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Single nucleotide polymorphism of candidate genes in non-descript local goats of Sri Lanka

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ABSTRACT

In the present study, genetic polymorphism in exon 4 of kappa casein (*k-CSN3*), exon 2–3 of alpha lactalbumin (*LALBA*) and exon 1 of gonadotropin releasing hormone receptor (*GnRHR*) genes were analyzed as candidate genes for milk production, milk quality and prolificacy in non-descriptive local goats in Sri Lanka. Altogether eleven, one and three single nucleotide polymorphisms (SNPs) were identified in *k-CSN3, LALBA* and *GnRHR* gene fragments, respectively utilizing the DNA sequencing technique for the first time in Sri Lanka. Seven polymorphic sites out of eleven in *k-CSN3* gene fragment and the recorded variable site in *LALBA* gene fragment were homozygous while all three polymorphic sites in *GnRHR* gene fragment were heterozygous. Two of the SNPs recorded in the present study are known to be unique for Sri Lankan non-descript goat population at G203T and A730G in *k-CSN3* and *GnRHR* genes, respectively. The study records another two SNPs in *GnRHR* gene, which are already known to be correlated with higher fecundity in goats (G757A and G891T). Results of the present study will be extremely important in future attempts to develop markers to improve the milk production, milk composition and litter size of non-descript local goats in Sri Lanka.

Key words: DNA sequencing, *GnRHR*, *k-CSN3, LALBA,* Non-descript local goats, SNPs

Sri Lankan non-descript local goats are highly localized, heterogeneous group which covers the majority of the goat population in the country (National Livestock Breeding Policy 2010) and are currently known to be under the threat of extinction due to intensive cross breeding with exotic breeds (Silva 2010). These non-descript goats are much more important at local circumstances as they bear invaluable genetic resources for high adaptability to local environmental conditions, disease resistance (Kirishnasanth 2013) and a high degree of prolificacy (National Livestock Breeding Policy 2010) though, they are poor in production parameters compared to exotic breeds (Silva 2010). Low performance in production of non-descript goats may be the result of poor management practices in rural areas and genetic makeup of animals (Azevedo *et al.* 1994). However, SNPs in production related genes affect significantly on the phenotypic characters, though they arise at a lower rate in a population (Cargill *et al.* 1999) and production parameters can be greatly enhanced at a minor expense by stimulating these mutations. Consequently, modern researchers are at an inspired interest on association analysis of SNPs and related phenotypic characters such as milk, meat and wool production and prolificacy traits. *k-CSN3* and *LALBA* genes are milk trait related and *GnRHR* is a fecundity trait related gene which are collectively widely studied genes for genetic variability. Many researchers have reported the association between *k-CSN3* genetic variability and milk composition (Chiatti *et al.* 2005, Marletta *et al.* 2007) and milk production (Marletta *et al.* 2007). Further studies have revealed the association between *LALBA* gene polymorphism and nutrient content of milk (Lan 2007, An 2009, Zidi 2014) and relationship between genetic variability of *GnRHR* gene and prolificacy trait (Ming-Xing *et al.* 2009, Yang *et al.* 2011). The inclusive objective of this study was to identify SNPs in Caprine *k-CSN3*, *LALBA* and *GnRHR* genes aiming to genetically characterize non-descript local goats of Sri Lanka, thereby to provide genetic clues for future association analysis.

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MATERIALS AND METHODS

Animals, sample collection and DNA extraction: A sample of 112 non-descript local goats from Eastern (49), Northern (15), Northwestern (37), and Southern (11) provinces of Sri Lanka was used for the study. Blood was collected from animals into sterile vacutainer tubes containing 100 μg/ml Ethylene Diammine Tetraacetic Acid (EDTA) using jugular puncture method. DNA extraction from collected blood samples was done using salting-out protocol (Jawdat *et al.* 2011) and commercially available kits (DNA Purification Kit). Extracted genomic DNA was visualized on Ethidium bromide stained 1% agarose gels

and quantified using a DNA spectrophotometer.

PCR amplification and purification of PCR products: The gene fragments focused in this study include exon 4 of Caprine *k-CSN3* (458 bp), exon 2–3 of *LALBA* (667 bp) and exon 1 of *GnRHR* (746 bp) genes and PCR was performed as described by Kiplagat *et al.* (2010), Jain *et al.* (2009) and Yang *et al.* (2011), respectively. The PCR was carried out in the presence of sense and antisense primers of *k-CSN3* (F'- 5'-TATGTGCTGAGTAGGTATCC-3' and R' 5'-TTGTCCTCTTTGATGTCTCC-3'), *LALBA* (F'- 5'-CCAGTGGTTATGACACACAAGC-3' and R'-5' TCCAG AATCTTCTTGACACACA-3') and *GnRHR* (F'- 5' CTCGTTCGCTTTAGCACCC-3' and R'-5'-CTGTGGT CCAGCAAAGATG-3') and 20, 61 and 30 samples were analyzed for each gene, respectively. The reaction was done in a 25 µl reaction mixture containing 50 ng genomic DNA, 0.5 µM of each primer, 10× PCR reaction buffer, 1.5 mM of $MgCl_2$, 200 µM of dNTPs, 0.625 units of *Taq* DNA polymerase and water. Amplification of all three genes were carried out in a 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. Following the visualization, interested PCR band was excised out from the agarose gel and PCR product was purified using PCR gel clean-up kit and quantified.

Sequencing of PCR amplification product: Amplified products of all 112 samples were sequenced for screening genetic variations using the same primers used for PCR. Sequencing reaction was performed utilizing Bigdye terminator chemistry in a genetic analyzer.

Sequence data analysis: All sequences were aligned, base ambiguities were visualized and edited manually using CodonCode Aligner 5.1.5 and poor allelic phase estimates were excluded from the analysis. Polymorphic sites were visualized using Sequencher (Gene Codes Corp. v 5.3). Haplotype list was inferred through visual observations and their frequencies were calculated with the aid of MS Excel. DNA sequences were translated to amino acid sequences using ExPASy translate tool (Gasteiger *et al.* 2003).

RESULTS AND DISCUSSION

The specific primers amplified *k-CSN3* exon 4 (60 bp - 518 bp) (reference to GenBank accession no X60763), exon 2–3 region of *LALBA* including exon 2 (1327–2060 bp), intervening intron region (1416–1890 bp) and a part of exon 3 (1891–2060 bp) (reference to GenBank accession no M63868) and *GnRHR* exon 1 (458 bp-1204 bp) (reference to GenBank accession no L42937.1). Analysis of sequence data revealed the presence of one, four and eleven SNPs in analyzed *LALBA*, *GnRHR* and *K-CSN3* gene fragments respectively.

Previous studies have revealed the presence of 16 polymorphic sites within the complete exon 4 of *k-CSN3* gene in domestic goats which comprises 13 protein variants and 3 synonymous mutations (Prinzenberg *et al.* 2005). In this study, we recorded 11 polymorphic sites in *k-CSN3* exon 4 gene fragment with 9 non-synonymous variations. Only 4 sites out of 11 were heterozygous (Fig. 2) while 7 sites were homozygous (Table 1). Even at the heterozygous sites, the dominant genotype was represented by homozygous genotype and a minority of the population was represented by the heterozygous genotype. All three genotypes (GG, AA, AG) of *k-CSN3* gene were observed at A274G and G309A variable sites, whereas at A471G and T591C sites only two genotypes were found (Table 2).

Within the *k-CSN3* gene fragment studied, altogether nine haplotypes were recorded and the most frequently found haplotype at heterozygous polymorphic sites was GG/GG/AA/TT (35%) followed by AG/GG/AA/TT (15%) and AA/GG/AA/TT (15%). Altogether, those three genotypes covered up to 65% of the variability in the whole population and the remaining 35% of variability divided among other six haplotypes. According to recorded variability, 10 possible allelic combinations were speculated and among them GGAT was the dominant haplotype at a frequency of 0.46875 and second most common haplotype was AGAT

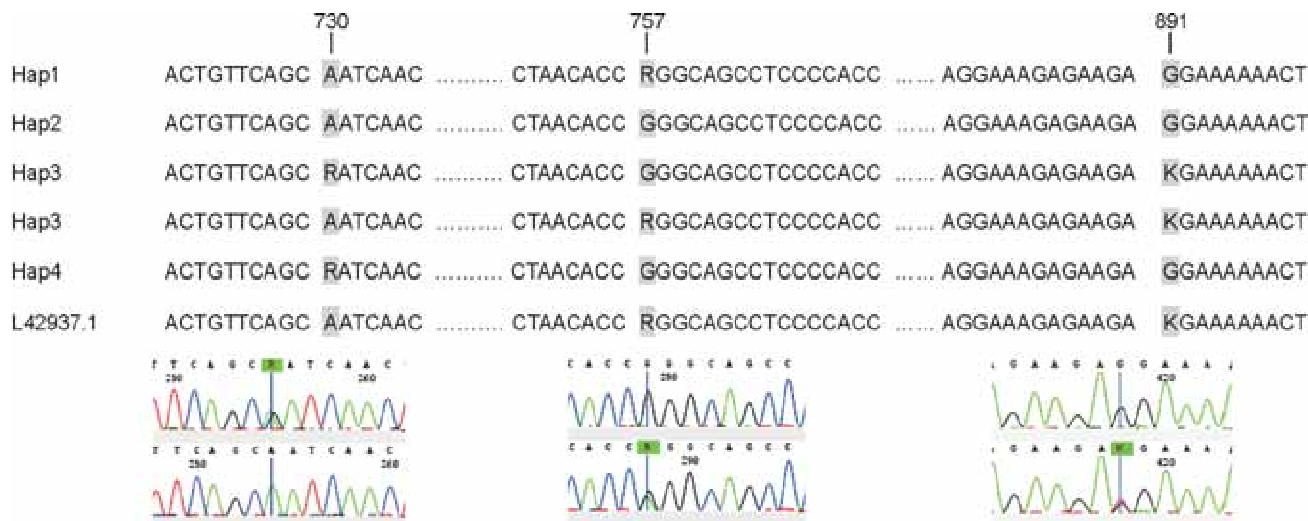

Fig. 1. Sequence alignment of *GnRHR* gene fragment compared to GenBank accession no. L42937.1

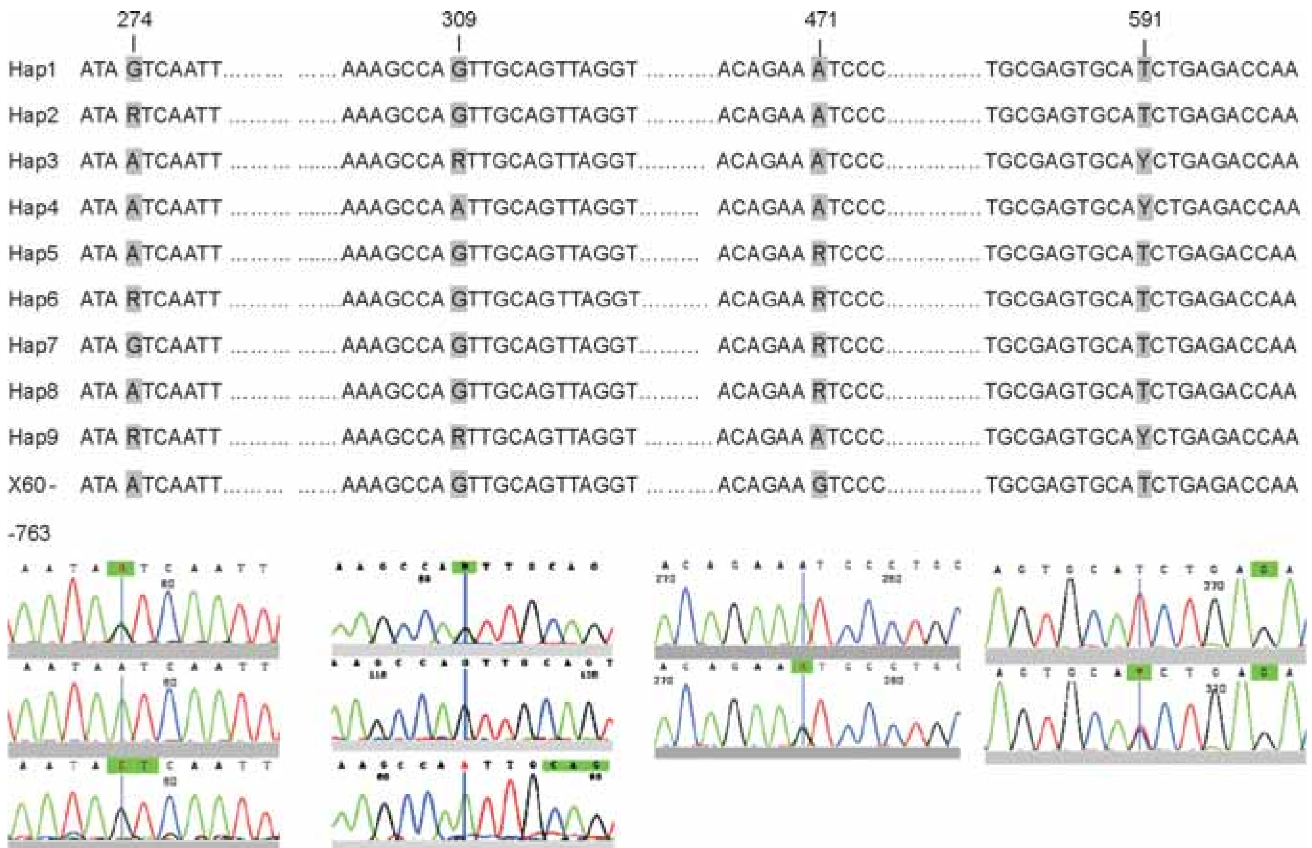

Fig. 2. Sequence alignment of *k-CSN3* gene fragment compared to GenBank accession no. X60763.

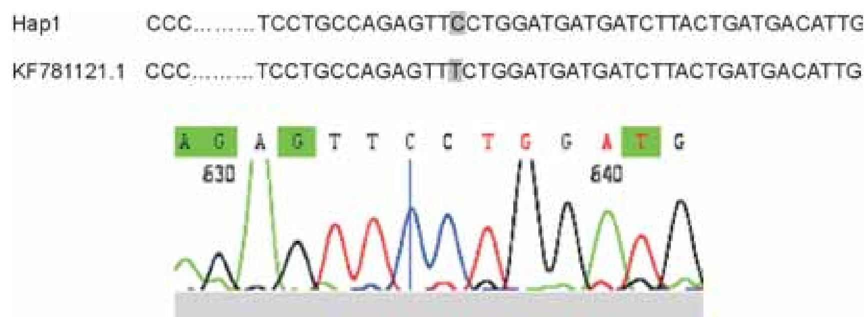

Fig. 3. Sequence alignment of *LALBA* gene fragment compared to GenBank accession no. KF781121.

at a frequency of 0.30625.

Analysis of the deduced amino acid sequences showed point mutations at each polymorphic site and all of them were non-synonymous variations except for the site T245C and A284G where mutation was synonymous (Table 1). At the heterozygous polymorphic site A274G, mutation of nucleotide substitution from A to G is responsible for variation in amino acid from Asn to Ser while the same variation at the A309G and A471G sites change amino acid sequence from Ile to Val. Furthermore, T to C variation at the 591 site is liable for Ser to Pro variation in amino acids. Locally adapted goats in Sri Lanka showed T, G, A, T, C and C/T nucleotides at 203, 384, 385, 550, 583 and 591 sites, respectively and each of these were responsible for amino acid variations compared to already published sequences in GenBank database (Table 1). Locally adapted goats demonstrated an entirely new polymorphic site at the position of 203 and it is also responsible for deviations of G to T and Gln to His in nucleotide and amino acid sequences, respectively. At the site 384, local animals demonstrated nucleotide G (codes for amino acid Asp) while some other exotic breeds showed A (codes for Asn) at the same site (Prinzenberg *et al.* 2005). The nucleotides found in local goats at 385, 550, 583 sites were A, T and C respectively and exotic breeds showed G (Prinzenberg *et al.* 2005), C and T (Yahyaoui *et al.* 2001) at the same positions.

Many researches (Yahyaoui *et al.* 2001, Caroli *et al.*

Table 1. *k-CSN3* and *GnRHR* genetic variability among locally adapted goats in Sri Lanka

GenBank accession no./	Nucleotide position in *k-CSN3* gene											GenBank accession no./ Reference	Nucleotide position in *GnRHR* gene		
	203	245	274	284	309	384	385	471	550	583	591		730	757	891
X60763	G (Q)	T (Y)	A (N)	G (L)	G (V)	G (D)	A (D)	G (V)	T (V)	C (A)	T (S)	Yang *et al.* (2011)	-	A/G (P)	G/T (R/M)
AF485340								A(I)				This study	A(A)		G
AY090466		C(Y)					G(G)	A			C(P)	This study	A	G	G
AF521022			G(S)					A				This study	A/G(A)	G	
AY350425		C		A(L)	A(I)			A		T(V)		This study	A		
AY428577		C				A(N)		A	C(A)			This study	A/G	G	G
This study	T(H)		G					A							
This study	T		A/G					A							
This study	T				A/G			A			C/T				
This study	T				A			A			C				
This study	T							A							
This study	T		A/G					A/G							
This study	T		G					A/G							
This study	T							A/G							
This study	T		A/G		A/G			A			C/T				

Table 2. The allelic and genotypic frequencies, recombined genotypic and haplotypic frequencies at the heterozygous polymorphism sites of *k-CSN3* gene in non-descriptive goats in Sri Lanka

Locus	Genotype	Genotype frequency	Allele/ Haplotype frequency
A274G	GG (9)	0.45	G 0.525
	AA (8)	0.4	A 0.475
	A/G (3)	0.15	
G309A	GG (17)	0.85	G 0.9
	AA (1)	0.05	A 0.1
	A/G (2)	0.1	
A471G	AA (16)	0.8	A 0.9
	A/G(4)	0.2	G 0.1
T591C	TT (17)	0.85	T 0.925
	C/T (3)	0.15	C 0.075
Haplotype	GG/GG/AA/ TT (7)	0.35	GGAT 0.46875
	AG/GG/AA/ TT(3)	0.15	AGAT 0.30625
	AA/AG/AA/ CT (1)	0.05	AAAC 0.06875
	AA/AA/AA/) CT (1	0.05	AGAC 0.01875
	AA/GG/AA/ TT (3)	0.15	AAAT 0.01875
	AG/GG/AG/ TT (1)	0.05	AGGT 0.0625
	GG/GG/AG/ TT (1)	0.05	GGGT 0.0375
	AA/GG/AG/ TT (2)	0.1	GAAC 0.00625
	AG/AG/AA/ CT (1)	0.05	GAAT 0.00625
			GGAC 0.00625

2001, Yahyaoui *et al.* 2003, Prinzenberg *et al.* 2005) reported the silent mutation at 254 site. The synonymous mutation at 284 site found in this study was previously described by Yahyaoui *et al.* (2001), Prinzenberg *et al.* (2005) and Jann *et al.* (2004) in different goat breeds. Further, the variabilities at 471 and 591 positions were formerly described in a single paper by Yahyaoui *et al.* (2001).

Although there were no variable sites in the exon 2 region of the *LALBA* gene fragment studied, a non-synonymous point mutation was recorded by a nitrogenous base-exchange form T to C in the exon 3 (Fig. 3). The reported mutation is not responsible for amino acid sequence variation and was previously described by Lan *et al.* (2007) and Cosenza *et al.* (2003).

GnRHR exon 1 region from 30 goat samples was analyzed and three polymorphic sites (Fig. 1) were identified in non-descriptive local goats and all three polymorphic sites were heterozygous (Table 1). Heterozygous genotypes were recorded at only minor frequencies within each polymorphic site while the majority of animals showed homozygous genotypes. The most common haplotype; AA,GG,GG was found from 57% of the population and there were two haplotypes (AA,A/G,G/T and A/T,GG,GG) with similar frequencies in this population representing the scarcest recombinations (3%). Among possible allelic recombinations, AGG and ATT were the highest and lowest documented recombinations respectively from the analyzed population (Table 3).

Inferred amino acid sequences for the base variations in A730G and G757A sites showed presence of silent mutations, however, the reported G891T variation is responsible for a codon change from Arg to Met (Table 1).

Studies by Yang *et al.* (2011) have reported the same

Table 3. The allelic and genotypic frequencies, recombined genotypic and haplotypic frequencies for the polymorphism of *GnRHR* gene in non-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	AAG 0.13
	AA,GG,GG (17)	0.57	AGG 0.76
	A/G,GG,G/T (04)	0.13	AGT 0.06
	AA,A/G,G/T (01)	0.03	AAT 0.02
	A/T,GG,GG (01)	0.03	ATG 0.02
			ATT 0.01

two SNPs at G757A and G891T. In their study, Boer goats with genotype GG and GT at G757A and G891T sites, respectively have shown significantly positive correlations with litter size in first, third and fourth parities ($P<0.05$). Other than the two previously identified SNPs, our study reported a novel SNP at A730G and was a synonymous mutation. Since silent mutations can influence the gene expression by regulating the transcription, splicing and translation events (Goymer 2007), the synonymous mutation reported in this study may have significant effects on related production traits. The absence of some genotypes at polymorphic sites may be due to the negative correlation with reproduction traits and elimination of them by consecutive artificial selection (Yang *et al.* 2011).

This study is a preliminary report on SNPs in *k-CSN3*, *LALBA* and *GnRHR* genes of non-descript local goats in Sri Lanka by DNA sequencing technique. However, results of the current study reveal high genetic variability of Sri Lankan non-descript goats compared to exotic breeds; reporting absolutely novel polymorphic sites at G203T and A730G for *k-CSN3* and *GnRHR* genes, respectively. Non-synonymous mutations found in this study can be utilized to develop molecular markers and even silent mutations which are closely adjoined with non-synonymous sequences can be used for the purpose to be used in marker assisted selection and association analyses. However, further studies are essential to evaluate physiological role of different genetic haplotypes found in the current study in relation to productive traits of non-descript goats of Sri Lanka.

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