Identification of a single nucleotide polymorphism in POU1F1 gene (exon 6) and its association with Early body weights in native goats of kerala

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ABSTRACT

Information on genetic diversity in particular of genes that control economic traits like growth is needed for the genetic improvement and development of desi breeds of goat. Pituitary-specific transcription factor –1 (POU1F1) is known to be an important candidate gene in livestock growth by regulating the expression of anterior pituitary hormones like growth hormone, prolactin, TSH and itself. The present study aimed at the identification of genetic polymorphisms in the exon 6 fragment (251 bp) of POU1F1 gene in 100 Malabari and 60 Attappady Black goats of Kerala and its possible association with early body weights at birth, three, six, and nine months of age. PCR-SSCP analysis revealed two genotypes CC and TC with genotypic frequencies of 0.30 and 0.70 in Malabari and 0.77 and 0.23 in Attappady black. C was the dominant allele in both Malabari (0.65) and Attappady (0.91) populations. Sequencing revealed a synonymous single nucleotide polymorphism (sSNP) of C T transition at the 180th position of the fragment (g.180C>T). The observed and expected heterozygosity in Malabari goat population (0.6970 and 0.4564) were higher than in Attappady (0.1724 and 0.1603). HWE ($\chi 2$) test showed that the genotypic distribution of POU1F1 locus in Malabari was in agreement with Hardy-Weinberg equilibrium while it was not in equilibrium in the Attappady goat population ($p<0.01$). The animals with TC genotype had significantly higher body weight at six weeks of age than those with CC genotype ($p<0.05$). The study points out that the SNP (g.180C>T) identified in the locus significantly contributed to variation in early body weight at six months of age in goats and hence suggests the possibility of using this polymorphism in the marker-assisted selection (MAS) and breeding programmes for the genetic improvement of Malabari and Attappady goats of Kerala.

Keywords: POU1F1, SNP, early body weights, goats
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INTRODUCTION

Goat rearing is a low input enterprise with assured higher output and hence preferred by the rural poor as an alternative source of supplementary income and milk (Kumar, 2007). Malabari and Attappady Black are the two native goat genetic resources of Kerala. Malabari is a dual purpose breed well known for prolificacy and milk yield. Attappady Black goats are meat type that stands out for their hardy nature and faster growth rate (Stephen et al., 2005). Candidate gene approach is widely employed for the early selection of goats for economic traits and a total of 271 candidate genes have been identified in goats (Supakorn, 2009). POUIF1, mainly expressed in the anterior pituitary of vertebrates is considered as a good candidate gene in small ruminants (Bastos et al. 2006). It is located on chromosome 1q21-22 with 16078 base pairs consisting of six exons and five introns. The product of this gene is a pituitary-specific transcription factor-1 also known as PIT-1 or GHF-1 that plays an important role in the transcriptional regulation of the genes coding for anterior pituitary hormones like growth hormone, prolactin, thyroid-stimulating hormone and POU1F1 itself (Daga et al., 2013). Accordingly, mutations on POU1F1 gene result in the deficiency of these

hormones (Cohen et al., 1997). The genetic polymorphisms in the exons and flanking regions of POU1F1 are also found to be associated with birth weight, body weights, milk and wool traits in sheep and goats (Lan et al., 2007a; Bai et al., 2016; Zhou et al., 2016). Hence, the present study was undertaken with the objective of identifying single nucleotide polymorphism in the exon 6 of POU1F1gene and its association with early body weights in Malabari and Attappady Black goats of Kerala.

MATERIALS AND METHODS

Sampling

A 100 Malabari goats and 60 Attappady Black goats belonging to the University Goat and Sheep Farm, Mannuthy were utilized for the study. Jugular blood samples of 2 ml each were collected in EDTA vacuitainers, rapidly transported to the laboratory at low temperature and stored at-20° C.

DNA extraction and amplification

DNA was extracted from whole blood using the standard phenol-chloroform method (Sambrook and Russell, 2001) and frozen at -20 C until use. Purity of DNA was detected by 2% agarose gel electrophoresis. Genomic DNA was used for the amplification of 251 bp fragment (exon 6) of POU1F1 gene using primers designed by the Primer 3 software (V.0.4.0) (http://bioinfo.ut.ee/primer30.4/). The PCR primers for POU1F1 exon 6 were synthesized (Sigma-Aldrich, USA) as follows: POU1F1-F: 5'-CCTCCCAGTATTGCTGCTAA-3' and POU1F1-R: 5'-CACAATGGGAGACCTATCTG-3'. The PCR was conducted in a 20µL reaction mixture containing 1µl of DNA, 2mM of $MgCl_2$, 0.4µl of dNTPs, 1µl of 10 pmol of each primer and 0.2 µl of Taq DNA polymerase. The PCR protocol involved an initial denaturation at 95° C for 30s followed by 35 cycles of denaturation at 95° C for 15s, annealing at 63° C for 15s, extension at 72° C for 30 s and final extension at 72° C for 5 min (Bio-Rad Thermal cycler, USA). Electrophoresis of PCR products were performed in 2% agarose gel in parallel with 50 bp DNA marker (Fermentas) in 1x TBE buffer at a constant voltage of 80 V for 45 min. After ethidium bromide coloration, products were visualized by ultraviolet transillumination (Bio-Rad, USA).

PCR-SSCP analysis

The PCR product was subjected to single-strand conformation polymorphism (SSCP) analysis. Aliquots of 10 µL of PCR products were mixed with a 20 µL of denaturing solution (containing 9.5 ml of deionised formamide, 0.4 ml of 0.5M EDTA, 2.5 mg of xylene-cyanole and 2.5 mg bromophenol blue) centrifuged, denatured by heating at 95° C for 10 min and immediately chilled on ice. SSCP analysis was done using vertical electrophoresis (Hoefer, USA). Denatured amplicons were loaded on 12% PAGE gel in 1x TBE buffer at a constant voltage of 130 V for 18 h. The gel was stained by a silver staining method (Sanguinetti and Simpson, 1994).

DNA sequencing analysis

The PCR products from different SSCP patterns were sequenced using a commercial service (SciGenom Labs Pvt. Ltd. Cochin) in forward and reverse directions. Nucleotide sequence alignments and comparisons were carried out using reference sequence NC_030808.1 in GenBank using BLASTn (http://www.ncbi.nlm.nih.gov/blast) and EMBOSS: merger (http://emboss.bioinformatics.nl/cgi-bin/emboss/merger). The NCBI (National Centre for Biotechnology Information) Blast algorithm was used to search the NCBI GenBank database (http:/www.ncbi.nlm.nih.gov/) for homologous sequences.

Statistical analysis

The allelic and genotypic frequencies in both breeds were calculated by the standard procedure (Falconer and Mackay, 1996). The observed (H_0) and expected (H_e) heterozygosity and chi-square test for Hardy-Weinberg equilibrium were estimated using PopGene 3.1 software. One-way ANOVA was used to analyze the association of POU1F1 variants with early body weights at birth (BW0), three (BW3), six (BW6) and nine (BW9) months of age and their effects were examined using the following General Linear Model using SPSS (Version 21):

$$Y_{ij} = \mu + POU1F1_{i} + Breed_{j} + e_{ij}$$

where Y_{ij} is the observed trait (body weight); µ is the overall mean; $POU1F1_i$ is the fixed effect due to POU1F1 genotype (i = CC, TC); $Breed_j$ is the fixed effect due to breed (j = Malabari, Attappady) and e_{ij} is

the random error. Least squares means and their standard errors were computed for all genotype effects.

RESULTS AND DISCUSSION

The PCR-SSCP analysis of the exon 6 fragment of POU1F1 revealed two distinct banding patterns in Malabari and Attappady goat populations indicating that the populations were polymorphic for the locus. Two genotypes CC and TC were identified in the breeds, with alleles C and T in different proportions (Fig. 1 and 2).

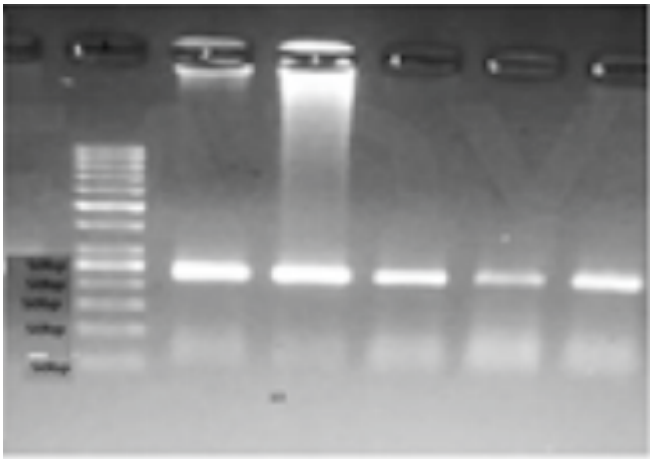

Figure 1. *PCR amplification of exon 6 (251 bp) fragment of POU1F1 gene Lane 1: 50 bp DNA marker; Lane 2 -5: 251 bp product*

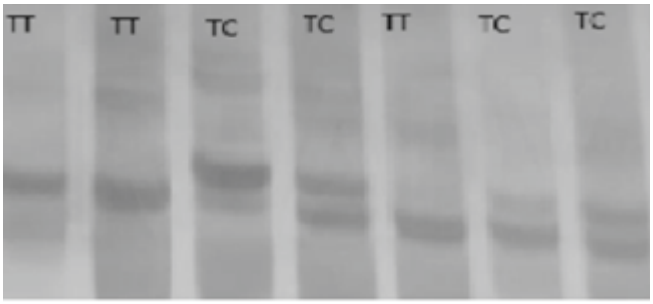

Figure 2*. SSCP pattern of 251 bp fragment of POU1F1 gene (exon 6) with CC, TC genotypes*

The genotypic frequencies of CC and TC genotypes in Malabari were 0.30 and 0.70 respectively (Table 1). The TC genotype was more predominant than CC genotype in Malabari. The allelic frequencies for C and T alleles were 0.65 and 0.35 respectively. In Attappady goats, the common genotype was CC with a frequency of 0.77 and the heterozygous TC genotype was less frequent with a frequency of 0.23. The C allele was most common (0.88) when compared to T allele (0.12). The allele C was most common in both the genetic groups. The TT genotype was not found in either of these indigenous goat populations and this could possibly be due to genetic drift operating in small populations (Dagong *et* al. 2016). The rarity of TT genotype for this *POU1F1* locus in local goat populations has been reported in Indian Barbari goats (Sharma *et al*., 2013).

The observed and expected heterozygosity in Malabari goat population were 0.6970 and 0.4564 respectively. The estimates in Attappady goats were 0.1724 and 0.1603 respectively. The observed heterozygosity (H_o) was significantly higher ($p<0.01$) than the expected heterozygosity (H_e) in Malabari giving an indication of the low level of inbreeding. However, the observed and expected heterozygosity levels did not differ significantly in Attappady. The heterozygosity estimates were also found to be lower in Attappady compared to Malabari indicating a lower genetic diversity in the Attappady Black goat population for the locus under study.

HWE ($\chi 2$) test showed that the genotypic distribution of the POU1F1 locus under study was in agreement with Hardy-Weinberg equilibrium in Malabari goats ($p<0.01$) while it was in HW disequilibrium in the Attappady goat population. A plausible reason for this deviation of Attappady population from Hardy-Weinberg equilibrium could be the presence of a moderate selection pressure for economic traits in such smaller populations accompanied with the loss of certain less favoured alleles of the locus during artificial selection (Li et al., 2013).

Representative PCR products were gel-eluted and sequenced, which confirmed the presence of an SNP at 180th position of the fragment (15804th position of ORF) with a C T transition. On further analysis, the SNP (g.180C>T) was found to be synonymous (sSNP) having a codon change of AGC to AGT coding for the amino acid, Serine (Fig 3 and 4). The SNP does not result in a change in amino acid sequence and hence it can be inferred that it does not modify the specific POU1F1 domain (Daga et al., 2013).

Table 1. Genotype frequencies, allelic frequencies and the population indices of the SNP of 251 bp fragment (exon 6) of POU1F1 based on SSCP pattern in Malabari and Attappady , goats (** p<0.01)

Attributes	Malabari (100)	Attappady Black (60)
Genotype Frequency CC	0.30 (30)	0.77 (46)
Genotype Frequency TC	0.70 (70)	0.23 (14)
Allele frequency C	0.65 (130)	0.88 (106)
Allele frequency T	0.35 (70)	0.12 (14)
Observed Heterozygosity Ho	0.6970	0.1724
Expected Heterozygosity He	0.4564	0.1603
χ2 27.84	0.203	
HWE p value	0.000**	0.652

HWE (χ^2) test showed that the genotypic distribution of the *POU1F1* locus under study was in agreement with Hardy-Weinberg equilibrium in Malabari goats ($p<0.01$) while it was in HW disequilibrium in the Attappady goat population. A plausible reason for this deviation of Attappady population from Hardy-Weinberg equilibrium could be the presence of a moderate selection pressure for economic traits in such smaller populations accompanied with the loss of certain less favoured alleles of the locus during artificial selection (Li *et al.,* 2013).

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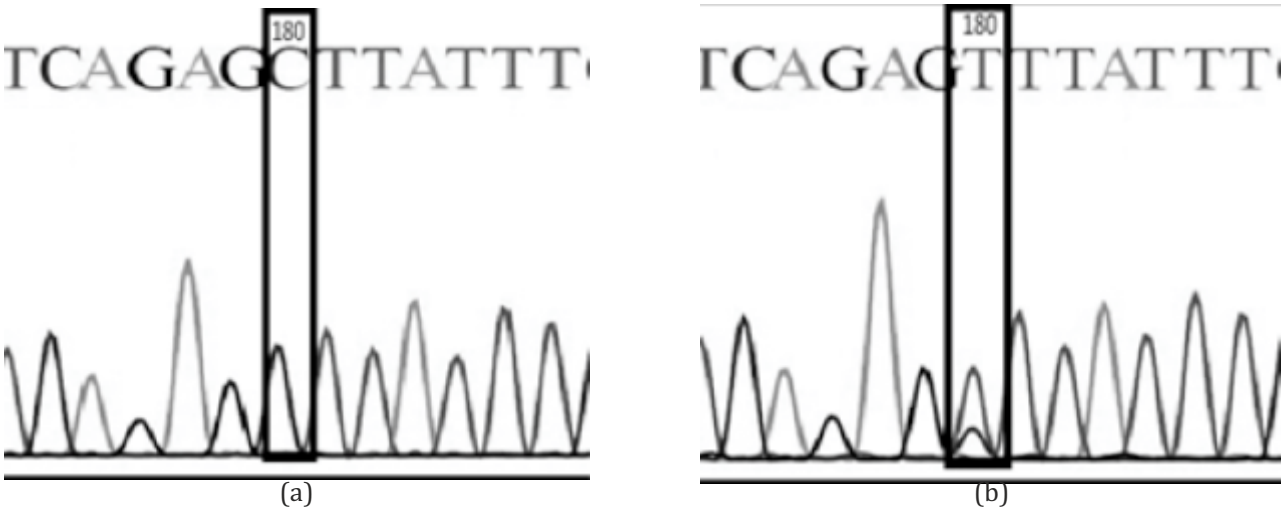
The *POU1F1* exon 6 polymorphism was examined for association with early body weights at birth (BW0), three (BW3), six (BW6) and nine (BW9) months of age in both the genetic groups (Table 2). There was no significant difference in CC and TC genotypes for birth weight and body weights at three and nine months of age. This is in agreement with the earlier reports in which birth weight and weaning weight were not associated with *POU1F1* genotypes (Sadhegi *et al.,* 2014). However, the genotypes differed significantly in body weight at six months of age in both Malabari and Attappady populations ($p<0.05$). Animals carrying TC genotype (13.71±



(a) (b)

Figure 3*. Sequence map of genotypes a) CC genotype, b) TC genotype*

Table 2. Association of the POU1F1 exon 6 genotypes with early body weights in Malabari and Attappady goats

Body weights	Malabari (100)		Attappady Black (60)	
	CC	TC	CC	TC
BW0 (Kg)	1.86± 0.05	1.82± 0.04	1.99± 0.06	2.05 ± 0.11
BW3 (Kg)	6.80± 0.23	6.82± 0.09	6.48± 0.21	6.23 ± 0.18
BW6 (Kg)	12.65± 0.54b	13.71± 0.33a	11.66± 0.39c	12.88 ± 0.33b
BW9 (Kg)	20.67± 0.73	20.13± 0.78	19.79± 0.40	19.30 ± 0.35

Means with different superscripts within the same row differ significantly (p<0.05)

0.33) showed higher body weight at six months (BW6) than those with CC genotype (12.88 ± 0.33). The association of *POU1F1* genotypes with body weight at six months of age can be considered as a molecular marker and this would facilitate the early selection of goats.

CONCLUSION

The PCR-SSCP analysis of the exon 6 (251 bp fragment) of POU1F1 gene revealed two distinct banding patterns in Malabari and Attappady goats indicating that the populations were polymorphic for the locus. Two genotypes, CC and TC could only be observed in the populations. Attappady had less heterozygosity and hence a lower genetic diversity with respect to the locus when compared to Malabari ($p<0.01$). A synonymous SNP was observed at the 180^{th} position of the fragment with a C T transition. The SNP (g.180C>T) was found to be associated with body weight at six months of age and the TC genotype had significantly higher body weight at this stage than the CC genotype ($p<0.05$). The results of the study imply that the POU1F1 locus significantly affect body weight during growing stages in indigenous goats. The single nucleotide polymorphism in POU1F1 exon 6 can be used as a molecular marker for the early selection of goats for body weight at six months of age.

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