ASSOCIATION OF SNPs IN EXON 3 OF LEPTIN (*LEP*) GENE WITH GROWTH TRAITS IN NILAGIRI SHEEP OF TAMIL NADU

D. Cauveri
Assistant Professor
Dept. of Animal Genetics and Breeding
Madras Veterinary College, Chennai

Contents

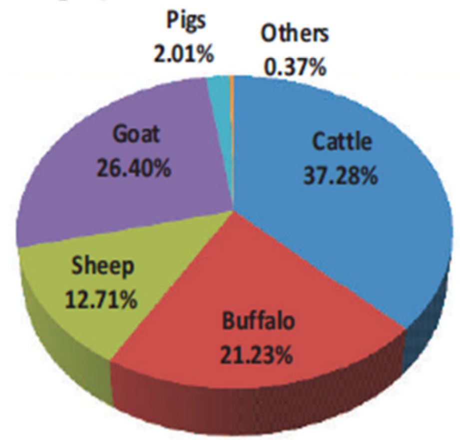
Introduction

Sheep population in India

ALL INDIA LIVESTOCK CENSUS (in thousands)			
CATEGORY			
	2007	2012	% Change
Sheep			
❖ Exotic/Crossbred			
• Male	1,144	1,207	5.51
• Female	2,586	2,574	-0.46
❖ Total Exotic/Crossbred	3,730	3,781	1.37
❖ Indigenous			
• Male	16,730	13,916	-16.82
• Female	51,098	47,372	-7.29
❖ Total Indigenous	67,828	61,288	-9.64
Total Sheep	71,558	65,069	-9.07

Courtesy: 19th LIVESTOCK CENSUS-2012 ALL INDIA REPORT

Graph 3.1:Distribution of Livestock



Sheep breeds of Tamil Nadu

Coimbatore

Kilakarsal

Madras Red

Mecheri

Sheep breeds of Tamil Nadu

Nilagiri

Ramnad White

Tiruchy Black

Vembur

Nilagiri sheep

- ❖Native to the Nilgiris of Tamil Nadu
- ❖Evolved during 19th century
- ❖Contains unknown levels of inheritance of Coimbatore, Tasmanian Merino, Cheviot and South Down breeds of sheep (Rao *et al.,* 1960).
- ❖Medium-sized animals having white coat colour with a convex face line giving them a typical Roman nose.
- ❖Both sexes are polled.
- ❖Dual purpose breed used for meat and fine wool production

Single Nucleotide Polymorphisms

- Substitution
- Addition
- Deletion of one or few nucleotides

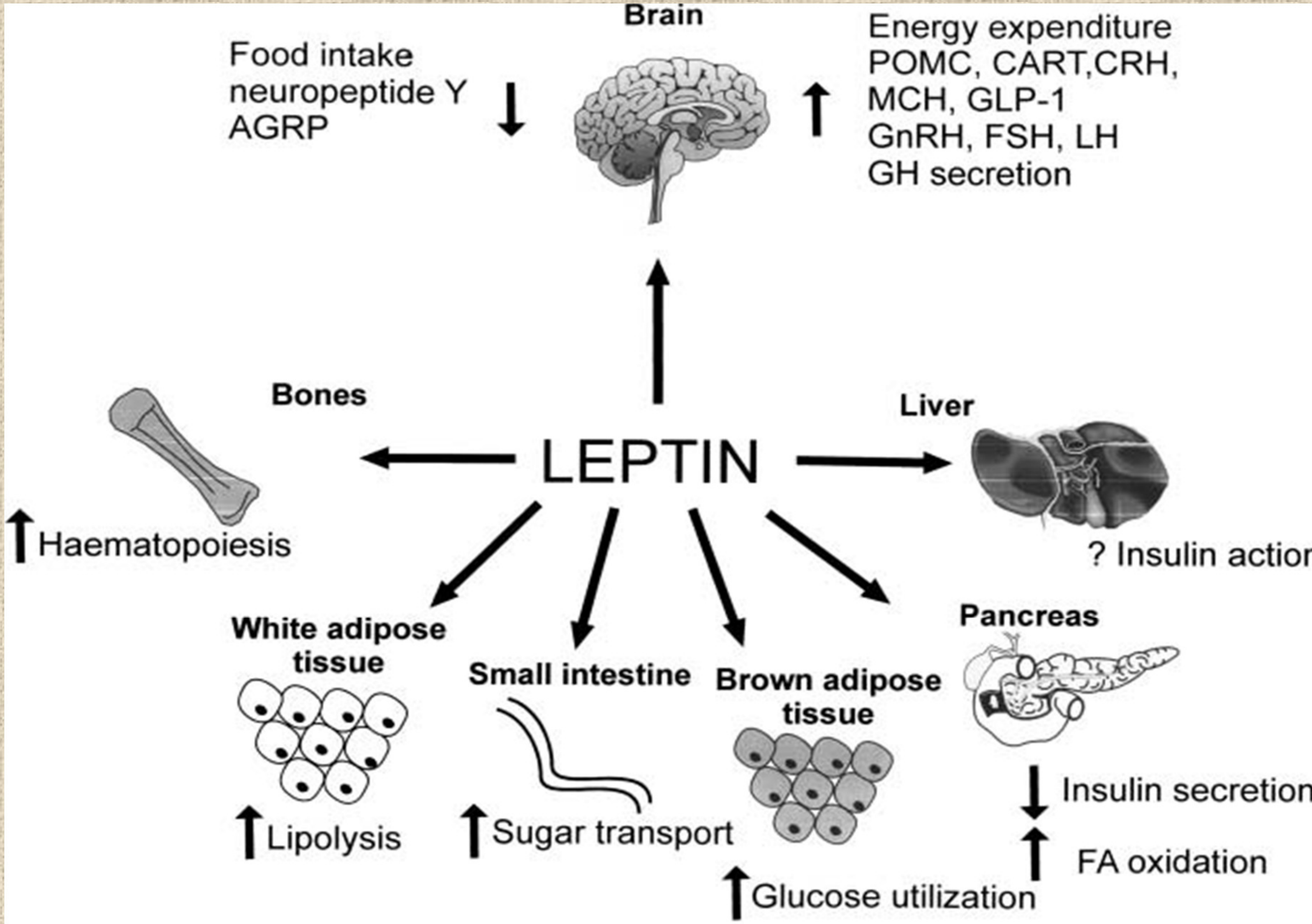
Characteristics

- Prevalent
- Stably inherited
- More suitable for long-term selection markers

ABOUT LEPTIN GENE

- Zhang *et al*. identified leptin gene in 1994 by positional cloning
- *LEP* maps on OAR4q32 (Perucattia *et al.* 2006) and CHI 3q33 in goats (Supakorn, 2009)
- The leptin gene contains three exons which cover approximately 15 kb of genomic DNA.
- The mature hormone, which contains 146 amino acid residues, is translated mainly from the third exon.

CENTRAL AND PERIPHERAL ACTIONS OF LEPTIN



BIOLOGICAL EFFECTS OF LEPTIN

- Regulates body-weight homeostasis and energy balance
- Growth – mutation of leptin gene leads to reduced plasma GH levels (Clement *et al*. 1998)
- Reproduction – Maturation of the reproductive system-regulating the onset of puberty
- Link between nutritional state and the immune system

Objectives

Objectives

- Studying the polymorphism of Leptin (*LEP*) gene in Nilagiri sheep of Tamil Nadu
- Establishing the association of the leptin genotypes with growth traits
- Analyzing the potential of this gene as genetic marker for growth traits in Nilagiri sheep

Design of the experiment

Design of the experiment

- Collection of blood samples and isolation of DNA from Nilagiri sheep
- Sequences of *LEP* gene downloaded from NCBI
- Designing of primers, standardisation and PCR amplification of the gene
- Sequencing representative sample
- SNPs screening
- Genotyping the SNPs
- Collection of growth data
- Association of SNPs with growth traits
- Identification of markers that could be used for selection

Materials and methods

Materials and Methods

- Breed – Nilagiri
- Isolation of DNA using DNAzol
- Design of primers (http://frodo.wi.mit.edu/primer3/)
  - *LEP* gene - NC_019461 and Gene ID 443534
- PCR amplification of genes
- Sequencing representative samples by outsourcing
- Analysis by using Seqman program by LASERGENE
- Genotyping of the SNPs with PCR-RFLP
- Association with growth traits using the least-squares model

$$Y_{ijk} = \mu + S_j + G_k + e_{ijk}$$

Sequence of the *LEP* gene

```
(- 69)      TTGCGCTTCG GCGGCTATAA GAGGGGCGGG CAGGCATGGA GCCCCGGAGG GATCGAGGAA TCGCGGCGC
( +1)
                                                                                  C AGCAGCGGCG  AGGTAAGTGC
(+ 18601)   INTRON 1~11400 bp  CTCGTTGTTA TCCGCATCCA AAGACGTGGA  TGTGGGTGGT AACGGAGCTC ATGGGTGTTC TCGGAGAGCT GGCGATGTGC CATGTGTGGT
(+ 18711)   TTCTTCTGTT TTCAGGCCCC AGAAGCCCAC CCTGGGAAGG AAAATGCGCT GTGGACCCCT GTACCGATTC CTGTGGCTTT GGCCCTATCT CTCCTACGTG GAGGCTGTGC
CCATCCGCAA GGTCCAGGAT GACACCAAAA CCCTCATCAA GACGATTGTC ACCAGGATCA ATGACATCTC ACACACGGTA GGGAAGGACA GGGAGATGAG GTAAAACCGT
GGCCATCCCG TGGGGGACCC CAGAGGTTGG CGGAGGAGGC TGTGCAGCCT TGCACAGGGC CCCAGCGGCC TGGACGCCCC CACTAGCGTA CAGACAACTC CTCTCCTCAT
CCACTTCCCT TGCCTCCCAC CTTCTCACTC TCCTCCCTCC CAGACCGGAA TCCTAGTGCC CAGGCCCAGA AGGAGTCACA AAGGTCCCGG  INTRON 2 ~ 1800 bp

           GGGGAGGAAG CACCTCTACA CTCGAGGGAA AGGCGGAGTT GGGGGAGCTC TGAGGAGCTG CCCTCTCTCC CACTGAGCTC TTGATGTCCC CTTCCTCCTG
CATAGCAGTC CGTCTCCTCC AAACAGAGGG TCACTGGTTT GGACTTCATC CCTGGGCTCC ACCCTCTCCT GAGTTTGTCC AAGATGGACC AGACATTGGC AATCTACCAA
(+ 15781)   CAGATCCTCG CCAGTCTGCC TTCCAGAAAT GTGATCCAAA TATCTAATGA CCTGGAGAAC CTCCGGGACC TTCTCCACCT GCTGGCCGCC TCCAAGAGCT GCCCCTTGCC
(+ 15881)   GCAGGTCAGG GCCCTGGAGA GCTTGGAGAG CCTGGGCGTC GTCCTGGAAG CCTCCCTCTA CTCCACCGAG GTGGTGGCCC TGAGCCGGCT ACAGGGGTCT CTACAGGACA
TGTTGCGGCA GCTGGACCTC AGCCCTGGGT GCTGAAGCCT TGAAGGCCTC TCTTCCCAAA GTCCAGGGAA GAAACCTGAG CTTCTGGCTG TCTGCAGAAG AGAGCCTATG
TGGGCATCCT TTATGCAGGC CAGCGGGCCA TTTCTCTCTT GCTCCTCTCA GCTGCTCTTC CAAAGGCAGA AAACTGCGAG CCAGGAAACC AAAGATATAA ATACAGGTTC
CATGCCCACC AGGAAGGGGG GCCCATCCAG CAAACAGTAG ACCGGAGCTG GGATTTTCAC AGCAGTCTTC CTCCCTGTTC CAGCTCCCTC TCACTGCATG CTTCAGCATG
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ACGATGGAAG CATTTATTTA TTAATTATGC ATTTTATTCT GAATGGATTT GAAGCAAGAC ACCAGCTTTT CCAGGCTTTT TGGGGTCAGC TGGGGCGAGG GATGCTACTG
GGGTGCCCAT CGACAGGCCT CGGCGAGGCA AACCCATTTT GAGTGACTTG AGGGCTCTCA AGTTTGTTCT CCAGGGACTG GCTTTGTTTC TACTGTGACT GACTTTAAAT
TACAGTGTTT GCAATGGCAT TGCTCTGAAT GGATCTCGAA GGACCAAGTT ATTTTAAAAA GAAGAAGAAG AATTTTGTCA AGTGTAATAT ATTGCTGGGT ATGCCCAGAG
GTGGGAAATG TGTTGATGGA AGGGGGGGAG ATCCAGAATG TGTTTTCTGA ATAACATTTT TGTGATGGAC TCTTTGGATG GGGTAAGTCA TCTTCTCATC TTTGCAGTTT
TCATGAAGAG GAGATGACTC CTTCGGGGGG GATTGTGGGG GCTTGCTAAC CATCCATGGA TCAAGTGGTG GGGGTACTGA AGCTGAAGGC CATTGGGATA GTGGTGAGCT
CTGGCCTTCT CTGACTGTTA GAGAGTGGTC TTGCTCATCA GGAAGTGAGG ACCCCACACT GGAAATGGTG ATCCCCAGAA CAGGGGTCCT TGGTGTGAAC GGTCTGGGTT
GACCCCACGT TGTATTGATA ACATGGTCAT GACCTTCTTT GGGATTTGCA TGCTCACCCA AAGCAAGGCC ATGCTTCCCA TCCATTTGGG AAGGATTTTT ATTCCAGTGG
GAGGGGGAAG TATTCCAGCG TGGGCTTCAG TGGATGGTCC CTCGACCTGG GTCAGCAATG GGTCAGTTGA GGCCCAAGAC CCCAGGACCA GCCCCCAGGA GCCTCCTTCT
CACTAGTGGT CATGTGCAGC AGAACAAAGG AGGAGGCTTG GGTTTCCCAC CATCCTGCCA TTGTGATGCA GCCATCACAC GACAGGAGGT GGATCGGTCC AAGGAAATTG
GAGTCTAAGC AACCAATTTT AAGACTGAGC ACCTACTTGT GCTCAGCCTC AACTGGTGCT ATGGGCTGAG AAGCTCACCA AATAAATATT AAAATGCAAG CCCTGCCCTC
AGGGACCTTG CATTCCAGAT GGTAGAATCC CACTCACCAG CATGCAAAGG CTGCCGTTTC ACCATGGCAA CTGAGCAGCT GAGACAGTGC AGTCCTCAGC AGGTGGGAAA
TGCTGAGCTG TGGAGGGCAG TGCCCAGGGG CCTACAGGCT AACTGTGCTT GCACTTGGTA GCATTTTTAC TTTTCAGGGC ACGTCAGCAT CTATTACTGT GATGCCACAT
CCCTTTGAAG CGGGATAACT AAGAAATTAA TAAGAAGAAA ATACCTAAGA CCATATCAGC AGACAGGGGG CAAAACCAAG ACTAGAATCC CGGTCCTCTG ACCTCCAGAG
TGCGCCTTGA GCCAGGTGGT AGTCTCTGGA GATGTGAACA AAGTAGGGCA GGGAGGGCAG GAGTGCTGCA GGAAGAGAGG AGTTCTGAGG CCAATTTTGC AGGTGGTGAG
GGAAGTGAAT TGCCTGGAGG GAGGAGGCTG TTTTGTTTGA AGCTTTGGTC TGAGACACCG AGGGGAGGTG ATAGAGTGAG TAGCTAGTTA CAAAGAAAGG CAGAGAAAGG
AAAGATGGGA GGGAAGGGTT ATGTTGAAGA GGACTTCAGG GGCAAAGATG TTTGCTACTG AGAGAGATAA GGGTGAAAGT TCTAGAACGA GACTCATGTG ATGGACAGAG
(+ 18521)   TCAGACCTGC TCGAGAAGAT ATATCCTGAT GATCACAGCC CAGTCAGGCC AGGATGTTTT AAGCCTTTTG CTCACAAAGC CTGGCATGGC ACTAGGGCTC GTTCCCGGAG
(+ 18631)   CGTGAAACTT CCAAAATGTA AATGATTGTG TTTTTCTGTA ACTTAAAACA ATTTTTTTTG TTCCAAAAAG TCCAAATAAA TGACCTTTGC CCCTTGGTCC TTGTTTTTGG
TCGTCCGCTG TGGAGGGGCT CCAGAAGGGC TGCCTGTGTG TGACAGGGCA CCGCCTGGCT GGCCCCAGTC CATCCCTGGG ATCAGCTCTG
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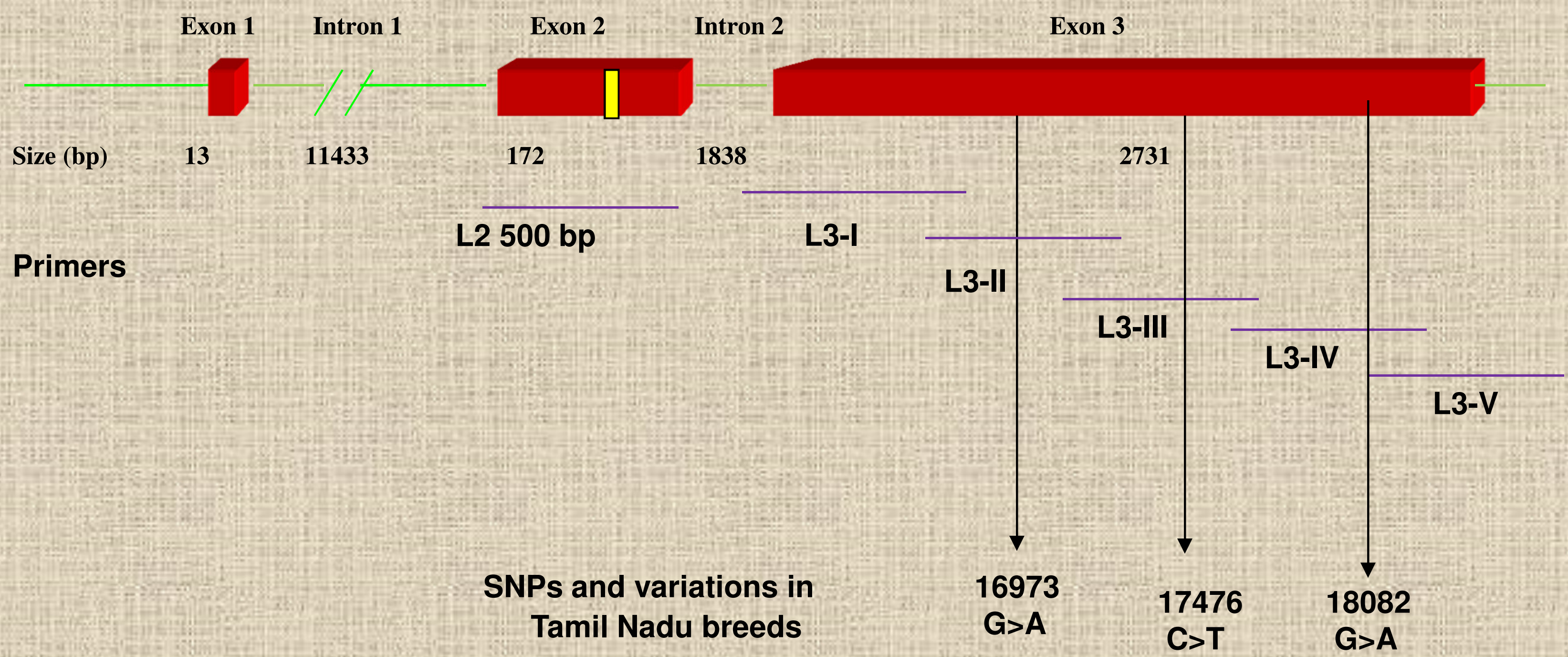
LEP Gene



Number	Sequence (bp)	Exon (bp)	Intron (bp)
1	2430 - 2442	13	11433
2	13876 - 14047	172	1838
3	15886 - 18616	2731	

Primers for *LEP* gene

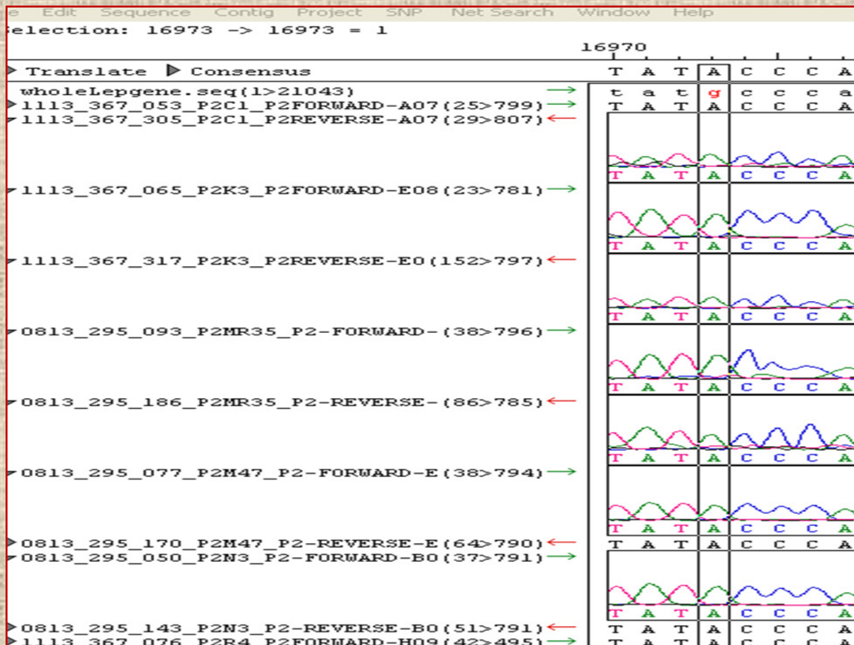
Region	Primer Sequence (5'-3'end)		Annealing Temperature (°C)	Product size
E3	Forward	ctgaggagctgccctctct	59.8	832 bp
	Reverse	gcttccatcgtgtgttgtgt		
E3	Forward	agtagaccggagctgggatt	61.6	840 bp
	Reverse	agaccgttcacaccaaggac		
E3	Forward	tggactctttggatggggta	61.4	797 bp
	Reverse	ctgcactgtctcagctgctc		
E3	Forward	tggactctttggatggggta	62.4	849 bp
	Reverse	ctgcactgtctcagctgctc		
E3	Forward	gccaggtggtagtctctgga	63.6	686 bp
	Reverse	ctttgggggaatcctttagc		

Results

SNPs and variations found in the Exon 3 of *LEP* gene

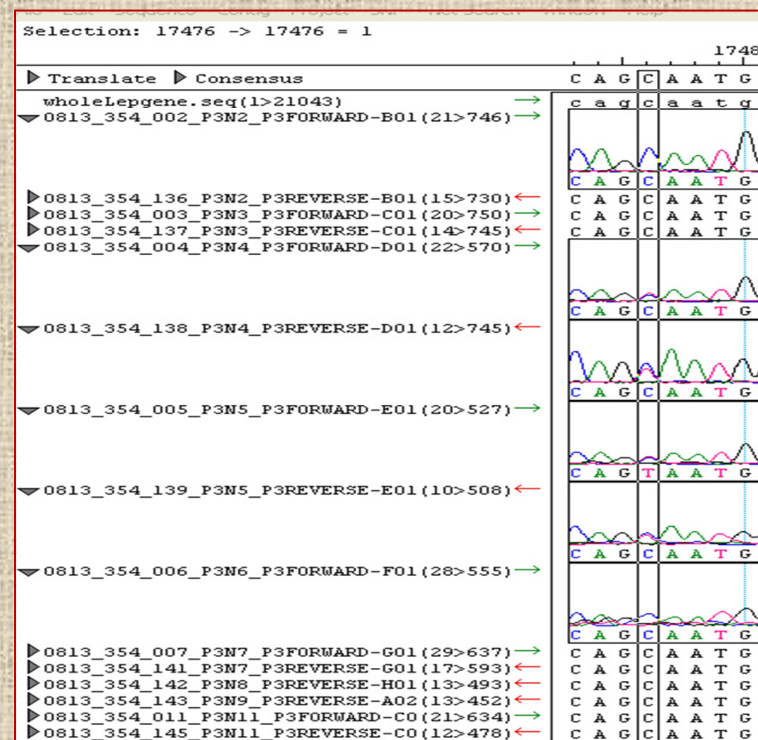
Locus (position in bp)	Reference Sequence	Sheep breeds							
		Coimbatore	Kilakarsal	Madras Red	Mecheri	Nilagiri	Ramnad White	Tiruchy Black	Vembur
Exon 3									
16973 G>A	G	Replaced by A in all samples							
17476 C>T	C	Only CC genotypes				CC (10) CT (3)	Only CC genotypes		

Chromatogram of SNPs identfied in the Exon 3 of *LEP* gene



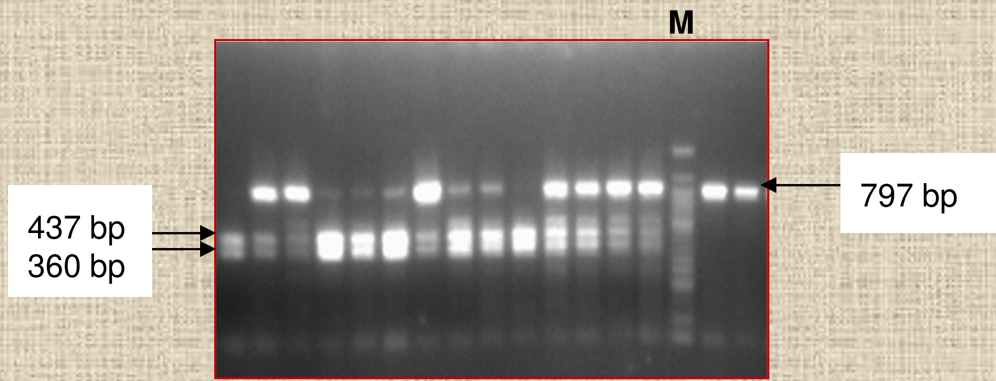
SNP L1
16973 G>A

SNP L2
17476 C>T



Genotyping of SNPs in *LEP* gene by PCR-RFLP

SNP-L2 genotyping using *Bsr*DI in Nilagiri



Regions	SNPs	Genotype	Genotype frequency	Alleles	Allele frequency
			Nilagiri		Nilagiri
Exon 3	17476 C>T (SNP- L2)	CC	**0.73**	C	**0.87**
		CT	0.27	T	0.13

Association of the SNP L2 with growth traits in Nilagiri sheep

Genotypes	Least-squares means ± S.E. (kg) for the effect of SNP L2 on body weights of Nilagiri sheep				
	Birth	Weaning	6-months	9-months	Yearling
Overall	2.52 ± 0.13 (116)	11.48 ± 0.49 (114)	15.86 ± 0.48 (114)	19.06 ± 0.74 (112)	22.84 ± 0.86 (112)
CC	2.52 ± 0.11 (84)	11.21 ± 0.45 (82)	15.97 ± 0.44 (82)	19.40 ± 0.76 (80)	23.75 ± 0.88 (80)
CT	2.51 ± 0.23 (32)	11.76 ± 0.81 (32)	15.76 ± 0.79 (32)	18.73 ± 1.21 (32)	21.93 ± 1.40 (32)

Figures in parentheses indicate number of observations

** P<0.01; *P<0.05; NS – Not significant; Subclass means with different superscripts are significantly different from each other

Association of the SNP L2 with growth traits in Nilagiri sheep

Genotypes	Least-squares means ± S.E. (g) for the effect of SNP L2 on ADG of Nilagiri sheep	
	Pre-weaning	Post-weaning
Overall	99.71 ± 4.62 (114)	41.75 ± 2.23 (112)
		*
CC	96.60 ± 4.24 (82)	**46.88[a] ± 2.28 (80)**
CT	102.81 ± 7.61 (32)	36.63[b] ± 3.62 (32)

Figures in parentheses indicate number of observations

** P<0.01; *P<0.05; NS – Not significant; Subclass means with different superscripts are significantly different from each other

Conclusions

CONCLUSIONS

- The exon 3 of *Lep* gene was polymorphic in the sheep breeds of Tamil Nadu.
- SNP L1 was found in all the sheep breeds of Tamil Nadu at 16973 bp, G replaced with A where only AA genotypes were found for the reference G.
- Nilagiri breed showed a breed specific allele SNP L2 at 17476 bp, a C>T transition with a CT frequency of 0.27.
- SNP L2 is found to influence the post-weaning ADG with the C allele to be considered as a favourable allele for marker assisted selection

THANK YOU