



Association of kisspeptin gene (*KISS1*) with litter size in migratory Gaddi goats in western Himalayan state of Himachal Pradesh

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ABSTRACT

Migratory goat farming, practiced by nomadic communities is common in Himalayan regions. Gaddi also known as 'White Himalayan goat' is the predominant goat breed constituting 60–65% of total goats in the state. The Kisspeptin gene (*KISS1*) that encodes kisspeptin protein is considered to be a candidate gene affecting multiple birth in goats. The present study was carried out to identify polymorphism at kisspeptin (*KISS1* gene) and its association with litter size in migratory Gaddi goat. Polymorphism of *KISS1* gene was investigated in Gaddi goats (89) using PCR-RFLP and DNA sequencing approach. PCR-RFLP analysis at *KISS1* locus revealed 3 different genotypes, viz. AA with undigested one fragment at 377 bp, TT with two digested fragments at 256 and 121 bp, and AT with three fragments at 377, 256 and 121 bp. The frequencies of AA, AT and TT genotypes were 0.17, 0.52 and 0.31, respectively while the frequency of A and T alleles was 0.43 and 0.57. AT genotype was predominant genotype while AA genotype was having least frequency among the goats screened. The observed allele number (N_o) and effective allele number (N_e) were 2 and 1.96, respectively. Observed heterozygosity (H_{obs}), expected heterozygosity (H_{exp}) and PIC values estimated were 0.52, 0.49 and 0.37, respectively. H_{obs} and PIC values indicated that sufficient genetic variation exist at the locus. Sequencing of representative sample of different genotypes confirmed presence of SNP (T125A) as detected by PCR-RFLP. The mean litter size for animals belonging to AA, AT and TT genotypes were 1.12 ± 0.08 , 1.34 ± 0.11 and 1.73 ± 0.15 kids, respectively. Significant association of genotypes was observed with litter size in Gaddi goat. The study detected association between allele T in *KISS1* gene and litter size. Study on additional data based on more number of animals in diversified flocks should be carried out for validation of the preliminary findings.

Key words: Bone morphogenetic protein, Gaddi breed, Himachal Pradesh, Kisspeptin, Litter size, Population genetics index

Small ruminants, sheep and goats, play a vital role not only in meeting increased human demand for animal origin protein foods but also in sustainability of overall farming system in general and livestock production system in particular. Sheep and goat production is a predominant livestock activity in harsh climatic regions of the country particularly in hilly areas and arid zones with the little arable agricultural land. Gaddi, also known as 'White Himalayan goat', is the predominant goat breed constituting 60–65% of total goats in the state. Gaddi, is reared both for wool and mutton production by migratory nomads/tribe 'Gaddi' (Sankhyan *et al.* 2016). The breed is primarily reared in transhumant/migratory/pastoralist production system. Compared to intensive and semi-intensive production system, migratory production system result in low productivity/animal due to sub-optimal management. Thus in order to improve the productivity under migratory

production system, breeding and optimum management are important aspects to improve production efficiency. Improving the breeding efficiency of goats under migratory production system is an important area of concern for increasing the economic returns. Unfortunately, the genetic mechanism of caprine prolificacy is not as understood as those in sheep but the tendency of twinning and triplet births is inherited and similar in both (Hua *et al.* 2008). Bone morphogenetic protein receptor type-IB (*BMPRI-IB*) and bone morphogenetic protein 15 (*BMP15*) are widely screened loci associated with prolificacy in sheep, however in goats these loci are not linked with prolificacy as in sheep. Thus, other loci are being identified/screened for prolificacy in different goat populations. In case of widely explored caprine fecundity genes, *KISS-1/GPR54* system is universally considered as a key regulator and a catalyst for the puberty onset, and is fundamental gatekeeper of sexual maturation in mammals. Polymorphisms at *KISS1* gene plays an important role in reproductive functions including age at sexual maturity and litter size (Maitra *et al.* 2014, Ahlawat *et al.* 2015). Therefore, the present study was

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carried out with the objective of identification of polymorphism at kisspeptin (*KISS1*) gene and its association with litter size in migratory Gaddi goats.

MATERIALS AND METHODS

Gaddi goat breed is primarily reared for chevon and fibre by migratory tribe 'Gaddis'. Its breeding tract lies in Chamba, Kangra, Mandi Kullu, Kinnaur and Lahaul & Spiti in HP extending to adjoining areas of J&K and Uttarakhand. Geographically the breeding tract lies in Western Himalayan ranges at latitude 31°06'–33°05'N and longitude 74°54'–78°10'E, at altitude varying from 500–3800 m above mean sea level.

Collection of data: The study was conducted in five adopted flocks in different migratory route in the Himalayan ranges of Himachal Pradesh, India. The reproductive data recording was done only for goats although the flocks were mixed with sheep and goat. All the animals were tagged and identified, and baseline data was generated for reproductive traits. After generation of baseline data, these flocks were monitored periodically over five years (2011–2016) for their reproductive performance and 89 samples were collected after screening of reproductive records from five adopted flocks from different migratory routes.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis: The genomic DNA was isolated by phenol-chloroform extraction procedure (Sambrook and Russel 2001). Primer used and PCR conditions for studying *KISS1* gene polymorphism are presented in Table 1. For amplification, 25 µl of PCR reaction was prepared by adding 10 pmole of each primer, 100 µM of each dNTPs, 1.5 mM MgCl₂, 10× PCR buffer, 100 ng DNA template and 0.5 Unit Taq DNA polymerase. The amplification was carried out using a thermal cycler (BioRad, USA) with the following conditions: initial denaturation of 5 min at 94°C followed by 30 cycles of denaturation at 94°C for 45 sec, annealing at 56°C for 30 sec and extension at 72°C for 45 sec, and lastly the final extension of 5 min at 72°C. The primers amplified 377 bp fragment from intron 1 of *KISS1* gene. *KISS1* polymorphism in goat populations was studied using PCR-RFLP using *XmnI* restriction enzyme. PCR product (10 µl) was subjected to overnight digestion with 1.5 U of restriction enzyme at 37°C. The restriction fragments were subjected to

electrophoresis in 2% agarose ethidium bromide gel in 1× TBE buffer. Gels were visualized under UV light and documented in GelDoc (Bio-Rad).

Genetic diversity analysis and test for Hardy-Weinberg equilibrium: Population genetic indices, gene and genotypic frequencies, gene heterozygosity (He), polymorphism information content (PIC), effective allele numbers (Ne) and inbreeding estimate (F_{IS}) were calculated using Popgene 32 (ver1.32), Microsoft Windows-based freeware for population genetic analysis (Yeh *et al.* 1999). Chi square (χ²) analysis was applied to test population for genetic equilibrium utilizing Popgene 32 software.

Association of *KISS1* gene polymorphism with litter size in Gaddi goats: The following fixed effects model was employed for analysis of litter size in Gaddi does and least squares mean was used for multiple comparison in litter size among different genotypes

$$Y_{ijklm} = \mu + H_i + LS_j + P_k + G_l + e_{ijklm}$$

where Y_{ijklm} , observed phenotypic value for litter size; μ , population mean; H_i , fixed effect of i th herd; LS_j , fixed effect of the j th kidding season ($j = 1, 2, 3, 4$); P_k , fixed effect of the k th parity ($k = 1, 2, 3$); G_l , fixed effect of the l th genotype ($l = 1, 2, 3$), and e_{ijklm} is the random residual effect of each observation. Analysis was done using the general linear model procedure of SAS (ver 9.3) (SAS Institute Inc, Cary, NC, USA). Mean separation procedures were conducted using a least significant difference test.

RESULTS AND DISCUSSION

Detection of PCR-RFLP genotypes: The amplified fragments obtained from all tested Gaddi goats were of 377 bp. Similar amplicon sizes were reported by An *et al.* (2012) in 3 Chinese goat breeds, Maitra *et al.* (2014) in 9 Indian goat breeds, Othman *et al.* (2015) in Egyptian small ruminant breeds and El-Tarabany *et al.* (2017) in Egyptian goat breeds. These PCR amplified fragments (377 bp) were digested with *XmnI* endonuclease. Depending on the presence or absence of restriction site (GAANN[^]NNTTC) (N = A or T or C or G) at position 121[^]122 different digestion patterns were observed. At *KISS1* locus, 3 different genotypes were observed, viz. AA with undigested one fragment at 377 bp, TT with two digested fragments at 256 and 121 bp, and AT with three fragments at 377, 256 and 121 bp. Similar to present investigation, 3 different digestion patterns were observed by An *et al.* (2015) in 3 Chinese goat breeds. Gupta *et al.* (2015) also observed 3 digestion patterns in Black Bengal goat. Othman *et al.* (2015) also reported similar digestion patterns in Egyptian small ruminant breeds. Similar to present investigation, polymorphism in *KISS1* gene was also reported by Feng *et al.* (2009) in Chinese goat breeds, Cao *et al.* (2010) in Jining Grey goat, Hou *et al.* (2011) in Chinese goat populations and Gupta *et al.* (2015) in Black Bengal goat.

Genetic diversity analysis and test for genetic equilibrium: The allele and genotypic frequencies and

Table 1. Primer used for amplification of the *KISS1* gene and PCR-RFLP reaction parameters

Gene (Mutation)	KISS1
Primers (5'–3')	FP CCCGCTGTAAGTAGAGAAAG RP CATCCAGGGTGAGTGATA CT
Temperature condition	56°C (30 cycles for 60 sec)
Restriction digest	<i>XmnI</i> restriction enzyme (37°C, overnight)
Expected product size (bp)	377 bp
Digestion fragments (bp)	377, 256 and 121 bp

various other measures of genetic diversity as observed for Gaddi goats are presented in Table 2. The frequencies of AA, AT and TT genotypes were 0.17, 0.52 and 0.31, respectively while the frequency of A and T alleles was 0.43 and 0.57. AT genotype was predominant genotype while AA genotype was having least frequency among the goats screened. An *et al.* (2013) reported that frequency of T allele ranged from 0.55 to 0.60 and frequency of A allele ranged from 0.40 to 0.45 in 3 tested Chinese goat breeds. Similar to present investigation, Gupta *et al.* (2015) in Black Bengal goat reported gene frequency for T and A allele as 0.61 and 0.39, with genotypic frequency of TT, TA and AA genotypes as 0.14, 0.50 and 0.36, respectively. Othman *et al.* (2015) observed frequencies of AT and TT genotypes as 0.55 and 0.45, respectively while allele frequency observed for A and T alleles were 0.27 and 0.73.

The observed allele number (No) and effective allele number (Ne) were 2 and 1.96, respectively. Observed heterozygosity (Hobs), expected heterozygosity (Hexp) and PIC values estimated were 0.52, 0.49 and 0.37, respectively in Gaddi goat breed. Hobs and PIC values indicated that sufficient genetic variation exist at the locus. The PIC value suggested the usefulness of marker since for bi-allelic locus maximum PIC value is 0.375. In study of An *et al.* (2013), observed heterozygosity (He), effective allele number (Ne) and PIC values were 0.48, 1.93 and 0.37, respectively, which were of similar magnitude as observed in present study. The F_{IS} estimate was observed as -0.06 indicating heterozygous excess at the studied locus. The test for genetic equilibrium was carried out by comparing observed genotypic frequencies with expected calculated from gene frequencies. Chi square (χ^2) analysis revealed that $\chi^2_{(cal)} < \chi^2_{(tab)}$ at 5% level of significance and 1 df indicating that screened population of Gaddi goats was found in Hardy-Weinberg Equilibrium (HWE). Maitra *et al.* (2014) also observed that Black Bengal goats were in state of HWE for same locus. El-Tarabany *et al.* (2017) in Egyptian small ruminant breeds reported that Damascus and Zaraibi goats were in a state of HWE, however, Baladi population was in disequilibrium ($P < 0.01$) for the T/A loci.

DNA sequencing and analysis: Representative samples (18) of different genotypes as revealed by PCR-RFLP were sequenced after purification of respective PCR product. Comparison of 18 representative sequences from Gaddi goats confirmed the presence of T/A substitution obtained by PCR-RFLP. Sequence has been deposited with gene bank

repository (Accession No. MH397145). The chromatogram representing the substitution is depicted in Fig.1. Earlier studies (Cao *et al.* 2010, Sharma *et al.* 2012, An *et al.* 2013, Maitra *et al.* 2014, Othman *et al.* 2015) also reported same SNP in KISS1 gene. Sequence alignment for wild type and mutant alleles corresponding to reference sequence retrieved from NCBI database using MegAlign programme of DNASTAR software confirmed presence of A/T base change at 125 bp (Fig. 1). Apart from this sequence analysis of representative samples also revealed 3 nucleotide changes at 56 bp (C/A), 197 bp (G/C), and 271 (T/C).

Effect of KISS1 gene polymorphism on litter size: The mean litter size belonging to AA, AT and TT genotypes was 1.12 ± 0.18 , 1.34 ± 0.11 and 1.73 ± 0.15 , respectively. Significant ($P < 0.05$) association of genotypes was observed with litter size. The study detected association between allele T in KISS1 gene and litter size. Earlier studies (Chu *et al.* 2012, An *et al.* 2013) indicated that KISS1 gene may have some association with prolificacy. An *et al.* (2015) studied the genetic polymorphism of KISS1 gene in 3 Chinese goat breeds and recorded the association of T-A substitution with the litter size. The frequency of T allele observed in present study was 0.52, which was consistent with earlier report of An *et al.* (2015) in different Chinese goat populations having frequency of T allele in the range of 0.55–0.60.

In present study, an important observation recorded was the presence of T allele in Gaddi goat breed which have been reported to be associated with greater litter size in Jining Grey (Cao *et al.* 2010), Boer (An *et al.* 2013) and some Indian goat breeds (Maitra *et al.* 2014). The significant association of TT genotype with superior litter size in Gaddi goats was also consistent with the previous results obtained by Hou *et al.* (2011) and An *et al.* (2013). Gupta *et al.* (2015) also observed that variations in KISS1 gene may play an

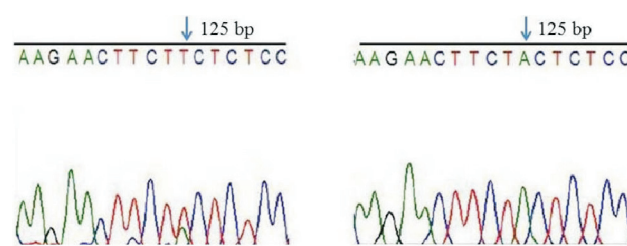


Fig. 1. Chromatogram representing T/A substitution in KISS1 gene in Gaddi goats.

Table 2. Measures of genetic diversity and test of equilibrium for KISS1 gene in Gaddi goats

Genotypic frequency		Allele frequency		Ne	H _{obs}	H _{exp}	PIC	F _{IS}	Chi-square
Genotype	Frequency	Allele	Frequency						
AA	0.17 (15)	A	0.43	1.96	0.52	0.49	0.37	-0.06	0.39 (P= 0.53 ^{NS}) (d.f=1)
AT	0.52 (46)	T	0.57						
TT	0.31 (28)								

No, observed no of allele; Ne, effective no of alleles; He, heterozygosity; PIC, polymorphic information content; FIS, fixation index.

Table 3. Least squares means and standard error for litter size of different genotypes of *KISS1* gene in Gaddi goats

Genotypes	Number	Litter size
AA	15	1.12±0.18 ^a
AT	46	1.34±0.11 ^b
TT	28	1.73±0.15 ^b

Means with different superscripts differ significantly ($P < 0.05$).

important role in litter size in native Black Bengal goats but further research on a large population will be required to confirm the linkage with increased prolificacy. El-Tarabany *et al.* (2017) reported significant association of TT genotypes with superior litter size in Damascus and Zaribi breeds and indicated that prolific TT genotype in both Damascus and Zaribi breeds had superior estradiol 17 β level at estrus phase and progesterone level at both early and mid-luteal phases of the estrous cycle.

In the present study, PCR-RFLP assay revealed polymorphism in the amplified product of the intron 1 of kisspeptin (*KISS1*) gene in migratory Gaddi goat population. DNA sequencing confirmed one nucleotide mutation (T125A) in intron 1 region of *KISS1* gene with allelic frequency of alleles A and B as 0.43 and 0.57, respectively. In present study, significant association had been observed with T allele for litter size in screened migratory Gaddi goats. Since the investigation is of preliminary nature therefore the study on additional data based on more number of animals in diversified flocks should be carried out for validation of the preliminary findings.

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