Original Article

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OXIDIZED LOW DENSITY LIPOPROTEIN RECEPTOR 1 (OLR 1) GENE POLYMORPHISM IN MEHSANA BUFFALOES (*Bubalus bubalis*)

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ABSTRACT

Oxidized low density lipoprotein receptor 1 **(**OLR1) is the major protein that binds, internalizes and degrades oxidized low-density lipoprotein. It is reported that SNP 8,232 in the 3′-UTR in OLR1 was associated with milk fat yield and percentage. This study was aimed to reveal the PCR-RFLP pattern of OLR1 (3'UTR) in the Mehsana breed of buffaloes. A fragment of OLR1-288 bp was amplified by PCR, and subsequently, RFLP study was carried out to identify genotypes of the animals with *PstI* restriction enzyme. It revealed monomorphic patterns.

Further representative samples were cloned and in vector and after sequencing sequence variation in nucleotide sequences of OLR1was analysed. On comparison with published cattle sequence the nucleotide sequence variation between cattle and buffalo was present at nine nucleotide positions i.e. 85, 91, 116, 129, 151, 168, 171, 217, 240.

Keywords: Mehsana buffaloes, *Bubalus bubalis* PCR-RFLP, OLR1 gene, polymorphism

INTRODUCTION

The use of polymorphic markers in breeding programmes could make selection more accurate and efficient. The studies which are attempted to detect genes affecting quantitative traits via linkage to genetic markers can be divided into two categories: analysis of candidate genes and genome scans based on within-family genetic linkage. The candidate gene approach consists of the study of different genes potentially involved in the physiological process (e.g., milk proteins synthesis, milk fat synthesis) and identification for each gene of the allele responsible for desired phenotype. OLR1 is identified as a candidate gene for milk production traits (Khatib *et al*., 2007). Khatib *et al*. (2006) reported that SNP 8,232 in the 3′-UTR was associated with milk fat yield and percentage in Italian Brown Swiss.

OLR1 is the major protein that binds, internalizes and degrades oxidized low-density lipoprotein. The oxidized form of the low-density lipoprotein (oxLDL) is involved in endothelial cell injury, dysfunction, and activation, all of which are implicated in the development of atherosclerosis (Mehta and Li, 1998). It has been shown that oxLDL and its lipid constituents have numerous damaging effects on secretary activities of the endothelium, including induction of apoptosis (Imanishi *et al*., 2002). OLR1 was initially identified in bovine

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aortic endothelial cells by Sawamura *et al*. (1997). In addition to binding oxLDL, OLR1 removes aged and apoptotic cells from blood circulation (Oka *et al*., 1998). Information on OLR1 gene polymorphism in Mehsana buffalo is very scanty; hence, the present study was undertaken to reveal the PCR-RFLP pattern of the OLR1 locus in the Mehasana breed of buffaloes.

MATERIALS AND METHODS

Buffalo population, sampling and DNA extraction

To analyze the status of OLR1/ *Pst1* polymorphism, blood samples were collected randomly from 60 unrelated buffaloes of the Mehsana breed registered under the progeny testing programme of the Dudhsagar Research and Development Association, Mehsana, Gujarat state. DNA was extracted using standard protocol by the phenol: chloroform extraction procedure (Sambrook *et al*., 1989).

Molecular genotyping

Primers reported by Khatib *et al*. (2006) for *Bos taurus* could not amplify the OLR1 3' UTR region in the Mehsana buffalo. Hence, new primer pairs were designed (**F:** 5'CTG G AGGAAAA GAAGGAAACC3' **R:** 5'TGCTGTGA CCTTGAGTTAGGC3') using Bioinformatics tools, Primer 3.0 and Primer Express softwares (http://www.genome.wi.mit.edu/cgi_bin/primer/primer3) on the basis of gene sequences available in the data base NW_174132.2 to amplify the desired fragment.

PCR was carried out in a final reaction volume of 25 µl. Amplification cycling conditions involved initial denaturation 94°C for 10 minutes, followed by 35 cycles at 94°C for 1 minute, 54°C for 45 seconds and 72°C for 1 minute with a final extension at 72°C for 10 minutes.

For the PCR-RFLP analysis, 10 µl of each PCR amplified product was digested with 5 units of the *PstI* (CTGCA↓G) in a 30µl total reaction and incubated in a water bath at 37°C for 16 h. The digestion products were separated by electrophoresis on a 2% agarose gel in 0.5% TBE buffer.

Cloning and sequencing

PCR products from a representative 288 bp OLR1 3'UTR sample was purified and cloned in pTZ57R/T vector (InsT/Aclone™ kit). Ligated recombinant vector was transformed in competent E. coli (DH5-α) cells. Recombinant plasmids were extracted and used for cycle sequencing and subjected to automated DNA sequencing on ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, USA). Sequencing was carried out using BigDye® Terminator v3.1 Cycle sequencing kit (Applied Biosystems, USA). Sequence data obtained was analyzed *in silico* by employing software tools *viz.* NCBI BLAST, SeqScape and ClustalW to access the genetic variation.

RESULTS AND DISCUSSION

A 288 bp fragment of OLR1 gene loci was amplified by PCR, using the designed primers. PCR amplicons were digested with restriction enzyme *PstI*. It was expected OLR1 had a *PstI* restriction site at 215 bp and would produce two fragments of 215 and 73 bp. On screening the OLR1/*Pst1* in the 60 Mahsana buffaloes, all the samples showed an identical restriction pattern with the absence of restriction site producing 288 bp fragment only

(Figure 1). All the animals revealed only one genotype (BB) and a monomorphic pattern with B allele fixed in the Mehsana buffalo.

The molecular basis of OLR1/*Pst1* polymorphism is mutation C→A in recognition site of *Pst1* restriction enzyme.

After sequencing and assembling the sequences it was observed that the sequence has a mutation at the site of *Pst1*i.e at nucleotide position 215. The recognition site of *PstI* 5'-C **T** G C A^G-3' is mutated, T is deleted, and A added. Hence the sequence observed is 5'–C- G **A** C A^G-3'(5'-C **T** G C A^G-3' →5'-C **T** G C A^G-3') hence, the absence of the site in the fragment.

The results of the present study, i.e. the monomorphic pattern in the buffalo, are not in accordance with Khatib *et al.* (2006).

A consensus sequence of 288 bp OLR1 UTR 3' was obtained by assembling the forward and reverse compliment sequence using the Seq Scape software programme. The consensus sequences were then aligned with known sequences for OLR1 UTR 3'in GenBank using NCBI BLAST and ClustalW programme. The nucleotide sequence alignment of OLR1 with published sequences revealed 96% to 99% homology (Table 1).

The nucleotide sequence variation between cattle and buffalo for OLR1 (3'UTR)

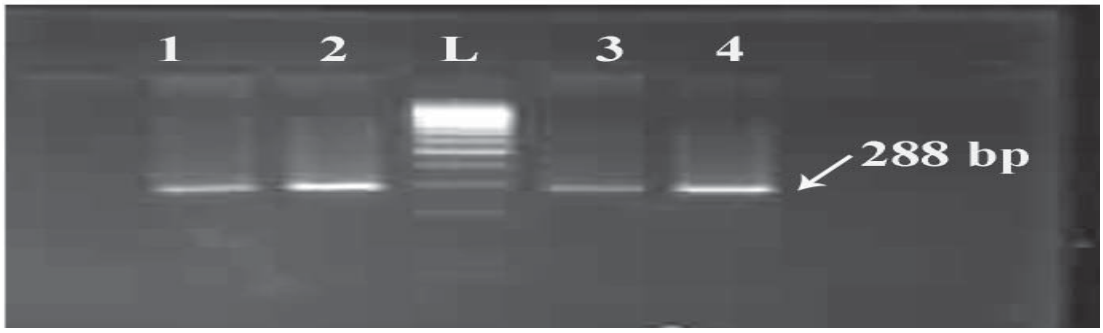

Figure 1. OLR/Pst1 PCR-RFLP of OLR 1 gene 3' UTR 288bp OLR 1 3' UTR gene PCR fragment in Mehsana buffalo digested by pstI.
Lane 1, 2 : OLR/Pst1 digest single fragment of 288 bp showing absence of site in the fragment
Lane 3 : 100 bp Ladder
Lane 5 : PCR product of OLR1 of 288 bp

Table 1. *Bubalus bubalis* OLR 1 - Blastn in GenBank + EMBL + DDBJ.

Sr. No	Accession	Description	Location/ Source	Max Indent
1	BT029784.1	Bos taurus oxidised low density lipoprotein (lectin-like) receptor 1 (OLR1), mRNA, complete cds	(1-288) 862-1149	96 %
2	D89049.1	Bos taurus mRNA for lectin-like oxidized LDL receptor, complete cds	(1-288) 858-1145	96 %

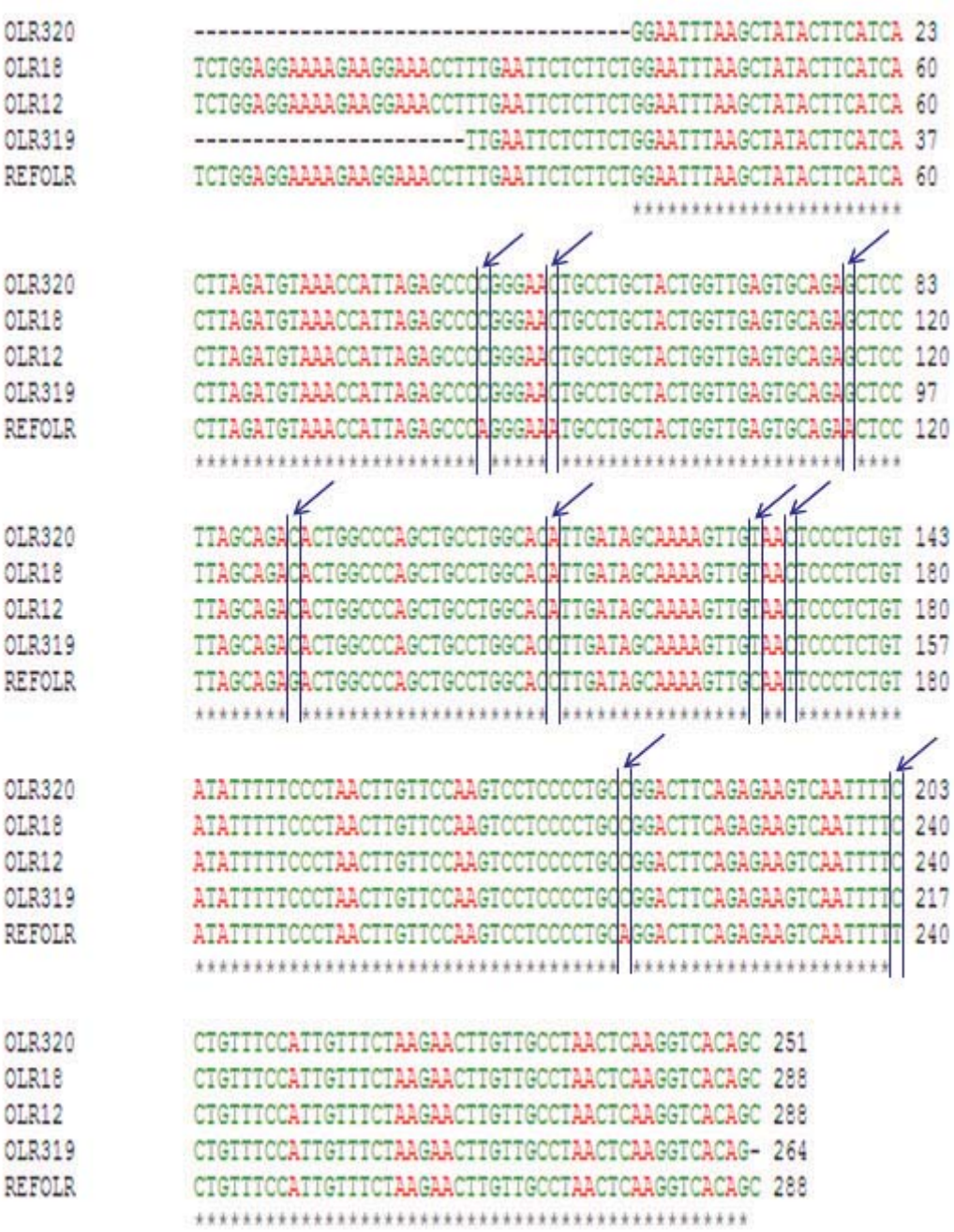

Plate 1. Clustal W results of OLR1 3'UTR.

was observed at nine nucleotide positions i.e. 85, 91,116,129,151,168,171,217,240 (Plate 1).

No buffalo sequences were available in the GenBank database for OLR1 3'UTR before preparing this manuscript. One genomic nucleotide sequence of 288 bp consisting of OLR1 3'UTR was submitted to Genbank of NCBI database accession number GQ385226.

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