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NOVEL POLYMORPHISM IN OLR1 GENE IS ASSOCIATED WITH HIGH MILK FAT CONTENT IN RIVER BUFFALO

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Milk production traits have been focus of livestock breeders and animal geneticist for the selection of dairy animals with superior phenotypic and genotypic tendencies. Oxidized low density lipoprotein receptor 1 (OLR1) has been found controlling high milk fat content in dairy cattle of many regions but no association has been reported in buffaloes so far. Buffalo being an efficient converter of poor quality roughages into valuable products as milk and meat is known as black gold of Asia. Present research was conducted for genetic exploration of OLR1 gene in river buffalo breed of Pakistan (Nili-Ravi). Animals were categorized in high and low milk fat content (threshold ~ 8% milk fats). Genetic analysis revealed a total of fifteen variations in the sequences of OLR1 gene. Chi square test revealed only one variation obeying hardy Weinberg equilibrium. This novel mutation (P17H) was further analyzed by genotyping using *AluI* restriction enzyme. AA genotype of this variation was associated with high milk fat content (8.86%). Results of this study illustrated the significance of this marker as selection signature in our quest for genetically superior dairy buffaloes to enhance production potential of our animals.
Keywords: Association, buffalo, genotyping, milk fat %age, OLR1, SNP.

INTRODUCTION

Genetic association of production traits with candidate genes has been the most promising application of gene based technologies to enhance the production capacities of dairy animals. Many of the breeds have been improved by genetic selection on the basis of novel selection signatures in significant parts of the genes and still our quest for new markers is in the run. Oxidized low density lipoprotein receptor 1 (OLR1) is a type-II membrane surface protein that belongs to C-type Lectin family (Khatib *et al*., 2006). This receptor acts as the major cell surface receptor for oxidized low density lipoprotein (Ox-LDL) (Kataoka *et al*., 2000). The *OLR1* gene is located in BTA5, it encodes the lectin-like oxidized low-density lipoprotein receptor, a protein that binds, internalizes and degrades oxidized low-density lipoproteins (oxLDL). Several studies with dairy cattle had shown significant effects of the marker rs109019599 (also descripted as g.8232C>A or C223A) in 3′ untranslated OLR1 gene with milk fat production (Ates *et al*., 2014; Wang *et al*., 2012). Data on cattle genome maps have provided its location on bovine chromosome number 5 (Chen *et al.*, 2001). There are five exons found in the gene that encode a total of 270 amino acid residues protein. Bovine chromosome-5 is very strong and well characterized region controlling dairy potentials (De Koning *et al.*, 2001; Olsen *et al.*, 2002; Heyen *et al*., 1999; Awad *et al*., 2010; Schopen *et al*., 2011; Rodriguez-Zas *et al.*, 2002). OLR1 gene is also present in this location and has recently gained much of dairy geneticists attention for presence of imperative genetic markers that can be helpful in selection of animals with superior dairy potentials. The present study investigated the association of a single nucleotide polymorphism (SNP) in the OLR1 gene with milk fat content in 198 river buffaloes of Nili-Ravi breed. PCR-RFLP technique was performed for genotyping the animals. A total of fifteen SNPs were identified. Out of these, genotype AA of first polymorphism was found to have strong association with high milk fat content (8.8% age). Identified selection signature can serve as genetic marker for section of superior dairy buffaloes to enhance the milk fat content of our dairy animals.

MATERIALS AND METHODS

***Sampling strategy*:** A total of 298 animals of Nili-Ravi buffalo breed were selected from government and private livestock farms (Buffalo Research Institute, Pattoki; Livestock experimental Station, Okara). Animal were categorized into two groups. Group-1 included animals in first month of their second lactation with milk fat content more than 8% (n=189). In group-2, animals were selected with same cyclic stage (first month of second lactation) but with milk fat content less than 8% (n=109). Then selected animals were subjected to blood sampling. 10mL blood was collected from each animal in EDTA added vaccutainer. Blood was immediately transferred to the ice cooler and was shifted to

Table 1. List of Primers of *OLR1* gene.

Sr#	Primer Name	Tm	GC %	Product Size	Primer Sequence (5'-3')
1	*OLR1* F1	59.8	47.6	385	CA CACAGATTCACCACTTTCCCTTCC
2	*OLR1* R1	58.7	50.1		CCACACCCAGGCATTGTAGTT
3	*OLR1* F2	57.6	42.9		GATATTGAATCCCAGCTCCT
4	*OLR1* R2	57.9	42.7	465	CATCTTCCCATTCACTCCTA
5	*OLR1* F3	56.8	48.0		GTTGGGTTGATTTGTTGTC
6	*OLR1* R3	55.4	42.3	508	GGACCTCTAATGTAGAACCTG
7	*OLR1* F4	59.2	47.8		AGTCTGGGTGTAATTCTGAC
8	*OLR1* R4	60.1	55.0	490	CTTTACAGCGATGTCTAGTG
9	*OLR1* F5	55.5	43.1		GCTCCACTAGACATCGCTG
10	*OLR1* R5	56.4	41.0	390	CAGTGAGAAGCCCACACATC
11	*OLR1* F6a	59.5	47.9	512	CCAACTCTCTCACACAAGGAC
12	*OLR1* R6a	60.3	45.0		GGACTTGGAACAAGTTAGGG
13	*OLR1* F6b	58.8	40.9		GGATCTGGAGGAAAAGAAGG
14	*OLR1* R6b	58.3	40.9	497	GCAAAGGCAATGTAGTGA
15	*OLR1* F6c	56.7	42.5		CCTAACTCAAGGTCACAGC
16	*OLR1* R6c	57.1	42.9	453	GGACAAGCCAATTTAAGAC
17	*OLR1* F6d	57.4	40.8		CTTGGAATCACATGGTAGT
18	*OLR1* R6d	57.2	42.0	574	GAGATTCTAGTCCATGAAATC

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Genomic DNA extraction, PCR amplification and sequencing: DNA was extracted by using organic DNA extraction protocol reported by Maryam *et al*. (2012) with some modifications. Specific sets of primers (Table 1) were used to amplify the all exons of OLR1 gene (Accession no. NC_007303.3). For amplification, strand template, $MgCl_2$, Buffer, Primers set, Taq Polymerase and dNTPs were used. Reaction temperatures have been mentioned in Table 1. Then PCR amplicons were purified and sent for DNA sequencing.

Bioinformatics analysis: Identified sequences were aligned with OLR1 gene sequence reported in cattle (AC_000162.1) and total of fifteen SNPs were identified (Table 2, Fig. 1). These variations were tested for Hardy Weinberg Equilibrium and only one (P17H) was found obeying HWE and was selected for association analysis.

Genotyping: P17H was further genotyped by restriction digestion by using *AluI* enzyme. Reaction mixture composition has been given in Table 3. Restriction map for this variation has been given in Fig. 2. A total of 198 animals were genotyped for OLR1 and genotypic and allelic frequencies were calculated. This data was used for the association analysis.

Statistical analysis: Because of tracing back pedigree information for three generations, the total number of animals included in this experiment reached 496. For the association studies, the trait of interest (fat %age) was analyzed using statistical programs e.g. POPGENE version 1.32 for Hardy–Weinberg equilibrium and VassarStats to check the final effects of genotypes on the milk fat %age:

Table 2. Polymorphic sites detected in the *OLR1* region.

Genetic variants	Transition/ Transversion	Chi test
P17H	Transversion	1.2051**
80942C>A	Transition	0.0010*
S57Y	Transversion	0.0005*
A68G	Transversion	0.0013*
84396A>C	Transversion	0.1306**
K89E	Transversion	0.0913**
84454T>C	Transition	0.0011*
84484A>C	Transversion	0.0010*
84569C>T	Transition	0.0015*
89859T>C	Transition	0.1014**
90332T>C	Transition	0.2403**
90355T>C	Transition	0.0000
90986G>A	Transition	0.1410**
91007G>A	Transition	0.0021*
R181T	Transversion	0.0002*

**Significant, **Non-significant*

Table 3. Reaction recipe for restriction digestion (Incubation Temp=37°C for 24 hrs.).

Amplified Template	10µl
Restriction buffer	2µl
Restriction Enzyme	0.7µl
H_2O	17.3µl

$$Y=\mu+hsy+L+G+\alpha+e$$

Y = performance data of fat %age of animals, µ=overall mean, hys = herd- season - year effect, L = fixed effect of lactation, G = fixed effect of polymorphisms corresponding to the genotype, α = random polygenic effect for all known pedigree relationships, e=random residual.

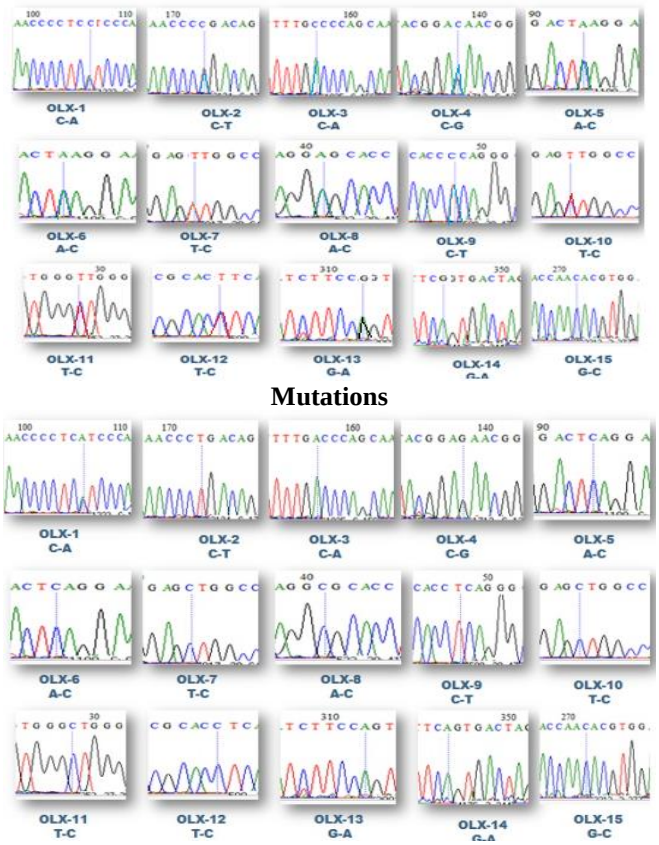

Figure 1. Sequences of *OLR1* gene showing DNA sequence variation.

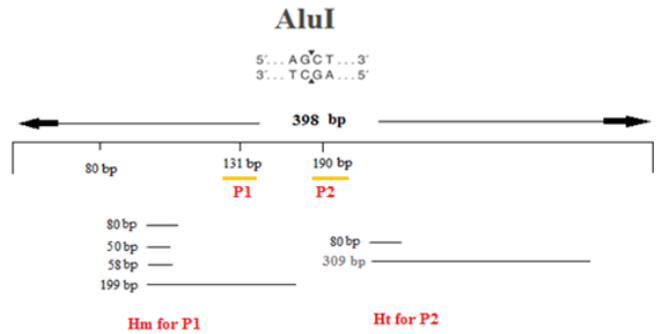

Figure 2. Restriction Map of *OLR1*.

RESULTS

OLR1 gene was studied for the identification of biomarkers. Khatib *et al*. (2006) identified the genetic variation in this gene that was associated with milk fat content. In present study, total fifteen polymorphisms were identified (Table 2, Fig. 1). Out of these 15, four were intronic and remaining eleven were exonic. From these eleven, five were synonymous and were not changing any amino acid. Remaining six were non-synonymous. Ratio of transition and transversion is 1.15:1.

Results of single marker analysis depict the distribution of alleles indicated that 84396A>C[P= 0.1306 >0.05] , K89E [P= 0.0913>0.05], 89859T>C [P= 0.1014>0.05], 90332T>C [P= 0.2403>0.05] and 90986G>A [P= 0.1410>0.05] were non-significant and following Hardy-Weinberg equilibrium indicating that the alleles were randomly distributed throughout the population, no migration had occurred, no bottlenecks happened. While for loci P17H [P= 0.0001<0.05], 80942C>A [P= 0.0010<0.05], S57Y [P= 0.0005<0.05], A68G [P= 0.0013 <0.05], 84454T>C [P= 0.0011<0.05], 84484A>C [P= 0.0010<0.05], 84569C>T [P= 0.0015<0.05], 91007G>A [P= 0.0021 <0.05], and R181T [P= 0.0002 <0.05] as probability value of Chi-square test was below 0.05, suggesting that population at these polymorphic sites was indicating significant deviations from Hardy-Weinberg equilibrium. These results have been given in Table 2. All of mutations were novel and were not reported before. Most of variations were identified in exon-4. Khatib *et al*. (2006) also reported associated mutations in exon-4.

DISCUSSION

Out of total fifteen variants, one was found obeying HWE and was selected for association analysis. Description of identified variations has been mentioned in Table 2. Chi square testing was performed on these variations and P-value (>0.05) was calculated. P17H was selected for genotypic analysis to study the association of this probable marker (Fig. 3).

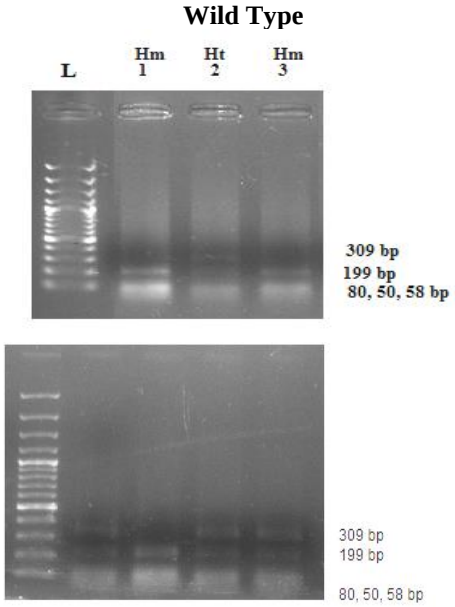

Figure 3. Electrophoretic pictorial of the restriction digestion.

It was found that AA genotype was more frequent and animals carrying this genotype were having high milk fat %age (8.86%). Results of genotypic and allelic frequencies are given in Table 4 and 5. Outcomes of association analysis has been given in Table 6.

Table 4. Allele Frequency for all Loci of *OLR1*.

SNP ID	Allele Frequency		Minor Allele Frequency
P17H	**C**	**A**	0.2771
	0.7229	0.2771	
84396A>C	**A**	**C**	0.0120
	0.9880	0.0120	
K89E	**A**	**C**	0.0723
	0.9277	0.0723	
89859T>C	**T**	**C**	0.2530
	0.7470	0.2530	
90332T>C	**T**	**C**	0.0241
	0.9759	0.0241	
90986G>A	**G**	**A**	0.2289
	0.7711	0.2289	

Table 5. Genotypic Frequency for all Loci of *OLR1*.

0.2683	0.1951	0.5366
0.5484	0.0645	0.3871
0.4516	0.0968	0.4516
0.2439	0.5854	0.1707
0.2195	0.5122	0.2683
0.5366	0.1951	0.2683

The allele frequencies identified at this position were not in-accordance with those reported by Khatib *et al*. (2006), Wang *et al.* (2012) and Komisarek & Dorynek (2009), who reported 0.46, 0.43 and 0.42 for allele A and 0.54, 0.57 and 0.58 for allele C in US, Polish and the Israeli Holstein cattle populations, respectively. However, they are consistent with the frequencies reported by Schennink *et al*. (2009) with 0.71 and 0.29 for alleles A and C in an experiment with a Dutch Holstein population. Allele C was found associated with high milk fat %age.

Association analysis revealed one significant association of identified loci with milk fat %age. Restriction digestion was performed to calculate genotypic and allele frequency of the P17H mutation. For P17H, BB genotype was more frequent with value of 0.5366. Kataoka *et al*. (2000) studied OLR1 and found similar genotypic frequency. The present study is an example of candidate gene approach to find some novel variations at population level. This study is first step in finding some probable markers for milk fat %age in Nili-Ravi buffalo that can be used in future selection and breeding program.

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Table 6. Single marker association by One Way ANOVA.

Genetic Variations	AA (Mean ± SE)	AB (Mean ± SE)	BB (Mean ± SE)	P-value (0.05)
P17H	n=26 6.1167± 0.14 [b]	n=19 5.55±0.2958[b]	n=53 8.86±0.0282[a]	<.0001
84396A>C	n=54 8.26±0.5671	n=06 8.85±1.2527	n=38 7.26±0.3881	0.351946
K89E	n=45 9.26±0.6794	n=09 6.35±1.2659	n=45 8.06±1.0328	0.171322
89859T>C	n= 24 5.86±0.3655	n= 58 7.35±0.4113	n= 17 7.12±0.6272	0.114209
90332T>C	n= 21 5.86±0.3655	n= 51 6.6±0.238	n= 26 7.92±0.8974	0.087217
90986G>A	n= 53 6.66±0.7935	n= 19 8.1±0.8406	n= 26 8.92±0.4954	0.106808

Notes: P-value refers to the results of association analysis between each SNP and milk Fat %age. Means within a row with different superscripts differ ($P < 0.05$).

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