0.43 <sup>b</sup>   | 1.62±0.52 <sup>b</sup>    | 1.83±0.39 <sup>b</sup>   | 1.60±0.42 <sup>b</sup> |
|                    | BB        | 2.17±0.85 <sup>a</sup>   | 2.53±1.01 <sup>a</sup>    | 2.60±1.22 <sup>a</sup>   | 2.28±0.93 <sup>a</sup> |
| Taihang black goat | BB        | 1.19±0.14 <sup>a</sup>   | 1.50±0.25 <sup>a</sup>    | 1.67±0.48 <sup>a</sup>   | 1.45±0.41 <sup>a</sup> |
|                    | AB        | 1.23±0.12 <sup>a</sup>   | 1.70±0.08 <sup>a</sup>    | 1.77±0.16 <sup>a</sup>   | 1.57±0.28 <sup>a</sup> |

Values with different superscripts within the same column differ significantly at  $p < 0.05$ .

selected for high prolificacy population in our sample collection areas.

## DISCUSSION

Recently, the genetics of goat litter size has been well documented, with some important prolificacy genes of sheep such as BMP15 also studied in goats. So far, there have been 8 mutants of BMP15 gene detected, they are FecX<sup>I</sup>, FecX<sup>H</sup>, B1, FecX<sup>G</sup> (B2), B3, FecX<sup>B</sup> (B4), FecX<sup>L</sup>, FecX<sup>R</sup> (Hanrahan et al., 2004; Davis et al., 2005; Bodin et al., 2007; Martinez-Royo et al., 2009). A 17 bp deletion of the reading frame of the functional gene was also reported (Monteagudo et al., 2009). It is well known that BMP15 increased ovulation in heterozygous mutant ewes because the altered proteins result in increased sensitivity of granulosa cells to FSH, which leads to accelerated follicular development and precocious ovulation of small follicles (Moore et al., 2004; Moore and Shimasaki, 2005). Ovulation rates in BMP15 mutants are high in the heterozygotes while the homozygous mutants show a primary ovarian failure resulting in complete sterility (Galloway et al., 2000; Hanrahan et al., 2004; Bodin et al., 2007; Monteagudo et al., 2009). Based on those theories, original studies of BMP15 as candidate genes for fecundity in sheep were well documented in the Belclare/Cambridge sheep, the Lacaune sheep, and Small Tailed Han sheep (Bodin et al., 2002; Liu et al., 2003; Kumar et al., 2006; Jiao et al., 2007).

In recent years, increased attention has been given to studying candidate genes for fecundity in goats. Jiao et al. (2007) and Chu et al. (2007) reported that exon 2 of BMP15 have A963G and C1050G mutants, which leads to the amino acid changes in S300G and L329V, respectively. It was reported by Feng et al. (2009) that the A963G mutant of BMP15 gene exon 2 in Jining Grey goats has three genotypes (AA, AG and GG) while only AA genotype was found in Liaoning cashmere and Inner Mongolia Cashmere goats. Boar goat have two genotypes (AG and GG) while Angora and Inner Mongolia Cashmere goats have only AA genotype. Feng et al. (2009) also found that genotype distributions of BM P15 gene were significantly different among those goat breeds and preliminarily indicated that the BM P15 gene is either a major gene which affects the prolificacy in Jining Grey goats or a molecular marker in close linkage with such a gene. Lin et al. (2007) reported that the mutation of FecX<sup>G</sup> was not detected in all samples from the white goat of Guizhou. FecX<sup>B</sup> mutation was found in female goats with triplets and in male goats. All of the FecX<sup>B</sup> mutations were identified in heterozygous genotype (AB) and the frequency of mutated B allele was 4.5%. It suggested that FecX<sup>B</sup> mutation was related to the regulation of fecundity in white goats of Guizhou. The increased

prolificacy in six goat breeds (Boer, Haimen, Huanghuai, Nubi, Matou and Jining Grey) were not associated with any known point mutations in BMP15 gene (Chu et al., 2007; Hua et al., 2008). On the other hand, goats with low fecundity (Yunling Black goat) showed no relationship to point mutations in BMP15 (Cui et al., 2009).

In the present study, the high fecundity breed, Funiu white goats, and low fecundity breed, Taihang black goats were genotyped for BMP15 and the exon 2 of BMP15 gene was found to be polymorphic. Three genotypes (AA, BB and AB) were detected in Funiu white goats; this is consistent with previous studies of the Jining grey goat. Two genotypes (AB and BB) were detected in Taihang black goats and is similar to that found in Boar goats. Sequencing revealed that four mutations (456T→G, 466C→G, 510C→T, 511T→C) in genotype BB of Funiu White Goat resulted in amino acid substitution of V155G and S171P. No similar mutation was detected in Taihang black goats. Mean litter size of Funiu white goat tended to increase in later parities with values of 1.61, 2.28 and 2.39 from the first to third parity, respectively. Similarly, litter size increased in Taihang black goats to 1.20, 1.57 and 1.70, respectively. In this study, correlation between genotype and litter size from the first to the third parity was analyzed. In goat production, the litter size during a doe's lifetime is very important factor, as it affects economic benefits to goat producers. However, the litter size at second kidding is often a valuable index to determine whether a goat is prolific. The Funiu white goat with genotype BB had 0.68 or 0.66 kids for the average first litter size, more than those with AA or AB, respectively, and had 0.82 or 0.91 kids at the second kidding, more than those with AA or AB, respectively ( $p < 0.05$ ). The difference of the least squares means for litter size between AA and AB at different parities was not significant ( $p > 0.05$ ) in Taihang black goat. This appears to agree with the previous view that goats with low fecundity show no correlation with point mutations in BMP15, while high fecundity may be associated the mutations. The present study shows that litter size in Funiu white goats was significantly influenced by sire, kidding season and parity ( $p < 0.05$ ,  $p < 0.05$  and  $p < 0.05$ , respectively). Because of the lack of functional data and small population size used, the conclusion requires further studies of seasonal effects on litter size in different parities and we are presently undertaking this research.

## CONCLUSION

The results of this study preliminarily concluded that in Funiu white goats and Taihang black goats, the BMP15 gene was polymorphic; the BM P15 gene may be a major gene which affects the prolificacy in Funiu white goats. The association of the polymorphism of BMP15 exon 2 with

litter size of Chinese indigenous local goats in this study suggests its feasibility as a molecular breeding marker. Funiu white goats with genotype BB had more kids than other genotypes and could be used for the development of new breeds of prolific goats. Further research on a large number of animals is required to confirm the link with increased prolificacy in Funiu white goats.

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