GENETIC POLYMORPHISM OF THE GONADOTROPIN RELEASING HORMONE RECEPTOR GENE IN NON-DESCRIPTIVE LOCAL GOATS IN SRI LANKA

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ABSTRACT

In the present study genetic polymorphism in exon 1 of Gonadotropin Releasing Hormone Receptor (GnRHR) gene was analyzed as a candidate gene for prolificacy in non-descriptive local goats in Sri Lanka. Three Single Nucleotide Polymorphisms (SNPs)- A730G, G757A and G891T (Arg/Met) were identified by DNA sequencing for the first time in Sri Lanka. All three polymorphic sites were heterozygous and dominated by homozygous genotypes (GG and AA). At the G757A and G891T sites GG genotype and at the A730G site AA genotype were dominant with the frequencies of 0.77, 0.80 and 0.87 respectively. Two of the SNPs recorded in the study are silent mutations (A730G and G757A) and the other one is responsible for amino acid change from Arg to Met. We are reporting the mutation at the A730G polymorphic site in the GnRHR gene for the first time among all goat breeds of the world. Results of the present study will be extremely important for developing markers to improve the litter size of local goats.

Keywords: GnRHR Gene, Non-Descriptive Goats, SNPs

INTRODUCTION

Sri Lankan goat population mainly entailed with locally adapted animals and their crosses with exotic breeds. Locally adapted goats are found in dry zone of the country and are mainly composed of non-descriptive type goats. These animals are characterized by small body size and low performances. Records on fecundity of locally adapted animals are precious as they have both conservation and economic importance. GnRHR gene has been studied as a major candidate gene for litter size in cattle (Yang *et al*, 2011), goat (Yang *et al*) and sheep (Jie*et al*, 2008) and the trait is known to significantly influence by polymorphism at various sites. However, currently we have extremely limited amount of records on genetic polymorphism in local goats (Lokugalappatti*et al*, 2011) and this study was intended to provide information on SNPs in GnRHR gene (exon 1) for developing markers to utilize in future assisted selection programs.

METHODOLOGY

A sample of 30 goats representing phenotypically nondescript animals from Eastern, North western and Southern provinces of Sri Lanka was included in this study. Approximately 5ml of blood was collected from each animal to sterile vacutainer tubes containing 100 µg/ml Ethylenediamminetetraacetic Acid (EDTA) using jugular puncture method and genomic DNA was extracted using a commercially available kit (Wizard Genomic DNA Purification Kit). Extracted DNA was quantified using a DNA spectrophotometer (BIOMATE 3).Exon 1 of Caprine GnRHR gene with a target of 746bp fragment (683bp- 1429bp)was amplified using a pair of primers (F'-5'-CCAGTGGTTATGACACACAAGC-3' and R'-5'-TCCAGAATCTTCTTGACACACA-3'). PCR was performed in a 25 µl reaction mixture containing 50 ng genomic DNA, 0.5

µM of each primer, 1× PCR reaction buffer, 1.5 mM of MgCl2, 200 µM of dNTPs and 0.625 units of Taq DNA polymerase. The thermal cycling protocol was 4 min at 95°C, 32 cycles of denaturation at 94°C for 30 s, annealing at 62.0°C for 40 s, extension at 72°C for 40 s, with a final extension at 72°C for 10 min and amplifications were carried out in an ABI2720 (Applied Biosystems®) Thermal Cycler. Amplified PCR products were then visualized on 1% Agarose gel stained with Ethidium Bromide (1 µg/ml) and verified using a 100 bp ladder (Vivantis 6x).All 30 samples were sequenced for screening genetic variations using Bigdye terminator chemistry in an ABI 3500 DNA analyzer available at Faculty of Science, University of Peradeniya.

Sequence Data Analysis

Sequenced data were initially checked against caprine genome sequences already published in Genbank and poor allelic phase estimates were excluded from the analysis. Multiple sequence alignment of sequences were performed, bases ambiguities were visualized and manually edited using Codon Code Aligner 5.1.5.Polymorphic sites were visualized with the help of Sequencher (v 5.3) and DNA sequences were translated to amino acid sequences using ExPASy translate tool. Haplotype list was inferred and their frequencies were calculated manually.

RESULTS AND DISCUSSION

The region of the GnRHR gene was amplified as expected (*figure 1*). GnRHR exon 1 region from 30 goat samples was analyzed and three polymorphic sites were identified in non-descriptive local goats. All three polymorphic sites were heterozygous and two of the sites were represented by G/A and the other was G/T alleles (*Table 1*).

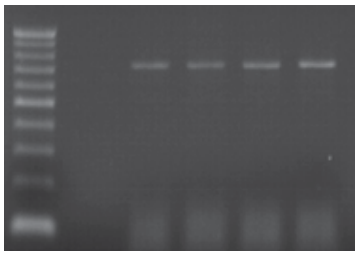

Figure 1.Electrophorogram of the Amplified GnRHR Gene Fragment by PCR and Visualized on 1% Agarose Gel (w/v). The Strands with 746 bp (Lanes 3-6) Show the Desired Fragment and M Indicates the Marker with 100 bp DNA Ladder

Heterozygous genotypes found at the polymorphic sites were represented by a minor population of goats (0.13,0.23 and 0.20 at AG, GA and TG positions respectively) while the majority of animals were represented by homozygous genotype (0.87, 0.77 and 0.80 at AA, GG and GG genotypes respectively). At both G757A and G891T loci G was the predominant allele whereas at locus A730G, A was predominant and there frequencies were 0.88, 0.90, 0.93 respectively. The most commonest haplotype AA,GG,GG was represented by 14 goats out of 30 (57%). There were two haplotypes (AA,A/T,G/T and A/T,GG,GG) with similar frequency of occurrence in this population represented the scarcest combination (3%). Among possible allelic recombination, AGG (76%) and ATT (1%) were the respectively highest and lowest documented recombination from the goat population analyzed in this study (*Table 1*).

Analysis of the deduced amino acid sequences in A730G and G757A sites showed presence of silent mutations as they were positioned at the end of each codon. However, the reported G891T variation is responsible for codon changes from Arg to Met (*Table 2*).

Table 1: The Allelic and Genotypic Frequencies, Recombined Genotypic and Haplotypic Frequencies for the Polymorphism of GnRHR Gene in Nnon-Descriptive Goats in Sri Lanka

Locus	Genotype	Genotype Frequency	Allele/Haplotype Frequency
A730G	AA (26)	0.87	A 0.93
	AG (04)	0.13	G 0.07
G757A	GG (23)	0.77	G 0.88
	GA (07)	0.23	A 0.12
G891T	GG (24)	0.80	G 0.90
	GT (06)	0.20	T 0.10
Haplotypes	AA,A/G,GG (07)	0.24	AAG0.13
	AA,GG,GG (14)	0.57	AGG0.76
	A/G,GG,G/T (04)	0.13	AGT0.06
	AA,A/T,G/T (01)	0.03	AAT0.02
	A/T,GG,GG (01)	0.03	ATG0.02
			ATT0.01

Studies by Yang and others (Yang *et al*, 2011) have reported the same two SNPs at G757A and G891T. Further, they have found GG genotype as the predominant genotype at above two loci (0.848, 0.902 respectively) in Boer goats which showed a significantly positive correlation with the litter size at G757A position. The present study is consisted with the findings of Yang *et al* by demonstrating the GG genotype (at G891T andG757A) and not reporting the TT genotypes at G891T sites. The absence of the genotypes may be due to the negative correlation with reproduction traits and elimination by consecutive artificial selection. Other than the two previously identified SNPs our study reports a naïve SNP at A730G and was a synonymous mutation. Even silent mutations can have positive effects on prolificacy effecting the GnRHR gene expression which can result in increase or decrease of gonadotrophins concentration there by, changing the density of GnRHR on the gonadotropes (Schubert *et al*., 2000). Further, silent mutations which are closely adjoined with non-synonymous sequences can be directly used to develop makers for marker assisted selection. A more reliable link between the non-synonymous mutation reported in this study and its effects on fecundity parameters can be found through an association study along with phenotypic data.

Table 2: DNA Sequence Variations at Polymorphic Sites and Corresponding Protein Sequence Variation in Non-Descriptive Goats in Sri Lanka

Locus	Variation in DNA Sequence	Protein Sequence
A730G	AAT,GAA,AAC,CAC,TGT,TCA,GCA,ATC or AAT,GAA,AAC,CAC,TGT,TCA,GCG,ATC	Asn,Glu,Asn,His,Cys,Ser,Ala,Ile Asn,Glu,Asn,His,Cys,Ser,Ala,Ile
G757A	AGC,AGC,ATC,CTA,CTA,ACA,CCG,GGC or AGC,AGC,ATC,CTA,CTA,ACA,CCA,GGC	Ser,Ser,Ile,Leu,Leu,Thr,Pro,Gly Ser,Ser,Ile,Leu,Leu,Thr,Pro,Gly
G891T	ACT,CAG,AGG,AAA,GAG,AAG,AGG,AAA or ACT,CAG,AGG,AAA,GAG,AAG,ATG,AAA	Thr,Gln,Arg,Lys,Glu,Lys,Arg,Lys Thr,Gln,Arg,Lys,Glu,Lys,Met,Lys

CONCLUSION

Our results identified three new polymorphic sites (G757A, G891T, and A730G) in GnRHR gene for non-descriptive local goats in Sri Lanka. Two of which were previously reported (G757A and G891T) by other researchers and we found a novel site which not has been previously reported for any goat breed (A730G).However, a study on larger sample for many generations along with phenotypic characters will reveal more reliable association between reported SNPs and reproductive traits.

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