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Polymorphisms in GDF9 Gene and Its Relationship with Litter Size in Five Breeds of Black Goats

Short Communication

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ABSTRACT

In this study, we investigated the relationship between the genetic polymorphism of growth differentiation factor 9 (GDF9) genes and the litter size in 384 individuals of five breeds of black goats. Four pairs of primers were designed to detect single nucleotide polymorphism of GDF9 gene in goats by PCR-SSCP. The least square was used to analyze the relation between different genotypes and the litter size. The results showed that the PCR products from primer pair 1 (P1) displayed polymorphisms in three genotypes (AA, AB and BB) in big foot (BF) and Jintang (JT) black goats. For primer pairs of P2, P3 and P4, there was no polymorphism. The sequencing results revealed that there was a single nucleotide mutation (A792→G) in exon 2 of GDF9 gene in BF and JT black goats, and this mutation resulted in an amino acid change: valine→isoleucine. In BF and JT black goats, the average litter size in the third parity was significantly higher in genotype AA than both genotypes of AB and BB while the average little size of genotype AB was higher than that of genotype BB in the same parity. GDF9 gene could be therefore considered as a candidate gene for marker-assisted selection of litter size trait in goats.

KEY WORDS GDF9, genetic polymorphism, goat, litter size, PCR-SSCP.

INTRODUCTION

The ovulation rate and litter size are results of well regulated interactions of endocrine and paracrine mediators in mammals. Even though the tendency of twining and triplicate is common in both sheep and goats, how the litter size is precisely controlled remains a critical question in reproductive biology.

Growth differentiation factor 9 (GDF9) was first identified as an oocyte-derived growth factor required for ovarian somatic cell function (Dong *et al*. 1996), the $FecG^H$ mutation leads to the substitution of serine with phenylalanine at position 77 of the mature GDF9 peptide S77F, (Hanrahan *et al*. 2004). A close homolog of GDF9 was discovered later, namely, GDF9B (BMP15), and it is also expressed in the oocyte from the primary follicle stage continuing through ovulation (Dube *et al*. 1998). Heterozygous ewe carriers of the $FecG^H$ (high fertility) allele exhibit one to two additional ovulations compared with non-carriers, whereas homozygous mutant ewes are sterile, and have small, flattened streak ovaries containing only follicles that do not develop up to the primary (type 2) stage (Hanrahan *et al*. 2004; Galloway *et al*. 2000; Mc Natty *et al*. 2005; Bodin *et al*. 2007).

The genetic basis of caprine prolificacy remains to be explored. Recent studies on prolific goat breeds like Boer, Haimen, Huanghuai, Nubi, Matou and Jining Grey suggested that genetic control of higher prolificacy in goats is

different from sheep (Chu *et al*. 2005; Chu *et al*. 2007; Hua *et al*. 2008; Hua and Yang 2009).

The known point mutations of BMPR1B (FecB) and BMP15 ($FecX^H$, $FecX^I$, $FecX^G$ and $FecX^B$) genes are monomorphic in the prolific goat breeds (Hua *et al*. 2008). Two new point mutations in the BMP15 gene linked with a higher prolificacy in the Jining Grey goat, therefore BMP15 gene was suggested to be the major gene that influences prolifically in this breed (Chu *et al*. 2007).

The black goats are indigenous species with different fecundities in China. The big foot (BF) black goat in Chongqing city and Jintang (JT) black goat in Sichuan province are of high fecundity, with average litter size of 2.89 and 2.46, respectively. The Hainan (HN) black goat in Hainan province is of middle fecundity, the average litter size is 1.75. The fecundity of Guizhou (GZ) black goat in Guizhou province and Taihang (TH) black goat in Henan province are low, the average litter sizes are 1.52 and 1.43, respectively, which provides a very good natural variation to study the reproductive differences between prolific and non-prolific black goats. The objective of this research is to analyze the polymorphisms of GDF9 gene and to investigate the relationship between different genotypes and litter sizes in these five breeds of black goats.

MATERIALS AND METHODS

Experimental goat breeds and samples

A total of 384 adult female individuals from five breeds of black goats were examined in this study, including big foot black goats (BF, n=96), Jintang black goats (JT, n=81), Hainan black goats (HN, n=59), Guizhou black goats (GZ, n=58) and Taihang black goats (TH, n=90).

Approximately 10 mL blood was collected aseptically from the jugular vein into sterile tubes which contained 0.5 mL EDTA (0.5 M) as anticoagulant reagent. All samples were taken back to the laboratory in icebox. The genomic DNA was extracted from white blood cells using standard phenol–chloroform extraction procedure (Sambrook and Russel, 2002).

The DNA samples were dissolved in TE buffer (pH 8.0) to a final concentration of 100 ng/μL, DNA samples were checked by Nano drop 2000 (thermo scientific) for purity and stored at −20 °C for use.

Information of primer sequences and PCR conditions based on the complete GDF9 gene coding sequence (GenBank accession number EF446168.2), four pairs of primers were designed to amplify partial intron, exon 2 and 3'-UTR of the GDF9 gene. The sequences of the four pairs of primers are listed in Table 1. The 12 μL PCR amplification system, containing 1.2 μL reaction buffer (10X), 50 ng genomic DNA, 10 pmol/L each primer, 1.2 μL dNTPs (0.25 mmol/L each), $MgCl_2$ (4.0 mmol/L or 3.0 mmol/L) and 0.5 U *Taq* DNA polymerase (MBI), was used. The cycling procedure is as follows: 4 min at 95 °C; 35 cycles of 94 °C 30 s; 61 °C 1 min and 72 °C 1.5 min; with a final extension at 72 °C for 10 min. Polymorphisms were detected by 12% PAGE (82 mm×82 mm×1.0 mm) using constant voltage (200 V) for 2.5 h. The gel was stained with 1% silver nitrate exactly as described by (Ji *et al*. 2007).

Table 1 Primers information and the optimal reaction conditions for the amplification of GDF9 gene

Name	Sequence (5'→3')	Product (bp)	Tm (°C)	Amplicon locations*
P1	F: CCATGACTT TAGACTTAGC R: TGGTTTTACT TGACAGGAG	324	58. 1	3958-4281
P2	F: GTTGGATTG TTTTTCTTCT R: CTCTTTTATC ACCAGGTTG	171	57	3272-3442
P3	F: GCTTTTGTAT CTGAACGAC R: GCTAAGTCT AAGTCATGG	289	57	3688-3976
P4	F: CACCTGGTG ATAAAAGAG R: GTCGTTCAGA TACAAAAGC	283	57	3424-3706

* Nucleotide locations corresponding to the sequence EF446168.2.

Statistical analysis

The average heterozygosity (He) and polymorphism information content (PIC) of GDF9 gene in the five breeds were analyzed by the cluster analysis software (poultry institute of the Chinese academy of agricultural sciences).

The relationship between GDF9 genotypes and litter size in five breeds of black goats were analyzed using GLM (general linear model) method, which was performed by SPSS software (version 13.0). Linear model was:

$$Y_{ij} = \mu + M_i + e_{ij}$$

Where:

Y_{ij}: the litter size trait measured on each of the ij^{th} animal.

μ: the overall population means.

M_i: the fixed effects associated with i^{th} genotype.

e_{ij}: the random error.

RESULTS AND DISCUSSION

Analysis of GDF9 gene by PCR-SSCP

SSCP analysis showed that only primer pair P1 detected polymorphisms in two out of the five breeds with three genotypes as AA, AB and BB (Figure 1). Other three primer pairs P2, P3 and P4 did not detect polymorphism in all the five breeds of black goats.

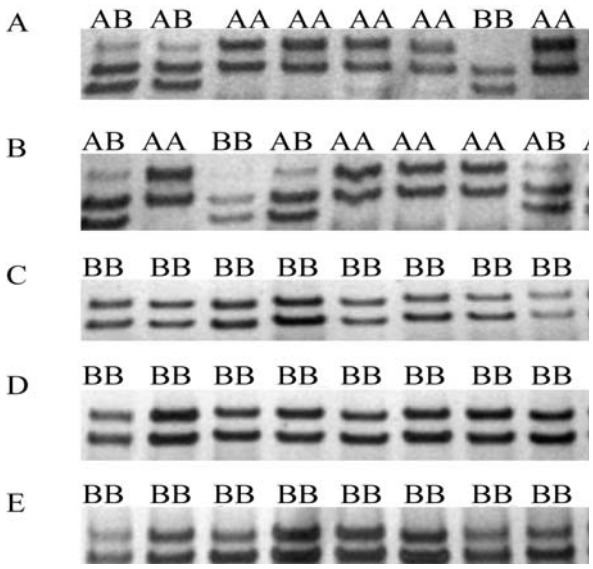

Figure 1 PCR-SSCP patterns of GDF9 by the primer P1 in 5 breeds black goats

A: big foot black goat; B: Jintang black goat; C: Hainan black goat; D: Guizhou black goat and E: Taihang black goat

Different genotypes (AA, AB and BB) from different individuals are labeled on top of each lane

Confirmation of polymorphisms by sequencing

To confirm the polymorphic results from PCR-SSCP of P1 primer pair and check the specific polymorphisms in DNA sequences, the polymorphic fragments were sequenced in both directions.

Part of the sequencing profiles of the three observed genotypes are shown in Figure 2. A single nucleotide mutation (G792→A) in exon 2 of GDF9 gene was detected, and this mutation resulted in an amino acid change: valine→isoleucine.

Association of GDF9 genotypes with average litter size in the third parity in BF and JT goats

In both BF and JT goats, the litter size of genotype AA at third parity was significantly greater than that of the goats of both genotypes AB and BB, while genotype AB had significantly greater LS than that of genotype BB.

The results showed that the different genotypes of GDF9 gene could significantly affect the litter size in BF and JT breeds. GDF9 gene may be therefore considered as a candidate gene for litter size selection for BT and JT goats.

Table 2 Genetic structure of the GDF9 gene (P1) for BF and JT breeds

Breed	Genotypes	Genotype frequencies	Allele frequencies	He	PIC
BF	AA (33) AB (17) BB (46)	0.34 0.17 0.47	A= 0.43 B= 0.56	0.49	0.37
JT	AA (44) AB (21) BB (16)	0.54 0.25 0.19	A= 0.67 B= 0.32	0.44	0.34

The natural mutation related to the increase of ovulation-rate was only reported in sheep (Hanrahan *et al*. 2004) and goat (D *et al*. 2008; Lin *et al*. 2007). The regulation of GDF9 gene on ovulation rate appeared to be more sensitive on species with low ovulation rate phenotypes (such as goat and human) than those with higher ovulation rate vertebrates (rat, mouse, dog and pig), especially for species of laying eggs, such as chicken and fish (Elis *et al*. 2007; Johnson *et al*. 2005; Clelland *et al*. 2006; Liu and Ge, 2007).

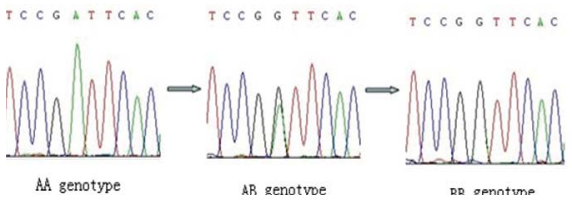

Figure 2 Partial sequencing maps of AA, AB and BB genotypes in black goats GDF9 gene amplification products by P1

BMP15 and BMP receptors have been detected in goat ovarian follicles at all stages (Silva *et al*. 2004). Polymorphisms of GDF9 of white goat in Guizhou province had been confirmed in previous investigations (Du *et al*. 2008; Lin *et al*. 2007). Studies have found that different genotypes have significant impact on the average little size ($P<0.05$) in small tail Han sheep (Li *et al*. 2003; Chu *et al*. 2004; Yang *et al*. 2006). Jining Grey goats have 1.04 and 0.75 more average litter size with genotypes AA and AB, respectively, than that of genotype BB ($P<0.01$), and the average litter size of genotype AA is 0.29 (Table 3) more than that of AB ($P<0.05$) (Wu *et al*. 2005).

Table 3 Association of GDF9 (P1) genotypes with third LS traits in BF and JT breeds

Breeds	Genotypes	Sample size	LS at parity 3
BF	AA	33	2.96 ± 0.25^{a}
	AB	17	1.84 ± 0.42^{ba}
	BB	46	1.66 ± 0.27^{b}
JT	AA	44	2.77 ± 0.41^{a}
	AB	21	1.78 ± 0.28^{ba}
	BB	16	1.58 ± 0.27^{b}

The means within the same column with at least one common letter, do not have significant difference ($P>0.05$).

We have investigated the mutations in GDF9 gene of five breeds black goats and confirmed that the mutations of GDF9 were present in two black goat populations. Our re-

search found that mutation of GDF9 gene exists in two high fecundity breeds of five breeds black goats investigated, and this mutation (allele) contributes to the greater litter size of the two polymorphic breeds. GDF9 gene can be therefore considered as a candidate gene of litter size for selecting BF and JT black goats.

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