Exploration of genetic variability in regulatory and coding regions of Oxidized low density lipoprotein receptor1 (*OLR1*) gene in Murrah buffaloes

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Abstract

The identification of oxidized low-density lipoprotein receptor1 (*OLR1*) gene polymorphisms in Murrah buffaloes opens possibilities for understanding the genetic basis of lipid metabolism and its impact on economically important traits. The objective of the present study was to explore genetic polymorphism in *OLR1* gene in 100 Murrah buffaloes. Two regions of *OLR1* gene i.e. 146 bp and 385 bp fragment of 3'UTR and exon 1, respectively were amplified via PCR and screened for genetic variation using restriction fragment length polymorphism (RFLP) technique. The fragments of 3'UTR and exon 1 region of *OLR1* gene were digested using restriction enzymes viz., *Pst*1 and *Alu*1, respectively. Monomorphic patterns were revealed for both the regions of *OLR1* gene through RFLP. Further, a total 50 samples of exon 1 region of *OLR1* gene were Sanger sequenced. BioEdit software was used for multiple alignments of sequenced samples. Sequencing results were compared with reference sequence of *Bos taurus* (*NC_037332.1)* and nucleotide changes were observed at total five positions in Murrah buffaloes, out of which three were transition and two were transversion changes. Conclusively, in the present study, loci screened through PCR-RFLP technique in Murrah buffaloes were found to be monomorphic indicating absence of genetic variability at these loci. Nucleotide changes observed at different locations through sequencing in Murrah buffaloes as compared to *Bos taurus* can be used as selection signatures.

Keywords: Buffalo; Murrah; *OLR1;* PCR-RFLP; Polymorphism; Sequencing

Introduction

Buffalo may prove as a more viable option in the dairy industry as compared to cattle due to their adaptability to a variety of ecological conditions because of greater milk yield and higher fat content (Dongre *et al.,* 2023). Buffalo produce 6.63 liters of milk on average per day, with an average fat, protein, and lactose content of 7.5, 4.2, and 5%, respectively (BAHS, 2024). India has 22 registered breeds of buffaloes and Murrah is among the best buffalo breeds (NBAGR, 2025). Murrah buffaloes play a crucial role in dairy farming systems, contributing to food security, livelihoods and the agricultural economy in many parts of the world. Originating from the districts of Rohtak, Hisar, Jind, Nabha, and Patiala in the Indian states of Haryana and Punjab, as well as the Pakistani province of Punjab, the Murrah breed has also been utilized to increase the milk output of dairy buffaloes in various nations, including Italy, Bulgaria, and Egypt.

Direct selection of genes or genomic areas that influence economic qualities in animals is one way that marker assisted selection (MAS) may increase genetic improvement in livestock. By increasing the frequency of a marker allele linked to a beneficial QTL, selection for that allele can improve dairy performance. The ability to identify a dairy animal's marker genotypes shortly after birth is one possible advantage of molecular marker-based selection. In order to increase genetic gain in a herd for milk and its constituent traits, marker information can be utilized to predict an animal's genotype before actual performance recording for a trait is available. This significantly shortens the generation interval.

The breakdown of oxidized low-density lipoprotein (ox LDL), which damages the vascular endothelium, is facilitated by oxidized low-density lipoprotein receptor 1 (*OLR1*) (Imanishi *et al.,* 2002). This important protein was first discovered in the endothelial cells of the bovine aorta. *OLR1* not only impasses ox LDL but also eliminates apoptotic and aged cells from circulation. The bovine *OLR1* gene encrypts 270 amino acids and shares 72% similarity with the human protein. The *OLR1* gene encodes the type II membrane protein *OLR1*, which is a member of the C-type lectin family (Chen *et al*., 2001). The gene has five exons and has been estimated to be situated between 106 and 108 cM of bovine chromosome 5 based on integrated data from various cattle maps (Khatib *et al*., 2006). This gene has 11306 bp in total length (Javed *et al*., 2013). Numerous QTL influencing milk production traits in dairy animals, such as milk yield (De Koning *et al*., 2001) and milk fat yield (Olsen *et al*., 2002), have been identified in *OLR1* gene. The *OLR1* gene has been considered a potential gene influencing milk production traits in dairy cattle because of its function in lipid metabolism and Ox-LDL breakdown (Khatib *et al.,* 2006). Holstein Friesian cattle having a heterozygote *OLR1* genotype were shown to have a reduced age at first calving (Ardicli *et al*., 2019). However, till date, there are no reports pertaining to study of polymorphism in *OLR1* gene in Murrah buffaloes, therefore, the present study was undertaken to identify genetic variations in 3'UTR and exon 1 regions of *OLR1* gene in Murrah buffaloes.

Materials and methods

Sample collection and location of study

Blood samples were taken from 100 Murrah buffaloes kept at Buffalo Farm, Department of Livestock Production Management, LUVAS, Hisar, in order to explore the polymorphism in the 3'UTR and exon 1 region of *OLR1* gene. Hisar is located at 29° 10' N latitude, 75° 40' E longitude, and 215.2 meters above sea level. Due to its semi-arid location, the city experiences sub-tropical weather.

Ethics statement

The authors confirm that the relevant ethical review committee approval has been obtained. Permission for collection of blood samples was taken from the Institutional Animal Ethics Committee (IAEC), LUVAS, Hisar (VCC/IAEC/376-97, 03-02-22).

DNA extraction, quality and quantity check of DNA

DNA was isolated using Maxwell RSC genomic DNA extraction kit which uses tiny magnetic particles to extract DNA from samples. DNA quality was assessed in 1% agarose (w/v) suspension in 1X TAE buffer (pH 8.0) containing1% ethidium bromide while quantity was assessed using a Nanodrop spectrophotometer (260/280 nm). The following formula was used for checking quantity of DNA:

Quantity of DNA (µg/ml) = OD260 × 50 × Dilution Factor

For each sample, the OD260 to OD280 ratio was noted. DNA samples having a range of 1.7 to 1.9 were considered as good and used for the further study.

Primers used and PCR conditions

The PCR reaction mixture (25 µl) consisted of 2.0 µl DNA template, 1.5 µl each of forward and reverse primers, 12.5 µl green master mix (Promega), and 7.5 µl nuclease-free water. Amplification was performed using primers specific to the *OLR1* gene, as described by Mashhadi *et al.* (2017) and Javed *et al.* (2018) (Table 1). The PCR conditions included an initial denaturation at 95°C for 3min, followed by 34 cycles of denaturation at 95°C for 45s,

Table 1. Primers used for amplification of targeted regions of *OLR1* gene in Murrah buffaloes

Gene	Target region	Primersequence(5′-3′)		Annealing Temp.(˚C)	Amplicon size(bp)
OLR1	3'UTR	F	TCCCTAACTTGTTCCAAGTCCT	54	143
		R	CTCTACAATGCCTAGAAGAAAGC		
	Exon 1	F	CACACAGATTCACCACTTTCCCTTCC	60	385
		R	CCACACCCAGGCATTGTAGTT		

annealing at the optimized temperatures for 3'UTR and exon 1 region (54°C and 60°C) for 30s, and extension at 72°C for 1min. A final extension step at 72°C for 10 min was included.

RFLP conditions

Five units of *PstI* (CTGCA↓G) and *AluI* (AG↓CT) were added to 10 µl of each PCR amplified product in a 30 µl total reaction, and the mixture was incubated for 16 hours at 37°C in a water bath for RFLP analysis. After being separated by electrophoresis on a 2% agarose gel in 1% TAE buffer, the digestion products were visualized on gel documentation system.

Sanger sequencing

BioEdit and ClustalW tools were used to perform multiple sequence alignment on fifty PCR-amplified samples of exon 1 region using the reference sequence of *Bos taurus* (*NC_037332.1*).

Results

The study involved PCR amplification of specific fragments of the *OLR1* gene in Murrah buffaloes. Two regions were targeted: a 385 bp fragment of exon 1 and a 146 bp fragment of the 3'UTR region. Gradient PCR was used to optimize the annealing temperatures for the primers, which were determined to be 60°C for 30 seconds for the exon 1 region and 54°C for 30 seconds for the 3'UTR region.

Restriction Digestion Analysis

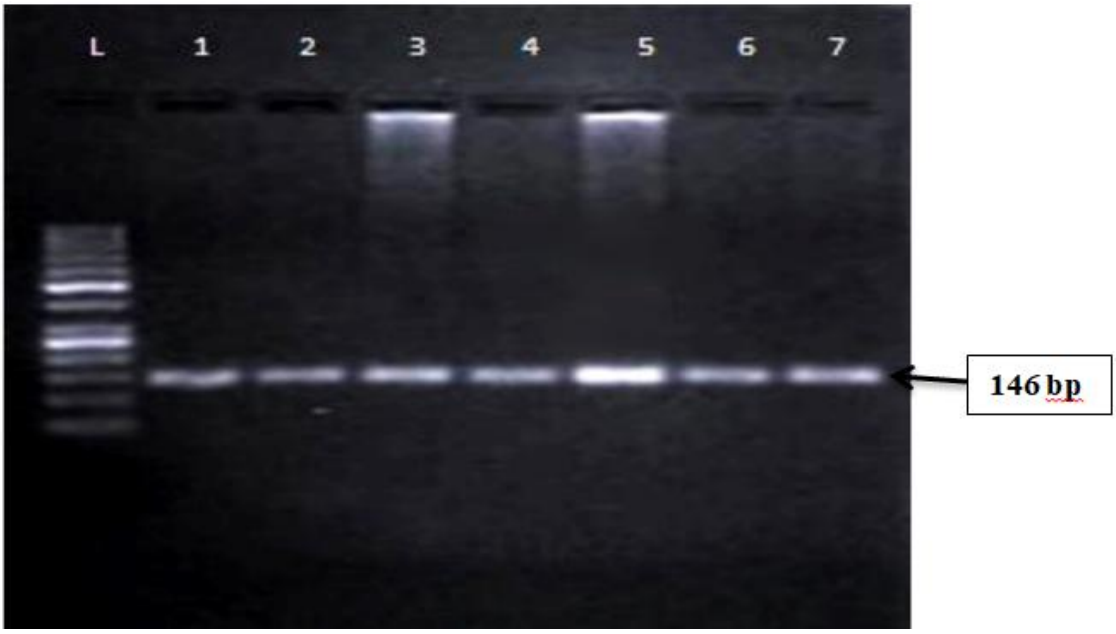
The PCR products were then subjected to restriction enzyme digestion using *PstI* and *AluI*. For the 3'UTR region, it was anticipated that *PstI* digestion would yield fragments of 118, 30, and 143 bp (though mentioned 143 bp in anticipation, it seems there might be a slight discrepancy in the fragment sizes expected). However, upon screening 100 Murrah buffalo samples, all showed an identical restriction pattern, with only a single 146 bp fragment observed, indicating the absence of the *PstI* restriction site (Figures 1 and 2). Similarly, for the exon 1 region, *AluI* digestion was expected to produce fragments of 309, 199, 80, 50, and 58 bp. Nevertheless, screening the *OLR1/AluI* products from the same 100 samples revealed a uniform restriction pattern, with only a 385 bp fragment detected, suggesting the absence of the *AluI* restriction site (Figures 3 and 4).

Sequencing Analysis

To further investigate the genetic variations, 50 PCR-amplified products of the exon 1 region were sequenced using Sanger sequencing. Multiple sequence alignment was performed using BioEdit software, and the results were compared with the reference sequence of *Bos taurus* (NC_037332.1). The analysis revealed nucleotide changes at five positions: g.1C>T, g.71A>G, g.108C>A, g.148C>T, and g.168C>G. Among these changes, three were transitions, and two were transversions (Figures 5 & 6).

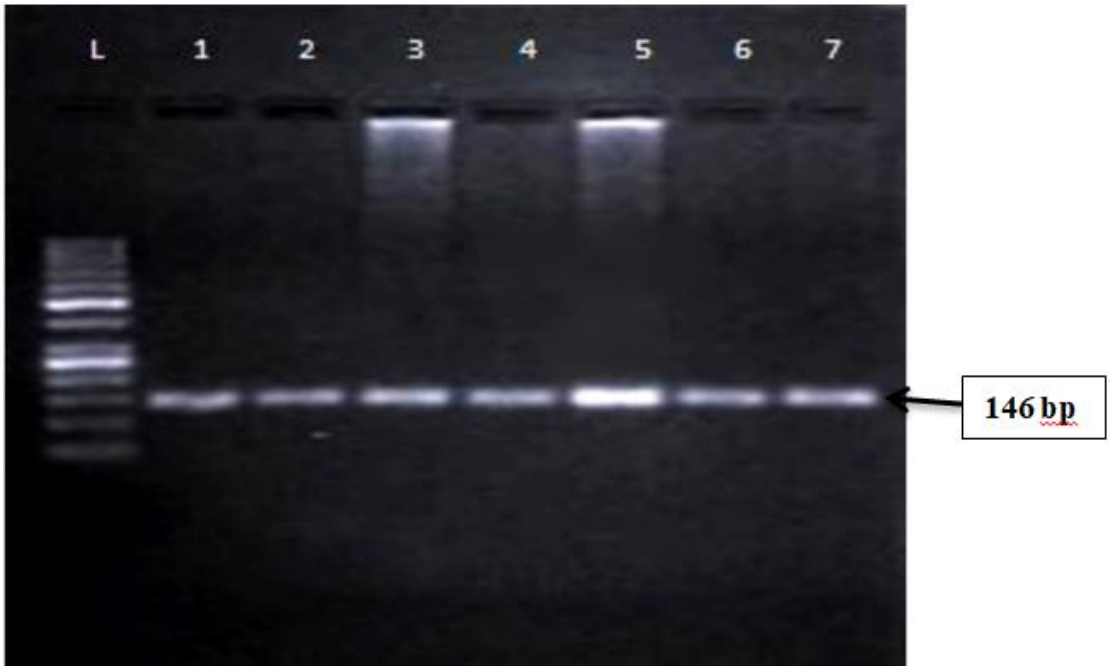
Discussion

PCR was performed using known primers to amplify a 385 bp fragment of exon 1 region and a 146 bp fragment of the 3'UTR region of *OLR1* gene. The annealing temperatures for the primers were optimized using gradient PCR. Optimum annealing temperature was 54°C for 30 seconds for the 3'UTR region and 60°C for 30 seconds for the exon 1 region. However, earlier researchers reported different temperature and time combinations for annealing of primers i.e. 57°C/30 seconds (Khatib *et al.,* 2006), 62°C/30 seconds (Komisarek and Dorynek., 2009), 54°C/45 seconds (Deshpande *et al.,* 2013), 60°C/30 seconds (Fontanesi *et al.,* 2015) and 56°C/75 seconds (Shabir *et al.,* 2011). The PCR products were digested with the restriction enzymes *PstI* and *AluI*. It was anticipated that *OLR1* gene would have a *PstI* restriction site at the 146 bp fragment, which would produce fragments of 118, 30, and 143 bp. However, when the *OLR1*/*PstI* products from 100 Murrah buffalo samples were screened, all showed an indistinguishable restriction pattern with absence of the restriction site, resulting in a single 146 bp fragment (Figures 1 and 2).On contrast, Khatib *et al.* (2006) observed one genetic variation in 3'UTR region of the gene in Holstein dairy cattle and found positive effect of allele C on fat and protein percentage in addition to fat yield ($p<0.05$). Soltani-Ghombavani *et al.* (2013) reported that the *OLR1* gene, particularly its 3'-UTR region, plays a significant role in determining milk fat and protein percentage in Iranian Holstein cattle, however they also observed more milk yield in animals of CC and AC genotype as compared to AA genotype although the association was not significant statistically. Komisarek



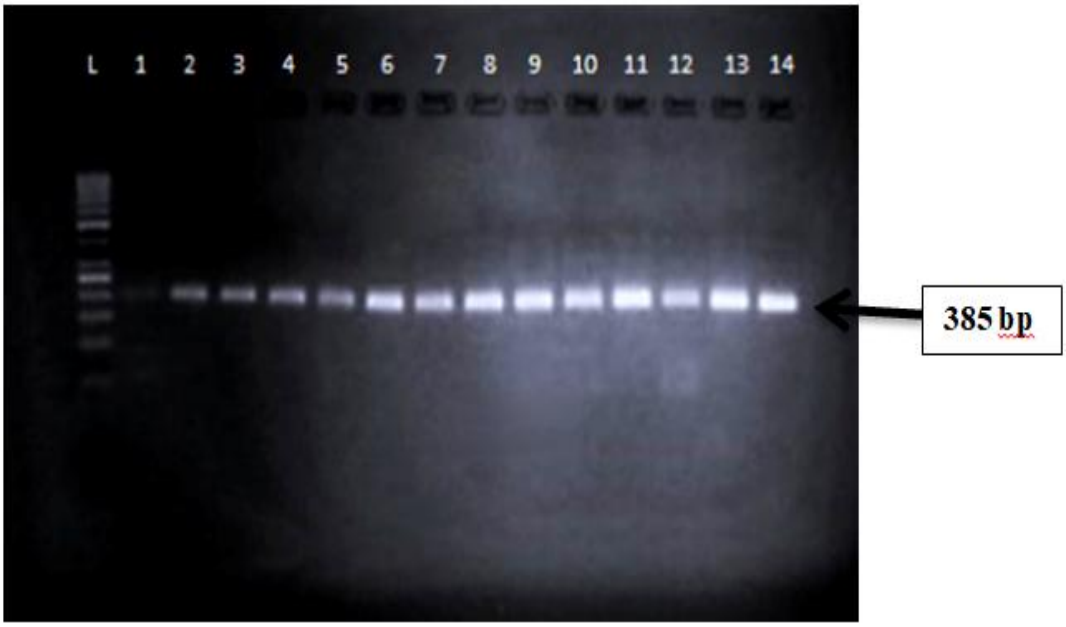
Lane: 1-7 Ladder: 50 bp

Fig 1: PCR amplified product of 3'UTR region of *OLR1* gene in Murrah buffaloes



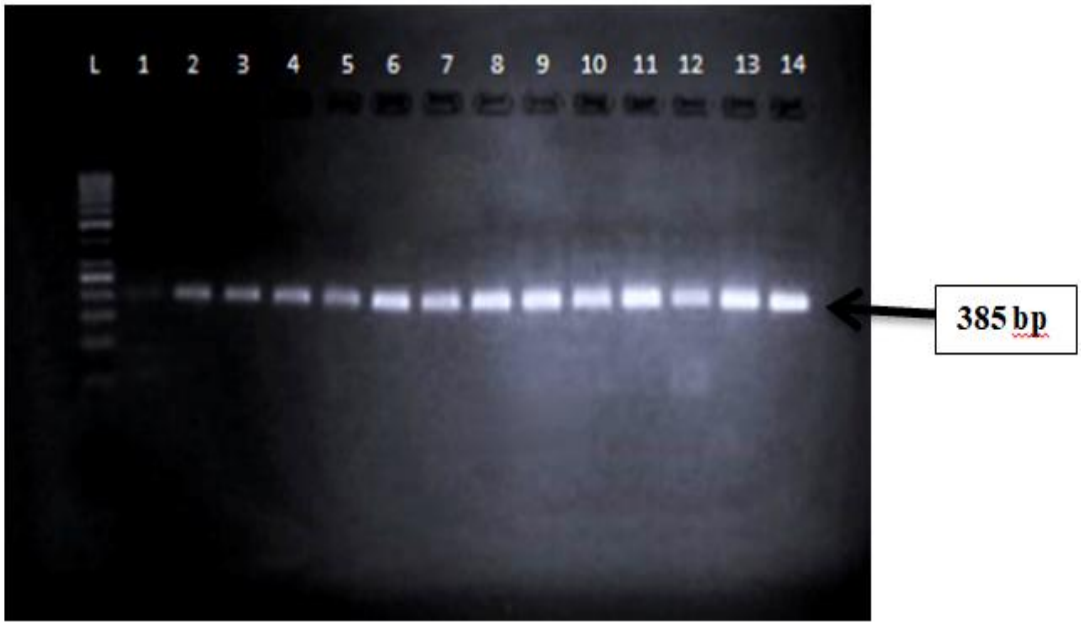
Lane: 1-7 Ladder: 50 bp

Fig 2: PCR-RFLP product of 3'UTR of *OLR1* gene (*Pst1*) in Murrah buffaloes



Lane: 1-14 Ladder:100bp

Fig 3: PCR amplified product of exon 1 region of *OLR1* gene in Murrah buffaloes



Lane: 1-14 Ladder: 100 bp

Fig 4: PCR-RFLP product of exon1 of *OLR1* gene (*Alu1*) in Murrah buffaloes

Fig 5: ClustalW analysis of amplified *OLR1*gene sequence in Murrah buffaloes compared with reference sequence of *Bos taurus*

and Dorynek (2009) explored 3'UTR region of *OLR1* in Holstein Friesian bulls and found three types of genotypes i.e. CC, AC and AA. They observed decreased milk fat percentage in AA genotyped animals and reported substantial association of C allele with fat yield and fat percentage. However, Schennink *et al.* (2009) reported that there is no association between *OLR1* gene and fat percentage in Dutch Holstein Friesian cows. Similar to present findings, in a Czech Fleckvieh population, there was no association among *OLR1* polymorphism and either milk production or reproduction traits (Rychtarova *et al.,* 2014). The polymorphism of *OLR1* gene in Mehsana buffaloes was investigated by Deshpande *et al*. (2013) using a 288 bp fragment of the 3'UTR region. They found monomorphic patterns, but when compared to the published cattle sequence, they found that the nucleotide sequences of cattle and buffalo differed at nine different positions viz., 85, 91, 116, 129, 151, 168, 171, 217, and 240.

In present study, 385 bp fragment of exon 1 region of *OLR1* gene was digested using *AluI* enzyme. It was expected that the *OLR1* gene would have an *AluI* restriction site at 385 bp fragment, which would produce fragments of 309, 199, 80, 50, and 58 bp. However, screening the *OLR1*/*AluI* products from the same 100 Murrah buffalo samples revealed an alike restriction pattern, suggesting the absence of restriction site and yielding only 385 bp fragment (Figures 3 and 4). On contrary, Javed *et al.* (2018) reported polymorphisms in exon 1 region of the gene and its significant association with the milk fat content in Nili-Ravi buffaloes.

Further, PCR-amplified products of a total of 50 samples of exon 1 region of *OLR1* gene were get sequenced using Sanger sequencing. Multiple sequence alignment was done with the help of BioEdit software. On comparing

Figure 6: Clustal W analysis of amplified *OLR1* genesequence in Murrah buffaloes compared with reference sequence **of *Bos taurus***

the sequencing results with the reference sequence of *Bos taurus* (*NC_037332.1*), nucleotide changes were observed at five positions: g.1C>T, g.71A>G, g.108C>A, g.148C>T, and g.168C>G. Among these, three were transition mutations, and two were transversions. Figures 5 and 6 are representing nucleotide changes in Murrah buffaloes as compared to *Bos taurus* reference sequence (NC_037332.1). In Jaffarabadi and Surti breeds of buffaloes, Shabir *et al.* (2011) found three genetic variations (SNPs) i.e. 423 (T>C), 843 (T>C) and 866 (T>A) in intron 1 of *OLR1* gene.

The present study employed the PCR-RFLP technique to screen specific loci in Murrah buffaloes, revealing a monomorphic pattern across all loci examined. This indicates the absence of genetic variation at these loci within the studied population. However, subsequent nucleotide sequencing of samples uncovered nucleotide changes at various positions in Murrah buffaloes compared to *Bos taurus*. These variations can serve as selection signatures, highlighting regions of the genome that may have undergone positive selection in Murrah buffaloes.

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