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Short Communication
Molecular Characterization of Acyl CoA: Diacylglycerol O-acyltransferase 1 (DGAT1) in Sheep and its Comparison with Other Ruminants

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Abstract

Background: Triacylglycerols are quantitatively most important storage form of energy for eukaryotic cells. Acyl CoA: Diacylglycerol O-acyltransferase 1 (DGAT1, EC 2.3.1.20) is a microsomal enzyme that catalyzes the terminal and only committed step in triacylglycerol synthesis by using diacylglycerol and fatty acyl CoA as substrates. **Objective:** The present study was undertaken with the objectives to study the molecular differences in DGAT1 among different ruminants. **Materials and Methods:** The DGAT1 was partially amplified (411 bp) by designing gene specific primers and confirmed by sequencing the amplicon and its comparison with DGAT1 gene of other ruminants (i.e., bovine, buffalo and goat). **Results:** Comparative study of DGAT1 among different ruminants reveals different level of mutation with respect to its gene sequence (0.0-0.4%) and protein sequence (0.0-1.2%). **Conclusion:** Molecular differentiation among different ruminants reveals that bovine and buffalo are very close to each other and sheep is related to goat. Further study is underway to detect polymorphism and associated them with milk fat trait in sheep.

Key words: Diacylglycerol, ruminant, polymorphism, milk, triglycerides, DGAT1

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diacylglycerol O-acyltransferase 1 (DGAT1) is one of the key enzymes in controlling the synthesis rate of triglycerides (TAG) in adipocytes. TAG is essential for many physiological processes and its metabolism is widely conserved in nature[1]. The DGAT1 enzyme also plays a significant role in dietary TAG absorption in the small intestine[2]. The DGAT1 is the only enzyme in the pathway that is thought to be exclusively committed to TAG synthesis and it is considered a key enzyme in this reaction[3]. Since, this gene maps to the QTL for milk fat percentage on the centromeric region of BTA14 it has been studied as a candidate gene for association with milk fat content in bovine. Mutation analysis in cattle has revealed 19 polymorphic sites within the DGAT1 gene[4]. Among these polymorphisms, the GC/AA exchange at positions 10433/10434 (GenBank accession No. AJ318490) in exon 8, resulting in the K232A amino acid exchange was found to affect the milk fat content[5-7] and has been extensively studied in several cattle populations[8]. The effects of DGAT1 and K232A on milk fat content differed across populations[6,9] suggesting that further variations in the genomic region of DGAT1 may be involved. In this context, polymorphism in the promoter region have also been reported to have functional relevance for DGAT1 transcription[7,10]. In this study, attempt has been made to study the molecular differences in DGAT1 among different ruminants that have different percentage of milk fat. Furthermore, the impact of variation in gene and protein sequence if any detected among various ruminants was also assessed.

MATERIALS AND METHODS

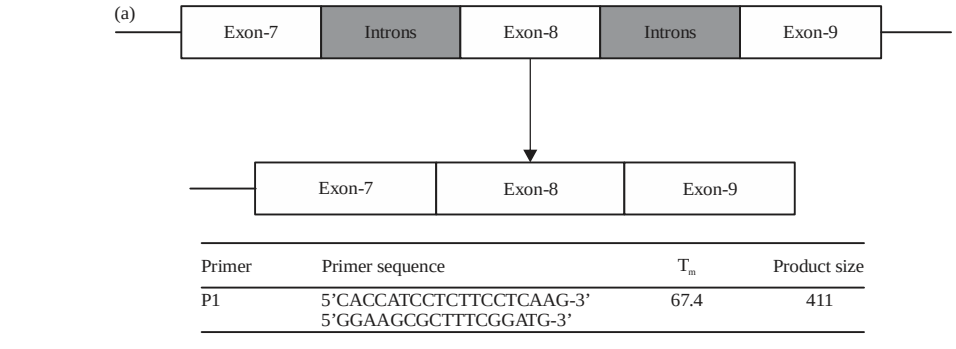
Ethical approval: The experiment was approved by Institutional Animal Ethics Committee.

Experimental materials: The present study was conducted on a total of about 20 animals belonging to Indian sheep breed. The blood samples were taken from the breeds reared in different places of Haryana.

Blood collection and DNA isolation: Approximately, 10 mL venous blood was collected from each animal using 0.5 mL of 2.7% EDTA as an anticoagulant. The sample were brought to the laboratory in a double walled ice box containing ice with cool pack and stored at -20°C till the isolation of DNA. Genomic DNA was isolated from blood following the protocol of Sambrook and Russell[11]. The quality and purity of the isolated DNA was determined by agarose gel electrophoresis and spectrophotometry, respectively. The DNA samples giving a single band on agarose gel with A_{260}/A_{280} around 1.8 were used for further analysis. The DNA was quantified by comparing with Lambda DNA of known concentration in 0.8% agarose gel.

Amplification of DGAT1 gene: A fragment of 411 bp of the DGAT1 gene containing the K232A substitution was amplified by using a set of forward (f: 5'CACCATCCTCTTCCTCAAG-3') and reverse (R: 5'GGAAGCGCTTTCGGATG-3') primers[7]. For amplification, 50 µL of PCR reaction was prepared by adding 10 pM of each primer, 0.2 µM of each dNTPs, 1.5 mM $MgCl_2$, 10xPCR assay buffer, 130 ng DNA template and 1 U Taq DNA polymerase. The amplification was carried by using a programmable thermal controller (PTC-100, MJ Research) with the following conditions: Initial denaturation of 5 min at 94°C followed by 35 cycles of 94°C for 45 sec, 53°C for 45 sec and 72°C for 45 sec with a final extension step of 72°C for 10 min. For sequencing, the PCR products were run in 1.5% agarose gel and product band was eluted using QIA quick gel extraction kit for purification. The amplified and purified product were sequenced by Sanger method by outsourcing (Xclaris lab, Ahmedabad, India).

Comparison of DNA and protein sequence: The gene sequences obtained (based on forward and reverse primers) from different ruminants were assembled utilizing overlapping sequences (consisting of 411 bp) and edited to a 244 bp of partial DGAT1 gene (excluding introns) comprising exon 7, 8 and 9 (Fig. 1a). These sequences were aligned using multiple alignment programs, Clustal W (http://www.ebi.ac.uk/Tools/msa/clustalw2/) with DNA weight matrix and multiple parameters like gap opening 10.0, gap extension 0.20 and transition weight 0.50. A phylogenetic tree based on similarity coefficients generated by neighborhood joining method was performed[12]. Furthermore, all DNA sequences obtained were translated to protein sequence using EMBL transseq (http://www.ebi.ac.uk/Tools/st/). These protein sequences were further aligned using multiple alignment programs, Clustal W (http://www.ebi.ac.uk/Tools/msa/clustalw2/) with protein weight matrix and default parameters for gap opening and extension. A phylogenetic tree based on similarity coefficients was also generated[13].



Primer	Primer sequence	T_m	Product size
P1	5'CACCATCCTCTTCCTCAAG-3' 5'GGAAGCGCTTTCGGATG-3'	67.4	411

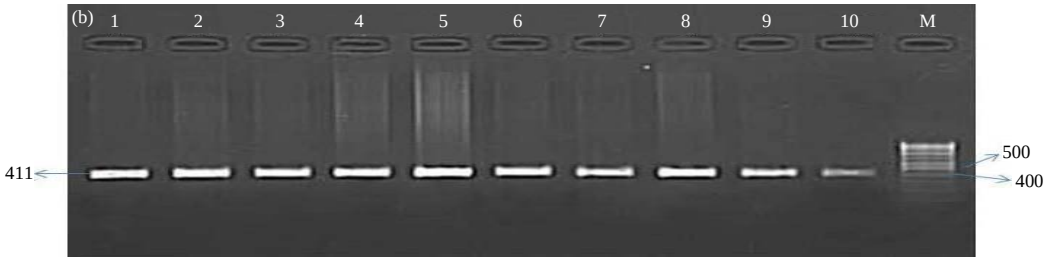


Fig. 1(a-b): Primer and partially amplified DGAT1 from different ruminants, (a) A gene specific primer was designed based on exon 7, 8 and 9 sequence from bovine with a product size of 411 and (b) A representative gel picture showing the amplified product of DGAT1 from sheep and other animals, lane 1-5 corresponds to amplified gene form sheep, 6-8 from goat and 9-10 from buffalo, while M is 100 bp DNA ladder

RESULTS AND DISCUSSION

The DGAT1 plays a fundamental role in TAG synthesis by using diacylglycerol and fatty acyl CoA as substrates. In cattle, a non synonymous substitution in DGAT1 influences enzyme activity having a major effect on milk composition[14]. These features make DGAT1 an interesting candidate gene to explain the phenotypic variation of fat percentage and other milk related traits in other ruminants species such as sheep and goats. The sequence of the Chinese water buffalo (*Bubalus bubalis*) DGAT1 gene has recently been reported[15]. The GC/AA substitution in exon 8 of the DGAT1 gene in cattle has been associated with milk fat content[4,14,16]. The sequence analysis of DGAT1 region in six breeds of cattle (*Bos indicus*), five breeds of buffalo (*Bubalus bubalis*) revealed fixed DGAT1K allele. The sequence of desired region of control individuals revealed heterozygous condition for reported mutation. No variation was observed in all the 11 breeds of cattle and buffalo in the study reported by Tantia *et al.*[17]. Genetic deletion of DGAT1 in mice revealed that this enzyme is not essential and that DGAT1 knockout (DGAT1 KO) mice have reductions in TG levels in many tissues including adipose tissue, when fed a high-fat diet[18]. In the recent study, exon 7, 8 and 9 of DGAT1 gene from different Indian sheep breed was partially amplified using DGAT1 specific primers. High reproducible and distinct band of size 411 was obtained (Fig. 1b). The amplified products were confirmed by nucleotide sequencing (FJ-415875) using the same set of primers and compared with the reference gene of DGAT1 from bovine. The amplified fragments of DGAT1 correspond to part of exon 7, 8 and 9 (Fig. 1a). A relatively small variation was detected among the DGAT1 gene sequences obtained from different ruminant species. All the gene sequences were compared using multiple sequence alignment programs and level of variation in gene sequences is represented in Fig. 2a. Genomic variation among various ruminants ranges from 0.0-0.8% in comparison to DGAT1 reference sequence obtained from bovine. The goat has the minimum percentage of variation (0.0%) followed by bovine and buffalo (0.4%). Furthermore, the sequences were used to generate a dendrogram (Fig. 2b). It reveals that buffalo and reference are very close to each other, similarly sheep and goats are closely related. Based on the comparison of protein sequences of DGAT1, a relatively small variation was detected among the different ruminants (Fig. 3a). Overall the rate of variation varies in between 0.0-1.2%, with bovine (EU077528), buffalo (FJ-014704) having the highest variation (1.2%) and followed by goat (FJ-415876) with no variation. The dendrogram (Fig. 3b) obtained from pairwise comparison of protein sequence also reveals similar findings as mentioned above:

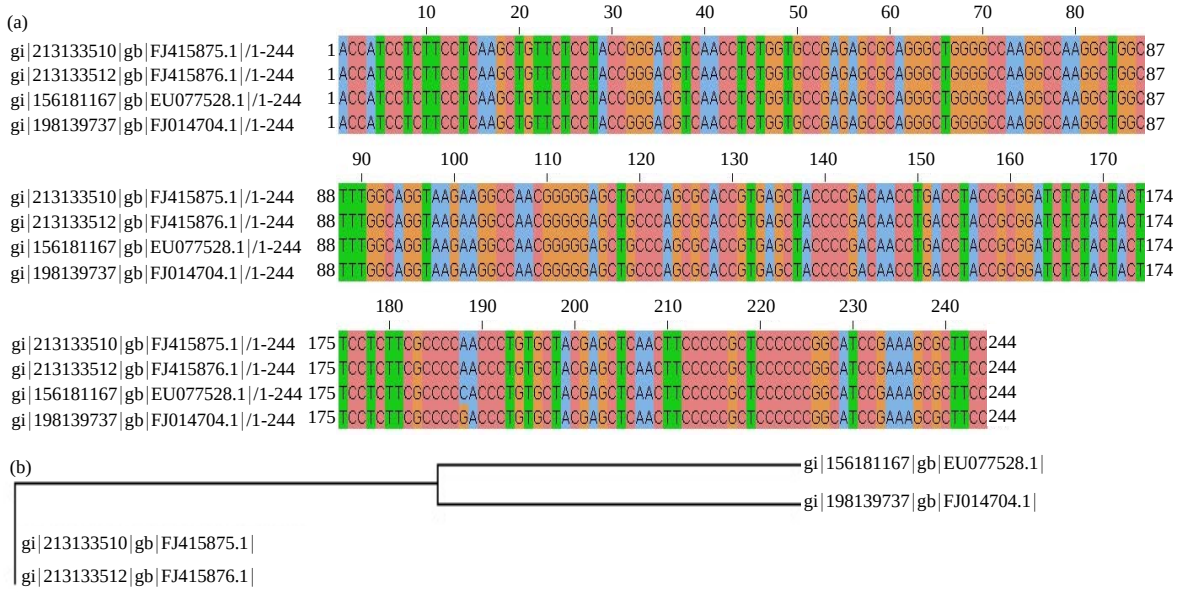


Fig. 2(a-b): Comparison of DGAT1 gene sequences, (a) Multiple sequence alignment of DGAT1 sequences of different ruminants were aligned using program, changed nucleotide were represented in white box, only few mutations were detected between different ruminats and (b) The dendrogram representing the similarity between DGAT1 gene sequences obtained from different ruminants

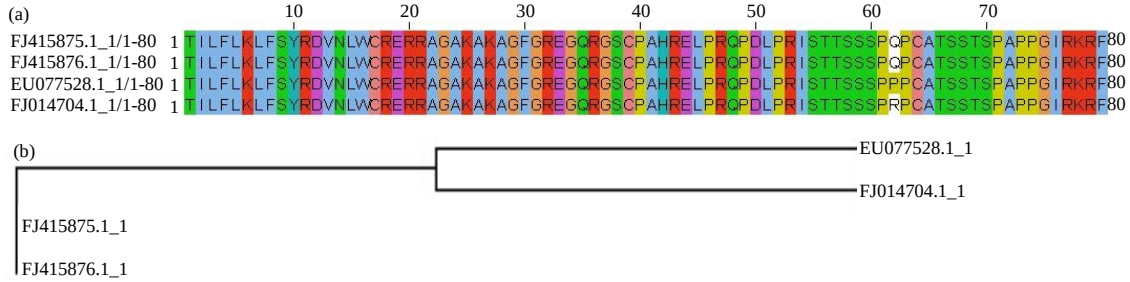


Fig. 3(a-b): Comparison of DGAT1 Protein sequences, (a) The DGAT1 sequences obtained from different ruminants were aligned using multiple sequence alignment program, changed amino acids were represented in white box, only few mutations were detected between different ruminats and (b) The dendrogram representing the similarity between DGAT1 protein sequences obtained from different ruminants

Buffalo and reference are very close to each other, similarly, goat and sheep are closely related. The variation in gene as well as protein sequences of DGAT1 might be associated with variation in milk fat content. The significant variation in binding site among different breeds of buffaloes may be associated with variation in milk content.

CONCLUSION

The findings described here should be useful in determining the role that DGAT1 plays in regulating milk content.

SIGNIFICANCE STATEMENT

The goat has the minimum percentage of variation followed by bovine and buffalo. It reveals that buffalo and reference are very close to each other, similarly sheep and goats are closely related.

Overall the rate of variation varies in between 0.0-1.2%, with bovine (EU077528) and buffalo (FJ-014704) having the highest variation (1.2%) and followed by goat (FJ-415876) with no variation.

The variation in gene as well as protein sequences of DGAT1 might be associated with variation in milk fat content.

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