Veterinary Research 6 (3): 68-71, 2013
ISSN: 1993-5412

Single Nucleotide Polymorphism in DGAT1 Locus of Indian Cattle and Buffalo Breeds

R.T. Venkatachalapathy, Arjava Sharma, T.K. Bhattacharta and Soumi Sukla
Animal Genetics Division, Indian Veterinary Research Institute,
Izatnagar, Bareilly, UP 243122, India

Abstract: An investigation was carried out to identify single nucleotide polymorphism in DGAT locus of Indian cattle and buffalo breeds. DNA samples were collected from 368 animals belonging to four breeds of riverine buffalo (*Bubalus bubalis*) and three breeds of Indian cattle (*Bos indicus*). Polymerase chain reaction: Single Strand Conformational Polymorphism was carried out to detect single nucleotide polymorphism in 189, 201, 178, 257, 231 and 411 bp fragments from four breeds of buffalo. Two SSCP patterns, CC and CT were observed in the 411 bp fragment of buffalo and Indian cattle. Frequency of CC ranged from 0.41-0.59 in buffalo and 0.73-0.82 cattle breeds. The overall frequency of CC and CT genotypes were 0.51, 0.49 in buffalo and 0.76, 0.24 in cattle, respectively. Frequency of C allele was 0.74 and 0.88 in buffalo and cattle population, respectively. Sequencing of different alleles revealed a transition mutation C to T at 14th position of intron-8. PCR-SSCP of 189, 201, 178, 257 and 231 bp fragments showed monomorphic patterns in all the breeds of buffalo and cattle.

Key words: *DGAT1* gene, polymorphism, buffalo and SSCP, cattle, India

INTRODUCTION

Diacylglycerol O-Acyltransferase 1 (DGAT1) belongs to a gene family of three known members, the other two represent ACAT1 (Acyl-CoA:cholesterol acyltransferase and ACAT2 (Acyl-CoA:cholesterol acyltransferase. DGAT1 was initially mapped to human chromosome 8q and in mice on 15q by FISH analysis (Cases *et al*., 1998). Several genome scans in cattle (Coppieters *et al*., 1998; Heyen *et al*., 1999; Ashwell *et al*., 2001) revealed a putative QTL on the centromeric end of bovine chromosome 14 with a strong effect on milk fat yield and percentage as well as for milk yield and milk composition. Subsequently, the chromosome segment harboring the QTL was fine mapped to <9.5 cm (5 cm) flanked by the closest non-identical markers ILSTS039 and BULGE004 (Riquet *et al*., 1999). The interval was refined to a 3 cm segment on the centromere, flanked by the markers BULGE09 and BULGE11 (Looft *et al*., 2001; Farnir *et al*., 2002). The comparative positional cloning led to the identification of DGAT1 as candidate gene within the region of a QTL on bovine chromosome 14 for milk fat percentage and other milk yield traits (Grisart *et al*., 2002). Expression of mRNA and activity of DGAT1 were ubiquitous in mouse and human tissues with the highest levels in mammary gland, liver, small intestine and adipose tissue (Cases *et al*., 1998; Farese *et al*., 2000; Smith *et al*., 2000). *DGAT1* gene encodes the Acyl CoA:Diacylglycerol O-Acyltransferase (DGAT), an integral membrane bound protein in plays a fundamental role in the metabolism of cellular glycerolipids.

MATERIALS AND METHODS

DNA samples were collected from 548 animals belonging to four breeds of Indian riverine buffalo (*Bubalus bubalis*) and three breeds of Indian Zebu cattle (*Bos indicus*) from government farms situated in different geographical locations of the country. Genotyping was carried out by SSCP to find out different allelic variants in DGAT1 locus.

Collection of blood samples: Total 10 mL of venous blood was collected under sterile conditions, from the jugular vein of animal in a 15 mL polypropylene centrifuge tube containing 0.5 mL of 2.7% EDTA solution as anticoagulant. The tube was tightly capped and shaken gently to facilitate thorough mixing of blood with the anticoagulant. Blood samples were transported to the laboratory in an icebox containing ice packs and stored in the refrigerator at -20°C till the isolation of DNA.

DNA preparation: Genomic DNA was isolated from the frozen blood samples using phenol-chloroform extraction

Corresponding Author: R.T. Venkatachalapathy, Centre for Pig Production and Research,
Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, 680651 Kerala, India

Table 1: Primer sequences for *DGAT1* gene amplification

Sequence (5'-3')	Name	Temperature (°C)	Region amplified	Fragment size (bp)
GACACAGACAAGGACGGAGA	F	59	Partial E1 and partial I1	189
ACAGCTGTGCACCAAGGTC	R	60	- do -	
AAGCAACGCACGGTTATTTC	F	60	E3 to partial E4	201
CAATGACCAGGCACAGAGC	R	60	- do -	
CCAGGTGGTGTCTCTGTTCC	F	60	Partial E4-E5	178
CAGCCACGGCAAAGATATTG	R	61	- do -	
GGAGCTCTGACGGAGCAG	F	60	E6 to partial E7	257
CCGGTAGGAGAACAGCTTGA	R	60	-do-	
GCACCATCCTCTTCCTCAAG	F	62	Partial E7 to partial E-9	411
GGAAGCGCTTTCGGATG	R	62	- do -	
ACATGGACTACTCCCGCATC	F	60	Partial E12-E13	231
CACCAGTCCCGGTAGAACTC	R	59	- do -	

method (Sambrook *et al*., 2001) and samples were checked for its quality, purity and concentration.

Polymerase chain reaction: Primers for amplification of the genomic DNA were designed using DNASIS MAX Software (Hitachi Miraibio Inc., USA) from the cattle nucleotide sequence (NCBI, Acc. No. AY065621). Six fragments (189, 201, 178, 257, 231 and 411 bp) spanning from exon-1-13 of *DGAT1* gene were amplified using specific set of primers (Table 1). PCR was performed in a thermal cycler (PTC 200, MJ Research Ltd. San Francisco, USA). PCR reactions were carried out in a 25 μL volume using 50 ng of genomic DNA, 1x PCR buffer containing 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 pm of each primer, 5% DMSO and 0.5 U Hotstar Taq polymerase (QUIAGEN, Hilden, Germany). Addition of 5% DMSO was facilitated the proper amplification of GC rich regions of *DGAT1* gene. PCR condition included 15 min at 95°C, 35 cycles of 60 sec at 94°C, 60 sec at 62°C, 60 sec at 72°C and final extension at 72°C for 411 bp fragment. The other five fragments were amplified with same profile with reduction in the initial denature for 12 min and cyclic time to 45 sec each. Amplified PCR products were loaded into the wells of agarose gel with a standard 100 bp DNA ladder (GeneRuler, MBI Fermentas, Germany) as a marker to check the size of the fragment. Electrophoresis was carried out at the rate 6 Volts/cm in 1X TBE buffer. Gels were stained with ethidium bromide and visualized under UV light.

Single stand conformational polymorphism: The amplified fragments were mixed with SSCP loading buffer in the ratio of 1:4 (3 μL sample with 12 μL dye), denatured at 95°C for 5 min and immediately snapped in ice. The products were run in 12% polyacrylamide gel at 4°C for 22 h at 200 V for 411 bp fragment and 10 h for other fragments (189, 201, 178, 257 and 231 bp). The composition of poly acrylamide gel was 30% acrylamide/Bis-acrylamide (49:1)-12 mL, 10% Ammonium per sulphate-100 μL, TEMED-20 μL, 5% Glycerol-1.5 mL and 1x TBE-16.5 mL.

Gels were stained with silver nitrate as per the procedure described by Bassam *et al.* (1991) and SSCP fragments were visualized directly. The genotypes were detected directly by observing SSCP pattern of each sample in the gels. The allele and genotype frequencies were estimated by direct counting method (Falconar and Mackay, 1998).

Representative samples from different genotypes were sequenced to find out the nucleotide differences between different alleles by automated sequencer (ABI prism) using Sanger's dideoxy chain termination method.

RESULTS AND DISCUSSION

Six fragments, 189, 178, 257, 201, 231 and 411 bp were amplified and five shown monomophic pattern in all Indian cattle and buffalo breeds. Three fragments 189, 178 and 257 bp showed four typical bands (Fig. 1a-c). The other two fragments, 201 and 231 bp showed typical three band pattern (Fig. 2a and b). PCR-SSCP of 411 bp fragment, corresponding to the partial exon 7-9 regions exhibited polymorphism and the pattern with two bands was identified as CC and three bands as CT genotypes (Fig. 3). The genotypic and allelic frequencies based on PCR-SSCP pattern are presented in the Table 2. The sequencing analysis of different alleles revealed a transition mutation C to T at 14th position of intron-8, correspond to 275th position of the fragment.

These patterns were identified as CC and CT genotypes. CC genotype was the lowest in Surti (0.41) and the highest in Bhadawari (0.59) whereas CT genotype was the highest in Mehsana (0.51) and the lowest in Bhadawari (0.41). The frequency of A allele ranged from 0.66 (Surti) to 0.80 (Bhadawari) with the overall frequency being 0.74. The frequency of C allele ranged from ranged from 0.86-0.91 in cattle.

The sequencing of representative samples revealed the transitional mutation of C to T at 275th position of fragment which corresponds to 14th position of intron-8. The detected SNP has not been reported in cattle or other species. The nucleotide substitution from C to T observed

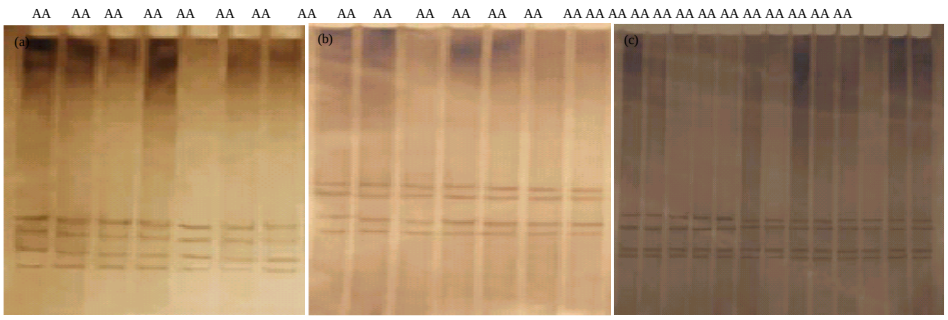

Fig. 1: a) PCR-SSCP pattern of 189 bp; b) 178 bp and c) 257 bp fragments of *DGAT1* gene 12% PAGE

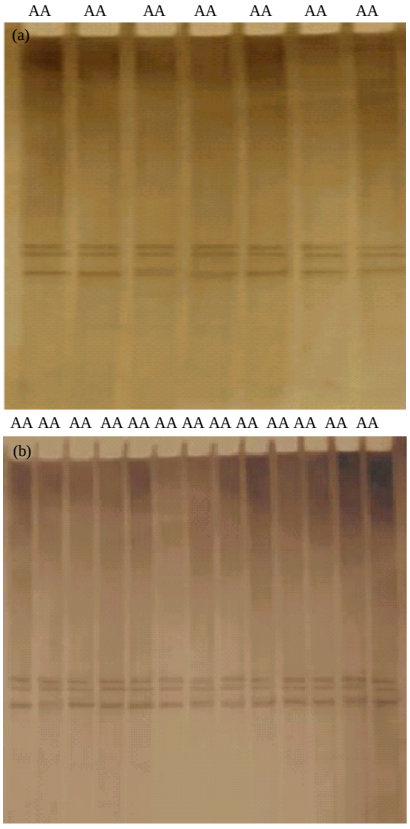

Fig. 2: a) PCR-SSCP pattern of 201 bp and b) 231 bp fragment of *DGAT1* gene 12% PAGE

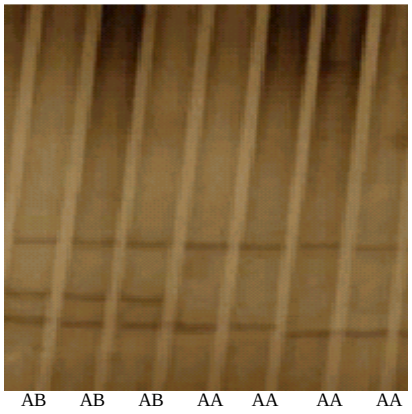

Fig. 3: PCR-SSCP pattern of 411 bp fragment of *DGAT1* gene 12% PAGE

Table 2: Genotype and allelic frequencies of 411 bp fragments of *DGAT1* gene in buffaloes based on PCR-SSCP pattern

	Genotypic frequency		Allelic frequency	
Species and breed	CC	CT	C	T
Buffalo (*Bubalus bubalis*)				
Bhadawari (54)	0.59 (32)	0.41 (22)	0.80	0.20
Mehsana (71)	0.49 (35)	0.51 (36)	0.74	0.26
Murrah (57)	0.54 (31)	0.46 (26)	0.77	0.23
Surti (56)	0.41 (23)	0.49 (33)	0.66	0.34
Overall	0.51 (121)	0.49 (117)	0.74	0.26
Cattle (*Bos indicus*)				
Hariana (40)	0.73 (29)	0.27 (11)	0.86	0.14
Sahiwal (40)	0.75 (30)	0.25 (10)	0.87	0.13
Nimari (40)	0.82 (32)	0.18 (08)	0.91	0.09
Overall (120)	0.76 (91)	0.24 (29)	0.88	0.12

Numbers in the parentheses denote the sample size

in smaller proportion of cattle compared to buffaloes. No polymorphism was detected in the screened exonic regions of *DGAT1* gene. Five out of six fragments showed monomorphic patterns indicating highly conserved nature of this gene. Similar findings were also made in zebu breeds using PCR RFLP (Kaupe *et al*., 2004). This also shows the fundamental role of the gene in highly important physiological functions such as intestinal fat absorption, lipoprotein assembly, adipose tissue formation and lactation. If the gene shows high polymorphism, this may alter the functioning of the DGAT enzyme to a great extent and the vital processes of milk secretion might have been affected as evidenced in the *DGAT1* gene deficient mice that were not able to secrete milk, though survived (Smith *et al*., 2000). This might be the probable reason behind the conserved nature across the species and its evolutionary importance. Supportive evidences were also found in some studies involving cattle *DGAT1* gene (Grisart *et al*., 2002; Winter *et al*.,

2002) where 17 haplotypes were found but only two in the exonic region and one being silent. Though nucleotide substitution were reported in 22 positions of buffalo DGAT1 coding sequence from that of cattle only seven resulted in the amino acid replacement in the peptide sequence (Venkatachalapathy *et al*., 2008). The detected SNP was located in non-coding region without a direct effect on peptide sequence, it maight influence the gene expression affecting rate of mRNA splicing (Hastings and Krainer, 2001). Hence, a critical association study encompassing all regions of the *DGAT1* gene in a large population might reveal its importance in buffalo and Zebu cattle.

CONCLUSION

DGAT catalyses the only committed final step in the triglyceride synthesis and presumed to be rate-limiting in lipid metabolism (Cases *et al*., 1998). It is an important microsomal enzyme in higher eukaryotes for physiological processes involving triacylglycerol metabolism such as intestinal fat absorption, lipoprotein assembly, adipose tissue formation and lactation. Surprisingly, DGAT1 knockout mice were not able to secrete milk, most likely because of deficient triglyceride synthesis in the mammary gland (Smith *et al*., 2000). Thus, both functional and positional data made DGAT1, a promising candidate gene for milk production traits and in particular for fat content in bovines. India buffaloes and cattle breeds are well known for its milk quality, chiefly for fat content, they are able to thrive under harsh climatic conditions and resistant to many diseases. Therefore, the present investigation was carried out to find out variable positions at DGAT1 locus in cattle and buffalo population.

REFERENCES

Ashwell, M.S., C.P. van Tassell and T.S. Sonstegard, 2001. A genome scan to identify quantitative trait loci affecting economically important traits in a US Holstein population. J. Dairy Sci., 84: 2535-2542.

Bassam, B.J., G. Caetano-Anolles and P.M. Gresshoff, 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem., 196: 80-83.

Cases, S., S.J. Smith, Y.W. Zheng, H.M. Myers and S.R. Lear, 1998. Identification of gene encoding an acyl CoA: Diacyl glycerol acyltransferase, a key enzyme in triacylglycerol synthesis. Proc. Natl. Acad. Sci., 95: 13018-13023.

Coppieters, W., J. Riquet, J.J. Arranz, P. Berzi and N. Cambisano *et al*., 1998. A QTL with major effect on milk yield and composition maps to bovine chromosome 14. Mamm. Genome, 9: 540-544.

Falconar, D.S. and T.F.C. Mackay, 1998. Introduction to Quantitative Genetics. 4th Edn., Addison Wesley Longman Ltd., England pp :1-2.

Farese, R.V.Jr., S. Cases and S.J. Smith, 2000. Triglyceride synthesis: Insights from the cloning of diacylglycerol acyltransferase. Curr. Opin. Lipidol., 11: 229-234.

Farnir, F., B. Grisart, W. Coppieters, J. Riquet and P. Berzi *et al*., 2002. Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: Revisiting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. Genetics, 161: 275-287.

Grisart, B., W. Coppieters, F. Farnir, L. Karim and C. Ford *et al*., 2002. Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine *DGAT1* gene with major effect on milk yield and composition. Genome Res., 12: 222-231.

Hastings, M.L. and A.R. Krainer, 2001. Pre-mRNA splicing in the new millennium. Curr. Opin. Cell Biol., 13: 302-309.

Heyen, D.W., J.I. Weller, M. Ron, M. Band and J.E. Beever *et al*., 1999. A genome Scan for QTL influencing milk production and health traits in dairy cattle. Physiol. Genomics, 1: 165-175.

Kaupe, B., A. Winter, R. Fries and G. Erhardt, 2004. DGAT1 polymorphism in *Bos indicus* and *Bos taursus* cattle breeds. J. Dairy Res., 71: 182-187.

Looft, C., N. Reinsch, C. Karall-Albrecht, S. Paul and M. Brink *et al*., 2001. A mammary gland EST showing linkage disequilibrium to a milk production QTL on bovine Chromosome 14. Mamm. Genome, 12: 646-650.

Riquet, J., W. Coppieters and N. Cambisano, 1999. Fine mapping of quantitative trait loci by identity by descent in outbred populations: Application to milk production in dairy cattle. Proc. Nat. Acad. Sci. USA., 96: 9252-9257.

Sambrook, J., E.F. Fritsch and T. Maniatis, 2001. Molecular Cloning: A Laboratory Manual. 3rd Edn., Cold Spring Harbor Laboratory Press, New York.

Smith, S.J., S. Cases, D.R. Jensen, H.C. Chen and E. Sande *et al*., 2000. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. Nat. Genet., 25: 87-90.

Venkatachalapathy, R.T., Sharma, Arjava, Sukla, Soumi and T.K. Bhattacharya, 2008. Cloning and characterization of DGAT1 gene of Riverine buffalo. Mitochondrial DNA, 19: 177-184.

Winter, A., W. Kramer, F. Werner, S. Kollers and S. Kata *et al*., 2002. Association of a lysine232/alanine polymorphism in a bovine gene encoding acyl-coA: Diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. Proc. Natl. Acad. Sci., 99: 9300-9305.