RESEARCH ARTICLE

Genetic Polymorphism of Insulin-Like Growth Factor 1 and Prolactin Receptor Genes in Surti and Mehsana Goats by PCR-RFLP

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Abstract

The present study was planned with the objective to amplify 5'NCR Insulin-like growth factor 1 (IGF1) and 3'UTR Prolactin receptor (*PRLR*) genes using caprine specific primers by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) in Surti and Mehsana goats. IGF1 gene 5'NCR was found to be monomorphic on restriction digestion with *HaeIII*, which revealed only one genotype GG in both Surti and Mehsana goats. The allele frequency of G was found to be 1.00 in both the breeds. Restriction digestion of PRLR gene 443 bp fragment with AluI also showed monomorphic pattern. Only one genotype CC was found with an allele frequency of 1.0 in both the Surti and Mehsana goats.

Keywords: Goat, Insulin-like growth factor 1(IGF1), Prolactin receptor (PRLR), PCR-RFLP, Polymorphism.

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Introduction

India is a mega-biodiversity, contributing a proportionate share of livestock breeds to the world (Sadana and Pandey, 2004). Goat meat (chevon) is a good source of protein with low fat and cholesterol content, suitable for human consumption (Banskalieva *et al.,* 2000). India has 34 well defined breeds of goats out of which 5 breeds, Surti, Mehsana, Kutchi, Zalawadi and Gohilwadi are native to Gujarat (ICAR-NBAGR, 2021). The Surti and Mehsana goat breeds are kept for both milk and meat purpose which are found in two different parts of Gujarat, Surat and Mehsana, respectively.

Growth traits determine economic worth of the animal. Animal growth is controlled by Hypothalamic-pituitary-somatic (HPS) axis, which is responsible for the secretion of the growth hormone (GH) from the pituitary gland and stimulation of insulin-like growth factor 1 (IGF1) from the liver (Wang *et al.,* 2004). The IGF1 gene sequence in goats is located on chromosome 5 (Schibler *et al.,* 1998) and is composed of 6 exons. IGF1 aids in cell differentiation, embryogenesis, reproduction, foetal development, regulation of metabolism and growth (Adam *et al.,* 2000; Shen *et al.,* 2003). Due to the various roles of IGF1 gene in animal growth, it has been considered as a candidate marker associated with growth and carcass traits in various domestic livestock species.

IGF1 gene polymorphism have been reported in various goat and sheep breeds by various researchers including Jamunapari and Sirohi goats (Sharma *et al.,* 2013), Egyptian sheep and goat breeds (Othman *et al.,* 2016), Iranian Markhoz goat (Rasouli *et al.,* 2017), Malabari and Attappady Black goats (Naicy *et al.,* 2017), Assam Hill goat (Sarmah *et al.,* 2019), Indonesian Kejobong goat (Lestari *et al.,* 2020).

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Prolactin (PRL) is an anterior pituitary peptide hormone which regulates more than 300 activities in vertebrates, including endocrinology and metabolism, brain and behaviour, reproduction, immune regulation and protection (Bole-Feysot *et al.,* 1998; Binart *et al.,* 2010). Its lactotrophic potential is facilitated in combination with its receptor, PRLR is detected in various tissues including brain, ovary, placenta and uterus in several mammalian species. The PRLR gene was mapped to chromosome 20 in goats and 16 in sheep, and is composed of 10 exons (Lu *et al.,* 2011). PRLR is a strong candidate gene for milk production and reproduction (Parihar *et al.,* 2017).

Several PRLR gene polymorphism have been reported in different breeds, including Chinese Liaoning cashmere goat (Zhou *et al.,* 2011), Jining Grey goat (Ran *et al.,* 2011), Xinong

Saanen, Guanzhong, and Boer goat breeds (Hou *et al.,* 2014), Chinese Hu sheep (Wang *et al.,* 2015), Chinese Haimen goat (Dejun Ji *et al.,* 2016), Gaddi goat (Sankhyan *et al.,* 2019), Shaanbei white cashmere goat (Xin Feng Liu *et al.,* 2019).

The present study was undertaken to detect genetic polymorphism in 5'NCR of IGF1 gene and 3'UTR of PRLR gene in Surti and Mehsana goats.

Materials and Methods

Ethical Statement: Technical program was approved by Institutional Ethical Committee.

Genomic DNA Extraction

Blood samples were collected from 50 Surti goats maintained at Livestock Research Station, NAU, Navsari and 50 Mehsana goats maintained at Livestock Research Station, SDAU, Sardarkrushinagar, Gujarat, respectively. About 5 mL of the blood was collected from the jugular vein of each animal into sterile 10 mL vacutainer containing 0.5M EDTA as anti-coagulant and stored at -4°C till further processing. Genomic DNA was extracted from whole blood samples using the standard phenol-chloroform extraction method in the laboratory (Sigma and Himedia Ltd.) as described by John *et al.* (1991). Purity and DNA concentration was checked using Nanodrop spectrophotometer (ND-2000c) at optical density (OD) 260 nm and 280 nm. The quality of genomic DNA was checked by 0.8% agarose gel electrophoresis at 80 V for 60 min.

Polymerase Chain Reaction (PCR)

Oligo primers specific to caprine IGF1 gene as specified by Othman *et al.* (2016) and PRLR gene as specified by Hou *et al.* (2014) were synthesized and supplied by Eurofins Genomics and utilized to amplify the desired fragments in the present study (Table 1).

Table 1: Primer sequence, Region and PCR product size for IGF1 and PRLR genes

Gene	Primer sequence	Region	PCR product size
IGF1	F-5'TGAGGGGAGCCAATTACAAAGC3' R-5'CCGGGCATGAAGACACACACAT3'	5'NCR	294 bp
PRLR	F-5'AGTGAGAGTTATGGAAGGATG3' R-5'AAGGTTAAGCAACTGGTCTT3'	3'UTR	443 bp

PCR was performed in a total reaction volume of 20 µL consisting of 10 µL of 2X master mix (Takara), 2.5 µL genomic DNA (75 ng), 0.8 µL (8 pmole) of each forward and reverse primer and 5.9 µL of nuclease free water.

PCR amplification of 5'NCR of IGF1 gene was carried out with initial denaturation at 94°C for 5 min which was followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 64°C for 15 s and extension at 72°C for 15 s and final extension at 72°C for 5 min. Amplification of 3'UTR of PRLR gene consisted of similar protocol with annealing temperature as 54°C.

PCR products were analysed on 2% agarose gel electrophoresis for 60 min at 80 V. 50 bp DNA ladder (Qiagen) was used as a molecular size marker. The bands were visualized under UV light after staining with ethidium bromide and documented by gel documentation system (Alpha Imager HP).

Restriction Fragment Length Polymorphism (RFLP)

The Restriction Enzymes used in the study were procured from Takara Bio Inc and Genei Laboratories Private Limited. Restriction digestion of the amplified PCR products were carried out in a total reaction volume of 20 µL containing 10 µL PCR products, 2 µL 10X RE buffer, 7.7 µL nuclease free water and 0.3 µL restriction enzyme. Incubation and inactivation protocol of PCR products for restriction enzymatic digestion is presented in Table 2. Digested products were run on 2% agarose gel in 0.5X TBE buffer for 70 min at 80 V, visualized under UV light and photographed by gel documentation system. 50 bp DNA ladder (Qiagen) was used as a molecular size marker.

Table 2: Protocol for restriction enzymatic digestion of PCR products

Restriction enzyme	Recognition Site	Sample	Incubation	Inactivation
HaeIII	5'...GG▼CC...3' 3'...CC▲GG...5'	IGF1(294 bp)	37° for 1 h	80°C 10 min
AluI	5'...AG▼CT...3' 3'...TC▲GA...5'	PRLR (443 bp)	37° for 1 h	80°C 10 min

Results and Discussion

On amplification of 5'NCR of IGF1gene and 3'UTR of PRLR gene, PCR products of 294 and 443 bp, respectively were observed in both the breeds (Plates 1, 2, 3 and 4).

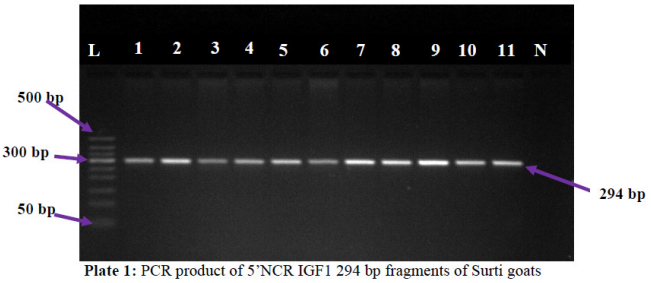

Plate 1: PCR product of 5'NCR IGF1 294 bp fragments of Surti goats

Lane-50 bp Ladder, 1 to 11-294 bp PCR products, N-Negative control

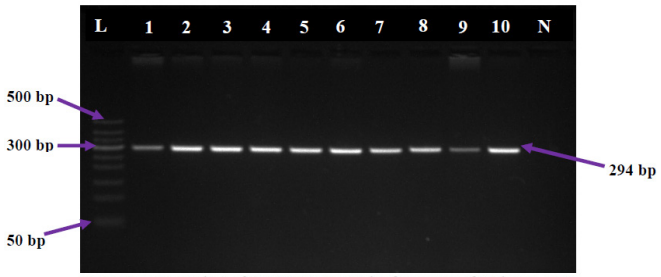

Plate 2: PCR product of 5'NCR IGF1 294 bp fragments of Mehsana goats

Lane-50 bp Ladder, 1 to 10-294 bp PCR products, N-Negative control

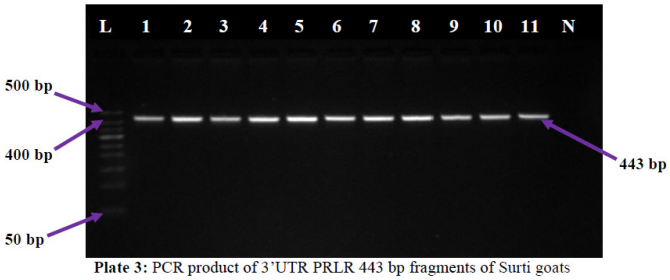

Plate 3: PCR product of 3'UTR PRLR 443 bp fragments of Surti goats

Lane-50 bp Ladder, 1 to 11-443 bp PCR products, N-Negative control

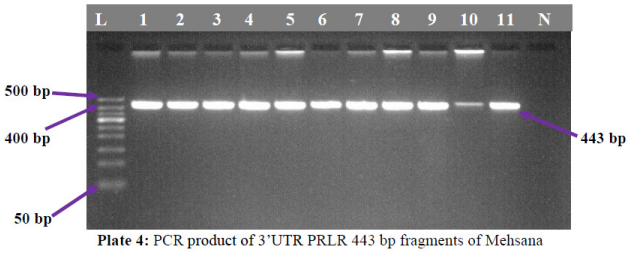

Plate 4: PCR product of 3'UTR PRLR 443 bp fragments of Mehsana goats

Lane-50 bp Ladder, 1 to 11-443 bp PCR products, N-Negative control

Restriction digestion of 294 bp 5'NCR of IGF1 gene with *HaeIII* revealed only one genotype GG (Plate 5 and 6). The frequency of genotype GG and allele G were observed 1.0 each in both the Surti and Mehsana goats. Therefore, 5'NCR of IGF1 gene was found to be monomorphic in both the breeds of goats. Similar results were obtained for restriction digestion of PRLR gene 443 bp (3'UTR) fragment with AluI showing monomorphic pattern (Plate 7 and 8). Only one genotype CC was found with an allele frequency of 1.0 in both the Surti and Mehsana goats.

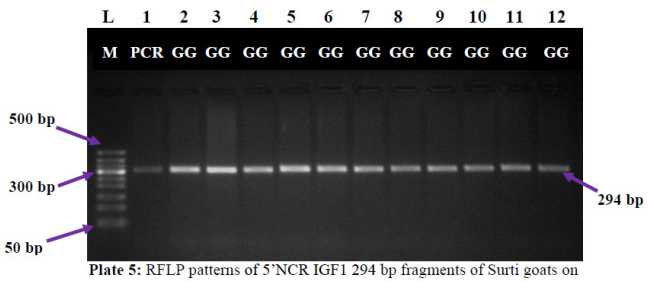

Plate 5: RFLP patterns of 5'NCR IGF1 294 bp fragments of Surti goats on digestion with HaeIII

Lane-50 bp Ladder, 2 to 12-294 bp RFLP products, 1-PCR product

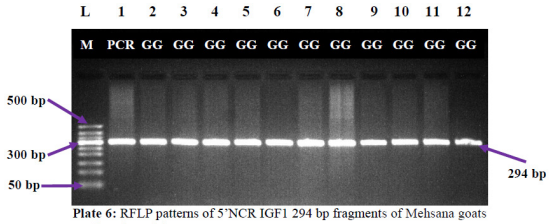

Plate 6: RFLP patterns of 5'NCR IGF1 294 bp fragments of Mehsana goats on digestion with HaeIII

Lane-50 bp Ladder, 2 to 12-294 bp RFLP products, 1-PCR product

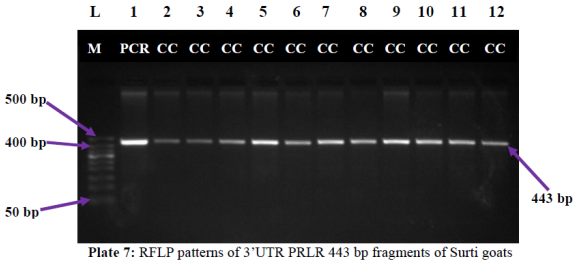

Plate 7: RFLP patterns of 3'UTR PRLR 443 bp fragments of Surti goats on digestion with AluI

Lane-50 bp Ladder, 2 to 12-443 bp RFLP products, 1-PCR product

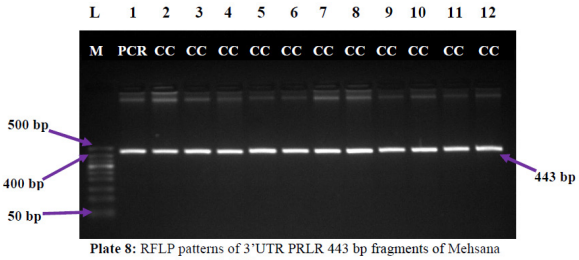

Plate 8: RFLP patterns of 3'UTR PRLR 443 bp fragments of Mehsana goats on digestion with AluI

Lane-50 bp Ladder, 2 to 12-443 bp RFLP products, 1-PCR product

The RFLP pattern for 5'NCR of IGF1 gene was not in agreement with Othman *et al.* (2016) who reported three genotypes; GG (234, 48 and 38 bp); CG (272, 234, 48 and 38 bp) and CC (272 and 48 bp) in Egyptian sheep and goat breeds. The genotype frequencies for GG, CG and CC were 0.55, 0.29 and 0.16, respectively. The allelic frequencies for G and C were 0.70 and 0.30, respectively. Naicy *et al.* (2017) also reported two genotypes GG (96 bp, 72 bp) and AG (268 bp, 196 bp, 72 bp) in two native goat breeds of Kerala. The genotype frequencies for GG and AG were 0.62 and 0.38, respectively. The allelic frequencies for G and A were 0.81 and 0.19, respectively.

Similarly, the RFLP pattern for 3'UTR of PRLR gene was not in agreement with Hou *et al.* (2014) who reported two RFLP bands of 443 and 383 bp in three goat breeds of China. They reported two genotypes CC and CT and their genotype frequencies were 0.67 and 0.33, respectively. The allelic frequencies for C and T were 0.83 and 0.17, respectively. Zhou *et al.* (2011) also reported results in Liaoning cashmere goats of China with two fragments 136 and 26 bp. They reported genotypes CC and CT and the genotype frequencies were 0.86 and 0.14, respectively. The allelic frequencies for C and T were 0.93 and 0.07, respectively.

Conclusions

In the present study, regions of IGF1 294 bp (5'NCR) and PRLR 443 bp (3'UTR) successfully amplified with caprine specific

primers. Absence of polymorphism regions of IGF1 (5'NCR) and PRLR (3'UTR) alleles at both the loci was observed in Surti and Mehsana goats breeds. Both these goats breed populations were observed to be fixed for the selected regions of the IGF1 (5'NCR) and PRLR (3'UTR) which reveals substantial conservation of genome organization among the higher vertebrates.

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