



Established SNPs in DGAT1 gene of exotic goat is absent in indigenous goat breeds

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DGAT1 (acyl CoA: diacylglycerolacyltransferase1) is important in higher eukaryotes for physiologic processes involving triacylglycerol metabolism. The DGAT1 gene is located on chromosome 14 in cattle (Grisart *et al.* 2002, Winter *et al.* 2002) and plays a key role in triacylglycerol synthesis by catalyzing the final step of triglyceride synthesis. A non conservative lysine to alanine substitution at amino acid position 232 in the mature polypeptide chain (K232A) could explain at least 50% of the QTL variation for some milk production traits in cattle (Winter *et al.* 2002). In particular, the presence of K allele was associated with higher fat yield and both fat and protein percentages, whilst the A-allele was responsible for higher milk yield. Angiolillo *et al.* (2007) characterized complete coding region of caprine DGAT1 gene in exotic goat but no SNP was identified. However, a single SNP involving a T to C substitution in intron 16 of caprine DGAT1 was reported by them. Thus in this work we characterized a 307bp region encompassing completely intron 15, exon 16, intron 16 and partially the 3' end of exon 15, and 5' end of exon 17 in the indigenous goat DGAT1 gene with the aim of seeing whether we are able to detect the reported SNP as well as some new one in DGAT1 of indigenous goat.

Blood samples (135), 45 each from Barbari, Beetal and Ganjam goat were randomly collected from several villages in their respective breeding tracts. Blood sample (5–6 ml) was obtained from jugular vein puncture in vacutainer tubes pretreated with 0.25% ethylene di amine tetra acetic acid (EDTA). The genomic DNA for PCR amplification was isolated from white blood cells using the standard phenol–chloroform extraction method (Sambrook *et al.* 1989) with minor modification. After checking the quality and quantity, DNA was diluted to final concentration of 100 ng/μl in water and stored at 4°C. The utilized primer pair (F: ctctgctcttccatgagg R: atgagtgcagccacacagc) and PCR

conditions were as described by Angiolillo *et al.* (2007). The PCR amplification was performed in a total volume of 25 μl containing ~ 100 ng of genomic DNA, 10pmol of each primer, 200μM of each dNTP, buffer with 1.5 mM MgCl₂ and 1U *Taq* DNA polymerase. Amplification was performed using thermal cycler with initial denaturation at 95 °C for 5 min followed by 35 cycle of 94°C for 45s, 60°C for 45s and 72°C for 45s with final extension of 5 min at 72°C. Amplified products were checked on 2% agarose gel (w/v) in 1X TAE buffer.

Sequence variation was visualized by PCR-SSCP (polymerase chain reaction –single strand conformation polymorphism). Ten microliters of each PCR product was added to 16 μl of loading dye (96% formamide, 0.01 M EDTA pH 8, 0.05% bromophenol blue, 0.05% xylene cyanol) and 10 μl of water. The samples were heated at 96°C for 10 min, cooled on ice for at least 5 min and loaded to a 10% polyacrylamide gel (38: 1 acrylamide: bisacrylamide). Electrophoresis was carried out at 4°C, 3200 v/h in 0.5X TBE buffer. The gels were subsequently fixed in 5% ethanol, stained with 0.2% AgNO₃ and revealed with 2% NaOH. All polymorphisms identified by SSCP were confirmed by direct sequencing in both directions on ABI 3100 genetic analyzer using BigDye terminator sequencing kit according to the manufacturer's instruction. The same primers used for the initial amplification of amplicons were used for this direct sequencing.

Nucleotide sequence of investigated region of DGAT1 (307bp) in Indian goat has been submitted to NCBI GenBank (Accession number GU908132). Multiple sequence alignments were performed with MegAlign program of LASERGENE software to identify SNPs. Cluster analysis of obtained sequence with that of other species was performed following Neighbour-Joining procedure using CLC Free Workbench software. The BLAST algorithm was used to search the NCBI Genbank (<http://www.ncbi.nlm.nih.gov>) databases for homologous sequences.

Intra-muscular fat content, also assessed as marbling of meat, represents important meat quality trait of economic importance. Gene coding DGAT1 has been mapped within

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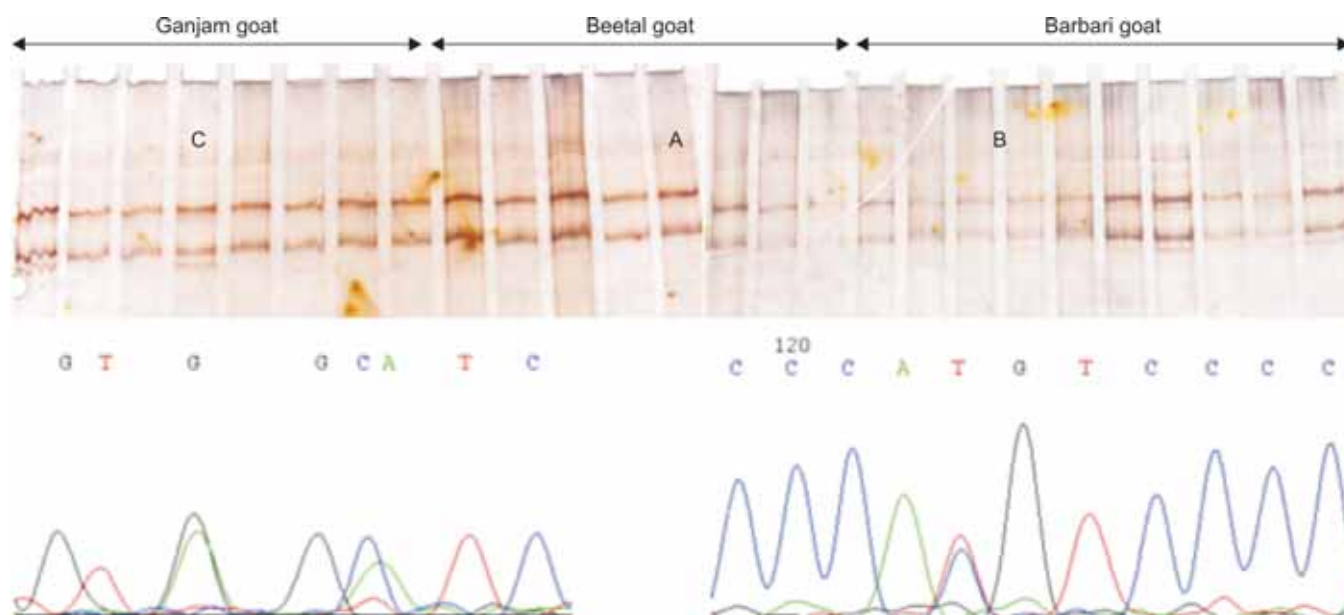


Fig 1. Band pattern for different SSCP variants and heterozygous nucleotides at position 4 (G/A) and 123 (T/C).

the marbling QTL of cattle in centromeric region of bovine chromosome14 (Thaller *et al.* 2003). Given the very limited number of QTL identification studies in small ruminants to date, utilizing observations from studies performed in cattle and applying the findings to small ruminants could be an alternative way that may provide insight into common genetic markers (Miltiadou *et al.* 2010). The PCR products of 45 DNA samples each from Barbari, Beetal and Ganjam goat breeds were analyzed on SSCP gels to identify variation in 307bp region as this constitutes the only reported polymorphic site in exotic goat. SSCP showed 3 different variants of this region in investigated Indian goat breeds. Out of these, A and C were observed in Ganjam, A in Beetal and B in Barbari breed (Fig. 1). However, all the 3 patterns of the SSCP came out to be the same on sequencing and sequence submitted to NCBI database (GenBank Accession No. GU908132). On finer inspection of chromatograms heterozygous sites were also observed. These were unambiguous with the presence of dual peaks in the chromatogram at position 4 (G/A) and 123 (T/C) of Ganjam goat. As discussed by Kathiravan *et al.* (2009) heterozygous nucleotides might cause different mobility during SSCP analysis resulting in ambiguous band patterns.

Number of nucleotide changes were recorded in caprine 307bp region as compared to cattle (AJ318490), which included 21 variations, 5 deletion and 3 additions. Transitions are much higher (90.5%) as compared to transversions

(9.5%). However, comparison of the observed sequences from different Indian goat breeds exhibited 100% similarity with the exotic goat (DQ380250). Polymorphism of the goat DGAT1 gene seems to be rather limited as sequencing of 1552bp of the caprine DGAT1 cDNA from 3 different exotic breeds did not reveal any polymorphic site too (Angiolillo *et al.* 2007). However, sequencing of 1 kb genomic fragment (from exon12–17) in these goats detected a single SNP at position 703 of the DQ380250 sequence. This SNP was a T to C transition located at intron16. It is important to note that even this SNP was not found in the investigated Indian goat breeds, where all individuals were monomorphic for the T nucleotide residue. Polymorphism in human DGAT1 gene is also moderate with two exonic synonymous SNPs and eight SNPs in non-coding regions (<http://www.ensembl.org>). In strong contrast to human and goat, there is remarkable level of polymorphism in the bovine DGAT gene with as much as 17 single base exchanges (Winter *et al.* 2002) of which 10 are exonic. The maintenance of a larger pool of DGAT1 alleles in cattle than in human or goats might be due to the fact that several of these bovine alleles including K232A polymorphism, are positively selected due to their favorable associations with milk traits (Kuhn *et al.* 2004). Explored 307bp sequence covering completely intron 15, exon 16, intron 16 and partial exon15 and exon 17 shared a high nucleotide identity with other

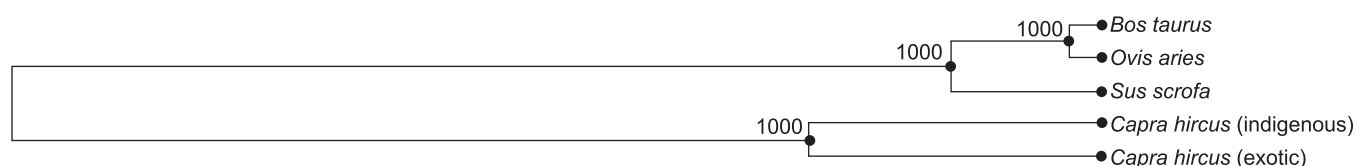


Fig 2. Phylogenetic tree between indigenous goat and other ruminants based on sequenced DGAT1 region.

mammalian orthologous sequences such as bovine (92%) and pig (80%).

Construction of phylogenetic tree based on sequenced region of indigenous goat DGAT1 gene revealed the clustering of exotic and indigenous goat on one node whereas, pig formed an altogether different node.

SUMMARY

The essential role of DGAT1 in fat metabolism makes this gene an interesting candidate for explaining the genetic variation of fat content in milk or meat. To the best of our knowledge, this is the first study conducted with 3 different breeds of indigenous goat demonstrating absence of only SNP reported in the DGAT1 gene of exotic goat breeds. In the near future this knowledge will help for investigating the polymorphism of this gene in other Indian goat breeds aiming to identify some new variants of this gene and association with meat quality traits based on fat content in goat meat.

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