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Characterization and association of the *Pit-1* gene in the Indian buffalo

RAKESH C PARIKH[1] and DHARMSHIBHAI N RANK[2]

Anand Agricultural University, Anand, Gujarat 388 001 India

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ABSTRACT

Pituitary-specific transcription factor (Pit-1) is responsible for pituitary development and hormone expression in mammals. In present study, a comparison of buffalo Pit-1 gene sequences with *Bos taurus* revealed no nucleotide changes however, 7 single nucleotide polymorphisms (SNPs), one of which was non-synonymous were found within Indian buffalo breeds (*Bubalus bubalis*). SNP c.160 T>A alters the encoded amino acid from methionine to lysine. Association of all SNPs with milk production traits was assessed in Mehsana buffalo populations and the results demonstrated that SNP c.160 T>A was significantly associated with the milk fat percentage (P=0.0358). Sequence analysis showed that the buffalo Pit-1 protein shares high homology with cattle (*Bos taurus*) (100%), goat (99%), sheep (99%), human (96%) and pig (98%). Polymorphisms detected in buffalo *Pit-1* gene are expected to have functional significance and might be useful as genetic markers in association studies for milk fat and milk yield in buffalo.

Key words: Association, Indian Buffalo, Milk yield, *Pit-1* gene, SNP

India ranks first in respect of buffaloes and second in cattle population in the world and about 53% of the total milk production of the country is contributed by buffaloes (Anonymous 2012). India has large genetic diversity in buffaloes represented by 13 recognized breeds besides several lesser-known populations comprising about 27% of the total bovine population of the country (http://www.nbagr.res.in/regbuf.html).

POU1F1 (also known as PIT-1or GHF-1) is a tissue-specific transcription factor and belongs to the large family of POU domain proteins (Bodner *et al.* 1988). Expression of *Pit-1* gene is mainly found in the anterior pituitary and is responsible for pituitary development and hormone expression in mammals (Andersen and Rosenfeld 1994). In cattle, sheep and goat, *Pit-1*gene is located on chromosome 1 (Moody *et al.* 1995). *Pit-1* cDNA was cloned in several domestic animals like bovine (Bodner *et al.* 1988), swine (Tuggle *et al.* 1993), ovine (Thomas *et al.* 2000) and dog (Lantinga-van Leeuwen *et al.* 2000) including other species like rat (Bodner *et al.* 1988*,* Ingraham *et al.* 1988), mouse (Li *et al.* 1990), chicken (Tanaka *et al.* 1999) etc. Therefore, Pit-1 gene could be a good candidate gene for genetic markers for different economic traits in domestic animals. Functional domains of Pit-1protein are encoded by six separate exons (Theill *et al.* 1992). However, as per our knowledge, molecular characterization of Pit-1gene in water buffalo (*Bubalus bubalis*) has yet not been reported.

It is essential to understand the genetic structure of potential genetic marker (like *Pit-1*) and its correlation with various economic traits in water buffalo. Hence, the current study was designed to characterize and to identify genetic polymorphisms in the *Pit-1* gene within Indian buffalo breeds.

MATERIALS AND METHODS

The *Pit-1* gene was characterized in a panel of 40 animals drawn from 10 diverse Indian water buffalo breeds (Mehsana, Surti, Jaffarabadi, Banni, Murrah, Pandharpuri, Toda, Bhadawari, Chilika and Nili Ravi). Sampling and breeding sites of the buffalo breeds used in this study are shown in Fig. 1. Genomic deoxynucleic acid (DNA) was isolated from 5 mL of blood by the phenol-chloroform extraction method (Sambrook and Russel 2001). The quality and purity of the isolated DNA were determined by agarose gel electrophoresis and spectrophotometry, respectively, and diluted to optimum concentration for further analysis. For association study 130 blood samples from Mehsana buffalo were also collected from their breeding tract and DNA extracted.

No data were available about the nucleotide sequence for the buffalo *Pit-1* gene in GenBank hence the genomic region corresponding to the putative buffalo *Pit-1* gene was amplified by the polymerase chain reaction (PCR) using 6 primer pairs (Table 1) flanking exons designed from *Bos taurus* GenBank NC_007299.4 DNA sequence. Primers were

Present address: [1]Student (drrakesh81@rediffmail.com), [2]Professor and Head (dnrank@gmail.com), Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry.

Fig. 1. Sampling sites of the 10 Indian buffalo breeds used in this study.

synthesized at a private firm. PCR reaction was prepared in 25 µL volume containing 90 ng of genomic DNA, 10 pmol of each primer, 12.5 µL 2× PCR master mix (including 4 mM $MgCl_2$, 1.6 mM dNTPs and 0.05 U of Taq DNA polymerase) and amplification was performed in the variety thermal cycler. The PCR amplification protocol was standardized to cyclic condition consisting of initial denaturation at 94 °C for 5 min followed by 32 amplification cycles of denaturation at 94 °C for 30 sec, annealing temperature as mentioned in Table 1 for 45 sec and extension at 72 °C for 45 sec, with a final extension at 72 °C for 10 min. PCR amplicons were resolved on 2% agarose gel to confirm the desired size and purified by GenElute agarose spin columns.

Purified PCR products were subjected to cycle sequencing reactions in a 10 µL reaction volume using the BigDye Terminator v3.1 Cycle sequencing kit following manufacturer's instructions. Sequencing was carried out using forward and reverse primers (Table 1) on a ABI PRISM 310 genetic analyzer and the sequences obtained for each fragment were aligned. The SeqScape program was used to obtain a Pit-1 consensus sequence using *Bos tautus* sequence as reference. The single nucleotide polymorphisms (SNPs) detected in the Indian water buffalo *Pit-1* gene were further confirmed in additional samples from the panel of 40 Indian water buffalo and the frequencies of the SNPs estimated.

The allele and genotype frequencies were estimated by direct counting. The relationships between Pit-1 genotypes and milk yield traits in Mehsana buffaloes (n=130) were evaluated using general linear model (GLM) procedure of SAS version 9.2 software. Following linearity model was applied to analyze the association of variations of *Pit-1* gene with milk yield traits in Mehsana buffalo.

$$Y_{ijk} = \mu + S_i + G_j + e_{ijk}$$

where, Y_{ijk} is the observation of the milk yield traits of the progeny of ith sire; S_i is sire families; G_j, fixed effects of genotypes of each SNP; e_{ijk} is the random residual effect.

RESULTS AND DISCUSSION

A 2, 117 base genomic sequence of the buffalo *Pit-1* gene including exons and part of introns was submitted to the NCBI GenBank (accession number JN871198.1). Our sequence contained 116 bp upstream from the ATG start codon and 876 bp coding sequence comprised all 6 exons.

Comparison of the Indian water buffalo Pit-1sequence with the *Bos taurus* sequence revealed no any nucleotide changes in the exonic as well as in partial intronic regions indicating highly conserved gene. Among the 10 Indian buffalo breeds 7 SNPs (belonged to Exons 1, Intron 1, Exon 2 and Intron 2 regions) were observed with varying

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

Serial number	5'→3' primer sequences	T_a	Position (from/to)*	size(bp)	Region
1.	F: AgCATgCgCTCTCTTggTgC R: AAggCgCAgCCgCATgTAgA	54°C	-116 to 207	323	5' upstream–Ex 1–Int 1
2.	F: TggTCAgTCACgCCAACCCA R: TgCATCCAAgCgTCCCCAAACT	58°C	3334 to 3752	419	Int 1–Ex 2–Int 2
3.	F: ACAgggAgCTTAACCCCTTgTCT R: TgCAgACggAgTTgTgTgTCCC	10777 to 11049	60°C	273	Int 2–Ex 3–Int 3
4.	F: TgCAAAACTgAAgCTCATggCC R: ACggTgTgTgCGAATgCTCTgg	54 °C	13420 to 14008	589	Int 3–Ex 4–Int 4
5.	F: CCAgAgCATTCgCACACACCgT R: TggggTCTTTCCCggAgCAA	58°C	13987 to 14778	792	Int–4–Ex 5–Int 5
6.	F: AgTCAgTgCCCTCTTgTggCA R: AgCTgATggCAAgCAggACATC	58 °C	15189 to 191 (3' downstream)	954	Int 5–Ex 6–3' down stream

F, forward; R,Reverse; T_a, annealing temperature; Ex, exon; Int, intron; bp,base pair. *, nucleotide positions according to GenBank accession number NC_007299.4.

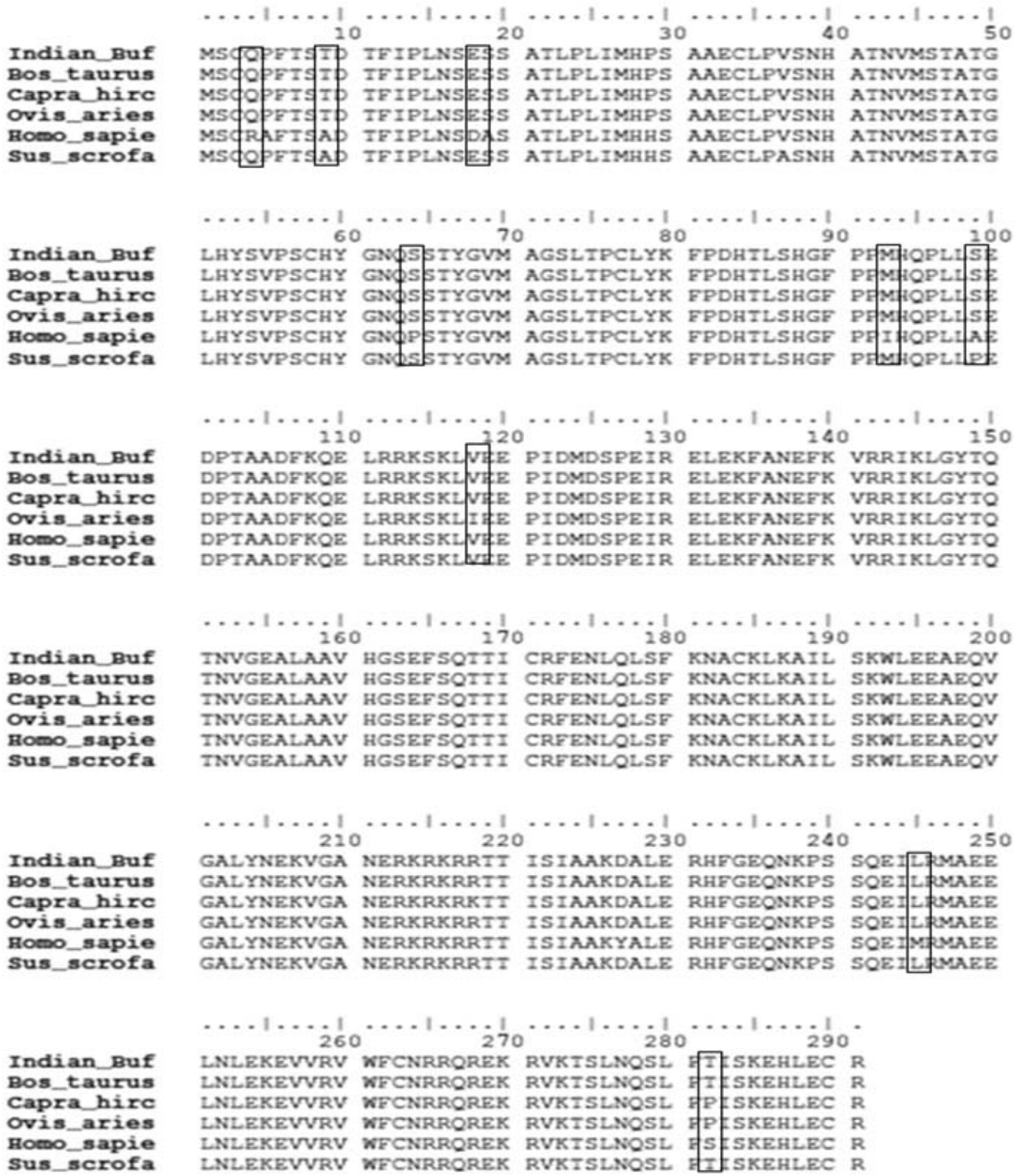

Fig. 2. Multiple sequence alignment of the Pit 1 amino acid sequences among different species. The box indicates the inter species variation in amino acid sequence.

Table 2. The Pit-1 polymorphism with amino acid variation in Indian buffalo (*Bubalus bubalis*) breeds. Data shown for exon 1, 2 and partial intron 1, 2 regions

Region	Nucleotide position*	Changes (from / to)	Type	Frequency (%)	Amino acid variation
Exon1	160	T to A	Transversion	13.25	M45K
Intron1	343134423483	C to T	Transition	47.5	
		G to A	Transition	13.75	
		C to G	Transversion	2.5	
Exon2	3519	C to T	Transition	20	silent
Intron2	37403741	T to C	Transition	42	
		A to G	Transition	30	

* Nucleotide positions according to GenBank accession number NC_007299.4. M, Methionine; K, lysine.

frequencies and amino acid substitution (Table 2) of which 2 were transversions and 5 transitions. No SNPs were found in exons 3, 4, 5 and 6. SNP c.160T>A in exon 1 is non synonymous causing methionine to lysine amino acid substitution. These SNPs in Pit-1gene of Indian buffalo breeds are reported here for the first time as a novel SNP.

To ascertain the association of Pit-1 SNP genotypes with milk yield traits in buffalo, first lactation 305 day standard milk yield (MY) and milk fat % (MF) were compared among different SNP genotypes in 130 Mehsana buffalo. SNP c.160T>A in Exon 1 showed significant difference among genotypes with respect to milk fat percentage in Mehsana buffalo. Least squares means (LSM) of milk fat percentage of SNP c.160T>A for genotypes TT = 6.9495 and TA = 8.1995 showed significant difference at 5 % level (P=0.0358). None of the other SNPs had any significant influence on milk yield or milk fat percentage.

Predicted protein sequence comprised 291 amino acids of buffalo Pit-1 was compared and it revealed high homology with cattle (100%) (*Bos taurus*) (accession number: DAA33622.1), goat (99%) (accession number ABZ81984.1), sheep (99%) (accession number: CAD70238.1), human (96%) (accession number: AAA60093.1) and pig (98%) (accession number: NP_999328.1) (Fig. 2).

The polymorphisms for *Pit-1* gene and its association with milk and growth rate traits were studied in *Bos taurus* (Hori-Oshima and Barreras-Serrano 2002, De Mattos *et al.* 2004, Dybus *et al.* 2004, Javanmard *et al.* 2005) and *Bos indicus* cattle (Mukesh *et al.* 2008, Selvaggi and Dario 2011). In the bovine *Pit-1* gene, the restriction fragment length polymorphism (for the *Hinf*I restriction enzyme – *Pit-1 /Hinf I*) was first identified by Woollard *et al.* (1994). Later on, Renaville *et al.* (1997) could associate A allele at *Pit-1/Hinf I* locus with superior milk and protein yields and inferior for fat percentage in dairy cattle. Zwierzchowski *et al.* (2002) also showed that the allele A of the Pit-1 locus has positive effect on daily milk yield and milk composition in Polish Black-and-White cows. Recently, Ozdemir (2012) also found similar observation in Holstein cows. All these efforts were concentrated mainly on *Bos taurus* and *Bos indicus* cattle breeds. Association of *Pit-1* gene polymorphism with growth and carcass traits was also reported in cattle (Zhao *et al.* 2004, Kai *et al.* 2006, Selvaggi *et al.* 2011 and Tang *et al.* 2012) and pig (Stancekova *et al.* 1999, Sun *et al.* 2002). However, polymorphism in *Pit-1* gene and association studies in buffalo are scanty, Misrianti *et al.* (2010) detected polymorphism in exon 6 of *Pit-1* gene in FH cattle (n=45) however no polymorphism was detected in Indonesia buffaloes (n=320).

The present study provided preliminary information related to SNPs in Pit-1 gene and their allelic and genotypic distribution pattern and association with milk production traits in Indian buffaloes although with limited data. The study need to be extended on larger number of animals to get a better understanding of the effect of these SNP on production traits in buffalo.

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