

Investigation of Genetic Variation in Bmp4 Gene in Local Indigenous and Jamnapari Crossbred Goats in Damana Veterinary Service Division Sri Lanka

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Introduction

Small ruminants, such as goats (*Capra hircus*), constitute an important livestock resource in most countries and are essential for the livelihood of many farmers (Baker *et al.*, 2003). Application of molecular genetics approaches for the genetic progress of quantitative economic traits such as growth and reproduction in goats is an effective way of increasing their production as these methods could lead to finding of genetic markers useful for improved selection. Molecular genetics approaches have been used in the world for goat production in the recent past, and these strategies are yet to be established in Sri Lanka since they require high knowledge and capital investments. Therefore, this study was conducted as a preliminary step for the application of molecular genetics approaches in selection of goats for improved production in Sri Lanka. Single Stranded confirmation Polymorphism (SSCP) analysis is one such powerful genetic screening method to identify the sequence variation in Polymerase Chain Reaction (PCR) amplified products. In the present study, we investigated the PCR-SSCP genetic variation in the intron 2 of Bone Morphogenetic Protein 4 (BMP4) gene, which plays a major role in growth and reproduction. The study was focused on Local types (LT) and Jamnapari crossbred (JC) goats in Damana Veterinary Service (VS) division in the Ampara district of Sri Lanka.

Materials and methods

Venous jugular blood samples were collected from total of 72, LT (18) and JC (54) goats in 10 farms from the Damana VS division of the Ampara district. Genomic DNA was extracted using QIAamp DNA Blood Mini Kit and target region was amplified using previously published (Fang *et al.* 2009) forward (5'-CTGGGGAAATGTTTGGTA 3') and reverse (5'-GCTAAGAGTTG GGTGATGAG 3') primers. The PCR cycling protocol was 3 min at 94°C, 40 cycles of 94°C for 45 sec, 49°C annealing for 45 sec, 72°C for 1 min, with a final extension at 72°C for 30 min. SSCP method was used to investigate different conformation pattern in the BMP4 gene with single stranded fragment movements. Aliquots of 3.5 µl PCR products were mixed with equal volume of loading solution (95% formamide deionized, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 4 min at 100°C and chilled in ice immediately. Denatured DNA was subjected to 12% PAGE (polyacrylamide gel electrophoresis) in 1X TBE buffer at a constant temperature of 4°C. Amplified fragments were separated initially at 300V for 5 min followed by 130V, 5W and 6mA current for 18 hours. At the end of the electrophoresis gels were stained with 0.1% silver nitrate. The DNA banding patterns were observed, recorded and photographed with GeneSys gel documentation system (Syngene). Frequencies of each conformational pattern were calculated for both LT and JC animals separately.

Results

Polymorphisms were detected in intron 2 region of BMP4 gene in both LT and JC goats in Damana VS division. Altogether three different conformation patterns were observed and the three patterns were designated as A, B and C (figure 1).

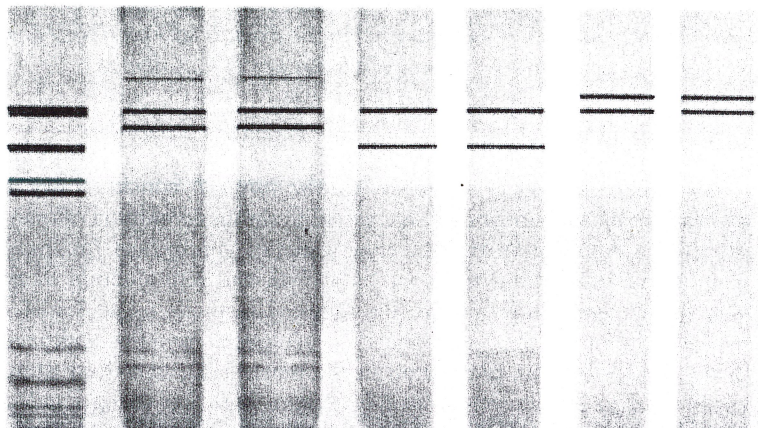


Figure 1: The electrophoresis patterns of PCR-SSCP for intron 2 of goat BMP4 gene. English letter corresponds to the conformational pattern. First lane indicates the 1kbp size standard. The area demarcated by line represents the schematic view of each conformational pattern.

All the three conformational patterns (A, B and C) were found in both Local Indigenous and Jamnapari crossbred animals. Pattern A was predominantly found in both local indigenous (66.67%) and Jamnapari crosses (72.22%) in all the ten farms. Next to pattern A, pattern B has the highest frequency, whereas pattern C was found to be at lowest frequency in both breeds (Table 1).

Table 1: Frequency distribution of each conformational pattern resulted in PCR-SSCP of intron 2 of goat BMP4 gene

Breed	Frequency of Conformational Patterns		
	Pattern A	Pattern B	Pattern C
Locals	66.67%	27.78%	5.56%
Jamnapari Cross	72.22%	18.52%	9.26%
Total frequency of each pattern	70.83%	20.833%	8.33%

Discussion

This study was carried out as a part of a detailed study aiming at genetic characterization of indigenous goats in Sri Lanka for economically important traits, mainly growth and reproduction using molecular markers. Several studies have been conducted on BMP4 gene in different species including human, mice and bovine worldwide (Fang *et al.*, 2008), and such previous studies conducted in China (Fang *et al.*, 2009 and Chu *et al.*, 2010), has described three conformational patterns (genotypes) with two alleles for the same gene fragment in three goat breeds for growth and reproduction traits. Similarly gene sequencing of the three observed conformational patterns to identify the genotypes and the alleles present is being pursued in order to determine whether any of the three conformational patterns observed in the study are corresponding to the previously reported polymorphism.

According to data obtain from the study, patterns A and B were shown by both local and Jamnapari crossbred goats. But pattern C was mainly shown by Jamnapari crosses, except one local goat in farm 4. But pattern C was not identified in farm 10 where only local goats are being reared. Since pattern C cannot be seen in animals from farm 10 and as it was mainly seen in Jamnapari crosses, we can predict that this pattern may be inheriting from Jamnapari goats rather than locals. Same time we can predict that the local goat that showed pattern C in farm 4, may be a Jamnapari cross where Jamnapari characteristics are not well expressed. But to obtain accurate results and to prove these predictions, we need to sequence the three patterns. However, these results and conclusions should be considered as preliminary ones, and further investigations will be essential for detecting the polymorphism of this gene in all part of the country.

Conclusions

Based on results obtained, it can be concluded that goats in the study area are polymorphic for the intron 2 of BMP4 gene and possess at least three genotypes, alleles or allelic groups. However these conclusions were preliminary and sequence variation based on Single Nucleotide Polymorphisms (SNPs) of these populations is yet to be analyzed.

References

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