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Short Communication

# Characterization of the *DGAT1* gene in the Indian buffalo (*Bubalus bubalis*)

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## Abstract

The positional candidate diacylglycerol *O*-acyltransferase (*DGAT1*) gene affecting milk fat percentage is reported in Indian buffaloes (*Bubalus bubalis*). A comparison with Chinese buffalo (*Bubalus bubalis*) revealed eight exonic single nucleotide polymorphisms (SNPs), five of which were non-synonymous. A total of 19 SNPs were observed among diverse buffalo breeds in India. A Unique 22 base insertion has been reported in the intron between exon ten and eleven.

*Key words: Bubalus bubalis*, *DGAT1* gene, diacylglycerol *O*-acyltransferase, Indian water buffalo, single nucleotide polymorphisms (SNPs).

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Diacylglycerol *O*-acyltransferase (DGAT1; EC 2.3.1.20) 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 (Cases *et al.* 1998). The *DGAT1* gene is a positional candidate gene for milk fat percentage with K232A substitution associated with higher fat percentage in *Bos taurus* (Winter *et al.* 2002; Grisart *et al.* 2004; Kuhn *et al.* 2004). Kaupe *et al.* 2004 reported the frequency of this substitution in various cattle breeds and grouped them from very low frequency to fixation in *Bos indicus* cattle breeds.

There are 10 recognized breeds of water buffalo (*Bubalus bubalis*), which are adapted to different climates and show large variation in size and have milk productivity ranging from 600 kg to 2500 kg per lactation with 6 to 8 percent fat. The Indian water buffalo (*Bubalus bubalis*) is an important dairy animal in the Indian subcontinent, with buffaloes producing more than 50% of the total milk in India. In the last two decades the buffalo population in India has registered an annual growth of 1.93%, reaching 97 million in 2003 (Annual Report, 2006) and continuing to be the mainstay of the Indian dairy industry and replacing cattle in several milk producing areas.

The *DGAT1* gene has been shown to be important for milk production, with Smith *et al.* (2000) having demonstrated defective lactation in *DGAT1* -/- female mice. The sequence of the Chinese water buffalo (*Bubalus bubalis*) *DGAT1* gene has recently been reported (NCBI GenBank AY999090). Since buffaloes yield higher milk fat than *Bos indicus* or *Bos taurus* cattle we felt that the characterization of *DGAT1* in Indian buffalo was necessary. In the research reported in this paper the Indian water buffalo *DGAT1* gene sequence was generated and compared with the AY999090 from Chinese buffalo and *Bos taurus* cattle to record variations.

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The *DGAT1* gene was characterized in a panel of 24 animals drawn from 6 diverse Indian water buffalo breeds (Murrah, Bhadawari, Tarai, Pandharpuri, Marathwada and Mehsana). The Murrah breed is a Northern Indian large dairy breed while the Bhadawari and Tarai are small breeds adapted to extensive production systems. The Pandharpuri and Marathwada breeds are medium sized buffaloes from central India and the Mehsana breed is a western Indian dairy breed. The DNA was isolated from blood samples and diluted to optimum concentration. The genomic region corresponding to the putative buffalo *DGAT1* gene was amplified by the polymerase chain reaction (PCR) with 16 primer pairs (Table 1) designed for the *Bos taurus* GenBank AJ318490 DNA sequence. The PCR reactions were performed in 25 µL reaction mixtures containing 50 ng of genomic DNA, 4 pmol of each primer, 1.5 mM $MgCl_2$, 0.2 µM of each dNTP and 1 unit of Taq DNA polymerase (Sigma) and in a model 9700 GeneAmp Cycler (Applied Biosystems, USA) using an amplification protocol consisting of 5 min denaturation at 94 °C followed by 30 amplification cycles of 94 °C for 45 s, 57 °C to 55 °C for 45 s and 72 °C for 45 s, with a final extension at 72 °C for 10 min. The PCR products were separated on 2% (w/v) agarose gel (Promega, USA) for examination of specific products of expected sizes. The PCR products were purified using a Montage column (Millipore) and subjected to

cycle sequencing reactions in a 10 µL reaction volume using the BigDye system (Applied Biosystems, USA) following the manufacturer's protocol. Sequencing was carried out using forward and reverse primers (Table 1) on a 3100 Avant automated sequencer (Applied Biosystems, USA). In addition 8 primers (Table 2) were designed for primer walking and used to sequence the larger PCR products. The sequences obtained for each fragment were aligned to obtain a *DGAT1* consensus sequence using the *B. taurus* sequence as reference and the SeqScape program v.2 (Applied Biosystems, USA). The single nucleotide polymorphisms (SNPs) detected in the Indian water buffalo *DGAT1* gene were further confirmed in additional samples from the panel of 24 Indian water buffalo and the frequencies of the SNPs estimated.

The sequence of 8717 bases for the Indian water buffalo *DGAT1* gene has been submitted to the NCBI GenBank with accession number DQ886485. We found that the gene in buffalo coded for 489 amino acids which is similar to Chinese buffalo, cattle and pig. Comparison of the Chinese and Indian water Buffalo sequences revealed eight changes in exonic regions, five of which were non-synonymous at amino acid numbers 98, 154, 367, 370 and 407 (Figure 1). Comparison of the Indian water buffalo *DGAT1* sequence with the *B. taurus* sequence revealed 10 synonymous changes in the coding sequence CDS and two non-synonymous changes. The first non-conservative change was a double SNP at nt6962 and 6963 (adenine/adenine to guanine/cytosine) resulting in the previously reported change of K232A in *B. taurus* which has been associated with increased fat yield and reduced milk yield (Spelman *et al.* 2002; Thaller *et al.* 2003; Weller *et al.* 2003). All the buffalo breeds (Indian and Chinese) as well as *Bos* indicus cattle (Kaupe *et al.* 2004; Lacorte *et al.* 2006; Tantia *et al.* 2006) have a fixed K allele, which may be responsible for higher milk fat in buffalo and *B. indicus* cattle.

**Table 2** - Additional primers designed for Primer walking.

| Serial number | 5' - 3' sequence | Position | Type |
|---|---|---|---|
| 1 | gTCCTTCCTgTgggCTATgA | 657-676 | Forward |
| 2 | TCTggAgCTgATCCCAAAAC | 1145-1164 | Reverse |
| 3 | gAAgCTgCTCCTgTCCTgTT | 1601-1620 | Forward |
| 4 | CAggCACAgCCTTCTAgTCC | 2089-2108 | Forward |
| 5 | gTCAgggAgAgggTgACAgA | 2428-2447 | Forward |
| 6 | CTgTTCCAAATTgCCCTCAT | 4425-4444 | Forward |
| 7 | gAACAgACAggCTgCTgAgg | 4960-4979 | Reverse |
| 8 | gAgCTgACTCTgCgCTTTTT | 7384-7403 | Forward |

Rincón *et al.* 2006 found that Uruguayan Creole cattle (*B taurus*) had *DGAT1* allele frequencies (A allele = 0.89; K allele = 0.11) similar to *B taurus* beef breeds. The *B. taurus* breeds selected for milk production show more variation in *DGAT1* with allele frequencies of some breeds being close to 0.50 for each allele (*e.g.* German Holstein, A allele = 0.58; K allele = 0.42) or higher for the K allele (*e.g.* New Zealand Holstein-Friesian, A allele = 0.40; K allele = 0.60; and Jersey, A allele = 0.31; K allele = 0.69), which is most likely the result of artificial selection for increasing milk yield and/or high milk fat content (Spelman *et al.* 2002; Kaupe *et al.* 2004).

The second amino acid change (R431G) reported by us was the result of two transversions at 8168 and 8170 (CGC → GGA). Grisart *et al.* 2002 a reported CGC

**Table 1** - Primer pairs for amplification of the Indian buffalo (*Bubalus bubalis*) *DGAT1* gene.

| Serial number | 5' → 3' forward primer | 5' → 3' reverse primer | Position (from/to) | Size | Region |
|---|---|---|---|---|---|
| 1 | ACggCAgTggCgTAgTAgAg | ACgTCTCCgTCCTTgTCTgT | 1 to 286 | 285 | 5' upsteam-Exon 1 |
| 2 | AgAggAggTgCgggATgT | AAATCCAgTTCCCCAggAgT | 218 to 1500 | 1282 | Exon 1-Intron 1 |
| 3 | ggCACACAgTTCACCTgAgA | ggTggCACCTCATTCAACCT | 1295 to 2337 | 1042 | Intron 1 |
| 4 | ACTCCTggggAACTggATTT | CAATCACCATCTgCATgTCC | 1481 to 2893 | 1412 | Intron 1 |
| 5 | TTggCAggTTgTAgCATgAg | gCAAggCCTCCAgTTTTgTA | 2891 to 3394 | 503 | Intron 1 |
| 6 | ggCCTCTCCCCTTACAAAAC | CACACACCAATTCAggATgC | 3363 to 4024 | 661 | Intron 1/Exon 2 - Exon 2 |
| 7 | CgTCTCCACTCTCCAggTgT | AgCAgCAgCAAAggACAgAT | 3930 to 5463 | 1533 | Intron 1- Intron 2 |
| 8 | TggCATCCTgAATTggTgTg | gAAATAACCgTgCgTTgCTT | 4003 to 6003 | 2000 | Exon 2 - Exon 3 |
| 9 | gCAgCAggTTTCTTCTgTCA | gTgAgggCACTgCTTACCAC | 5730 to 6346 | 616 | Intron 2 - Exon/Intron 5 |
| 10 | AAgCAACgCACggTTATTTC | TTCAggAACAgAgACACCACCT | 5984 to 6146 | 162 | Exon 3 - Exon 4 |
| 11 | ATCCAggTggTgTCTCTgTT | AACAgCTTgAggAAgAggATgg | 6121 to 6785 | 664 | Exon 4 - Exon 7 |
| 12 | CATCCTCTTCCTCAAgCTgTTC | TgCCAgAAgTAggTgATggACT | 6765 to 7872 | 1107 | Exon 7 - Exon 14 |
| 13 | CCCTgTgCTACgAgCTCAAC | ggTgATAgACTCggAgTTCCTg | 7120 to 7861 | 741 | Exon 9 - Exon 14 |
| 14 | TgAgCTCATgCAgTTTggAg | CACCAggTACTgAggggAgA | 7710 to 8155 | 445 | Exon 13 - Exon 16 |
| 15 | CTCCCCTCAgTACCTggTgA | CCCgATgATgAgTgACAgC | 8137 to 8370 | 233 | Intron 15/Exon 16- Exon 17 |
| 16 | gATAgTgggCCgCTTCTTC | TgCACAgCACTTTATTgACACA | 8304 to 8717 | 413 | Exon 17-3' downstream |

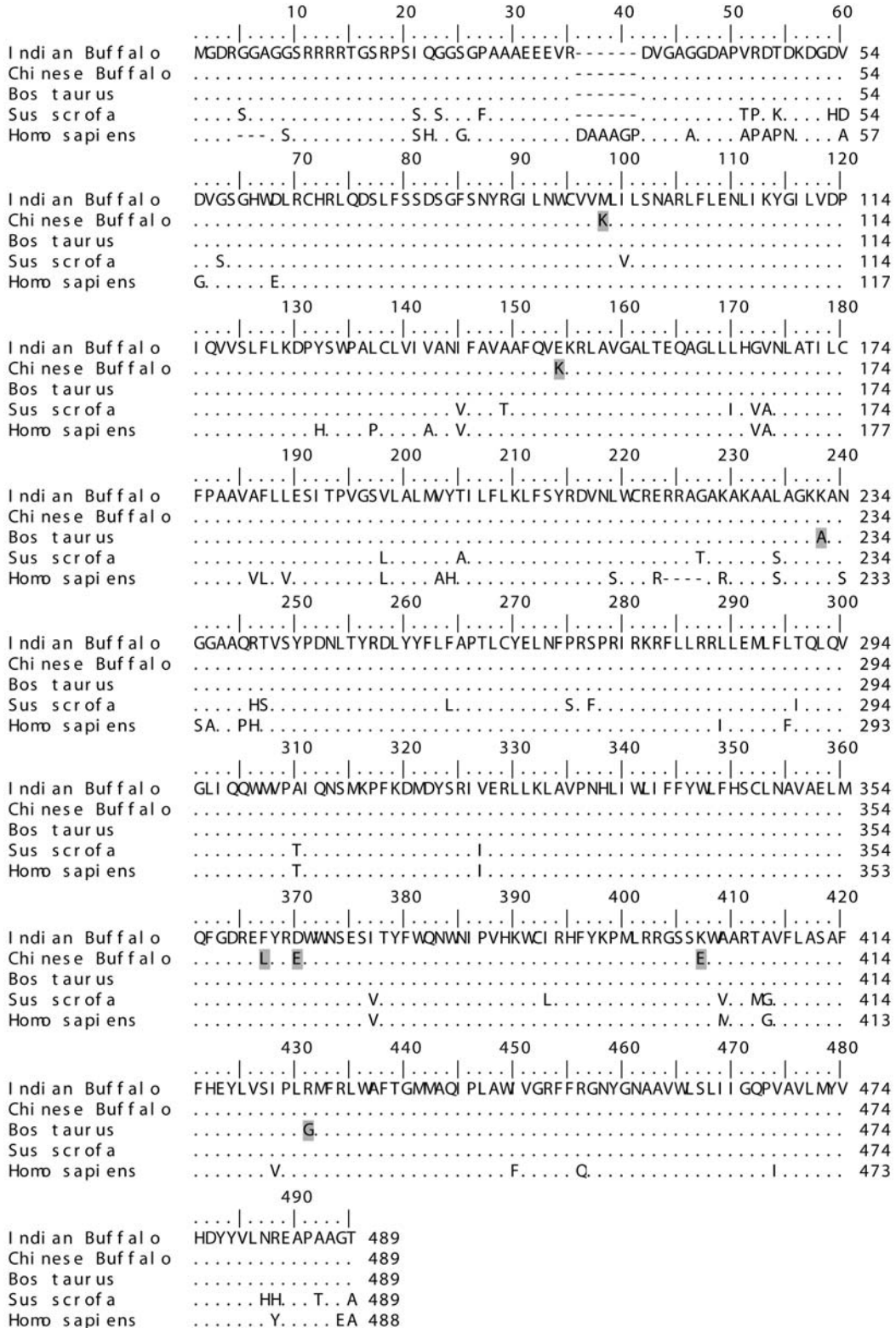


**Figure 1** - Clustal-W alignment of the DGAT1 amino acid sequence.

(AY065621) change while Winter *et al.* (2002) reported a GGA (AJ318490) change in *B. taurus* breeds, whereas Indian and Chinese buffalo had a CGC change coding for Arginine

We found that the Indian buffalo *DGAT1* gene sequence was quite close to the Chinese buffalo sequence, with a striking insertion of 22 bases in intron 10 which was unique to Indian buffalo and was also not present in *B*

**Table 3** - The DGAT1 polymorphism in Indian buffalo (*Bubalus bubalis*) breeds. Data shown for introns and the 3' untranslated region (UTR).

| Region and nucleotide | Position (from/to) | Frequency | Region and nucleotide | Position (from/to) | Frequency |
|---|---|---|---|---|---|
| Intron 1 | | | Intron 2 | | |
| 1179* | A to T | 0.12 | 5545 | T to C | 0.20 |
| 1195 | C to T | 0.11 | | | |
| 1606 | T to C | 0.06 | Intron 3 | | |
| 1784 | G to A | 0.07 | 6067 | C to T | 0.06 |
| 1875 | G to A | 0.13 | | | |
| 2141* | G to T | 0.04 | Intron 15 | | |
| 2217 | C to T | 0.14 | 8087 | C to T | 0.08 |
| 2394 | C to T | 0.08 | | | |
| 3057 | A to G | 0.21 | Intron 16 | | |
| 3096* | G to T | 0.18 | 8259 | G to A | 0.41 |
| 3627 | C to T | 0.20 | | | |
| 3674 | G to A | 0.16 | 3' UTR | | |
| 3741* | G to C | 0.11 | 8426 | C to T | 0.13 |
| 3815* | C to A | 0.16 | | | |

*Transversion.

*taurus*. Among the Indian buffalo breeds 19 SNPs were observed with varying frequencies (Table 3). Only 5 changes were transversions and 14 transitions. These SNPs in Indian buffalo breeds are novel and are reported here for the first time. Although these SNPs are not expected to have any functional significance but they may be useful as genetic markers in association studies for milk fat and milk yield.

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