

Polymorphism of the growth hormone (*GH*) gene and its association with growth traits in the Kilakarsal breed of sheep

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ABSTRACT

The goal of this study was to investigate the single nucleotide polymorphism of the growth hormone gene and its association with growth traits in the Kilakarsal breed of sheep. Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) of the growth hormone gene (422 bp) with *Hae*III restriction enzyme revealed an A781G transition mutation at the exon 2 and the existence of two genotypes, viz., AA (366 bp and 56 bp) and AB (422 bp, 366 bp and 56 bp). The occurrence of the B allele is due to the A781G transition in the exon 2 of the growth hormone gene, which causes an amino acid change from Serine to Glycine. The genotype BB was absent in the Kilakarsal sheep population. The absence of the BB genotype at the A781G locus of the growth hormone gene in the Kilakarsal sheep population indicates that the natural selection process might be acting against this particular genotype through reduced viability or early embryonic death. Further, the statistical analysis revealed the significantly higher yearling weight of the AB than the AA genotype ($P=0.038$), and an appreciable difference of 1.40 kg in 9-month weight. The highly significant ($P<0.001$) Chi-square value (29.94) showed that the population is not the under Hardy-Weinberg equilibrium. This indicates that molecular markers associated with body weight should be explored for their use in marker assisted selection in Kilakarsal population.

Key words: Kilakarsal sheep; GH gene; polymorphism; lethal mutation; growth traits

Introduction

The efficiency of mutton sheep production enterprises can be improved by enhancing litter size and lamb weight (MONTOSI et al., 2013). In India, demand for meat products has increased

in the last two decades, and will continue to rise due to the increase in the human population (DEVI et al., 2014). With the development of molecular biology and biotechnology, scientists are able to

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achieve more accurate and efficient selection goals by marker-assisted selection (RAJCAN et al., 2011; COLLARD et al., 2008). The growth hormone is a peptide encoded by a single gene, consisting of five exons and four intervening introns. The growth hormone (GH) gene plays an important role in postnatal growth and development, tissue growth, lactation, reproduction, as well as protein, lipid and carbohydrate metabolism (AKERS 2006; AYUK et al., 2006). With its functional and positional potential, the growth hormone gene has been widely used as a marker in several livestock species, including cattle (BEAUCHEMIN et al., 2006; KATOH et al., 2008; SODHI et al., 2007; THOMAS et al., 2006; THOMAS et al., 2007), sheep (GORLOV et al., 2017; MARQUES et al., 2006) and goats (BOUTINAUD et al., 2003, MALVEIRO et al., 2001). Kilakarsal, a mutton type sheep breed of southern India, is characterized by medium size and dark tan coat dorsally, with black colouration in the ventral region. It is hardy, heat tolerant and has the capacity to utilize coarse feed materials efficiently. It has traits which are beneficial in drought prone areas (RAVIMURUGAN et al., 2012). The present study aimed to detect polymorphism of the growth hormone gene, and if any, its association with body weight at various ages, viz., birth, 3, 6, 9 months and yearling weights in Kilakarsal sheep.

Materials and methods

Blood sample collection and DNA isolation. Blood samples of the Kilakarsal (99 sheep) breed were collected from a sheep farm maintained at the Veterinary College and Research Institute, Tirunelveli, India. Genomic DNA was extracted using the standard Phenol-Chloroform extraction procedure (SAMBROOK et al., 1989) with slight modifications using a DNAzol reagent, instead of SDS and proteinase K. The growth traits considered were birth, 3, 6, 9 months and yearling weights. Data on all body weight traits were available for only 39 Kilakarsal sheep and were used in the association studies.

Primer synthesis and PCR-RFLP reactions. The published primer sequences were used for amplification of the growth hormone gene (HUA et al., 2009):

GH1-F 5'-CTCTGCCTGCCCTGGACT-3'

GH1-R 5'-GGAGAAGCAGAAGGCAACC-3'

PCR reactions were performed in a 20 µl mixture containing 10 pmol forward and reverse primers each, a 10 µl master mix which contained 200 M dNTP (deoxyribonucleotide phosphate), 1.5 mM MgCl₂, 1 unit of Taq-DNA polymerase (Sigma), and 50 ng genomic DNA as the template. Gradient PCR was used to optimize the reaction accuracy: 95 °C for 7 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 40 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 7 min. RFLP analysis was conducted to detect SNPs. The PCR amplicons were digested with *Hae*III restriction endonuclease and checked in 2% agarose gel with a 50 bp DNA ladder in a gel documentation system. The genotypes were assigned on the basis of the restriction digestion pattern of the PCR products.

Statistical analysis. The allele and genotype frequencies were calculated using the standard formula and the population was tested for the Hardy-Weinberg equilibrium using the Chi-square test (FALCONER and MACKAY 1996). Associations of the genotypes with growth traits were calculated by analyzing the variance of growth traits, viz. birth, 3, 6, 9 months and yearling weights. Data were grouped into two periods of births, viz. period 1 (2009-2010) and period 2 (2011-2012); two seasons of birth, viz. season 1 (Jan-May) and season 2 (July-Nov), and two genotypes at the A781G locus, viz. AA and AB. Sex was another non-genetic factor considered in the analysis. The data were corrected for the fixed effects, viz. period of birth, season of birth, genotypes, the sex of the animal, and the weight of the dam at mating, as covariables using least-squares procedures (HARVEY 1990) with the following model:

$$Y_{ijkl} = \mu + P_i + C_j + S_k + G_l + b(WM_{ijkl} - WM) + e_{ijkl}$$

where, Y_{ijkl} was the phenotypic value of traits; μ , the population mean; P_i was the fixed effect of the period of lambing; C_j was the fixed effect of the season of lambing; S_k was the fixed effect of sex; G_l was the fixed effect of genotype; $b(WM_{ijkl} - WM)$, regression of Y on the dam's weight at mating and e_{ijkl} , residual random error, NID (0, σ^2).

Results

The region of the growth hormone gene, which includes exon 2 (422 bp), was amplified from ovine genomic DNA. Restriction digestion of the amplified region by the endonuclease, *Hae*III with the recognition sequence – GG/CC, showed polymorphism, and revealed the existence of two genotypes, viz. AA (366 bp and 56 bp) and AB (422 bp, 366 bp and 56 bp), characterized by the presence of two alleles, namely A and B (Fig. 1).

The BB genotype was absent. The genotypic frequencies for AA and AB genotypes were 0.29 and 0.71, respectively. The allelic frequencies were 0.65 and 0.35 for the A and B alleles, respectively. This variation is due to the A781G transition of exon 2 of the growth hormone gene, which caused an amino acid change from Serine to Glycine. The highly significant ($P < 0.001$) Chi71 square value (29.94) showed that the population is not under the Hardy-Weinberg equilibrium.

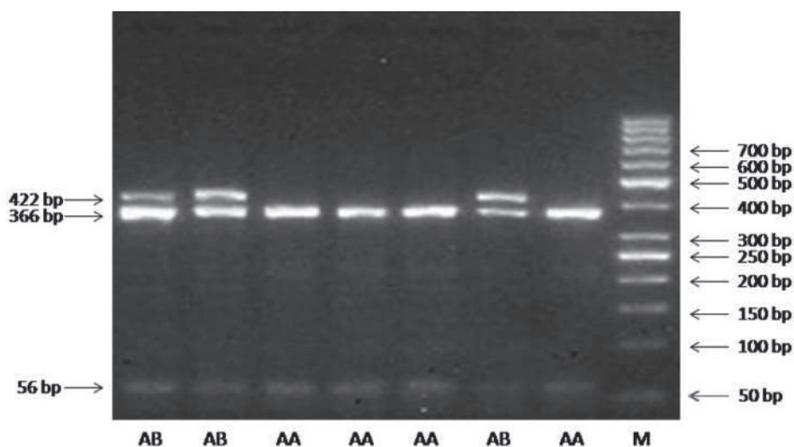


Fig. 1: Representative genotyping of the growth hormone gene at locus A781G by agarose gel electrophoresis. Strands with 366 and 56 for AA genotype, 422, 366 and 56 for AB genotype appeared at this locus. M represented a marker with 50 bp DNA ladder (50 bp, 100 bp, 150 bp, 200 bp, 250 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp).

The analysis revealed that animals with the AB genotype had significantly higher yearling weight than the AA genotype ($P=0.038$), and there was an appreciable difference of 1.40 kg in 9-month weight between AA and AB genotypes, though it was not

significant ($P=0.135$). There was no significant difference in mean birth weight, weaning weight (3-months), 6-month weight and 9-month weight between AA and AB genotypes (Table 1).

Table 1: The effect of genotypes at the A781G locus of the growth hormone (GH) gene on body weight in Kilakarsal sheep

Traits	Least squares means \pm S.E. of genotypes		P-value
	AA	AB	
Birth weight (kg)	2.68 \pm 0.08 ^a (14)	2.73 \pm 0.05 ^a (25)	0.553
Weaning weight (kg)	8.10 \pm 0.51 ^a (13)	8.21 \pm 0.33 ^a (25)	0.867
6-months weight (kg)	11.58 \pm 0.63 ^a (13)	11.80 \pm 0.41 ^a (25)	0.761

Table 1: The effect of genotypes at the A781G locus of the growth hormone (GH) gene on body weight in Kilakarsal sheep (continued)

Traits	Least squares means \pm S.E. of genotypes		P-value
	AA	AB	
9-months weight (kg)	13.30 \pm 0.80 ^a (12)	14.70 \pm 0.50 ^a (25)	0.135
12-months weight (kg)	16.72 \pm 0.57 ^a (12)	18.11 \pm 0.35 ^b (25)	0.038

Means in the same row bearing different superscripts differ significantly ($P \leq 0.05$)
 Figures in parentheses indicate the number of observations

Discussion

In this research, the growth hormone gene polymorphism was mapped in 99 Kilakarsal sheep down to the A781G transition. The BB genotype was absent in this study. Similar results were reported in Vembur sheep (n=112) (SEEVAGAN et al., 2015), and Kenguri sheep (n=60) (VAISHALI et al., 2017). KUMARI et al., (2014a) (n=364) also reported the absence of the BB genotype at the A781G locus in 9 Indian sheep breeds, but the author did not conclude anything about the lethal effect of this mutation. Similar results were reported in LuBei white goats (n=50) (LI et al., 2004), Chengdu-Ma (n=37) (BAI et al., 2005) and in Boer goats (n=154) (HUA et al., 2009). The recessive lethal gene causes death in a homozygous condition. The semi-dominant lethal gene causes death in the homozygous condition and phenotypic deformities in the heterozygous condition. In this research, heterozygous animals were phenotypically normal. The role of the *GH* gene in oogenesis, follicular development and embryogenesis was confirmed by earlier reports of SIROTKIN et al., (2003) and SILVA et al., (2009). Therefore, the 'B' allele at the A781G locus of the *GH* gene might be a recessive lethal allele. However, experimental research with more animals and other sheep breeds is required to confirm this hypothesis. The departure from Hardy-Weinberg equilibrium is also due to the complete absence of one genotype, possibly due to natural selection.

The growth hormone is released from the anterior lobe of the pituitary gland, where its main effects

are associated with the stimulation of the growth of bones and skeletal muscles, through the action of insulin-like growth factor (IGF-1) (AN et al., 2011). AKERS (2006) reported that high-yielding animals reveal greater GH levels in comparison to low-yielding ones. This study showed that animals with the AB genotype performed better compared to those with the AA genotype, as was also observed by HUA et al., (2009) who associated the AB genotype with higher weaning weight (80 days) and yearling weight in Boer goats. KUMARI et al., (2014b), while studying the GH gene loci A781G and A1575G in Avikalin (n=114) and Malpura (n=164) sheep breeds, reported that the ABCD haplotype individuals were about 19 to 46 % heavier compared to the AACC haplotype at different stages of growth. Similar results were reported by RAKESH et al., (2023) in Assam Hill and Sirohi goats, which indicated that the AB and CD genotypes displayed slightly higher values in most morphometric traits compared to the AA and CC genotypes at loci A781G and A1575G of the GH gene. However, VAISHALI et al., (2017) reported a non-significant difference between the AA and AB genotypes for body weight, height at wither, body length and chest girth, both in Kenguri sheep. THOMAS et al., (2007) also indicated that a heterozygous genotype might be advantageous for growth hormone gene polymorphism for traits of muscularity and adiposity in Brangus bulls.

Conclusions

The absence of the BB genotype at the A781G locus of the growth hormone gene in Kilakarsal sheep population indicates that the natural selection process might be acting against this particular genotype through reduced viability or early embryonic death. However, further experimental research is required to validate the role of the B allele as a recessive lethal allele. It is concluded that this mutation could serve as a molecular marker and thus can be used for MAS (marker-assisted selection) in Kilakarsal sheep population to avoid embryonic losses. The study shows that animals with the AB genotype performed better compared to those with the AA genotype. However, more extensive data are required to validate this association of the genotype AB with better performance than AA in Kilakarsal sheep. It was concluded that new molecular markers associated with ovine growth traits should be explored further for their potential use in MAS and more genetic improvement of the Kilakarsal population.

Conflict of Interest

The author(s) declare no potential conflicts of interest in the research, authorship, and/or publication of this article.

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SAŽETAK

Cilj ovog rada bio je istražiti polimorfizam pojedinačnog nukleotida gena hormona rasta i njegovu povezanost sa svojstvima rasta u ovaca pasmine Kilakarsal. Lančana reakcija polimerazom – polimorfizam duljine restrikcijskih fragmenata (PCR-RFLP) gena hormona rasta (422 bp) s restrikcijskim enzimom *HaeIII* otkrila je A781G mutaciju na eksonu 2 i postojanje dvaju genotipova: AA (366 bp i 56 bp) i AB (422 bp, 366 bp i 56 bp). Navedena mutacija, osim što uzrokuje pojavu alela B, uzrokuje i promjenu aminokiseline serin u aminokiselinu glicin. Genotip BB u istražene pasmine ovaca nije pronađen. Odsutnost genotipa BB na lokusu A781G gena hormona rasta u ovaca pasmine Kilakarsal upućuje na to da prirodni process selekcije može djelovati protiv tog određenog genotipa putem smanjene sposobnosti preživljavanja ili rane embrionalne smrti. Nadalje, statistička je analiza otkrila znakovito veću tjelesnu masu genotipa AB od genotipa AA ($P=0,038$) i znatnu razliku od 1,40 kg u 9-mjesečnoj tjelesnoj masi istraženih ovaca. Visoko znakovita vrijednost ($P<0,001$) hi-kvadratnog testa (29,94) pokazala je da ova populacija ovaca ne odstupa od Hardy-Weinbergova zakona ravnoteže. To nas upućuje na zaključak da treba istražiti molekularne biljege povezane s tjelesnom masom ovaca pasmine Kilakarsal radi njihove primjene u selekciji potpomognutoj biljezima.

Ključne riječi: ovce pasmine Kilakarsal; gen hormona rasta; polimorfizam; letalna mutacija; svojstva rasta
