Small Ruminant Research 132 (2015) 92–98

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Association analysis of novel SNPs in *BMPR1B, BMP15* and *GDF9* genes with reproductive traits in Black Bengal goats

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ARTICLE INFO

Article history:
Received 6 July 2015
Received in revised form 3 August 2015
Accepted 7 October 2015
Available online 22 October 2015

Keywords:
BMPR1B
BMP15
GDF9
Black Bengal goat
Fecundity
Precocity

ABSTRACT

Black Bengal goats are an interesting genetic material to underpin the genetic mechanism of reproduction due to high prolificacy and sexual precocity. In the present study, novel SNPs in *BMPR1B, BMP15* and *GDF9* genes were genotyped to evaluate their association with the reproductive traits. PCR-RFLP and Tetra primer ARMS-PCR based protocols were developed for genotyping the six novel SNPs viz. T(-242)C in *BMPR1B*, G735A and C808G in *BMP15* and C818T, A959C and G1189A in *GDF9*. Linear mixed model for association of these SNPs with litter size and linear fix model for other traits were employed. The effect of season and parity was highly significant ($p \leq 0.01$) on litter size which varied with change in locus combination. However, there was no significant effect of genotype and year of birth on the litter size. Similarly, age at first heat, age at first service and age at first kidding were not affected by the year of birth as well as the genotype. Season of kidding did not affect the age at first heat and age at first service. However, significant ($p \leq 0.05$) association was observed between the season of kidding and the age at first kidding. The regression of age at sexual maturity on the age at first service and regression of age at first service on the age at first kidding was highly significant ($P \leq 0.01$). Further studies involving more number of breeds as well as animals may be fruitful for exploring the association of novel SNPs with caprine reproductive traits.

1. Introduction

India is endowed with a wide diversity of goat genetic resources, which form the backbone of its rural livelihood security by providing meat, milk, fur and cashmere (Ahlawat et al., 2014). It is an established fact that an animal producing twins or triplet contributes more than 1.5 times towards meat than the animals producing single offspring per kidding. Hence, improvement of reproductive traits has been of increasing interest in goats. There is dire need to identify genes responsible for more kids per conception and also in the life time of the animal. Selection aimed at increasing the frequency of alleles with a positive effect on a given trait was initiated by geneticists in recent years. Marker Assisted Selection (MAS) in conjunction with traditional selection methods is considered to be most effective for improvement in traits that are expressed later in life, are sex-limited, and of low heritability, such as litter size (Dekkers, 2004; Williams, 2005). Better production of prolific breeds in sheep has been achieved following this approach (Mishra et al., 2009).

Genetics of prolificacy in sheep emphasizes the importance of three major fecundity genes, namely, Bone Morphogenetic Protein Receptor type IB (*BMPR1B*) or *FecB*, Growth Differentiation Factor 9 (*GDF9*) or *FecG* and Bone Morphogenetic Protein 15 (*BMP15*) or *FecX*. All the three genes are members of transforming growth factor β (TGFβ) superfamily and are intra-ovarian regulators of folliculogenesis in mammals (Davis et al., 2005). *BMPR1B (FecB)* was identified as the first major gene for prolificacy in sheep. *BMPR1B* has been implicated as one of the type 1 receptors, downstream of *BMP15* in sheep, mouse and rat granulosa cells (Mulsant et al., 2001). The Booroola sheep mutation *FecB* is a Q249R mutation in the kinase domain of *BMPR1B*, which increases the fertility of ewes (Wilson et al., 2001). The *FecB* locus is autosomal with codominant expression, which is additive for ovulation rate (Souza et al., 2001; Wilson et al., 2001).

BMP15, an oocyte-derived growth factor is involved in ovarian follicular development. It is a critical regulator of many granulosa cell (GC) processes such as proliferation and steroidogenesis

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http://dx.doi.org/10.1016/j.smallrumres.2015.10.010
0921-4488/

Table 1
Genetic variants associated with prolificacy phenotype in sheep.

Gene	Mutation	Amino acid change	Founder breed	Reference
BMPR1B	*FecB*	Q249R	Booroola Merino, Garole and Javanese	Mulsant et al. (2001) Souza et al. (2001) Wilson et al. (2001)
BMP15	$FecX^{I}$	V299D	Romney	Galloway et al. (2000)
	$FecX^{H}$	Q291ter	Romney	Galloway et al. (2000)
	$FecX^{B}$	S367I	Belclare	Hanrahan et al. (2004)
	$FecX^{G}$	Q239ter	Belclare and Cambridge	Hanrahan et al. (2004)
	$FecX^{L}$	C321Y	Lacaune	Bodin et al. (2007)
	$FecX^{R}$	17 bp deletion	Rasa Aragonesa	Martinez-Royo et al. (2008) Monteagudo et al. (2009)
	$FecX^{Gr}$	T317I	Grivette	Demars et al. (2013)
	$FecX^{0}$	N337H	Olkuska	Demars et al. (2013)
GDF9	$FecG^{H}$	S395F	Belclare and Cambridge	Hanrahan et al. (2004)
	$FecG^{T}$	S427R	Icelandic	Nicol et al. 2009
	G1	H87R	Garole	Polley et al. (2010)
			Bayanbulak	Zuo et al. (2013)
	$FecG^{E}$	F345C	Santa Inês	Silva et al. (2011)
	G1111A	V371M	Norwegian White Sheep	Vage et al. (2013)

(Shimasaki et al., 2004; Juengel and McNatty, 2005). Till date, 8 mutations related to high prolificacy have been identified in the sheep *BMP15* gene (Table 1). Six mutations ($FecX^{I}$, $FecX^{H}$, $FecX^{B}$, $FecX^{G}$, $FecX^{L}$, $FecX^{R}$) produce the same phenotype i.e., increased ovulation rate or litter size in heterozygous carriers and sterility in homozygous carriers. However, for $FecX^{Gr}$ and $FecX^{0}$ mutations, homozygous ewes are highly prolific.

GDF9 plays a critical role in mammals as a growth and differentiation factor during early folliculogenesis. Five mutations in *GDF9* have been associated with fertility in sheep viz. $FecG^{H}$, $FecG^{E}$, *FecTT*, *G1* and *G1111A* (Table 1). $FecG^{H}$ causes increased ovulation rate in heterozygous ewes while homozygous ewes are sterile. Interestingly, $FecG^{E}$ does not cause sterility in the homozygous individuals but leads to significantly higher prolificacy as compared to heterozygous individuals. Similarly, ewes homozygous for the *G1111A* allele are also reported to be fertile.

We have previously screened the Indian goats for prolificacy associated markers of sheep. It was observed that none of the associated mutations in the candidate genes (*BMPR1B*, *BMP15* and *GDF9*) in sheep were present in the Indian goats (Ahlawat et al., 2013). Two novel SNPs viz. T(-242)C and G(-623)A in the promoter region of *BMPR1B* gene (Ahlawat et al., 2014), two novel SNPs (G735A and C808G) in exon 2 of *BMP15* gene and three non-synonymous SNPs (C818T, A959C and G1189A) in exon 2 of *GDF9* gene were reported (Ahlawat et al., 2015a) by our group in Indian goats. These novel polymorphisms need to be associated with caprine reproductive traits for identification of molecular markers that can be used to identify prolific and early maturing animals at an early stage of life.

Black Bengal is a famous breed of goat in India and is known for its high prolificacy, early sexual maturity, low kidding interval and extremely good adaptability (Dhara et al., 2008). It is a major meat producing animal in eastern states of India desirable for its superior chevon quality. Being a prolific breed, 47.5% animals produce twins and 7% animals give birth to triplets. This makes this breed an interesting genetic material to underpin the genetic mechanism of prolificacy and sexual precocity. Hence, the objectives of the present study were to genotype Black Bengal goats for novel SNPs in *BMPR1B*, *BMP15* and *GDF9* genes and to evaluate the association of the these mutations with the reproductive traits.

2. Materials and methods

2.1. Animal selection, sample collection and DNA isolation

One hundred and fifty eight Black Bengal goats were utilized for association of identified genotypes in *BMPR1B*, *BMP15* and *GDF9* genes with the litter size (first, second, third and fourth parity). For other reproductive traits viz. age at sexual maturity (days), age at first service (days) and age at first kidding (days), data was recorded for sixty animals. Blood samples were collected from goats maintained at Kotulpur goat cum fodder farm, West Bengal, India. Five milliliter blood per goat was collected aseptically from the jugular vein in EDTA containing vacutainer tubes (B.D. Bioscience, Germany). All samples were delivered back to the laboratory in an ice box. Genomic DNA was extracted from white blood cells using standard phenol-chloroform extraction protocol (Sambrook and Russell, 2001).

2.2. Genotyping of the identified SNPs

SNPs reported earlier by our group in *Fec* genes of Indian goat viz. T(-242)C in the promoter region of *BMPR1B* gene, G735A and C808G in exon 2 of *BMP15* gene and C818T, A959C and G1189A in exon 2 of *GDF9* gene were genotyped. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was employed for the mutation A959C and G1189A of *GDF9* and C808G of *BMP15* gene, whereas, remaining three SNPs were genotyped using tetra-primer Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR).

Primers for PCR-RFLP (Table 2) of *GDF9* and *BMP15* mutation were from Feng et al. (2011) and Ahlawat et al. (2013), respectively. Polymerase chain reaction (PCR) was carried out in a final reaction volume of 25 μl on i-cycler (BIO-RAD, USA). PCR cocktail consisted of 50–100 ng of genomic DNA, 200 μM of each dNTPs, 50 pM of each primer, 0.5 units of *Taq* DNA polymerase and *Taq* buffer having 1.5 mM $MgCl_2$ for each reaction. The PCR cycle was accomplished by denaturation for 1 min at 94 °C; 30 cycles of denaturation at 94 °C for 45 s, annealing at specific temperature for 45 s, extension step at 72 °C for 45 s with a final extension at 72 °C for 5 min. The PCR products were visualized following electrophoresis through a 1.8% Ethidium bromide stained agarose gel. PCR-RFLP genotyping protocol was developed for the mutation C808G of *BMP15* and A959C and G1189A of *GDF9* gene using the NEB cutter online program (http://tools.neb.com/NEBcutter2). PCR-RFLP was performed with *BssSI*, *BstNI* and *MspI* on PCR products corresponding to mutations C808G of *BMP15*, A959C and G1189A of *GDF9* respectively (Table 2) as per manufacturer's instructions (Fermentas International, Inc.) by mixing 5 μL of the PCR product with enzyme buffer and one unit of respective enzyme in a final volume of 15 μL. Digested fragments were separated by electrophoresis on 3% agarose gel stained with Ethidium bromide (2 μL/100 mL). The fragments were photographed under Gel Documentation Unit and their sizes were