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SNP Genotyping of DGAT1 Gene in *Bubalus bubalis*

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Present study was conducted to validate six out of nineteen earlier reported [20] Single Nucleotide Polymorphisms (SNPs) in acyl-CoA: diacylglycerol acyltransferase1 (DGAT1) - intron I gene fragment in Mehsana buffalo, to explore some novel SNPs in the selected gene fragment of this species and finally analyze the correlation of the considered and explored SNP's with milk fat percentage and milk yield. Animals were ranked according to their production in terms of milk yield and milk fat percentage and clubbed into two tail classes. DGAT1- intron I of Mehsana buffalo was amplified from nucleotide position 1511 to 2657, giving an amplicon size of 1146bp, to cover six out of nineteen earlier reported SNPs at nucleotide positions viz. 1606, 1784, 1875, 2141, 2217 and 2394. All the SNPs except 2217 were found to be monomorphic and SNP 1606 exhibited allele C instead of wild allele T as reported in an earlier study (Mishra et. al. 2007). Four novel SNPs at nucleotide positions 2099, 2214, 2219 and 2370 were detected. All the SNPs were correlated with MFP and MY, however, no significant association of any SNP considered and explored in the present study was found with any of the two milk production traits. The study suggests that the SNPs analyzed do not have any influence on milk yield and milk fat percentage and might be used as genetic markers for Mehsana buffaloes.

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INTRODUCTION

Milk yield as well as milk components are subjected to considerable individual variation within a particular breed of species. Since Milk Fat Percentage (MFP) is a quantitative trait, better selection and breeding methods for the Indian buffalo breeds can increase the milk production to meet the growing demand of expanding human population of India. Presence of Quantitative Trait Loci (QTL) in the centromeric end of chromosome 14 has been reported to have a major effect on milk fat contents in dairy cattle [1-5]. One of the important genes located in the proximal region of chromosome 14 in cattle is acyl-CoA: diacylglycerol acyltransferase1 (DGAT1), which has been recently identified as a strong candidate gene for milk production traits [6-7]. DGAT1 is a microsomal enzyme catalyzing the addition of fatty acyl Co A to 1, 2, diacylglycerol to yield CoA plus triglycerol and is important in lipogenesis in many tissues, including mammary gland [8]. Nucleotide sequence analysis of this gene has revealed that the bovine DGAT1 gene includes 17 exons of variable size encoding a 489 amino acid protein [9]. Several workers have independently reported

the association of K232A (Lysine to alanine) substitution in exon 8 of DGAT1 gene with milk production traits especially MFP and Milk Yield (MY) in different breeds of *Bos taurus* [10-18]. However, absence of K232A substitution in Indian buffalo breeds was recently reported [19-20]. Interestingly, 19 SNPs in intron region of DGAT1 gene in buffalo have been reported in various Indian buffalo breeds which suggest a clue that these SNPs might be useful as genetic markers or associate with MFP and MY [20]. Keeping this perspective in view, the present work was aimed to ascertain the prevalence of six (1606, 1784, 1875, 2141, 2217 and 2394) out of 19 reported SNPs in DGAT1 gene in Mehsana buffaloes and assess their association with MFP and MY.

MATERIALS AND METHODS

Blood samples from 100 Mehsana buffaloes, unrelated and randomly selected, were collected under the project "Parentage Verification of Progeny Tested Daughters" in collaboration with DURDA (Dudhsagar Research and Development Association), Dudhsagar dairy, Mehsana. The animals were ranked according to their MFP and MY into two tail classes viz. Highest (n = 25) and lowest MY class (n = 25) and highest (n = 25) and lowest MFP class (n = 25). Statistical analysis revealed a significant difference ($p < 0.05$) between high and low producing groups for milk yield as well as milk fat percentage (Table 1). Since some of animals were common to MFP and MY groups, total animals studied were 80 instead of 100. Genomic DNA was extracted from blood by Phenol: Chloroform method. The concentration and the purity of the DNA obtained were assessed by spectrophotometery and electrophoresis in 0.8% agarose gel respectively. The 1146bp DGAT1 gene segment of *Bubalus bubalis* was amplified by PCR using a pair of primers designed using a reference sequence (NCBI accession number DQ886485). The primers, Forward - TGG ATT TGG GGT CAC TTT ; Reverse- GTC CCT CTA CCA GCC TTC C, were designed to include six earlier reported SNPs located at 1606, 1784, 1875, 2141, 2217 and 2394 nucleotide positions of DGAT1 gene in buffalo. Approximately 90 ng of genomic DNA was amplified in 25µl PCR reaction consisting of 2X Master mix (MBI Fermentas). Thermal cycling was performed on Veriti (Applied Biosystems) with an initial denaturing step at 950C for 10mins followed by 40 cycles of denaturation at 95^0C at 60 seconds, primer annealing at 65^0C for 45 seconds, and primer extension at 72^0C for 60 seconds and final extension at 72^0C for 10 mins. The PCR products were confirmed on 2% agarose in 0.5 X Tris Boric acid Ethylene diammine tetra acetic acid (TBE) buffer, visualized under ultraviolet light and documented by gel documentation system (SyngENE, Gene Genius Bio Imaging System, UK) {Fig. 1}. The PCR productes were purified by Perfectprep PCR cleanup protocol purification (Perfectprep PCR cleanup 96- cat no. 955156013-Eppendorf kits) using a vacuum pump and a filter plate. Purified products were again electrophoresed on 2% agarose in 0.5 X Tris Boric acid Ethylene diammine tetra acetic acid (TBE) buffer (Fig. 2) subjected to cycle sequencing using BigDye terminator system (Applied Biosystems, USA) following the manufacturer's protocol and the cycle sequencing products obtained were purified by vacuum manifold using AcroPrep™ 96 filter plates (Omega 3K, Pall Corporation, USA) and run on ABI-PRISM automated DNA sequencer. Sequences obtained were analysed and curated on Sequencing Analysis Software, Version 5.2.0 (Applied Biosystems, USA). Forward and reverse sequences were aligned along with a reference sequence from *Bubalus bubalis* on Seq Scape Software v.2.5 (Applied Biosystems, USA) to generate the consensus sequence of each sample. Each consensus sequence was then subjected to local alignment with Genebank database sequences using BLASTn protocol available at NCBI to confirm the homology. The SNPs were identified by double peaks at a specific locus during sequence analysis signifying their heterozygous nature. Genotypic and allelic

frequencies were calculated among trait groups and pooled group and Chi-square test was performed to assess differences in allele frequencies at each SNP between breed pairs and in total. The data generated was analyzed using standard statistical protocols for association of all the considered and newly explored SNPs in DGAT1 gene of Mehsana buffalo with MFP and MY, and the findings thus obtained were examined to draw the conclusions. All the sequences were submitted to National Center for Biotechnology Information (NCBI), USA and they were assigned accession numbers (GU562627- GU562693).

RESULTS

SNPs 1606, 1784, 1875, 2141 and 2394 in DGAT1 (Intron 1) were found to be monomorphic in Mehsana buffalo and nucleotides C, G, G, G and C respectively were fixed at these nucleotide positions (Fig. 3). Additionally, SNP 1606 exhibited allele C instead of wild allele T (Fig. 3) as reported in an earlier study [20]. SNP 2217 was found to be polymorphic (Fig. 4) and its genotypic and allelic frequencies was derived among high and low performance group to reveal predominance of particular genotype in a specific group, however, no particular genotype favored high MY or MFP. Apart from reported SNPs there were four other SNPs explored at positions 2099, 2214, 2219 and 2370 (Fig. 4). Genotypic and allelic frequencies of all the SNPs, considered and explored, were derived among high and low performance groups to reveal predominance of particular genotype in a specific group; however, again no such significant association was found (Table 3 and Table 4). The frequency of minor allele T was found to range between 0.08 and 0.10 in high and low MY groups and between 0.06 and 0.1 in high and low MFP group respectively with overall frequency of 0.08. Statistical analysis indicated that there was no significant difference ($p < 0.05$) in MY and MFP among various SNP genotypes.

DISCUSSION

A study characterized DGAT1 gene in six different Indian buffalo breeds including Mehsana breed [20], however, it is yet not clear whether the monomorphic nature of five out of six considered SNPs was observed in Mehsana breed of buffalo. The monomorphic nature of five SNPs in the present study in Mehsana buffalo suggests that these SNP's could be breed specific which can aid in breed identification studies. Allele C was observed at SNP 1606 instead of wild allele T which is not is agreement to the one detected earlier work [20] where in presence of wild allele T instead of C was reported, however, this difference of this allele in this particular breed can again be a useful marker in terms of breed identification. The frequency of minor allele T ranged between 0.08 and 0.10 in high and low MY groups and between 0.06 and 0.1 in high and low MFP groups respectively with overall frequency of 0.08 in the present study which is comparable to that reported study [20] where in the observed frequency of minor allele at these SNPs ranged from 0.06 to 0.13 in a group of six breeds of *Bubalus bubalis*. Patel et al. (2009a) and Patel et al. (2009b) recently genotyped several DGAT1 SNPs -8087, 8259, 8426 and 3627, 3674, 3741, 3815 in Mehsana buffalo but no significant correlation of these SNPs with MY and MFP was found which is in agreement with the present work. The non-association of the studied SNPs with MFP and MY in DGAT1 – intron I gene of Mehsana buffalo rules out their functional significance, however, these SNPs can be used as genetic markers along with the four novel SNPs explored in Mehsana buffalo.

To conclude, five out of six SNPs were found to be monomorphic in Mehsana buffalo. There was no significant difference in MY and MFP among different SNP genotypes and no association of the SNPs genotyped was observed with MY and MFP in Mehsana buffalo. Four novel SNPs were observed in DGAT1- intron I gene, however again these SNPs had no influence on MY and MFP. Thus, these SNP do not have any functional

significance in terms of milk production traits but could be useful as genetic markers.

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TABLES

Table 1: Average first lactation milk yield and milk fat percentage in high and low producing groups of Mehsana buffalo

Trait group	First lactation milk yield Mean ± S. E.	Milk fat % Mean ± S. E.
High (25)	2498.40 ± 35.81[a]	8.04 ± 0.05[a]
Low(25)	1333.96 ± 31.57[b]	6.14 ± 0.035[b]

Note: Means with different superscripts within a column differ significantly ($p < 0.05$)

Table 2: Average MY and MFP and their association with each SNP genotype.

SNP	Trait	SNP nucleotide	Mean ± S.E. of mean	Std. deviation	F-value
2099	Milk yield	CC (33)	1872.70 ± 104.586	600.803	0.247
		CT (13)	2011.77 ± 177.691	640.673	
		TT (04)	1964.25 ± 370.795	741.590	
	Fat%	CC (33)	7.158 ± 0.1682	0.9663	0.755
		CT (12)	6.792 ± 0.2891	1.0013	
		TT (05)	7.320 ± 0.5122	1.1454	
2214	Milk yield	AG (47)	1836.00 ±513.82195	889.96573	0.8843
		GG (03)	1921.29 ± 87.91993	602.74870	
	Fat%	AG (46)	6.750 ± 0.5515	1.1030	0.5621
		GG (04)	7.115 ± 0.1450	0.9832	
2219	Milk yield	CC (46)	1932.65 ± 89.100	604.306	0.6303
		CT (04)	1726.75 ± 379.397	758.794	
	Fat%	CC (45)	7.140 ± 0.1460	0.9797	0.3090
		CT (05)	6.600 ± 0.4528	1.0124	
2217	Milk yield	CC (42)	1940.57 ± 97.300	630.578	0.4752
		CT (08)	1788.13 ± 182.137	515.160	
	Fat%	CC (42)	7.0619 ± 0.15643	1.01380	0.6722
		CT (08)	7.2125 ± 0.30905	0.87413	
2370	Milk yield	GT (04)	1726.75 ± 379.397	379.397	0.6303
		TT (46)	1932.65 ± 604.306	89.100	
	Fat%	GT(05)	6.600 ± 0.4528	1.0124	0.3090
		TT (45)	7.140 ± 0.1460	0.9797	

Table 3: Genotypic and allelic frequencies of SNP 2099, 2214 and 2219 among high, low and pooled performance groups

SNP	Trait	Group	Genotypic frequency			Allelic frequency	
2099			CC	CT	TT	C	T
	Milk Yield	High	0.64 (16)	0.28 (07)	0.08 (02)	0.78	0.22
		Low	0.68 (17)	0.24 (06)	0.08 (02)	0.80	0.20
	Fat %	High	0.68 (17)	0.20 (05)	0.12 (03)	0.78	0.22
		Low	0.64 (16)	0.28 (07)	0.08 (02)	0.78	0.22
		Pooled	0.66 (66)	0.25 (25)	0.09 (09)	0.79	0.21
2214			GG	AG	AA	G	A
	Milk Yield	High	0.96 (24)	0.04 (01)	0.00 (0)	0.98	0.02
		Low	0.92 (23)	0.08 (02)	0.00 (0)	0.96	0.04
	Fat %	High	0.96 (24)	0.04 (01)	0.00 (0)	0.98	0.02
		Low	0.88 (22)	0.12 (03)	0.00 (0)	0.94	0.06
		Pooled	0.93 (93)	0.07 (07)	0.00 (0)	0.96	0.04
2219			CC	CT	TT	C	T
	Milk Yield	High	0.96 (24)	0.04 (01)	0.00 (0)	0.98	0.02
		Low	0.88 (22)	0.08 (02)	0.04 (01)	0.92	0.08
	Fat %	High	0.96 (24)	0.04 (01)	0.00 (0)	0.98	0.02
		Low	0.84 (21)	0.12 (03)	0.04 (01)	0.90	0.10
		Pooled	0.90 (90)	0.08 (08)	0.02 (02)	0.94	0.06

Table 4: Genotypic and allelic frequencies of SNP 2217 and 2370 among high, low and pooled performance groups

SNP	Trait	Group	Genotypic frequency			Allelic frequency	
2217			CC	CT	TT	C	T
	Milk Yield	High	0.88 (23)	0.08 (02)	0.04 (01)	0.92	0.08
		Low	0.80 (20)	0.20 (05)	0.00 (0)	0.90	0.10
	Fat %	High	0.80 (20)	0.20 (05)	0.00 (0)	0.90	0.10
		Low	0.88 (23)	0.12 (03)	0.00 (0)	0.94	0.06
		Pooled	0.84 (43)	0.15 (07)	0.01 (01)	0.92	0.08
2370		GG	TG	TT	G	T	GG
	Milk Yield	High	0.00 (0)	0.04 (01)	0.96 (24)	0.02	0.98
		Low	0.04 (01)	0.08 (02)	0.88 (22)	0.08	0.92
	Fat %	High	0.00 (0)	0.04 (01)	0.96 (24)	0.02	0.98
		Low	0.04 (01)	0.12 (03)	0.84 (21)	0.10	0.90
		Pooled	0.02 (02)	0.08 (08)	0.90 (90)	0.06	0.94

FIGURES

Fig. 1. : A 1146bp amplicon of DGAT 1 - Intron I gene fragment electrophoresed on 2% agarose in 0.5 X TBE buffer

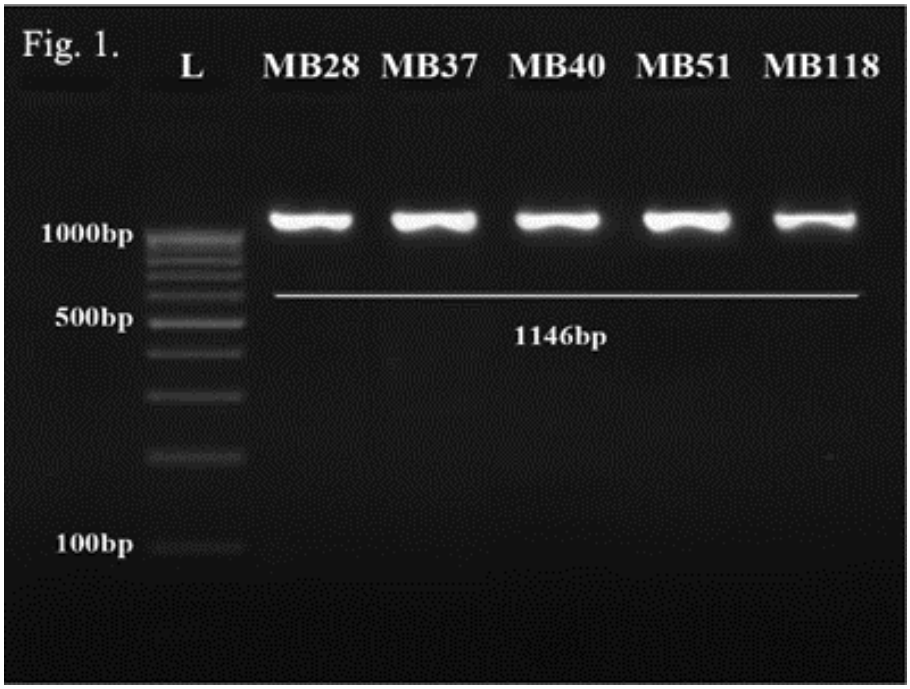

Fig. 2. : A 1146bp purified PCR product of DGAT 1 - Intron I gene fragment run on 2% agarose in 0.5 X TBE buffer

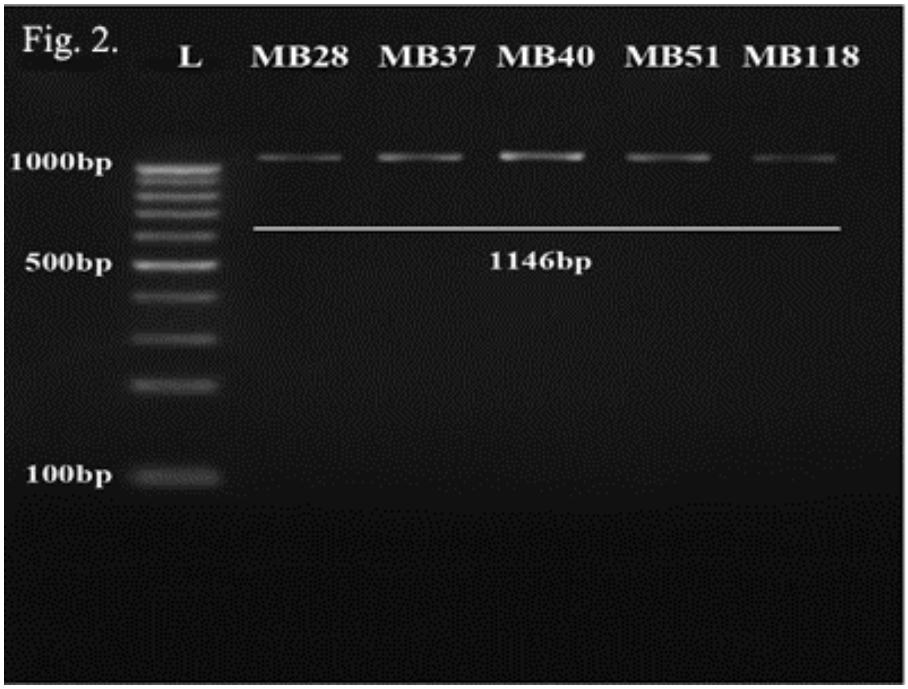

Fig. 3. : DGAT 1- Intron I gene fragment sequences showing monomorphic SNPs at nucleotide positions 1606, 1784, 1875, 2141 and 2394

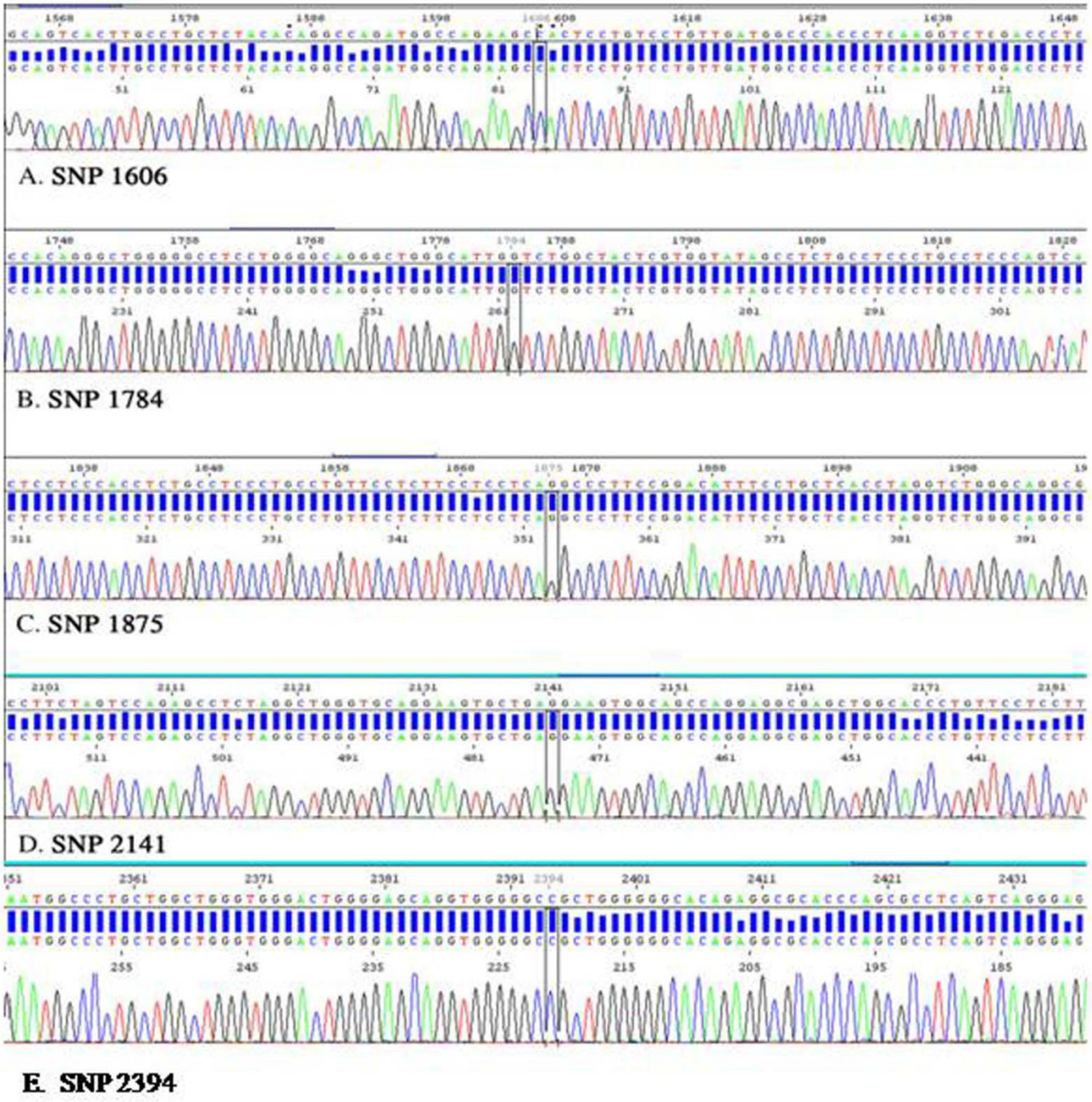

Fig. 4. : DGAT 1- Intron I gene fragment sequences showing SNP at nucleotide positions 2099, 2214, 2219, 2370 and 2217