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Characterization of third exonic region of POU1F1 gene in the Osmanabadi breed of goat

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Caprine, ovine and bovine pituitary specific transcription factor 1gene (POU1F1 also known as PIT-1 or GHF1) is considered as one of the candidate gene for the growth in various livestock species. It regulates expression of GH, PRL, TSH-β genes and is essential for differentiation, reproduction and survival of somatotropes, lactotropes and thyrotropes. The inhibition of POU1F1 synthesis leads to a marked decrease in proliferation of cell lines producing PRL and GH (Beigi *et al*. 2010, Selvaggi and Dario 2011, Heidari *et al.* 2012). The POU1F1 polymorphisms have been found to be associated with birth weight, body weight, milk yield, milk proteins, fat yields and litter size mainly in cattle and goat (Lan *et al*. 2007a,b; Lan *et al*. 2009a,b,c,d; Jiang-Zuo 2010, Daga *et al.* 2013, Ma *et al*. 2017).

Osmanabadi is the predominant meat purpose goat breed, popular for its high prolificacy and adaptability to harsh climatic conditions. There is wide variation in the growth rate of Osmanabadi goats even though they are raised under uniform environmental conditions. It is important to find out whether the variation in the growth rate is a reflection of underlying genetic variation. Hence, considering the potential effect of POU1F1 gene on growth traits in various livestock, present research was undertaken to investigate caprine POU1F1 gene polymorphism and its association with the body weights of Osmanabadi goat.

Total 217 blood samples and the records of body weights at birth, 3rd and 6th month were collected from Osmanabadi goats of either sex maintained at various farms of Punyashlok Ahilyadevi Maharashtra Sheep and Goat Development Corporation Ltd., Pune. The blood samples were processed for genomic DNA isolation, using Phenol: Chloroform: Isoamylalcohol method (Sambrook and Russel 2001). The 225 bp fragment of POU1F1 (exon-3) gene was amplified using the custom synthesized primer pair (F:5’GGAGCTTAACCCCTTGTCTTTATAAGT3’ and R: 5’ CTAGCTTAATTCTTCTCACTTTAAACT3’) (GenBank Acc. No. AJ549206.1). The optimised thermal cycler conditions were: initial denaturation for 5 min at 95°C, 35 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec, extension at 72°C for 1 min, and final extension at 72°C for 10 min.

For SSCP, PCR products were denatured using denaturing solution (95% formamide deionized, 25 mM EDTA, 0.025% xylene- cyanole and 0.025% bromophenol blue). The denatured DNA were subjected to 12% native PAGE (polyacrylamide gel electrophoresis) in 0.5% TBE buffer at a constant voltage of 200V for 3–4 hrs. The polyacrylamide gels were stained with 0.23% silver nitrate for studying different SSCP band pattern.

The representative PCR products of each unique PCR-SSCP variants were sequenced directly using standard cycle conditions by Sanger’s dideoxy chain termination method with standard sequencing primers, viz. T7 and SP6. The sequence obtained was subjected to BLAST analysis (www.ncbi.nlm.nih.gov/BLAST) and aligned using the ClustalW method of MegAlign Programme of Lasergene Software (DNASTAR). The association of the observed allelic variants/ genotypes with body weight of the animals was performed using two mean Students ‘t’ test described by W. Gosset (Snedecor and Cochran 1994).

The PCR amplification of extracted DNAs from goat blood samples yielded a locus specific single band. The custom designed primers were annealed at 57°C and yielded 225 bp exon-3 amplicon of POU1F1 gene in all samples. The PCR-SSCP analysis of these amplicons exhibited 3 different band patterns, viz. single band, two bands and four bands, suggesting nucleotide variation at two loci. However, direct sequencing of representative PCR products with two SSCP bands revealed ‘C’ > ‘T’ transition at nucleotide position 42. While, PCR products having one

Table 1. Gene and genotypic frequencies at exon-3 locus of Osmanabadi POU1F1 locus

Genotypes and frequencies			Allele frequencies	
TT	CC	CT	T	C
0.286	0.714	00	0.286	0.714

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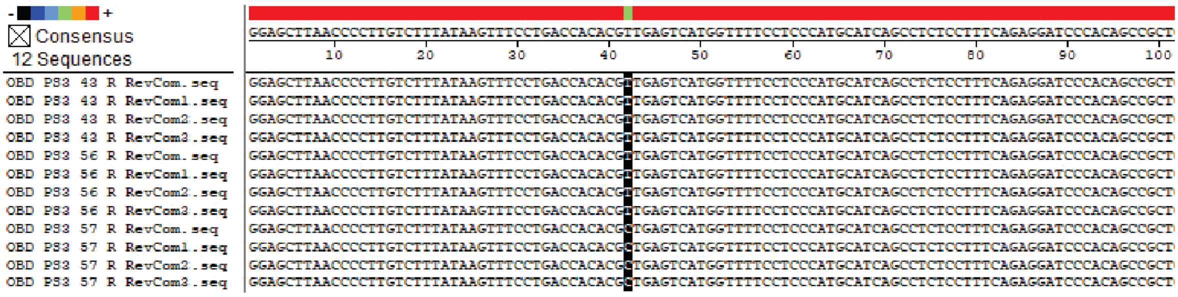

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Consensus                 GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGTTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 43 R RevCom.seq   GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGTTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 43 R RevCom1.seq  GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGTTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 43 R RevCom2.seq  GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGTTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 43 R RevCom3.seq  GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGTTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 56 R RevCom.seq   GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGTTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 56 R RevCom1.seq  GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGTTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 56 R RevCom2.seq  GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGTTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 56 R RevCom3.seq  GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGTTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 57 R RevCom.seq   GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGCTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 57 R RevCom1.seq  GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGCTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 57 R RevCom2.seq  GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGCTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
OBD PS3 57 R RevCom3.seq  GGAGCTTAACCCCTTGTCTTTATAAGTTTCCTGACCACACGCTGAGTCATGGTTTTCCTCCCATGCATCAGCCTCTCCTTTCAGAGGATCCCACAGCCGCT
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Fig. 1. Alignment report of POU1F1 exon-3 Osmanabadi goat sample Nos. 43, 56 and 57.

or four SSCP bands exhibited no nucleotide variation (Fig. 1). Accordingly, Osmanabadi goats were genotyped as 'TT' and 'CC' and observed gene and genotypic frequencies were 0.286 ('T' allele) and 0.714 ('C' allele), respectively. The observed nucleotide variation in exon-3 at position 42; C>T, did not result in amino acid change.

These findings are in agreement with Daga *et al.* (2013), who observed similar C>T transition in Sarda goats. However, Osmanabdi goats are lacking C>T transition at nucleotide position 92 of POU1F1 exon-3 as observed in Sarda goats. In addition to exon-3, other nine exonic and seven non-coding region SNPs within the Sarda goat POU1F1 gene were also reported (Daga *et al.* 2013). Lan *et al.* (2009^{d}) reported 12 novel SNPs within the Inner Mongolian white cashmere goat POU1F1 gene, out which 6 SNPs were identified in exon-3. Besides these, polymorphisms were also reported at exon-3 region of POU1F1 gene in Iranian sheep breeds by Bastos *et al.* (2006), Negahdary *et al.* (2013), Jalil-Sarghale *et al.* (2013) and Mostafa *et al.* (2014). Polymorphism with two genotypes AA (78%) and AB (22%) in Romney sheep and AA (64%) and AB (36%) in Merino sheep was recorded by Ekegbu *et al.* (2018).

The statistical analysis (Table 2) did not reveal any significant differences between body weights of Osmanabadi goats belonging to TT and CC genotypes at birth, 3 and 6-months age. It indicated that there was lack of significant association between genotypes (TT and CC) at exon-3 locus of POU1F1 gene and body weights in Osmanabadi goat. However, comparatively higher body weights were recorded at birth, three-month and six-month body weight of TT goats. Daga *et al.* (2013) reported similar non-significant association between POU1F1 exon-3 polymorphism (C>T at nucleotide position 42) and milk production traits in Sarda goat. However, significant positive influence of POU1F1 exon-3 polymorphism on body weights at different age of Iranian sheep have been reported by Bastos *et al.* (2006), Negahdary *et al.* (2013), Jalil-Sarghale *et al.* (2013) and Mostafa *et al.* (2014).

Table 2. Association of the POU1F1 exon-3 genotypes with body weight of Osmanabadi Goat (mean±S.D.)

Body weight	Genotype TT (n = 62)	Genotype CC (n = 155)
Birth weight (kg)	2.329±0.495^{NS}	2.302±0.468^{NS}
3-month body weight (kg)	7.847±1.752^{NS}	7.708±1.798^{NS}
6-month body weight (kg)	11.999±1.495^{NS}	11.824±1.513^{NS}

NS, ($P<0.05$).

In Chinese goats, several POU1F1 (especially exon-6 and its flanking region) gene polymorphisms associated with growth, milk yield and wool fibre traits have been reported. Significant positive associations between POU1F1 gene and growth traits in sheep and goats were reported by Jiangzuo (2010), Wang *et al.* (2013), Ma *et al.* (2017) and Han *et al.* (2019). In addition to growth traits, POU1F1 gene variants were found to be associated with fibre production in Cashmere goats (Lan *et al.* 2009a, Lan *et al.* 2009d); wool traits in Makooei sheep (Negahdary *et al.* 2014); sheep and goat milk production (Lan *et al.* 2009^{c}, Mura *et al.* 2012 and Ozmen *et al.* 2014). Similarly, its association with litter size in Awassi sheep and Shaanbei White Cashmere goat was reported by AL-Khuzai and AL-Anbari (2018) and Zhu *et al.* (2019), respectively.

The POU1F1 gene mutations also exhibited a positive association with milk protein of cattle (Yan *et al.* 2011); growth and carcass traits of swine (Getmantseva *et al.* 2017). Even in chicken, POU1F1 gene polymorphisms were found to be associated with various production traits (Jiang *et al.* 2004, Nie *et al.* 2008, Manjula *et al.* 2018).

These research findings emphasize the significance of POU1F1 gene in augmenting productivity of various livestock species. However, scanty literature is available regarding association between POU1F1 gene and important economic traits of Indian small ruminants. Hence, it is essential to identify various genetic markers associated with production traits across Indian goat breed populations and incorporate them in goat breeding programme.

SUMMARY

The POU1F1 gene has been identified as one of the major gene for productivity, and its polymorphism is known to be associated with important production traits of various livestock. The present study was designed to investigate POU1F1 (exon-3) gene polymorphism and its association with body weight in Osmanabadi goat (n=217). The PCR-SSCP and DNA sequencing revealed single 'C' > 'T' transition at nucleotide position 42 in the third exonic region (225 bp) POU1F1 gene of Osmanabadi goat.

Genotyping revealed ‘TT’ and ‘CC’ genotypes with predominance of ‘C’ allele in the Osmanabadi goat population. Though no association between the said SNP and body weight was observed, these findings can be extended to find the genetic variability of POU1F1 gene associated with growth traits of various Indian goat breeds.

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